American Peptide Symposium

26th

11th International Peptide Symposium

June 22nd – June 27th, 2019
Monterey, CA

Catch the New Wave of Peptide Science!

APS
MONTEREY - 2019

Co Chaired by
Paramjit Arora
& Anna Mapp

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THE 26TH AMERICAN PEPTIDE SYMPOSIUM

26th American Peptide Symposium

WELCOME!

Welcome to Monterey and the 26th American Peptide Symposium, APS2019! We are delighted that this meeting is being held in conjunction with the 11th International Peptide Symposium.

APS2019 will present new developments guiding the chemistry and biology of peptides. Peptides have historically served as leads for drug design but are now increasingly accepted as drug candidates. Peptides are also attractive as materials as innovative synthesis and bioconjugation methods enable large-scale preparation of novel architectures. Of course, these new opportunities present new challenges that require out of the box thinking. We sought to construct a meeting program that reflects the modern landscape of the field, with recent successes, new opportunities and unmet challenges all represented. The field of peptide science is highly diverse in content and practitioners. The innovation and energy in the field is a direct result of this diversity. The APS has always served as a forum for bringing together an engaging and supportive group of participants; we hope you will find this year to be no different.

The 2019 APS program will have two keynote lectures from renowned scientists. Professor and Nobel Laureate Frances Arnold of the California Institute of Technology will open the symposium at 6 PM on Saturday, June 22, with her lecture "Innovation by Evolution: Bringing New Chemistry to Life," followed by a reception in the Serra Ballroom of the Monterey Conference Center. Exhibits will be open during this social event. The closing talk on Thursday, June 27, will be by Professor Helma Wennemers of ETH-Zurich on "Controlling Supramolecular Assemblies with Peptidic Scaffolds." The closing banquet will start that evening at 7:00 PM in the De Anza Ballroom.

The conference will feature talks by scientists at the leading edge of peptide science and at a variety of career stages. This includes seminars by winners of the American Peptide Society Awards. The Merrifield Award lecture will be presented on Sunday, June 23 by Professor Lila Gierasch of the University of Massachusetts, Amherst. On Monday, June 24, Professor Fernando Albericio of the University of KwaZulu-Natal will deliver the Goodman Lecture. Professor Xuechen Li, University of Hong Kong, will give the Makineni Award lecture on Tuesday, June 25. Thursday, June 27 will feature two du Vigneaud Award lectures by Professors Annette Beck-Sickinger of Leipzig University and Hiroaki Suga of the University of Tokyo. Make sure to look for the inaugural Early Career Award Lectureships presented by Professor Jevgenij Raskatov, University of California–Santa Cruz and by Professor Monika Raj, University of Auburn. Finally, a key part of the scientific program are the two poster sessions, which will include remarkable young scientists who are part of the Young Investigator Poster Competition.

We greatly appreciate the generous support of our sponsors and exhibitors who make this conference possible. We strongly encourage you to interact with them and learn about the exciting products, services and equipment they have available. Without their support, the exciting scientific program as well as the social events would not be possible.

Thank you for joining and participating in the conference! We hope that you will have a wonderful time, both scientifically and socially!

Paramjit Arora, Co-Chair

Anna Mapp, Co-Chair

26th American Peptide Symposium

A Message from the President of the American Peptide Society

On behalf of the American Peptide Society, welcome to the 25th American Peptide Symposium and 11th International Peptide Symposium! We are excited that you are here to "Catch the New Wave of Peptide Science" in Monterey, CA. Peptide science is indeed having a new wave, with exciting advances in basic, translational, and pharmaceutical research. We will see examples of all of these areas, while covering a broad range of topics connecting chemical, materials, biological, pharmaceutical and medical science. The symposium will no doubt get off to a fantastic start with the opening lecture by Nobel Laureate Frances Arnold (Caltech) and close with inspiring research by Helma Wennemers (ETH). The impressive program also includes representative research from a diverse group of people from around with world. This biennial symposium assembles national and international peptide scholars, researchers, educators and students together with corporate exhibitors and sponsors to learn, discuss, and connect around our common interests.

The Society has undergone a number of exciting changes since our last meeting two years ago — we have a new logo and a new webpage to reflect the new wave of peptide science (if you love the new logo, you can thank the entire council for their input; if you hate it, you can blame me!) I encourage you to check out the new website (www.americanpeptidesociety.org) with up-to-date highlights of recent research by society members – we hope it will be your go-to site to learn about exciting research in the field. I also encourage you to become a member of the society if you are not already — membership is free and a great way to stay connected! The APS also partners with Wiley to support our society journal, Peptide Science, which publishes both original articles and reviews that cover all aspects of peptide science. The journal was recently split from Biopolymers, so now is our chance to make it the top journal in the peptide field! Please consider publishing your latest work there.

During this meeting we will celebrate the achievements of peptide scientists at every stage of their career. I look forward to recognizing this year's recipient of our highest honor, the Merrifield Award, Lila Gierasch, on Sunday afternoon. I also congratulate the recipients of the du Vigneaud Award, Annette Beck-Sickinger and Hiroaki Suga, given for achievements in mid-career; the Makineni Award recipient, Xuechen Li, given for a recent advancement in peptide science; and the Goodman Award recipient, Fernando Abericio, given for lifetime achievement. In addition, I am pleased to celebrate our inaugural Early Career Lecturers, Monika Raj and Jevgenij Raskatov, for their impressive contributions to peptide science.

Our co-chairs Bobby Arora and Anna Mapp have assembled an outstanding and inclusive program covering a breadth of exciting topics. I congratulate them on their successful efforts to include both established Society members as well as many new faces. Chairing the symposium requires a tremendous amount of time dedication so, on behalf of the entire membership, I thank Anna and Bobby for their outstanding service to the Society. The conference could not take place without considerable effort from our colleagues who have been involved in the planning and execution of the Symposium as well as those presenting lectures and posters and hosting the exhibits. I look forward to a stimulating week and hope everyone enjoys the symposium. In addition to learning about much great science, I hope you have time to mingle, network, and enjoy the beautiful Monterey Peninsula!

Marcey Waters

26th American Peptide Symposium

APS SYMPOSIA CHRONOLOGY

Symposium	Year	Chair(s)	Location
First	1968	**Saul Landa** Yale University, **Boris Weinstein** University of Washington-Seattle	Yale University New Haven, CT
Second	1970	**F. Merlin Bumpus** Cleveland Clinic	Cleveland Clinic Cleveland, OH
Third	1972	**Johannes Meinhofer** Harvard Medical School	Children's Cancer Research Foundation Boston, MA
Fourth	1975	**Roderich Walter** University of Illinois Medical Center-Chicago	The Rockfeller University and Barbizon Plaza Hotel, New York, NY
Fifth	1977	**Murray Goodman** University of California-San Diego	University of California-San Diego San Diego, CA
Sixth	1979	**Erhard Gross** National Institutes of Health	Georgetown University Washington, DC
Seventh	1981	**Daniel H. Rich** University of Wisconsin-Madison	University of Wisconsin-Madison Madison, WI
Eighth	1983	**Victor J. Hruby** University of Arizona	University of Arizona Tucson, AZ
Ninth	1985	**Kenneth D. Kopple** Illinois Institute of Technology **Charles M. Deber** University of Toronto	University of Toronto Toronto, Ontario, Canada
Tenth	1987	**Garland R. Marshall** Washington University School of Medicine, St. Louis	Washington University St. Louis, MO
Eleventh	1989	**Jean E. Rivier** The Salk Institute of Biological Studies, LaJolla	University of California-San Diego San Diego, CA
Twelfth	1991	**John A. Smith** Massachusetts General Hospital	Massachusetts Institute of Technology Cambridge, MA
Thirteenth	1993	**Robert S. Hodges** University of Alberta-Edmonton	Edmonton Convention Center Edmonton, Alberta, Canada
Fourteenth	1995	**Pravin T.P. Kaumaya** The Ohio State University	The Ohio State University Columbus, OH
Fifteenth	1997	**James P. Tam** Vanderbilt University	Nashville Convention Center Nashville, TN

Symposium	Year	Chair(s)	Location
Sixteenth	1999	**George Barany** University of Minnesota-Minneapolis **Gregg B. Fields** Florida Atlantic University	Minneapolis Convention Center Minneapolis, MN
Seventeenth	2001	**Richard A. Houghten** Torrey Pines Institute for Molecular Studies, CA **Michal Lebl** Illumina, Inc., CA	Town and Country Resort Hotel San Diego, CA
Eighteenth	2003	**Michael Chorev** Beth Israel Deaconess Medical & Harvard Medical School, MA **Tomi K. Sawyer** ARIAD Pharmaceuticals Inc., MA	Boston Marriott Copley Place Boston, MA
Nineteenth	2005	**Jeffery W. Kelly** The Scripps Research Institute, CA **Tom W. Muir** Rockefeller University, NY	Town and Country Resort Hotel San Diego, CA
Twentieth	2007	**William D. Lubell** University of Montreal **Emanuel H. F. Escher** University of Sherbrooke	Palais des Congres Montreal, Canada
Twenty-first	2009	**Richard DiMarchi** Indiana University **Hank Mosberg** University of Michigan	Indiana University Bloomington, Indiana
Twenty-second	2011	**Philip Dawson** The Scripps Research Institute **Joel Schneider** National Cancer Institute	Sheraton San Diego San Diego, CA
Twenty-third	2013	**David Lawrence** UNC Chapel Hill **Marcey Waters** UNC Chapel Hill	Hilton Waikoloa Village Waikoloa, Hawai'i
Twenty-fourth	2015	**Ved Srivastava** GlaxoSmithKline **Andrei Yudin** University of Toronto	Hyatt Regency Grand Cypress Orlando, FL
Twenty-fifth	2017	**Jonathan Lai** Albert Einstein College of Medicine **John Vederas** University of Alberta	Whistler Conference Centre Whistler, BC, Canada
Twenty-sixth	2019	**Paramjit Arora** New York University **Anna Mapp** University of Michigan	Portola Hotel and Monterey Conference Center Monterey, CA

26th American Peptide Symposium

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SYMPOSIUM-AT-A-GLANCE

Saturday, June 22, 2019

1:00 PM – 6:00 PM	Registration	De Anza Foyer
1:00 PM – 4:30 PM	APS Council Meeting	Bonsai II
4:00 PM – 5:30 PM	**WORKSHOP**	Cottonwood Room
6:00 PM – 7:00 PM	**OPENING REMARKS AND OPENING PLENARY KEYNOTE**	De Anza Ballroom
7:00 PM – 10:00 PM	Opening Reception with Exhibitors	Serra Ballroom

Sunday, June 23, 2019

7:30 AM – 4:30 PM	Registration	De Anza Foyer
8:00 AM	Group 1 Poster Set up	Serra Ballroom
8:00 AM – 8:10 AM	Opening Remarks: APS 2019 Co-Chairs	De Anza Ballroom
8:10 AM – 10:05 AM	**SESSION 1: PEPTIDE DRUG HUNTERS: REGULATING RECEPTORS WITH PEPTIDE HORMONES**	De Anza Ballroom
10:05 AM – 10:30 AM	Coffee with Exhibitors & Posters	Serra Ballroom
10:30 AM – 12:10 PM	**SESSION 2: PEPTIDE DRUG HUNTERS: TAMING DIFFICULT TARGETS**	De Anza Ballroom
12:10 PM – 1:45 PM	Lunch with Exhibitors *(provided)*	Serra Ballroom
12:10 PM – 1:45 PM	International Liaison Meeting	Bonsai II
1:50 PM – 4:00 PM	**SESSION 3: DYNAMIC PROTEINS AND FORCES THAT GUIDE THEM**	De Anza Ballroom
4:00 PM – 4:15 PM	Break	
4:15 PM – 4:45 PM	**EARLY CAREER LECTURESHIP AWARD I**	De Anza Ballroom
4:45 PM – 5:30 PM	**MERRIFIELD AWARD LECTURE**	De Anza Ballroom
7:00 PM – 9:00 PM	Student Mixer	Club Room

Monday, June 24, 2019

7:30 AM – 4:30 PM	Registration	De Anza Foyer
8:00 AM – 9:55 AM	**SESSION 4: TARGETING PROTEIN-PROTEIN INTERACTIONS**	De Anza Ballroom
9:55 AM – 10:20 AM	Coffee with Exhibitors & Posters	Serra Ballroom
10:20 AM – 10:50 AM	**EARLY CAREER LECTURESHIP AWARD II**	De Anza Ballroom
10:50 AM – 12:10 PM	**SESSION 5: TOPOLOGICALLY COMPLEX PEPTIDE MACROCYCLES**	De Anza Ballroom
12:10 PM – 1:45 PM	Lunch with Exhibitors *(provided)*	Serra Ballroom
1:45 PM – 3:50 PM	**SESSION 6: CONTROLLING SECONDARY AND TERTIARY STRUCTURES**	De Anza Ballroom
3:50 PM – 4:05 PM	Break	
4:05 PM – 5:15PM	**SESSION 7: DESIGNER PROTEIN MIMICS**	De Anza Ballroom
5:15 PM – 5:45 PM	**GOODMAN LECTURE**	De Anza Ballroom
5:45 PM – 8:00PM	**GROUP 1 POSTER SESSION & RECEPTION**	Serra Ballroom

Tuesday, June 25, 2019

7:30 AM – 12:30 PM	Registration	De Anza Foyer
8:00 AM	Group 2 Poster Set up	Serra Ballroom
8:00 AM – 9:55 AM	**SESSION 8: PROTEIN ENGINEERING AND EVOLUTION**	De Anza Ballroom
9:55 AM – 10:25 AM	Coffee with Exhibitors & Posters	Serra Ballroom
10:25 AM – 10:55 AM	**MAKINENI LECTURE**	De Anza Ballroom
11:00 AM – 12:45 PM	**SESSION 9: EXPLORING COMPLEX SYSTEMS**	De Anza Ballroom
12:45 PM – 2:45 PM	Dr. Bert L. Schram Young Scientists' Lunch & Mixer *(Graduate students & post-docs only)*	Portola Room
12:45 PM – 2:00PM	Peptide Science Editorial Board Lunch & Meeting *(Editors and Editorial Advisory Board)*	Bonsai II
3:00 PM – 5:00 PM	**WORKSHOP ON CAREER DEVELOPMENT**	Cottonwood Room

Wednesday, June 26, 2019

7:30 AM – 4:00 PM	Registration	De Anza Foyer
8:00 AM – 9:55 AM	**SESSION 10: CONSTRUCTION OF MODIFIED PEPTIDES AND PROTEINS**	De Anza Ballroom
9:55 AM – 10:20 AM	Coffee with Exhibitors & Posters	Serra Ballroom
10:20 AM – 12:15 PM	**SESSION 11: NEW FRONTIERS IN THE SYNTHESIS OF PEPTIDE MACROCYCLES**	De Anza Ballroom
12:15 PM – 12:30 PM	Announcement of 27th APS	De Anza Ballroom
12:30 PM – 1:45 PM	Lunch *(On your own)*	
12:30 PM – 1:45 PM	**WORKSHOP**	Bonsai I and II
12:30 PM – 1:45 PM	Vincent du Vigneaud Award Lunch *(by invitation)*	Portola Room
1:45 PM – 3:45 PM	**SESSION 12: METHODOLOGICAL INNOVATIONS IN PEPTIDE SCIENCE**	De Anza Ballroom
3:45 PM – 4:00 PM	Break	
4:00 PM – 5:40 PM	**SESSION 13: PEPTIDES AS CELLULAR REAGENTS**	De Anza Ballroom
5:40 PM – 6:00 PM	APS Dedications	De Anza Ballroom
6:00 PM – 8:00 PM	**GROUP 2 POSTER SESSION RECEPTION**	Serra Ballroom

Thursday, June 27, 2019

7:30 AM – 10:30 AM	Registration	De Anza Foyer
8:00 AM – 8:30 AM	**DU VIGNEAUD LECTURE 1**	De Anza Ballroom
8:30 AM – 10:00 AM	**SESSION 14: CONTEMPORARY APPROACHES TO PEPTIDE LIGAND SCREENING**	De Anza Ballroom
10:00 AM – 10:25 AM	Coffee break	De Anza Foyer
10:25 AM – 12:15 PM	**SESSION 15: METHODOLOGICAL INNOVATIONS IN PEPTIDE SCIENCE, PART 2**	De Anza Ballroom
12:15 – 1:45 PM	Lunch Break *(On your own)*	
1:45 PM – 2:15 PM	**DU VIGNEAUD LECTURE 2**	De Anza Ballroom
2:15 PM – 3:40 PM	**SESSION 16: NANOMATERIALS AND PEPTIDE ASSEMBLIES**	De Anza Ballroom
3:40 PM – 4:25 PM	**CLOSING PLENARY KEYNOTE LECTURE**	De Anza Ballroom
4:25 PM – 4:40 PM	**CLOSING REMARKS**	De Anza Ballroom
7:00 PM – 10:00 PM	Closing Banquet	De Anza Ballroom

26th American Peptide Symposium

SCHEDULE OF EVENTS

Saturday, June 22, 2019

1:00 PM – 6:00 PM — Registration.....De Anza Foyer

1:00 PM – 4:30 PM — APS Council Meeting .....Bonsai II

4:00 PM – 5:30 PM — **WORKSHOP**
Sponsored by *Biotage* .....Cottonwood Room

6:00 PM – 7:00 PM — **OPENING REMARKS AND OPENING PLENARY KEYNOTE** .....De Anza Ballroom
L01 Frances Arnold, *California Institute of Technology*
"Innovation by Evolution: Bringing New Chemistry to Life"

7:00 PM – 10:00 PM — Opening Reception with Exhibitors .....Serra Ballroom

Sunday, June 23, 2019

7:30 AM – 4:30 PM — Registration.....De Anza Foyer

8:00 AM — Group 1 Posters Set Up.....Sierra Ballroom

8:00 AM – 8:10 AM — Opening Remarks: APS 2019 Co-Chairs .....De Anza Ballroom

8:10 AM – 10:05 AM — **SESSION 1**.....De Anza Ballroom
PEPTIDE DRUG HUNTERS: REGULATING RECEPTORS WITH PEPTIDE HORMONES
Session Chairs: Ravi Nargund, *Janssen R & D*
Beate Koksch, *Freie Universität Berlin*

8:10 AM – 8:35 AM — L02 Robert Garbaccio, *Merck & Company, Inc.*
"Investigation of a Site-Specific Antibody-Peptide Conjugate as a Long Acting GLPR1r/ GCGr Agonist"

8:35 AM – 8:55 AM — L03 Ved Srivastava, *Intarcia Therapeutics*
"Peptide Therapeutics: A Novel, Highly Selective Human Glucagon Receptor Agonist, ICA349 for Treatment of Obesity"

8:55 AM – 9:20 AM — L04 Krishna Kumar, *Tufts University*
"Molecular Design of Peptide Therapeutics for Global Diseases"

9:20 AM – 9:40 AM — L05 Elisabetta Bianchi, *IRBM*
"A Discovery Case Study: Early Implementation of an Appropriate Screening Funnel is Key to Success for Peptide Therapeutics"

SCHEDULE OF EVENTS

9:40 AM –10:05 AM	**L06**	Les Miranda, *Amgen Inc.* "Data Reduction and Visualization Technologies for the Design & Optimization of Therapeutic Peptide and Nucleic Acid Derivatives"
10:05 AM – 10:30 AM	Coffee with Exhibitors & Posters .....Serra Ballroom	
10:30 AM – 12:10 PM	**SESSION 2**.....De Anza Ballroom **PEPTIDE DRUG HUNTERS: TAMING DIFFICULT TARGETS** **Session Chairs:** Tomi Sawyer, *Merck & Co., Inc.* Meritxell Teixidó, *Institute for Research in Biomedicine, Barcelona*	
10:30 AM – 10:55 AM	**L07**	David Lane, *Agency for Science, Technology, and Research, Singapore* "Stapled Peptides from Bench to Bedside"
10:55 AM – 11:20 AM	**L08**	Rami Hannoush, *Genentech* "Challenges and Emerging Approaches in Peptide Drug Discovery: Applications in Modulating Stem Cells"
11:20 AM – 11:45 AM	**L09**	Alonso Ricardo, *Ra Pharmaceuticals* "Macrocyclic Peptide Inhibitors of Complement C5 for the Treatment of Systemic and CNS Immune Disorders"
11:45 AM – 12:05 PM	**L10**	Paul Alewood, *University of Queensland* "A Single Stable Topological Stereoisomer of a Two Disulfide-Bonded Peptide Demonstrates Full Potency on the Human Norepinephrine Transporter and Blocks Chronic Neuropathic Pain"
12:05 PM – 12:10 PM	Remarks on Peptide Drug Discovery, Tomi Sawyer, *Merck & Co., Inc.*	
12:10 PM – 1:45 PM	Lunch with Exhibitors (provided) .....Serra Ballroom	
12:10 PM – 1:45 PM	International Liaison Meeting.....Bonsai II	
1:50 PM – 4:00 PM	**SESSION 3**.....De Anza Ballroom **DYNAMIC PROTEINS AND FORCES THAT GUIDE THEM** **Session Chairs:** Catherine Grimes, *University of Delaware* Joshua Price, *Brigham Young University*	
1:50 PM – 2:15 PM	**L11**	Jane Dyson, *The Scripps Research Institute* "Intrinsically Disordered Proteins – Vital Peptide Cogs in Metabolic Machines"
2:15 PM – 2:35 PM	**L12**	Marina Rubini, *University College of Dublin* "The Impact of Fluoroproline Isomers on the Folding and Thermodynamic Stability of Globular Proteins"

2:35PM – 3:00 PM — **L13** Radhakrishnan Mahalakshmi, *Indian Institute of Science Education and Research, Bhopal*
"Peptide–Based Reverse–Mapping of Membrane Protein Aggregation Loci"

3:00 PM – 3:20 PM — **L14** David Aitken, *Université Paris-Sud*
"The Role of Heteroatoms for the Control of Conformational Preferences in Peptide Foldamers"

3:20 PM – 3:45 PM — **L15** Tobin Sosnick, *University of Chicago*
"SAXS Finds that Water is a Good Solvent for Many Disordered Proteins but the Addition of Fret Fluorophores Can Alter this Property"

3:45 PM – 4:00 PM — **L16-YI** Stephen Joy, *University of Michigan*
"A Highly Potent and Selective Inhibitor of the Myb-KIX Interaction"

4:00 PM – 4:15 PM — Break

4:15 PM – 4:45 PM — **EARLY CAREER LECTURESHIP AWARD I** .....De Anza Ballroom
Introduction by Philip Dawson, *The Scripps Research Institute*

L17-AW Jevgenij Raskatov, *University of California, Santa Cruz*
"Chirality, Alzheimer's Disease and Amyloid Beta"

4:45 PM – 5:30 PM — **MERRIFIELD AWARD LECTURE** .....De Anza Ballroom
Introduction by Robert Hodges, *University of Colorado, Denver*

L18-AW Lila Gierasch, *University of Massachusetts, Amherst*
"How Peptides Led the Way to Folding in the Cell"

7:00 PM – 9:00 PM — Student Mixer.....Club Room

Monday, June 24, 2019

7:30 AM – 4:30 PM — Registration.....De Anza Foyer

8:00 AM – 9:55 AM — **SESSION 4**.....De Anza Ballroom
TARGETING PROTEIN-PROTEIN INTERACTIONS
Session Chairs: Deborah Heyl-Clegg, *Eastern Michigan University*
Jevgenij Raskatov, *University of California, Santa Cruz*

8:00 AM – 8:25 AM — **L19** Michelle Arkin, *University of California, San Francisco*
"Tackling Challenging Drug Targets using Fragment-based Ligand Discovery"

8:25 AM – 8:50 AM — **L20** Adrian Whitty, *Boston University*
"Peptide and Nonpeptide Macrocycles as Promising Chemotypes for Inhibiting Protein-Protein Interactions"

8:50 AM – 9:10 AM — **L21** Jody Mason, *University of Bath*
"A High Throughput Screen to Derive Functional Antagonists of Transcription Factors"

SCHEDULE OF EVENTS

9:10 AM – 9:30 AM
L22 Eileen Kennedy, *University of Georgia*
"Allosteric Targeting of LRRK2 in Parkinson's using Constrained Peptides"

9:30 AM – 9:55 AM
L23 Hyun-Suk Lim, *Pohang University of Science and Technology*
"Targeted Protein Degradation via the N-End Rule Pathway"

9:55 AM – 10:20 AM
Coffee with Exhibitors & Posters .....Serra Ballroom

10:20 AM – 10:50 AM
EARLY CAREER LECTURESHIP AWARD II .....De Anza Ballroom
Introduction by Marcey Waters, *University of North Carolina, Chapel Hill*

L24-AW Monika Raj, *Auburn University*
"Chemical Tools for Selective Detection of Monomethyl Lysine PTMs"

10:50 AM – 12:10 PM
SESSION 5.....De Anza Ballroom
TOPOLOGICALLY COMPLEX PEPTIDE MACROCYCLES
Session Chairs: R. Mahalakshmi, *Indian Institute of Science Education and Research Bhopal and*
Marcos Pires, *Lehigh University*

10:50 AM – 11:15 AM
L25 Christina Schroeder, *The University of Queensland*
"Rethinking Voltage-Gated Sodium Channel Inhibition"

11:15 AM – 11:30 AM
L26-YI Solomon Gisemba, *University of Florida*
"Synthesis of Bicyclic Analogs of the Kappa Opioid Receptor Antagonist Arodyn"

11:30 AM – 11:50 AM
L27 A. James Link, *Princeton University*
"Genome Mining for Lasso Peptides"

11:50 AM – 12:10 AM
L28 Sonia Henriques, *Queensland University of Technology*
"Is the Mirror Image a True Reflection? Challenging the Chirality Paradigm in Peptide-lipid Interactions"

12:10 PM – 1:45 PM
Lunch with Exhibitors (provided) .....Serra Ballroom

1:45 PM – 3:50 PM
SESSION 6.....De Anza Ballroom
CONTROLLING SECONDARY AND TERTIARY STRUCTURES
Session Chairs: Andrew Jamieson, *University of Glasgow*
Eileen Kennedy, *University of Georgia*

1:45 PM – 2:10 PM
L29 Dek Woolfson, *University of Bristol*
"Augmenting Biology through de novo Protein Design in Cells"

2:10 PM – 2:30 PM
L30 Amelia Fuller, *Santa Clara University*
"Using Peptoids for Molecular Recognition in Water"

2:30 PM – 2:45 PM

L31-YI Samuel Melton, *University of Pennsylvania*
"Variation in the Xaa and Yaa Positions of Collagen Mimetic Peptides Containing Aza-Glycine"

2:45 PM – 3:05 PM

L32 Seth Horne, *University of Pittsburgh*
"Protein Tertiary Structure Mimetics through Systematic Alteration of Backbone Covalent Connectivity"

3:05 PM – 3:25 PM

L33 Bradley Nilsson, *University of Rochester*
"Rippled beta-Sheets from Sequence Mismatched L- and D-Amphipathic Peptides"

3:25 PM – 3:50 PM

L34 Samuel Gellman, *University of Wisconsin-Madison*
"Foldamer Catalysis"

3:50 PM – 4:05 PM

Break

4:05 PM – 5:15PM

SESSION 7.....De Anza Ballroom
DESIGNER PROTEIN MIMICS
Session Chairs: Monika Raj, *University of Auburn*
Andy Wilson, *University of Leeds*

4:05 PM – 4:30 PM

L35 Philip Kim, *University of Toronto*
"Integrating Computational Design with Screening in Mammalian Cells for Novel Peptide Therapeutics"

4:30 PM – 4:55 PM

L36 Meritxell Teixidó, *Institute for Research in Biomedicine Barcelona*
"Gate2Brain: Peptide Shuttles for the Delivery of Drugs to the Brain"

4:55 PM – 5:15 PM

L37 Dehua Pei, *The Ohio State University*
"Development of a Peptidyl Calcineurin-NFAT Interaction Inhibitor that Prevents Permeability Pulmonary Edema in a Mouse Model of Acute Respiratory Distress Syndrome"

5:15 PM – 5:45 PM

GOODMAN LECTURE.....De Anza Ballroom
Introduction by Paul Alewood, *The University of Queensland*

L38-AW Fernando Albericio, University of KwaZulu-Natal
"Dissecting the SPPS Methodology: Resins, Handles, Coupling Reagents, Protecting Groups, Reagents, and Strategies"

5:45 PM – 8:00PM

GROUP 1 POSTER SESSION & RECEPTION.....Serra Ballroom

SCHEDULE OF EVENTS

Tuesday, June 25, 2019

7:30 AM – 12:30 PM — Registration.....De Anza Foyer

8:00 AM — Group 2 Poster Set up .....Serra Ballroom

8:00 AM – 9:55 AM — **SESSION 8**.....De Anza Ballroom
PROTEIN ENGINEERING AND EVOLUTION
Session Chairs: David Chenoweth, *University of Pennsylvania*
Wendy Hartsock, *Allergan*

8:00 AM – 8:25 AM — **L39** Ian Wilson, *The Scripps Research Institute*
"Broadly Neutralizing Antibody Assisted Design of Peptide-based Inhibitors of Influenza Virus"

8:25 AM – 8:50 AM — **L40** Catherine Grimes, *University of Delaware*
"Peptidoglycan and You: Perfect Together?"

8:50 AM – 9:10 AM — **L41** Jonathan Lai, *Albert Einstein College of Medicine*
"Structure-guided Design of Immunogens based on Flavivirus Glycoprotein E Domain III (EDIII)"

9:10 AM – 9:35 AM — **L42** David Liu, *Harvard University, Broad Institute*
"Base Editing: Chemistry on a Target Nucleotide in the Genome of Living Cells"

9:35 AM – 9:55 AM — **L43** Jutta Eichler, *University of Erlangen-Nurnberg*
"Antibody Paratope Mimetic Peptides"

9:55 AM – 10:25 AM — Coffee with Exhibitors & Posters .....Serra Ballroom

10:25 AM – 10:55 AM — **MAKINENI LECTURE** .....De Anza Ballroom
Introduction by Thomas Kodadek, *The Scripps Research Institute*

L44-AW Xuechen Li, *The University of Hong Kong*
"New Methods and Strategies for Protein Chemical Synthesis and Modifications"

11:00 AM – 12:45 PM — **SESSION 9**.....De Anza Ballroom
EXPLORING COMPLEX SYSTEMS
Session Chairs: Richard Cheng, *National Taiwan University*
Marina Rubini, *University College Dublin*

11:00 AM – 11:25 AM — **L45** Robert Tycko, *National Institute of Diabetes and Digestive and Kidney Diseases*
"Insights into Amyloid Formation from Solid State NMR"

11:25 AM – 11:40 AM — **L46-YI** Shehrazade Jekhmane, *Utrecht University*
"The Mode of Action of Antibiotic-peptide Plectasin in Native Membranes"

11:40 AM – 12:00 PM	L47	Neal Zondlo, *University of Delaware* "Perfluoro-tert-butyl Amino Acids to Sensitively Probe Protein Function"
12:00 PM – 12:20 PM	L48	William Pomerantz, *University of Minnesota* "Inspiration from Fluorination: Chemical Biology Approaches for Analysis of Protein-Protein Interactions"
12:20 PM - 12:45 PM	L49	Norelle Daly, *James Cook University* "Development of Granulin Derived Peptides as Wound Healing Agents"

12:45 PM – 2:45 PM — Dr. Bert L. Schram Young Scientists' Lunch & Mixer .....Portola Room
(Graduate students & post-docs only)

12:45 PM – 2:00PM — Peptide Science Editorial Board Lunch & Meeting .....Bonsai II
(Editors and Editorial Advisory Board)

3:00 PM – 5:00 PM — **WORKSHOP ON CAREER DEVELOPMENT**.....Cottonwood Room

Wednesday, June 26, 2019

7:30 AM – 4:00 PM — Registration.....De Anza Foyer

8:00 AM – 9:55 AM — **SESSION 10**.....De Anza Ballroom
CONSTRUCTION OF MODIFIED PEPTIDES AND PROTEINS
Session Chairs: Helma Wennemers, *ETH Zurich*
Frank Kotch, *Pfizer*

8:00 AM – 8:25 AM	L50	Dorothea Fiedler, *Leibniz-Forschungsinstitut für Molekulare Pharmakologie* "Inositol Phosphates and Protein Pyrophosphorylation – Challenges in Signal Transduction"
8:25 AM – 8:40 AM	L51-YI	Nina Hartrampf, *Massachusetts Institute of Technology* "Rapid, High-Fidelity Chemical Synthesis of Biologically Active Proteins and Enzymes"
8:40 AM – 9:05 AM	L52	Beate Koksch, *Freie Universität Berlin* "Bacterial Life Based on Fluorinated Amino Acids"
9:05 AM – 9:30 AM	L53	Michelle Chang, *University of California, Berkeley* "Synthetic Biology Approaches to Bioorthogonal Chemistry"
9:30 AM – 9:55 AM	L54	Ronald Raines, *Massachusetts Institute of Technology* "Ghost Proteins"

9:55 AM – 10:20 AM — Coffee with Exhibitors & Posters .....Serra Ballroom

10:20 AM – 12:15 PM — **SESSION 11**.....De Anza Ballroom
NEW FRONTIERS IN THE SYNTHESIS OF PEPTIDE MACROCYCLES
Session Chairs: Steven Castle, *Brigham Young University*
Christina Schroeder, *The University of Queensland*

SCHEDULE OF EVENTS

10:20 AM – 10:45 AM	**L55**	Jayanta Chatterjee, *Indian Institute of Science* "Can Amide Bond Thionation Improve the Pharmacological Landscape of Therapeutic Peptides?"
10:45 AM – 11:10 AM	**L56**	Masayuki Inoue, *The University of Tokyo* "Development of a Strategy for Discovery of Superior Analogues of Antimicrobial Natural Products"
11:10 AM – 11:30 AM	**L57**	William Lubell, *Université de Montréal* "Conception of Prostaglandin F2α Receptor Allosteric Modulators that Delay Preterm Birth by Harnessing the Paired Utility of Aza-Amino Acyl Proline and Indolizidinone Amino Acid Residues for Peptide Mimicry"
11:30 – 11:55 AM	**L58**	Max Cryle, *Monash University* "Understanding the Biosynthesis of the Glycopeptide Antibiotics"
11:55 AM – 12:15 PM	**L59**	David Perrin, *University of British Columbia* "The Total Synthesis of alpha-Amanitin and Natural-Product Inspired Peptides"
12:15 PM – 12:30 PM	Announcement of 27th APS	De Anza Ballroom
12:30 PM – 1:45 PM	Lunch (On your own)	
12:30 PM – 1:45 PM	**WORKSHOP**.....	Bonsai I and II
	A SUSTAINABLE SYNTHETIC METHODOLOGY FOR PEPTIDES AND PEPTIDOMIMETICS Sponsored by *CEM* (pre-registration required)	
12:30 PM – 1:45 PM	Vincent du Vigneaud Award Lunch	Portola Room
	Sponsored by BACHEM, (by invitation)	
1:45 PM – 3:45 PM	**SESSION 12**	De Anza Ballroom
	METHODOLOGICAL INNOVATIONS IN PEPTIDE SCIENCE **Session Chairs:** Sonia Henriques, *Queensland University of Technology* Jonathan Lai, *Albert Einstein College of Medicine*	
1:45 PM – 2:05 PM	**L60**	Joshua Kritzer, *Tufts University* "Cell Penetration Profiling for Biotherapeutics"
2:05 PM – 2:30 PM	**L61**	Tom Muir, *Princeton University* " Painting Chromatin with Synthetic Protein Chemistry"
2:30 PM – 2:55 PM	**L62**	Brandon Ruotolo, *University of Michigan* "Gas-Phase Structural Biology: New Technologies for the Rapid Assessment of Protein Complex Sequence, Structure, and Stability"

2:55 PM – 3:20 PM — **L63** Kathrin Lang, *Technical University of Munich*
"Expanding the Genetic Code – Protein Chemistry in Living Systems"

3:20 PM – 3:45 PM — **L64** Peng Chen, *Peking University*
"Bioorthogonal Cleavage Reactions in Space and Time: From Living Cells to Living Animals"

3:45 PM – 4:00 PM — Break

4:00 PM – 5:40 PM — **SESSION 13** .....De Anza Ballroom
PEPTIDES AS CELLULAR REAGENTS
Session Chairs: Qiang Zhang, *University at Albany*
Kamaljit Kaur, *Chapman University*

4:00 PM – 4:25 PM — **L65** Jaehoon Yu, *Seoul National University*
"Cell Penetrating Peptides to Increase Passive Permeability of Larger Cyclic Peptides as Inhibitors for Protein-Protein Interactions"

4:25 PM – 4:40 PM — **L66-YI** Stephanie Berger, *University of Washington*
"Computational Design of Structured Peptide PROTACs"

4:40 PM – 5:00 PM — **L67** Yftah Tal-Gan, *University of Nevada, Reno*
"Chemical Approaches to Study Cell-Communication in Streptococci"

5:00 PM – 5:20 PM — **L68** Shiroh Futaki, *University of Kyoto*
"Redesigning Cationic Lytic Peptides to Promote the Delivery of Biomacromolecules into Cell Interiors"

5:20 PM – 5:40 PM — **L69** Christopher Chang, *University of California, Berkeley*
"Activity-Based Sensing and Activity-Based Proteomics Approaches to Decipher Metal and Redox Biology"

5:40 PM – 6:00 PM — APS Dedications .....De Anza Ballroom
Philip Dawson, *The Scripps Research Institute*

6:00 PM – 8:00 PM — **GROUP 2 POSTER SESSION RECEPTION** .....Serra Ballroom

Thursday, June 27, 2019

7:30 AM – 10:30 AM — Registration.....De Anza Foyer

8:00 AM – 8:30 AM — **DU VIGNEAUD LECTURE 1** .....De Anza Ballroom
Introduction by Jean Chmielewski, *Purdue University*

L70-AW Hiroaki Suga, *The University of Tokyo*
"Revolutionizing the Discovery Processes of *de novo* Bioactive Peptides and Biologics"

SCHEDULE OF EVENTS

8:30 AM – 10:00 AM

SESSION 14..... .....De Anza Ballroom

CONTEMPORARY APPROACHES TO PEPTIDE LIGAND SCREENING

Session Chairs: Mark Lipton, *Purdue University*
Eranthie Weerapana, *Boston College*

8:30 AM – 8:55 AM — **L71** Eranthie Weerapana, *Boston College*
"Chemical-Proteomic Strategies to Investigate Cysteine Reactivity"

8:55 AM – 9:15 AM — **L72** Ratmir Derda, *University of Alberta*
"Synthesis and Discovery in Genetically-encoded Peptide Space"

9:15 AM – 9:40 AM — **L73** Ali Tavassoli, *University of Southampton*
"Platforms for the Generation and High-throughput Screening of Cyclic Peptide Libraries"

9:40 AM – 10:00 AM — **L74** Thomas Kodadek, *The Scripps Research Institute*
"Synthesis and Screening of Libraries of Conformationally Constrained, Peptoid-Inspired Oligomers"

10:00 AM – 10:25 AM — Coffee break.....De Anza Foyer

10:25 AM – 12:15 PM

SESSION 15..... .....De Anza Ballroom

METHODOLOGICAL INNOVATIONS IN PEPTIDE SCIENCE, PART 2

Session Chairs: Beatriz G de la Torre, *University of KwaZulu-Natal*
Amelia Fuller, *Santa Clara University*

10:25 AM – 10:50 AM — **L75** Lauren Goodrich, *Nimble Therapeutics*
"High-throughput Discovery and Parallel Optimization of Peptide Therapeutics"

10:50 AM – 11:10 AM — **L76** Andy Wilson, *University of Leeds*
"Development and Exploitation of Tools to Study Protein-Protein Interactions"

11:10 AM – 11:30 AM — **L77** Caroline Proulx, *North Carolina State University*
"In situ Oxidation of N-Phenylglycinyl Peptides for Oxime Bond Formation at Neutral Ph"

11:30 AM – 11:50 AM — **L78** Jonathan Collins, *CEM Corporation*
"A New One-Pot Process Improving the Greeness of FMOC SPPS"

11:50 AM – 12:15 PM — **L79** Andrea Peier, *Merck & Company, Inc.*
"Nanoclick Assay: A High Throughput, Target-Agnostic Permeability Assay that Combines Nanobret Technology with Intracellular Click Chemistry"

12:15 – 1:45 PM — Lunch Break (On your own)

1:45 PM – 2:15 PM

DU VIGNEAUD LECTURE 2 .....De Anza Ballroom
Introduction by Ronald Raines, *Massachusetts Institute of Technology*

L80-AW Annette Beck-Sickinger, *Leipzig University*
"Insights in the Binding Mode of Peptides, Activating G Protein Coupled Receptors"

2:15 PM – 3:40 PM

SESSION 16 .....De Anza Ballroom
NANOMATERIALS AND PEPTIDE ASSEMBLIES
Session Chairs: Caroline Proulx, *North Carolina State University*
Ali Tavassoli, *University of Southampton*

2:15 PM – 2:40 PM

L81 Leonard Prins, *University of Padova*
"Self-assembly of Peptides on Gold Nanoparticles for Molecular Recognition and Catalysis"

2:40 PM – 3:05 PM

L82 Nicole Sampson, *Stony Brook University*
"Control of Alternating Polymer Backbones in ROMP: Toward Sequence Control"

3:05 PM – 3:20 PM

L83-YI Sivan Nir-Luz, *The Hebrew University of Jerusalem*
"Tailoring the Self-assembly of a Tripeptide for the Formation of Antimicrobial Surfaces"

3:20 PM – 3:40 PM

L84 Jean Chmielewski, *Purdue University*
"Metal-promoted Assembly of Peptide-based Materials for Regenerative Medicine"

3:40 PM – 4:25 PM

CLOSING PLENARY KEYNOTE LECTURE .....De Anza Ballroom
Introduction by Marcey Waters, *University of North Carolina, Chapel Hill*

L85 Helma Wennemers, *ETH Zurich*
"Controlling Supramolecular Assemblies with Peptidic Scaffolds"

4:25 PM

CLOSING REMARKS .....De Anza Ballroom
Ved Srivastava, *Intarcia Therapeutics*

7:00 PM – 10:00 PM

Closing Banquet .....De Anza Ballroom

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EXHIBITOR LIST

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Teledyne ISCO	34
Tianjin Nankai Hecheng S&T Co. Ltd.	1
WuXi AppTec	51
Zhejiang Ontores Biotechnologies Co., Ltd	14

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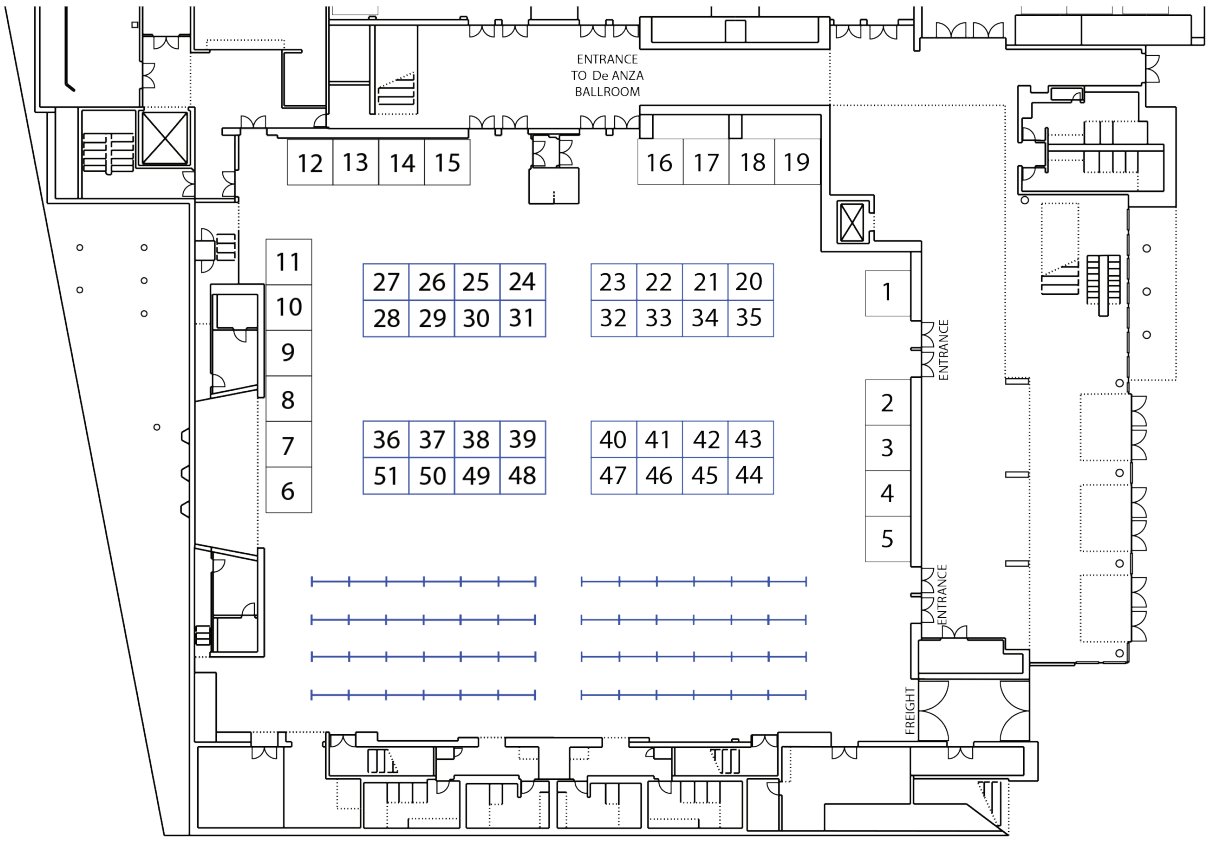
EXHIBITOR LAYOUT



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26th American Peptide Symposium

The Merrifield Award Winners*

The Merrifield Award, presented at the biennial symposia, recognizes the lifetime achievement of a peptide scientist, whose work exemplifies the highest level of scientific creativity.

2019 – Lila Gierasch, University of Massachusetts, Amherst

2017 – Charles M. Deber, University of Toronto
Robert Hodges, University of Colorado, School of Medicine

2015 – Horst Kessler, TU München Institute for Advanced Study

2013 – James P. Tam, Nanyang Technological University

2011 – Richard DiMarchi, Indiana University

2009 – Stephen B.H. Kent, University of Chicago

2007 – Isabella L. Karle, Naval Research Laboratory

2005 – Richard A. Houghten, Torrey Pines Institute for Molecular Studies

2003 – William F. DeGrado, University of Pennsylvania, School of Medicine

2001 – Garland R. Marshall, Washington University Medical School

1999 – Daniel H. Rich, University of Wisconsin – Madison

1997 – Shumpei Sakakibara, Peptide Institute, Inc.

1995 – John M. Stewart, University of Colorado – Denver

1993 – Victor J. Hruby, University of Arizona – Tucson

1991 – Daniel F. Veber, Merck Sharp & Dohme, Inc.

1989 – Murray Goodman, University of California, San Diego

1987 – Choh Hao Li, University of California, San Francisco

1985 – Robert Schwyzer, Swiss Federal Institute of Technology

1983 – Ralph F. Hirschmann, Merck Sharp & Dohme, Inc.

1981 – Klaus Hofmann, University of Pittsburgh, School of Medicine

1979 – Bruce Merrifield, The Rockfeller University

1977 – Miklos Bodansky, Case Western Reserve University

* Previously, the Alan E. Pierce Award sponsored by the Pierce Chemical Company (1977-1995). The Merrifield Award was established in 1997 by an endowment from Rao Makineni.

The 2019 R. Bruce Merrifield Award

Lisa M. Gierasch

Lila M. Gierasch earned her A. B. in 1970 from Mount Holyoke with a major in chemistry and obtained her Ph.D. in Biophysics from Harvard University in 1975, under the direction of Elkan Blout. She has held faculty positions at Amherst College, the University of Delaware, and the University of Texas Southwestern Medical School, where she held the Robert A. Welch Chair in Biochemistry. In 1994, she joined the University of Massachusetts as Head of the Chemistry Department and subsequently served as Head of the Department of Biochemistry & Molecular Biology. In 2004 she returned to full-time research and teaching. She is currently Distinguished Professor of Biochemistry & Molecular Biology and Chemistry at UMass Amherst. She was Editor-in-Chief of *Biopolymers: Peptide Science* from 2004 to 2008, and in 2016 she became the Editor-in-Chief of the *Journal of Biological Chemistry*. Professor Gierasch's research focuses on protein folding and the cellular machinery that helps maintain folded, functional proteins. In 2006 she received a NIH Director's Pioneer Award to tackle the challenging aspects of protein folding in vivo. Among her other honors are A. P. Sloan Fellowship in 1984, Guggenheim Fellowship in 1986, Vincent du Vigneaud Award from the American Peptide Society in 1984, D.Sc Honoris Causa from Mount Holyoke College in 2002, the 2006 Garvan-Olin Medal of the American Chemical Society, the 2012 Dorothy Hodgkin Award of the Protein Society in 2010, the 2014 Mildred Cohn Award of the American Society of Biochemistry & Molecular Biology, and the 2018 Ralph F. Hirschmann Award in Peptide Chemistry from the American Chemical Society. She was named a fellow of the Biophysical Society in 2014 and of the American Academy of Arts & Science in 2016.

L18-AW How Peptides Led the Way to Folding in the Cell

Lila M. Gierasch
Departments of Biochemistry & Molecular Biology and Chemistry, University of Massachusetts Amherst, Amherst, MA 01002 USA

The protein folding problem was originally framed as the challenge of understanding how the amino acid sequence of a protein encodes its complex native structure. Early protein folding research focused on determining the pathway by which an unstructured polypeptide forms structure characteristic of the native state. This view of protein folding relied heavily on relationships between amino acid sequences and their preferred structural motifs such as helices, sheets and turns, many of which were established using model peptides. For example, my studies of cyclic peptides, begun in the Blout laboratory, were centered on improving our understanding of sequence preferences for reverse turns. A paradigm shift in protein folding research took place with the recognition that the folding reaction must be described in terms of ensembles of states and conformational search over a funnel-shaped energy landscape. Ironically, at this point little attention was paid to the physiological context of protein folding. My laboratory's eyes were opened to the in-vivo protein folding problem by our studies of signal sequences and their role in targeting proteins to the secretory pathway or to membrane locations. This work brought us into the cell biology of protein biosynthesis, localization, and quality control just at the time that the molecular chaperone concept was born. Our work with peptides enabled us to be swept up in the exciting science that ensued: the intersection of the fundamental principles of protein folding and the extremely challenging environment of the cell. From this point on, we have sought to understand how molecular chaperones recognize clients that are unfolded (fully or partially) and the mechanisms whereby chaperones play roles in many processes in the cell, including but not

restricted to the folding reaction. Most recently, we have focused on a central molecular chaperone family, the Hsp70s, which facilitate folding, inhibit aggregation, maintain proteins in unfolded states, and partner with other chaperones, all through a deceptively simple mechanism. This lecture will briefly chronicle my path from research on model peptides to studies of folding in the cell and describe our current emphasis: the substrate binding and allosteric mechanism of Hsp70s.

26th American Peptide Symposium

The Rao Makineni Lectureship*

The Makineni Lectureship recognizes an individual who has made a recent contribution (within 2 years) of unusual merit to research in the field of peptide science.

2019 – Xuechen Li, The University of Hong Kong

2017 – Thomas Kodadek, The Scripps Research Institute

2015 – Paramjit Arora, New York University

2013 – Samuel H. Gellman, University of Wisconsin

2011 – Jeffery W. Kelly, Scripps Research Institute

2009 – William F. DeGrado, University of Pennsylvania

2007 – Ronald T. Raines, University of Wisconsin - Madison

2005 – Robin E. Offord, Centre Medical Universitaire, Switzerland

2003 – James P. Tam, Vanderbilt University

* Endowed by PolyPeptide Laboratories and Murray and Zelda Goodman (2003)

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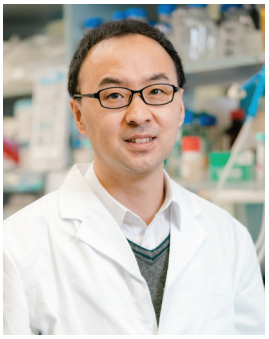
The 2017 Makineni Lectureship

Xuechen Li



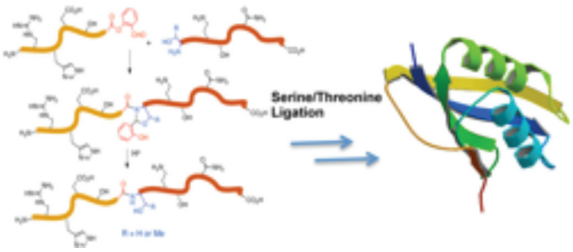
Professor Xuechen Li received his BSc degree from Nankai University, China, his MSc from the University of Alberta, Canada, and, in 2007, his PhD from Harvard University, USA. After postdoctoral work in the laboratory of Prof. Samuel Danishefsky at the Memorial Sloan Kettering Cancer Center in New York, he joined the Department of Chemistry at the University of Hong Kong as an Assistant Professor in 2009, and was promoted to Associate Professor in 2014 and Professor in 2018. He was granted the Croucher Senior Research Fellowship in 2018.

The central theme of Dr. Li's research focuses on the chemical biology of biomolecules (i.e., peptides/proteins and glycoproteins) with the aim of studying fundamental biological questions and developing potential therapeutic applications. His laboratory has developed various methods for protein chemical synthesis, peptide cyclization and protein modifications, including Ser/Thr ligation, P-B peptide desulfurization and phthalimidine protein bioconjugation. Furthermore, he has been working on the synthesis and medicinal chemistry studies of complex antibacterial cyclic peptides, with completion of the total synthesis of daptomycin and teixobactin.

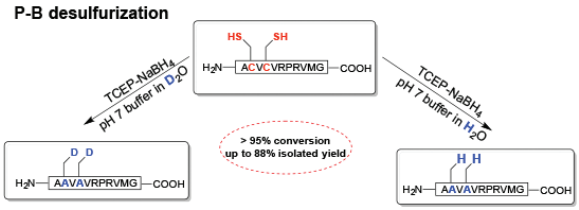
L44-AW New Methods and Strategies for Protein Chemical Synthesis and Modifications

Xuechen Li
Department of Chemistry, The University of Hong Kong; xuechenl@hku.hk

Over the past years, our laboratory has developed Serine/Threonine Ligation (STL) for protein chemical synthesis, in which an N-terminal serine or threonine of one unprotected peptide can ligate with a second unprotected peptide with the C-terminal salicylaldehyde ester to afford an *N,O*-benzylidene acetal linked peptide that upon acidolysis generates the natural peptidic Xaa-Ser/Thr linkage at the ligation site. Considering the high abundance of serine and threonine residues in natural proteins/peptides, Ser/Thr ligation will offer new options for convergent peptide and protein synthesis. Our laboratory has used Ser/Thr ligation in the synthesis of cyclic peptides including daptomycin and teixobactin, and proteins including human erythrocyte acylphosphatase, MUC1 glycopeptide, glycosylated interleukin-25, phosphorylated HMGA proteins and so on.



In addition, we recently developed an unprecedentedly mild system (TCEP/$NaBH_3$ or TCEP/$LiBEt_3H$) for chemoselective peptide desulfurization for extending native chemical ligation-desulfurization strategy in protein chemical synthesis. This method, termed P-B desulfurization, features usage of common reagents, simplicity of operation and versatile functionality compatibility. Furthermore, this method can readily incorporate deuterium into the peptide after cysteine desulfurization.



In this presentation, updated development of these methods and their applications will be discussed.

References:

1 Enabling N-to-C Ser/Thr Ligation for Convergent Protein Synthesis via Combining Chemical Ligation Approaches. Lee, C. L.; Liu, H.; Wong, C.; Chow, H. Y.; Li, X.* *J. Am. Chem. Soc.* 2**016**, *138*, 10477.
2 P-B Desulfurization: An Enabling Method for Protein Chemical Synthesis and Site- Specific Deuteration. Jin, K.; Li, T.; Chow, H. Y.; Liu, H.; Li, X.* *Angew. Chem. Int. Ed.* **2017**, *56*, 14607.
3 Serine/Threonine Ligation: Origin, Mechanistic Aspects and Applications. Liu, H.; Li, X.* *Acc. Chem. Res.* **2018**, *51*, 1643

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The Vincent du Vigneaud Award*

The Vincent du Vigneaud Awards recognize outstanding achievement in peptide research at mid-career.

2019	**Annette Beck-Sickinger,** Leipzig University
	Hiroaki Suga, The University of Tokyo
2017	**Roland T. Raines,** University of Wisconsin-Madison
	Wilfred van der Donk, University of Ilinois at Urbana-Champaign
2015	**Jean Chmielewski,** Purdue University
	David Craik, University of Queensland
2013	**Michael Chorev,** Harvard Medical School
	Kit S. Lam, University of California Davis Cancer Center
2011	**Fernando Albericio,** University of Barcelona
	Morten P. Meldal, Carlsberg Laboratory, Copenhagen
2010	**Philip Dawson,** The Scripps Research Institute
	Reza Ghadiri, The Scripps Research Institute
2008	**Tom W. Muir,** Rockefeller University
	Jeffery W. Kelly, The Scripps Research Institute
2006	**Samuel H. Gellman,** University of Wisconsin-Madison
	Barbara Imperiali, Massachusetts Institute of Technology
2004	**Steven B. H. Kent,** University of Chicago
	Dieter Seebach, ETH Zurich
2002	**Robert S. Hodges,** University of Colorado-Denver
	Horst Kessler, Technical University, Munich

2000	**Charles M. Deber,** University of Toronto **Richard A. Houghten,** Torrey Pines Institute for Molecular Studies
1998	**Peter W. Schiller,** Clinical Research Institute of Montreal **James A. Wells,** Genentech, Inc.
1996	**Arthur M. Felix,** Hoffmann-La Roche, Inc. **Richard G. Hiskey,** University of North Carolina
1994	**George Barany,** University of Minnesota-Minneapolis **Garland R. Marshall,** Washington University-St. Louis
1992	**Isabella L. Karle,** Naval Research Laboratory **Wylie W. Vale,** The Salk Institute for Biological Studies
1990	**Daniel H. Rich,** University of Wisconsin-Madison **Jean E. Rivier,** The Salk Institute for Biological Studies
1988	**William F. De Grado,** DuPont Central Research **Tomi K. Sawyer,** The Upjohn Company
1986	**Roger M. Freidinger,** Merck Sharpe & Dohme **Michael Rosenblatt,** Massachusetts General Hospital **James P. Tam,** The Rockefeller University
1984	**Betty Sue Eipper,** The Johns Hopkins University **Lila M. Gierasch,** University of Delaware **Richard E. Mains,** The Johns Hopkins University

* Sponsored by BACHEM Inc.

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The Vincent du Vigneaud Award*

Hiroaki Suga

Professor Hiroaki Suga was born in Okayama City, Japan in 1963. He received his Bachelor of Engineering (1986) and Master of Engineering (1989) from Okayama University, and Ph. D. in Chemistry (1994) from the Massachusetts Institute of Technology. After three years of post-doctoral work in Massachusetts General Hospital, he was appointed as a tenure-track Assistant Professor in the Department of Chemistry in the State University of New York at Buffalo (1997) and promoted to the tenured Associate Professor (2002). In 2003, he moved to Research Center for Advanced Science and Technology in the University of Tokyo as an Associate Professor, and soon after he was promoted to Full Professor. In 2010, he changed his affiliation to the Department of Chemistry, Graduate School of Science. His research interests are in the field of bioorganic chemistry, chemical biology and biotechnology related to RNA, translation, peptides and pseudo-natural products. He is the recipient of Akabori Memorial Award 2014, Japanese Peptide Society, Max-Bergmann Gold Medal 2016, and Nagoya Medal Silver 2017. He is also a founder of PeptiDream Inc, a publicly traded company in the Tokyo First Stock Exchange Market, which has many partnerships with pharmaceutical companies in worldwide. He also recently started MiraBiologics Inc.

L70-AW Revolutionizing the Discovery Processes of *de novo* Bioactive Peptides and Biologics

Hiroaki Suga
Department of Chemistry, Graduate School of Science, The University of Tokyo, Japan; hsuga@chem.s.u-tokyo.ac.jp

Macrocyclic peptides possess a number of pharmacological characteristics distinct from other well-established therapeutic molecular classes, resulting in a versatile drug modality with a unique profile of advantages. Macrocyclic peptides are accessible by not only chemical synthesis but also ribosomal synthesis. Particularly, recent inventions of the genetic code reprogramming integrated with an in vitro display format, referred to as RaPID (Random non-standard Peptides Integrated Discovery) system, have enabled us to screen mass libraries (>1 trillion members) of non-standard peptides containing multiple non-proteinogenic amino acids, giving unique properties of peptides distinct from conventional peptides, e.g. greater proteolytic stability, higher affinity (low nM to sub nM dissociation constants), and superior pharmacokinetics. The field is rapidly growing evidenced by increasing interests from industrial sectors, including small start-ups as well as mega-pharmas, toward drug development efforts on macrocyclic peptides, which has led to several *de novo* discovered peptides entering pre-clinical and clinical trials. This lecture discusses the aforementioned RaPID system and several showcases of therapeutic potentials of macrocyclic peptides generated by the Suga's laboratory and collaborations with other laboratories.

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H. Yu, P. Dranchak, Z. Li, R. MacArthur, M.S. Munson, N. Mehzabeen, N.J. Baird, K.P. Battalie, D. Ross, S. Lovell, C.K. Carlow, H. Suga*, J. Inglese,. "Macrocycle peptides delineate locked-open inhibition mechanism for microorganism phosphoglycerate mutases" *Nature Communications, (***2017***) Apr. 3*, 14932.

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T. Katoh; I. Wohlgemuth; M. Nagano; M.V. Rodnina; H. Suga "Essential structural elements in tRNA(Pro) for EF-P-mediated alleviation of translation stalling." *Nature communications, 7*, 11657 (**2016**)

Y. Iwane; A. Hitomi; H. Murakami; T. Katoh; Y. Goto; H. Suga*, "Expanding the amino acid repertoire of ribosomal polypeptide synthesis via the artificial division of codon boxes", *Nature Chemistry, 8*, 317–325 (**2016**)

K. Ito; K. Sakai; Y. Suzuki; N. Ozawa; T. Hatta; T. Natsume; K. Matsumoto; H. Suga "Artificial human Met agonists based on macrocycle scaffolds" *Nature Communications, 6*, 6373 (**2015**)

26th American Peptide Symposium

The Vincent du Vigneaud Award*

Annette G. Beck-Sickinger

Annette G. Beck-Sickinger studied chemistry and biology at the University of Tübingen (Germany) and received her Ph. D. in organic chemistry. She was post-doc with E. Carafoli (Laboratory of Biochemistry, ETH Zürich) and appointed as assistant professor of Pharmaceutical Biochemistry at ETH Zürich. Since October 1999, she is full professor of Biochemistry and Bioorganic Chemistry at the University of Leipzig. She spent a sabbatical at Vanderbilt University (Nashville, TN) as visiting professor.

Annette Beck-Sickinger was a member of the Board of the German Chemical Society (Gesellschaft Deutscher Chemiker, 2004-2012; Vice-President 2006-2008) and of the DFG panel "Biochemistry" (2004-2012). Since 2017 she is member of the Board of the German Society for Biochemistry and Molecular Biology (gbm) and Vice-President. She has been awarded with many prices including the Leonidas Zervas Award of the European Peptide Society, the gold medal of the Max-Bergmann-Kreis (2009), the Leipzig Science Award (2016) and the Albrecht Kossel Award of Biochemistry of the GDCh (2018). She was honoured with the membership of the Saxonian Academy of Science in 2009 and in 2012, she became an elected member of the German National Academy of Sciences Leopoldina. In 2017, she was awarded with the Saxonian Order of Merit.

Her major reseach fields include structure-activity-relationships of peptide hormones and G protein coupled receptors and protein modification to study function and interaction. A tight connection of chemical methods, bioorganic synthesis and molecular biology tools, including cloning, receptor mutagenesis, protein expression and cell biochemistry is applied. Her interests include further the identification of novel targets, novel therapeutic concepts and innovative approaches to modify proteins as well as concepts for improved enzyme catalysis and biomaterials.

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L80-AW Insights in the Binding mode of Peptides, Activating G Protein Coupled Receptors

Annette G. Beck-Sickinger
Institute of Biochemistry, Faculty of Life Science, Leipzig University, Brüderstr, 34, D 04103 Leipzig

Peptides hormones play an important role in the regulation of manifold activities in the body. Many of them transmit their activity through G-protein coupled receptors (GPCR), which are among the most promising drug targets nowadays. By means of ligand modification, receptor mutagenesis, NMR spectroscopy of recombinantly produced receptors and molecular modelling, we have identify the ligand binding site of agonists and antagonists of peptide GPCRs[1,2]. Furthermore, in combination with X-ray crystallography, cell-free receptor expression and photo¬cross-linking we identified the different binding modes of peptide agonists and non peptide antagonists[3,4] as well as different downstream pathways[5]. Knowing the specific binding mode allows the selection of appropriate sites for chemical modification of the ligand, including fluorescence labelling, lipidisation and PEGylation, which significantly modify the activity of the ligand[6,7] and leads to effective compounds *in vivo*[8]. Current application expand the direct effect of the peptide as drugs but allow using them as shuttling systems for intracellular targets e. g. in cancer or metabolic diseases [9].

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26th American Peptide Symposium

The Murray Goodman Scientific Excellence and Mentorship Award*

The Goodman Award recognizes an individual who has demonstrated career-long research excellence in the field of peptide science. In addition, the selected individual should have been responsible for significant mentorship and training of students, post-doctoral fellows, and/or other co-workers. The Awards Committee may also take into account any important contributions to the peptide science community made by the candidate, for example through leadership in the American Peptide Society and/or its journals

2019 – Fernando Albericio, University of KwaZulu-Natal

2017 – Paul Alewood, University of Queensland

2015 – George Barany, University of Minnesota

2015 – George Barany, University of Minnesota

2013 – Robert S. Hodges, University of Colorado-Denver

2011 – Victor J. Hruby, University of Arizona

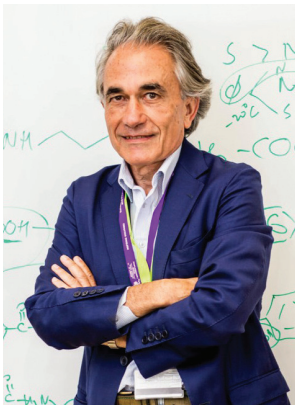
2009 – Charles M. Deber, University of Toronto

* Endowed by Zelda Goodman (2007)

26th American Peptide Symposium

The Murray Goodman Scientific Excellence and Mentorship Award

Fernando Albericio



Fernando Albericio got his Ph.D. in Chemistry at the University of Barcelona (UB) under the guidance of Ernest Giralt. After several postdoctoral stays at Tufts University (Victor Najjar); Université d'Aix-Marseille (Jurphass van Rietschoten); and University of Minnesota (George Barany) he returned to Barcelona in 1985 as an Associate Professor at the UB. From 1992-94, he was Director of Peptide Research at Millipore in Boston. He rejoined the UB after this period and was promoted to Full Professor in 1995. From 2003-2015, he was Group Leader at the Institute for Research in Biomedicine (IRB). From 2014-2015, he was founder Rector of Yachay Tech, a university in Ecuador. Since 2016, he is Research Professor at the University of KwaZulu-Natal (Durban, South Africa), where he has created the Peptide Science Laboratory.

Fernando has published more than 900 refereed papers, book chapters, and proceedings, edited 1 book, filed 60 patents, and has served as advisor for 72 Ph.D. students in different universities.

His major research interests cover practically all aspects of peptide synthesis (new reactions, building blocks, coupling reagents, solid phase supports, protecting groups, and linkers), as well as synthesis of peptides and small molecules with therapeutic activities (cancer and infectious diseases). Furthermore, his group is also involved in developing new systems for drug delivery and strategies for diagnostics as well. Lastly, he was working in the development of multicomponent platforms for the preparation of bioconjugates. More than 30 of the commodities developed by his group has reached the market: PAL-linker, PEG-PS-resin, ChemMatrix resin, Cys(Phacm), Cys(Dpm), Cys(SDmp), OxymaPure, K-Oxyma, Oxyma-B, COMU, PyAOP, PyClock, PyOxim, allyl based orthogonal chemistry, and green chemistry technologies associated to solid-phase peptide synthesis, among others. Fernando is deeply involved in the creation of the Entrepreneurial University in Spain, in Latinoamerica, and, presently, in South Africa. He participated in the foundation of the Barcelona Science Park (BSP), taking on various responsibilities, and served as General Director of this organization from 2005-2012. During his tenure at the BSP, he catalyzed the creation of almost 100 start-up companies. He is also co-founder of Biotechnology Incubator Durban (BID).

L38-AW Dissecting the SPPS Methodology: Resins, Handles, Coupling Reagents, Protecting Groups, Reagents, and Strategies

F. Albericio[a,b]
[a]School of Chemistry & Physics, University of KwaZulu Natal, Durban 4001, South Africa; Department of Organic Chemistry, University of Barcelona, 08028-Barcelona, pain

Recent years have witnessed a significant increase in the number of peptides based on Active Pharmaceutical Ingredients (APIs). Furthermore, peptides on the market have evolved from being small and linear, with approximately 10 amino acids, to being much more complex. Thus, regulatory agencies are increasingly approving cyclic peptides, longer peptides (20-40 amino acids), and peptides containing fatty acids. In research and industrial sectors, all these peptides are prepared using the solid-phase peptide synthesis (SPPS) strategy, which was developed by Merrifield in the early 60s.

This renaissance of peptides in the drug market has been, in part, possible thanks to the continuous work carried out by several groups working on diverse methodological aspects of SPPS.

In this presentation, I will go over the most important contributions to SPPS methodology made by my group in recent years. I will pay special attention to discussing resins, handles, coupling reagents, protecting schemes, and synthetic strategies for both research and industrial purposes.

26th American Peptide Symposium

Early Career Leadership Award

Jevgenij Raskatov

Jevgenij Raskatov was born in Moscow (where he grew up as a cellist) and, following the family move to Germany in 1994 studied Chemistry at the University of Heidelberg. He then moved to Oxford to pursue his graduate studies in organic chemistry with focus on asymmetric catalysis and chiral ion pairing. This was followed by a research stay at Caltech with Peter Dervan as a Humboldt postdoctoral fellow. Dr. Raskatov has started his independent research career at UCSC in 2014. At UCSC, Dr. Raskatov decided to focus his attention on the aggregation-prone peptide and the believed culprit of Alzheimer's Disease, Amyloid β. He used his strong background in physical organic chemistry and molecular stereochemistry, combined with his insight into bioactive peptides that he gained at Caltech, to create a paradigm-challenging molecular approach to convert toxic Aβ oligomers to non-toxic fibrils, using molecular symmetry logic. For this pioneering work, together with his other studies that use amino acid chirality to probe Aβ structure-function relationships he was awarded the 2018 New Investigator Award of the Boulder Peptide Society. His research program employs an unusually wide set of experimental techniques, ranging from molecular modeling and NMR to neuron biology and electrophysiology.

L17-AW Chirality, Alzheimer's Disease and Amyloid Beta

Jevgenij A. Raskatov[a],*
[a]*Department of Chemistry and Biochemistry, Physical Science Building 356, 1156 High Street, University of California Santa Cruz, Santa Cruz, CA 95064, USA;* Correspondence should be addressed to: jraskato@ucsc.edu*

Aggregation-prone polypeptides are produced by living systems, often as cleavage products of substantially larger protein precursors. Whereas their functions in health are challenging to study and not always well understood, an imbalance between their production and clearance can produce diverse pathological conditions, including Alzheimer's Disease. The believed seminal etiological agent of AD, amyloid beta forms aggregates of different size and shape, with distinctions frequently made between oligomers, protofibrils and fibrils. Oligomers are believed to be particularly harmful, whereas fibrils appear to represent an aggregation endpoint that may be relatively benign.

Through stereochemical argu¬ments, we envisioned that racemic amyloid beta should exhibit increased fibril formation and reduced toxicity. We synthesized the two enantiomers and found indeed that their equimolar mixture exhibited pronounced acceleration of fibril formation, as compared to the enantiopure coun¬ter¬parts. This led to substantial suppression of oligomer formation and inhibition of toxicity in model cell-based systems. The underlying molecular mechanisms that lead to the differences in biophysical and biological properties observed between enantiopure and racemic Aβ42 remain subject of active research in our laboratory.

26th American Peptide Symposium

Early Career Leadership Award

Monika Raj

Dr. Monika Raj is an Assistant Professor in the Department of Chemistry and Biochemistry at Auburn University. She obtained her Ph.D. in Organic Chemistry at the Indian Institute of Technology, Kanpur. She then pursued post-doctoral studies at the University of Pennsylvania and New York University. In the fall of 2014, she joined the faculty of Chemistry & Biochemistry at Seton Hall University at New Jersey before moving to the Auburn University in August 2017. Dr. Raj works in the field of chemical biology, which involves the development of organic chemistry tools for solving the problems in the field of biology and medicine. This includes the development of new chemical reactions including catalysts for the synthesis of chemical sensors, proteins, peptides and bioconjugates. Her laboratory has also made important contributions for the development of methods for the activation of amide bonds for late-stage diversification of peptides and proteins. Recently, Dr. Raj has focused on the development of a novel bioconjugation technique for the detection of monomethyl lysine containing posttranslational modifications (PTMs) to understand the role of these PTMs in various cellular processes and diseased states. This work was recognized in 2018 by NSF CAREER award

L24-AW Chemical Tools for Selective Detection of Monomethyl Lysine PTMs

Monika Raj, Yonnette Sim, Ogonna Nawjiobi,
Auburn University, Department of Chemistry and Biochemistry Auburn AL 36830

Selective modification of biomolecules provides scientists with an effective tool for a multitude of bioanalytical, therapeutic, biological and bioengineering applications. However, chemical strategies that can target a particular functional group at a single site in the presence of reactive amino acid side chains on protein surfaces are limited. We have developed a multicomponent bioconjugation approach for selective labeling of proteins containing secondary amines. This method does not require any genetic engineering of the protein target and protection of the side chains of other amino acids. The resulting bioconjugation reaction leads to the formation of a highly stable C-C bond at the site of the conjugation. The broad utility of the bioconjugation reaction is demonstrated by conjugation of various probes such as dye, peptides, and PEG on different proteins containing a proline at the N-terminus such as creatine kinase and aldolase. This method is employed for labeling monomethyl lysine containing posttranslational modifications (PTMs) on proteins with various cargoes. The dysregulation of monomethyl lysine PTMs has been linked to a variety of different biological malfunctions, yet the chemical methods for selective detection of mono methyl lysine PTMs are still lacking. This selective tagging methodology can effectively detect monomethyl lysine PTMs thus has a potential to further our understanding of the role of monomethylated lysine containing PTMs in regulating various cellular signaling processes.

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Young Investigator Poster Competition

Emel Adaligil, Genentech
Zoe Adams, The Scripps Research Institute
Maziar Ardejani, The Scripps Research Institute
Mónica Aróstica, USM/PUCV
Margaryta Babych, Université du Québec à Montréal
Christopher Baehr, University of California, Davis
Ross Ballantine, Queen's University Belfast
Shivani Bansal, University of California, Davis
Iraj Behroz, Technische Universität Berlin
Stephanie Berger, University of Washington
Christopher Bérubé, Université Laval and PROTÉO
Jannis Beutel, Friedrich-Alexander-Universität Erlangen Nürnberg
Chowdhury Raihan Bikash, University of Nevada Reno
Michael Bird, The Scripps Research Institute
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Tobias Schnitzer ..... ETH Zürich
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Dillon Flood ..... Scripps Research Institute
Quibria Guthrie ..... North Carolina State University
Bhavesh Khatri ..... Indian Institute of Science

GENERAL INFORMATION

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(De Anza Foyer)

Registration Hours

Saturday	1:00 pm – 6:00 pm
Sunday	7:30 am – 4:30 pm
Monday	7:30 am – 4:30 pm
Tuesday	7:30 am – 12:30 pm
Wednesday	7:30 am – 4:00 pm
Thursday	7:30 am – 10:30 am

POSTER INFORMATION

This year we have two poster sessions in the Serra Ballroom.

Session One

Set-up:

Sunday	8:00 am

Posters will be on display during the hours the Exhibits are open.

Session One Posters defend

Monday	5:45 pm – 8:00 pm

Posters must be removed at 8:00pm

Session Two

Set-up:

Tuesday	8:00 am

Posters will be on display during the hours the Exhibits are open.

Session Two Posters defend

Wednesday	6:00 pm – 8:00 pm

Posters must be removed at 8:00pm

EXHIBIT INFORMATION

Set-up:

Saturday	1:00 pm – 06:00 pm

Exhibit Hours:

Saturday	7:00 pm – 10:00 pm
Sunday	8:00 am – 5:30 pm
Monday	8:00 am – 8:00 pm
Tuesday	8:00 am – 12:50 pm
Wednesday	8:00 am – 8:00 pm

Exhibit Teardown:

Wednesday	08:00 pm

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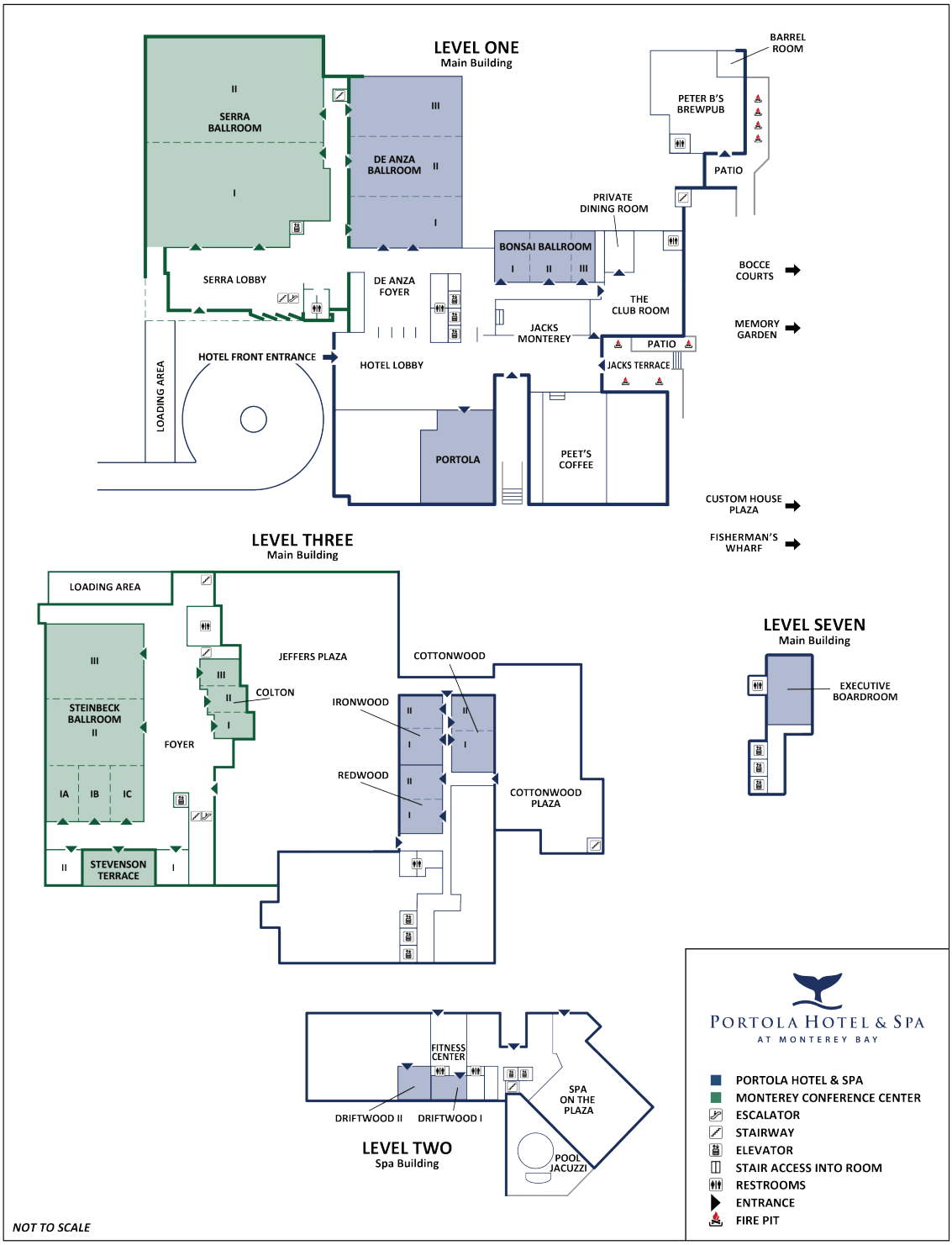
To access the WiFi in the Serra Ballroom, select APS EXHIBITS, passcode: peptides

FACILITIES MAP



LECTURE ABSTRACTS

LO1 Innovation by Evolution: Bringing New Chemistry to Life

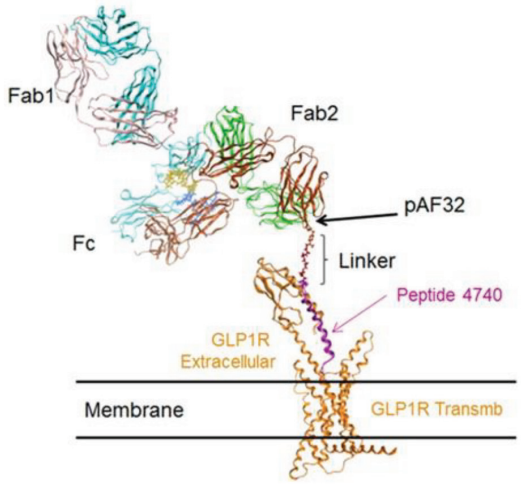
F. H. Arnold
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena CA, USA

Not satisfied with nature's vast catalyst repertoire, we want to create new protein catalysts and expand the space of genetically encoded enzyme functions. We use the most powerful biological design process, evolution, to optimize existing enzymes and invent new ones, thereby circumventing our profound ignorance of how sequence encodes function.

LO2 Investigation of a Site-Specific Antibody-Peptide Conjugate as a Long Acting GLPR1r/ GCGr Agonist

Robert M. Garbaccio[1], Paul Carrington[2], Maribel Beaumont[3], Gulesi Ayanoglu[3], Wolfgang Seghezzi[3], Shraddha Sadekar[3], Isabel Figueroa[3], Mohammad Tabrizifard[3], Grigori Ermakov[3], Xiaoyan Du[3], Yaoli Song[3], Michael Judo[3], Sheena Mumick[4], Dennis Gately[5], Anthony Manibusan[5], Nick Knudsen[5], Elisabetta Bianchi[6], Federica Orvieto[6]
[1]Merck Global Chemistry, Kenilworth, NJ, USA; [2]Merck Biology, Diabetes, San Francisco, CA, USA; [3]Merck Biologics, Palo Alto, CA, USA; [4]Merck In Vitro Pharmacology, Kenilworth, NJ, USA; [5]Ambrx, San Diego, CA, USA; 6IRBM, Pomezia, Italy

A long-acting dual agonist of the GLP1/glucagon receptors was developed through site-specific conjugation of an active peptide to a silent antibody through PEG linkers of different length. It was observed that the stability and potency of the resulting conjugates could be optimized by the nature of the linker as well as the position of conjugation on the antibody. A set of conjugates were evaluated in rodent pharmacokinetic and disease model studies and demonstrated to have potent and long acting activity reflecting their in vitro profile. Thorough metabolic characterization of the conjugates in vivo revealed peptide metabolism to potentially be limiting to this strategy.



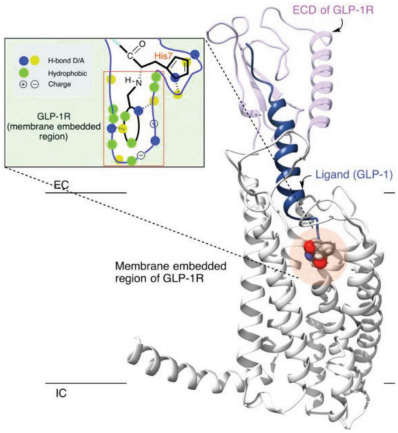
LO3 Peptide Therapeutics: A Novel, Highly Selective Human Glucagon Receptor Agonist, ICA349 for Treatment of Obesity

Ved Srivastava
Ved Srivastava, Ph.D., Vice President of Chemistry, Intarcia Therapeutics Inc, 6 Davis Drive |Research Triangle Park, NC 27709; Mobile: 858-216-6154

We have developed ICA349, a novel, peptidase resistant 38 amino acid peptide which is highly selective for the human glucagon receptor. ICA349 in combination with exenatide (GLP-1 agonist) demonstrated a significant reduction in body weight and composition, food intake, HbA1c, glucose and metabolic parameters in obesity/T2D rat models. The ICA6150349/ exenatide combination in this model achieved weight loss of up to 38% with a 70% reduction in fat mass and a near-normalization of lipids. In the male ZDF rat, the ICA6150349/ exenatide combination resulted in a significant decrease in HbA1c (-1.5%). The strategy and tactics for discovery and medicinal chemistry, pre-clinal data, and a sustained delivery method will be presented.

LO4 Molecular Design of Peptide Therapeutics for Global Diseases

Kathleen Sicinski[1], Vittorio Montanari[1], Krishna Kumar[1,2,3]
[1]Department of Chemistry, Tufts University, Medford, MA 02155; [2]Department of Biomedical Engineering, Tufts University, Medford, MA 02155; [3]Cancer Center, Tufts Medical Center, Boston, MA 02110



Obesity, Type 2 Diabetes, T2D, and related metabolic disorders afflict hundreds of millions worldwide. State of the art treatments include analogues of the endogenous gut peptide hormones called "incretins." The two principal peptides that form this class, Glucagon-like Peptide 1, GLP-1, and Glucose-dependent Insulinotropic Peptide, GIP, stimulate their cognate receptors, GLP-1R and GIPR, in different tissues with the primary function of maintaining glucose homeostasis in addition to having neuro- and cardioprotective effects. These peptides however suffer from poor metabolic stability and are rapidly degraded by the ubiquitous serine protease, dipeptidyl peptidase IV, DPP-4.

We describe here the design and development of potent peptide analogues that are completely refractory to hydrolytic enzyme action while retaining full biological activity, potency,

and efficacy. As general modulators of the gut-brain axis, these peptide hormones have also high promise for untreated neurological indications such as Alzheimer's and Parkinson's diseases, and traumatic brain injury, TBI. Furthermore, the platform allows for the design of hundreds of derivatives with the ability to tune the onset and duration of action, potency, efficacy, and providing a method for modulating gut and blood brain barrier, BBB, penetration. This lecture will describe the fundamental design principles, molecular pharmacology and in vivo data. Some of the compounds described here rival or better the compounds used in the clinic today and could serve as a model platform for discovery of clinically relevant molecular entities.

LO5 A Discovery Case Study: Early Implementation of an Appropriate Screening Funnel is Key to Success for Peptide Therapeutics

E. Bianchi[a], R. Ingenito[a], S. Esposito[b], L. Orsatti[b], M. Gallo[c], A. Di Marco[d], E. Monteagudo[b]
IRBM, [a]Peptide Chemistry, [b]DMPK/ADME, [d]Pharmacology, [e]NMR via Pontina km 30,600, 00071 Pomezia (Roma), Italy

A set of important tools to early support peptide discovery studies have been implemented at an early stage so to correctly drive our process of development of peptide therapeutics. In particular in vitro approaches have been set up to identify peptide metabolites, not only in plasma but also in the sub cutaneous tissue, to mimic metabolism at the site of injection that correlates with improved *in vivo* bioavailability. Peptide drugs are also at risk of raising safety issues, in fact injection, by IV or sc route, can often trigger a non-IgE mediated pseudo allergic reaction. This effect is caused by mast cell activation and consequent histamine release and therefore early implementation of screening of our peptide leads in a human mast cell line is key to dial out potential safety concerns that might become evident only in late development. The aggregation potential and the oligomerization propensities are also very important issues that can impact PK/PD, solubility and immunogenicity. This presentation will cover details of the repertoire of studies that we found to be necessary and indispensable to support our peptide drug discovery efforts.

1 S. Esposito, ML de Leonibus, R. Ingenito, E. Bianchi, L. Orsatti, E. Monteagudo. *J Pharm Biomed Anal.* **2018**, *159*, 449-458.

LO6 Data Reduction and Visualization Technologies for the Design & Optimization of Therapeutic Peptide and Nucleic Acid Derivatives

Les Miranda & Roxanne Kunz
Amgen Research, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA

The therapeutic discovery process generates large volume multi-dimensional datasets which are difficult to effectively manage to facilitate scientific decision-making. In support of large molecule discovery, the current state of bioinformatics capabilities is not well suited to uniquely register, and in turn, intuitively reveal key compositional differences versus an array of drug performance attributes. Moreover, the ability to unambiguously identify, compare, and communicate molecular compositions is integral to the scientific process, increasing efficiency of downstream data analysis including development of AI/ML methods. Here, we report our approach to the structure-based registration of peptide, nucleic acid, and biological entities with the capability of atomic-level differentiation. We also reveal data reduction and data visualization approaches to allow for fluid data interactivity and analysis of multivariate datasets related to therapeutic peptide discovery campaigns.

LO7 Stapled Peptides from Bench to Bedside

Simon Ng[a], Yu-Chi Juang[b], Arun Chandramohan[b], Hung Yi Kristal Kaan[b], Ahmad Sadruddin[b], Tsz Ying Yuen[c], Fernando J. Ferrera, Xue'Er Cheryl Lee[c], Liew Xi[c], Charles W. Johannes[c], Christopher J. Brown[a], Srinivasaraghavan Kannan[d], Pietro G. Aronica[d], Nils Berglund[d], Chandra S. Verma[d], Lijuan Liu[e], Alexander Stoeck[e], Tomi K. Sawyer[e], Anthony W. Partridge[b,1], David P. Lane[a,1]
*[a]p53 Laboratory, A*STAR, Singapore; [b]MSD, Singapore; [c]Institute of Chemical and Engineering Sciences, A*STAR, Singapore; [d]Bioinformatics Institute, A*STAR, Singapore; [e]Merck & Co., Inc., Boston, MA, USA*

Stapled peptides have been described since the landmark paper Greg Verdine in the year 2000. In 2019, four clinical trials of ALRN6924 are in progress as the first stapled peptide begins the long journey to clinical approval. Our own studies on stapled peptides have encompassed a number of targets and have given us great insight into the positive properties of this new class of molecules but also helped us to understand why they often fail and why the literature surrounding their use is so confused. In the case of a study developing stapled peptides towards the eIF4E complex, we developed molecules that blocked the binding of eIF4G and 4EBP1 with exceptional affinity. However, very careful biological assays built around the nanoBret and nanoBit technologies demonstrated that these peptides completely failed to enter cells and access their targets. Permeabilising the cells allowed us to show that these molecules were capable of target engagement and potent biological activity so that the only barrier to their function was a failure of cell permeability. In contrast, studies on inhibitors of the p53-MDM2 interaction yielded many molecules capable of biological activity both in tissue culture systems, and in whole animal models. The properties that allow the successful uptake of stapled peptides and their engagement with their target have been explored in extensive peptide series incorporating many unnatural amino acids and modified stapling chemistries. Eventually we have produced extraordinarily stable and potent inhibitors of MDM2. At the same time, we have realized that many published observations on stapled peptides have been overoptimistic in their interpretation of the data. Particular pitfalls include non-specific membrane engagement leading to cell lysis and the binding of the molecules to plasticware and other surfaces invoking false positive signals in a variety of biochemical assays. Currently, the published RAS binding peptides fall into this category. This experience has allowed us to develop a series of critical technologies that can quickly discover the off- target effects of apparently promising molecules reflecting what has been seen for many years in the field of small molecule discovery where molecules aptly named as pan-assay interference compounds (PAINs) have long been seen as beguiling false positives.

LO8 Challenges and Emerging Approaches in Peptide Drug Discovery: Applications in Modulating Stem Cells

Rami Hannoush
Department of Early Discovery Biochemistry, Genentech South San Francisco, CA

This talk will describe our group's efforts in discovering and optimizing peptide-based scaffolds and will highlight some

of the challenges and novel technologies for peptide lead identification and development. A case study on selectively targeting the Frizzled (FZD) receptor class will be discussed. In particular, FZD7 receptor is enriched in LGR5+ intestinal stem cells and plays a critical role in their self-renewal. FZDs interact with Wnt signaling proteins via, in part, a lipid-binding groove on the extracellular cysteine-rich domain (CRD) of the FZD receptor. The presentation will highlight our lab's recent efforts in providing new biochemical and structural insights into the molecular arrangement of members of the FZD receptor family and their mode of regulation by cis-unsaturated fatty acids. We also describe the identification of a potent peptide that selectively binds to the FZD7 CRD at a previously uncharacterized site and alters the conformation of the CRD and the architecture of its lipid-binding groove, revealing a new approach for targeting specific FZD isoforms. Treatment with the FZD7-binding peptide impaired Wnt signaling in cultured cells and stem cell function in intestinal organoids.

L09 Macrocyclic Peptide Inhibitors of Complement C5 for the Treatment of Systemic and CNS Immune Disorders

Alonso Ricardo
Chief Technology and Innovation Officer, Ra Pharmaceuticals

Complement component 5 (C5) is a member of the terminal complement pathway, and its cleavage results in the formation of two fragments: C5a, an anaphylatoxin, and C5b, the precursor for the membrane attack complex formation (MAC or C5b-9). Abnormal activation of the terminal complement cascade leads to pathological conditions such as autoimmune disease, intravascular hemolysis, and vascular injury. More recently, excessive activation of C5 has also been associated with inflammasome formation and the development of neuroinflammation.

Using its proprietary Extreme Diversity™ platform, Ra Pharma discovered and developed zilucoplan, a 15-amino acid macrocyclic peptide, designed to bind and inhibit the cleavage of complement C5. Zilucoplan exhibits a favorable safety and tolerability profile and has achieved clinically meaningful and statistically significant efficacy endpoints in Phase 2 clinical studies in patients with generalized myasthenia gravis (gMG) and paroxysmal nocturnal hemoglobinuria (PNH).

In addition to zilucoplan, Ra Pharma has used its proprietary passive, permeable peptide platform to discover a new class of short macrocyclic peptide C5 inhibitors; these molecules are orally bioavailable and have achieved CNS exposure in rodents. Ra Pharma believes these results validate the enormous potential of Ra's discovery engine for the identification of potent, selective, cell-permeable, and bioavailable peptides for the treatment of systemic disease.

L10 A Single Stable Topological Stereoisomer of a two Disulfide-bonded Peptide Demonstrates Full Potency on the Human Norepinephrine Transporter and Blocks Chronic Neuropathic Pain

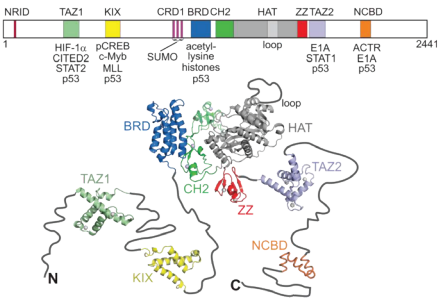
P. Wilhelm[a], S. Mohammadi[b], L.J. Whish[a] ,M. Bongers[a], A. Brust[a], Å. Anderson[a], L. Ragnarsson[a], M. Mobli[c], R.J. Lewis[a], M.J. Christie[b], and P. Alewood[a]*.
[a]Institute of Molecular Bioscience, The University of Queensland, St Lucia QLD 4072 (Australia); [b]Discipline of Pharmacology, University of Sydney, Sydney, NSW 2006 (Australia); [c]Centre for Advanced Imaging, The University of Queensland, St. Lucia QLD 4072 (Australia)

Conotoxin χ Mr1a is a 13 residue peptide with two disulfide bonds that noncompetitively inhibits the human norepinephrine transporter (hNET) leading to reduced allodynia in a mouse models of neuropathic pain. We grafted the 4-residue pharmacophore of Mr1a into a smaller disulfide framework that subsequently led to the discovery of an equipotent mimetic with a unique topological fold. Selective chemistry led to the discovery of two stereotopological isomers one of which in the globular fold was >1000 fold more potent than the other both in vitro and in vivo. These unusual isomers represent an additional level of structural complexity in multiply disulfide bonded peptides that are rarely observed and have implications for drug design, chemical synthesis and a deeper understanding of the structural diversity of cysteine rich peptides.

L11 Intrinsically Disordered Proteins – Vital Peptide Cogs in Metabolic Machines

H. Jane Dyson and Peter E. Wright
Department of Integrative Structural and Computational Biology, Scripps Research, 10550 North Torrey Pines Road, La Jolla CA 92037

Disordered proteins and disordered segments of larger proteins play a vital role in protein- protein interactions in many different metabolic processes. Cellular signaling is vitally dependent on the presence of disorder in the interacting proteins[1] — the same polypeptide can make interactions with many partners, leading to pathway cross-talk, and combinatorial post-translational modifications turn signals on and off. We study disordered proteins and their interactions in solution, primarily using NMR spectroscopy. Our interest has been in the molecular basis of the interactions and the mechanisms by which cellular signaling is controlled. Signaling pathways such as the hypoxia response[2] and the role of viral oncoproteins in virus-induced oncogenesis[3,4] are examples that show the central importance of disorder in cellular signaling pathways.



References:

1 Intrinsically disordered proteins in cellular signalling and regulation. P.E. Wright and H.J. Dyson (**2015**) *Nat. Rev. Mol. Cell Biol. 16*, 18-29.

2 Hypersensitive termination of the hypoxic response by a disordered protein switch. R.B. Berlow, H.J. Dyson and P.E. Wright (**2017**) *Nature 543*, 447-451.

3 The high-risk HPV16 E7 oncoprotein mediates interaction between the transcriptional coactivator CBP and the retinoblastoma protein pRb. A.L. Jansma, M.A. Martinez-Yamout, R. Liao, P. Sun, H.J. Dyson and P.E. Wright (**2014**) *J. Mol. Biol. 426*, 4030-4048.

4 Structural basis for cooperative regulation of KIX-mediated transcription pathways by the HTLV1 HBZ activation domain. K. Yang, R.L. Stanfield, M.A. Martinez-Yamout, H.J. Dyson and P.E. Wright (**2018**) *Proc. Natl. Acad. Sci. USA 115*, 10040-10045.

L12 The Impact of Fluoroproline Isomers on the Folding and Thermodynamic Stability of Globular Proteins

Roderer D.[a,b], Glockshuber R.[a], Rubini M[c].
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4-substituted proline analogues are of particular interest for investigating protein folding kinetics and thermodynamic stability, as substituents at the C^{γ} atom of proline have a strong effect on the two main conformational equilibria of proline residues in polypeptides, namely the *endo/exo* ring puckering of the proline ring and the *cis/trans* equilibrium of prolyl peptide bonds. The installing of electron-withdrawing substituents on the pyrrolydine ring shows to elicit a predictable effect on coiled-coil peptides. However, their impact in the context of the tertiary structure of globular proteins is contradictory.

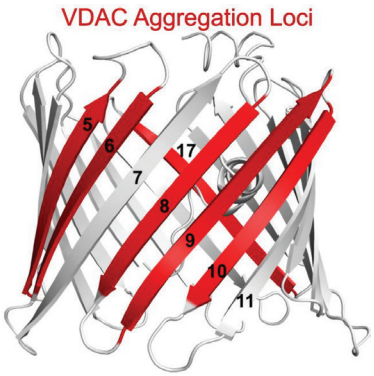
We have analyzed the influence of (4R)– and (4S)–fluoroproline (Flp) on the thermodynamic stability and on the rate–limiting *trans–to–cis* isomerization of the Ile75–Pro76 peptide bond in the folding of Trx1P, an *Escherichia coli* thioredoxin (Trx) variant. We showed that both Flp isomers adopted the endo pucker in the tertiary structure of Trx1P and that both exert a similar effect on the thermodynamic stability of the protein.1 However, while (4R)–Flp at position 76 had no effect on the isomerization rate in the context of the intact tertiary structure, (4S)–Flp accelerated the folding reaction ninefold. Similarly, tenfold faster *trans–to–cis* isomerization of Ile75–(4S)–Flp76 relative to Ile75–Pro76 was observed in the unfolded state.2 Our results show that the replacement of *cis* prolines by non–natural proline analogues can be used for modulating the kinetics of the rate–limiting folding step of proteins with *cis* proly–peptide bonds in the native state.

1 Rubini M., Schärer MA, Capitani G, Glockshuber R. *ChemBioChem,* **2013***, 14*, 1053-1057.
2 Roderer D, Glockshuber R, Rubini M. *ChemBioChem,* **2015***, 15*, 2162-6.

L13 Peptide–Based Reverse–Mapping of Membrane Protein Aggregation Loci

Radhakrishnan Mahalakshmi
Molecular Biophysics Laboratory, Department of Biological Sciences, Indian Institute of Science Education and Research, Bhopal. India

Mitochondria are the powerhouses of the cell. In the mitochondrial outer membrane, pore-forming β-barrel proteins called voltage-dependent anion channels (VDACs) execute metabolite transport across the membrane, regulate homeostasis, and control apoptosis. Owing to their tendency to oligomerize and aggregate with amyloidogenic proteins, VDACs are increasingly implicated in proteotoxicity and neurodegenerative diseases. However, the intrinsic aggregation sites of VDACs, and the molecular elements that switch a folded functional channel to toxic aggregates, were not known. By employing a reverse-mapping strategy involving peptide analogs of VDACs and a heuristic approach involving biophysical, biochemical, *in silic*o analysis, we have now successfully mapped the oligomerization loci of all three human VDAC isoforms. We find that all three VDACs possess similar oligomerization and aggregation hotspots on strands β5-β11 of its 19-stranded structure. These zones are intrinsically destabilized in all VDACs. Comprehensive thermodynamics and aggregation kinetics measurements using full-length VDAC protein further validates our findings from the peptide-based reverse-mapping approach. Our findings provide molecular insight on how VDACs associate in the cell, opening avenues for developing VDAC-based targeted therapeutics for neurodegenerative aggregates.



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Lella, M and Mahalakshmi, R. Direct structural annotation of membrane protein aggregation loci using peptide–based reverse–mapping. *J. Phys. Chem. Lett.* **2018***, 9*:2967-2971 (DOI: 10.1021/acs.jpclett.8b00953).
Gupta, A and Mahalakshmi, R. Helix–strand interaction regulates stability and aggregation of the human mitochondrial membrane protein channel VDAC3. *J. Gen. Physiol.* **2019**, jgp.201812272 (DOI: 10.1085/jgp.201812272).

L14 The Role of Heteroatoms in Dictating the Conformational Preferences of Peptide Foldamers

David J. Aitken, Zeynab Imani and Sylvie Robin
CP3A Group, ICMMO, Université Paris-Sud, Université Paris-Saclay, 91400 Orsay, France

For two decades, peptides and peptidomimetic architectures have been front-stage in the fast-developing area of foldamer science. Regular helical folding patterns have been discovered, allowing others to be designed, in which the conformational preferences are governed by N–H•••O=C hydrogen bonding networks akin to those found in native peptide secondary structure. We have been examining the effects of other types of hydrogen bonds as tools to assist and/or direct helical peptide folding, in order to generate new and more sophisticated foldamer axioms in a controlled and predictable manner. In this presentation we will assess the lessons learned from recent studies conducted on short oligomers of cyclic β-amino acids which bear a nitrogen or oxygen heteroatom which is strategically implicated in N–H•••N(sp3) •and N–H•••$O_{(sp3)}$ • hydrogen bonding networks, then present out most recent observations on the previously unknown role of sulfur in sustaining predictable and tunable folding patterns in novel short peptide sequences.

L15 SAXS Finds that Water is a Good Solvent for Many Disordered Proteins but the Addition of FRET Fluorophores can Alter this Property

Joshua A Riback[a], Micayl[a] A Bowman[b], Adam M Zmyslowski[c], Kevin W Plaxco[d], Patricia L Clark[b]*, Tobin R Sosnick[c]*
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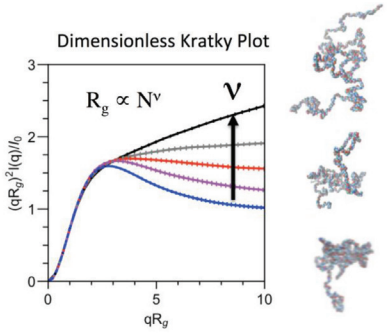
The dimensions that unfolded and intrinsically disordered proteins (IDPs) adopt at low or no denaturant remains controversial. We recently developed an analysis procedure for small-angle X-ray scattering (SAXS) and found that even relatively hydrophobic IDPs remain nearly as expanded as the chemically denatured ensemble (Riback et al., Science 2017). Hence, water should be considered a good solvent for most unfolded states and an early collapse phase in not obligatory in the folding of many proteins. In contrast, FRET studies (and simulations) often find disordered conformations to be compact. We achieve reconciliation by showing that the addition of FRET fluorophores reduces the disordered protein's dimensions (Riback et al., PNAS 2019). We also tested the suggestion that FRET and SAXS results can be reconciled if the $R_{gyration}$ and $R_{end\text{-}to\text{-}end}$ are "uncoupled" (i.e., no longer proportional), unlike random walk homopolymers. We find, however, that these two measures remain proportional. We conclude that mild chain contraction and fluorophore-based interactions, along with improved analysis procedures for both SAXS and FRET, can explain the preponderance of existing data regarding the dimensions of unfolded polypeptides. We propose that having disordered and unfolded proteins be solvated and expanded is biologically beneficial as these properties reduce unwanted misfolding and aggregation *in vivo*.



target the KIX domain and assess transcriptional effects in both AML cells and potentially other cancers dependent on aberrant Myb or MLL behavior.

L16-YI A Highly Potent and Selective Inhibitor of the Myb-KIX Interaction

Stephen T. Joy[1], Matthew J. Henley[2], Yanira Rodriguez Valdes[2], Junius E. Thomas[2], Jennie Lin[3], Dr. Matthew S. Beyersdorf[2], Dr. Anna K. Mapp[1,2]
[1]Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109 2Program in Chemical Biology, University of Michigan, Ann Arbor, MI 48109; [3]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

Acute myeloid leukemia (AML) is dependent on the native interaction between the transcriptional activator Myb and the transcriptional coactivator CREB-binding protein (CBP). The CBP coactivator interacts with a variety of different transcription factors through its flexible KIX domain, allowing CBP to control transcription of many genes. Unfortunately, the conformational plasticity and the presence of multiple binding sites on KIX make it challenging to target using small molecule inhibitors. Notably, the transactivation domains of Myb and MLL bind to two different sites on KIX, and fusing these two domains via a flexible linker produces a peptide (MybLL-tide) that has picomolar affinity for KIX. MybLL-tide has higher affinity for KIX than any previously reported compounds while also possessing 15,000-fold selectivity for the CBP KIX domain over other similar coactivator domains. Further modification of the MybLL-tide with a nuclear localization signal and a cell penetrating peptide moiety yield a modified MybLL-tide with cellular activity that potently modulates downstream gene expression and also inhibits AML cell viability. These promising results show that MybLL-tide can be an effective, modifiable tool to selectively

L17-AW Chirality, Alzheimer's Disease and Amyloid Beta

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Aggregation-prone polypeptides are produced by living systems, often as cleavage products of substantially larger protein precursors. Whereas their functions in health are challenging to study and not always well understood, an imbalance between their production and clearance can produce diverse pathological conditions, including Alzheimer's Disease. The believed seminal etiological agent of AD, amyloid beta forms aggregates of different size and shape, with distinctions frequently made between oligomers, protofibrils and fibrils. Oligomers are believed to be particularly harmful, whereas fibrils appear to represent an aggregation endpoint that may be relatively benign.

Through stereochemical argu¬ments, we envisioned that racemic amyloid beta should exhibit increased fibril formation and reduced toxicity. We synthesized the two enantiomers and found indeed that their equimolar mixture exhibited pronounced acceleration of fibril formation, as compared to the enantiopure coun¬ter¬parts. This led to substantial suppression of oligomer formation and inhibition of toxicity in model cell-based systems. The underlying molecular mechanisms that lead to the differences in biophysical and biological properties observed between enantiopure and racemic Aβ42 remain subject of active research in our laboratory.

L18-AW How Peptides Led the Way to Folding in the Cell

Lila M. Gierasch
Departments of Biochemistry & Molecular Biology and Chemistry, University of Massachusetts Amherst, Amherst, MA 01002 USA

The protein folding problem was originally framed as the challenge of understanding how the amino acid sequence of a protein encodes its complex native structure. Early protein folding research focused on determining the pathway by which an unstructured polypeptide forms structure characteristic of the native state. This view of protein folding relied heavily on relationships between amino acid sequences and their preferred structural motifs such as helices, sheets and turns, many of which were established using model peptides. For example, my studies of cyclic peptides, begun in the Blout laboratory, were centered on improving our understanding of sequence preferences for reverse turns. A paradigm shift in protein folding research took place with the recognition that the folding reaction must be described in terms of ensembles of states and conformational search over a funnel-shaped energy landscape. Ironically, at this point little attention was paid to the physiological context of protein folding. My laboratory's eyes were opened to the in-vivo protein folding problem by our studies of signal sequences and their role in targeting proteins to the secretory pathway or to membrane locations. This work brought us into the cell biology of protein biosynthesis, localization, and quality control just at the time that the molecular chaperone concept was born. Our work with peptides enabled us to be swept up in the exciting science

that ensued: the intersection of the fundamental principles of protein folding and the extremely challenging environment of the cell. From this point on, we have sought to understand how molecular chaperones recognize clients that are unfolded (fully or partially) and the mechanisms whereby chaperones play roles in many processes in the cell, including but not restricted to the folding reaction. Most recently, we have focused on a central molecular chaperone family, the Hsp70s, which facilitate folding, inhibit aggregation, maintain proteins in unfolded states, and partner with other chaperones, all through a deceptively simple mechanism. This lecture will briefly chronicle my path from research on model peptides to studies of folding in the cell and describe our current emphasis: the substrate binding and allosteric mechanism of Hsp70s.

L19 Tackling Challenging Drug Targets using Fragment-based Ligand Discovery

Michelle Arkin
University of California, San Francisco

Many potential drug targets are known to be relevant for disease, but have been 'undruggable' by current approaches. Our lab seeks to understand to what extent this failure has been due to the features of the biological target vs the methodologies used to discover small-molecule modulators. In particular, we have focused on protein-protein interactions and the larger networks controlled by these interactions. This presentation will describe the use of disulfide trapping to discover first-in-class compounds that modulate 'challenging' targets, including protease inhibitors and protein-protein stabilizing compounds.

L20 Peptide and Nonpeptide Macrocycles as Promising Chemotypes for Inhibiting Protein-Protein Interactions

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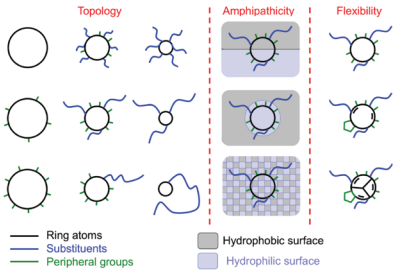
There is a wealth of compelling protein-protein interaction (PPI) targets that cannot readily be inhibited using conventional druglike small molecules. Consequently, there is strong interest in noncanonical drug chemotypes as inhibitors of PPIs including, for example, peptide and nonpeptide macrocycles, peptide foldamers, and covalent inhibitors. We are exploring the hypothesis that macrocyclic compounds (MCs) represent a privileged chemotype for inhibiting intracellular PPI targets. Large MCs can potentially exploit binding energy hot spots at a PPI surface site that are too widely spaced to be spanned by a smaller compound. Equally importantly, there is evidence that appropriately structured MCs can confer improved pharmaceutical properties — including, in particular, passive membrane permeability — compared to acyclic compounds of comparable molecular weight. Here we describe approaches to identifying PPIs that constitute promising targets for macrocyclic inhibitors, as well as progress toward the development of design guidelines for macrocycles for use in drug discovery, based in part on a Machine Learning analysis of structural features and physicochemical properties relevant to passive membrane permeability.



L21 A High Throughput Screen to Derive Functional Antagonists of Transcription Factors

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There are many library-based approaches that can screen for protein-protein interaction (PPI) inhibitors. However, none guarantee functional loss of the target protein. Using the oncogenic transcriptional regulator Activator Protein-1 (AP-1) as our exemplar, we have developed a novel intracellular Transcription-Block Survival (TBS) screening assay, which can ensure functional loss of the target protein. TBS works by ensuring that gene transcription is blocked, which is only restored if inhibitors bind AP-1 and prevent its DNA binding activity to restore cell viability. This ability to distinguish between non-functional binders and those capable of blocking DNA-binding function is unique and addresses a problem that has hampered the search for 'functionally active' inhibitors. Intracellular screening additionally removes toxic, insoluble, protease susceptible, or non-specific compounds from the screen, accelerating progress towards clinical trials.

L22 Allosteric Targeting of LRRK2 in Parkinson's using Constrained Peptides

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Parkinson's disease (PD) is a neurodegenerative disorder affecting more than five million people worldwide and only palliative treatment currently exists for the disease. Mutations in Leucine-rich repeat kinase 2 (LRRK2) are the most frequent cause of late-onset and idiopathic PD. LRRK2 belongs to the group of Roco proteins, which are characterized by the presence of a Ras-like G-domain (Roc), a C-terminal of Roc domain (COR), a kinase and several protein-protein interaction domains. LRRK2 has a complex activation mechanism, involving intra-molecular signaling, dimerization and protein-protein interactions. Significantly, several PD mutations in LRRK2 have been linked to decreased GTPase activity and increased kinase activity. However, it is not well understood how LRRK2 activity is regulated and how mutations in nearly every domain of the protein can alter the protein activity and function. Further, although mutations in LRRK2 are the most frequent cause of late-onset and idiopathic PD, each of the different but commonly occurring PD mutations in LRRK2 likely trigger different defects in LRRK2 function. As a strategy to investigate LLRK2 regulation and function, we sought to develop hydrocarbon-constrained peptides to disrupt LRRK2 dimerization. These dimerization disruptors were found to be cell permeable and could significantly inhibit LRRK2 dimerization and kinase activity in cells. Further, unlike many LRRK2 kinase inhibitors, these allosteric compounds do not induce altered localization of LRRK2 in cells. Overall, the inhibitors may serve as an effective strategy to downregulate LRRK2 kinase function in cells and may also serve as templates for the development of therapeutic agents for PD.

L23 Targeted Protein Degradation via the N-End Rule Pathway

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A proteolysis targeting chimera (PROTAC) is a heterobifunctional molecule having two small-molecules connected by a linker. One small-molecule binds to a target protein (e.g., a disease target protein) while the other small-molecule engages an E3 ubiquitin ligase. Thus, this chimeric molecule is able to recruit the target protein to the E3 ligase, thereby leading to ubiquitination and subsequent degradation of the target protein by the 26S proteasome. Due to the unique mode of action capable of effectively depleting disease-associated proteins, PROTAC technology is emerged as a novel, promising therapeutic strategy. Here, we present a new class of PROTAC molecules that degrade a target protein via the N-end rule pathway. The N-end rule is a conserved proteolytic system that degrade cellular proteins having N-terminal degradation signals (N-degrons), which are recognized by the ubr box proteins, E3 ligases. So the N-terminal amino acid residues of a protein determines its half-life, and the N-end rule pathway governs the rate of protein degradation. We designed chimeric molecules in which a staple peptide targeting NCOA-1 and a ligand for ubr box proteins covalently linked by various linkers. This bifunctional molecule was found to effectively degrade the target protein, NCOA-1 (a transcriptional co-activator), in cells, thereby inhibiting cancer cell migration and metastasis. This new PROTACS strategy could be particularly useful when targeting cells that do not express the E3 ligases targeted by current PROTACs (e.g., celebron).

L24-AW Chemical Tools for Selective Detection of Monomethyl Lysine PTMs

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Selective modification of biomolecules provides scientists with an effective tool for a multitude of bioanalytical, therapeutic, biological and bioengineering applications. However, chemical strategies that can target a particular functional group at a single site in the presence of reactive amino acid side chains on protein surfaces are limited. We have developed a multicomponent bioconjugation approach for selective labeling of proteins containing secondary amines. This method does not require any genetic engineering of the protein target and protection of the side chains of other amino acids. The resulting bioconjugation reaction leads to the formation of a highly stable C-C bond at the site of the conjugation. The broad utility of the bioconjugation reaction is demonstrated by conjugation of various probes such as dye, peptides, and PEG on different proteins containing a proline at the N-terminus such as creatine kinase and aldolase. This method is employed for labeling monomethyl lysine containing posttranslational modifications (PTMs) on proteins with various cargoes. The dysregulation of monomethyl lysine PTMs has been linked to a variety of different biological malfunctions, yet the chemical methods for selective detection of mono methyl lysine PTMs are still lacking. This selective tagging methodology can effectively detect monomethyl lysine PTMs thus has a potential to further our understanding of the role of monomethylated lysine containing PTMs in regulating various cellular signaling processes.

L25 Rethinking Voltage-Gated Sodium Channel Inhibition

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Voltage-gated sodium channels (Na_V) are integral in almost all aspects of human physiology, including cardiac and muscle function and pain perception, and sodium channel subtype $Na_V1.7$ have been genetically validated to be involved in nociception. Peptide toxins isolated from venomous creatures are potent inhibitors of human voltage-gated sodium channels and venom peptides selective against $Na_V1.7$ are showing great potential as therapeutic pain leads, inhibiting Na_V activity by blocking the pore domain (pore blockers) or by binding to the membrane-embedded voltage sensor domain of the sodium channel (gating-modifier toxins). However, despite intensive research efforts into $Na_V1.7$ inhibitors there has been little in the way of translation probably due to our lack of understanding how to achieve subtype selectivity and complete block of the $Na_V1.7$ subtype and how to move from effective *in vitro* to *in vivo* inhibitors. We are delineating the mechanism of action behind venom peptide inhibition of voltage-gated sodium channel on a molecular and global level in order to engineer peptides achieving subtype specific and complete inhibition of this therapeutically relevant sodium channel subtype to ultimately unlock the potential of these potent venom peptides as therapeutic leads for the treatment of pain.

L26-YI Synthesis of Bicyclic Analogs of the Kappa Opioid Receptor Antagonist Arodyn

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The acetylated dynorphin A analog arodyn (Ac[Phe^{1,2,3},Arg^4,D-Ala^8]Dyn A(1-11)-NH_2) exhibits potent and selective kappa opioid receptor antagonism.[1] While cyclization can impart metabolic stability, increase binding affinity, and/or improve permeability, bicyclization imparts additional conformational constraint which can further enhance metabolic stability.[2] There have been increasing reports and interest in bicyclic peptides, but to the best of our knowledge there are currently no reports of bicyclic opioid peptides, possibly due to the short length of the most extensively studied opioid peptides.[3] Based on structure-activity relationships of monocyclic arodyn analogs, we designed two bicyclic arodyn analogs using ring closing metathesis (RCM) and lactam chemistries. Here we report the synthesis and opioid receptor binding affinities of the bicyclic arodyn analogs. Research supported by NIDA grant R01 DA018832.

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L27 Genome Mining for Lasso Peptide

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Lasso peptides are a class of peptidic natural products typified by their threaded [1]rotaxane structure. The lasso structure is formed by a covalent bond, an isopeptide N-terminus to sidechain linkage, and a mechanical bond, via threading of the C-terminal tail through the covalent macrocycle. This unique structure endows lasso peptides with excellent protease resistance, and in many cases, resistance to thermal denaturation. Initial interest in lasso peptides stemmed from their potent narrow spectrum antimicrobial activities. The pace of lasso peptide discovery has been greatly accelerated in recent years due to the development of new genome mining tools (Maksimov *et al. PNAS* 2012). In this talk I will discuss how genome mining for lasso peptides has led to new insights into the biosynthesis and catabolism of lasso peptides. In addition I will present strategies for targeting genome mining to discover novel antimicrobial lasso peptides.

L28 Is the Mirror Image a True Reflection? Challenging the Chirality Paradigm in Peptide-lipid Interactions

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Peptide-lipid interactions are assumed to be independent of peptide chirality, probably because it is often ignored that lipid membranes are chiral. Thus, native and mirror image forms of peptides that act by binding to lipid are expected to have identical biological activities. Cyclotides, membrane-active peptides expressed in plants with specificity for phosphatidylethanolamine (PE)-phospholipids, challenge this paradigm: kalata B1, the prototypic cyclotide, is more active than its mirror image. Membrane chirality results from one chiral centre within the glycerol moiety of phospholipids; thus, to investigate whether the membrane chirality explains differences between native kalata B1 and its enantiomer, we synthesised phospholipids with non-natural chirality. We demonstrated that mirror image forms of kalata B1 prefer to bind to membranes of the same chirality. This study shows that the chiral environment of lipid bilayers can modulate the function of membrane-active peptides and challenges the view that peptide-lipid interactions are independent of membrane chirality.

L29 Augmenting Biology through de novo Protein Design in Cells

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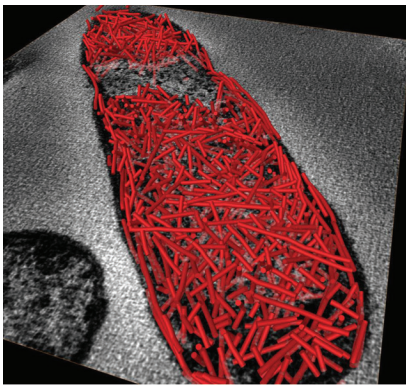
Protein design—i.e., the construction of entirely new protein sequences that fold into prescribed structures— has come of age: it is possible now to generate a wide variety stable protein folds from scratch using rational and/or computational approaches. A challenge for the field is to move from what have been largely in vitro exercises to protein design in living cells and, in so doing, to augment biology. This talk will illustrate what is currently possible in this nascent field using *de novo* α-helical coiled-coil peptides as building blocks.[1]



Coiled coils are bundles of 2 or more α helices that wrap around each other to form rope-like structures. They are one of the dominant structures that direct natural protein-protein interactions. Our understanding of coiled coils provides a strong basis for building new proteins from the bottom up. The first part of this talk will survey this understanding,[1] our design methods,[2,3] and our current "toolkit" of de novo coiled coils.[4-6]

Next, I will describe how the toolkit can be used to direct protein-protein interactions and build complex protein assemblies in bacterial cells. First, in collaboration with the Savery lab (Bristol), we have used homo- and hetero-oligomeric coiled coils as modules in engineered and de novo transcriptional activators and repressors.[7] Secondly, with the Warren (Kent) and the Verkade (Bristol) labs, we have engineered hybrids of a *de novo* heterodimer and a natural component of bacterial microcompartments to form a "cytoscaffold" that permeates $^{E.\ coli}$ cells (Figure).[8] This can be used to support the co-localisation of functional enzymes.

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L30 Using Peptoids for Molecular Recognition in Water

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New methods for the detection of small organic molecules in water will aid in the development of sensing and sequestration strategies for medical or environmental applications. We have initiated research aimed at understanding interactions between small organic molecules and water-soluble peptoids, *N*-substituted glycine oligomers. Peptoids are an attractive peptidomimetic scaffold for this application; their sequence-specific preparation is straightforward and accommodates the synthesis of a wide diversity of analogs, including those that exhibit stable structural features in aqueous solution. We have reported peptoids with putative amphiphilic helix structures that self-assemble in aqueous buffer. Recently, we reported that their self-association allows our water-soluble peptoids to form a supramolecular host for organic small molecules. New results to be presented here will demonstrate that peptoids with modulated self-association constitute a suite of supramolecular hosts that are able to detect and differentiate small molecules. This is a promising new application for peptoids, and an exciting new approach to the detection of small molecules in aqueous media.

L31-YI Variation in the Xaa and Yaa Positions of Collagen Mimetic Peptides Containing Aza-Glycine

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Protein folding is carefully regulated through intra- and intermolecular interactions between specific side chains and backbone functional groups of amino acids. Hydrogen bonding, along with a host of other weak interactions, serve to stabilize protein structure. Unnatural modifications to the protein backbone and side chains are commonly used to modulate the properties of proteins. An important class of unnatural peptide modifications are aza-amino acids, which are characterized by substitution of the α-carbon or some other adjacent position with nitrogen. Some of these amino acid analogs exhibit a higher degree of planarity and restricted dihedral angles compared to natural amide linkages, making them valuable tools in peptide mimicry and structural studies. Mimetic peptides are indispensable for studying natural proteins such as collagen, the most abundant protein in mammals. The quaternary structure of collagen involves three individual protein strands intertwining to form a tightly packed triple helical bundle. Collagen's primary structure are repeating (Xaa-Yaa-Gly) triplet sequences. Our group has previously shown the substitution of glycine residues with aza-glycine results in increased triple-helical thermal stability. Herein, we demonstrate the surprising contextual dependence of this increased stability based on the residues adjacent to the point of aza-glycine incorporation.

L32 Protein Tertiary Structure Mimetics through Systematic Alteration of Backbone Covalent Connectivity

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The structural diversity of proteins in nature is vast and the foundation for their unparalleled functional versatility. Inspired by nature's example, chemists have long sought to create synthetic molecular entities that fold in defined ways. Termed "foldamers," such agents have found applications in areas from biomedical to materials science. Progress in foldamer design and synthesis in recent years have led to bioinspired as well as abiotic secondary structures, high-affinity ligands for biomacromolecular surfaces, and complex multi-molecular assemblies. These impressive advances notwithstanding, the structural diversity shown possible to date still pales in comparison to the array of folding motifs found in nature. This talk will describe recent work toward a general strategy for creating foldamer mimics of protein tertiary structures through the systematic alteration of backbone covalent connectivity in natural sequences. Our approach is based on the idea that any protein can be thought of as having two sequences: one of amino acid side chains and another of backbone units that display those side chains. In work to date, we have leveraged this concept to create "heterogeneous-backbone" mimics of a range of prototype sequences with complex folded architectures. Collectively, results from these ongoing efforts expand the scope of structures and functions possible in foldamers and suggest new ways to control properties such as folded structure, folded stability, physiological stability, and dynamics.

L33 Rippled β-Sheets from Sequence Mismatched L- and D- Amphipathic Peptides

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Rippled β-sheets[1] are an emerging class of multicomponent supramolecular peptide biomaterial composed of enantiomeric L- and D-β-sheet peptides that coassemble in an alternating L/D pattern. While there have been several recent reports of rippled β-sheet formation from equimolar mixtures of enantiomeric L- and D-peptides,[2,3,4] the potential for a broader spectrum of rippled β-sheet assemblies in which the L- and D- coassembly partners are not only of opposite chirality but also differ in amino acid sequence has not yet been explored. We hypothesized that mismatched L- and D- amphipathic peptides that differ in hydrophobic amino acid identity would preferentially coassemble into two-component rippled β-sheets. Herein, we confirm this hypothesis by demonstrating the formation of rippled β-sheets from the coassembly of sequence mismatched L- and D- amphipathic Ac-$(FKFE)_2$-NH_2 and Ac-$(VKVE)_2$-NH_2 peptides. Mismatched coassembly of opposite chirality peptides results in peptide nanofibrils which are morphologically distinct from one-component pleated β-sheet fibrils or rippledβ-sheet assemblies of the parent enantiomers. Data is consistent with the formation of rippled β-sheet structures in which the L- and D- peptides are arranged in alternating fashion with high fidelity. This demonstration of rippled β-sheet coassembly between sequence mismatched amphipathic peptides of opposite chirality dramatically

broadens the scope of these multicomponent assemblies as rationally designed next-generation biomaterials.

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L34 Foldamer Catalysis

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The broad range of activities displayed by the "biofoldamers," proteins and RNA, establishes a set of functional goals to be approached with synthetic foldamers. Enzymes and ribozymes catalyze diverse reactions, often with extraordinary accelerations relative to background rates. The mechanisms by which rates are enhanced vary depending upon the reaction that is catalyzed, and a full accounting for enzymatic rate accelerations remains a subject of debate. Nevertheless, key principles are evident that suggest paths toward development of synthetic foldamer catalysts. Most enzyme active sites present arrays of reaction-facilitating functional groups that are spatially organized for coordinated action on the substrate(s). Our search for new foldamer catalysts focuses on achieving an optimal arrangement of just two reaction groups, i.e., optimal bifunctional catalysis.

Initial studies employed a simple crossed aldol reaction, in which formaldehyde is the obligate electrophile, to assay alternative arrangements of pairs of pyrrolidine units presented by distinct β- and α/β-peptide helices. The most effective foldamer contained an αββ backbone repeat with i,i+3 spacing between pyrrolidine-based β residues. Mechanistic analysis supported a catalytic cycle that features covalent activation of both substrates by a single foldamer molecule Recent work has focused on bifunctional foldamers that catalyze aldol reactions resulting in formation of macrocycles.

L35 Integrating Computational Design with Screening in Mammalian Cells for Novel Peptide Therapeutics

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I will present our technology platform that integrates computational library design with modern in-cell selection strategies to uncover novel peptide therapeutics. I will cover a number of different library designs and selection methodologies, including selections for phenotype or using genetic reporter systems and cell sorting, as well as methods to obtain highly stable mirror image peptides.

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L36 Gate2Brain: Peptide Shuttles for the Delivery of Drugs to the Brain

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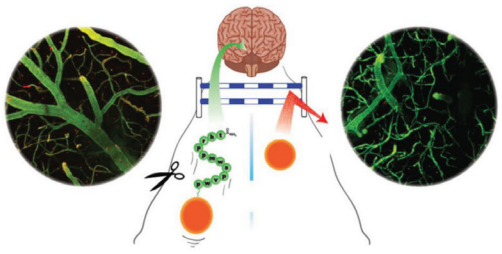
Gate2Brain shuttle peptides represent salvage for new or previously rejected CNS drug candidates by providing a way to cross the blood-brain barrier (BBB).

Gate2Brain technology consist on a toolbox of peptides able to cross the BBB and carry compounds covalently attached (including small molecules, peptides, proteins, antibodies, plasmids, siRNA or mRNA loaded nanoparticles, etc…) that cannot cross this barrier unaided. They have proofed to carry these cargoes in vitro and in vivo. These peptide shuttles use the existing transport mechanisms at the BBB without affecting the normal functioning of these mechanisms and preserving brain homeostasis.

By improving the delivery of therapeutic candidate to the CNS, we will ensure immediate impact in many CNS diseases patients. In addition, in a broader perspective, Gate2Brain technology may help to repurpose existing therapies previously rejected because of difficulty to reach the brain, accelerating the translation towards clinical development. Gate2Brain will also result in the application of lower concentrations of therapeutic agent, thereby significantly lowering systemic side effects and reducing the cost of the treatment.

Gate2Brain peptides combine protease resistance, capacity to carry a wide range of cargoes thanks to their versatility, low production costs, and low immunogenic risk. They provide a non-invasive, non-antigenic, permeable, stable, soluble and receptor-specific way to transport drugs across the BBB and into the CNS.



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L37 Development of a Peptidyl Calcineurin-NFAT Interaction Inhibitor that Prevents Permeability Pulmonary Edema in a Mouse Model of Acute Respiratory Distress Syndrome

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Acute respiratory distress syndrome (ARDS) is a frequently encountered acute lung inflammatory disease in critical care units with high morbidity and mortality. Supportive care is the only current treatment option for ARDS, as there are no specific pharmacologic agents available. A key step in the disease process is the activation of the nuclear factor of activated T cells (NFAT) c3 in lung macrophages, suggesting that inhibitors against the upstream protein phosphatase calcineurin should be effective for prevention/treatment of ARDS. We have developed a peptidyl inhibitor that selectively blocks the interaction between calcineurin and NFATc3, thereby preventing the dephosphorylation and nuclear translocation of activated NFATc3 without affecting many of the other calcineurin substrates. Starting from a previously reported peptidyl inhibitor, VIVIT, which has low potency and poor pharmacokinetic properties, we greatly improved the potency and proteolytic stability of VIVIT through computational and combinatorial chemistry approaches. Conjugation of the optimized inhibitor to a cyclic cell-penetrating peptide resulted in a potent (*K*D ~10 nM), metabolically stable, and orally bioavailable calcineurin inhibitor, CNI103, which specifically inhibits calcineurin signaling *in vitro* and *in vivo*. In a mouse model of acute lung injury/ARDS, intranasal delivery of CNI103 (3 mg/kg) prevented the development of permeability pulmonary edema, reduced cytokine production and lung inflammation in response to treatment with lipopolysaccharide with a highly favorable pharmacokinetic profile and minimal or no systemic toxicity. Based on these data, we expect that aerosolized CNI103 will replace current calcineurin blockers for the treatment of a variety of autoimmune and inflammatory lung diseases.

L38-AW Dissecting the SPPS Methodology: Resins, Handles, Coupling Reagents, Protecting Groups, Reagents, and Strategies

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Recent years have witnessed a significant increase in the number of peptides based on Active Pharmaceutical Ingredients (APIs). Furthermore, peptides on the market have evolved from being small and linear, with approximately 10 amino acids, to being much more complex. Thus, regulatory agencies are increasingly approving cyclic peptides, longer peptides (20-40 amino acids), and peptides containing fatty acids. In research and industrial sectors, all these peptides are prepared using the solid-phase peptide synthesis (SPPS) strategy, which was developed by Merrifield in the early 60s.

This renaissance of peptides in the drug market has been, in part, possible thanks to the continuous work carried out by several groups working on diverse methodological aspects of SPPS.

In this presentation, I will go over the most important contributions to SPPS methodology made by my group in recent years. I will pay special attention to discussing resins, handles, coupling reagents, protecting schemes, and synthetic strategies for both research and industrial purposes.

L39 Broadly Neutralizing Antibody Assisted Design of Peptide-based Inhibitors of Influenza Virus

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Influenza virus remains a constant threat to global health. The 1918 H1N1 pandemic caused around 50 million deaths worldwide and up to 30–50% mortality has been reported for emerging viruses, such as H5N1 and H7N9. Therefore, there is an urgent need to design a much more effective vaccine and therapies to protect against the multiple strains and types of influenza virus. Until relatively recently, it was thought that antibodies to influenza virus could protect only against highly related strains within the same subtype. However, since 2008, many potent human broadly neutralizing antibodies (bnAbs) have been isolated that target the hemagglutinin glycoprotein (HA). These antibodies bind to highly conserved functional sites on the HA — the stem (fusion domain) and receptor binding site — and have revealed common motifs for HA recognition despite different antibody origins and germlines. This structural and functional characterization of human bnAbs against the HA is now providing exciting new opportunities for design of novel vaccines and new therapeutics that afford greater protection against influenza virus. I will discuss antibody-inspired design, structure and properties of peptides as inhibitors of influenza virus. Some of the work[1] was done in collaboration with Janssen Prevention Center, Leiden, Netherlands.

[1] Kadam RU, Juraszek J, Brandenburg B, Buyck C, Schepens WBG, Kesteleyn B, Stoops B, Vreeken RJ, Vermond J, Goutier W, Tang C, Vogels R, Friesen RHE, Goudsmit J, van Dongen MJP, Wilson IA. Potent peptidic fusion inhibitors of influenza virus *Science* **2017***; 358*:496–502.

L40 *Peptido*glycan and You: Perfect Together?

Catherine L. Grimes
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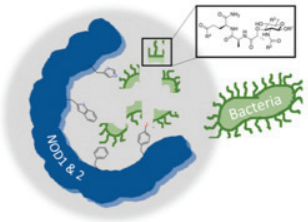
The bacterial cell wall, a polymer of carbohydrate and peptides, makes an excellent antibiotic target for two reasons: **1** it is essential for bacteria and **2** humans do not have bacterial cell walls — thus the drugs do not harm human cells. In addition to serving as a target for antibiotics, the human innate immune system uses the bacterial cell wall as a molecular calling card to recognize their presence and subsequently generate the appropriate immune response. We are interested in understanding how the bacterial cell wall is processed both by bacteria and the human host and propose new methods and tools for the characterization of this important polymer.



Both commensal and pathogenic bacteria are believed to produce peptidoglycan fragments and misrecognition can lead to the development of inflammatory bowel disease, IBD, such as Crohn's disease, CD, asthma and gastrointestinal, GI, cancers. Importantly, a long-standing debate around the biological relevance of the immunoactive synthetic fragment muramyl dipeptide, MDP, remains unclear due to a lack of NAM-based probes. We hypothesize that there are unidentified enzymatic targets and bacterial cell wall fragments that will be useful in the design of novel antibiotics and anti-inflammatory therapies.

We have taken a two-pronged approach towards testing this hypothesis. From the small molecule side, we have established an in vitro assay, which allows us to assess the affinity of Nod2, an innate immune receptor that binds to bacterial cell wall fragments. This assay has allowed us to tease apart binding from activation and we have begun to derive rules for molecular recognition by intracellular innate immune receptors. From the larger polymer side, we have embedded carbohydrates with small modifiable tags into the bacterial cell wall.

L41 Structure-guided Design of Immunogens Based on Flavivirus Glycoprotein E Domain III (EDIII)

Jonathan R. Lai
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The flaviviruses are globally significant vector-borne viruses that include Dengue virus (serotypes 1-4, DENV1-4), Zika virus (ZIKV), and Powassan virus (POWV). DENV and ZIKV are spread by *Aedes aegypti* and *Aedes albopictus* mosquitos, are endemic to subtropical regions, and can cause millions of infections per year. POWV is spread by ticks and is less common, but is associated with a severe and sometimes fatal encephalitis. We are using phage display and protein engineering to develop novel immunogens based on EDIII from DENV, ZIKV, and POWV. EDIII is an attractive target for vaccine design because it is relatively small (~80 residues), adopts a well-defined Ig-like fold, and is the target for protective neutralizing antibodies. We have engineered "resurfaced" EDIIIs from both DENV and ZIKV, whereby non-neutralizing epitopes are masked by mutation. Furthermore, we have developed protein nanoparticles containing EDIIIs from DENV, ZIKV, and POWV using spycatcher conjugation technology. We will describe these design strategies and their potential for development of novel subunit vaccines.

L42 Base Editing: Chemistry on a Target Nucleotide in the Genome of Living Cells

David R. Liu
Broad Institute of Harvard and MIT, Harvard University, and HHMI

Point mutations represent most known human genetic variants associated with disease but are difficult to correct cleanly and efficiently using standard nuclease-based genome editing methods. In this lecture I will describe the development, application, and evolution of base editing, a new approach to genome editing that directly converts a target base pair to another base pair in living cells without requiring double-stranded DNA breaks or donor DNA templates. Through a combination of protein engineering and protein evolution, we recently developed two classes of base editors (CBE and ABE) that together enable all four types of transition mutations (C to T, T to C, A to G, and G to A) to be efficiently and cleanly installed or corrected at target positions in genomic DNA. The four transition mutations collectively account for most known human pathogenic point mutations. Base editing has been widely used by many laboratories around the world in a wide range of organisms including bacteria, fungi, plants, fish, mammals, and even human embryos. We have recently expanded the scope of base editing by enhancing its efficiency, product purity, targeting scope, and DNA specificity. By optimizing base editor expression, we developed "max" versions of cytosine and adenine base editors with greatly increase editing efficiency in mammalian cells. We also show that base editing can function *in vivo* in post-mitotic somatic cells that do not support homology-directed repair. To improve the targeting scope of base editing, we used our phage-assisted continuous evolution (PACE) system to rapidly evolve Cas9 and base editor variants with broadened PAM compatibility, higher DNA specificity, and enhanced editing capabilities. Finally, we integrated several of these developments to address cell and animal models of human genetic disease. Base editing can be used to correct pathogenic point mutations, introduce disease-suppressing mutations, and create new models of genetic diseases.

Key References

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L43 Antibody Paratope Mimetic Peptides

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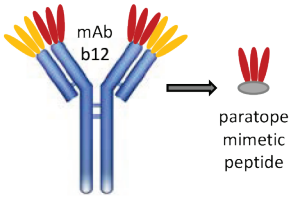
The design of synthetic antibody paratope mimics presenting one or more of the antibody's complementarity determining regions (CDRs), is a promising strategy to preserve antibody binding specificity in smaller molecules.[1] The broadly neutralizing HIV-1 antibody b12 recognizes the CD4 binding site of the HIV-1 envelope glycoprotein gp120, and efficiently neutralizes HIV-1 infections in vitro and *in vivo*. Based on the 3D structure of a b12 – gp120 complex,[2] we have designed an assembled peptide that presents the three heavy chain CDRs of b12, which contain the contact sites of the antibody for gp120 (Figure).[3]



This b12 mimetic peptide, as well as a truncated peptide presenting only two of the three heavy chain CDRs of b12, were shown to recognize gp120, as well as inhibit HIV-1 infection, in a b12-related fashion, demon-strating a functional mimicry of b12 by the paratope mimetic peptides.

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L44-AW New Methods and Strategies for Protein Chemical Synthesis and Modifications

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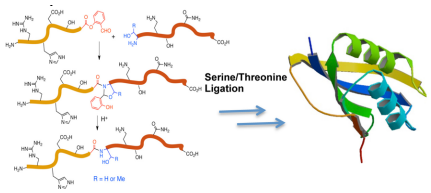
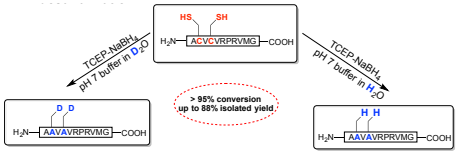
Over the past years, our laboratory has developed Serine/Threonine Ligation (STL) for protein chemical synthesis, in which an N-terminal serine or threonine of one unprotected peptide can ligate with a second unprotected peptide with the C-terminal salicylaldehyde ester to afford an *N,O*-benzylidene acetal linked peptide that upon acidolysis generates the natural peptidic Xaa-Ser/Thr linkage at the ligation site. Considering the high abundance of serine and threonine residues in natural proteins/peptides, Ser/Thr ligation will offer new options for convergent peptide and protein synthesis. Our laboratory has used Ser/Thr ligation in the synthesis of cyclic peptides including daptomycin and teixobactin, and proteins including human erythrocyte acylphosphatase, MUC1 glycopeptide, glycosylated interleukin-25, phosphorylated HMGA proteins and so on.



In addition, we recently developed an unprecedentedly mild system (TCEP/$NaBH_3$ or TCEP/$LiBEt_3H$) for chemoselective peptide desulfurization for extending native chemical ligation-desulfurization strategy in protein chemical synthesis. This method, termed P-B desulfurization, features usage of common reagents, simplicity of operation and versatile functionality compatibility. Furthermore, this method can readily incorporate deuterium into the peptide after cysteine desulfurization.



In this presentation, updated development of these methods and their applications will be discussed.

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3 Serine/Threonine Ligation: Origin, Mechanistic Aspects and Applications. Liu, H.; Li, X.* *Acc. Chem. Res.* **2018**, *51*, 1643

L45 Insights into Amyloid Formation from Solid State NMR

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Amyloid fibril formation by various polypeptides is a biophysically interesting and biomedically important phenomenon, any understanding of which depends on molecular structural information. I will describe my lab's efforts to elucidate the molecular structures of amyloid-β, Aβ, fibrils, including fibrils that develop in brain tissue of Alzheimer's disease, AD, patients, primarily using advanced solid state nuclear magnetic resonance, ssNMR, methods. Importantly, Aβ fibrils exhibit self-propagating, molecular-level structural polymorphism, which shows up clearly in ssNMR data as well as in electron microscope images.

Thus, we are interested in the possibility that structural variations in Aβ fibrils may correlate with variations in severity, progression rate, or other aspects of neurodegeneration in AD. Published data will be reviewed (see Qiang *et al., Nature 541*, 217-221, **2017**) and more recent data will be presented.

I may also describe our efforts to characterize and understand amyloid fibril formation by low- complexity protein sequences that lack hydrophobic residues, especially the low-complexity domain of the FUS protein (see Murray *et al., Cell 171*, 615-627, **2017**). In this case, major mysteries surround the nature

of interactions that drive amyloid formation, which we are currently addressing by ssNMR experiments.

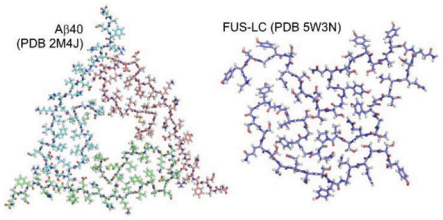

Figure: Structural models for brain-derived 40-residue amyloid-β fibrils (left) and the core of FUS low-complexity domain fibrils (residues 37-97), developed from ssNMR data, as viewed down the fibril growth direction.

L46-YI The Mode of Action of Antibiotic-peptide Plectasin in Native Membranes

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[a]NMR Spectroscopy group, Department of Chemistry, Utrecht University The Netherlands; [b]Veterinary Medicine, Department of Infectious Diseases and Immunology, Molecular Host Defence, Utrecht University, The Netherlands; [c]Biomolecular Biochemistry, Department of Chemistry, Utrecht University, The Netherlands

The rise of multi-drug resistant pathogens urgently calls for the design of powerful novel antibiotics that are robust to resistance development. Ideal templates for such next-generation antibiotics would be peptides that target the highly conserved cell-wall precursor lipid II, also known as the "Achilles' heel of bacteria". Such antibiotics would kill the most refractory pathogens at nanomolecular concentrations. However, due to the challenge of studying small membrane-embedded drug–receptor complexes in native conditions, the structural correlates of the medically relevant binding modes are unknown.

Here we present the physiologically relevant binding mode of the fungal antibiotic Plectasin, a highly promising template for drug design[1]. Previous structural studies presented a model of the plectasin — lipid II complex in micelles,[2] which often poorly mimic physiological membrane conditions and may critically alter the binding mode.[3] We revisit the plectasin — lipid II complex at close-to-physiological conditions using a cutting-edge solid-state NMR approach[3]. Our study reveals drastic differences in the plectasin — lipid II complex in native conditions and unravels, so far unknown, critical lipid II-binding features. Our insights provide a new foundation for the design of next-generation antibiotics using plectasin and other comparable antibiotics as templates.

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L47 Perfluoro-*tert*-butyl Amino Acids to Sensitively Probe Protein Function

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NMR spectroscopy is limited both by sensitivity and by signal-to-noise. We have synthesized perfluoro-*tert*-butyl amino acids (ethers of tyrosine, 4R-hydroxyproline, 4S-hydroxyproline, and homoserine) and examined their application to probe protein structure and function. Perfluoro-*tert*-butyl amino acids have 9 equivalent fluorines, which present as a singlet by ^{19}F NMR. The presentation of 9 fluorines as a singlet renders perfluoro-*tert*-butyl amino acids particularly sensitive as chemical probes. Within peptides, perfluoro-*tert*-butyl amino acids may be detected by simple pulse sequences in 30 seconds at 500 nM concentration, appropriate for the interrogation of function at biologically relevant concentrations. Perfluoro-*tert*-butyl tyrosine exhibits particularly sharp linewidths. Perfluoro-*tert*-butyl homoserine was incorporated at the site of a recognition leucine, yielding similar affinity in a coactivator peptide-estrogen receptor protein-protein interaction and allowing quantification of complex affinity by ^{19}F NMR spectroscopy. 4*R*- and 4S-perfluoro-tert-butyl hydroxyprolines exhibit distinct conformational preferences. 4*R*- and 4S-perfluoro-tert-butyl hydroxyprolines were incorporated in peptides with optimized recognition sequences for the protein kinases PKA and Akt. Phosphorylation of these peptides resulted in ^{19}F chemical shift changes, allowing the quantification of protein kinase activity by ^{19}F NMR in solution and in HeLa cell extracts. The distinct conformations of these amino acids were differentially recognized by the enzymes, demonstrating conformational selection by protein kinases. In addition, 4,4-difluoroproline was demonstrated to be an exquisite probe of proline conformation, allowing the development of new understanding of the mechanism of polyproline I/polyproline II helix interconversion, the determinants of stability of the polyproline I helix, and bases for the stabilization of *cis* amide bonds.

L48 Inspiration from Fluorination: Chemical Biology Approaches for Analysis of Protein-Protein Interactions

William Pomerantz
University of Minnesota

The estimated 650,000 protein-protein interactions (PPIs) in human cells are essential nodes in all biological processes. Detailed understanding of the molecular mechanisms of PPIs and their dynamic regulation is thus necessary for determining the fundamental rules governing their function in the cell. In this talk, I will first describe a rapid protein-based 19F NMR method for characterizing native protein interactions at protein surfaces using synthetic peptides. We label the aromatic amino acids with the highly sensitive fluorine atom, due to the high conservation of aromatic residues at protein interfaces, and recently developed methods for simultaneous labeling of proteins with two different types of fluorinated aromatic amino acids. Through our analysis of the KIX domain of the coactivator, CBP, we have begun to study its allosteric network through the lens of the aromatic side chains. In the second half of the talk, I will describe our application of fluorinated proteins for screening for inhibitors of PPIs. This method for NMR screening has recently led to the discovery of a new binding site distinct from those used by endogenous transcription factors, providing an alternative way to allosterically regulate KIX PPIs.

L49 Development of Granulin Derived Peptides as Wound Healing Agents

Mohadeseh Dastpeyman, Paramjit S. Bansal, David Wilson, Paul J. Brindley, Claudia Cobos Caceres, Javier Sotillo, Julia Seifert, Alex Loukas, Michael J. Smout, Norelle L. Daly
Centre for Molecular Therapeutics, Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, QLD Australia

Granulins are a family of growth factor proteins, which generally contain six disulfide bonds and are found in most organisms. An orthologue of granulin from the human parasitic liver fluke Opisthorchis viverrini, known as Ov-GRN-1, induces angiogenesis and accelerates wound repair. However, recombinant Ov-GRN-1 production is complex, and poses an obstacle for clinical development. We have developed peptides, based on the N-terminal region of Ov-GRN-1, that have potent in vivo wound healing activity and high oxidative folding yields. Peptides derived from Ov-GRN-1 are leads for novel wound healing therapeutics, as they are likely less immunogenic than the full-length protein and more convenient to produce. In addition to the potential therapeutic applications, these studies have identified unique folding pathways in granulins including structural roles for non-native disulfide bonds.

L50 Inositol Phosphates and Protein Pyrophosphorylation – Challenges in Signal Transduction

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Protein phosphorylation is one of the most commonly used mechanisms in signal transduction. Beyond phosphorylation, protein pyrophosphorylation — a posttranslational modification mediated by inositol pyrophosphate messengers — emerged over ten years ago as a potential additional layer of regulation for cellular signal transduction cascades.[1] However, while the inositol pyrophosphates have been linked to numerous important cellular processes,[2] the function of protein pyrophosphorylation remains poorly characterized because suitable reagents and detection methods are lacking.
Our group recently developed a mass spectrometry (MS) method to directly detect and identify pyrophosphopeptides in complex samples.[3] Excitingly, this MS method has enabled us to uncover the first endogenous pyrophosphorylation sites from mammalian cell lysates. The triggered MS method allowed for the definitive assignment of over 30 sites. The modified proteins span a range of processes and compartments and included histone deacetylase 2 (HDAC2) and cytoplasmic phosphoglucomutase 1 (PGM1). Our efforts to now to illuminate the effects of this modification on protein structure and function will also be discussed.

In sum, our novel MS method for the analysis of endogenous pyrophosphopeptides will enable the systems-wide study of this modification in a cellular context. Complemented by chemical tools for the generation of stoichiometrically pyrophosphorylated proteins,[4] the function and regulation of this poorly annotated PTM can be elucidated in the future.

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4 Marmelstein, A. M. *et al.* Pyrophosphorylation via selective phosphoprotein derivatization. *Chem. Sci. 9*, 5929-5936 (**2018**).

L51-YI Rapid, High-Fidelity Chemical Synthesis of Biologically Active Proteins and Enzymes

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The field of biopharmaceuticals is rapidly expanding, requiring new methods for on-demand high-fidelity production of chemically modified proteins. Even after decades of optimization, standard solid-phase peptide synthesis (SPPS) can't reliably produce peptides containing more than 50 amino acids in length. Larger peptides and proteins are produced by biological techniques, which limits their chemical composition. Here we report a method that closes the gap between SPPS and biological techniques. Using fully-automated continuous flow-based peptide synthesis (AFPS), we are able to routinely produce long peptides, proteins and protein-like polymers of up to 200 amino acids in a few hours of synthesis time. Complete control of every incorporated amino acid is opening the chemical space to a theoretically unlimited amount of modifications, such as incorporation of functional handles, glycoproteins, post-translational modifications and the synthesis of D-proteins. To validate the general applicability of this new technique, we report the synthesis and biological evaluation of selected examples.

L52 Bacterial Life Based on Fluorinated Amino Acids

F. Agostini, L. Sinn, D. Petras, P. C. Dorrestein, J. Rappsilber, N. Budisa, B. Koksch
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In a league of its own, fluorine enables us to engineer biopolymers with highly desirable properties. The particular challenge in using it as a tool lies in our ability to juggle the interplay between the specific properties of the fluorinated building block and its responsiveness to the environment it is exposed to.[1,2]

One of our current projects studies the way in which not just biomolecules in the laboratory, but whole living organisms accommodate fluorine. Fluorine incorporation into single proteins via related amino acid analogues has become common practice. However, an essential question remains: Can fluorinated amino acids generally be used to build up biomass or does the presence of large amounts of fluorine in cells render them nonviable? To gain information about the effect of long-term exposure of living cells to fluorine, we constructed an experimen based on bacterial adaptation in artificial fluorinated habitats. We propagated *Escherichia coli (E. coli)* in the presence of either 4- or 5-fluoroindole as essential precursors

for the in situ synthesis of tryptophan analogues. We illustrate the proteome-wide incorporation of 4- or 5-fluorotryptophane via translation in response to more than 20000 UGG codons in *E. coli*. Genomic, proteomic and metabolomics analyses reveal that full adaptation requires astonishingly few genetic mutations but is accompanied by large rearrangements in regulatory networks, membrane integrity and quality control of protein folding and this talk will introduce some of our results.[3]

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3 F. Agostini, L. Sinn, D. Petras, P.C. Dorrestein, J. Rappsilber, N. Budisa, B. Koksch. *submitted*

L53 Synthetic Biology Approaches to Bioorthogonal Chemistry

Michelle Chang
University of California at Berkeley

Nature has developed biosynthetic routes to the production of a wide range of small molecules. We have been exploring the biosynthesis of a terminal alkyne amino acid made by soil bacteria and its application to bioorthogonal chemistry.

L54 Ghost Proteins

Ronald T. Raines Department of Chemistry, Massachusetts *Institute of Technology, Cambridge, MA*

The surface of mammalian cells is highly anionic. Accordingly, Coulombic repulsion prevents anionic molecules from entering cells. We have tuned the reactivity of a diazo compound to elicit the efficient O-alkylation of carboxylic acids in water.[1] Such esterification enables proteins to traverse the plasma membrane directly, like a small-molecule prodrug.[2,3] As with prodrugs, the nascent esters are substrates for endogenous esterases that regenerate native proteins in the cytosol. The hydrolysis of nascent esters is necessary for membrane traversal. [This work was supported by grant R01 GM044783 (NIH).]

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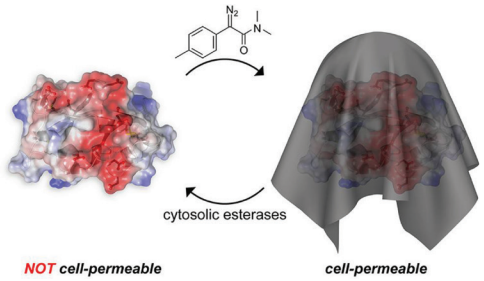


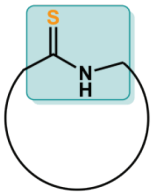
L55 Can Amide Bond Thionation Improve the Pharmacological Landscape of Therapeutic Peptides?

Jayanta Chatterjee
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

Solvation of an amide bond is a crucial factor that impedes the membrane permeability of peptides.[1,2] Therefore, desolvation of the amide groups either by direct chemical modification or via conformational control allows for improving their passive membrane permeability. The membrane permeability of cyclosporine A has been the source of inspiration to utilize N- methylation for improving the membrane permeability of cyclic peptides and has led to the identification of several orally bioavailable peptides and scaffolds.[3] However, curiously, the carbonyl oxygen (C=O) has never been utilized to assess its significance in amide bond desolvation.

Thus, we critically analyzed the desolvation of amide bonds by sequentially occluding both the hydrogen bond donor and acceptor property by N-methylation and thioamidation,[4] respectively. Desolvation of an amide bond enhanced the lipophilicity of model dipeptides, where surprisingly, thioamidation showed a stronger effect than N-methylation. Consequently, monothioamidated model macrocyclic peptides were synthesized that showed increased lipophilicity than the parent peptide. Remarkably, several monothioamidated analogs showed significantly improved membrane permeability than the all-oxo cyclic peptide. Conformational analyses indicated that a thioamide substitution increases the lipophilicity of the macrocyclic peptide due to a combination of thiopeptide-bond desolvation and amide H^N shielding due to conformational restriction.[5] The structural rigidity subsequently leads to the protection of the thioamidated macrocyclic peptides against proteolytic digestion in gastrointestinal fluids and blood plasma. We observed a good correlation between structural rigidity, transcellular- permeability, gastrointestinal stability and *in vitro* intrinsic clearance against liver microsomes of the monothioamidated macrocyclic peptides.

Therefore, a systematic single atom substitution (O to S) in the amide backbone of macrocyclic peptides show remarkable potential to increase their *half-life* in biological fluids and enhance their membrane permeability that will have direct impact on the oral bioavailability of macrocyclic peptides.



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L56 Development of a Strategy for Discovery of Superior Analogues of Antimicrobial Natural Products

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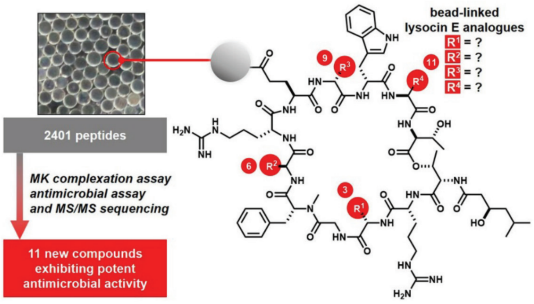
Here we report a novel strategy for the discovery of potent antibiotics by preparing thousands of analogues of a highly complex natural product. Lysocin E, **1**, a 37-membered natural peptide, induces rapid bacteriolysis of methicillin-resistant Staphylococcus aureus, MRSA. We designed the one-bead-one-compound approach, in which each bead carried a structurally unique analogue in submicrogram quantity, to produce a 1-based library consisting of 2401 bead-linked cyclic peptides.



By developing a new high throughput method that integrates submicrogram-scale solid-phase total synthesis, split-and-mix randomization, tandem mass spectrometry-sequencing, and miniaturized assays, we determined 26 candidates. Re-synthesis of these candidates in milligram scale disclosed that 11 artificial analogues exhibited antimicrobial activity more potent than or comparable to that of 1. Because of their high potency, newly discovered peptides will serve as highly promising seeds for the development of pharmaceuticals to treat various infectious diseases.

Citations

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L57 Conception of Prostaglandin F2α Receptor Allosteric Modulators that Delay Preterm Birth by Harnessing the Paired Utility of Aza-Amino Acyl Proline and Indolizidinone Amino Acid Residues for Peptide Mimicry

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Aza-amino acyl prolines and indolizidinone amino acids share common conformational preferences as demonstrated in second mitochondria-derived activator of caspase protein peptidomimetics, in calcitonin gene-related peptide antagonists, and in modulators of the prostaglandin-F2α receptor (FP).[1-4] Systematic study of the conformation and side chain functions of the central turn dipeptide residue of FP modulators has been used to demonstrate the sensitive relationships between activity and topology. Moreover, study of aza-Gly-Pro and aza-Phe-Pro analogs in a murine preterm labor model featuring treatment with lipopolysaccharide demonstrated their capacity to extend significantly (>20 h) the average time of delivery offering new prototypes for delaying premature birth. Our presentation will highlight the relevance of having two classes of scaffolds to mimic the same conformation and their use to facilitate lead development of FP modulators.[5]

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L58 Understanding the Biosynthesis of the Glycopeptide Antibiotics

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The glycopeptide antibiotics (GPAs) are a structurally complex and medically important class of peptide natural products that include the clinical antibiotics vancomycin and teicoplanin.[1] They contain a large number of non-proteinogenic amino acids and are produced by a linear non-ribosomal peptide synthetase (NRPS) machinery comprising seven modules.[2] Furthermore, GPAs are extensively crosslinked late in their biosynthesis on the NRPS assembly line by the actions of a cascade of cytochrome P450 enzymes, a process which contributes to the rigidity, structural complexity and activity of these compounds.[3] Due to the challenge of synthesizing GPAs, biosynthesis remains the only means of accessing GPAs for clinical use, which makes understanding the biosynthesis of GPAs of key importance.

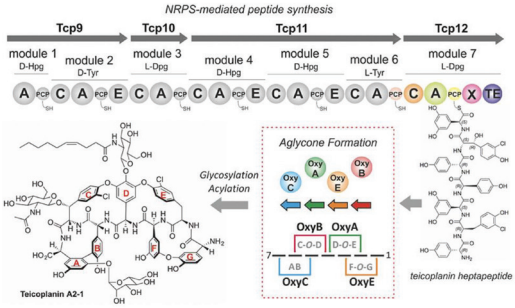

Figure 1: The biosynthesis of the glycopeptide antibiotics as exemplified for teicoplanin. In this process, a linear heptapeptide precursor is first assembled by the non-ribosomal peptide synthetase (NRPS) machinery, which is then cyclized by the actions of 3-4 cytochrome P450 (Oxy) enzymes prior to cleavage from the NRPS and subsequent structural diversification of the peptide aglycone.

In this presentation, I will detail results from our recent studies into the enzymology of the peptide assembly line, the P450-cyclisation cascade and the interplay of these two important biosynthetic processes during GPA biosynthesis. This includes the characterization of key enzymatic processes during NRPS-mediated peptide biosynthesis (chlorination, thioesterase activity and reconstitution of peptide synthesis)[4] as well as the P450-mediated cyclization cascade (substrate specificity of P450 enzymes and cascade reconstitution).[5] Overall, our results demonstrate how selectivity during GPA biosynthesis is mediated through the careful orchestration of critical modification steps and interactions between the peptide-producing NRPS machinery and trans-modifying enzymes.

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L59 The Total Synthesis of Amanitin and Natural Product Inspired Peptides

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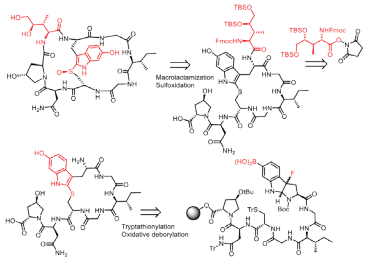
Alpha-amanitin is a classic natural product that was isolated 80 years ago[1] from the notorious death-cap mushroom, *A. phalloides*, which, since Roman times, has been an agent of murder and suicide. Alpha-amanitin, a potent, orally available, highly selective allosteric inhibitor of RNA polymerase II (Pol II), has been featured in thousands of publications, most notably: x-ray[2-4] and NMR total structure elucidation[5,6], affinity chromatography for the purification of RNA Pol II[7], co-crystallization with RNA Pol II[8], recent cryo-EM studies[9], and as a toxic payload for antibody-drug conjugates[10]. Its bicyclic octapeptide structure contains two key oxidized amino acids: trans-4-hydroxy-proline (Hyp) and notably (2S,3R,4R)-4,5-dihydroxy-isoleucine (DHIle). In addition, a crosslink comprising 6-hydroxy- tryptathionine-(R)-sulfoxide is unique among natural products. These key structural motifs have represented a long-standing synthetic challenge in total synthesis. By addressing a delicate three-fold oxidation of tryptophan to deliver the key 6-hydroxy-tryptathionine- (R)-sulfoxide along with the first enantioselective DHIle, we sealed the first total synthesis of amanitin in the synthetic record[11] (below). This work now provides access to derivatives to probe critical structure-activity relationships as well as a means of accessing scalable quantities of the toxin. Hence, we have applied aspects of this methodology to the synthesis of a prototypical phalloidin library as well as other monocyclic peptides of clinical interest[12]. The underlying methods that provided the synthesis of this venerated toxin along with other medicinally important peptides will be discussed.



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L60 Cell Penetration Profiling for Biotherapeutics

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Cell penetration is a major obstacle for developing peptide, protein and nucleic acid therapeutics. Many commonly used techniques for measuring cell penetration are qualitative, and most cannot distinguish cytosolic material from material trapped at the cell surface or in endosomes. We have devised a new technique, the chloroalkane penetration assay (CAPA), that measures penetration to the cytosol in a high-throughput, quantitative manner. Here, we describe the advantages and disadvantages of CAPA and demonstrate some of its applications. These include: determining structure-activity relationships for cell penetration of peptide therapeutics,

quantitating penetration into different subcellular organelles and compartments, and measuring cytosolic penetration across a wide range of bioactive molecules. Our latest efforts are leveraging CAPA to help define the most critical features for promoting cytosolic penetration for a variety of biomolecules and drug delivery systems.

L61 Painting Chromatin with Synthetic Protein Chemistry

Tom Muir
Princeton University

Understanding protein function is at the heart of experimental biology. Perhaps one of grandest contemporary challenges in this area is to catalogue and then functionally characterize protein posttranslational modifications (PTMs). Modern analytical techniques reveal that most, if not all, proteins are modified at some point; it is nature's way of imposing functional diversity on a polypeptide chain. Understanding the structural and functional consequences of all of these PTMs is a devilishly hard problem. While standard molecular biology methods are of limited utility in this regard, modern protein chemistry has provided powerful methods that allow the detailed interrogation of protein PTMs. In this lecture, I will highlight how these tools can be used to probe a series of problems in chromatin biology. In particular, I will discuss how histone PTMs regulate chromatin structure and function and how *dysregulation* of these processes can lead to disease.

L62 Gas-Phase Structural Biology: New Technologies for the Rapid Assessment of Protein Complex Sequence, Structure, and Stability

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Within each living organism proteins are at work, carrying out activities which impact every aspect of cellular function from synthesis to cell death. The next generation of medicines will rely heavily upon our ability to quickly assess the structures and stabilities of such complex macromolecular machines, as well as the influence of large libraries of conformationally-selective small molecule binders and protein-based biotherapeutics. Such endeavours are nearly insurmountable with current tools. In this presentation, I will discuss recent developments surrounding the activation of gas-phase protein complex ions aimed at bridging this gap in basic technology. One such development is collision induced unfolding (CIU), which uses ion mobility-mass spectrometry (IM-MS) to measure the stability and unfolding pathways of gas-phase proteins, without the need for covalent labels or tagging, and consuming 10-100 times less sample than almost any other label-free technology. In parallel with this approach, my lab are pursuing chemical modification strategies aimed at the improved liberation of sequence informative peptide fragments from intact protein complex precursor ions during collision induced dissociation (CID), enabling the assessment of protein quaternary structure and sequence simultaneously. Recent developments in understanding the mechanisms of protein CIU and CID, the ability of these tools to differentiate therapeutic antibodies and enable the discovery of conformationally-selective inhibitors, will be discussed.

L63 Expanding the Genetic Code – Protein Chemistry in Living Systems

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The desire to study and manipulate biological processes in their native environment has fuelled the development of approaches to endow proteins with new chemical functionalities in vivo. Genetic code expansion allows the site-specific incorporation of artificial amino acids into virtually any protein in living cells and animals. Together with significant developments in designing and re-discovering chemical reactions that are amenable to physiological conditions and applicable in living systems these methods have begun to have a direct impact on studying biological processes.

By site-specifically incorporating unnatural designer amino acids into proteins, we have developed tools to visualize proteins and modulate their enzymatic activity in living cells and to study protein-protein interactions as well as posttranslational modifications.[1-3]

In this talk I will present a novel approach that utilizes site-specifically incorporated designer amino acids as a platform for chemoenzymatic reactions. Combining genetic code expansion, Staudinger reduction and sortase-mediated transpeptidation we have developed a novel and generally applicable tool to ubiquitylate/SUMOylate target proteins in an inducible fashion. [4] Our approach allows the site-specific attachment of ubiquitin and ubiquitin-like modifiers — via a native isopeptide bond — to non-refoldable, multi-domain proteins under native conditions. It further enables for the first time the site-specific, inducible and E1/E2/E3-independent ubiquitylation of proteins in living mammalian cells, providing a powerful tool to dissect the biological functions of ubiquitylation with temporal control.

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L64 Bioorthogonal Cleavage Reactions in Space and Time: From Living Cells to Living Animals

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Employing small molecules or chemical reagents to modulate the function of an intracellular protein of interest, particularly in a gain-of-function fashion, remains highly desired but challenging. In this talk, I will introduce a "genetically encoded chemical decaging" strategy that relies on our developed bioorthogonal cleavage reactions to control protein activation in living systems with high spatial and/or temporal resolution. These reactions exhibited high efficiency and low toxicity for chemical decaging of the masked-lysine residue on intracellular proteins, which is complementary to the previously used photo-decaging reactions. We are currently employing this method to block specific kinase's activity in living cells, which allowed the subsequent gain-of-function study of individual kinase within the intracellular signaling transduction network. Our efforts on exploring the therapeutic potential of these novel reactions for pro-drug activation will also be discussed.

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L65 Cell Penetrating Peptides to Increase Passive Permeability of Larger Cyclic Peptides as Inhibitors for Protein-Protein Interactions

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Among developing drugs, the most promising new drug candidates may be targeting protein-protein interactions (PPIs) in cytosol space. Cyclic peptides are leading categorized compounds as inhibitors of PPIs for their structural rigidity and chemical stability. In order to inhibit efficiently, the cyclic peptides should be larger than conventional small molecule drugs to cover larger surface area of PPI. However, the larger candidate peptide to be, the less it permeable passively to get through cell membrane.[1]

We have been developing novel cell penetrating peptides (CPPs)[2] and investigating mechanisms.[3] One of our aims is to facilitate translocation of such large molecular weight PPI inhibitors that have intrinsically poor cell-permeability, when it compares with small molecules. As a proof of concept, we try to improve permeability of cyclosporine A (CsA), which has been known to inhibit a variety of intracellular PPIs through its unusual conformation-dependent passive permeability. By simply mixing CsA with our amphipathic CPPs, the drug is passing through membrane with higher diffusion coefficient measured by the parallel artificial membrane permeability assay (PAMPA).

Furthermore, a dose of the drug is significantly reduced, efficiently inhibiting secretion of cytokines using T cell. *In vivo* animal model studies show that 1/100 amount of CsA with our CPP is enough for efficacy to compare with CsA alone. Results suggest that the combination of CsA and our CPPs is an alternative therapeutic against dry eye syndrome with alleviated side-effects. Taken together, our CPPs can be efficient tools to deliver PPI inhibiting peptide drug candidates whose molecular weight is larger than 1,000.

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L66-YI Computational Design of Structured Peptide PROTACs

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Proteolysis-targeting chimeras (PROTACs) are often composed of two domains, one binding a molecular target and one binding an E3 ligase, connected by a flexible linker. Cooperative binding, enhanced by favorable interaction between target and ligase, increases the amount of ternary complex formed relative to non-cooperative binding at the same concentrations of each species. Cooperativity can be achieved by eliminating the flexible linker and rigidifying the PROTAC. We have recently developed computational methods to design structured macrocyclic peptides to simultaneously bind a relevant therapeutic target, KRAS, and an E3 ligase, VHL, and promote favorable KRAS-VHL interaction. We have shown that the designed peptides efficiently disrupt both interactions of KRAS with its native ligand B-Raf and VHL with its native ligand HIF1a with low micromolar IC50s. Ongoing work aims to further improve two-body and three-body binding interactions of existing designed PROTACs, and further develop these computational methods for additional target-ligase pairs.

L67 Chemical Approaches to Study Cell-Communication in Streptococci

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Quorum sensing (QS) is a communication mechanism in bacteria that regulates a variety of symbiotic and pathogenic phenotypes including root nodulation, biofilm formation, and virulence. As such, QS can be utilized to control bacterial behaviors — promote desirable processes, and attenuate detrimental phenotypes. The Streptococcus genus includes commensal and pathogenic Gram-positive bacteria, many of which are frequently found co-colonizing the same natural niches, such as the oral cavity and upper respiratory tract. Streptococci species utilize QS circuits that are centered on a peptide signal termed the competence stimulating peptide (CSP). The CSP-mediated QS circuits govern competence, bacteriocin production, biofilm formation and virulence factor production. We aim to develop CSP-based QS modulators that would be applied to study different streptococci species and assess the role of QS in bacterial competition, and presented here are our recent results on the *S. pneumoniae*[1-4] and *S. gallolyticus* subsp. *gallolyticus*[5] systems.

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L68 Redesigning Cationic Lytic Peptides to Promote the Delivery of Biomacromolecules into Cell Interiors

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One of the major research interests of our group is to design peptides for intracellular delivery based on the understanding of the molecular interplay between peptides and membranes. Thus, the spider toxin-derived peptide, L17E, was developed and was found capable of delivering biomacromolecules into cells[1]. This peptide was obtained by substituting a hydrophobic leucine (Leu) to a negatively charged glutamic acid (Glu) to reduce hydrophobic interactions, thereby attenuating the membrane lytic activity on cell surfaces. In the presence of L17E, the efficient intracellular delivery of a ribosome-inactivation protein (saporin), Cre recombinase and antibody (IgG) was accomplished, resulting in specific labeling of the cytosolic proteins and subsequent suppression of the glucocorticoid receptor-mediated transcription. Another example of a redesigned lytic peptide is the venom peptide Mastoparan X bearing a tri-glutamate unit and a Ni(II)-dipicolylamine at the N-terminus[2]. Using this peptide, various fluorescently labeled macromolecules were successfully delivered to the cytosol, enabling live-cell imaging of acetylated histones.

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L69 Activity-Based Sensing and Activity-Based Proteomics Approaches to Decipher Metal and Redox Biology

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Traditional strategies for development of chemoselective imaging reagents rely on molecular recognition and static lock-and-key binding to achieve high specificity. We are advancing an alternative approach to chemical probe design, termed activity-based sensing (ABS), in which we exploit inherent differences in chemical reactivity as a foundation for distinguishing between chemical analytes that are similar in shape and size within complex biological systems. This presentation will focus on ABS approaches to develop new fluorescent probes for transient reactive oxygen, sulfur, and carbon species and their signal/stress contributions to living systems, along with activity-based proteomics to identify novel targets and pathways that these emerging classes of chemical signals regulate.

L70-AW Revolutionizing the Discovery Processes of *de novo* Bioactive Peptides and Biologics

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Macrocyclic peptides possess a number of pharmacological characteristics distinct from other well-established therapeutic molecular classes, resulting in a versatile drug modality with a unique profile of advantages. Macrocyclic peptides are accessible by not only chemical synthesis but also ribosomal synthesis. Particularly, recent inventions of the genetic code reprogramming integrated with an in vitro display format, referred to as RaPID (Random non-standard Peptides Integrated Discovery) system, have enabled us to screen mass libraries (>1 trillion members) of non-standard peptides containing multiple non-proteinogenic amino acids, giving unique properties of peptides distinct from conventional peptides, e.g. greater proteolytic stability, higher affinity (low nM to sub nM dissociation constants), and superior pharmacokinetics. The field is rapidly growing evidenced by increasing interests from industrial sectors, including small start-ups as well as mega-pharmas, toward drug development efforts on macrocyclic peptides, which has led to several *de novo* discovered peptides entering pre-clinical and clinical trials. This lecture discusses the aforementioned RaPID system and several showcases of therapeutic potentials of macrocyclic peptides generated by the Suga's laboratory and collaborations with other laboratories.

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L71 Chemical-Proteomic Strategies to Investigate Cysteine Reactivity

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Cysteine residues are critical to the catalytic and regulatory functions of diverse proteins including proteases, oxidoreductases and kinases. Although the majority of catalytic cysteine residues within the human proteome are well annotated, the identity and endogenous functions of regulatory cysteines are poorly understood. These regulatory cysteines are often located distal to the catalytic or ligand-binding sites of proteins and typically regulate protein function through posttranslational modifications such as oxidation, nitrosation and lipidation. To identify and characterize regulatory cysteines, my lab has developed and utilized a suite of chemical-proteomic technologies that report on global changes in cysteine reactivity in vitro and in situ. Several key studies demonstrate the widespread applications of our cysteine reactivity-profiling platforms, including: (1) identifying sites of S-nitrosation in proteomes and characterizing two unannotated S-nitrosation-sensitive cysteine residues on HADH2 and CTSD; (2) developing caged cysteine-reactive probes to identify sites of cysteine oxidation directly in living cells during epidermal growth-factor (EGF) signaling; (3) enriching mitochondria to increase the number of identified mitochondrial cysteines by >10 fold over previous studies; (4) elucidating metal-binding sites on proteins, including cysteine residues involved in zinc and iron coordination; and, (5) characterizing cysteine-mediated protein activities that regulate lifespan through the insulin/IGF-1 signaling pathway. Together, these studies highlight the utility of our chemical-proteomic platforms for elucidating cysteine function in disparate biological systems.

L72 Synthesis and Discovery in Genetically-encoded Peptide Space

Ratmir Derda
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Genetically-encoded (GE) libraries of proteins are a major source of discovery of "biological" drugs generating a $200 billion in sales in 2017. In Chemistry, GE libraries of 109 polypeptides made of 20 natural amino acids represent an orthogonal "raw material for organic synthesis". Like canonical feedstock—petroleum-derived starting materials—GE-peptides are readily available but have limited structural diversity and practical utility. Like petroleum, peptides can be transformed to useful structures through multi-step organic synthesis. Departing from traditional diversification of low-functionality, achiral starting materials, we employ "late stage" modification of polar, functionality-rich, chiral molecules in water. Each transformation, when optimized, can routinely convert billion starting materials to billion products at once.

I will focus on recent developments from our group that expands the use of GE-technologies to Organic Chemistry and Chemical Biology. (1) Drug discovery for "undruggable targets" necessitates new chemical scaffolds of large surface area that do not break down in aggressive proteolytic environment encountered in serum or GI-tract. Using GE-libraries of peptides as a starting material for multi-step organic synthesis, we produce GE-libraries of novel bicyclic architectures that exhibit remarkable stability to proteolytic degradation. (2) We show that libraries of phage-displayed peptides can tackle fundamental physical-organic questions such as substrate control of Wittig reactions. (3) To permit building libraries with unnatural chemotypes / fragments / pharmacophores, I will describe new chemical strategy to late-stage functionalization of macrocyclic peptide libraries displayed on phage.

L73 Platforms for the Generation and High-throughput Screening of Cyclic Peptide Libraries.

Ali Tavassoli
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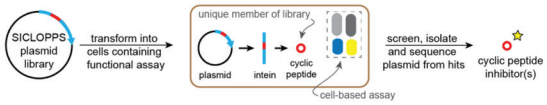
Cyclic peptide libraries hold significant potential when deployed against challenging targets, such as protein-protein interactions. SICLOPPS is a genetically encoded method for the rapid intracellular generation of cyclic peptide libraries of over a hundred million members. One advantage of SICLOIPPS is that is can be interfaced with a variety of cell-based functional assays (in E. coli, yeast or mammalian cells). We will discuss the use of this approach for the identification of inhibitors of a variety of challenging targets, including several first in class compounds.

We will also discuss approaches for taking cyclic peptides forward, beyond hits, into the clinic.



L74 Synthesis and Screening of Libraries of Conformationally Constrained, Peptoid-Inspired Oligomers

Paige Dickson[1], John Maina Ndungu[1], Irena Suponitsky[1] Hongchan An[1], Nicholas Paciaroni[1], Animesh Roy[2], Eric Koesema[2] and Thomas Kodadek[1]
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There is great interest in developing probe molecules and drug leads that target the so-called "undruggable proteome". This is perhaps better described as proteins that lack deep molecular crevices that can be targeted with "Rule of Five" small molecules. It is generally acknowledged that addressing difficult targets, such as those that function via protein-protein interactions (PPIs) will require larger molecules capable of making multiple interactions with the shallow pockets on these proteins. Several laboratories have made significant advances developing PPI inhibitors by structure-guided design of molecules that mimic specific secondary structures in binding partners, such as a-helices. We have embarked on a different approach in which we attempt to create large libraries of conformationally constrained molecules representing many different "folds". This presentation will cover recent developments in these efforts with a focus on new chemistry and screening strategies.

L75 High-throughput Discovery and Parallel Optimization of Peptide Therapeutics

Lauren Goodrich, PhD
Director, Research and Development, Nimble Therapeutics; www.nimbletherapeutics.com

We have developed a peptide synthesis and screening platform for the discovery of peptidic molecules that bind to protein targets of interest. This technique employs the use of digital micromirror devices to direct the maskless synthesis of millions of unique peptides in parallel on a microarray surface. Using a chemical catalog of over 300 amino acid building blocks (natural and non-natural), we can synthesize 18 million unique peptides in less than 48 hours. The method can be applied to synthesize both linear and cyclic peptidic molecules, enabling screens in a diverse chemical space. Here, we will describe the core technology and its application to rapidly and systematically evolve high-affinity, high-specificity binding peptidomimetics to protein targets in a reproducible and digitally controlled process.

L76 Development and Exploitation of Tools to Study Protein-Protein Interactions

Andrew J Wilson
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A key problem in life-sciences research is to understand protein-protein interactions (PPI) with molecular and temporal resolution. In this presentation we will describe our latest efforts to develop tools to analyse PPIs and map them using photo-activated peptide/protein labelling and cross-linking chemistry. We have developed a suite of 'tag and transfer' diazirine-based cross-linking reagents;[1] Diazirines are ideal cross-linking groups because upon excitation with UV light, they generate highly reactive carbenes capable of indiscriminate insertion into proximal bonds (Fig. 1a).[2] Here we will exemplify the power of these new reagents for structural proteomics analyses. In the second part of the presentation, focusing on the helix mediated NOXA-B/MCL-1 PPI[3] we will describe efforts to develop peptides conjugated to ruthenium (II) (tris)chelates as reagents that can perform photo- activated traceless protein-labelling reactions[4] —
a necessary first step in being able to label proteins in live cells without abrogating their normal function.

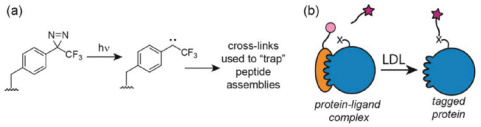

***Figure 1**. Diazirines for PIC (a) UV activated carbine formation (b) schematic depicting the use of photo-activated probes to tag proteins*

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L77 *In situ* Oxidation of N-Phenylglycinyl Peptides for Oxime Bond Formation at Neutral pH

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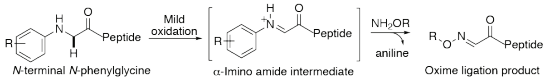
Oxime bond formation between a-oxo aldehydes and aminooxy groups has demonstrated widespread utility for the selective functionalization of biomolecules.[1] Current methods often rely on aniline catalysis to increase reaction rates at neutral pH *via* formation of a protonated aniline-Schiff base intermediate.[2] Here, we report an alternate route to access this reactive intermediate *via in situ* oxidation of *N*-phenylglycinyl peptide substrates under mild aqueous conditions in the absence of catalysts.[3] We demonstrate that the reaction conditions to trigger the tandem oxidation/oxime ligation depend on the electronics of the *N*-phenyglycine residue, are compatible with sensitive residues (e.g. Met), and exhibit improved orthogonality towards aldehydes.



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L78 A New One-Pot Process Improving the Greeness of FMOC SPPS

J. M. Collins, Sandeep K. Singh, Keith A. Porter, Michael J. Karney
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Improving the efficiency of solid phase peptide synthesis is important to meet the growing need for synthetic peptides. We have recently developed a new one-pot Fmoc SPPS process whereby the deprotection and coupling steps share the same solvent without any draining or delay between steps. This new process combined with microwave heating and optimized carbodiimide coupling reduces the entire cycle time to < 3 minutes while dramatically reducing overall waste generated. For instance, a 20mer peptide can be assembled in < 1 hour in high purity with < 200mL waste (0.1mmol scale). This process has been demonstrated on all types of peptides including long, difficult sequences as well as those containing important modifications (ex. phospho, glyco, N-methyl).

This technology achieves an improvement in the greenness of SPPS. The first aspect is that it provides an unprecedented reduction in solvent usage for SPPS. The second aspect is that it provides synthetic purities at the highest level with the use of optimized microwave heating and enhanced carbodiimide chemistry (CarboMAX). This simplifies subsequent purification and recovery, which also improves overall efficiency.

L79 NanoClick Assay: A High Throughput, Target-agnostic Permeability Assay that Vombines NanoBRET Technology with Intracellular Click Chemistry

Andrea Peier, Lan Ge, Nicolas Boyer, Kaustav Biswas, Chunhui Huang, Michael Garrigou, David Tellers, Sookhee Ha, Sir David Lane$, Chris Brown$, Charles Johannes$, Tsz Ying Yuen$, Ahmad Sadruddin, Brad Sherborne, Tomi Sawyer, and Anthony Partridge
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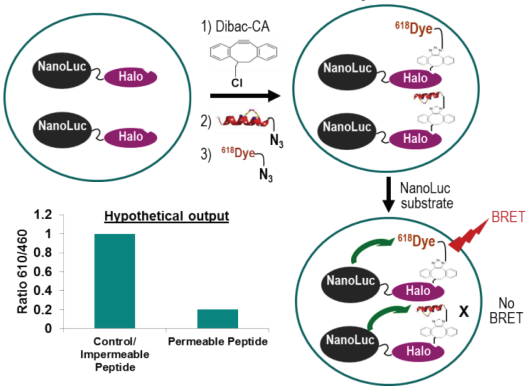
Macrocyclic peptides open new opportunities to target intracellular protein–protein interactions (PPIs) that are often considered non-druggable by traditional small molecules. Specifically, peptides have the potential to bind to shallow and highly expansive binding surfaces (orthosteric blocking) of such PPIs and/or other unique allosteric binding sites. However, their clinical development may be limited by their ability to efficiently penetrate into cells to modulate their cognate PPI targets. The ability to have a predictive, high-throughput assay to assess cell permeability is a critical tool to enable peptide drug discovery programs.

We developed a high throughput, target-agnostic cell permeability assay that quantitates the cumulative cytosolic exposure of a peptide in a concentration- and time-dependent manner. The assay has been named 'NanoClick' as it combines in-cell copper-free Click chemistry and monitoring of a NanoBRET signal in cells. The assay is based on cellular expression of the NanoLuc-HaloTag system and relies on the Click reaction of azide-tagged peptides with DiBac-chloroalkane (CA) anchored to the HaloTag. Subsequent introduction of an azido-dye followed by the NanoLuc substrate allows the detection of a BRET signal that is reduced by the presence of azide-functionalized peptides in the cytosol. The readout can be expressed as a permeability ratio of EC50s when compared to the response of a low permeability control.
We validated the assay using known cell penetrating peptides (CPPs) and were further able to demonstrate correlations to cellular activity using a p53/MDM2 model system. The assay has been applied across several MRL drug discovery programs and has been instrumental to guide and establish Structure – Permeability Relationships in the optimization of macrocyclic peptides for cellular potency across intracellular PPI target programs.

1. Schematic of NanoClick assay



L80-AW Insights in the Binding mode of Peptides, Activating G Protein Coupled Receptors

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Peptides hormones play an important role in the regulation of manifold activities in the body. Many of them transmit their activity through G-protein coupled receptors (GPCR), which are among the most promising drug targets nowadays. By means of ligand modification, receptor mutagenesis, NMR spectroscopy of recombinantly produced receptors and molecular modelling, we have identify the ligand binding site of agonists and antagonists of peptide GPCRs[1,2]. Furthermore, in combination with X-ray crystallography, cell-free receptor expression and photo¬cross-linking we identified the different binding modes of peptide agonists and non peptide antagonists[3,4] as well as different downstream pathways[5]. Knowing the specific binding mode allows the selection of appropriate sites for chemical modification of the ligand, including fluorescence labelling, lipidisation and PEGylation, which significantly modify the activity of the ligand[6,7] and leads to effective compounds *in vivo*[8]. Current application expand the direct effect of the peptide as drugs but allow using them as shuttling systems for intracellular targets e. g. in cancer or metabolic diseases [9].

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L81 Self-assembly of Peptides on Gold Nanoparticles for Molecular Recognition and Catalysis

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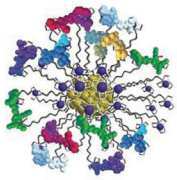

The exquisite properties of proteins in terms of molecular recognition and catalysis derive in large part from their size and structural complexity. Their size permits the presence of internal cavities, which are well-shielded from the bulk solvent, and permit additional advantages like allosteric control by secondary binding sites and multivalent interactions to drive protein-protein interactions. The fact that all proteins are composed of a very limited number of amino acids renders these structures even more fascinating. A strong impetus exists to develop synthetic structures able to match the size and complexity of proteins for applications in diagnostics, medicine and materials science. The advantage of synthetic structures is the ability to design the desired structure and the possibility to include non-natural building blocks. Here, we show that the self-assembly of peptides on Au nanoparticles is an attractive tool to obtain functional protein-like nanostructures for application in molecular recognition and catalysis.

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L82 Control of Alternating Polymer Backbones in ROMP: Toward Sequence Control

Nicole S Sampson, Jingling Zhang, Francis Boadi, Kathlyn A. Parker, Li Tan, Guofang Li, and Airong Song
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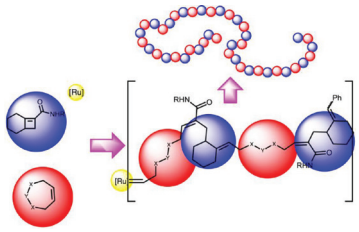
A desire to control the orientation and stereochemistry of monomer incorporation led to our design of new substrates for ruthenium-catalyzed ring-opening metathesis polymerization (ROMP). We demonstrated that cyclobutene-1-carboxylate esters, undergo alternating ROMP (AROMP or alt-ROMP) to give copolymers with precisely controlled sequence. Our alternating copolymer systems allow functionality to be placed along a polymer chain with larger than typical spacing.[1] We have used both homopolymers and alternating copolymers derived from cyclobutenes for mimicking antimicrobial peptides.[2] This AROMP protocol has been expanded to bicyclo[4.2.0] alkenes and cyclohexene that gives very long, linear copolymers with rigorous sequence alternation containing alternating unsaturated amides and 1-alkylidene-2-alkylcyclohexanes in a precisely controlled sequence.[3-5] We will also present new large cycloalkene6 monomers that may be used in place of cyclohexene and which provide polymers with heteroatom backbone functionalities that are degradable.



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L83-YI Tailoring the Self-assembly of a Tripeptide for the Formation of Antimicrobial Surfaces

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Bacteria settling on surfaces are currently one of the greatest concerns for the supply of proper health, water and energy. Bacterial accumulation on medical devices and implants, impair their function and can lead to severe infections and even death. Materials addressing this phenomenon are called antifouling materials. Different materials have been developed in the last 50 years, however, no optimal solution has yet to be found.

Here, we describe the self-assembly of a short peptide into two different types of supramolecular structures, depending on the pH of the solution. These particles are designed to reduce bacterial adhesion and at the same time release biocidal compounds. By using NMR and molecular dynamics (MD), we determined the structures of the peptide monomers and showed the forces directing the self-assembly of each structure under different conditions.

When adhered to a surface, the peptide particles modify its chemical and physical characteristics and confer it with the ability to resist biofouling. The inclusion of biocidal compounds (e.g. antibiotic, enzyme and anticancer drug) in the particles resulted in an improved antimicrobial activity of the surface. This approach and the detailed understanding of the processes are relevant for developing new sterile surfaces for health-care systems, water purification devices, food packaging or any environment that suffers from biocontamination.

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L84 Metal-promoted Assembly of Peptide-based Materials or Regenerative Medicine

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An innovative strategy to produce synthetic, three-dimensional, functionalized collagen and coiled-coil peptide-based materials will be presented. Strategically placed metal- binding sites within the peptides drive metal-ligand assembly, producing unique assemblies - including micron-sized florettes (Figure 1 left), 3D-crystals (Figure 1 right), cages, fibers, meshes and disks. The use of additional ligand binding sites within and on the surface of the peptide materials for inclusion of bioactive molecules will be described.

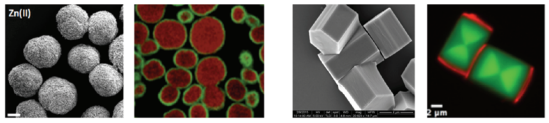

Figure 1. The inclusion of additional assembly signals with collagen (left) and coiled-coil (right) peptide trimers has led to their metal-promoted hierarchical assembly into micron-sized florettes and 3-D hexagonal crystals, both with included guests.

L85 Controlling Supramolecular Assemblies with Peptidic Scaffolds

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Self-assembly and selective recognition events involving proteins are critical in nature for the function of numerous different processes, for example, catalysis, signal transduction or the controlled formation of structural components such as bones. My group is intrigued by the question whether also peptides with significantly lower molecular weights compared to proteins can fulfill functions for which nature evolved large macromolecules. Specifically we ask whether peptides can serve as effective asymmetric catalysts, templates for the controlled formation of metal nanoparticles, hierarchical supramolecular assemblies, synthetic collagen based materials, or tumor targeting vectors.

The lecture will focus on our research interests in supramolecular assemblies and their application in chemical biology and material sciences.

POSTER ABSTRACTS

YI-P001 Development of Cell-permeable Macrocycles through mRNA Display

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Most biological processes are governed by protein-protein interactions (PPIs), and developing small molecules modulating these interactions is difficult due to lack of good surface complementarity for the "hot-spots" spread over a large and shallow interaction interface. On the other hand, biologics, such as antibodies, are ideal for targeting PPIs, but they lack cell-permeability. Macrocycles occupy an intermediate space between small molecules and biologics, and have the potential to harness both the extensive diversity and exquisite specificity of biological drugs and small molecules. While the benefits of macrocycles are vast, they have some challenges as potential drug leads, such as, low oral bioavailability and poor membrane permeability. We aim to develop cell-permeable macrocycle scaffolds utilizing NMR, chromatographic techniques, computational tools and biological assays by addressing what makes macrocycles cell- permeable, and then create macrocycle libraries through mRNA display composed of both natural and non-natural amino acids using the developed cell-permeable scaffolds.

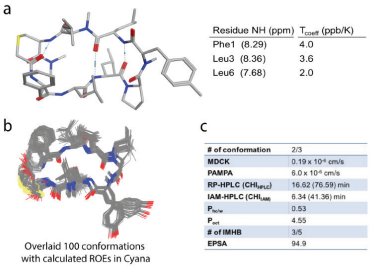

Figure. a. NMR analysis of macrocycle G825 for identification of intramolecular H-bonding network and solution-phase 3D structure driven from NMR analysis in chloroform. b. Overlaid 100 conformations in Cyana using NMR data. c. Properties of G825 in terms of cell-permeability calculated with analytical tools.

YI-P002 Structural Analysis of MAX Peptide Hydrogels via Carbon-Deuterium IR Spectroscopy

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Hydrogel scaffolds are becoming an increasingly popular platform for the design of injectable therapeutics and engineered tissues. One class of hydrogels that shows promise for its biocompatibility, biodegradability, and mechanical properties is the MAX peptides; comprised of two β-strands with a hydrophobic and a hydrophilic face, these hairpin peptides self-assemble into a rigid gel in basic conditions.[1] Hydrogels consisting of a combination of enantiomers of MAX have unique properties, including enhancement in rigidity of the gel.[2] Understanding the process through which the gel forms can inform design, especially for applications that necessitate that it can be shear-thinned and reformed. However, strategies for assessing the dynamics of the folding process are limited due to the self-associated nature of the product and intermediates. We find that changes in molecular environment associated with the peptide assembly can be detected using carbon-deuterium infrared spectroscopy. Using site-selective C-D IR we aim to observe the molecular environment in various MAX based peptide hydrogels and characterize the dynamic processes by which they adopt their hydrogel conformation.

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YI-P003 Multiscale Learning of Thermodynamic and Quantum Chemical Rules of Protein-Carbohydrate Interactions

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The complexity of interaction networks gives molecular systems useful emergent properties, yet makes them challenging to control and design. The interaction networks are only amenable to certain types of analysis depending on the scale at which they are studied. On a macro scale, for example, properties emerging from the protein complexity, such as cooperative folding, are accessible to experimental analyses.[1] On a micro scale, where many-electron interactions are of interest, quantum mechanical analyses can unravel the rules governing electronic interactions. Observations made at these two different scales then can be integrated using multiscale statistical models to indirectly verify the quantum mechanical rules. We employ this paradigm to experimentally and theoretically interrogate the electronic origins of protein-carbohydrate interactions in glycosylated proteins. We have generated a comprehensive experimental dataset comprising the unfolding thermodynamic information for fifty-two molecularly matched pairs of glycosylated and non-glycosylated proteins. The only varying structural fragments in this dataset are the protein and carbohydrate sidechains at two fixed positions. Using a multiscale machine learning approach, we have developed linear and nonlinear statistical models that quantitatively relate the electronic structures of the variable fragments to the experimentally observed interaction energies. This enabled us to identify previously unknown aspects of protein-carbohydrate interactions. Most importantly, we learned that protein-carbohydrate interactions in our model system are a function of the deep molecular orbital energy gaps of

the interacting fragments. Inclusion of deep molecular orbital overlaps in evaluation of previously considered non-covalent interactions should facilitate the accurate modelling and design of complex molecular systems.

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YI-P004 Antibacterial Activity of Short Peptides Derived from Rainbow Trout Cathelicidin

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Nowadays there is a big challenge in the search of new anti-infective agents with lesser effect on the resistome of pathogens. The use of antimicrobial peptides (AMPs) has been proposed as an alternative to conventional antibiotics, mostly because of their versatility and therapeutic properties. Among the AMPs already described in the APD3 database, those from aquatic organisms are interesting because of their spectrum of action, effectivity and because of being part of the immune system. Cathelicidin is an AMP from aquatic origin that presents high antimicrobial activity; however, its application as an antibacterial agent is reduced because of its molecular size (~60 aminoacid residues). The purpose of this work was the characterization and evaluation of functionality against Gram positive and Gram negative bacteria of peptides derived from rainbow trout cathelicidin. Short peptides of about 15 amino acid residues were designed by *in silico* analysis and produced by Fmoc solid phase peptide synthesis. Peptides were characterized by HPLC and mass spectrometry, and their secondary structure was determined by circular dichroism spectrometry in TFE30%, PBS, DMPG and SUV of bacterial membrane, and their antibacterial activity was determined on eight different bacterial strains. Short peptides were more active on Gram positive bacteria with a MIC of about 5μM. Short synthetic peptides with fairly high antimicrobial activity have been obtained based on an AMP of marine origin which is a component of the immune system of salmonids. Further studies will be aimed to evaluate the stability of such peptides and explore their immunomodulating properties.

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YI-P005 A Robust Enzymatic Nanoformulation Based on High Performance Lipase Immobilized onto Nanocellulose Fused Polypyrrole/graphene Oxide: Exquisite Stability and Catalytic Performance

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Herein as a promising nanozyme formulation, we have designed and fabricated a novel nanobiocatalyst based on high performance lipase immobilized onto nanocellulose impregnated polypyrrole/graphene oxide to explore its potential in industries to synthesize aromatic fruit flavour ester. The fabricated nanoformulation was characterized using Fourier transform infrared spectroscopy, differential thermal analysis, thermogravimetric analysis, X-ray diffraction, scanning electron microscopy, atomic force microscopy, transmission electron microscopy, and *Candida rugosa* lipase was immobilized onto nanocomposite through physical adsorption. The catalytic efficiency and operational stabilities of immobilized lipase were improved significantly compared to the free lipase. The reusability profile outcomes showed that the immobilized lipase formulation was an outstanding nanobiocatalyst as it retained 85% of its original catalytic activity after 10 cycles of application. The nanobiocatalyst was employed for the synthesis of the fruit flavour compound, ethyl acetoacetate. The immobilized lipase successfully synthesised flavour compound in solvent free media and n-hexane having 27.5% and 75.5% ester yields respectively. Moreover, these outcomes demonstrating graphene oxide modified carrier induced stabilization, amended solvent tolerance and operational stability of immobilized enzyme, will have quintessential influence on practical scale up of biotechnological industries.

P006 Synthetic Macromolecules as High Affinity PPIs Identified by uHTS Using Fiber Optic Array Scanning Technology (FAST)

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SRI International

Peptide libraries have been used for decades as powerful tools for probing protein-protein interac-tions for drug discovery, diagnostics and other biochemical pharmacology applications. One-bead one-compound (OBOC) libraries offer a powerful methodology for producing large combinatorial synthetic libraries. Current methods, flow cytometry for screening and MS/MS for sequencing, require the use of a minimum size beads of 30 μm with a moderate screening and sorting through-put, can be time consuming and are limited to peptides/peptoids building blocks and short oligo-mers. These limitations present a significant bottleneck for overall screening throughput and the ability to screen larger and more diverse OBOC libraries. We have developed a new methodology that enables the efficient production and screening of libraries of non-natural macromolecules with millions to billions of compounds with a reliable, automated sequencing method for developing high-binding-affinity reagents. The screening method utilizes a Fiber-optic Array Scanning Tech-nology (FAST), that is based on a laser scanning technique that enables screening of OBOC librar-ies with an unprecedented throughput of 5M beads per minute. We designed OBOC macromole-cule libraries to have novel and highly diverse building blocks that can be efficiently sequenced without complex MS/MS fragmentation analysis down to 1 fmol sensitivity. Combined with FAST, this

methodology enabled us to synthesize 1-billion compound library on 10 µm beads and demonstrate a screening throughput of 100M compounds per day. This method has been demon-strated to identify protein-protein interaction with sub nanomolar to picomolar affinities for high-impact targets, such as: KRAS, IL6 / IL6 receptor and Asialoglycoprotein receptor (ASGPR).

YI-P007 Self-Assembled Amyloidogenic Peptides as a Novel Nanovaccine Platform

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The design of nanoparticles exposing a high density of antigens constitutes a promising strategy to address safety concerns of conventional life-attenuated vaccines as well as to increase the immunogenicity of subunit vaccines. In this study, we developed a fully synthetic nanovaccine based on an amyloid peptide sequence with high self-assembling properties. The immunogenic epitope E2EP3 from the E2 glycoprotein of the Chikungunya virus was used to evaluate the potential of a 10-mer peptide derived from an endogenous amyloidogenic polypeptide as a novel vaccine platform. Chimeric peptides, comprising the peptide antigen attached to the amyloid core by a short flexible linker, were prepared by solid phase synthesis. As observed using atomic force microscopy, these polypeptides self-assembled into linear and unbranched fibrils with a diameter ranging from 6 to 8 nm. A quaternary conformation rich in cross-β-sheets characterized these assemblies, as demonstrated by circular dichroism spectroscopy and thioflavin T fluorescence. ELISA assays and transmission electronic microscopy of immunogold labeled- fibrils revealed a high density of the Chikungunya virus E2 glycoprotein derived epitope exposed on the fibril surface. These amyloid fibrils were cytocompatible and were efficiently uptaken by macrophages. Mice immunization revealed a robust IgG response against the E2EP3 epitope, which was dependent on self-assembly and did not require co-injection of the Alhydrogel adjuvant. These results indicate that cross-β-sheet amyloid assemblies constitute suitable synthetic self-adjuvanted assemblies to anchor antigenic determinants and to increase the immunogenicity of peptide epitopes.

YI-P008 Cell Penetrating Transformable Peptide Nanoparticles for Lysosomal Disruption and Cisplatin Sensitization in Non-Small Cell Lung Cancer

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In the United States non-small cell lung cancer (NSCLC) accounts for approximately 14% of all cancer deaths and is by far the largest contributor to cancer deaths annually. Traditional combination chemotherapies are toxic and chemotherapeutic resistances are often developed by tumors. Therefore, novel mechanisms by which to induce cancer cell death and chemotherapeutic sensitization are highly sought after. Recently, several drug candidates have been shown to selectively induce lysosomal membrane permeabilization (LMP) in aggressive cancer lines. And others like chloroquine, an anti-malarial drug, have been shown to sensitize NSCLCs to cisplatin in human trials. These drugs and others have led to great interest in LMP and lysosome dysregulation as a cancer therapeutic target. However, novel approaches are needed to overcome the two central limitations of most lysosomal inhibitors and LMP agents: low specificity and low potency. We have developed peptide amphiphiles which self-assemble into nanoparticles colocalize with the lysosome (Persons coefficient: 0.94), form aggregates due to the lysosomal pH shift, and induce LMP and cell death. We show that these transformable peptide nanoparticles are highly toxic to A549 cells with an IC50 in the low micromolar range and sensitize A549 cells to Cisplatin in the nanomolar range. Moreover, while these aggregates form in A549 lung carcinoma cells, they do not form in HEK 293, 3T3 fibroblasts or human cerebral microvascular endothelial cells. This is the first example of lysosome induced molecular self-assembly and it offers a promising new strategy for cancer therapy.

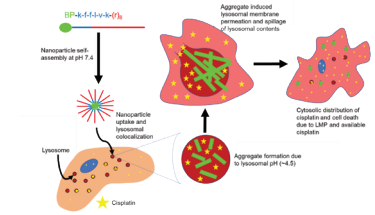

Figure 1: Schematic of CPTNP action *in vitro*. Briefly CPTNPs form peptide nanoparticles which are taken up by cells and traffic to the lysosome. Lysosomal pH shift induces nanofibril formation and lysosomal membrane permeation, thereby releasing the lysosomal contents into the cytoplasm and inducing LMP mediated cell death and cisplatin sensitization.

YI-P009 Improving Stability of Tridecaptins Towards D-Stereoselective Peptidases and Increasing Synthetic Cost Efficiency

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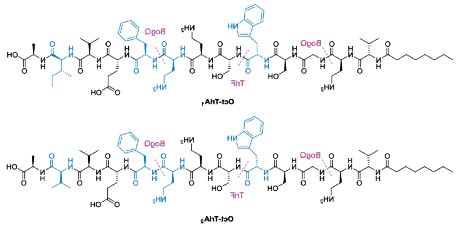

Tridecaptin A1 (TriA1) & TriA2 are non-ribosomal antimicrobial lipopeptides that have strong activity against multi-drug resistant (MDR) strains of Gram-negative bacteria.[1] It has recently been reported that the action of D-stereoselective peptidases could be a developing resistance mechanism against non-ribosomal antimicrobial peptides.[2] TriF, a D-stereoselective peptidase, targets and cleaves the tridecaptins at D-Trp-5, while BogQ can hydrolyse the peptide at D-Dab-2 and D-Dab-8, with the resulting fragments void of antimicrobial activity. Given the vital importance of antimicrobial peptides in killing MDR bacteria, such putative resistance mechanisms must be addressed.Recently, our lab has synthesized novel cyclic analogues of TriA1 that are resistant to cleavage

by TriF.[3] However, these peptides also have reduced antimicrobial activity. Using single amino-acid substitutions, we have synthesized new linear analogues of TriA2, which is substantially cheaper to synthesize than Oct-TriA1, that are resistant to TriF and retain full antimicrobial activity. Furthermore, we have also developed TriA2 analogues that are significantly more stable to hydrolysis by BogQ and retain the activity of the parent peptide. In an effort to improve the cost efficiency of the tridecaptins, we have also synthesised analogues wherein expensive and non-proteinogenic amino acids (Dab, D- Dab and *D-alle*) are substituted for cheaper alternatives, with full retention of antimicrobial activity. Cumulatively, this work has yielded new tridecaptin analogues with strong active against MDR Gram-negative bacteria, low haemolytic activity, high-resistance to degradation by D-peptidases and that are much cheaper to synthesize.

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P010 Cationic Amphipathic Triazines with Anti-bacterial and Anti-atopic Dermatitis Properties

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The emergence of multi-drug resistant bacteria forces the therapeutic world into a position, where the development of new and alternative kind of antibiotics is highly important. Herein, we present the development of triazine-based amphiphilic small molecular antibacterial agents as mimics of lysine- and arginine-based cationic peptide antibiotics (CPAs). These compounds were screened against a panel of both Gram-positive and Gram-negative bacterial strains. Further, anti-inflammatory evaluation of these compounds led to the identification of four efficient compounds, DG-5, DG-6, DL-5, and DL-6. These compounds displayed significant potency against drug-resistant bacteria, including methicillin-resistant *S. aureus* (MRSA), multidrug-resistant *P. aeruginosa* (MDRPA), and vancomycin-resistant *E. faecium* (VREF). Mechanistic studies, including cytoplasmic membrane depolarization, confocal imaging and flow cytometry suggest that DG-5, DG-6, and DL-5 kill bacteria by targeting bacterial membrane, while DL-6 follows intracellular targeting mechanism. We also demonstrate that these molecules have therapeutic potential by showing the efficiency of DG-5 in preventing the lung inflammation of lipopolysaccharide (LPS)-induced acute lung injury (ALI) mouse model. More interestingly, DL-6 exhibited impressive potency on atopic dermatitis (AD)-like skin lesions in BALB/c mice model by suppressing pro-inflammatory cytokines. Collectively, these results suggest that they can serve a new class of antimicrobial, anti-inflammatory and anti-atopic agents with promising therapeutic potential.

YI-P011 Discovery and Characterization of New Membrane Active Peptides

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Membrane permeability is the most capitalized feature of membrane active peptides (MAPs) for high uptake drug delivery systems. The nature of permeability, however, largely remains elusive and the permeability of peptides into cell are not cell specific. Permeability is complex and often described as occurring in multiple sequential steps. The process depends on the peptide itself, the cargo, the nature of the MAP-cargo bond, and the local chemical environment. Most of these MAPs are derived from known proteins found in nature, e.g. analogs of TAT peptide from the HIV-1 virus. While these motifs are certainly effective in their native configuration, they often fall short of clinical applicability due to their high cell toxicity, poor cell specificity, poor stability in vivo and insufficient activity at low doses. In general, rules of peptide entry also remain elusive and are an area of great interest. Many possible mechanisms of entry have been described, and it is likely that even a single type of peptide utilizes multiple pathways concomitantly. We propose that the interaction is additionally dependent on the chemical composition of the membrane itself. My goal is to develop and optimize a new approach to discover the next generation of MAPs by employing OBOC combinatorial methodology, where membrane activity is directly screened for, using biomimetic artificial membranes. In addition, synthetic combinational chemistry methods afford the use of unnatural amino acids. We believe such an approach may allow us to uncover classes of more detailed chemical and structural motifs that go beyond the current documented structures, as well as discover peptides that have a higher stability and potency in biological systems compared to peptides in the existing database. By sequentially screening OBOC libraries with various types of liposomes, we discovered 10 previously unreported lead peptides that selectively bind to fungal-like artificial vesicles and 6 new peptides that bind promiscuously to all three types of artificial vesicles. Using this system, we aim to understand the ability of peptides to distinguish between 2 types of membranes based on their lipid compositions and other properties of the peptides like their mechanism of interaction, cause of curvature and the kinetics of binding.

P012 Machine Learning-based Prediction of Antitubercular Peptides Using a Hybrid Feature Selection Protocol

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Mycobacterium tuberculosis is an insidious scourge to mankind, which acts as an etiological agent of tuberculosis (TB) infecting almost one-third of the world's population. In the recent years, the need to develop novel and effective alternatives for the treatment of TB has aroused with the high prevalence of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Alternative therapeutics such as peptide-based therapy has been substantiated to be persuasive due to several advantages, including diverse mechanisms of action, low immunogenicity, and selective affinity to bacterial cell envelopes. Moreover, identification of anti-tubercular peptides (AtbPs) *via* experimental techniques seem laborious

and expensive, hence the necessity for development of an efficient computational method in the prediction of anti-tubercular peptides aroused prior to both *in-vitro* and *in-vivo* analysis. Consequently, we developed a novel machine learning based predictor called AtbPpred for the identification of AtbPs. In this study, we explored five different machine learning algorithms using 47 feature encodings and developed their corresponding models. Since gradient boosting algorithm using hybrid features produced the best performance among various models, a two-step feature selection protocol was employed and identified the optimal features, which further improved its performance. Upon comparison with the state-of-the-art method, AtbPpred showed superior performance with an overall improvement of ~6% and 11% respectively in benchmarking and independent datasets. To facilitate the utility of our predictor, we have established a user-friendly webserver with the implementation of AtbPpred, which is currently available at http://thegleelab.org/AtbPpred.

P013 Evaluation of the "ResPep Continuous Flow Synthesizer" with Real-time UV-monitoring, Automated Feedback & Heating in Solid Phase Peptide Synthesis

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Automation in assembly of peptides via solid phase synthesis following the Fmoc-strategy[1] is a well-established method and commonly used for peptide synthesis today. Mostly, due to length of the peptide, the presence of hydrophobic stretches or sterically hindered amino acids, difficulties during peptide synthesis are sequence inherent.

Therefore, the choice of the proper conditions like coupling reagent and temperature have tremendous influence on the stepwise amide bond formation yielding the crude product in the highest purity possible. Here, we present examples of automated peptide synthesis of so called "difficult sequences"[2, 3, 4, 5] under demanding conditions on the new ResPep continuous flow (CF) synthesizer with real-time UV monitoring and feedback.

Test sequences

1. ACP-10mer: H-VQAAIDYING-NH2
2. JR-10mer: H-WFTTLISTIM-NH2
3. Exenatide: H-HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSS GAPPPS-NH2
4. Bivalirudin: H-(D)-Phe-PRPGGGGNGDFEEIPEEYL-NH2
5. Asn15-FBP28: H-YYNNRTLESTWEKPQELK-OH

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YI-P014 Computational Design of Structured Oeptide PROTACs

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Proteolysis-targeting chimeras (PROTACs) are often composed of two domains, one binding a molecular target and one binding an E3 ligase, connected by a flexible linker. Cooperative binding, enhanced by favorable interaction between target and ligase, increases the amount of ternary complex formed relative to non-cooperative binding at the same concentrations of each species. Cooperativity can be achieved by eliminating the flexible linker and rigidifying the PROTAC. We have recently developed computational methods to design structured macrocyclic peptides to simultaneously bind a relevant therapeutic target, KRAS, and an E3 ligase, VHL, and promote favorable KRAS-VHL interaction. We have shown that the designed peptides efficiently disrupt both interactions of KRAS with its native ligand B-Raf and VHL with its native ligand HIF1a with low micromolar IC50s. Ongoing work aims to further improve two-body and three-body binding interactions of existing designed PROTACs, and further develop these computational methods for additional target-ligase pairs.

YI-P015 Albicidin – Optimization and Structure-Activity Relationship Studies of a Highly Potent Class of Antibiotics

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Due to its remarkable activity against Gram-positive and Gram-negative bacteria, the peptide antibiotic albicidin, produced by the plant pathogen Xanthomonas albilineans, represents a promising lead structure in the search for a new class of therapeutically useful anti-infectives. Besides improving pharmacokinetic properties and enhancing bioactivity, structural optimizations are crucial to minimizing the effect of known resistance factors. The biological assessment of previous structural modifications, which included the introduction of *N*-terminal acyl groups and variations of the central amino acid, highlighted the prospect of further structural improvements. Albicidin's *C*-terminal dipeptide turned out to be a key pharmacophoric region of the molecule. A rational set of synthetic analogues was prepared and tested for minimum inhibitory concentrations (MICs) against various bacterial strains and DNA gyrase inhibition in an approach to investigate the structure-activity relationship of the molecule.

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P016 Assessing the Role of Hydrophobic Interactions in Competence Stimulating Peptide (CSP)-ComD Binding in *Streptococcus pneumoniae*

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A mechanism of density-dependent chemical communication known as quorum sensing (QS) regulates a variety of bacterial phenotypes, including competence in the *Streptococcus pneumoniae*, that forward pathogenicity and antibiotic-resistance formation. In gram-positive bacteria, QS is mediated by peptides. Competence-Stimulating Peptide-1 (CSP-1) in *S.pneumoniae* is an 17-amino acid amphipathic, α-helical peptide that contains a patch of hydrophobic residues thought to be critical in binding to its cognate receptor (ComD1) and initiating QS. Natural and non-natural amino acids were incorporated though conservative point mutations on the hydrophobic face of CSP-1 to assess the impact of each residue on CSP-1 helicity and QS activation. Our structure-activity studies demonstrate that an α-helical conformation must be maintained for effective receptor binding. In addition, our results reveal that several of the side chains on the native peptide are not optimized for ComD1 binding as more potent CSP-1 derivatives were identified. These findings provide critical insight in the development of potent modulators of *S.pneumoniae* QS.

YI-P017 Teaching an Old Dog a New Trick: Oxime Resin as Versatile Solid-support towards Various Cyclic Peptides Scaffolds

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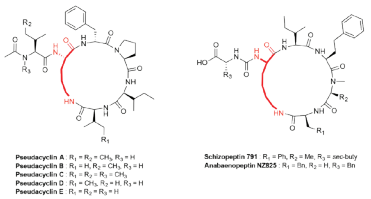
Discovered by Kaiser and DeGrado in the early 80's, Kaiser oxime resin possesses a unique labile oxime ester link that can be cleaved by aminolysis to release the peptide.[1] As oxime ester linkage is stable to acidic, as well as to non nucleophilic basic conditions, anchoring linear peptides as an oxime ester bond allows peptide elongation on solid-support using Boc strategy. After N-Boc removal, the susceptibility of oxime resin to nucleophilic attack of the free amino terminal group allows the release of the peptide via acid-catalyzed *N*-terminal macrocyclization. Based on groundwork from our lab towards large peptide macrocycles,[2-4] we will report our recent results on the chemical reactivity of the Kaiser oxime resin to prepare naturally occuring macrocyclic peptides, from pentapeptides to decapeptides, in a head-to-tail fashion.

We will report also the first on-resin head-to-side chain concomitant cyclization/cleavage using orthogonally protected amino acids (Boc/Cbz) to prepare natural pseudacyclins A-E as well as schizopeptin 791 and anabaenopeptin NZ825 macrocyclic peptide phytochemicals.[5-6]



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YI-P018 Use of Peptide Nucleic Acids for the Chemoselective Ligation of Biomolecules

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The DNA-like, base-pair-specific hetero-dimerization of peptide nucleic acids (PNA)[1] has been utilized for a range of applications, including the alignment and pre-organization of biomolecules, generating spatial proximity, and enabling otherwise unlikely covalent reactions.[2] Here, we explore the possibility of using complimentary PNAs as adapter domains for the chemo-selective, site-directed covalent ligation of intrinsically multi-reactive molecules, such as peptides.

In a first study using match and mismatch 7-mer to 10-mer PNAs, we could show that hybridization of matching sequences, with subsequent covalent stabilization of the resulting heterodimer through formation of a thioether bond, is a promising strategy for the highly selective and site-directed ligation of biomolecules.

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P019 *De novo* Design of Peptides for Enhanced Cell Permeability, Oral Bioavailability and Blood-brain Barrier Traversal

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[a]Department of Biochemistry, University of Washington, Seattle

Peptides offer attractive opportunities for drug design as they combine the stability of traditional small-molecule therapeutics with the specificity and potency of larger antibody-based drugs. The larger surface area and increased number of atomic interactions from peptides enable both better affinity and specificity for targets like protein-protein interfaces that have traditionally been considered "undruggable". We developed new computational tools for precise *de novo* design of diverse conformationally-restricted cyclic and disulfide-linked peptides (18 to 30 amino acids), and harnessed these tools to exhaustively sample the conformational space of smaller (7 to 14 amino acids) peptide macrocycles. These design tools extended the field of *de novo* design to incorporate non-canonical amino acids, and enabled sampling with vast chemical diversity for design of structural folds with shapes beyond natural evolution. *De novo* peptide design capabilities have also opened avenues for systematic exploration of biophysical rules that guide cell-permeability, oral bioavailability

and blood-brain barrier traversal among peptides. Overall, these new computational methods and designed peptides provide the basis of rational design of targeted

YI-P020 Rational Design of Quorum Sensing Modulators in *Streptococcus mutans*

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Streptococcus mutans, a Gram-positive major dental caries pathogen, uses quorum sensing (QS) to control biofilm formation, stress response and bacteriocin production. QS is a communication method bacteria use to regulate gene expression in relation to their population density.[1] S. mutans uses a 21-residue competence stimulating peptide pheromone (21-CSP) as QS regulator. 21-CSP is exported through an ABC transporter, followed by additional processing by a membrane bound protease, SepM, to convert 21-CSP into active 18-CSP that binds to the cognate ComD receptor.[2] Our goal is to find QS modulators that can either activate or inhibit the *S. mutans* QS circuitry. To achieve this goal, we conducted structure activity relationship (SAR) studies of 21-CSP and 18-CSP and found residues important for SepM processing and ComD receptor binding (Figure 1). We also established the minimal structural requirements of 18-CSP for QS activity.[3] Based on these studies we rationally designed highly potent QS activators with activities at the picomolar range.[4] Our studies will help to understand *S. mutans* QS and design therapeutics against S. mutans infections.

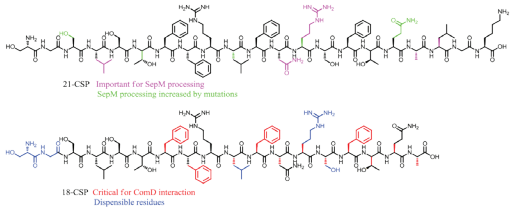

Figure 1. Structural requirements for 21-CSP:SepM processing and 18-CSP:ComD binding.

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YI-P021 Expedient on-Resin Synthesis of Peptidic Benzimidazoles: Applications in Synthesis of Peptide-like Small Molecules

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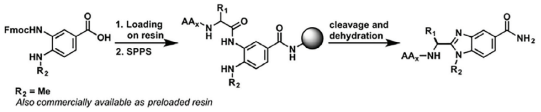
The benzimidazole moiety is a ubiquitous pharmacophore present in numerous anthelmintic, antibacterial, antiviral, antineoplastic, and antifungal drugs. While the polypharmacology of this heterocycle has spurred the development of numerous solution-phase syntheses, only a handful of disparate and inefficient methods detailing its synthesis on-resin have been reported. In 2018 we reported the concise and expedient syntheses of internal and C-terminal peptidic benzimidazoles — an emerging class of peptide deformylase (PDF)-inhibiting antimicrobials. This method benefits from being performed wholly on solid-phase at room temperature resulting in minimal purification and tolerance of temperature sensitive functionality. Here we will demonstrate the synthetic utility of this method through the synthesis of a number of small molecules with use as drugs or biological tools. Applying solid phase peptide synthesis methods and mild benzimidazole formation allows for easier handling and fewer purification steps for these molecules during synthesis.



P022 Solid-phase traceless-Ugi Multicomponent Reactions for One-pot Backbone Anchoring and Cyclic Peptide Synthesis

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On-resin head-to-tail macrocyclization implies the anchoring of the linear peptide precursor via a side chain functional group, a backbone amide or C-terminal bonding on latent inducible linkers (safety-catch) for cyclative cleavage. While side chain anchoring requires the presence of a trifunctional amino acid in the sequence, the cyclative cleavage and backbone anchoring strategies can be performed with any amino acid. The latter, known as the backbone amide linker (BAL) strategy, involves the coupling of an α-amino ester to an aromatic aldehyde linker by reductive amination followed by acylation of the generated benzylic amine with the next amino acid. In an effort to reduce reagent equivalents, reaction time and the number of steps, we have developed a new one-pot methodology to anchor peptides by their backbone to a solid support using an isocyanide-based multicomponent reaction.[1]

The developed approach uses a microwave-assisted Ugi four-component reaction to simultaneously condense and bind a Fmoc-protected amino acid and an amino ester to a supported aldehyde. Afterward, the generated backbone anchored dipeptide can be used in solid-phase peptide synthesis to prepare head-to-tail cyclic peptides. The backbone anchored peptides can be efficiently released from the resin by microwave-assisted acidolysis with trifluoroacetic acid. This straightforward one-pot anchoring approach was also applied to condense fragments and prepare a variety of linear and macrocyclic peptides. The development, optimization and use of the solid-phase traceless-Ugi reaction will be presented.

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P023 Structure-activity Studies of the Antimicrobial Peptide Bactofencin A and its Interaction with the Bacterial Membrane

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[a]Faculty of Pharmacy, [b]Institute of Nutrition and Functional Food, [c]STELA Dairy Research Centre, Université Laval, Quebec (QC), G1V 0A6 Canada; dLaboratory of Medicinal Chemistry, CHU de Québec Research Centre, Quebec (QC) G1V 4G2 Canada

Bacteriocins are a family of ribosomally-synthesized antimicrobial peptides produced by a wide variety of bacteria. With their attractive properties and activities, these peptides are promising alternatives to conventional antibiotics in the food and animal production industry as well as veterinary and human medicine. Among bacteriocins, we were particularly interested in bactofencin A, a peptide of 22 amino acids containing one disulfide bond able to inhibit the growth of several clinically relevant pathogens including *Listeria monocytogenes* and *Staphylococcus aureus*.

In this study, bactofencin A and several analogs were prepared to perform structure-activity relationship studies and investigate the mode of action of the peptide. We observed that synthetic bactofencin A was a potent inhibitor of *L. monocytogenes* and *S. aureus*, similar to the bacteriocin produced naturally by *Lactobacillus salivarius*. Of particular interest is that linear analogs lacking the disulfide bond found in bactofencin A were as potent and also active against several strains of methicillin-resistant *S. aureus* (MRSA) and one strain of vancomycin-resistant *S. aureus* (VRSA). Supported by the structure-activity relationship study, investigation of the interaction of bactofencin A with bacterial membrane by molecular dynamics simulations showed the importance of the positively charged N-terminal tail for peptide-membrane interaction. The synthesis, structure-activities studies and results from the molecular dynamics simulations will be presented.

P024 Engineered pH-responsive Peptide Based siRNA Transporters for Enabling Enhanced siRNA Delivery

Abhijit Biswas[#,1], Chiranjit Dutta[#,1], Kasturee Chakraborty[#,2], Sanchita Mukherjee[#,2], Paramita Gayen[2], Somnath Jan[2], Dhananjay Bhattacharyya[*,3], and Rituparna Sinha Roy,[*,2]
[1]Department of Chemical Sciences, IISER Kolkata; [2]Department of Biological Sciences, IISER Kolkata; [3]Computational Science Division, Saha Institute of Nuclear Physics, Kolkata; #: authors having equal contribution;*: corresponding authors

Cell-penetrating peptide based molecular transporters have gained significant importance for intracellular delivery of functional siRNA. The cellular internalization pathway of CPP-cargo complex is determined by the size, charge and concentration of CPP-cargo complexes and cell types used for the study and mainly occur by endocytic pathways and suffer from endosomal entrapment. The endosomal entrapment and inefficient release of cargo from endosome prevents high yield cytosolic delivery of functional siRNA. Designing clinically safe and effective transporter for cytosolic delivery of siRNA in biologically active form remains as key hurdle. To overcome these challenges, we have designed protease-resistant peptide based molecular transporters having Arg-(D)His-Arg moiety in the sequence and evaluated their physicochemical properties and gene silencing efficacies. Computational studies show that at physiological condition, Arg-His hydrogen bonding interaction stabilizes Arg-^{D}His-Arg template, whereas such interaction disappears at lower pH. pH-sensitive residue, histidine exhibits proton sponge effect in endosomal acidic environment and facilitates the release of entrapped CPP-cargo complexes to the cytosol. Interestingly, our designed histidine-rich molecular transporters demonstrated functional siRNA delivery in breast cancer cell lines like commercial transfection agent HiPerfect and exhibited significant gene silencing in MAPK/ERK signaling pathway as evidenced by RT-PCR and immunofluorescence studies. MAPK pathway promotes cell proliferation, metastasis and drug resistance in triple negative breast cancer cells, MDA-MB-231. We anticipate such molecular transporters might be translated to clinics for developing next-generation siRNA based nanotherapeutics against triple negative breast cancer.

P025 Development of a Potent and Highly Selective Glucagon Receptor Agonist

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Intarcia Therapeutics Inc., Discovery and Translational Medicine, Research Triangle Park, NC 27709, USA

The Glucagon receptor has been well- characterized over time. In recent years, glucagon receptor agonists have become the focus of several metabolic discovery programs due to glucagon's ability to increase energy expenditure and reduce fat mass. In combination with other agents such as GLP-1 agonists, glucagon receptor agonists have been shown to provide additional benefit such as reductions in weight-loss of up to 30%.

At Intarcia our approach was to develop a selective glucagon agonist that can be combined with a selective GLP-1 agonist in an appropriate therapeutic dose ratio. Initial hits-to-lead structure activity relationships (SAR) led to the discovery of ICA6160102A, a potent, selective glucagon receptor agonist. In vivo assessment of this compound as a single agent, as well as, in combination with exendin-4 (GLP-1 agonist) revealed an exceptional weight-loss profile with no indications of safety issues at either the macro or microscopic levels. SAR strategy, in vitro pharmacology, pharmacokinetics, and in vivo assessment of compounds in diet-induced obesity (DIO) rodent models of obesity will be presented. Strategically focused further SAR resulted in a clinical candidate ICA6150349A.

Compound ID	GCGR	GLP-1	Sequence ID
Glucagon (native)	11.3	9.9	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH
ICA6160102A	11.2	<7	YSQGTFTSDYSKYLDSK*RAQE*FVK*WLDE*T-OH

*Denotes location of lactam bridge

YI-P026 Peptide-Oligonucleotide Conjugates as Nanoscale Building Blocks

Josephine T. Boesen[a1], Kasper K. Sørensen[a], Peter W. Thulstrup[b],
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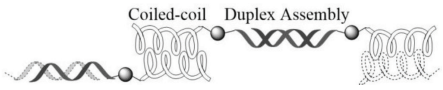
Directed self-assembly, using two bioorthogonal principles, offers the prospect of creating nanoscale building blocks

and synthetic peptides. Designed peptide-oligonucleotide conjugates (POCs) could attain a vital role in the delivery of drugs, as vehicles for drug transport.

We designed a POC where the oligonucleotide forms an antiparallel duplex and the peptide forms an antiparallel dimer. It can thus form large nano-assemblies. Dynamic light scattering, circular dichroism and small-angled X-ray scattering were used to investigate one such peptide-oligonucleotide conjugate. The methods indicated the formation of nanoscale assemblies, many times larger than the starting materials, with formation of higher-ordered structures.



P027 Controlling the Self-assembly and the Morphology of Peptide-based Nanostructures by Electrostatic Capping

Ximena Zottig, Soultan Al-Halifa, Margaryta Babych, Noe Quittot, Steve Bourgault
Chemistry Department, Université du Québec à Montréal, Montreal, C.P. 8888, Succursale Centre-Ville, Montreal, H3C 3P8QC, Canada

Proteinaceous supramolecular assemblies are of great interest for different biomedical applications. Peptides that self-assemble into cross-β-sheet amyloid assemblies constitute promising building blocks to construct ordered functional materials. However, the usage of amyloid assemblies is still limited by the difficulty of controlling and predicting the final supramolecular morphology as well as the intrinsic polymorphism of amyloid-based nanostructures. In this study, we harnessed electrostatic repulsions between N-terminal charges to control the architecture and the polymorphism. Our strategy relies on the addition of different charged capping units on a potent amyloidogenic β-sheet-forming sequence in order to confine the self-assembly energy landscape and to modulate protofilaments packing and growth. A small peptide library derived from the 20-29 segment of the islet amyloid polypeptide (SNNFGAILSS; I10) was designed. Different mesoscopic morphologies were obtained by tuning the charge and the electrostatic force of the capping unit, including short nano-rods, ribbon-like filaments and polymorphic rope-like fibrils. The addition of a positive capping unit led to monodisperse rod-like assemblies, with a unique control over the shape, height and length. These cytocompatible nanorods were very rigid for a biopolymer, with absence of twisting and bending. We proposed that electrostatic repulsions between N-terminus positive charges hinder β-sheet tape twisting, leading to a unique control over 1D amyloid length by protofilament growth frustration. The charged-capping unit strategy presented herein is promising to obtain monodisperse preparation of peptide-based assemblies and offers key molecular insights of the inherent polymorphism of amyloid fibrils.

YI-P028 Synthetic Lipopeptides as Probes in a Novel Fluorescence Resonance Energy Transfer Assay for Lipoprotein Signal Peptidase II

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[1]School of Chemistry and [2]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

Protein lipidation in Gram-negative bacteria is a post-translational modification which requires an essential enzyme, lipoprotein signal peptidase II (LspA). This endoprotease is responsible for cleaving a signal peptide from the N-terminus of the immature lipoprotein. As an essential prokaryotic enzyme, not present in eukaryotes, LspA is an ideal antibiotic target.[1] To screen potential inhibitors a Fluorescence Resonance Energy Transfer (FRET) based assay for LspA, suitable for high-throughput drug screening, was developed. The synthetic lipopeptide based FRET reporter is based on a conserved sequence containing an essential dipalmitoyl- glyceryl cysteine residue. Upon treatment of the peptide with LspA the peptide is hydrolysed and the resultant increase in fluorescence can be used to monitor the activity of the enzyme. During the synthesis of several generations of this FRET probe we have investigated multiple approaches towards peptide lipidation including linear incorporation during solid phase peptide synthesis and late stage lipidation strategies. This assay should aid in the development of new antimicrobial agents through the discovery of novel LspA inhibitors.

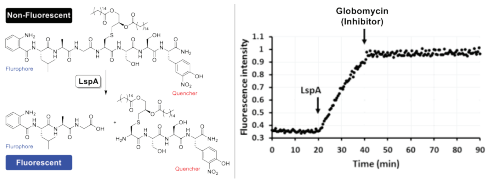

Figure 1: Enzymatic reaction of LspA and FRET peptide.

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P029 A Disulfide-scan of Insulin by [3+1] Methodology Exhibits Site-specific Influence on Bioactivity

Florence M. Brunel[*a], John P. Mayer[b], Vasily M. Gelfanov[a], Alexander N. Zaykov[a], Brian Finan[a], Diego Perez-Tilve[c] and Richard D. DiMarchi[a]
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Insulin is the principal hormone involved in the regulation of metabolism and has served a seminal role in the treatment of diabetes. While a miraculous substance, insulin has notable limitations as a medicine and consequently the quest for safer and more convenient therapy continues. We explored a disulfide scan in search of a single additional disulfide bond that could regulate bioactivity in a binary manner, presumably by reducing conformational flexibility. Building upon recent advances in insulin synthetic methodology we developed a straight-forward route to preparation of nearly two dozen novel analogs that employed a unique [3+1] strategy. Subsequent to formation of the three disulfide bonds in native insulin, an additional fourth site-specific disulfide was selectively formed from two S-Acm protected cysteines. The bioactivity was established for the constrained (4-DS) and unconstrained (3-DS) analogs by *in vitro* methods, and extended to *in vivo*

study for select peptides. All the 4-DS peptides (n=19) were successfully synthesized with insignificant differences in yield, regardless of the projected distance separating the cysteines constituting the fourth disulfide. Conversely, with only a single exception, the attempt to simultaneously form all four disulfide bonds was uniformly unsuccessful. These results demonstrate an unforeseen ability of native insulin to structurally accommodate an additional covalent tether when properly introduced by a two-step synthesis. We also identified a preferred anchor point where a single disulfide bond can significantly regulate insulin activity.

YI-P030 Heterogeneous-Backbone Mimics of Disulfide-Rich Peptide Scaffolds

Chino Cabalteja[1,2], Daniel Mihalko[1], W. Seth Horne[1,2]
[1]Department of Chemistry and [2]Molecular Biophysics and Structural Biology Graduate Program, University of Pittsburgh

Disulfide-rich peptides have garnered interest in the development of bioactive agents due to the diverse structural configurations and potent biological activities accessible to complex polycyclic chain topologies. However, such peptides can suffer drawbacks including low proteolytic stability and synthetic challenges regarding chain topology control. An approach to overcoming the former limitation that has been widely explored in other systems is to alter the connectivity of the peptide backbone in a way that maintains the native fold but hinders recognition by protease enzymes. Here, we advance this "heterogeneous-backbone analog" strategy in the context of a computationally-designed disulfide-rich mini-protein. Substitution of the helix-to-hairpin turn region of the prototype sequence with turn-inducing moieties led to variants that oxidized cleanly into a single product with the correct disulfide-bond connectivity. Increasing the degree of substitution to encompass the remainder of the domain yielded heterogeneous-backbone analogs that also exhibit clean oxidative folding and recapitulate the tertiary structure of the prototype. Moreover, these variants show measurably improved proteolytic resistance compared to that inherent in the topologically constrained scaffold. These results collectively demonstrate heterogeneous-backbone substitution as a robust method for engineering the properties of disulfide-rich peptides, as well as the mimicry of their structure, which may be applicable to the design of peptide-based bioactive ligands.

YI-P031 Applications of Covalent Crosslinking of DNA-Encoded Non-natural Peptides to Protein Targets in Living Cells

Bo Cai, Dongwook Kim, Saeed Akhand, Yixing Sun, Sijie Wang, Michael K. Wendt, and Casey J. Krusemark
Department of Medicinal Chemistry and Molecular Pharmacology, Purdue Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA

DNA-encoded chemical libraries (DELs) have recently emerged as an important tool in molecular discovery. DEL offers extremely high sensitivity and thereby high capacity for chemical diversity, allowing billions of small molecules to be tested as ligands to protein targets in a cost- and time-efficient manner. Selections of DELs, however, have largely been limited to biochemically pure protein targets, which presents a major hurdle for application of DELs against a large proportion of proteins. This includes proteins that are difficult to purify or express recombinantly and proteins that may require additional factors and post-translational modifications for full activity. To expand the utility of DELs, we are applying covalent crosslinking of DELs to target proteins for assessment of ligand binding either inside living cells or to integral membrane proteins on live cell surfaces. Here, we report: 1) selection of a library of DNA-encoded non-natural peptides to targets inside living cells for the first time using a cell penetrating peptide to deliver the DEL into cells. 2) selection of a non-natural peptide DEL via crosslinking to GPCR proteins on live cell surfaces.

YI-P032 Development of MC1R Selective Ligands and Conjugates for Selective Targeting of Melanocortin 1 Receptor Overexpressing Melanoma

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Melanoma is a lethal form of skin cancer. Despite recent breakthroughs of BRAF-V600E and PD-1 inhibitors showing remarkable clinical responses, melanoma tumors eventually become resistant to these therapies. To create opportunities to utilize cytotoxic drugs that have low cancer resistance issue but lack selectivity to melanoma cells, we developed ligand-targeted therapies by using MC1R selective ligands (MC1RL) to selectively target MC1R overexpressing melanoma. The drug-MC1RL conjugates were shown to maintain strong binding interactions to MC1R and induce selective drug delivery to A375 melanoma cells through its MT-II moiety *in vitro*. Furthermore, with camptothecin as the therapeutic drug, camptothecin-MT-II (compound 1) was demonstrated to effectively inhibit A375 melanoma cell growth with an IC50 of 16 nM. By providing selectivity to melanoma cells through its MT-II moiety, the approach of drug-MC1RL conjugates enable us to have much more options for cytotoxic drug selection, which can be the key to solve the cancer resistant problem for melanoma.

Support: GM108040

P033 Antibacterial Peptides from the Microalga *Isochrysis galbana*

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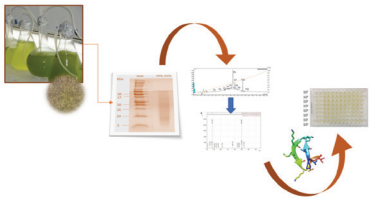
Microalgae are versatile unicellular eukaryotic photosynthetic organisms widely used in biotechnology (de Los Reyes et al 2016; Sun et al 2014). The problem generated by the bacterial resistance to antibiotics has prompted the search for products that can help in reducing its use. Within this context, marine microalgae are particularly interesting microorganisms having the potential to produce this kind of compounds due to the nature of their environment.

Isochrysis galbana is a marine microalga used in aquaculture as feed for bivalves and fish, because of its nutritional value and high content of lipids and unsaturated fatty acids (Aussant et al 2012). There are some reports that associate this microalga with antibacterial activity (Molina-Cárdenas et al 2014), but the products responsible for this activity have not been fully characterized.

In this report *Isochrysis galbana* was used to look for antibacterial activity related to peptide molecules. From

protein enriched fractions obtained from the alga and de novo sequencing, we obtained peptide sequences that were chemically synthesized by Fmoc solid-phase peptide synthesis, and tested against Gram + and Gram – bacteria.

Results showed that peptides from *Isochrysis galbana* were active against some of the target strains used, at concentrations comparable to the antibacterial peptide BTM P1 derived from *Bacillus thuringiensis*, used as positive control (Segura et al, 2006).



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P034 Synthetic Studies of Yaku'amide A and Analogs

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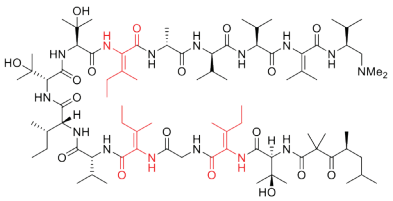
Yaku'amide A is an anticancer peptide natural product with a unique mode of action involving simultaneous suppression of ATP synthesis and promotion of ATP hydrolysis. The results of our synthetic studies targeting yaku'amide A will be presented. The β-tert- hydroxy amino acids were constructed via osmium-catalyzed aminohydroxylation utilizing a chiral mesyloxycarbamate reagent. The E- and Z-ΔIle residues were forged stereospecifically by combining *anti* dehydration, azide reduction, and O→N acyl transfer into a one-pot process. Convergent fragment assembly maximizes the efficiency of the synthetic route, which is still lengthy due to the complexity of the structure. Accordingly, readily accessible yaku'amide A analogs that closely mimic the natural product have been designed using molecular modeling. Progress towards synthesizing these compounds will be detailed.



Yaku'amide A (E- and Z-ΔIle residues shown in red)

YI-P035 Development of Mitochondrial Targeting Vectors

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Mitochondria are key organelles that perform essential cellular functions and exert both vital and lethal functions in physiological and pathological scenarios. Hence, they represent an attractive druggable target to treat metabolic, degenerative and hyperproliferative diseases. Targeting mitochondria with organelle-specific agents or pro-drugs have proven to be an effective therapeutic strategy[1-3]. The mitochondrial membrane potential ($\Delta\psi_m$), the electrochemical gradient built across the inner membrane by the respiratory chain complexes, constitutes a distinguishing feature of mitochondria that can be exploited for targeting of drugs to this organelle. In an effort to provide carriers for mitochondrial delivery of bioactive cargo, the Kelley laboratory at University of Toronto developed mitochondria-penetrating peptides (MPPs) that can efficiently traverse both the plasma membrane and mitochondrial membranes with a variety of attached cargoes[4-7]. Specifically, we focus on the development of chemical tools to perturb mitochondrial biochemistry, probes allowing precise measurement of mitochondrial function, and new techniques for high-throughput characterization of the mitochondrial proteome.

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YI-P036 Selective N-terminal Peptide Modification and Development of Glucose-responsive Insulin

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Over the years, significant progress for the treatment of type 1 diabetes (T1D) has being achieved; Insulin analogues, either fast-acting or long-lasting insulin, have shown improved ability to maintain glycemic homeostasis and reduce diabetes-associated complications compared to native insulin. However, the glucose-lowering action is not regulated in a glucose responsive fashion.[1] Hypoglycemia is the major consequence of insulin overdosing, because current insulin therapies remain bioactive even when blood glucose level fall to a dangerously low level. This can lead to the loss of consciousness, coma or even death. There is currently no glucose responsive insulin (GRI) in market, and only a small number of unimolecular GRI developments have been undertaken in the literature.[2] We aim

to develop a novel series of GRIs with a glucose dependent C-peptide based on the conformational restriction of insulin. The glucose-sensing element will be on the C terminal of the B-chain; while saccharide portion will be put on the N terminal of the A-chain. This will lead to an intramolecular cross-linking that will inhibit the binding between the analogue and insulin receptor at low glucose concentration; while the competitive displacement of this restriction at high glucose concentration will lead to high affinity receptor binding.

We successfully developed a highly selective N-terminal modification of native peptides and proteins based on reductive alkylation.[3] A series of functional groups can be facilely introduced at the N-termini of proteins. We further demonstrated that, in insulin modification, preserving the positive charge at the N-terminus of A chain using reductive alkylation instead of acylation leads to a 5-fold bioactivity increase.3 Taking advantage of this powerful tool, a series of insulin analogues with saccharide modification on the A-chain and glucose sensor modification on the B-chain have been synthesized. The bioactivity and glucose responsiveness of these synthetic insulin derivatives will be further evaluated.

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P037 Purity Determination of Peptide LVNEVTEFAK as a Calibrator for the Measurement of Albumin in Human Urine

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Albumin in human urine is an important bio-marker for the early detection of kidney damage. Accurate measurement of urinary albumin is important for the diagnosis and treatment of kidney disease. In our laboratory, an analytical method was developed for the measurement of urinary albumin using a signature peptide of albumin, LVNEVTEFAK (L-K), as the calibrator. To ensure metrological traceability of the measurement results for urinary albumin, the concentration of L-K ($C_{L\text{-}K}$) in the calibration solution was determined using "peptide impurity corrected amino acid analysis (PICAA)" approach.

In this approach, all peptides (L-K as well as other peptide impurities) in the L-K calibration solution were subjected to hydrolysis. The total concentration of peptides (C_{total}) was determined by amino acid analysis using isotope dilution mass spectrometry (AAA-IDMS). Two hydrolysis procedures (in gaseous and liquid phases), as well as different temperature conditions were investigated. Amino acid certified reference materials (CRMs) were used as the calibration standards for the quantification of C_{total}. The RSD of the AAA-IDMS measurement was less than 1.3% and the total RSD using different amino acids was 0.9%, demonstrating good precision.

To obtain $C_{L\text{-}K}$, the percentage of L-K ($_{PL\text{-}K}$) in total peptides was determined by HPLC-DAD. The total peptide impurities were found to be below 5%. $C_{L\text{-}K}$ was obtained by multiplying Ctotal with $P_{L\text{-}K}$. Using the $C_{L\text{-}K}$ value, our laboratory proceeded to develop the analytical method for urinary albumin and successfully provided the target values for this clinical marker in our External Quality Assessment (EQA) Programme.

P038 Discovery of Low-Molecular Weight Anti-PD-L1 Peptides for Cancer Immunotherapy

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Immunotherapy using checkpoint inhibitors, especially PD-1/PD-L1 inhibitors, has now evolved into the most promising therapy for cancer patients. However, most of these inhibitors are monoclonal antibodies, and their large size may limit their tumor penetration, leading to suboptimal efficacy. As a result, there has been a growing interest in developing low-molecular-weight checkpoint inhibitors.

Here, we developed a novel biopanning strategy named Precision Biopanning Procedure (PBP) and discovered anti-PD-L1 peptide inhibitors (12 aa, ~1.6 kDa) to block the PD-1/PD-L1 interaction. The peptides exhibit not only high affinity but also high specificity to human PD-L1 protein as well as PD-L1-positive human cancer cells MDA-MB-231 and DU145. Molecular docking studies indicate that the CLP002 peptide specifically binds to PD-L1 at the residues where PD-L1 interacts with PD-1. The peptide also blocks the CD80/PD-L1 interaction, which may further enhance the immune response of tumor-infiltrating T cells. The CLP002 peptide restores proliferation and prevents apoptosis of T cells that are co-cultured with cancer cells. The CLP002 peptide also inhibits tumor growth and increases survival of CT26 tumor-bearing mice, suggesting that the CLP002 peptide represents a promising low-molecular-weight checkpoint inhibitor for cancer immunotherapy.

Moreover, low-molecular-weight anti-PD-L1 peptides can be easily linked to a targeting ligand or encapsulated in a nanoscale delivery system to improve their accumulation in the tumor microenvironment, thus minimizing the non-specific blockade effect in other tissues expressing PD-L1.

P039 Effect of Citrulline Side Chain Length on Secondary Structure Formation, and on the TAR RNA Recognition and Cell Penetration of Tat-Derived Peptides

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Arginine (Arg) is a positively charged amino acid (with three methylenes) responsible for protein electrostatic interactions, which are important for structure stability and binding negatively charged biological entities such as nucleic acids and cell surface glycosaminoglycans (for cell penetration). Deimination of Arg forms citrulline (Cit) with a neutral urea side chain. Herein we report a series of systematic structural and functional studies to explore the effect of the number methylenes linking the Cit urea group to the backbone. Alanine-based peptides were used to explore the effect on helix forming

energetics by circular dichroism spectroscopy. Hairpin (strand-turn-strand) forming peptides were used to explore the effect on sheet forming energetics by NMR methods. The effect on the binding of the basic region of the HIV Tat protein, Tat(47-57), to TAR RNA was investigated by electrophoretic gel mobility shift assays. The effect on Tat(47-57) cell penetration into Jurkat cells was determined by flow cytometry. The structural stability studies showed the uniqueness of the Cit side chain length for helix formation, but altering the side chain length had minimal effect on sheet formation. Cit side chain length affected RNA binding in a position dependent manner, but the position of incorporation affected cell penetration more than the side chain length. These results should facilitate the incorporation of urea-bearing amino acids in the design of peptides with specific structure or function

YI-P040 A New Genre of Macrocyclic Peptidomimetics Targeting the Polo-box Domain of Polo-like Kinase 1

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Our work is focused on developing polo-like kinase 1 (Plk1) polo-box domain (PBD)-binding inhibitors with the intent of ultimately achieving constructs that may serve as potential new anti-cancer agents. Starting from a pentamer phosphothreonine (pThr)-containing peptide derived from a cognate Plk1 substrate, we have previously shown that introduction a -(CH2)8Phe moiety from the imidazole pi-nitrogen of a His residue can result in 1000-fold affinity enhancement.1 Herein we disclose compact pThr-containing macrocycles showing similar high affinity, yet in which the alkyl-His residue has been replaced by specifically designed Glu analogs. Variants have also been prepared containing phosphatase-stable pThr mimetics, including (2S,3R)-2-amino-3-methyl-4-phosphonobutanoic acid (Pmab). These analogs represent a new genre of PBD-binding ligand, which may provide new insights to the Field.

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YI-P041 Probing Teixobactin Binding Parameters and Enhancing its Activity Scope Using Adjuvants

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The prevalence of life-threatening, drug-resistant microbial infec-tions has challenged researchers to consider alternatives to cur-rently available antibiotics, especially since the discovery of novel classes of antibiotics has been a rarity in recent years. Teixobactin, a non-ribosomally synthesized peptide from *Eleftheria terrae*, was identified in 2015 by an innovative method of cultivating bacteria.[1] This depsipeptide has been found to be a potent antimicrobial agent with a wide range of activity, including against drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enter-ococci (VRE). It was proposed that teixobactin exerts its antimicrobial activity by binding to precursors required for cell-wall synthesis, such as lipid II.[2] However, the exact interactions which occur between teixobactin and lipid II upon binding are not fully understood. Our studies utilize isothermal titration calorimetry to probe the binding of this key peptide to various lipid II analogues, as well as expanding this strategy to include several teixobactin analogues. Furthermore, the activity of teixobactin against Gram-positive organisms is well documented. We have found evidence that, with the help of adjuvants which can perme-ate the membrane of Gram-negative organisms, teixobactin activity against a panel of Gram-negative organisms is enhanced.

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YI-P042 Development of SEC-HPLC for High Molecular Weight Proteins in Pramlintide Insulin Formulation

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Insulin and amylin are two hormones that are found to be deficient in people with diabetes. These hormones work together and are essential to maintaining blood sugar levels. Amylin is highly insoluble and even toxic to pancreatic beta cells because of deposition of fibrillary proteins. Pramlintide acetate, a soluble analogue is an aqueous, non-aggregating form of amylin established by substituting three proline amino acid residues. This form of amylin was found to be effective in reducing blood glucose level when given subcutaneously. The pramlintide-insulin combination therapy mimics the human body's natural interaction between insulin and amylin to regular blood sugar levels. Insulin is considered to be the main anabolic hormone of the body. Human insulin protein is a dimer of the A-chain (21 amino acids) and a B-chain (30 amino acids) linked together by disulfide bonds, and has a molecular mass of 5808 Da. Here we demonstrate the development of the Size Exclusion Method for the Determination of High Molecular Weight Species in Pramlintide/ Insulin Drug Product.

YI-P043 Akt Kinase Activation Mechanisms Revealed Using Expressed Protein Ligation

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Akt1 is a critical protein kinase that drives cancer proliferation and modulates glucose and insulin responses and is activated by C-terminal phosphorylations at Ser473 and Ser477/Thr479. Here, we employed expressed protein ligation (EPL) to produce

site-specifically phosphorylated forms of purified Akt1 that are well-suited for mechanistic analysis. Unexpectedly, we discovered that pSer473 binds intramolecularly to the Akt1 pleckstrin homolog (PH) domain-kinase domain linker to relieve Akt1 autoinhibition induced by PH domain. Moreover, we found that the more recently described modifications, pSer477/pThr479, employ an activation mechanism distinct from that of pSer473. However, the role of the PH-kinase linker to pSer473-induced Akt1 activation and the unidentified mechanism of activation by pSer477/pThr479 represent key gaps in our understanding of the Akt1 regulation. Thus, we have developed a sequential EPL method to generate semisynthetic segmentally $^{15}N/^{13}C/^{2}H$ isotopically labeled Akt1 containing distinct C-terminal phospho forms, and have applied protein NMR spectroscopy for assessing the conformational analysis of PH domain as a function of Akt1 C-tail phospho-forms. These results provide a new framework for understanding how Akt is controlled in cell signaling and suggest distinct functions for differentially modified Akt forms.

P044 Development of Novel Peptide Ligands for the Complement Receptor c5ar1 with Improved Selectivity and Pharmacological Properties

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The complement system is a key component of the innate immune response and contributes to protection against infection and recovery from injury. A key component of the complement system is the anaphylatoxin C5a, which acts via its receptor, C5aR1, to promote a range of inflammatory responses. However, aberrant activation of the C5a-C5aR1 system is implicated in a broad range of inflammatory-based diseases including many neurodegenerative conditions. A number of small peptide-based agonists and antagonists of C5aR1, primarily based on the C-terminus of C5a, have previously been developed, but ligands with improved selectivity, pharmacokinetic and pharmacodynamic profiles, and tissue targeting properties are desirable. In this presentation, I will describe the design and characterisation of several next-generation C5aR1 peptide ligands that address some of the limitations of the currently available molecules both as potential drug leads but also as valuable tools for further elucidating the physiological role of C5a/C5aR1.

P045 A Novel Methodology for the Development of Ruggedized Capture Receptors for Biological Assays

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As ubiquitous and wearable sensor technology becomes a standard method for biological detection and surveillance, there will be a significant need for the rapid development and production of biorecognition receptors capable of superior performance in multi-domain environments We report on the development of synthetic, thermally stable receptors using a novel discovery and multi-valent maturation strategy exemplified against the Chikungunya Virus (CHIKV) E2 surface protein capable of straightforward bioassay integration. An *in situ* 'click' screen of a propargylated protein against a library of peptide macrocycles was performed. With the guidance of *in silico* experiments, the down-selected peptide macrocycles were matured to produce multi-valent cooperative constructs, resulting in high selectivity for CHIKV E2 in up to 50% human serum and an over 200-fold improvement in affinity performance compared to the mono-valent macrocycles, outperforming a commercial polyclonal anti-E2 antibody. Additionally, the functional activity for one of the multi-valent receptors is retained after heating for 1 h at 90 °C in buffered solution. The extreme temperature stability, and most importantly functional activity following high temperature exposure, could enable more effective sensing in multi-domain environments and improved shelf-life. Aside from sensing applications, the overall methodology could also have a broad impact to the fields of diagnostics, therapeutics, and multifunctional materials.

YI-P046 Chemical Synthesis of Shiga Toxin B-Subunit

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Shiga toxin-producing bacteria (*Shigella* and Shiga-toxin producing E. coli) are responsible for over 100 million infections worldwide due to contaminated water or food. These toxins cause hemorrhagic colitis and potentially life-threatening hemolytic uremic syndrome in susceptible populations. Antibiotic treatment is typically not used due to increased toxin production, widespread resistance, and disruption of gut flora. Our goal is to develop an orally dosed D-peptide Shiga toxin inhibitor. This inhibitor will work directly on the Shiga toxin present in the intestines and block receptor binding to prevent or treat the symptoms mentioned above. To discover such D-peptide inhibitors, we will use mirror-image phage display to screen libraries of phage displaying L-peptides on their surface against a synthetic mirror-image target (composed of D-amino acids). The 69-residue Shiga toxin B-subunit (StxB) assembles as a pentamer and is responsible for binding to GB3 receptors on the gut wall to mediate toxin internalization. Thus we will require D-StxB as a target for use in mirror-image phage display. To develop and validate our synthesis scheme, we first synthesized L-StxB by breaking it into two segments using Fmoc solid-phase peptide synthesis. We then used native chemical ligation to generate the full-length StxB and folded it using a multistep oxidative dialysis process. Finally, we used size-exclusion chromatography, circular dichroism, and a GB3 binding ELISA to confirm the quality of our synthetic product. This same synthesis scheme is now being repeated using D-amino acids to produce D-StxB for screening in mirror-image phage display.

YI-P047 Utilizing the Metal-mediated Assembly of Coiled-coil Peptides to Incorporate his- Tagged Cargo into Designed, Three-Dimensional Peptide Crystals

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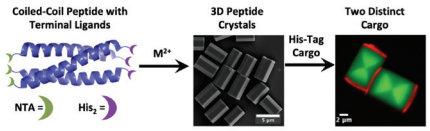
Protein crystals are a requirement for structural elucidation of biopolymers, and present exciting opportunities for drug delivery and biosensor development. Obtaining these crystals however, remains a formidable challenge. Here, we present a trimeric coiled-coil peptide, functionalized with metal-binding ligands that promote head-to-tail assembly into 3D peptide crystals upon addition of divalent metal ions. Variation of the metal ion concentration controls the morphology of the crystals, ranging from hexagonal disks to hexagonal rods. X-ray diffraction has elucidated the hexagonal anti-parallel

packing of the coiled-coil peptides within these metal-mediated assemblies. Unsatisfied ligands on the peptides were utilized to direct two distinct His-tagged cargoes to specific sections of the crystals. Small molecules and large biopolymers can be incorporated in the interior, as well as the external faces of the hexagonal structures in an ordered fashion. The ability to incorporate cargo with a wide range of sizes has greatly expanded the capabilities of the peptide crystals to be used as delivery agents or biosensors.



YI-P048 SNAC-tag for Sequence-specific Chemical Protein Cleavage

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Biocompatible chemical protein cleavage methods have been long-sought to replace enzymatic cleavages, but have yet to be realized. Here, we report the development of the SNAC-tag (Sequence-specific Nickel Assisted Cleavage) to achieve sequence-specific chemical protein cleavage under biocompatible conditions with comparable efficiency to enzymes. We demonstrate that the SNAC-tag can be inserted before both water-soluble and membrane proteins to achieve fusion protein cleavage, even when enzymatic cleavages fail.

YI-P049 Amyloid Peptide-based Hydrogels as 3D Cell-culture Scaffolds

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Amyloid fibrils are often cited for their association with neurodegenerative disease. Hydrogels based on similar fibrils derived from designed peptides, however, find applications as biomaterials. We investigate if short amyloidogenic peptides can be tuned to form hydrogels. The 16-22 residue stretch of β-amyloid peptide, Aβ16-22 (Ac-KLVFFAE-NH2), forms typical amyloid fibrils at neutral pH. The aromatic diphenylalanine motif is believed to play critical role. We studied the hydrogelating potential of Aβ16-22 peptide and its aromatic analogue Phe20Tyr. Aβ16-22 failed to cause hydrogelation up to 20 mM concentrations whereas Aβ16-22 (Phe20Tyr) formed hydrogel at concentrations as low as 2 mM 1. The gel is composed of distinct amyloid-like fibrils and Tyr hydroxyl group appears to be involved in H-bonding. The gel displays sustained release of the trapped doxorubicin and supports the growth of RIN-5F, HEK-293, BHK-21 and IMR-32. Even though Aβ16-22 turned out to be non-hydrogelating, tandem Aβ16-22 peptides connected through type I' or type II' β-turn supporting motifs (Ac-KLVFFAE-turn-KLVFFAE-am) turned out to be amyloidogenic 2 that caused gelation at 2 mM concentration. These gels also resulted in sustained drug release and supported the cell growth. These data establish the potential of short amyloidogenic peptides as functional biomaterials.

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YI-P050 Hydrazones as Submonomers in Solid Phase Peptoid Synthesis: Enabling the Efficient Incorporation of Trans-inducing *N'*-alkyl *N*-amino-glycine Residues

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Peptoids are a class of peptidomimetics in which the side chain is bonded to the backbone nitrogen instead of the α-carbon, giving *N*-substituted glycine oligomers.[1] Compared to peptides, peptoids exhibit increased resistance to proteases. However, these peptide mimics possess greater flexibility than their peptide counterparts because of the lack of chiral center and backbone hydrogen bond donor. Peptoid monomers that are structure-inducing, easy to incorporate, and tolerate diverse substitution patterns are especially valuable. To restrict conformational freedom in *N*-substituted glycines, incorporation of cis- and trans-inducing side chains have been reported. For example, *N*-aryl-, *N*-hydroxy-, *N*-alkoxy-, and N-acylamino-glycine monomers have all been shown favor $\omega \sim 180^0$. In this work, we demonstrate the use of benzaldehyde hydrazone as a submonomer in solid phase peptoid synthesis that can be incorporated in high yields. We demonstrate that this side-chain can be orthogonally deprotected to liberate an N-amino glycine intermediate, which can undergo reductive aminations with various aldehydes to create "libraries from libraries". Conformational analysis of model compounds will also be presented.

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P051 New Disulfide Reducing Reagent for Solid-Phase Peptide Synthesis

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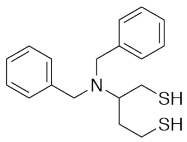
Although the amino acid Cys is not generally abundant in proteins, it is crucial for their stabilization. Furthermore, the presence of Cys in a large number of peptides with interesting biological activities has fueled the development of many Cys-containing Active Pharmaceutical Ingredients. Finally, the presence of a free thiol in a peptide or other organic molecule is often used for bioconjugation purposes. The manipulation or synthesis of Cys-containing peptides and proteins requires the use of disulfide-reducing agents. Here we describe the synthesis and application of a new reducing agent, DABDT, which belongs to the class of dithiol reducing agents, whose oxidized form is stabilized by a six-member ring such as DTT. The preparation of DABDT has been conveniently finely tuned, achieving outstanding performance in terms of purity and yield. DABDT is environment friendly due to the absence of odor, and its solubility in a broad range of organic solvents. DABDT has proved to efficiently remove the S-dimethoxylbenzylthio (SDMP) and also to reduce other disulfide bridges. SDMP is currently one of the most widely used thiol protecting groups in peptide synthesis that can be removed by reducing agents.



DABDT

We envisage that due the excellent properties of DABDT, it will be rapidly adopted by the scientific community as the disulfide reducing agent of choice.

P052 Towards a Totally Green Solid-Phase Peptide Synthesis (GSPPS)

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The demand for synthetic peptides is grow, and many efforts have been channelled into the optimization of the synthesis itself, i.e. coupling reagents, protecting groups, resins, automatization of the process, etc. However, little attention has been paid to making the process greener.[1] Here we will present our work on the complete substitution of the hazardous solvents currently used for peptide synthesis, namely DMF, DCM, and DEE/TBME.

The biomass-derived organic solvent GVL can substitute DMF in the coupling, deprotection, and washing steps of SPPS. Moreover, given the stability of GVL at high temperatures, it is compatible with the application of microwave-assisted automated SPPS. 2-MeTHF can replace DCM for the introduction of the first protected amino acid onto CTC resin. DCM can also be substituted by GVL and 2-MeTHF for the introduction of the first amino acid onto Wang resin. Finally, CPME and 2-MeTHF can substitute the hazardous ethers DEE and TBME during the precipitation step after global deprotection and cleavage from the resin carried out with TFA. The use of GVL, 2-MeTHF, and CPME, which are fully compatible with polystyrene and polyethylenglycol resins, will make a significant contribution to making SPPS greener.

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YI-P053 Interrogating Molecular Recognition Conservation of a Unique Coactivator Motif and its Homologs

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Protein-protein interactions (PPIs) between activators and coactivators at the site of transcription play a critical role in the regulation of gene through the assembly of transcriptional machinery. Found only in higher eukaryotic organisms, the Med25 subunit of the Mediator Complex is a unique coactivator that deviates from prototypical coactivator structures. Specifically, its activator binding domain, referred to as the Activator Interaction Domain (AcID), is comprised of a seven stranded b-barrel flanked by 3 α-helices. The AcID motif binds to a variety of activator binding partners using its two binding faces in order to elicit different gene responses. However, the nature of these interactions are poorly understood. Using a second AcID containing protein found in humans, in addition to a Med25 AcID homolog found in Arabidopsis thaliana, the purpose of this study is to probe these interactions using peptide mimics of the transcriptional activation domains of the activator binding partners AcID. This will allow for the interrogation of key residues and features of AcID responsible for binding interactions, as well as determine if this is conserved molecular crosstalk is conserved across AcID homologs. Probing the nature of these events will allow for the development of selective and specific modulators of AcID, ultimately leading for the bias of one AcID motif over another.

YI-P054 Expanding the Potential and Multivalency of the B_2T Synthetic Peptide Vaccine Against Foot-and-mouth Disease Virus

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Foot-and-mouth disease virus (FMDV) causes a highly transmissible infection of pigs and other animals, recognized as the animal disease with the direst economic effects worldwide. High incidence rates in large areas of Eurasia, Africa or Latin America stress the need for effective ways to control FMD, among which safe, marker vaccines (i.e., allowing to tell infected from vaccinated animals) are viewed as the most sensible option, with peptide-based vaccines receiving growing attention in this regard. In 2016 we reported the full protection of swine against FMD by vaccination with B_2T, a platform displaying two and one copies, respectively, of FMDV B- and T-cell peptide epitopes in a branched fashion.[1] To the known advantages of peptide vaccines (safety, marker nature, fine-tuning to various strains, easy shipping and storing), B_2T adds highly efficient synthesis by thiol-ene conjugation of prepurified modules, confering it fast adaptability in emergency responses to new outbreaks. We are now exploring the potential of the B_2T design to provide enhanced performance in terms of, e.g., lower dosage, longer-lasting protection and/or multivalency. To this end, strategies such as tandem display of >1 T-cell epitope, or back-to-back fusion of homologous or heterologous B2T units by chemoselective reactions (e.g., CuAAC) are being studied. Results from trial vaccinations of Swiss mice and swine will be discussed.

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YI-P055 De novo Lariat Based Cyclic Peptide Design for Targeting GPCRs

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G protein-coupled receptors (GPCRs) are the largest family of cell surface receptors and are important human drug targets. Of the 826 human GPCRs, 118 of them recognize endogenous peptide or protein ligands. Many of the GPCRs that remain unexplored as therapeutic targets are peptide- or protein-binding receptors. In these cases, identification of binders has been more challenging due to the difficulties involved in developing small-molecule binders based on the endogenous

ligand, which is a major obstacle for discovery of novel GPCR drugs. We have utilized computational methods to design stable cyclic and lariat-based structures for targeting GPCRs. We have also utilized peptide design as a framework for incorporating non-selective small molecule binders into a selective peptide motif; thus, increasing the affinity of the peptide and the selectivity of the small molecule. Here in we discuss progress towards targeting select GPCRs via de novo peptide design and optimization.

P056 Peptide Foldamers in Drug Discovery

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Helices in proteins are most abundant and important secondary structures that recognize macromolecules such as other proteins and DNA, and play an important role in a variety of fields such as biology, medicinal chemistry, and organic chemistry. Therefore, peptide-based helical foldamers have been developed in recent years. As tools for peptide-helix stabilization, non- proteinogenic amino acids such as α,α-disubstituted a-amino acids, cyclic β-amino acids, and cross-linked side chains are often utilized.

Herein we present secondary structural control of short peptides using the above non-proteinogenic amino acids.[1,2] Furthermore, we applied the stabilized helical peptides to the inhibitors of nuclear receptor (ER, VDR)-coactivator interaction,[3,4] to the protein-degradation inducers,[5] to the antimicrobial peptides,[6] and to the efficient cell-penetrating molecules.[7,8,9]

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YI-P057 Tick Cysteine-rich Protein Evasin-3 Regulates Chemotaxis through Disruption of Chemokine/GAG Interactions

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CXCL1 and CXCL8 are inflammatory chemokines that regulate neutrophil and leukocyte trafficking and play an important role in development of several type of cancer and atherosclerosis. Ticks as blood-sucking parasites interfere with the chemokine signaling system of the host, particularly secreting Evasins - small cysteine-rich chemokine-binding proteins. Here, we solved the structure of Evasin-3 and investigated structural determinants of its complex with CXCL1/8 by solution NMR spectroscopy. Data showed that Evasin-3 binds CXCL1 and CXCL8 in the same manner and it disrupts GAG-binding by blocking interaction with the α-domain. Recently, the second distinctive non-overlapping GAG-binding β-domain, which is present in CXCL1 but not in CXCL8, has been identified.[1] We exploited this finding and visualized development of atherosclerosis in mice carotid arteries by targeting CXCL1 deposited on activated endothelium using fluorescently labeled Evasin-3 variants. Moreover, from the native sequence of Evasin-3 we developed a novel 40 aa binder and its cyclic analog. Both peptides are easily accessible by solid-phase peptide synthesis, retain nM affinity to CXCL8 and have exceptional proteolytic stability. Taking into account structural homology of chemokines, obtained peptides could be used as a scaffold for further development of potent and selective chemokine-binding agents.

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YI-P058 New Enrichment Strategies for the Discovery of Plasma Membrane Permeable Peptide Therapeutics

Tanvi J Desai, Chengwei Wu, , Joseph Cannon, Kristen Kwasnjuk Amy Aslamkhan, Matthew Kuhls, Anthony Partridge, *Tomi Sawyer, Conrad Raab, Raymond Evers, Jerome Hochman Merck Research Laboratories (West Point, PA), Merck & Co., Inc., Kenilworth, NJ, USA*

Advances in high content synthesis have allowed screening of massively diverse libraries of peptides with high affinity to specific protein targets. Apart from target engagement, membrane permeability remains an important characteristic for increasing the probability of success for peptide drugs.

To identify peptides with target binding and permeability simultaneously we demonstrate three new approaches: for the first approach we prepared liposomes encapsulating copper free click chemistry reagent DBCO-Peg4-Biotin and show partitioning of permeable azide containing peptides by biotinylation. Our data show that known permeable peptides were biotinylated, whereas impermeable peptides did not. The second approach involves encapsulating a target, a tagged protein inside liposomes. Data we collected show that permeable and protein binding peptides partition through the lipid bilayer of protein containing liposomes and bind to the target protein. We were able to detect these peptides by target protein pulldown followed by LC-MS. The third approach screens one bead one compound(OBOC) libraries for membrane active peptides by assessing their binding to fluorescent large unilamellar vesicles (or liposomes). Permeable peptide showed higher binding to liposomes containing phosphatidylcholine.

YI-P059 Atropisomeric Amino Acids Alter the Energy Landscape of Peptide Macrocycles

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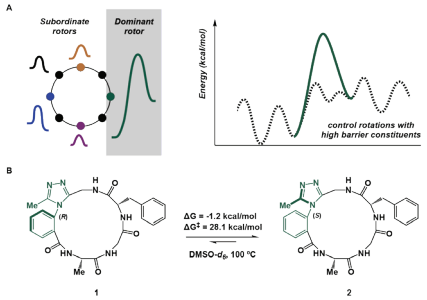
Macrocycles can be considered as sets of interconnected rotors each associated with a barrier to conformational

interconversion. The cooperativity among rotors is a likely reason for the multitude of conformational states in a given cyclic system. In cyclic peptides, rotors originate from amino acid amides with similar rotation barriers. This may explain the difficulty of maintaining distinct conformations in solution. As part of a program aimed at the discovery and application of synthetic methods that provide control over macrocycle conformation,[1,2] we considered the merits of installing dominant rotors into macrocyclic systems (Fig. A).[3] Such functionalities would possess a rotation barrier that is substantially larger than the rest (referred to as subordinate rotors). Our strategy targets new atropisomeric amino acid units to stabilize the solution conformations of cyclic peptides. The atropisomer units can be readily assembled using condensation protocols and later incorporated into a peptide chain using conventional Fmoc solid-phase peptide synthesis. The high barrier of rotation about the C_{aryl}–$N_{hetaryl}$ single bond in the resulting macrocycles prevents conformational interconversion at room temperature owing to the steric interactions about the chiral axis (Fig. B). NMR analysis of the conformational exchange between **1** and **2** at elevated temperatures allowed us to define the kinetic parameters *k* (rate constant) and $\Delta G^{\ddagger}$ (energy barrier of rotation), as well as the thermodynamic parameters K (equilibrium constant) and ΔG (thermodynamic barrier). These measurements will help parametrize the responses of diverse macrocyclic environments to substituents.



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YI-P060 Improving the Cell Penetration and Antibacterial Activity of Cationic Amphiphilic Polyproline Helices (CAPHs) Through Facile Modification at the N-Terminus

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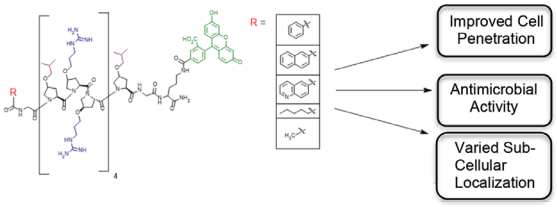

As our most powerful antibiotics become ineffective against drug resistant bacteria, there is a need to develop new therapeutic agents to combat this global crisis. To this end, we have previously designed a unique class of molecules called cationic amphiphilic polyproline helices (CAPHs). These peptides are composed of a rigid polyproline backbone with repeating hydrophobic and cationic functionalities, which grant the peptide amphiphilic properties. This amphiphilicity allows CAPHs to penetrate the cell, while still maintaining antibacterial activity. Here, we demonstrate a drastic improvement in the ability of the CAPHs peptide to penetrate mammalian cells through facile modification of the N-terminus with various hydrophobic moieties. Interestingly, even with improved cell penetration, these peptides remain non-toxic in mammalian cells *in-vitro*, while maintaining antibacterial activity. Furthermore, these N-terminus functionalities also direct the sub-cellular localization of CAPHs, providing a potential tunable feature to target bacteria that reside in different cellular compartments.

P061 Cysteine-rich Tick Protein Evasin-4 Neutralizes CCL5 and CCL5/CXCL4 Heterodimer

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Evasins are class of small cysteine-rich chemokine-binding proteins expressed by ticks to evade the host immune response during feeding. Evasin-4 identified in saliva of *Rhipicephalus sanguineus* is a 104 *aa* protein which binds a broad range of CC chemokines[1]. Binding to CCL5 is of particular interest due to involvement of CCL5 and CCL5/CXCL4 heterodimer in development of atherosclerosis[2]. Here, we solved the structure of Evasin-4 by X-ray crystallography and showed that despite only 27% sequence homology with Evasin-1 (PDB 3FPR), Evasin-4 adopts a similar fold with two orthogonally arranged β-sheets and a short α-helix. Formation of CCL5/Evasin-4 complex was studied by solution NMR spectroscopy using [13C, 15N] E66S CCL5. Obtained data indicated that Evasin-4 binds to the CCL5 N-terminus, the 30s loop and the β3-strand which comprise the CCL5 dimer and CCL5/CXCL4 heterodimer interface. Moreover, it has been proven that Evasin-4 blocks CXCL4 binding to CCL5 in NMR experiments. Structural determinants of CCL5/Evasin-4 interactions will be investigated further by X-ray crystallography and NMR. Evasin-4 activity *in vitro* will be studied by CCL5 and CCL5/CXCL4 induced monocyte adhesion and migration assays.

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YI-P062 Incorporation of Cysteine Disulfide to Stabilize the Bioactive Conformation: Studies on Short Antimicrobial Peptide Temporin-SHf

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Screening of short, broad spectrum and potent antimicrobial peptide of length less than 10 residues in antimicrobial peptide databases has revealed temporin–SHf as potent candidate. Temporin–SHf is a phenylalanine rich ultra short antimicrobial peptide derived from skin secretion of frog *Phelophylax Saharica*. It adopts a helical confirmation in micelles and unstructured in water. Important drawback of temporin-SHf is the susceptibility of bioactive conformation for denaturation and proteolytic degradation. To overcome these difficulties, an evolutionarily adopted disulfide engineering strategy has been employed to design the robust scaffold of temporin-SHf. The hydrophobic Leu4 and Ile7 residues located at i and i+3 position of the helical conformation was mutated with cysteine disulfide to yield [L4C, I7C]temporin-SHf. The designed peptide was synthesized, characterized using 2D-NMR spectroscopy and accessed the antimicrobial activity. [L4C, I7C]temporin-SHf, like temporin-SHf, adopts helical conformation from Phe3 to Phe8 residues but in the absence of micelles and exhibits the broad spectrum antimicrobial activity similar to that of native peptide. The reduction potential of cysteine disulfide of [L4C, I7C]temporin-SHf is -289 mV which less than the disulfides of -CXXC- motifs of peptides/proteins. Enzymatic digestion experiments using trypsin and serum have confirmed the advantage of disulfide in imparting proteolytic stability to temporin-SHf. Disulfide stabilized [L4C, I7C]temporin-SHf may servers as an attractive robust scaffold for structure-function studies and towards design of viable temporin-SHf based peptide antibiotics.

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YI-P063 Polyethylene Glycol and the Hydrophobic Effect

Steven R. E. Draper, Dallin Ashton, Anthony Carter, and Joshua L. Price

The hydrophobic effect (the exclusion of water in the folding of proteins, resulting in the burial of hydrophobic residues) contributed greatly to the protein folding process. This is because the removal of water from hydrophobic residues is entropically favorable and leads to a lower energy for the folded protein. Some proteins, especially therapeutics derived from fragments of a protein, have exposed hydrophobic patches. These patches can cause an otherwise effective therapeutic to aggregate and become inactive. The conjugation of polyethylene glycol (PEGylation) has been shown to be able to desolvate the residues around it. We have created an exposed hydrophobic patch in the WW domain by inserting leucine at positions 12 and 14 and inserted propargyloxy phenylalanine (PrF) at position 23, which is near both position 12 and 14. We PEGylated with a PEG azide using CLICK chemistry. We found that PEGylation at this site increased the stability of the peptide by -0.57 ± 0.03 kcal/mol. Next, we tried changing the solvent excluded volume of the residues by putting phenylalanine and cyclohexyl alanine in positions 12 and 14. Phenylaline was stabilized by -0.33 ± 0.02 kcal/mol and cyclohexyl alanine was stabilized by -1.00 ± 0.03 kcal/mol. Interestingly, cyclohexyl alanine had a large negative change in heat capacity, which implies the burial of hydrophobic surface area. When the ΔΔG of these compounds were plotted against the solvent excluded volumes of the hydrophobic residues, they correlated nicely.

YI-P064 A Novel Enzyme Involved in Cyclotide Biosynthesis

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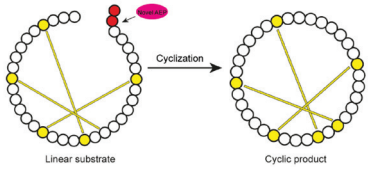
Cyclotides are plant-derived peptides with a well conserved cyclic peptide backbone in a cystine knotted arrangement. This has given cyclotides exceptional stability and has attracted huge interest in pursuing them as scaffolds for drug design. Asparaginyl endopeptidases (AEPs) are ubiquitous in plants and responsible for the proteolytic processing of seed storage proteins. These AEPs have been shown to facilitate backbone cyclization of cyclotides during their biosynthesis. In this study, two novel AEPs were identified from a cyclotide-producing plant. One of them was recombinantly expressed and it is the fastest cyclase known for backbone cyclization of a linear substrate. The novel AEP is able to cleave at the N-terminal site of a cyclotide precursor, suggesting that this enzyme is involved in both N- and C- terminal maturation of cyclotides from the plant. Overall, this study provides a proof-of-concept for the application of AEP to cyclize a reengineered peptide and this work demonstrates that the novel AEP has great potential as a useful cyclization tool for drug design application.



P065 pH-dependent Covalent Bond Cleavage in Creka Peptide-zerumbone Conjugate: A Release of Zerumbone into Breast Cancer Tumor

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Zerumbone (ZER) is a cyclic sesquiterpene derived from the tropical ginger with diverse biological activities including anti-cancer properties.

We investigated the effect of co-administration of ZER with TP5-iRGD tumor-penetrating peptide towards normal and breast cancer cell lines. The cell viability assay indicated that ZER

inhibited the growth of cancer cells: MCF7 MDA-MB-231and Hs27 normal breast cells at 72h treatment. The IC50 was found to be 7.51, 14.96 and >100 µg/ml respectively. The IC50 of co-administration of TP5-iRGD with ZER in the same cell lines was found to be 3.13, 0.49 and >100µg/ml respectively. The TP5-iRGD peptide showed no significant cytotoxicity. The results have been confirmed AO/PI staining under fluorescence microscopy.CREKA peptide (Cys-Arg-Glu-Lys-Ala) is a tumor homing peptide that are overexpressed in cancerous tissue and not in the normal tissue and it has a favorable targeting ability to tumor. Therefore, according to the above mentioned primarily unpublished findings of ZER co-administrated with TP5-iRGD, CREKA peptide will be conjugated with ZER via triazole ring formation as a pH responsive linker, and will be tested in vivo using breast cancer animal model, aiming at creating a bio-conjugate therapeutic strategy for breast cancer. The approach involving the release of ZER in tumor cells utilizing the extracellular tumor pH. Furthermore, the released ZER in the tumor will be analyzed by matrix assisted laser desorption/ ionization (MALDI) mass spectrometry using an aqueous tumor washing buffer with adjusted pH to the point whereby the ZER is insoluble. The unexpected immune system responses of the conjugated complex will be discussed.

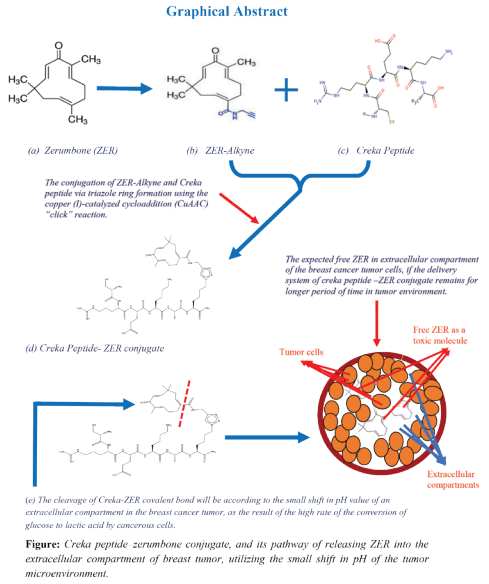

Figure: *Creka peptide zerumbone conjugate, and its pathway of releasing ZER into the extracellular compartment of breast tumor, utilizing the small shift in pH of the tumor microenvironment.*

YI-P066 A General Approach Towards Piperazic Acid (Piz)-Containing Natural Products and Peptidomimetics

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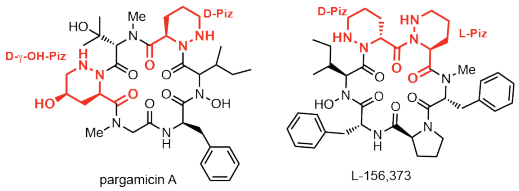
Piperazic acid (Piz) and its congeners are found in a number of structurally complex non-ribosomal peptide natural products that exhibit diverse biological activities. Although it is a cyclic residue, the hydrazino moiety in Piz strongly favors a *trans* amide conformation when acylated on Nα. This is in stark contrast to other naturally occurring cyclic residues such as proline and pipecolic acid, which display significant *cis* amide rotamer populations. Despite its structural attributes and presence in bioactive peptides, Piz is not widely employed in peptidomimetic applications, primarily due to synthetic challenges. Most approaches introduce pre-formed orthogonally protected Piz residues, prepared in several steps, onto a growing peptide chain. Here, we present the synthesis of Piz- containing natural products and their congeners via a sub-monomer- based electrophilic amination strategy starting from glutamic acid. Application of this strategy enables an efficient synthesis of L- 156,373, a potent oxytocin receptor antagonist isolated from *Streptomyces silvensis*. The pargamicins are a family of potent antimicrobial peptides featuring Piz and its congener γ-hydroxy Piz. Efforts towards the first total synthesis and structure activity relationship studies of pargamicin A will also be presented. The described approach provides a means to efficiently introduce Piz congeners into host peptides via late stage cyclization, and should find broad utility in residue scanning applications.



YI-P067 Protection of Dibenzocyclooctyne (DBCO) from Acid-Mediated Rearrangement Enables Click-Assisted Native Chemical Ligation

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Chemical protein synthesis, a method combining solid-phase peptide synthesis and native chemical ligation (NCL), enables large protein synthesis with complete atomic control. However, its scope is limited by the high peptide concentrations required for efficient NCL. Poorly soluble peptides undergo incomplete ligations, leading to complex purifications and low yields of ligated material. To overcome these challenges, here we introduce Click-Assisted NCL (CAN). In CAN, peptides are first functionalized with traceless lysine linkers[1] before addition of a strained cyclooctyne (DBCO) or azide. The strained click reaction forces peptides into close proximity, increasing their effective concentration for ligation. After ligation, gentle hydroxylamine treatment removes the clicked handles, affording the native ligated peptide. Since DBCO undergoes acid-mediated rearrangement, we used a copper(I) complex[2] to protect DBCO peptides during resin cleavage. Excitingly, low concentrations of clicked model peptides completely ligate after 4 h, ~120-fold faster than non-templated ligation, which also accumulates many undesired side products due to the long reaction time. We also demonstrate that the click, ligation, desulfurization, and handle-cleavage steps can be performed in one-pot to synthesize the *E. coli* ribosomal subunit L32. CAN is an essential first step towards our ultimate goal: multiple peptide ligations in one-pot via a traceless template.

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P068 Extending Drug Lifetimes with an Albumin-Binding D-Peptide

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D-peptides, composed of synthetic D-amino acids, are an emerging drug class with high target affinity and specificity. Their resistance to proteolysis grants them a much longer *in vivo* lifetime than their L counterparts; this benefit is limited, however, by rapid renal clearance due to their small size. By contrast, human serum albumin (HSA) is an extremely long-lived and abundant serum protein with a half-life of ~21 days. Conjugation to a HSA-binding tag is a well-validated strategy to extend drug lifetimes. However, existing tags are limited by poor solubility, steric bulk, proteolytic degradation, and/or potential immunogenicity. We aim to develop an HSA-binding D-peptide to slow the clearance of conjugated cargo, particularly D-peptide inhibitors. D-peptides that bind to a given target can be rapidly identified by mirror-image phage display, which requires total chemical synthesis of a mirror-image (D) form of the target protein. HSA is too large for current synthetic methods (585 aa), but contains three autonomously folding ~200-aa domains that represent more reasonable synthetic targets. Using our Aligator software[1], we have designed optimal strategies to synthesize domains I and III by Fmoc-SPPS and NCL. We have conducted scout syntheses of individual peptide segments and produced most in good quality, though several have poor solubility. We are currently working to improve solubility using a traceless "helping hand" linker[2].

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YI-P069 Automating Microfluidic mRNA Display for the Identification of Peptides with High Affinity to Cancer Targets

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The detection and treatment of cancer is becoming increasingly personalized. More and more cancer markers and targets are being identified in research labs that could be used to inform treatment or detect cancer earlier. Unfortunately, only a limited number of these new markers and targets can be tested in the clinic. This is largely because we have yet to develop efficient ways to detect or target these markers and targets. Existing reagent generating methods (hybridoma, phage display, yeast display) do not take advantage of advances in sequencing technology. mRNA display offers a unique platform to select high affinity peptides from trillion member libraries and identify the highest affinity clones through High Throughput Sequencing Kinetics, helping you identify a hit more quickly. Automating the selection process will allow us to rapidly progress through selection and identify peptide ligands to new markers and targets. Incorporating microfluidics will allow us to speed up the process further and enable us to select for higher affinity ligands.

YI-P070 Expanding the Scope of Scaffold Permeability Via Thioether Cyclization of Cyclic Peptides

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As our understanding of biological targets becomes more complex, so do efforts to reach such targets. Passive cellular permeability is a coveted goal amongst these efforts but can become difficult to achieve as molecular weight increases. In both natural and synthetic cases, cyclic peptides can provide the conformational dynamics needed to afford passive permeability through the membrane whilst retaining the capacity for intracellular solvation. Furthermore, unique cyclization methods can offer even more diversity in the pursuit of unique permeable conformations. Herein we describe a synthetic library made using split-pool technique and incorporating a thioether bond via a C-terminal cysteinyl side-chain as the cyclization point. The resulting synthetic peptides were subjected to a parallel artificial membrane permeability assay (PAMPA) to assess passive permeability through an artificial cell-membrane mimic. From these results, permeable leads are isolated and examined to determine scaffold structure and inherent intramolecular interactions that allow access to permeable conformations.

P071 The Cyclic Tetrapeptide JVA-4001 Demonstrates Multifunctional Opioid Antagonist-Agonist Activity and Prevents Reinstatement of Drug-Seeking Behavior in Mice

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While the opioid receptors (μ, δ, and κ) have long served as targets for the treatment of pain, recent research has revealed their potential as targets for the treatment of addiction. We are interested in developing novel compounds that treat addiction, given the ongoing opioid epidemic. Herein, we highlight select findings for the novel cyclic tetrapeptide JVA-4001, which displays potent dose-dependent κ antagonism and μ agonism when administered centrally (i.c.v.) or peripherally (i.p. or p.o.) to mice. When administered orally (p.o.) to mice, JVA-4001 blocked the activity of centrally-administered agonists, demonstrating that it is both orally bioavailable and blood-brain barrier permeable. JVA-4001 did not significantly impair locomotor activity or respiration at therapeutic doses when given peripherally (i.p. or p.o.). With these encouraging results, we sought to assess its utility in the conditioned place preference (CPP) assay, a model of drug seeking behavior. Following central (i.c.v.) or oral (p.o.) administration, JVA-4001 was without effect alone, but prevented both stress- and drug-induced reinstatement of extinguished morphine-CPP, highlighting its potential as a treatment for opioid abuse. In the future, we plan to further investigate the properties of JVA-4001 and its analogs to establish this scaffold's intrinsic activity and potential as an orally-administered treatment for opioid addiction.

P072 Synthesis of Peptide Alcohols Using New Fmoc Amino-Alcohols Resins

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Peptides containing a C-terminal alcohol function are an important class of compounds. Some exhibit antibiotic properties like the peptaibols. One of the most important peptide alcohol is the somatostatin analogue octreotide (Sandostatin), which is used clinically for the diagnosis and treatment of a variety of neuroendocrine tumors and gastrointestinal disorders.

The conventional solid-phase synthesis of C-terminal peptide alcohols are difficult due to the absence of a free carboxylic group to attach to the resin except when the chlorotrityl resin is used. However loading the chlorotrityl resins with Fmoc-amino alcohols can take hours even days and tend to be inconsistent. For this reason and the lack of commercially available resins, the use and testing of peptide alcohols (PAs) in SAR are limited.

At A*STAR, we have developed a set of resins (Pepcohol resins) that enable the synthesis of PAs using the conventional 9-fluorenylmethoxycarbonyl (Fmoc)/tBu SPPS strategy. As an example, we are presenting the synthesis of the well-known peptide alcohols Octreotide, Alameticin 30 and Trichogin GA IV using the new set of resins developed in our lab.

YI-P073 Design of Redox-active Antibacterial Peptide Hydrogels to Prevent Ymplant-related Infections

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Infection of medical implants represents a serious ongoing problem, caused by bacterial adhesion and subsequent biofilm formation on the implant interface. To prevent such infection, we designed a set of catechol-functionalized cationic peptide antibacterial hydrogels that were inspired by the lysine- and DOPA-rich mussel foot protein. These supramolecular gels are designed to be injected during surgery to the implantation site and directly adhere and coat the implant substrate. Furthermore, utilizing the redox-activity of catecholic-DOPA residues, these gels kill bacteria by two mechanisms: via a direct contact mechanism between the polycationic gel and the bacterial cell surface, causing membrane disruption, and by DOPA-mediated production of hydrogen peroxide (H_2O_2), a known antibacterial agent. We demonstrated that these gels exhibit high bactericidal activity against clinically isolated gram-positive bacteria, including the notorious multidrug resistant bacteria, MRSA. We further showed how amino acid composition and peptide sequence can modify the amount of generated H_2O_2, and consequently alter the antibacterial activity of the gel. Moreover, we characterized the ability of the gels to act as adhesives at the implant-tissue interface by utilizing lap-shear tensile strain tests.

Collectively, these results indicate that DOPA-containing hydrogels hold promise as antibacterial adhesives, suitable for implantation at the tissue-implant interface.

YI-P074 Selenomethionine as an Expressible Handle for Complex Bioconjugations

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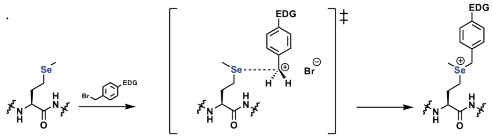

Site-selective chemical protein modifications are ubiquitous in the fields of chemical biology and biopharmaceuticals. Although numerous chemistries exist for the site and chemoselective modification of proteins, new methods that facilitate usability and large-scale production of biological conjugates are still of great interest. Herein we employ a physical organic study to develop the selenomethionine (SeM) alkylation as a useful protein bioconjugation strategy. SeM containing proteins are readily obtained through auxotrophic expression and SeM exhibits unique nucleophilic properties which allow it to be selectively modified even in the presence of other reactive nucleophilic residues such as cysteine or lysine. The resulting selenonium adduct has proven stable, and the chemistry is broadly tunable. We have developed a shelf stable, and readily modified linker for efficient conjugations to SeM, which exhibits a rate at least 1.5 orders of magnitude faster than any previously reported SeM conjugate linkers. We envision this addition to the chemical biology toolbox to find broad utility for the production of chemical biology probes and biopharmaceuticals.

YI-P075 Trapping and Characterization of a Non-toxic Aβ42 Oligomeric Species

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Amyloid β (Aβ) 42 is an aggregation-prone peptide and the believed seminal etiological agent of Alzheimer's Disease (AD). Intermediates of Aβ42 aggregation, commonly referred to as diffusible oligomers, are considered to be among the most toxic forms of the peptide. These fleeting supramolecular assemblies remain poorly understood at the structural level. We found that the physiologically relevant modification of Aβ42 through introduction of D-serine at residue 26 (i.e., S26s chiral edit) of the peptide stabilizes Aβ42 aggregation intermediates, yet abrogates toxicity. The use of fluorescence quenching assays, coupled with TEM, AFM, gel electrophoretic assays, conformation-specific antibodies and CD consistently pointed to the formation of soluble, pre-fibrillary species with β-sheet character. Subsequent molecular level structural work using a combined NMR- and DFT-computational approach revealed that the changes induced in the peptide through the S26s chiral edit, while only affecting the peptide conformation very locally, produce drastic changes in the ability of the peptide to adopt a fibril-seeding conformation by altering its H-bonding capacity and the curvature of the backbone.

YI-P076 Cell-penetrating Peptides Restricting Oligomerization of G Protein-coupled Receptors: The CB_1 R- HT_{2A}R Dimer

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G protein-coupled receptors (GPCRs), classically described as monomeric transmembrane (TM) receptors, form a ternary complex between a ligand, the GPCR and its associated G protein. However, it is now well accepted that many GPCRs form, in addition to functional monomeric structures, higher-order oligomeric complexes constituted by a number of equal (homo) or different (hetero) monomers. GPCR heteromers are defined as novel signaling units with functional properties different from homomers. For instance, when coexpressed in specific brain regions, CB_1R and $5HT_{2A}$R form heteromeric structures that have been related to tetrahydrocannabinol-linked effects such as memory impairment, anxiety and dependence.[1] In order to identify the functional properties of the CB_1R-$5HT_{2A}$R heterodimer, we used synthetic peptides with the amino acid sequence of the CB_1R TM domain, fused to a cell-penetrating sequence derived from HIV TAT, to disrupt the formation of the heteromer. These peptides were tested in bimolecular fluorescence complementation assays in cells expressing receptors fused to two complementary halves of YFP, and by their ability to modify cAMP and p-ERK1/2 signaling when both receptors are co-activated. In addition, using MD simulations of the CB_1R-$5HT_{2A}$R heterodimer we have identified hot-spots on the dimer interface and designed peptides, partially reproducing CB1R TM domains and fused to a Tat CPP sequence, to disrupt heterodimer formation. Both in vitro (e.g., bimolecular fluorescence complementation, cAMP) and *in vivo* (preventing amnesic effects, preserving analgesic effects) assays show the peptides as effectively interfering CB_1R-$5HT_{2A}$R cross-talk, hence a potential inroad to pathway-specific signaling therapeutics.

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P077 A Sugar Touch for Minimizing Diketopiperazine on SPPS

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A small chemical or a minimum structural change can have a significant impact on biological processes. An illustrative example is the partial double bond character of the peptide linkage with a direct influence on the overall conformation of the peptide/protein. Almost all peptide bonds prefer the most thermodynamically stable *trans* conformation, except for Pro residue, since steric constraints are similar in both *cis* and *trans* isomers. One of the chemical implications of this isomerization is the diketopiperazine (DKP) formation during SPPS, which is being favorable for *cis* conformer. Therefore, this annoying side reaction is especially critical when Pro is the first amino acid coupled to the resin. Herein, we envision that drawbacks can be overcome when the first Pro residue is shifted to trans conformation. Taking into account that in proteins it has been observed that *O*-glycosylated Ser/Thr adjacent to Pro favors *trans* conformation,[1] we hypothesized that the same idea could be translated in a minimization of the DKP formation. To shift the *cis* to *trans* conformation, we tested a series of glycosylated amino acids in the second position after the Pro. As a striking result, the Ser(βGlcNAc) after Pro avoided the DKP formation. Further NMR analysis and modeling studies shown that the glycan moiety favors the trans conformation due to the formation of intramolecular hydrogen bonds between the peptide backbone and the carbohydrate. We consider that this finding may be beneficial not only for efficient peptide synthesis but also to further investigate the impact of the partial double bond character of peptides and proteins.[2]

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P078 Peptides Targeting Breast Tumor Derived Exosomes as a Therapeutic Strategy for Metastatic Breast Cancer

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Cell-to-cell communication is essential for breast cancer growth and dissemination. Exosomes are important players in this process by transporting and transferring proteins and genetic material to sensitive cells located either at the primary tumor site or in distant organs such as the brain or bones. In this study we aim to develop a peptide with anticancer activity toward breast tumor cells and capable of interacting with breast tumor derived exosomes. By affecting directly the intercellular communication we expect to deliver a peptide with an innovative mechanism of action with increased selectivity when compared to conventional drugs.

Exosomes from breast tumor and healthy cell lines were isolated using a commercially available kit and further biophysically characterized with transmission electron microscopy (TEM), atomic force microscopy (AFM), flow cytometry, Western Blot and dynamic light scattering. The interaction of two anticancer peptides (ACPs), the plant defensin PvD1 and a newly designed peptide from a viral protein template, vCPP2319, with breast cells and/or exosomes was studied with surface plasmon resonance (SPR), dynamic light scattering and Western Blot analysis. Our results suggest that vCPP2319 peptide acts intracellularly leading to cell death and reveals that PvD1 is capable of modulating the exosomal membrane composition and to bind to these vesicles with high affinity. These effects

may consequently result in tumor growth arrest and condition tumor spreading.

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YI-P079 Proximity-driven Reactions of Unprotected Peptides

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This presentation highlights the role of proximity-driven activation in the reaction chemistry of unprotected peptides, using two current examples. First, we will describe our development of a new amide-forming chemical ligation reaction for protein total synthesis. The reaction leverages a hydroxamic acid nucleophile — incorporated into the side chain of aspartic acid — to react with a peptide-thioester, and to bring the resulting mildly-activated acyl group into proximity with an *N*α-group for amide formation by *O,N*-acyl transfer. This process constitutes a rare example of a facile *O,N*-acyl shift that proceeds across a medium-size ring ($t_{1/2}$ ~15 min), and illustrates the utility of hydroxamic acids for accessing *O*-acyl 'isopeptides' in water, without the need for side chain protection.

As a second example, we illustrate how proximity-driven activation emerged as a mechanistic solution during *in-vitro* selection for peptides that react with a small molecule, perfluoroaromatic 'probe'. These studies identified a 29-residue peptide— containing an internal cysteine residue—that reacts with the probe ~4,000-fold faster than a reference cysteine-peptide. A combination of biophysical and kinetics studies support a model in which a binding interaction brings the probe into proximity with the cysteine residue for covalent reaction. Remarkably, the reaction product exhibits a conformational stability of ~4 kcal/mol, which is unusual for peptides in this size regime (<30 residues, and without covalent cross-links). The 'bind-react' kinetic model invoked here could plausibly account for the rate enhancement exhibited by other reactive peptide 'tags' of current interest in bioconjugation.

YI-P080 Antimicrobial Coatings Derived from Mussel-Glue and Antimicrobial Peptides for the Prevention of Implant-Associated Infections

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Prevention of implant-associated infections has been one of the main challenges in orthopedic surgery. This challenge is further complicated by the concern about the development of antibiotic resistance as a result of using traditional antibiotics for infection prophylaxis[1]. In this study, three antimicrobial coatings were designed consisting of bifunctional peptides derived from mussel foot proteins (mfp), responsible of the marine Mytilus mussel adhesion[2], and an antimicrobial peptide (AMP) derived from phospholipase A2, present in Bothrops asper snake venom[3]. In order to study the deposition and stability of the resulting coatings, quartz crystal microbalance (QCM) measurements were carried out with titanium probes, the most widely used medical implant material. The adsorption of the peptides was achieved using a concentration of 0.1 mg/mL in buffer solutions at pH 3, 5.5 and 6.5, yielding coatings which adhered quantity and stability is pH depending as well as their capacity to resist buffer washing and modulate surface properties by strongly reducing bovine serum albumin adsorption.

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YI-P081 Yield Comparison of Deprotection Reagents in Fmoc Solid Phase Peptide Synthesis

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Although it is commonly understood that coupling is the most demanding reaction in the process of solid phase peptide synthesis (SPPS), the N-amino deprotection step is also crucial to secure the quality of the target peptide. Poor efficiency in deprotection will result in decreased yield and product quality[1]. Several aspects must be taken into consideration when selecting a deprotection reagent: its physicochemical behavior, basicity (pKa) and polarity, concentration and time of reaction, toxicity and disposability of residues and, finally, availability of reagents. In fact, piperidine has a current legal status as a controlled substance regulated by the Drug Enforcement Agency[2]. That is why we reported a yield comparison of three strategies for deprotection using Fmoc simultaneous multiple-peptide synthesis[3]. Three peptide sequences in high-demand production in our laboratory (NBC-112: FISEAIIHVLHSR, NBC-155: TLEEFSAKL and NBC-759: KKWRWWLKALAKK) were synthesized using Rink amide resin and 4-methylpiperidine (4MP), piperidine (PP), and piperazine (PZ) as Fmoc removal reagents. The use of 4MP as deprotection reagent showed a significantly higher yield in two of the synthesized peptide, especially in those sequences that, according to our experience, are difficult to synthesize. These differences in yield were not observed during the microwave-assisted synthesis of these sequences[4], possibly because the kinetics of deprotection reaction using 4MP, PP and PZ is equalized under heating conditions.

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YI-P082 Study of Bias Signaling of Inteleukin-1 Receptor Allosteric Modulator Peptide by the Synthesis and Application of β-Substituted α-Amino-γ-lactam Derivatives

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α-Amino-γ-lactams are commonly known as Freidinger-Veber lactams. They are valuable tools in peptide-based drug discovery, because they can facilitate molecular recognition by constraining φ, ω and backbone dihedral angles, and stabilizing turn conformations.[1] Potential to mimic side chain function with constraint of χ-dihedral angles arises from addition of β-substituents onto Freidinger-Veber lactams. A set of β-substituted α-amino-γ-lactam residues have been synthesized using a combination of Mitsunobu chemistry on the *trans* β-hydroxy-α-amino-γ-lactam (Hgl) residue,1 and nucleophilic ring opening of the cyclic sulfamidate derived from its cis lactam counterpart.[2] Lactams were introduced into the sequence of the inteleukin-1 receptor (IL-1R) allosteric modulator peptide 101.10 to study the structural requirements for regulating the activity and signaling of this key cytokine mediator of inflammasome activation.[3] Our presentation will describe the synthesis and insertion of β-substituted α-amino-γ-lactams into 101.10, *in vitro* experiments to study their influences on biased signalling, and in vivo results in models of preterm birth and oxygen-induced retinopathy.

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YI-P083 Increasing the Oral Bioavailability of Peptide Macrocycles by Thioamide Substitution

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Peptides are considered as poor drug leads owing to their compromised pharmacokinetics. Most of the marketed peptide drugs such as, Octreotide are administered parenterally, a route which often has low patient compliance. Two major obstacles to the effective oral delivery of macrocyclic peptides include low membrane permeability and rapid degradation in gastro-intestinal tract which has varying pH environment and enzymatic composition. Previous studies have revealed that passive membrane permeability can be enhanced by shielding of amide (-NH) groups by intramolecular Hydrogen bonding or by lowering their solvent accessibility. By far, the most common approach of desolvating the amide bond is N-methylation. But there are no studies where efforts have been put towards understanding the importance of carbonyl modification in this context. Thioamide is an ideal isosteric modification of the peptide bond, which is basically a single atom substitution ('O' to 'S') that minimally perturbs the backbone conformation of peptides. To understand the effect of this modification in enhancing the lipophility and permeability of macrocyclic peptides, we chose the well-studied cyclic hexapeptide model c(ILLpYL)[1] and sequentially substituted all the amides with thioamides. Out of all the variants, three thioamidated peptides showed higher retention time in reverse phase HPLC column, enhanced PAMPA (*in vitro*) and Caco-2 (*in cellulo*) permeability and significantly improved metabolic stability in human serum, microsome and simulated gastric and intestinal fluid compared to the reported tri-N-methylated variant of the same peptide, that has an oral bioavailability of 28%.[2] Hence, we believe that this unexplored class of peptidomimetics will open up new avenues for the design of orally bioavailable peptide drugs.

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P084 A Helping Hand for Solubility: Development of a Second-Generation, Traceless Solubility-Enhancing Modification

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The combination of solid-phase peptide synthesis and native chemical ligation allows the production of full-length proteins that cannot be made recombinantly, such as post-translationally modified or mirror-image proteins. However, chemical protein synthesis still faces many difficulties, such as slow reaction kinetics, complex synthetic routes, and poor solubility of segments and assembly intermediates. Many strategies have been implemented to combat poor solubility, including our previously reported site-specific, reversible "helping-hand" (HH) linker that allows for the attachment of solubilizing tags. Here we introduce an improved, second-generation HH linker with greater stability, a more cost-effective synthesis, and superior handling properties. To demonstrate its usefulness, we employed this linker in the synthesis of the difficult Shiga toxin B-subunit (StxB) protein. We envision widespread use of this improved linker in the synthesis of difficult proteins. More recently, novel linkers for solubilizing tags that take advantage of different functional groups are being developed to give more complete coverage for difficult peptide sequences. The addition of these new linkers will facilitate chemical protein synthesis by increasing the number of attachment sites and offering orthogonal control for their installation/removal.

YI-P085 Synthesis of Cyanobactin-Derived Photoaffinity Probes for Target Identification

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Patellamides are cyclic ribosomally synthesized and post-translationally modified peptides (RiPPs) of the cyanobactin superfamily, produced by *Prochloron* sp., an uncultured obligate symbiont of *L. patella*.[1] These natural products have been found to display a number of valuable biological activities, such as cytotoxicity and reversal of multidrug resistance in human leukaemia cells.[2] Other members of the cyanobactin family possess immunomodulating, antimalarial, antibacterial activity, but for many the exact cellular mechanisms are unconfirmed.

Biosynthetic machinery and structural features are highly conserved amongst cyanobactins. Of the seven genes of the patellamide pathway, *patE* encodes a 71-residue precursor peptide comprising of two core sequences, flanked by leader and follower sequences that act as recognition motifs. Previous work in the Naismith group has included the engineering of a modified heterocyclase that can process synthetic core peptides without any leader, as well as discerning minimal tripeptide recognition sequences (which are cleaved upon macrocyclization).[3,4]

These findings, combined with the natural promiscuity of the enzymes of the patellamide biosynthetic pathway, allow for the chemoenzymatic synthesis of a diverse range of macrocyclic peptides, including ones containing unnatural residues and non-amino acid moieties such as polyethers, polyketides and sugars.[5]

Photoaffinity labelling (PAL) is a powerful tool for the elucidation of protein-ligand and protein-protein interactions. Here, we present work on the synthesis of patellamide derived photoaffinity probes via the combination of solid-phase peptide synthesis (SPPS), enzymatic modification and chemical conjugation which can be used to determine cellular targets, verified through secondary assays.

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YI-P086 Synthesis of Bicyclic Analogs of the Kappa Opioid Receptor Antagonist Arodyn

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The acetylated dynorphin A analog arodyn (Ac[Phe^{1,2,3},Arg^{4},D-Ala^{8}]Dyn A(1-11)-NH_2) exhibits potent and selective kappa opioid receptor antagonism.[1] While cyclization can impart metabolic stability, increase binding affinity, and/or improve permeability, bicyclization imparts additional conformational constraint which can further enhance metabolic stability.[2] There have been increasing reports and interest in bicyclic peptides, but to the best of our knowledge there are currently no reports of bicyclic opioid peptides, possibly due to the short length of the most extensively studied opioid peptides.[3] Based on structure-activity relationships of monocyclic arodyn analogs, we designed two bicyclic arodyn analogs using ring closing metathesis (RCM) and lactam chemistries. Here we report the synthesis and opioid receptor binding affinities of the bicyclic arodyn analogs. Research supported by NIDA grant R01 DA018832.

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P087 Constrained Macrocyclic Melanocortin Peptides Added New Pieces to the Puzzle of the Melanocortin Receptor Selectivity

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The melanocortin peptide hormones are the endogenous bioactive ligands at the human melanocortin receptors (*h*MCRs), which are five GPCR subtypes. Although the melanocortin system is known to be involved in the regulation of different patho-physiological functions,[1] SAR knowledge still lacks of structural requirements needed to reach fine modulation of hMCRs.

On the tail of our previous macrocyclic melanocortin analogues,[2,3] herein we describe the design and synthesis of novel derivatives achieved by modifications of the cycle dimension and the incorporation of conformational constrains. This study led to the discovery of compounds with interesting activity and selectivity profiles, offering enhanced SAR information related to the melanocortin system knowledge.

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YI-P088 Unmasking of N-terminal N-aryl Amino Acids into Reactive α-imino Amide Intermediates Under Mild Conditions: Applications in Oxime Ligations

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Aniline catalysis has allowed oxime ligations between α-oxo aldehydes or ketones with aminooxy functional group to proceed at pH 7, which has increased their utility in bioconjugation reactions.[1] While formation of an aniline Schiff base intermediates is critical for reactivity, high concentrations of aniline can be toxic to cells and selectivity in the presence of endogenous ketones and aldehydes can be problematic.[1] Additionally, methods to install α-oxo-carbonyl functional groups on peptide substrates typically require $NaIO_4$ exposure, which may lead to side-reactions with sensitive residues (e.g. Met, Trp),[2] or the use of excess pyridoxal 5'-phosphate (PLP).[3] We recently reported that aerobic oxidation of certain N-phenylglycinyl peptides to give α-imino amide intermediates in situ was possible, which reacted with aminooxy groups to yield oxime products in the absence catalysts.[4] Here, we demonstrate that *N*-terminal *N*-aryl amino acids, possessing diverse substituents at the α-carbon, can also undergo tandem aerobic oxidation/oxime ligation reactions at neutral pH to afford substituted oxime bonds. The scope of this work will be discussed and mechanistic insight will be provided.

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YI-P089 Design and Evaluation of Fatty Acid Peptide Conjugates for siRNA Delivery and Silencing in Breast Cancer Cells

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Linear and cyclic fatty acyl-peptide conjugates were synthesized via solid-phase peptide synthesis for siRNA delivery and mRNA silencing in breast cancer cells. All peptides contained arginine, a positively-charged amino acid, a characteristic required to interact with the negatively charged siRNA. The peptides were conjugated with either palmitic acid (C16) or stearic acid (C18). The fatty acid component of the peptide was designed to improve the interaction with the hydrophobic residues in the phospholipid of the cell membrane. Therefore, the positively charged amino acid and the hydrophobic conjugates are designed to enhance the cell penetrating characteristics of the peptides. The attraction between the peptides and siRNA allows the peptide to encapsulate and stabilize the siRNA and protect it against early degradation. This is a crucial feature since, without the protection and stability that the peptide provides, siRNA would not be able to penetrate the cell membrane and reach the cytoplasm. All four peptides, LP-C16, LP-C18, CP-C16, and CP-C18 were successfully synthesized and purified using Reverse Phase High Performance Liquid Chromatography (RP-HPLC), and the molecular weights were confirmed using Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF). Future plans for this study include siRNA encapsulation and incorporation into Peptide Lipid Associated Nucleic Acids (PLANA), determination of siRNA delivery, and silencing effect of the PLANA complex.

P090 Novel Quinine Prodrugs as Anti-malaria Drug Candidates

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Malaria, which is transmitted to humans by the *Anopheles* mosquito, is a potent threat to human beings; moreover, the range of this mosquito is expanding owing to global warning. Malaria is a disease caused by parasitic protozoa of the genus Plasmodium. Recently, chloroquine-resistant strains of Plasmodium have been identified; thus, the use of quinine as malaria treatment has increased. However, patients with severe malaria require intravenous administration of quinine for 4 h, resulting in pain in patients.

Photaki et al. reported a decomposition reaction of arginine methyl ester[1]. In this reaction, the guanidino group of an arginine methyl ester attacks the ester carbonyl carbon of another arginine methyl ester, forming an arginine dimer. Next, the N-terminal amino group of the dimer attacks the guanidino-carbon within the molecule, forming a heterocyclic compound and ornithine methyl ester. We applied this reaction into a novel prodrug design strategy[2,3].

Here, we designed and synthesized a series of quinine prodrugs. The quinine prodrugs were stable in water and acidic media, and could release its parent drug rapidly and spontaneously under physiological condition. If the current quinine treatment regimen can be modified using our prodrug approach to consist of a single injection, the burden of malaria on many patients might be reduced.

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YI-P091 Exploring the Effects of Macrocyclic Tetrapeptides with Promising Anti-Proliferative Activity in Triple Negative Breast Cancer

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There is a need to develop novel targeted therapies for the treatment of triple negative breast cancer (TNBC), which is inherently difficult to treat due to the aggressive nature of the disease and the lack of HER2 and hormone receptor expression, rendering hormone and HER2-targeted therapies ineffective. The oncoprotein c-Myc is often overexpressed in TNBC and is involved in regulating cell growth and programmed cell death. Downregulation of c-Myc has been found to decrease cell proliferation and promote apoptosis and may be a promising approach for therapeutic development.

We have previously identified two macrocyclic tetrapeptides, CJ-15,208 and [D-Trp]CJ-15,208,[1] that inhibit proliferation and decrease c-Myc protein levels in c-Myc-overexpressing prostate cancer cell lines.[2] Since c-Myc protein levels are elevated in TNBC, we are exploring the effects of CJ-15,208, [D-Trp]CJ-15,208, and a more potent analog of CJ-15,208 on TNBC cell lines. Here we compare the anti-proliferative and cytotoxic activity of these peptides in breast cancer cell lines and examine the peptides' effects on c-Myc protein levels and proteins involved in regulating its expression. These peptides may show promise as potential treatments for TNBC.

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YI-P092 Identification of a Bacteriocin-Eegulating Quorum Sensing Circuit in *Streptococcus gallolyticus* subsp. *gallolyticus*

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Streptococcus gallolyticus subsp. *gallolyticus (Sgg)*, a member of the group D streptococci, is an opportunistic pathogen that is strongly suggested to be a bacterial driver of colorectal cancer (CRC), in addition to causing other diseases, such as infective endocarditis and septicemia. The mechanism by which *Sgg* causes CRC is not presently known. We determined that *Sgg* utilizes quorum sensing (QS), a cell-density mechanism that allows bacteria to determine their population density to activate certain genes synchronously, to outcompete other gut microbes (Figure 1). Furthermore, we identified the *Sgg* QS signaling peptide that we termed, gallocin-stimulating peptide (GSP), as this peptide regulates the production of a class II.b bacteriocin termed, gallocin.[1,2] This QS phenotype allows *Sgg* to kill other colonic bacteria such as Enterococcus faecalis, assisting *Sgg* to colonize particular niches within the human body. We have conducted structure-activity relationship studies on the GSP signal and uncovered unique features about this signal that differs from similar streptococci QS signals. This presentation will discuss our recent findings on this novel pathway.

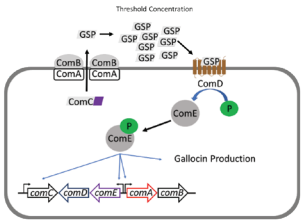

Figure 1. Streptococci *comABCDE* QS pathway. The *comC* gene produces a propeptide QS signal that gets cleaved and exported by the ABC transporter, ComAB, to create the gallocin-stimulating peptide (GSP). GSP will bind the histidine kinase, ComD, at a specific concentration level, which phosphorylates the response regulator, ComE. The phosphorylation of ComE leads to the activation of the pathway along with expression of Gallocin, a class II.b bacteriocin.

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YI-P093 Rapid, High-Fidelity Chemical Synthesis of Biologically Active Proteins and Enzymes

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The field of biopharmaceuticals is rapidly expanding, requiring new methods for on-demand high-fidelity production of chemically modified proteins. Even after decades of optimization, standard solid-phase peptide synthesis (SPPS) can't reliably produce peptides containing more than 50 amino acids in length. Larger peptides and proteins are produced by biological techniques, which limits their chemical composition. Here we report a method that closes the gap between SPPS and biological techniques. Using fully-automated continuous flow-based peptide synthesis (AFPS), we are able to routinely produce long peptides, proteins and protein-like polymers of up to 200 amino acids in a few hours of synthesis time. Complete control of every incorporated amino acid is opening the chemical space to a theoretically unlimited amount of modifications, such as incorporation of functional handles, glycoproteins, post-translational modifications and the synthesis of D-proteins. To validate the general applicability of this new technique, we report the synthesis and biological evaluation of selected examples.

P094 Inhibitory Effect and Molecular Docking Study upon Angiotensin I-converting Enzyme by Peptides from an Elastase-Treated Hydrolysate of Porcine Aortic Elastin

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Hypertension is one of the main risk factors causing cerebro and cardiovascular diseases. Angiotensin I-converting enzyme (ACE) which catalyzes the production of a vasoconstrictive peptide, angiotensin II from angiotensin I is an essential target for antihypertension. Elastin is an important macromolecular protein that exists widely in elastic tissues such as arteries, ligaments, lung, skin, etc. and has been shown to possess ACE inhibitory activity. In this study, we explored the novel peptides with ACE inhibitory activity derived porcine aortic elastin and investigated the relationship between the values of IC_{50} and calculated ACE Docking Scores using docking simulation of ACE inhibitory peptides.

Porcine aortic elastin was treated with pancreatic elastase and the hydrolysate was separated by the use of HPLC. The ACE inhibitory activity of fractions obtained was analyzed by ACE inhibition assay using hippuryl-L-His-L-Leu as a substrate (ACE inhibition assay). Nine peptides in ACE inhibitory fractions were separated and their sequences were identified by means of LC/MS/MS and chemically synthesized by solid-phase method. ACE inhibitory activities of synthetic nine peptides were examined by ACE inhibition assay. The calculation of Docking Scores of nine peptides against ACE was performed using Molecular Operating Environment (MOE) software.

The relationship between the values of IC50 and calculated ACE Docking Scores of synthetic nine peptides with ACE inhibitory activity is likely to correlate with interaction energy between these inhibitors and ACE active pockets.

The resulting ACE inhibitory peptides may be beneficial as ingredients of functional foods for preventing hypertension.

P095 Structure-Antibody Binding Affinity Relationship Study of Cyclic Disulfide Peptides

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Antibodies are widely utilized as therapeutic agents or research tools, due to the high target specificity. Hence, peptides that selectively bind to antibodies are attractive molecules, that can be applied to the antibody purification, preparation of antibody-drug conjugate (ADC)[1] and so on. Indeed, we previously developed an ADC preparation method mediated by cyclic antibody-binding peptide **1** (GPDC*AYHKGELVWC*TFH: *disulfide, K_d = 220 nM)[2]. Herein, the structure-activity relationship (SAR) study of peptide 1 was performed to obtain more potent peptide applicable to the efficient ADC preparation.

As an initial SAR study, Ala-scanning of peptide **1** was performed to evaluate the function of each residue. The substitutions of Tyr6, His7, Leu11, Val12 and Trp13 to Ala led a complete loss of antibody-binding affinity. Whereas, the N-terminal Gly1-Pro2 sequence were irrelevant for the strong binding, and the peptide **2** with the deletion of these residues retained a beneficial affinity (*K*d = 270 nM). In the further study of peptide **2**, we finally found the K8L peptide with a strong binding affinity (K_d = 8.1 nM).

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P096 "ON-RESIN" Disulfide Peptide Synthesis Using Methyl 3-Nitro-2-pyridinesulfenate

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"On-resin" disulfide bond formation is an ideal strategy for preparing disulfide-containing cyclic peptides in SPPS. In the present study, we challenged this strategy using a newly developed disulfide bond-forming agent, methyl 3-nitro-2-pyridinesulfenate (Npys-OMe; 1)[1], which facilitates intra-molecular disulfide bond formation between two cysteine residues. In oxytocin synthesis, after the construction of protected peptide resin, the tBu-thiol groups of two Cys residues were selectively deprotected by the reduction with 2-mercaptoethanol, and the resulting thiol groups were oxidized with **1** on the resin. The purity of crude oxytocin obtained after final deprotection was 91%. Further HPLC purification gave oxytocin in a total yield of 65% with >99% purity. In an attempt for the on-resin synthesis of a more complicated peptide "α-conotoxin" with four Cys residues, two disulfide bond formations using the combination of **1** and conventional iodine oxidation were successfully accomplished on resin in both manual and automatic protocols. This gave α-conotoxin in a total yield of about 2% with >99% purity.

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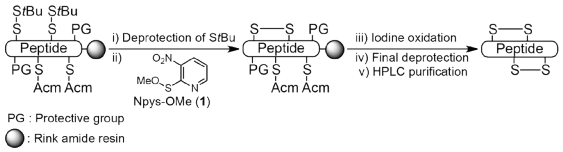

YI-P097 Discovery and Structure Study on a Multifunctional Peptide, QUB-3307, Derived from the Frog Skin Secretion of *Fejervarya limnocharis*

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Amphibian skin secretions are remarkable sources of the discovery of bioactive peptides. Herein, we reported a novel peptide through the combination of 'shot-gun' cloning and MS/MS fragmentation sequencing, namely QUB-3307. QUB-3307 exhibited potent board-spectrum antimicrobial activity against ESKAPE strains with severe cytotoxicity and hemolysis. To eliminate the side effect, we designed a truncated analog, QUB-2531, by removing the 8 N-terminal residues, which resulted in low cytotoxicity and hemolysis without decreasing the antimicrobial effect dramatically. Furthermore, another truncated analog, QUB-1367, demonstrated less antimicrobial potency. The membrane permeabilization assay showed that QUB-3307 disrupted the cell membrane of Gram-positive bacteria at 2×MIC. However, the other two analogs did not damage the cell membrane up to their respective bactericidal concentrations. Additionally, QUB-3307 showed strong anti-cancer activity on several cancer cells, though it may depend on the nonspecific membrane permeabilization. Interestingly, QUB-3307 and QUB-2531 exhibited an inhibitory effect on the enzymatic reaction of trypsin. However, QUB-1367 was failed to remain the inhibitory effect, indicating that the intact rana box domain could contribute to the trypsin inhibition. In summary, a novel multifunctional peptide was successfully discovered. The studies on the truncated analogs revealed the core domain of this peptide and eliminated the side effect, indicating that the hydrophobic N-terminus was significant for membrane penetration, while the C-terminal domain may kill microorganisms through non-membrane permeabilization pattern. Additionally, QUB-2531 exhibited both antimicrobial and trypsin inhibitory activity with high therapeutic index, which may provide new ideas for the generation of leads for the design of peptides with therapeutic applications.

P098 "Protagger: Rationally Designed Linker-free Protein Fusion Tags"

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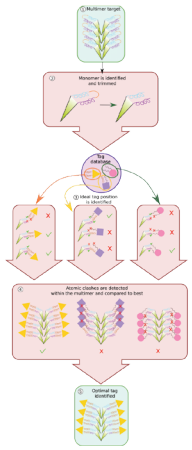
Cellular electron cryo-tomography (ECT) and subtomogram averaging has enabled unprecedented insight to the interior world of the bacterial cell and their molecular machines. These complex macromolecules enable a range of critical cell functions. For example, ECT has enabled detailed study of the dynamic, multi-membrane spanning motility machine of the bacteria, the flagellar motor. Currently, the primary limitation of *in situ* methods such as ECT are nanometer resolutions they can regularly achieve, precluding easy identification of specific proteins of interest within a larger macromolecular complex. To unambiguously identify proteins within macromolecules in subtomogram averages, I have developed Protagger to rationally add linker-free protein chains to act as localisation tags for ECT. By iterating through a database of crystal structures, Protagger identifies protein chains optimal for insertion at a user-identified tag-site through fused secondary structure elements. This is repeated throughout the multimer on every copy of the protein of interest, adding additional density without affecting the quaternary structure or function. As proof of principle, we demonstrate tagging of the *Salmonella enterica* flagellar filament,composed of 1000's of copies of the flagellin FliC. This ~50 kDa protein has been successfully tagged twice with linker free ~20 kDa protein tags and expressed *in situ*. The bacteria retain partial motility and produce WT length flagella when examined with EM, suggesting this new methodology is successful.



P099 Anticancer Activity of Truncated Cysteine-deleted Tachyplesin Analogs with a Hyaluronan Binding Sequence

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Cysteine-deleted tachyplesin (CDT) is a linear analog of tachyplesin, an antimicrobial peptide derived from the hemocytes of the Japanese horseshoe crab, which retains selective antibacterial properties. We have found that some CDT analogs also possess anticancer activity against A549 lung cancer cells and likely exert anticancer effects through both a membranolytic and a hyaluronic acid-mediated mechanism. In an effort to further explore this latter effect as well as find the minimal sequence required for activity, truncated CDT analogs of various lengths which retain a common hyaluronan binding sequence ([B(X_7)B] in which B is either R or K and X_7 contains no acidic residues and at least one basic amino acid) were synthesized in both D- and L-forms. The peptides were tested against both normal lung cells and A549 in the presence and absence of hyaluronidase. Effects on various liposome membrane models were also examined.

P100 Examining the Role of Hyaluronan and Heparin Binding Domains of Insulin-like Growth Factor Binding Proteins in Anticancer Activity

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There are six IGFBPs (Insulin-like Growth Factor Binding Proteins with highly conserved structures which regulate cell activity in diverse ways, including inhibition of mitogenesis, differentiation, survival, and other IGF-stimulated actions. Some of these proteins also exhibit IGF receptor-independent activity. IGFBP-3, in particular, has been associated with protection against lung cancer and inhibits the growth and survival of non-small cell lung cancer A549 cells. IGFBP-3 and -5 contain a C-terminal hyaluronan binding motif [B(X_7)B] in which B is either R or K and X_7 contains no acidic residues and at least one basic amino acid. IGFBP-3 and -5, and -6 have C-terminal heparin-binding domains of the type [B_3(X_2)B] where B is a basic residue. In order to assess the anticancer activity and possible mechanism of action of these proteins, 18 amino acid analogs, derived from the consensus C-terminal domains containing these motifs, of all six IGFBPs were synthesized. ELISA, dot-blotting, and MTT assays were then utilized to examine the activity of these peptides against cancer cells.

YI-P101 Self-assembled Peptide-based Materials Inspired by the Humboldt Squid's Sucker Ring Teeth

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Sucker ring teeth (SRT) of the Humboldt squid (Dosidicus gigas) are unusual hard tissues. The teeth are natural "biotools" that are both hard and robust, yet non-mineralized and fully proteinaceous (comprising of proteins named "suckerins"), and stabilized by supra-molecular interactions. Suckerins are highly modular at the primary structure level and exhibit a semi-crystalline structure of b-sheet forming repeats intervened by longer amorphous domains, similar to silk fibroins.

Over the years, different short peptide sequences (≤12 residues) from suckerin-19 have been found to self-assemble into supramolecular structures, such as stiff fibers with lengths in the order of millimeters, stiff spherical beads with high elastic modulus, and hydrogels with concentration-dependent modulus. 3 of these self-assembled materials will be presented together with the techniques employed to study their assembly mechanisms, structural and mechanical properties.

This presentation highlights the importance of molecular-scale interactions as well as self-assembly mechanisms in forming mechanically robust peptide-based materials, giving us both insight to the structure and property of the native sucker ring teeth as well as the self-assembled materials. The lessons we learn from a single structural protein here can equip us with a plethora of possibilities toward engineering new peptide-based biomimetic materials.

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YI-P102 Design and Synthesis of Potent Histone Lysine Demethylase Inhibitors Based on the Structure of 8-Hydroxyquinoline

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Methylation and Demethylation of histone are important epigenetic processes that control numerous transcriptional activities.[1] Histone lysine demethylase 4 (KDM4) is a member of the Jumonji domain 2 (JDJM2) family which is overexpressed in tumors such as prostate cancer.[2] Previously, we have identified 8-hydroxyquinoline (8HQ)-based compounds that showed high potency and selectivity on inhibiting KDM4B.[3] In an attempt to improve their biological activities, we have carried out virtual screening on the crystal structures of KDM4B with a large dataset of 8HQ-containing structures derived from a variety of natural and unnatural amino acids. The virtual screening was performed with PyRx program that employs AutoDock for calculating docking energy. The hit compounds with the most promising binding scores were selected for synthesis and biological evaluation. For facile production of a large number of hit compounds, we have developed an efficient solid-phase synthetic protocol. We have identified several potent compounds from biological assays on prostate cancer cell lines and carried out structure-activity studies for further improvement.

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P103 Evaluation of Properties to Support Peptide Transport for Intracellular Exposure and Transcellular Transport

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Peptide permeability is a loosely defined term often used to describe parameters associated with oral absorption but interchangeably used in the context of ability to engage intracellular targets. However, many peptides with activity against intracellular targets are not orally absorbed, and structural features compatible with intracellular exposure deviate from the boundaries required for oral absorption. Permeation across biological membranes can occur directly at the plasma membrane or following endocytosis and may be mediated by diverse mechanism including passive permeability, lipid partitioning, and cationic partitioning. To better understand properties that distinguish intracellular from transcellular transport we evaluated transcellular transport and intracellular uptake of peptides in Caco-2 cell monolayers grown on filter supports. Peptides selected evaluated covered different mechanism of transport (passive permeability, lipid partitioning and cationic partitioning) and a broad range of predicted physical chemical properties and included macrocyclic, linear and stapled peptides. Evaluation of the cell uptake and transport showed that in addition to impermeable peptides, some peptides showed significant cell uptake and transcellular, while other were trapped in the cells showing very little transepithelial transport. One characteristic associated with peptides showing high transcellular transport relative to peptides accumulated in the cells is higher intracellular unbound fraction. These results as well as physical chemical properties associated with cell uptake and transport will be presented.

P104 On the Road to Commercialization of Antimicrobial Peptides to Treat Gram-negative Bacterial Infections

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The extensive clinical use of classical antibiotics has led to the ever-growing emergence of medically resistant strains of bacteria. Indeed, there are now "Superbugs" that are resistant to all available antibiotics and development of a new class of antibiotics is now critical. Cationic antimicrobial peptides represent such a new class. We have designed *de novo* all D-conformation 26-residue amphipathic α-helical antimicrobial peptides (AMPs) to treat gram-negative pathogens (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*). Our AMPs successfully work against organisms with resistance to antibiotics. For testing we use 7 strains of Acinetobacter baumannii resistant to antibiotics of last resort (colistin and polymyxin B) and 20 *Acinetobacter baumannii* world-wide isolates from 2016 and 2017 with antibiotic resistance to 18 different antibiotics. These isolates come from four continents, 12 different countries and 17 different cities. Our AMPs have excellent antimicrobial activity and no toxicity as measured by the most stringent test of hemolysis of human red blood cells (18 hours at 37oC and up to 1000 µg/ml of AMP). We accomplished this by incorporation of the unusual amino acids (diaminobutyric (Dab) and diaminopropionic (Dap) residues) into our peptides on the polar face to replace Lys residues. In addition, we incorporated "specificity determinants," two positively charged Lys residues in the center of the non-polar face, which provide specificity for prokaryotic cells over eukaryotic cells. These specificity determinants also prevent unwanted high affinity binding to serum proteins as well as selectivity against gram-negative organisms. AMPs without specificity determinants are extremely hemolytic whereas peptides with specificity determinants show no hemolytic activity when Dab and Dap residues are used on the polar face. This study clearly shows the potential of amphipathic α-helical

AMPs with specificity determinants and the use of the unusual amino acids (Dab and Dap) as polar face positively charged residues. This is the first successful demonstration of the use of Dab and Dap residues in antimicrobial peptides. Our results show that these compounds have the potential to replace existing antibiotics as new therapeutics where the development of resistance is highly unlikely.

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P105 Long Recombinant Homogeneous Linkers for Dual Conjugations, to Replace Heterogeneous PEG

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Polyethyleneglycol linkers are classical tools for linking peptides to biomolecules or spectroscopic probes. Long linkers are sometimes preferred, but available PEG's longer than 5 kDa are heterogeneous. Long XTEN linkers (mixed sequences of GEDSTAP residues giving PEG-like properties) have been described with dual functionalities, thus enabling cross-linking of proteins etc. However, XTEN sequences are rich in Ser and Glu, and we found that Ser/Glu have drawbacks for our use. Our main expression platform is based on yeast, and sequences with multiple Ser gets partially O-mannosylated during yeast expression, thus leading to heterogeneous products. Also, we find that multiple Glu in linkers can increase the viscosity of solutions. To solve these problems, we designed linkers based on repeats like (GQAP)n. Such sequences could be expressed as either long extensions from proteins (proteins with "recombinant PEG"), or (GQAP)n could be equipped with orthogonally reactive terminals to be used as linkers for dual conjugations.

We exemplify this with H-(GQAP)50-Cys, which we expressed in high yield in yeast. The isolated product had mixed disulfides at Cys (Cys, glutahion and homodimer), but the mixture could be transformed to material with homogeneous disulfide using excess 4-mercaptophenylacetic acid. The N-terminal amine was then transformed to azide, the disulfide was reduced to free Cys, and the dually reactive linker was conjugated to iodoacetyl-insulin in one end, and conjugated as triazole in the azido end, to a trypsin inhibitor with alkyne functionality. Data for enzymatic stabilization of the insulin-trypsin inhibitor conjugate will be reported comparing to simple insulin/trypsin inhibitor mixtures.

YI-P106 Synthesis of Minimal Coiled Coils via a Covalent Crosslinking Strategy

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Binding epitopes featuring helical secondary and tertiary structures are commonly observed in natural and designed protein-protein interactions (PPIs). While many strategies exist for stabilization of the α-helical secondary structure, a general strategy to create epitope mimetics that imitate the diversity of helical tertiary structures as is lacking. We recently described a strategy to stabilize short antiparallel helix dimers by replacing an interhelical salt bridge with a covalent bond.[1] Here, we report an improved synthetic strategy that provides access to not only antiparallel dimers but also parallel heterodimers and heteromeric trimers. Optimized interhelical hydrophobic packing and careful placement of an optimized cross-linker were essential for helix stabilization.[2] We have applied this strategy to develop tertiary structure inhibitors for PPIs that are difficult to inhibit with α-helix mimics.

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P107 Design of Artificial Hydrolases Based on a Polyproline Scaffold

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Vulnerability of natural enzymes to environmental conditions gives rise to the design of artificial ones. Many simple oligopeptides were developed as minimalistic catalysts for mimicking the activity and selectivity of natural enzymes. Here, we used the polyproline helix as a scaffold to design a series of oligopeptides with a catalytic dyad incorporated to serve as hydrolases. Circular dichroism was used to characterize the peptide structure and the p-nitrophenyl acetate (*p*-NPA) assay was applied to measure the hydrolytic activity. These peptides exhibit hydrolytic activities with the k_{cat}/K_M value ranging from 0.56 to 1.23 M-1 s^{-1}. The results also indicate that a well-formed polyproline II structure can lead to a high catalytic efficiency. We further conjugated the short polyproline peptide containing a catalytic dyad of His-His or His-Ser to a self-assembling peptide MAX1, as new catalysts (H2H5 and H2S5) for ester hydrolysis. At high pH values, these two peptides can self-assemble into β-sheets and fibrils. From *p*-NPA assay, H2H5 exhibits a relatively high catalytic efficiency (k_{cat}/K_M = 49.7 M^{-1} s^{-1}) at pH 9.0 while H2S5 has an even better activity (k_{cat}/K_M = 121.6 M^{-1} s^{-1}) at pH 10.0, demonstrating that the self-assembled structures dramatically enhance their hydrolytic activity. Moreover, H2S5 has a stronger affinity with p-nitrophenyl-(2-phenyl)-propanoate (*p*-NPP) and p-nitrophenyl methoxyacetate (*p*-NPMA) than H2H5, leading to a higher activity on these substrates. Our results provided a new strategy for developing peptide-based enzymes and also revealed the necessity of maintaining an ordered structure and a well-organized catalytic site for effective hydrolases

P108 Peptide Design: From Structure to Function

Parisa Hosseinzadeh, Gaurav Bhardwaj, Timothy Craven, Vikram Mulligan, Stephen Rettie, Xinting Li, David Baker*

The emerging health crisis of the 21st century calls for a worldwide effort towards development of more effective therapeutics with fewer side effects. Peptides offer significant advantages over antibodies and small molecules, the two most commonly used therapeuticsc, as they combine the selectivity and modularity of antibodies with ease of modification and potential for permeability of small molecules. In particular, they have attracted attention as the molecule of choice for targeting protein-protein interfaces and "undruggable" targets. Much progress has been made towards advancing the field of peptide therapeutics, from library-based method to modifying constrained scaffolds. Yet, these methods cannot sample the vast space peptides and peptide-like molecules can cover. In addition, the process is in general lengthy and costly. In order to overcome these limitations, we are developing methods that enable computational design of peptides with custom-tailored shaped for specific targets of interest; the computational screening of hundreds of thousands of peptides increase the chance of obtaining a hit while testing fewer compounds. I briefly present our success in designing structured macrocycles and will then move on to different methods we're employing to obtain successful binders to multitude of targets, including low nM inhibitors of histone deacetylases.

YI-P109 The Design and Synthesis of Novel Peptide Macrocycles Inspired by Bacterial Genome Mining

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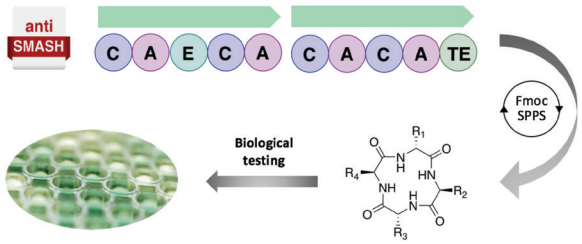
Antibiotic-resistant bacteria continue to pose one of the greatest global human health threats. In the U.S. alone, these infections effect more than two million people and cause nearly twenty-three thousand mortalities per year.[1] There is an urgent need to identify new small molecules capable of treating these infections. Efforts in our laboratory have focused on identifying such molecules by mining bacterial genomes for cryptic gene clusters. To accomplish this, the freely available software AntiSMASH[2] was used to identify peptide sequences encoded by non- ribosomal peptide synthetase (NRPS) biosynthetic gene clusters (BCG's) containing bacterial resistance genes. The resulting sequences were synthesized on the solid-phase in a cyclic fashion using the alkanesulfonamide "safety-catch" linker.[3] Cyclic peptides were chosen to harness the enhanced proteolytic stability and cell permeability properties of peptide macrocycles.[4] Finally, the antimicrobial efficacy of these compounds was tested against pathogenic bacteria.



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YI-P110 Single-Molecule Quantitation of Phosphorylation Modifications

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Proteomic studies have provided important findings about how cells respond during growth and diseases develop, however some disease biomarkers can be difficult to probe when the samples are derived from a biopsy, often due to limited tissue sample size. To probe these extremely low abundance proteins, we have developed a single-molecule approach to identify and quantify peptides that are found in zeptomole (10^{-21}) abundance. This technique, known as fluorosequencing, utilizes single-molecule TIRF microscopy to monitor fluorescently labeled peptides as they undergo Edman degradation. The loss of fluorescent signal at each cycle indicates that specific fluorescently tagged amino acids have been removed from a peptide, and after the loss of many fluorescent signals, a peptide motif can be generated to identify the parent sequence. With this technique, our team is developing methodology that will allow for the quantitation of the phosphorylation state of endogenous proteins. For this technique to be successful for a wide range of targets, it requires chemical labeling of amino acids that are highly specific. However, few published phosphate labeling chemistries can survive the trifluoroacetic acid that is used during the Edman chemistry. I will present our research showing the direct fluorescent labeling of phosphorylation modifications that survive the trifluoroacetic acid and can be sequenced at single molecule sensitivity, allowing us to directly quantitate the amount of modified and unmodified peptides

YI-P111 Coffeetides: Iron-chelating Cysteine-rich Peptides from Coffee Waste

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Coffee is the most widely consumed brewed drink and second largest commodity in the world. 50% of coffee waste was produced during coffee production[1]. The waste by-products include coffee pulps and husks which have limited applications. A sustainable approach to the waste management is to endow value-added products as useful pharmaceuticals and cosmetics. Here, we report the discovery and characterization of a novel class of iron-binding cysteine-rich peptides from the waste product of coffee husks, designated coffeetides. Combining genomic, transcriptomic and proteomic analysis, we identified 23 coffeetides from three common *Coffea* species, *C. arabica*, *C. canephora* and *C. rosmosa*. Coffeetides are 35-41 amino acids in length, containing eight cysteine residues and an unusual disulfide connectivity. Disulfide mapping, chemical synthesis and NMR determination confirmed a novel coffeetide structural fold with the N- and C-termini being topologically fixed by disulfide bonds, rendering coffeetides a highly compact

pseudocyclic structure that confers their resistance to heat, proteolysis, and acid and serum-mediated degradation. Coffeetides are non-toxic to human cells, but can bind to Fe^{3+} ion and could be explored as iron-chelating agents for treating diseases caused by iron overload such as hemochromatosis and thalassemia. Our work highlights explorations of value addition to coffee by-products in waste management and economical resources.

Acknowledgement:
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YI-P112 Stapled Peptides to Disrupt Ral signalling: An Achilles' Heel in Ras-driven Cancer?

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Occurring in over 30 % of human tumours, RAS oncogenes are the most commonly mutated in human cancers[1]. More than 30 years of attempts to target Ras therapeutically have yielded no efficient therapies, leading the proteins to be widely deemed 'undruggable'. In recent years there has been substantial evidence implicating the Ral proteins: RalA and RalB, which are activated downstream of Ras, as critical mediators of transformation in many Ras-mutant driven cancers including pancreatic and colorectal cancers[2]. Despite this growing evidence, the Ral proteins have not yet been targeted therapeutically. This work develops a novel strategy to target oncogenic Ras signalling by competitively disrupting Ral-effector interactions using α-helical stapled peptides based on the native Ral effector RLIP76. Initial work identified a lead stapled peptide with a Kd of 5 μM for RalB that could disrupt Ral-effector interactions and inhibit autophagy, a RalB-dependent process in mammalian cells[3]. This peptide has since been optimized to include amino acid substitutions which increase the affinity for RalB > 30-fold, taking the affinity into the low nanomolar range. The structural basis for this improved interaction has been investigated using X-ray crystallography and NMR techniques, which have also guided further optimization of the peptides including increasing the solubility to give more drug-like properties. The peptides are now being tested in mammalian cells to assess their suitability as potential lead therapeutics.

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P113 Exploiting Thioamide Reactivity in Peptide Synthesis

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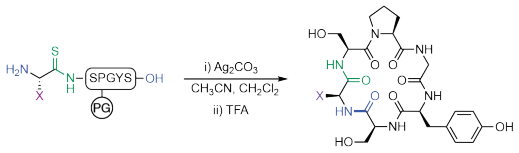
In recent years a range of novel amide ligation strategies have been developed, many incorporating sulfur-containing reagents as thioesters and other carboxylic acid surrogates. We recently reported a novel method for peptide synthesis based on the reaction of thioamides with carboxylic acids in the presence of silver(I) to generate isoimides, which can undergo acyl transfer to generate peptide imides.[1,2] Recent progress in this area will be highlighted, including novel strategies for macrocyclization and ligation of peptide thioamides.[3]



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YI-P114 Protein and Small Molecule Engineering towards An Orthogonal Chromatin Landscape

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Reversible methylation in DNA and histone has been nature's favorite epigenetic modification for controlling chromatin-dependent processes. Particularly important is the trimethylation of lysine 9 in histone H3 (H3K9Me3) that regulates heterochromatin organization and gene silencing. Mechanism and dynamics of heterochromatin formation and its spread in chromatin landscape are poorly understood. Employing structure-guided protein-ligand and protein-protein interface engineering, we have developed a set of orthogonal epigenetic modulators to construct a heterochromatin structure at a precise space and time. We have modified an enzyme involved in S-adenosyl methionine (SAM) synthesis to generate S-adenosyl benzyl-methionine (Bn-SAM) which is accepted by an engineered methyltransfrease to benzylate H3 which in turn is recognized by a modified 'reader' protein. The engineered methylation apparatus is capable to 'write' and 'read' benzylated H3 (H3K9Bn) in mammalian cells. We have established the integrity, catalytic efficiency and orthogonality of the modified system through detailed biochemical and structural studies. By applying the histone 'benzylation' apparatus, we plan to uncover the mechanism of heterochromatin formation with exquisite temporal control, and to understand how such structural changes affect gene expression and cellular processes.

P115 Reviving Old Protecting Group Chemistry for Site-selective Peptide-Protein Conjugation

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Cysteine's relatively low abundance in proteins coupled with the fact that it can be uniquely nucleophilic due to its low pKa (~ 8.2), makes it an ideal handle for site-selectively conjugating proteins to groups of interest via alkylation or crossed-disulfide formation. An advantage of the disulfide bond is that intracellular release of the moiety of interest is possible.[1] For effective reaction to occur, the protein's reaction partner needs to harbor a thiol group for oxidative reaction with the cysteine. In general, in order to reduce the formation of homodimeric disulfide species, either the protein cysteine or thiol partner is activated. We report herein a remarkably simple strategy for conjugating proteins bearing accessible cysteines to unprotected peptides containing a Cys(Scm) (Carbomethoxysulfenyl) protecting group.[2] The Scm group was introduced on-resin via a Cys(Acm) (Acetamidomethyl) building block.[3] The peptides employed for this proof of principle study were highly varied and structurally diverse and underwent multiple on-resin decoration steps prior to protein conjugation. The methodology was applied to three different proteins and proved to be site-selective. Moreover, the overall synthesis is facile, efficient and versatile, and takes advantage of solid phase peptide chemistry strategies for the decoration of proteins with free Cys residues.

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P116 Streamlined Total Chemical Synthesis of Protein Targets

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The rate and scale of proteins prepared by total chemical synthesis has increased over the last several years—propelled by native chemical ligation (NCL), new chemical protecting group strategies, and automated solid-phase peptide synthesis (SPPS).

Navigen Pharma employs these technologies to synthesize non-natural D-protein targets for selection by mirror-image phage display in order to develop D-peptide-based drugs. In this talk, we will highlight therapeutic protein targets that we have recently prepared by total chemical synthesis. These targets include monomeric C5a (74 aa), and trimeric CD40L (149 aa) and TNFα (157 aa). First synthesized in L-form, the synthetic proteins are folded and functionally evaluated in parallel with functional, recombinantly-expressed proteins, prior to D-form synthesis. To streamline complex protein synthesis, we are pioneering two new assembly tools: Helping Hands and Pullover Peptides. Helping Hands are semi-permanent tags at Lys residues, which improve peptide segment solubility and handling during assembly. Pullover Peptides are recently-developed tools that simplify the chromatographic purification of complex, long protein segments during the assembly of larger synthetic proteins.

YI-P117 Rapid Discovery of Illuminating Peptides for Instant Detection of Opioids in Blood

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In 2017 Opioid overdose caused more than 72000 deaths in the US alone. Current approaches for opioid identification and quantification in body fluids include immunoassay and chromatographic methods (e.g., LC-MS, GC-MS) which requires expensive instrumentation and extensive sample preparation. We proposed developing a fluorescence-based sensor chip to sensitively detect different opioid drugs in blood using a homogenous immunoassay without any washing steps. We synthesized nine one-bead one-compound (OBOC) combinatorial libraries containing a large number of random peptides while incorporating a molecular rotor dye, Malachite Green (MG), to the Lys side chain amine group at different positions of peptide sequences. A subsequent three-step fluorescent screening assay using confocal microscope were employed to track beads with change in fluorescence intensity.

Positive beads meeting the desired criteria were isolated for decoding.

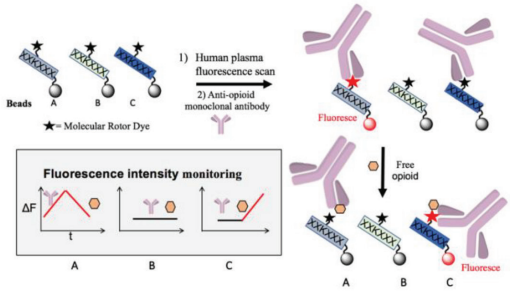

Figure 1. Three-step screening of illuminating-peptide library

Selected illuminating peptides were immobilized onto glass slide, using cupper free click chemistry, to form fluorescence-based sensor chip and tested for morphine in plasma. We have demonstrated that this proof-of-concept platform can be used to develop fluorescence-based sensors against morphine. More importantly, this technology can be applied to discovery of other novel fluorescence-based sensors in fluids, e.g., anti-doping and cancer/infectious biomarkers

P118 Design, Synthesis and Characterisation of Potent Conotoxin Peptidomimetics Incorporating a Triazole Disulfide Bond Mimic

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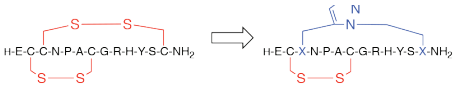
The potency and selectivity of conotoxin peptides for neuropathic receptors has made them attractive lead compounds in the development of new therapeutics.[1] However, these disulfide bond rich peptides are limited as lead drug compounds due to their unfavourable physicochemical properties.

We have used a peptidomimetic triazole disulfide bridge surrogate to replace each disulfide bridge in turn within α-conotoxin GI.[2] This produced a mimetic with an order of magnitude increase in blood plasma stability, whilst retaining full biological activity. Our studies were performed in human CN21 cells expressing human AChRs, making them applicable towards drug development in humans.

Bespoke force field descriptions of the triazole mimetic were developed that allowed us to determine the solution structure by NMR spectroscopy, we discovered significant similarities in conformation between the mimetic and the peptide bioactive toxin.



nAChR IC_{50} 9.8 nM **nAChR IC_{50}** 8.2 nM

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YI-P119 The Mode of Action of Antibiotic-peptide Plectasin in Native Membranes

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The rise of multi-drug resistant pathogens urgently calls for the design of powerful novel antibiotics that are robust to resistance development. Ideal templates for such next-generation antibiotics would be peptides that target the highly conserved cell-wall precursor lipid II, also known as the "Achilles' heel of bacteria". Such antibiotics would kill the most refractory pathogens at nanomolecular concentrations. However, due to the challenge of studying small membrane-embedded drug–receptor complexes in native conditions, the structural correlates of the medically relevant binding modes are unknown.

Here we present the physiologically relevant binding mode of the fungal antibiotic Plectasin, a highly promising template for drug design[1]. Previous structural studies presented a model of the plectasin – lipid II complex in micelles,[2] which often poorly mimic physiological membrane conditions and may critically alter the binding mode.[3] We revisit the plectasin – lipid II complex at close-to-physiological conditions using a cutting-edge solid-state NMR approach[3]. Our study reveals drastic differences in the plectasin – lipid II complex in native conditions and unravels, so far unknown, critical lipid II-binding features. Our insights provide a new foundation for the design of next-generation antibiotics using plectasin and other comparable antibiotics as templates.

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P120 A*STAR Peptide Engineering Platform (PEP): Targeting Macrocyclic Modalities for Protein-Protein Interactions

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Challenges in the discovery of macrocyclic peptide modalities are being met by the emergence of new technologies and creative multidisciplinary approaches. As a result peptide research is now experiencing a remarkable transformation. This talk will focus on how A*STAR has embraced this revitalization and is evolving technologies to enable the discovery and development of new peptide modalities for protein-protein interactions. Highlights will include our recent advances in diversity, screening, design, chemistry and formulation with examples targeting the p53 and translational initiation (EIF4F) pathways for oncology.

YI-P121 Hierarchical Self-assembly of a Cross-linked Coiled Coil Peptide for Biomaterial Applications

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Self-assembling peptides have been used in recent decades for a variety of medical applications, including but not limited to tissue engineering, drug delivery, and biosensing. One peptide of particular interest, the leucine-zipper of the transcription factor GCN4, can exist in multiple oligomeric states based on the hydrophobic interface. Through functionalizing these GCN4 peptides with metal-binding ligands on the hydrophilic faces of the coiled coil, metal ion-mediated self-assembly can be promoted radially,[1] and by altering the C- and N-termini of the peptide, self-assembly can be facilitated linearly.[2] A design which merges these two modifications to create crosslinked coiled coil biomaterials will be reported.

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YI-P122 A Highly Potent and Selective Inhibitor of the Myb-KIX Interaction

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Acute myeloid leukemia (AML) is dependent on the native interaction between the transcriptional activator Myb and the transcriptional coactivator CREB-binding protein (CBP). The CBP coactivator interacts with a variety of different transcription factors through its flexible KIX domain, allowing CBP to control transcription of many genes. Unfortunately, the conformational plasticity and the presence of multiple binding sites on KIX make it challenging to target using small molecule inhibitors. Notably, the transactivation domains of Myb and MLL bind to two different sites on KIX, and fusing these two domains via a flexible linker produces a peptide (MybLL-tide) that has picomolar affinity for KIX. MybLL-tide has higher affinity for KIX than any previously reported compounds while also possessing 15,000-fold selectivity for the CBP KIX domain over other similar coactivator domains. Further modification of the MybLL-tide with a nuclear localization signal and a cell penetrating peptide moiety yield a modified MybLL-tide with cellular activity that potently modulates downstream gene expression and also inhibits AML cell viability. These promising results show that MybLL-tide can be an effective, modifiable tool to selectively target the KIX domain and assess transcriptional effects in both AML cells and potentially other cancers dependent on aberrant Myb or MLL behavior.

P123 A Design Strategy for Creating a Small and High-Affinity Antibody Mimetics by Double-CDR Grafting

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Small mimetics that can bind to the same epitopes as their parental antibodies (Abs) with comparable affinity and specificity are cheap and promising alternatives for the treatment of various diseases. Epitope-binding sites (paratope) of Abs are found in the complementarity-determining region (CDR) loops. Our previous study showed that CDR peptides structurally constrained within a scaffold exhibited higher affinity and specificity for their target compared to freely fluctuating peptides. Based on this, we developed a novel strategy for the development of small Ab mimetics: Paratope are reconstituted via the grafting of multiple CDR peptides into a structurally constrained position in a small protein scaffold. In the current study, we describe the design and validation of a double CDR-grafted anti-HER2 Ab mimetics, D-FLAP. Two hundred D-FLAP candidates were first designed in silico by replacing hexapeptide at two different loops of FN3 scaffold (10 kDa) with two hexapeptides in the CDRs of HER2 Ab. Then, we found 48 and 46 candidates in them were predicted to keep constrained hexapeptides and reconstitute paratope of parental Ab, respectively, and 13 candidates had both features. After in silico binding energy calculation, ten candidates were predicted to bind to HER2 in a similar mode as parental Ab. Finally, the binding affinity of purified candidates were evaluated by ELISA. D-FLAP-147 exhibited high affinities for HER2 (K_D = 58 nM). Future endeavors will focus on increasing the performance of HER2-binding D-FLAPs and extending the design strategy to create other high-affinity antibody alternatives.

YIP-P124 Selective Peptide Binders for Human Programmed Death Ligand-1 (PD-L1) as an Immune Checkpoint Inhibitory Ligand in Cancer

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Programmed death ligand 1 (PD-L1) and its receptor (PD-1) are involved in a critical immune check point pathway that allows the tumor to escape from the immune system. Currently, there are no routine and reliable method available to non-invasively monitor the PD-L1 status and to allow patient stratification based on PD-L1 expression level. Our goal is to develop peptide based diagnostic agents that can selectively and specifically bind to PD-L1 for evaluating PD-L1 status in tumor cells and for monitoring the immunotherapy.

An *in vitro* selection strategy called mRNA display was used for selecting from more than a trillion different peptide sequences for a desired binding affinity towards PD-L1 as the target. Using this method, an initial random library of mRNA-peptide fusions with nine fully randomized amino acid residues was prepared and several rounds of selection were performed. The binding level was monitored at the end of each cycle until a desirable binding affinity was achieved. The library was then analyzed through next generation sequencing and the top peptide sequences were synthesized and individually tested. A top sequence with significant binding affinity towards PD-L1 was used to design a new doped library and underwent several additional rounds of selection. The final selected pool was again sequenced by illumina sequencing and more peptide sequences with an enhanced binding affinity towards PD-L1 were detected and were characterized. The significant binding level of selected peptides for PD-L1 demonstrates their potential as a starting point for developing desired diagnostic agents to trace PD-L1 positive tumor cells.

P125 Peptide Cross-Linked Hyaluronan Hydrogels

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As very promising, injectable hydrogels can be used in non-invasive surgical procedures. These biomaterials are able to fill the defect area completely with a good permeability. The technique can be effectively applied to provide a wide range of bioactive substances, such as drugs, proteins, growth factors and even living cells.[1]

Hyaluronic acid (HA) is an interesting candidate for the tissue engineering because of its unique physicochemical and biological properties. HA could be cross-linked by synthetic reagents, linkers or specific reaction conditions.[2] Based on natural compounds such as desmosin[3] and its analogs, peptides can be used as cross-linking agents due to their potential biodegradability and biocompatibility. The specific application and controlled density of cross-link network could be modulated by the primary sequence of peptides.

A new peptide cross-linking strategy for preparation HA hydrogel from its oxidized form was developed. N-terminal modification of peptide by aminooxyacetic acid[4] led to the insertion of moiety used to attach peptide to HA-aldehyde and resulted in stable linkage –CH=N-O-CH_2– . We prepared group of peptides with different chain lenght, i.e. different distance of reaction centers and then we prepared corresponding HA-peptide hydrogels. In the terms of gelation process and mechanical properties, we have not observed significant improvement in comparison to commonly used POA (*O,O'*-1,3-propanediylbishydroxylamine). However, it is possible to prepare modified peptides with such sequence which allow to prepare hydrogels including covalently attached peptides motifs applicable in tissue engineering.

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P126 Carbon-Carbon Bond Forming Reactions for the Synthesis of Posttranslationally Modified Peptides

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Most of the natural proteins and peptides undergo posttranslational modifications (PTM) which improves their stability, alters their function or enables their transport across the membranes in living organisms. One of the most common sites for modification is side chain of asparagine and aspartic acid. These modifications include alkylations of heteroatoms, hydroxylations of the C-H bonds, glycoslytion, as well as formation of succinimide. In order to understand the role of these modifications it is necessary to access these modified peptides and proteins in a chemically pure form. Majority of the PTMs involve formation of the carbon-heteroatom bonds that are catalyzed by variety of enzymes. Typically, these enzymes modify the same protein or peptide in multiple ways leading hard to separate mixtures.

Recently, chemical methods have been used to prepare pure PTMed proteins and peptides. However, these methods often require extensive protection/deprotection steps of the peptide side chain functional groups. We have developed an alternative strategy for accessing peptides with PTMs at aspartic acid and asparagine side chains by addition of aminoacyl and alkoxy acyl radicals to dehydroalanine that was preinstalled in the peptide backbone using chemical and enzymatic methods. We have shown that peptides that contain N-methylated and N-glycosylated asparagine, as well as succinimide can be synthesized using this method. Overall, this new C-C bond forming strategy will provide access to peptides with modified side chains that resemble the postranslation modifications performed by enzymes in many living organisms. This in turn will enable studies on understanding the biology of such peptides.

YI-P127 Triple-tagged Peptides Targeting Intracellular Proteins

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In medicinal chemistry, peptide labeling has been widely utilized for applications which include fluorescent tags for in vitro or in vivo imaging or poly-lysine tails for cell permeability. Many ligation methods are known, including: oxime/hydrazone formation, Staudinger ligation, thiol-maleimide Michael addition, alkyne-azide "click" chemistry, and Suzuki-Miyaura cross-coupling. These methods have been shown to efficiently ligate or singularly label peptides, but their combined use for applying multiple labels to a peptide for biological study has not been well studied. Therefore, we have developed an efficient synthetic strategy for multiple labeling of peptides via various orthogonal coupling reactions, including Suzuki-Miyaura cross-coupling, alkyne-azide cycloaddition, and thiol-maleimideMichael addition. The compatibility of these chemistries was tested with different combinations with respect to the order in which they were performed, and here we report an optimized synthetic strategy which allows for all three chemistries to be utilized with minimal side reactions.We have constructed a short Bak peptide carrying a fluorescent tag, an affinity tag, and a cell permeable peptide via Suzuki-Miyaura cross-coupling, alkyne-azide click chemistry, and thiol-maleimide Michael addition reactions. The multiple labeled peptide was found to be permeable to the cell membrane, as seen under fluorescent microscopy, and was able to pull down a protein that it binds to.

P128 Peptide-based Biosensor for Detection of Listeria Monocytogenes-Can Bacteriocins Make It?

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The high mortality rate from infections by Listeria monocytogenes, a Gram-positive foodborne pathogen, designate this microbe as a high threat to humans. A biosensor, which is rapid, sensitive, portable, and not labor-intensive, for detection of *L. monocytogenes* can help diminish some of the drawbacks associated with this pathogen. We have used a class IIa bacteriocin, Leucocin A, as a molecular recognition motif in conjunction with different transduction methods to develop a biosensor for *L. monocytogenes*.[1-3] Leucocin A is a potent antimicrobial peptide that displays specific activity against listeria and other closely related bacteria. In this presentation, the results from a peptide-based platform where Leucocin A is immobilized on surface, and impedance spectroscopy, fluorescence spectroscopy or microfluidic cantilever is used to detect listeria will be discussed.

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YI-P129 Membrane-permeable Lariat Peptides

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Medicinal chemists are wary of the chemical space beyond the rule of 5. Membrane permeability tends to drop off as molecular weight and polar group count increase. Many cyclic peptides in this chemical space are passively permeable and show promising bioactivity, inspiring investigators to delineate the basic principles underlying the passive permeability of cyclic peptides. Although lariat peptides comprise a large portion of bioactive cyclic peptide natural products, exploration of permeability in the lariat space has been limited to the space immediately surrounding complex natural products. In order to gain a better understanding of how the lariat tail will impact passive membrane permeability, we generated a mass-encoded library of lariat peptides. We determined the passive membrane permeability for over 1000 lariat peptides and identified stereochemical features associated with permeability. Some sublibraries elicited interesting morpholigal and cell cycle phenotypes in HeLa cells.

YI-P130 Expanding The Limit of Hydrogen Bond Strength through Thioamide Substitution

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Hydrogen bonds play a pivotal role in maintaining the protein structure. The energy of a hydrogen bond contributes around 5-6 kcal/mol towards the stabilization of an isolated bond and 0.5-1.5 kcal/mol for proteins in solution due to the hydration of peptide bonds.[1] However, this contribution is context dependent and it has been extensively studied through amide to ester or an olefin mutation,[2,3] however, although they provide useful information by eliminating a H-bond donor or acceptor, their conformational features significantly differ from an oxoamide making the interpretation complex. We examined the influence of enhanced intramolecular H-bond strength and the desolvation of the amide bond through thioamide substitution on protein structural stability. Towards this goal, we targeted the solvent exposed carbonyl of the Pin1 WW domain, a small β-sheet protein, and replaced selected backbone amide bonds with thioamide. We observed that all the O to S (amide to thioamide) mutated variants folds into the same β-sheet structure as the wild type. Furthermore, thioamide incorporation at a sterically allowed, solvent exposed hydrogen bond displayed almost no effect on the protein stabilization despite its better H-bond donor property. Finally, utilizing the double mutant cycle we observed that a thioamide acts synergistically with the amino acid side chain in effective solvent shielding of a hydrogen bond present in the core of a protein. With a single atom substitution, we could achieve around 15 ^{0}C enhancement in thermal stability (T_M) compared to the wild type Pin1 protein. Our results suggest that this approach will open new avenues towards engineering stable protein scaffolds.

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YI-P131 Differential Control of Protein Stability by n→π* Interaction

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A protein conformation is defined by an assortment of various covalent and noncovalent interactions. Here, we probe the role of recently identified n→π* interaction in guiding the structural stability of proteins utilizing model thioamide containing folded β-sheet peptides and synthetic proteins in solution using NMR and circular dichroism spectroscopy. We found that an n→π* interaction between C=Si and C=Oi+1 result in decreased ϕ dihedral angle between the donor (*i*) and the acceptor (*i*+1) residues. We further observed that the van der Waals repulsion between the Cγi and C=Si (donor atom) in an amino acid can enhance the strength of an n→π* interaction, indicating that the extent of n→π* interaction varies with amino acid side chain. Our experimental results suggest that the local kink in the peptide chain induced by this interaction either stabilizes secondary structures like β-turn (and α-helix) or destabilizes β-sheets, affecting the overall stability of the protein. Our observation suggests that an amide bond can potentially involve in a competing interaction between hydrogen bonding and n→π* interaction through HN (H-bond donor) and C=O (n→π* donor or acceptor), respectively, the relative strength of which dictates the local torsion angles ϕ and ψ about the C$^{\alpha}$. Further, we were successful in engineering an n→π* interaction in the reverse-turn of the protein GB1and detect it via X-ray crystallography. Our finding clearly indicates that n→π* interaction is operative in proteins and has a critical role in protein folding and governing its structure.

P132 Structure, Activity, and Mechanism of Action of Cyclic Lipopeptides Produced by *Paenibacillus elgii*

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Paenibacillus elgii strain having antimicrobial activity was isolated from agricultural soil collected from Jeonnam province in South Korea. The *P. elgii* strain was cultured in M9 media at 30 ºC for 48 h. After cultivation, the culture supernatant was prepared by centrifugation. Multiple antimicrobial substances were included in the culture supernatant. LC-MS analysis suggested that the antimicrobial substances belong to pelgipeptin (PGP) family which is cyclic lipopeptide by 9-amino acid with a short lipid chain. We purified the PGP peptides using *Diaion* HP-20 and RP-HPLC chromatography. The amino acid sequence of the purified PGPs was determined by LC-MS/MS, suggesting PGP-A, -B, -C, and -D. The complete molecular structures of the PGP peptides were determined by 2D NMR spectroscopy. The antimicrobial activity of PGP peptides determined by minimal inhibitory concentration showed broad and strong antimicrobial activities against Gram-negative and Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) with 1~16 MICs (μg mL^{-1}). The mechanism of action of PGPs was investigated by fluorescence experiments. The calcein dye leakage and membrane depolarization profiles of PGPs exhibited a gradual increase in the fluorescence intensity, which suggests that these peptides are targeting bacterial membrane by disrupting cytoplasmic membrane. In particular, PGP-A showed very low hemolysis activity than other PGP peptides. Therefore, PGP-A can be a highly potential peptide antibiotics with a broad antimicrobial activity and low cytotoxicity.

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Y1-P133 Decoration of a Polylactide Chain with Isolated Amino Acid Moieties through Organocatalytic Ring-opening Copolymerization: A Route Towards Bioactive Degradable Polymers for Tissue Engineering Applications

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Hybrid polypeptide-based polyesters have emerged as a promising material for applications within the biomedical field, offering the advantage of processability and degradability as well as the ability to integrate with biological systems.[1] Our group is developing three dimensional porous polyester-based scaffolds for tissue regeneration.[2] To increase the cell-interaction properties of the main chain polyesters, we have developed a strategy for the random inclusion of isolated α-amino acid units along a polylactide chain at low reaction temperatures. This was achieved by thorough elucidation on the kinetic and thermodynamic features of the organocatalytic ring-opening copolymerization system of L-lactide and a cyclic ester-based monomer bearing a latent α-amino acid moiety. The copolymerization system was shown dependent on the catalyst loading, monomer concentration, solvent polarity, and temperature. Consequently, the reaction could be tuned to allow up to 13 % incorporation of isolated α-amino acid units with control over molecular weight (Mn≈ 7.3 kg mol-1) and dispersity (Đ≤1.27). These findings provide a thorough synthetic platform for tunable incorporation of peptidic moieties along the polyester backbone and provides a mild and selective methodology for the preparation of well-defined scaffold materials combining high hydrolyzability with structural features that mimics polypeptides.

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P134 p16INK4a-Derived Peptides Inhibit CDK4/6 and Mesothelioma Proliferation

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Introduction: Malignant mesothelioma is a devastating cancer with a very poor prognosis. Up to 90% of mesothelioma tumors exhibit low expression of the endogenous CDK4/6 (cyclin-dependent kinases 4 and 6) inhibitor p16INK4a (p16). Current small molecule CDK4/6 inhibitors exhibit activity against other CDKs. More selective inhibition of CDK4/6 will aid in further study of the roles of these proteins and the development of anti-neoplastics. To develop such inhibitors, we are designing and evaluating stabilized peptide inhibitors of CDK4/6. The starting point for our research is based on truncated peptides derived from p16 that inhibit CDK4/6 with potency similar to the full-length p16. Here we describe the early development of foldamer CDK4/6 inhibitors that incorporate non-natural amino acids.

Hypothesis: Targeting CDK4/6 via selective stabilized helical peptides will lead to development of more effective therapy against mesothelioma.

Results and Discussion: We have previously described utilization of olefin-tethers and a TAT-leader sequence to promote conformational stabilization of p16-derived peptides. To surpass the activity of these peptides, we designed and evaluated several peptides with systematic replacement of key amino acids with beta-amino acids. We have identified multiple peptides with beta-amino acid substitutions that have inhibitory activity against CDK4/6 (IC_{50} values in the low μM range, i.e., 2.2 μM to 8.5 μM for one key peptide) and activity against mesothelioma proliferation (IC_{50} values 3.1 μM to 34.6μM for one key peptide depending on cell line subtype). We are conducting molecular dynamic studies to see if helicity of these peptides correlates with activity.

YI-P135 Improving upon Nature: A Medicinal Chemistry Optimization of Cordyheptapeptide A and B

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Pursued for their rich structural diversity as well as the compelling array of biological activities and targets, cyclic peptides have long provided fertile ground as a source of novel bioactive scaffolds. Many cyclic peptide natural products, in particular those enriched in aliphatic, N-methyl, and D-amino acid residues, are highly active in mammalian cells, prompting studies by our group and others into the factors that govern cell permeability in these large, non-"druglike" molecules. We synthesized and investigated the properties of cordyheptapeptide A (**1**), a cyclic peptide natural product. Originally isolated from *Cordyceps*, a fungal genus widely valued for its pharmaceutical potential, the cordyheptapeptide family, including cordyheptapeptides B and C, are reported to show toxicity toward bacteria, fungi, as well as a variety of cancer cell lines, although mostly in the micromolar range. Although a

solution-phase total synthesis for **1** has been described, and its crystal structure has been solved, the biological target(s) and/or mechanism of action of 1 remain unknown. Here we describe a novel phenotypic activity for 1 in HeLa cells with an EC_{50} of 4 nM, and an efficient solid-phase synthesis approach which enabled further optimization to produce the derivative 30 with cellular EC_{50} of 0.9 nM. Cytological profiling of **1** and clustering its fingerprint with a 2035-member library of compounds of "known bioactives" suggest that **1** may have an interesting and potentially novel unique MOA.

YI-P136 Identification of Cell-Permeable Cyclic Peptides in a DNA Encoded Library through Reverse Phase Chromatography

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Small molecule combinatorial chemical libraries have been an essential tool utilized by medicinal chemists for over twenty years to effectively sample large areas of chemical space. Over the past decade, DNA-encoded libraries (DNAELs) have emerged as a promising new strategy to greatly increase the size and diversity of these chemical libraries. In the past several years, DNAELs with sizes ranging from 10^6-10^{12} have been reported. These massive libraries often manage to produce bioactive hits and can be decoded by amplifying and sequencing the DNA barcode. Although DNA Encoded Libraries can produce bioactive compounds consistently, the hits have no guarantee to access their targets in vivo due to issues with metabolism and cell permeability, especially with the recent push towards larger drugs. Our lab has been focused on developing a simple screen based on RP-HPLC to identify cell permeable members from a large, complex DNA Encoded Library. Inspired by recently published findings from our lab, we already have acquired encouraging preliminary data and are currently synthesizing a test library. Our preparative screen would be able to identify permeable members from a large (>10^6) and diverse DNA-Encoded Library.

YI-P137 Chemoselective Pd-catalyzed Modification of Cysteine-containing Peptides and Proteins

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Many proteins are posttranslationally modified, which is important for their function and localization within cells. Among different modifications such as phosphorylation, glycosylation and lipidation, the attachment of prenyl-groups is especially important for the association of certain proteins to specific membranes.[1]

As this prenylation mostly occurs within the last C-terminal amino acids of proteins, semisynthetic approaches to obtain prenylated protein variants have been developed.[2] In these approaches, prenylated peptides are linked to a target protein by chemoselective modification reactions. The difficulty of this method is that the attachment of lipid chains to C terminal peptides occurs mostly via thioethers and thioesters, chemistry not easily compatible with standard Fmoc-based solid phase peptide synthesis and cleavage conditions, which in turn leads to quite complex synthesis strategies.[1-3] Modified peptide and protein variants can also be obtained by using selective chemical reactions, which can lead to natural posttranslational modifications or the introduction of fluorophores or reactive tags.[4] However, most of these reactions problems generate severe challenges when it comes to selectivity, reaction conditions and yield.[4,5]

Here we demonstrated that the Pd-catalyzed Tsuji-Trost-allylation can be used for the prenylation of Cys-containing peptides and proteins. By this, prenylated peptides and proteins can be accessed, with high n/iso ratio and excellent chemoselectivity. Furthermore, it is possible to introduce other modifications such as a fluorophore, an affinity tag or staples into peptides by this reaction.

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P138 Conformational Restriction of Coiled-coil Based Fusogens

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Fusion of cellular membranes has attracted considerable scientific attention, not only for being ubiquitous and vital in living organisms (e.g. neurotransmitter release, fertilization, and material exchange), but also for its potential to be used for *in vivo* applications.

Despite the diversity in fusion systems, the fusion cascade shares a few common features: first, contact between two membranes is developed and accompanied by disruption of the contact site. This is followed by fusion of the proximal leaflets and lipid mixing, which culminates in opening of a fusion pore and content mixing.

In recent years we developed a pair of heterodimeric coiled-coil forming lipopeptides to mimic the function of natural SNARE proteins and induce membrane fusion between liposomes, and between liposomes and cells both in vitro and *in vivo*.[1-4] The fusogens consist of a set of two complementary lipopeptide molecules, comprised of a coiled-coil recognition domain conjugated to cholesterol via a poly(ethylene glycol) spacer.

Here, intramolecular crosslinking of one coiled-coil peptide was employed to locally change peptide helicity and stability without affecting hydrophobic and electrostatic interactions which are crucial for coiled-coil formation. In this manner, the effect of lipopeptide structure on coiled-coil binding dynamics, liposomal interactions and fusogenicity was studied. This approach enables us to gain insights as to the mechanism of peptide-based membrane fusion and expands the possibilities of using this system for the delivery of therapeutics.

References:
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P139 On-DNA Medicinal Chemistry of Peptidomimetic Ligands to the Polycomb Chromobox Family of Chromodomains

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DNA-encoded chemical libraries have emerged as new approach for rapid ligand discovery. A major advantage of this approach is the ability to assess many molecules collectively in an in vitro selection. We present the synthesis of both combinatorial and scanning positional DNA-encoded libraries of peptides and peptidomimetics for targeting the trimethyllysine-binding domains (chromodomains) of chromobox proteins. We present a quantitative evaluation of affinity-based selection assay robustness using DNA-linked ligands of known affinity to CBX7 and CBX8. Statistical analysis of assays indicated low DNA tag bias and adequate robustness for both ligand discovery and determination of quantitative structure activity relationships. Highly optimized assays allowed iterative "design, build, test" cycles of DNA-encoded libraries, which were panned in parallel against all members of the polycomb CBX family. This approach allowed ready identification of ligand selectivity and potency across this protein family by DNA sequencing. We additionally explore the utility of covalent crosslinking to improve selection assays and allow selections to be conducted within live mammalian cells. We present progress with novel, isoform selective ligands in biochemical and cellular assays, including growth inhibition of select cancer cell lines. Throughout this work, we highlight the benefits of a DNA-based approach over more traditional peptide library approaches such as cellulose and micro arrays.

YI-P140 Elucidating the Biophysical and Biological Properties of the p3 Peptide in Alzheimer's Disease

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Alzheimer's Disease is the 6th leading cause of death in the U.S, affecting 10% of seniors over the age of 65. The disease is characterized by elevated extracellular β-Amyloid (Aβ) accumulation and intracellular tau protein tangles. Aβ is an intrinsically disordered, aggregation-prone peptide produced from the transmembrane protein, Amyloid Precursor Protein (APP). Aβ aggregates through a complex and multi-faceted pathway to form soluble oligomers, followed by insoluble, β-sheet-rich fibrillar structures. While amyloid plaques isolated from Alzheimer's patients contain a wide variety of Aβ peptides and other proteins, the current amyloid model only seeks to understand the properties of two predominant isoforms — containing 40 or 42 amino acids. A C-terminal fragment of Aβ, p3, has been described as soluble and void of amyloidogenic properties since its discovery, despite minimal scientific evidence to support this. Despite this, my results indicate that not only does p3 rapidly aggregate to form oligomers and fibrils morphologically similar to those of Aβ, but also expedites Aβ aggregation through seeding. I have additionally found that, despite the established toxicity of p3, it exhibits minimal cellular uptake compared to Aβ. Given that recent literature suggests a strong relationship between cellular uptake and toxicity for Aβ, this suggests a possible unique mechanism of toxicity for p3. This calls for a more complex and heterogenous amyloid model that involves collective contribution of different Aβ peptides to the pathology of Alzheimer's Disease.

YI-P141 The Ghrelin Receptor with a Heavy Appetite for Ghrelin Peptidomimetics: Applications in Imaging and Therapy

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The ghrelin receptor (GR) is expressed in many human tissues (most notably the hypothalamus) and causes an increase in appetite upon activation by its endogenous ligand; ghrelin. The GR is differentially expressed in malignant compared to benign tumours (e.g. prostate). Therefore, there is an interest in developing GR-targeted peptides as novel drugs to modulate signaling (e.g. cancer cachexia and obesity) and image the receptor (e.g. disease diagnosis and progression). The initial compound G-7039, a peptidomimetic ghrelin agonist (IC_{50} 5.2 nM/EC50 0.18 nM) underwent structure-activity relationship (SAR) studies to generate ligands and positron emission tomography (PET) agents to study/image the GR. The first generation peptidomimetic [1-Nal[4],Lys[5] (4-fluorobenzoyl (4-FB)]G-7039 (IC_{50} 69 nM/EC_{50} 1.1 nM) was radiolabeled with ^{18}F in a decay-corrected radiochemical yield of 48%, radio purity of ≥ 99%, and molar activity of ≥ 34 GBq/μmol. Despite its success as a PET imaging agent, its solubility (cLogP = 5.28) and binding affinity needed improvement. The second generation peptidomimetic [Tyr[4],Lys[5](2-fluoropropionyl (2-FP)]G-7039 (IC_{50} 0.28 nM/EC_{50} 0.12 nM) had improved binding and lipophilicity (cLogP = 4.36). Labeling of this ligand was low yielding, however it was identified as a partial agonist because of a unique H-bond interaction to the GR. The third generation required a modified prosthetic group (2FP to ammonium methyltrifluoroborate-$AMBF_3$) in order to radiolabel in higher yields, resulting in [Tyr[4],Lys[5](AMBF3)] G-7039 (IC_{50} 0.85 nM). This compound was radiolabeled in a single step, with a decay-corrected radiochemical yield of 28%, radiopurity of ≥ 99%, and molar activity ≥ 15 GBq/μmol. Finally, the fourth generation, a homobivalent G-7039 ligand (IC_{50high} 0.43 nM:IC_{50low} 0.42 pM /EC50 1.8-2.1 nM) was found to bind to the GR homodimer and was designed to study GR homo/heterodimerization. Four generations of unique peptidomimetics eliciting differences in receptor binding and activation have been synthesized with potential applications towards imaging and therapy.

P142 Synthesis of Glycosylated Amino Acid Building Blocks via Acylation of β-Glycosylhydrazides

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Glycosylated asparagine residues are useful building blocks for the generation of N-linked glycopeptides and hydrolytically-stabilized analogs of O-linked glycopeptides. Glycosylated asparagines are often generated via β-glycosylamines, intermediates that can be problematic to generate and handle. We report here our efforts to pioneer a new strategy for the generation of glycosylated amino acid building blocks using β-glycosylhydrazides as nucleophiles instead of the corresponding amines.

YI-P143 Palladium Prompted On-Demand Cysteine Chemistry for the Synthesis of Peptides and Proteins

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Peptides and proteins that bear site-selective modifications are of great interest for various biochemical studies. Total chemical and semi-synthesis of proteins based on chemoselective ligation of unprotected peptides enable the preparation of modified peptides/proteins while overcoming the limitations of molecular biology approaches. The thiolate functionality of the Cys residue coupled with the wise selection of orthogonal protecting groups (PGs) has been employed to control the directionality of synthesis and/or the modification sites. Nevertheless, the deprotection conditions of the Cys PGs reduce the efficiency and applicability of the syntheses. Recently, we reported an unprecedented palladium chemoselectivity for on-demand deprotection of the Cys PGs; thiazolidine, acetamidomethyl (Acm) and tert-butyl under aqueous conditions.[1–5] These novel tools were applied for the synthesis of complex peptides and proteins such as the activity-based probe of ubiquitinated histone H2A, which was cross-linked with Calypso/ASX heterodimer deubiquitinase to form a stable covalent nucleosome-enzyme complex. These findings also prompted us to exploit the use of palladium chemistry for the preparation of peptides and proteins bearing disulfide bridges. We developed a strategy for the direct disulfide bond formation directly from Cys(Acm) by applying palladium and diethyldithiocarbamate. The utility of this chemistry that was performed in a one-pot and highly efficient manner was exemplified in the synthesis of oxytocin peptide and in the first total chemical synthesis of Thioredoxin-1 protein. These discoveries open exciting avenues for the efficient preparation of unique and complex peptides and proteins.

1 M. Jbara†, S. Laps†, M. Morgan, G. Kamnesky, G. Mann, C. Wolberger, A. Brik*, *Nat. Commun.* **2018**, DOI: 10.1038/s41467-018-05628-0 (†equally contribution).
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P144 Development of the Stable, Fast Acting Glucagon Hypopen Analogue NN9513 for Clinical Testing

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Glucagon is effective as emergency treatment of insulin-induced hypoglycaemia. The hyperglycaemic effect of glucagon is achieved by stimulating glycogenolysis and gluconeogenesis in the liver. The commercial glucagon kit consists of a vial with freeze dried glucagon and a syringe prefilled with water. The reconstitution process is prone to error when carried out by individuals - often relatives under stress with limited training. There is therefore an unmet need for a safer and more convenient prefilled ready-to-use glucagon containing device for the treatment of severe hypoglycaemia.

A stable glucagon solution is pivotal, but native glucagon is only soluble for a few hours at pH 3 due to aggregation and it is therefore necessary to develop a new analog. It is also necessary to introduce major improvements relative to native glucagon in order to avoid chemical degradation and achieve long term storage stability. For this purpose we developed the glucagon analogue NN9513 for clinical testing. Introduction of a short chain of glutamic acid moieties on the lysine sidechain in position 24 increased not only the solubility but also the physical stability dramatically. Several amino acid substitutions were required to achieve the required chemical stability; Asp^{15}, Asp^{21}, Met^{27} and Asn^{28} were replaced with amino acids that reduced degradation significantly, but to obtain further stability additional N-terminal modifications were required. In addition to stability data, in vitro and preclinical *in vivo* PK and PD data will be presented.

The resulting clinical candidate NN9513 showed extraordinary physical and chemical stability in neutral aqueous formulations.

YI-P145 Synthetic Cyclic Peptomers as Virulence Blockers that Suppress Pathogenicity of Antibiotic Resistant Bacteria

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There is an urgent demand for new antibiotics and anti-infective drugs to address multidrug resistant bacteria and new pathogens. Treatment with antibiotics not only leads to resistance, but it also destroys our healthy microbiota. In contrast to traditional antibiotics, which kill bacteria, virulence blockers suppress the pathogenicity of bacteria without killing or inhibiting growth. Our chemical biology approach to blocking virulence will provide new opportunities to understand microbe interactions and may pave the way for new therapeutic strategies. We aim to improve the physical properties and potency of novel cyclic peptide inhibitors to combat the virulence of aggressive gram negative bacteria by inhibiting the Type 3 Secretion System (T3SS). The T3SS is a conserved needle-like appendage that bacteria use to secrete toxins

(Yops) into the host cell to suppress its defenses against the bacteria (Cornelis and Van Gijsegem, 2000). A screen for T3SS inhibitors in collaboration between the Lokey and Auerbuch-Stone labs yielded a peptoid containing analog of cyclic peptide epipheropeptin D (EpD). Peptide-to-peptoid substitutions in EpD led to increased activity relative to the parent compound and yielded compounds that were more potent than the published inhibitors (Duncan, M. C. et al., 2014) in an NF-kappaB luciferase assay designed to report on T3SS-mediated insertion of inflammatory proteins into mammalian cells. These initial results confirm that the position and nature of the peptoid group influenced activity. Further investigations aim to **1**) Improve the physical properties and potency of EpD and **2**) Identify the target of this cyclic peptoid.

P146 Cell-penetrating Peptides Engineered from a Human Defense Protein have Antimalarial and Anticancer Properties

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Host defense molecules are produced by a wide variety of organisms and many of these have provided valuable templates for developing new peptide-based drugs. The human defense protein platelet factor 4 (PF4) has antimicrobial and antimalarial properties. Inspired by PF4, we engineered a stable cyclized peptide – cPF4PD – that retained the critical structure and antimalarial activity of the parent protein. Here we show that the selective membrane-dependent mechanism of action of cPF4PD allows recognition and entry into diseased cells, including malaria parasite-infected and cancerous cells, while remaining non-toxic to healthy cells. cPF4PD is unique in its origin from a human protein, which together with its selective and non-disruptive entry into cells, makes it a very exciting molecule for safely targeting intracellular components of diseased cells.

P147 Development of a Potent Inhibitor of Estrogen Receptor-Coregulator Interactions in ER-Positive Breast Cancer

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Estrogen receptor-α (ERα) coregulators participate ER signaling by modulating the transcriptional activity of ER and are frequently overexpressed in ER-positive breast cancer. Targeting the interface between ERα and coregulators instead of the ligand binding pocket of ERα may provide an alternative therapeutic strategy for breast cancer and thereby overcome the resistance to the classical antiestrogens.

The α-helical LXXLL motifs of coregulators are responsible for the ER–coregulator interaction and ER activation. In order to emulate the LXXLL motif and disrupt the ERα–coregulator interactions,[1] we have designed tris-benzamide-based α-helix mimetics. This peptidomimetic features a rigid and preorganized scaffold which can project the substituents in the same spatial arrangement as an α-helix does for its side chains. In this study, we report chemical optimization of the tris-benzamide scaffold to achieve the affinity and selectivity for ERα by mimicking the extended region encompassing the LXXLL motif and the flanking sequences. ERX-41 was found to disrupt the ERα–coregulator interactions and effectively inhibit transcription and cell growth of ER-positive breast cancer cell lines with IC50 values ranging from 100 to 125 nM.

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YI-P148 Amino Acid Scanning at P5' within the Bowman–Birk Inhibitory Loop Reveals Specificity Trends for Diverse Serine Proteases

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Serine proteases from immune cells have been known to be implicated in various autoimmune and inflammatory disorders which account for a great portion of morbidity, mortality and health care cost worldwide. To block the activity of these proteases in disease conditions, one of the strategies is to develop potent and selective pharmacological inhibitors. Sunflower trypsin inhibitor-1 (SFTI-1) is a 14-amino acid backbone cyclic peptide that has been widely used as a template to generate serine protease inhibitors due to its small molecular size and rigid conformation. To develop potent and selective inhibitors, it is important to understand how each contact residue within the inhibitor's binding loop affects its activity. In this study, we designed a SFTI-based library with diverse P5' residues to characterize the P5' specificity of various serine proteases including the neutrophil protease cathepsin G. Screening the library against 11 serine proteases revealed that a subset of amino acids is generally favored by most proteases. However, there are still several proteases that show unique preference including kallikrein-related peptidase 5 (KLK5), matriptase, factor XIIa and thrombin. Guided by this information, we modified existing inhibitors and generated new peptide variants with improved potency and selectivity over other structurally similar proteases. These findings reveal the potential of the P5' residue in serine protease inhibitor engineering when using SFTI-1 or Bowman-Birk inhibitors as a template.

YI-P149 A Rationale Design and Development of a Strychnine Antidote

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Strychnine (Fig 1a), a poisonous alkaloid present in the seeds of the *strychnos nux vomica* tree. Strychnine is a neurotoxin which acts as an antagonist of glycine receptor. The victim will have spastic muscle contractions, resulting in death by

anoxia and exhaustion. Unfortunately, there is no antidote for strychnine. There is a need to develop an effective antidote for the early treatment of strychnine poisoning. Therefore, we used the OBOC combinatorial library method to discover the high-affinity capturing agent for strychnine. Four rationale 3-prong cholic acid-based peptidomimetic OBOC libraries were constructed and 33 D- and L-amino acids (natural and unnatural) were used as the building blocks. As shown in Fig 1b, the 3-prong compound folded upon itself to form a folded-structure that can capture strychnine. We used [^{3}H]-labeled strychnine as probe and autoradiography to detect strychnine binding. One of the positive peptidomimetics, was confirmed binding to strychnine, with the binding affinity (KD) of 50μM. We confirmed that ip. injection of mice with strychnine solution by caused seizures, followed by death, in all mice at the dose of 2mg/Kg. However, no seizures were observed in the mice injected with strychnine-antidote peptide complex; all the mice survived. (Fig 1c, d) Therefore, our findings in this study indicated that the 3-prong peptidomimetic peptide can be used as a potential strychnine antidote for the treatment of acute strychnine poisoning.

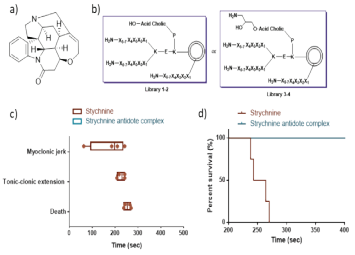

Fig 1 a) Chemical structure of strychnine; b) rational design of 3-prong OBOC libraries; c) seizures and death occur time after injection of strychnine and strychnine-antidote complex through *ip*; d) survival curve of strychnine and strychnine-antidote complex groups. (strychnine dose: 2mg/Kg)

P150 A Phe-to-Tyr Switch Changed the Conformation and Affinity of the Cyclic Endomorphin Analogs

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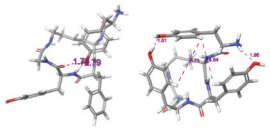
Peptide-based ligands at the mu-opioid receptor (MOPr) may be developed into non-/low liability analgesics for the treatment of pain. We have reported a series of cyclic peptides, which have a general sequence of Tyr^1-c[D-Lys^2-Xxx^3-Tyr^4-Gly^5], for their affinity and functional activity at the MOPr. Among these compounds, Tyr^1-c[D-Lys^2-Phe^3-Tyr^4-Gly5^j (Compound-1) exhibits the highest affinity with a Ki,μ of 0.99 nM and agonist potency with a EC50 of 2.3 nM. However, Compound-2, which has a Tyr^3 group at the Xxx^3 position, has a dramatically lower affinity (Ki= 114 nM) and agonist potency (EC50= 70.4 nM) at the MOPr. The two peptides only differ from one residue or a hydroxyl group, but both mimic the sequence of endomorphin-2, Tyr-Pro-Phe-Phe-NH2.

To understand what drives the difference of affinity toward the MOPr, we studied their conformations in solution by NMR. We then sampled the two cyclic peptides in water. The minimum energy ensembles of both peptides reproduced the conformations suggested by the NMR experiments. With the additional 4'-OH group at Phe^3 (Compound-2, Tyr^3), a hydrogen bond formed between this 4'-OH and the protonated amino group of Tyr1 at the N-terminus, rotating the (O)C—C$^\alpha$(H) of the Tyr1 as well as the C(H) — C$^\beta$(H) of the Tyr^3. The formation of the hydrogen bond affected the orientation of its side chains and the backbone conformation. This study shows that peptide conformation has a controlling effect on the ligand-receptor interaction. Removing/Adding a hydroxyl group may significantly change the conformation of a peptide and affect its biological activity.



YI-P151 Small Molecule Oligopeptides Isolated from Walnut (*Juglans regia L.*) and their Anti-Fatigue Effects in a Rat Model of Combined (Physical and Mental) Fatigue

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Fatigue can be classified as physical and mental depending on the cause. However, in our daily lives, combined fatigue, which is the combination of physical and mental fatigue, is most often experienced. Walnut (*Juglans regia L.*) is unique for its extensive biological activities and pharmaceutical properties. There are few studies on walnut oligopeptides (WOPs), which are small molecule peptides extracted from walnuts. This study aimed to evaluate effects of WOPs on combined fatigue and explore the underlying mechanism. Fifty male Sprague-Dawley rats were randomly divided into five experimental groups: normal control group, model control group, and three WOPs intervention groups. The normal control group and model control group were administered distilled water, and the three intervention groups administered WOPs by orally gavage at a dose of 220, 440, and 880 mg/kg of body weight, respectively. To produce an animal model of combined fatigue, rats were kept in a cage filled with water to a height of 2.2 cm for 5 d. To evaluate the extent of fatigue, the rats swam with a load of steel rings that weighed approximately 5% of their body weight and were attached to their tails. After exhausted swam test, serum biochemical indicators, inflammatory cytokines and expression of oxidative stress markers were measured. It was observed that fatigued rats treated with WOPs (440 and880 mg/kg) for 5 d could swim longer than fatigued animals given distilled water. WOPs also markedly decreased the activities of alanine aminotransferase, aspartate aminotransferase, tumor necrosis factor α, interleukin-6 and interleukin-1β in serum and enhanced the glycogen storage of liver. Moreover, WOPs treatment inhibited fatigue induced oxidative stress by increasing the activity of superoxide dismutase, glutathione peroxidase and decreasing the content of malondialdehyde in serums. These findings suggested that WOPs have a significant protective effect on combined fatigue rats, and the mechanism possibly mediated by the partial inhibition of oxidative stress and inflammatory response in serum.

YI-P152 Radioprotective Effect of Walnut Oligopeptides Against Gamma Radiation-induced Splenocyte Apoptosis and Intestinal Injury in Mice

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Walnut oligopeptides (WOPs) intake is associated with augment of antioxidant defense system and immune system.The study was aimed to evaluate the radioprotective effect of walnut

oligopeptides extracted from walnut seed protein against $^{60}Co\gamma$-irradiation induced damage in mice. Female BALB/c mice were administered WOPs through drinking water for 14 days until a single dose of whole-body $^{60}Co\gamma$-irradiation. The 30-day survival test was carried out in the first group (8 Gy), and the other two groups (3.5 Gy) were sacrificed at 3 days and 14 days post-irradiation. Blood and organ samples of mice in the three groups were collected, the histopathological analysis and immunohistochemistry were conducted, and the number of peripheral blood leukocytes, bone marrow DNA content, inflammatory cytokines, antioxidant capacity and intestinal permeability were measured. We found that the administration of WOPs augmented antioxidant defense system, accelerated of hematopoietic recovery and showed the significant trend toward higher survival rate and less weight loss compared with non-administrated control mice. In addition, WOPs administration appeared to be important for limiting IR-induced splenocyte apoptosis and inflammatory cascade as well as reducing intestine epithelial barrier dysfunction and promoting epithelial integrity. These results suggest that pre- and post-treatment of WOPs may help to ameliorate acute damage and accelerate its recovery which is induced by ionizing radiation in mice.
Keywords: walnut oligopeptides; antioxidant; epithelial barrier; immunosuppression; splenocyte apoptosis

YI-P153 Hypoglycemic Rffects of Oat Oligopeptides in High-Calorie Fiet/STZ-induced Fiabetic Rats

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Diabetes is a complex and refractory disease with high morbidity, high disability and high mortality which can cause many complications. Past studies have found that oats and many bioactive peptides have hypoglycemic effects. This study was aimed to determine whether oat oligopeptides (OOPs) could regulate hyperglycemia in Sprague–Dawley (SD) rats with type 2 diabetes mellitus (T2DM). A model of diabetes was induced by a 45-day high-calorie diet and streptozotocin intraperitoneal injection (30 mg/kg BW, twice). SD rats with T2DM were assigned to OOPs treatment groups (0.25, 0.50, 1.00 and 2.00 g/kg BW), whey protein control group (1.00 g/kg BW), metformin positive control group (Initial dose is 50 mg/kg BW, then increase by 50 mg/kg BW every 2 weeks to a maintenance dose 250 mg/kg BW), model control group, and normal control group randomly. After 12-weeks intervention, fasting blood glucose (FBG), oral glucose test tolerance (OGTT), serum insulin, antioxidant level, and hepatic enzymes were measured. In addition, micturition frequency of diabetic rats was recorded in this study for the first time. It was observed that the administration of OOPs (2.00 g/kg BW) resulted in a significant decrease in FBG since 6th week and a significant decrease in the OGTT-AUC on 6th and 10th week. In addition, the administration of OOPs (2.00 g/kg BW) reduced HOMA-IR index and 24-h urine volume significantly whereas increased SOD activity significantly. These results suggested that OOPs may have a hypoglycemic effect in diabetic rats.

YI-P154 Anti-inflammation Effect of Small Molecule Oligopeptides Isolated from Panax Ginseng C. A. Meyer in Rats

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Present study was designed to investigate the anti-inflammatory effects of Small Molecule Oligopeptides Isolated from *Panax ginseng C. A. Meyer* (GOPs). For the anti-inflammatory activity, dextran-induced paw edema and granuloma models were used in Sprague-Dawley rats (180-200g, 12 weeks old, n=10). Rats were treated orally with GOPs (0, 62.5, 125, 250 and 500 mg/kg) for both prophylactic studies. It was evaluated that the level of TNF-α, IL- 1β and IL-10 in serum of granuloma model; while the level of TNF-α, PGE2, LTD4 and RAF in paw tissue were detected. The effect of GOPs on the expression of MAPK and NF-kB was assessed by PCR. We found that oral administration of GOPs inhibited inflammation caused by cotton pellet and dextran. GOPs significantly inhibited the edema formation via MAPK and NF-kB. These finding suggested that GOPs have beneficial effect on acute and chronic inflammation, and the mechanism possibly mediated by inhibiting gene expression involved in inflammation and downregulating inflammatory mediators.

P155 Experimental Research of Walnut Oligopeptides on Moisturizing Intestine to Relieve Constipation

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To assess the effects of walnut oligopeptides on moisturizing the intestine to relieve constipation, and to provide basis for application of walnut oligopeptides in the treatment of constipation. ICR adult male mice were randomized into 7 groups: control group, model group, whey protein group (440mg/kg bw) and 3 walnut oligopeptides groups (220; 440; 880mg/kg bw). Test substances were gavaged continuously for 7 days. Constipation model was established with loperamide hydrochloride. The propulsion rate of ink in small intestine, the time when the first black fences granule was excreted, the number, weight and water content of black feces granules, the content of substance P, vasoactive intestinal peptide, endothelin, somatostatin, motilin, gastrin in serum, and the length of small intestinal villus was determined. Compared with whey protein group, The propulsion rate of ink in small intestine was significantly increased [(0.69 ± 0.10) vs (0.92 ± 0.14)], the time when the first black fences granule was excreted was shrotened [(272.60 ± 24.83) vs (241.50 ± 29.88) min], and the number and weight of feces granules was increased [defecation grain number: (5.00 + 1.41) vs (6.80 + 1.75), defecate weight: (0.06 + 0.02) vs (0.12 + 0.03)g] in high dose of walnut oligopeptides group; The content of ET and motilin in serum was significantly increased while the level of somatostain was significantly decreased. Futhermore, the intestinal villi injury that loperamide hydrochloride caused was reduced. Walnut oligopeptides has the function of moisturizing the intestine to relieve constipation, and its mechanism may be related to that walnut oligopeptides can promote the expression of gastrointestinal hormone endotoxin, motilin and inhibit the expression of somatostatin in serum of mice.

P156 Oat Oligopeptides: Immunomodulatory Effects on Innate and Adaptive Immunity in Mice via Cytokines Secretion, Antibody Production and Th cells Stimulation

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The study aimed to investigate the immunomodulatory activity of oligopeptides derived from oat (Avena Nuda L.) (OOPs). Healthy female BALB/c mice were randomly assigned to five groups, given deionized water (control), whey protein (0.5 g/kg body weight (bw)), and WOP 0.25, 0.5, 1.0 and 2.0 g/kg bw daily through drinking water for 30 days, respectively. Seven assays were performed to determine the immonomodulatory effects of OOPs on immune organs ratios, cellular and humoral immune responses, macrophage phagocytosis, and NK cell activity. Spleen T lymphocyte subpopulations (by flow cytometry), serum cytokine and immunoglobulin levels (by multiplex sandwich immunoassays), and intestinally secretory immunoglobulin (by ELISA) were determined to evaluate how OOPs affected the immune system. Our results showed that OOPs could significantly improve innate and adaptive immune responses in mice through the enhancement of cell-mediated and humoral immunity, macrophage phagocytosis capacity and NK cell activity. We conclude that the immunomodulatory effects might be attributed to increased T and Th cells percentages, IL-1α, IL-4, IL-6, IL-10, IFN-γ, TNF-α and GM-CSF secretion as well as IgA, IgG, IgM production. These results indicate that dietary OOPs intake could be considered as a promising immunomodulator with dosages range from 0.25 to 2.0 g/kg bw.

P157 Oat Oligopeptides (Avena Nuda L.) Supplement Alleviate Exercise-induced Fatigue in Mice

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Oat oligopeptides (OOPs) was derived from oat (Avena Nuda L.) and investigated for its anti-fatigue effects on swimming performance and related biochemical parameters. Male ICR mice were randomly divided into 5 groups including control group and four OOPs groups at doses of 0.5、1.0、2.0 and 4.0g/kg bw respectively, and treated with intragastric intervention for 30 days. The loading swimming endurance, blood lactic acid, serum urea nitrogen, lactate dehydrogenase activity, hepatic glycogen and muscle glycogen levels were determined. The results showed that the loading swimming endurance of mice extended significantly in all four OOPs groups compared with that in control group ($P < 0.05$). The under curve area of blood lactic acid of mice in all four OOPs were significantly reduced than that in control group ($P < 0.05$). Moreover, compared with control group, the muscle glycogen levels and lactate dehydrogenase activity of mice in OOPs 2.0 g/kg bw group increased significantly. These results suggested that OOPs can alleviate physical fatigue via reducing blood lactic acid accumulation and increasing liver glycogen with dosages range from 0.25 to 2.0 g/kg bw.

P158 A Photolabile Backbone Amide Linker for the Solid-Phase Synthesis of Cyclic Peptides and Peptide Thioesters

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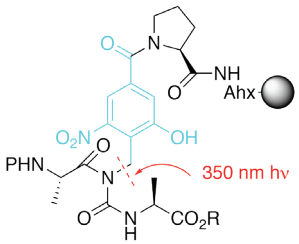
In this work we build upon the backbone amide linker (BAL) strategy introduced by Albericio and Barany[1] that permits C-terminal functionalization and head-to-tail cyclization of the resin-bound peptide by introducing an additional level of orthogonality to the procedure via photochemical release of the peptide from the linker. This modification overcomes several of the limitations of the original BAL strategy including reduced yields and an inability to make thioesters.

The photolabile BAL strategy uses a 2-hydroxy-6-nitrobenzyl motif (**1**) that facilities loading of the residues onto the linker as well as eventual photolysis at 350 nm. The problem of diketopiperazine formation experienced with the original BAL strategy has also been overcome using our photolabile linker in conjunction with a new C-terminal protecting group developed in our labs.[2] Additionally, peptide thioesters have been conveniently made on solid support using our linker. Several examples of cyclic peptides and peptide thioesters made using this strategy will be presented.



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2 Hostetler, M. A. and Lipton, M. A. *J. Org. Chem.* **2018**, *83*, 7762-7770.

YI-P159 New Pwpriswea from de nova Assembly Transcriptome of the Skin of Treefrog *Boana pugnax*

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Colombia presents a biotechnological potential by having the second world biodiversity with approximately 62,000 species. Besides, it has the second place in amphibians, which is one of the largest suppliers of peptides with many activities, especially on their skin. However, due to the effects of environmental pollution, the number of individuals decreases, making it difficult to work with a large number of them to obtain the peptides using proteomics. In addition, it is thanks to the sequencing with RNA-seq, that this problem is being overcome, since with only the skin tissue of four individuals of the species *Boana pugnax*, it has been possible to assemble its de novo transcriptome and get a background of 375 candidates. Which were selected based on the best characteristics of the cationic antimicrobial peptides and that contained the penetrability motif R/KXXR/K. Therefore, we managed to get four peptides that we named pugnins, from one to four. These were synthesized in solid phase and their antimicrobial activity was also tested in two pathogenic strains, both gram-positive and gram-negative, obtaining a growth inhibition of 100% with almost all except

E. coli. For this reason, we performed molecular dynamics to understand the mechanism of action of pugnin with the best result on E.coli, using a membrane model of four POPE: one POPG. We found that the peptide remained stable and rotated on its own axis generating a pore that facilitates the entry of water into the bacteria.

P160 Developing Peptide Analogs for Skin Cancer Treatment

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Nonmelanoma skin cancer, which includes squamous cell carcinoma (SCC), is the most common cancer diagnosed in the United States. Cytoplasmic CDC25A binds to a heterodimeric 14-3-3ε protein and suppresses SCC cell apoptosis. Dimerization of 14-3-3 proteins is central to their function as scaffolding proteins. As such, interference of 14-3-3ε heterodimerization has therapeutic potential for the prevention and treatment of skin cancer. We docked a virtual tetrapeptide library to the heterodimerization domain of 14-3-3ε using Molegro Virtual Docker package 6.0. The top ten poses were submitted to micro second-scale molecular dynamics simulations using the GROMACS package. Two peptides that formed the most stable complexes were synthesized on solid phase using Fmoc-chemistry. The peptide analogs were tested in vitro to evaluate their effect of blocking 14-3-3ε heterodimers on human SCC12B.2 cell survival. Of the two analogs, one had an IC_{50} of 20 μM and the other was not active. The active peptide reduced 14-3-3ε heterodimerization with 14-3-3γ and 14-3-3ζ as determined by using co-immunoprecipitation assay. Additionally, the active analog increased apoptosis in xenografted SCC by 51% when compared to vehicle-treated tumors. These data present a novel, effective means of targeting 14-3-3ε heterodimers in skin cancer.

P161 Ribosomal Formation of Thioamide Bond in Polypeptide Synthesis

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It is well established that the ribosome can catalyze the formation of amide, *N*-methyl-amide and ester bonds during polypeptide synthesis. Here we demonstrate a strategy for the preparation of amino(thio)acyl-tRNA and its use in the ribosome-mediated catalysis of a thioamide bond in an mRNA dependent manner. Both, linear and thioether-cyclized peptides having one thioamide backbone were synthesized by the ribosome in a custom-made cell-free translation system in the combination with flexizymes. Moreover, ribosomal synthesis of an *N*-methyl-thioamide bond was shown for the first time. This method enables us to express a peptide library containing thioamide backbone at designated sites, possibly leading to such ligands against a protein target of interest.

YI-P162 Discovery and Characterization of Monoclonal Antibodies Targeting β-amyloid Trimers

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Alzheimer's disease (AD) is characterized by the progressive accumulation of beta amyloid peptide (Aβ) aggregates. Emerging evidence suggests that soluble, oligomeric forms of Aβ, which are precursors to amyloid fibrils, are key mediators of neurodegeneration in AD. Understanding the structures of these oligomers is critical for elucidating the pathogenesis of the disease, and may lead to better diagnostics and therapies to combat or prevent AD. Here, we generated monoclonal antibodies (mAbs) against a stable, structurally defined Aβ trimer derived from the central and C-terminal regions of Aβ. Using single B cell sorting technology, we isolated a panel of novel mAbs from immunized rabbits as well as human patients with AD. Binding studies of these mAbs demonstrated strong reactivity and specificity for Aβ trimers. Preliminary imaging studies in mouse and human brain tissue suggest these mAbs can detect endogenous Aβ species. Further investigation will explore the potential of these mAbs as chemical probes, diagnostic tools, and disease modifying agents to study AD. These studies will help shed light on the longstanding challenge of correlating the structures of Aβ oligomers with their function in neurodegeneration, and hold promise in aiding the development of AD biomarkers and immunotherapies.

YI-P163 Computational Analysis and Prediction of Insect Neuropeptides by Exploring Informative Features in an Integrative Machine-learning Framework

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One of the potential targets for the pest control is the sensing of insect neuropeptides via their associated receptors. Therefore, computational identification of such neuropeptides from the sequence information is a demanding task in the field of immunoinformatics. Currently, only a few computational methods have been developed to accomplish this task; however, the existing methods have been developed using redundant sequences and also employed basic features to train the model in a simple and coarse-grained manner. In this work, we are the first to construct a high-quality benchmark dataset and comprehensively analyze a wide range of features and assess their individual performances using four different machine-learning algorithms (support vector machine, random forest, extremely randomized tree, and eXtreme gradient boosting tree). Subsequently, we developed an ensemble framework by integrating single method-based models, which further improves the prediction performance and model robustness on the independent dataset. Based on our proposed ensemble model, we further developed a user-friendly online webserver, thereby maximizing convenience for experimental scientists toward insect neuropeptide prediction.

YI-P164 Development of a Green Solid-Phase Peptide Synthesis Protocol

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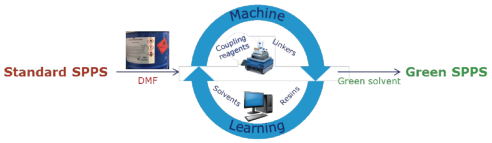
At Novo Nordisk we are striving to reduce our environmental impact and improve the synthesis of our products. In that context, eliminating environmentally problematic solvents used in the production of therapeutic peptides, such as N,N-dimethylformamide (DMF) and dichloromethane (DCM), is a major objective. The European Chemicals Agency (ECHA) has classified DMF, DCM and other solvents as Substances of Very High Concern (SVHCs) and their use is likely to be restricted in the near future. The purpose of this project is to develop new solid-phase peptide synthesis (SPPS) protocols which will be using green solvents instead of DMF and DCM without decreasing the efficiency of the synthesis.

We are applying machine learning algorithms to our work, where we are focusing on the evaluation and optimization of randomized reaction parameters such as dissolution properties of standard SPPS reagents, resin swelling, linker compatibility and coupling cycle efficiency. It is the hope that the machine-learning tool will enable us to process vast amounts of data in order to identify robust, scalable and routinely applicable protocols.

When optimised and greener SPPS procedures are identified, these will be evaluated in head-to-head comparisons with current standard SPPS protocols using challenging peptide sequences.



P165 Concerted Biophysical and Biological Evaluation of Pseudomonas Lipopeptides as a Premise to Unlock their Application Potential

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Cyclic lipodepsipeptides (CLiPs) are secondary metabolites with a broad array of biological functions, mostly produced by Bacillus and Pseudomonas. First recognized for their antimicrobial action, their involvement as natural agents in plant protection has already led to their application in plant biocontrol and crop protection. Scattered literature reports have indicated their anticancer activity as well. With more than 100 CLPs from Pseudomonas (Ps-CLPs) currently reported, a detailed investigation of biological activity reports for 51 Ps-CLPs reveals little tangible information with respect to structure-activity relationships may be extracted from these, given the diversity of assays and assay conditions used.1 We have advocated that a concerted research action should be undertaken if the full potential of the different classes of Ps-CLPs is to be realized.2 We present the results of our steps towards this goal.
A novel and simplified nomenclature scheme is introduced to organize the large diversity of CLPs from Pseudomonas. Using subsequent sequence alignment, all Ps-CLPs can be assigned to 15 different groups, each containing multiple members Using newly determined solution structures from representatives from 7 different groups and 2 available from literature we propose that all Ps-CLPs adopt one of three distinct folds. The added level of organization – 3 fold families rather than 15 Ps-CLPs groups, provides critical guidance in selecting Ps-CLPs for further biophysical and biological evaluation. Screening of a selection of 8 Ps-CLPs against a variety of cancer cell lines as well as permeability tests are used to discuss links between trends in activity and overall amphipathicity.

1 N. Geudens, J. C. Martins, Review in *Frontiers in Microbiology* **2018**, *9*, art. 1867.
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P166 In situ of Oligopeptide Formation for Modification of Hair Shape

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Coating of tri-peptide (KCL) ethyl ester to hair keratin by oligomerization reaction was performed. α-Chymotrypsin was used as catalyst under a controlled pH (8.5-9) and temperature (25ºC-55ºC). The proposed tri-peptide oligomerization was verified by MALDI-TOF spectra. After oligopeptide coating morphological changes of hair keratin was observed qualitatively and quantitatively by picture imaging and by perming efficiency, respectively. Herein we demonstrate an oligopeptide coating which promotes the change of Asian hair shape as alternative to harmful chemicals. Hair damage, health and environment hazards are deeply reduced with this approach, allying low costs associated with enzyme catalysis of peptide synthesis could lead to a sustainable material industry development.

P167 Sequence-specific Folding of Cucurbit[8] Uril-bound Peptides

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In order to achieve supramolecular control of peptide conformation, and with the future aim of host-directed supramolecular peptide assembly, we have endeavored to investigate the interactions of cucurbit[8]uril (CB[8]) with peptides bearing binding motifs at noncontiguous positions. Herein we describe the molecular recognition of peptides by CB[8] through inclusion of noncontiguous residue sidechains accompanied by peptide folding. We present evidence of recognition of N-terminal aromatic residues and Lys or Leu residues of natively unfolded peptides by the hydrophobic CB[8] cavity and concomitant changes in peptide structure. This system is a first step toward the development of ternary supramolecular β-strand complexes, pontentially providing a strategy for the self-assembly of discrete modular miniproteins.

YI-P168 An Octopus-like N-glucosylated Adhesin Peptide-dendrimer: Toward a Selective Apheresis for Personalized Treatment of Multiple Sclerosis

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We recently demonstrated that a set of defined N-Glc moieties (up to 9) in adhesin proteins, expressed by the emerging pathogen non-typeable Haemophilus influenzae, is crucial to recognize high-affinity IgM antibodies in a disease form of multiple sclerosis (MS). This result is the first instance that exposure to infectious agents may be associated with increased MS risk and progression and linked to exogenous, bacterially-derived, antigens mimicking mammalian cell surface glycoconjugates triggering autoimmune responses in MS.[1] With the idea in mind that circulating IgMs are perpetuating a non-self recognition of CNS myelin triggering MS disease activity, we developed a collection of synthetic N-glucosylated adhesin peptides to correlate 3D structure with IgM recognition. Short unstructured N-Glc peptides displaying the highest IgM affinity (IC_{50} 10^{-10}-10¬$^{-8}$) were selected to load onto different polymeric scaffolds to obtain an octopus-like N-Glc-adhesin dendrimer ideally trapping serum IgMs. This challenging task is appearing a promising stratagem to characterize and purifying specific IgMs (an unmet need in chemical immunology) with the final aim to set up a selective apheresis technology for a personalized MS treatment.

[1] M. T. C. Walvoort, C. Testa *et al., Scientific Reports,* **2016**, *6*: 39430.

P169 Template-Driven Peptide Assembly Yields Fluorous Nanoemulsions Capable of Ultrasound-Guided Cytosolic Protein Delivery

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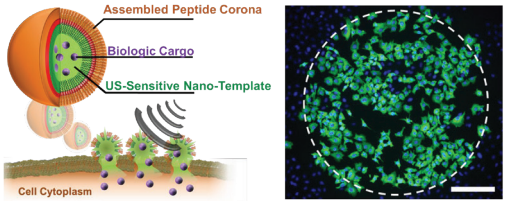
Fluorous nanoemulsions are emergent materials that have enjoyed accelerating clinical and research interest due to their potential as multimodal contrast agents and spatiotemporally controlled delivery devices. However, despite recent progress, challenges remain in controlling their material properties and acoustic activation, as well as overcoming the poor loading efficiency and delivery of biologic cargo from the carrier. Here, we describe the design and synthesis of a new class of phase-changing nanoemulsions prepared via templated peptide assembly, we term a 'nano-peptisome'. Nano-peptisome architecture develops from the spontaneous assembly of *de novo* peptide amphiphiles around an ultrasound (US)-sensitive fluorinated droplet as the template. Utilizing peptide-assembly allows facile particle synthesis, direct incorporation of bioactive sequences displayed from the peptide corona, and the ability to encapsulate biologics during particle preparation using a mild solvent exchange procedure. Nano-peptisome size can be precisely controlled by simply modulating the starting peptide and fluorinated solvent concentrations during synthesis, leading to programmable acoustic properties of the final carrier. Biomacromolecular cargo, including peptides and proteins, present during particle assembly are encapsulated within the particle core and directly delivered to the cytoplasm of cells upon US-mediated rupture of the carrier. Parallel US imaging studies reveal that nano-peptisomes can be tracked and guided using clinically relevant B-mode modalities, while Doppler imaging allows for real-time monitoring of particle activation and rupture in tissue mimetic gels. These results establish nano-peptisomes as a novel theranostic platform capable of image-guided delivery of bioactive macromolecules into cells with spatial and temporal precision.



YI-P170 Variation in the Xaa and Yaa Positions of Collagen Mimetic Peptides Containing aza-Glycine

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Protein folding is carefully regulated through intra- and intermolecular interactions between specific side chains and backbone functional groups of amino acids. Hydrogen bonding, along with a host of other weak interactions, serve to stabilize protein structure. Unnatural modifications to the protein backbone and side chains are commonly used to modulate the properties of proteins. An important class of unnatural peptide modifications are aza-amino acids, which are characterized by substitution of the α-carbon or some other adjacent position with nitrogen. Some of these amino acid analogs exhibit a higher degree of planarity and restricted dihedral angles compared to natural amide linkages, making them valuable tools in peptide mimicry and structural studies. Mimetic peptides are indispensable for studying natural proteins such as collagen, the most abundant protein in mammals. The quaternary structure of collagen involves three individual protein strands intertwining to form a tightly packed triple helical bundle. Collagen's primary structure are repeating (Xaa-Yaa-Gly) triplet sequences. Our group has previously shown the substitution of glycine residues with aza-glycine results in increased triple-helical thermal stability. Herein, we demonstrate the surprising contextual dependence of this increased stability based on the residues adjacent to the point of aza-glycine incorporation.

YI-P171 Determination of the Intracellular Targets of Cationic Amphiphilic Polyproline Helices (CAPHs) in Gram-Positive Bacteria

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Antimicrobial peptides are a class of antibiotics that generally act by targeting the microbial cell membrane, resulting in cell lysis. We have designed and synthesized unnatural, proline-rich peptides, termed cationic amphiphilic polyproline helices (CAPHs). One such CAPH, **P14LRRFI**, is a non-membrane lytic, broad spectrum antimicrobial with efficient mammalian cell penetration. Biotin-NeutrAvidin pulldown and subsequent proteomics analysis revealed its target in Staphylococcus aureus as the 47 kDa glycolytic enzyme, enolase. Further kinetic analysis with the acylated analogue (**P14LRRAc**) demonstrated that the peptide acts as an uncompetitive inhibitor of this target. Applying these same methods to other Gram-positive bacteria, *Streptococcus pneumoniae* and *Bacillus anthracis*, revealed the same enolase target.

YI-P172 At the Crossroad between Integrins and p53:RGD Peptidomimetics to Challenge Glioblastoma Multiforme

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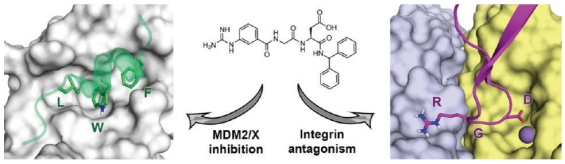
In the fight against Glioblastoma Multiforme (GBM), recent literature data have demonstrated that α5β1 integrin and p53 are convergent pathways in the control of glioma apoptosis. These outcomes prompted us to seek a molecule able to simultaneously modulate both target families. Thus, we present a successful lead optimization campaign of RGD mimics, which led to the discovery of one peptidomimetic acting as a potent MDM2/4 and α5β1/αvβ3 blocker.[1] Additionally, NMR and modeling studies defined the molecular basis of interaction with its targets.



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YI-P173 Organometallic Gold(III) Reagents for Cysteine Arylation

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Cysteine bioconjugation has emerged as a powerful tool that allows for the introduction of a diverse array of substrates to biomolecules through the formation of robust covalent linkages. We have studied a series of organometallic gold(III) complexes that serve as efficient and versatile reagents for chemoselective cysteine arylation of peptides and proteins. The bioconjugation reactions proceed rapidly (<5 min) in a wide pH range (0-14), at ambient temperature, and in low micromolar concentrations. This work will present a series of water- and air-stable, isolable gold(III) organometallic complexes that facilitate the incorporation of a wide range of both organic and inorganic moieties— which include heterocycles, PEG, a fluorescent dye, and an anti-cancer drug— to peptides and proteins. We will present examples of facile cysteine arylation where bioconjugation processes targeting macrocyclic peptides, protein-polymer conjugates, and other hybrid protein-based constructs will be highlighted. This presentation will also describe the spectroscopic and structural characterization of all organometallic complexes in addition to studies relevant to the utilization of the target conjugates for biological applications.

YI-P174 Protein Kinase C Beta II Peptide Inhibitor Elicits Potent Effects on Attenuating Myocardial Ischemia/Reperfusion Injury

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Reperfusion injury contributes to myocardial tissue damage following a heart attack partly due to the generation of reactive oxygen species (ROS) upon cardio-angioplasty. Protein kinase C beta II (PKCβII) inhibition occurring during reperfusion with peptide inhibitor (N-myr-SLNPEWNET; PKCβII-) decreases ROS release and leukocyte infiltration in rat hind-limb and myocardial ischemia/reperfusion (I/R) studies, respectively. However, the role of activating PKCβII during reperfusion is not known. In this study, we hypothesize that myristoylated (myr)-PKCβII- will decrease infarct size and improve post-reperfused cardiac function compared to untreated controls, whereas PKCβII peptide activator (N-myr-SVEIWD; myr-PKCβII+) will show no improvement compared to controls. Isolated perfused male rat hearts were subjected to global I(30min)/R(50min) and infused with myr-PKCβII+ (20μM; n=7), myr-PKCβII- (20μM; n=7) or plasma (control; n=9) during the first 5min of reperfusion. Left ventricular (LV) cardiac function was measured using a pressure transducer. At the end of reperfusion, hearts were frozen (-20oC), sectioned and stained using 1% triphenyltetrazolium chloride to differentiate necrotic (dead) tissue. Infarct size was calculated as percent dead tissue vs. total heart tissue weight. Myr-PKCβII- significantly improved left ventricular end-diastolic pressure to 45±9 mmHg compared to control (66±3; $p<0.01$) and myr-PKCβII+ (58±5; $p<0.05$) Myr-PKCβII- significantly reduced infarct size to 16±3% compared to control (28±4%; $p<0.05$), whereas myr-PKCβII+ (25±3%) showed no difference. The data suggests PKCβII was activated similarly in control and myr-PKCβII+ hearts indicating that myr-PKCβII+ did not exacerbate I/R injury, whereas myr-PKCβII- may be effective to reduce I/R injury when given to heart attack patients during cardio-angioplasty.

P175 Using Designed Electrostatic Interactions to Control Hydrogel-mediated Protein Delivery *in vivo*

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Protein therapeutics have become a vital source of new drugs, but they can't be administered orally and the need for constant systemic injections can complicate treatment. New protein drug delivery modalities could therefore be transformative clinical tools for improving treatment efficacy, patient compliance, and reducing overall costs. Here, we describe a new method for controlling protein release from syringe-injectable peptide hydrogels that can be administered locally to relevant tissue. This approach utilizes a small, ionic fusion domain (pArg-tag) that can be easily installed onto the terminus of a protein during recombinant expression. The pArg-tag can interact with the complementary charge displayed on the surface of the hydrogel fibers and protein release rates can then be controlled by adjusting the number of charged residues within the tag. We first validated this approach in vitro by demonstrating improved protein stability and highly tunable release timelines for model proteins. Molecular dynamics simulations were performed to provide mechanistic insight into the pArg-tag/peptide fiber interaction, which helped create a model for protein release. The pArg-tagged proteins do not affect the mechanical properties of the hydrogel, allowing for the materials to retain their capability for local delivery by syringe injection. Furthermore, we demonstrate that the pArg-tag can be used to control protein release in vivo and that a single administration can slowly release protein from the hydrogel over the course of several weeks. We're currently applying this fusion-tag strategy to improve the delivery of a variety of therapeutic proteins.

YI-P176 Investigating Bacterial Interspecies Communication among Oral *Streptococci*

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Streptococci are believed to be among the earliest commensal colonizers of the oral microflora, maintaining either synergistic or antagonistic interactions with several pathogenic bacteria.[1] These interactions are important because they influence the rate of antibiotic resistance development and pathogenicity of members in the microbial community.[2] *Streptococcus pneumoniae*, an opportunistic pathogen, and Streptococcus mitis are prototypes of commensal bacteria in the mitis group and share >80% of their genes. Both *S. mitis* and *S. pneumoniae* are naturally competent, and competence is regulated by the competence stimulating peptide (CSP)-based quorum-sensing (QS) system.3Our goal is to examine the interspecies interactions between *S. pneumoniae* and *S. mitis* and utilize the CSP of *S.mitis* as a template for the development of inhibitors of the *S. pneumoniae* competence regulon. Most of the strains of *S. pneumoniae* use one of two pheromones, CSP1 or CSP2, that interact with their cognate receptors, ComD1 and ComD2, respectively.3Based on the reported systematic structure-activity-relationship (SAR) analysis of both the CSP1 and CSP2 scaffolds in *S. pneumoniae*,[4] several single, double and triple mutant analogs of *S. mitis*-CSP were chemically synthesized and the ability of these analogs to modulate the competence regulon of *S. pneumoniae* was examined. Understanding the interactions between *S. pneumoniae* and *S. mitis* within the microbiome may thus create new avenues for the development of new treatments for pneumococcal infections.

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YI-P177 Peptide-based Efflux Pump Inhibitors Against Pathogenic Bacteria

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Antibiotic resistant bacteria have acquired multiple mechanisms to evade the lethal effects of current therapeutics. One such mechanism involves membrane-embedded multidrug efflux pumps that can effectively expel an array of substrates, including common antibiotics and disinfectants. Among these pumps, small multidrug resistant (SMR) efflux proteins consist of four transmembrane helices (TMs), with TMs 1-3 comprising the substrate binding pocket, and TM4 containing the binding motif to form the functional antiparallel homodimer. Principal substrates of SMRs are Quaternary Ammonium Compounds (QACs), which include dyes such as ethidium bromide (EtBr) and biocides including benzalkonium chloride (BZK), a commonly used hospital disinfectant. We have designed peptides to target the binding interface of the TM4-TM4 helix-helix interaction to competitively disrupt SMR dimerization within the membrane. In the present work, we show that a peptide corresponding to TM4 of Psmr, the SMR of *Pseudomonas aeruginosa* — an opportunistic pathogen associated with lung infections in cystic fibrosis patients — significantly inhibits Psmr-mediated EtBr efflux. The designed peptide has sequence acetyl-Ala-(Sar)3–LLGIGLIIAGVLV–KKK-NH2, with the conserved 'GG7' heptad motif underlined. When used in combination with sub-lethal concentrations of biocides, the *P. aeruginosa* TM4 peptide was also able to inhibit growth of *E. coli* expressing the *P. aeruginosa* SMR. Additionally, the hydrophobic Psmr TM4 peptide did not induce nonspecific membrane disruption, as monitored through minimal uptake of a membrane impermeable dye. These results show promise for a new approach to tackle the increasing threat of antibiotic resistant bacteria.

PYI-178 Optimizing Cell Penetration and Anti-Bacterial Activity of Cationic Amphiphilic Polyproline Helices by Modifying Unnatural Cationic Residues

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Bacterial pathogens such as Mycobacterium, *Salmonella, and Brucella* have evolved resistance to antibiotics is by establishing replication niches inside mammalian phagocytic macrophages Due to their inability to penetrate cell membranes, many common classes of antibiotics, such as aminoglycosides and beta-lactams show low levels of accumulation inside mammalian cells Recently a designed peptide class based on a repeated unnatural amino acid triad that forms a cationic amphiphilic polyproline helix (CAPH) has been reported.[1,2] CAPHs exhibit clearance of intracellular

bacterial and concentration dependent co-localization within mammalian cells.[1,2] In efforts to optimize the cell penetration and antimicrobial activity of CAPHs we have focused on the structure of the unnatural, cationic amino acid and have made several modifications to the original CAPH, **P14LRR**. Herein we demonstrate that by modifying the length between the proline backbone and the guanadinium moieties, we can modulate the cell penetration and antimicrobial activity of the CAPHs. We will describe the creation of a CAPHs library, focused on the cationic residue, to optimize cell penetration and antimicrobial activity, while minimizing cytotoxicity.

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YI-P179 Expanding the Phosphorylation Code: Endogenous Protein Pyrophosphorylation in Human Cell Lines

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Protein phosphorylation is a key signaling mechanism employed by simple and complex organisms alike. It has been proposed that phosphoserine residues can be further elaborated through additional non-enzymatic phosphorylation to form protein pyrophosphates.[1] High energy metabolites known as the inositol pyrophosphates act as the phosphate donor in this reaction and have been implicated in insulin secretion, cancer progression and telomere lengthening. While protein pyrophosphorylation has previously been observed in a biochemical setting, it has never been detected directly. Here we present a proteomics-based approach to site-specifically characterize endogenous protein pyrophosphorylation. Exploiting our access to pyrophosphorylated peptide standards,[2] a proteomics workflow leveraging SIMAC enrichment and SAX fractionation coupled to data-driven EThcD-fragmentation mass spectrometry[3] was developed. Using this approach, endogenous pyrophosphorylation sites on more than 30 proteins were detected in HEK293T and HCT116 cell lines. These proteins exhibit diverse cellular function and localization, and include HDAC2, HSP90A, Tau (MAPT), and NDPK (NME1). With the direct detection of endogenous pyrophosphorylation sites, biochemical characterization of the function of this modification can now be performed.

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YI-P180 Studies Toward Construction of the Bicyclic Theonellamide Skeleton

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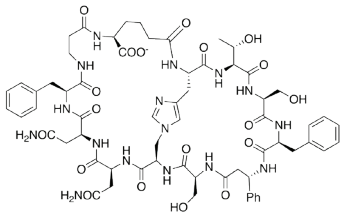
Theonellamides A-G,[1] theonegramide[2] and theopalauamide,[3]isolated from marine sponges of the *Theonella* genus, are a class of novel bicyclic dodecapeptides known to inhibit the growth of prototypical fungi and cancer cell lines. The compounds are characterized by a bridging τ-histidinoalanine (τ-HAL) residue and several other post-translationally modified residues. We have previously reported the synthesis of some key building blocks, *viz.erythro*-hydroxyasparagine,[4]τ-histidinoalanine5 and (2*S*,4*R*)-2- amino-4-hydroxy-adipic acid.[6] In this presentation, our progress toward the controlled assembly of the bicyclic system will be described. Lessons learned in regard to the optimal order of events, protecting group strategy and progress-to-date will be discussed for the assembly of "theonellamide X," a non-natural congener composed of commercially available amino acids.



theonellamide X

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YI-P181 Generation of Bioactive Peptides from Fermented Plant-based Substrates for Food Applications as Natural Preservatives

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Halal food industry has high demand for natural preservatives with broad range of applications. Therefore, generation of bioactive peptides in plant-based substrate for food preservation have high potential for commercialization. In this study, Kenaf seeds were subjected to bio-transformation via lacto-fermentation to generate peptides for food preservation. Seeds were defatted and protein was extracted to produce the Defatted Kenaf Seed Protein (DKSP). DKSP was subsequently fermented using three Lactic Acid Bacteria (LAB) strains for 24, 48 and 72 hours at 37 °C, and the antibacterial activity was evaluated using 96 wells micro-titer plate method. The fermented DKSP was subjected to fractionation and peptide identification using RP-HPLC and LC-MS, respectively. The DKSP fermented for 48 hours by the strain Lactobacillus casei demonstrated the highest antibacterial activity 87%, 100%, 99%, and 97% towards *Salmonella typhimurium* ATCC14028, *Escherichia coli* ATCC11229, *Staphylococcus aureus* ATCC6538, and *Bacillus subtilis* ATCC6633, respectively. The MIC value was 30 mg/mL against all the tested pathogens while the MBC value was in the range of 40 to 60 mg/mL for *S. typhimurium* and *P. aureginosa*, 50 mg/mL for B. subtilis,

40 mg/mL. A total of 15 peptide sequences were identified from fractions 17, 27 and 43 and showed different charge and molecular weight. The findings of this first study demonstrated high potential for the kenaf seed protein fermented using *Lactobacillus casei* as a source of natural preservatives for broad range food applications.

YI-P182 Development of Peptide-Based High-Affinity Vancomycin Inhibitors to Attenuate the Selection of Vancomycin Resistance in Enterococci

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The rapid spread of antibiotic resistance genes and the increasing number of multi-drug resistant (MDR) bacterial pathogens are among the most pressing issues in modern medicine. Last resort antibiotics with the ability to treat these pathogens have been identified, and their use is tightly regulated to prevent the development of resistance. One of these last resort antibiotics, vancomycin, is critical for the treatment of many MDR pathogens. While transferable vancomycin resistance is rare, the commensal bacteria Enterococcus possesses vancomycin resistance genes that are transferable to MDR pathogens such as methicillin-resistant Staphylococcus aureus (MRSA).

Vancomycin resistance occurs when active vancomycin makes its way to the colon, where it persists for weeks or months, decimating the colonic microbiome and allowing for the selection of resistance genes. To prevent the development of resistance it is critical to develop a methodology that allows vancomycin to retain its activity against MDR pathogens, while neutralizing the undesired accumulation of active vancomycin within the colon. Here we demonstrate that modification of the vancomycin L-Lys-D-Ala-D-Ala tripeptide binding site can be used to produce small peptides with enhanced binding affinity to vancomycin, competitively binding vancomycin and reducing its activity against Enterococcus. Several N-terminal modifications have produced compounds with enhanced activity over the native tripeptide, including several analogs that were designed to covalently bind vancomycin, thereby acting as suicide inhibitors.

P183 Computational Design of Folding,Functional Synthetic Heteropolymers using the Rosetta Software Suite

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Proteins possess the remarkable ability to fold spontaneously into precise and intricate three-dimensional structures that are responsible for their functions. The amino acid sequence of a protein uniquely determines its fold, and many examples now exist of successful computational prediction of fold from sequence, and of computational design of sequences producing desired folds. Of the tools available, the Rosetta software suite has earned a reputation for robustness and versatility. In recent years, I have worked to generalize Rosetta's algorithms and architecture, permitting structure prediction and design of folding heteropolymers built from non-natural chemical building-blocks, with folds inaccessible to natural proteins. This has required the development of unbiased sampling methods that do not rely on databases of known structures as templates, and physics-based scoring methods that do not depend on statistics from known structures. I will present examples of folded peptides and polypeptides ranging from 8 to 60 amino acid residues, and incorporating L- and D-amino acids, non-natural side-chains, exotic cross-linkers, and metal ligands as structural components. Included among these will be examples of peptide folds with symmetries involving mirror operations that are inaccessible to natural proteins, designed peptides that undergo changes in conformation in response to a stimulus, peptides engineered to have catalytic activity, and peptides designed to bind to and inhibit target proteins of therapeutic interest. Finally, I will describe ongoing work to harness new computational techniques, such as current machine learning approaches, to improve the robustness reduce the computational expense of Rosetta's peptide design and structure prediction pipeline.

P184 Highly Efficient Synthesis of Pyrrole-Imidazole Amide Sequence for Application to DNA-Binding Polyamides

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Pyrrole-imidazole polyamides (PIPs) are cell-permeable DNA-binding ligands which can be designed to recognize specific base sequences in the minor groove of DNA.[1] The essential components of PIPs are N-methylpyrrole amino acid (Py) and N-methylimidazole amino acid (Im), but the preparation of Py-Im sequence has difficulty due to the coupling of electron-rich electrophile (Py) with electron-poor nucleophile (Im). To overcome this problem, it was necessary to use uncommon starting materials,[2] highly activating reagents,[3] or heating condition in some cases despite of thermal lability of nucleophile.[4] In this time, we have developed highly efficient condensation condition to obtain the Py-Im sequence with appropriate activated ester, amine and solvent. This method can be applied not only to dimer synthesis but also elongation reaction by monomer or polyamide fragments. Eventually, we have manufactured 10-mer of PIP which has the sequence of Py-Im on gram-scale using our manner. We would like to discuss the detail and utility of this method in our poster presentation.

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P185 Novel Unimolecular Amylin-adrenomedullin Dual Agonists

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Obesity is a global health concern due to the disturbing rise in prevalence and extent. With cardiovascular diseases being the lead cause of mortality in patients there is a substantial unmet medical need to develop novel drugs targeting not only obesity, but also obesity associated cardiovascular diseases such as hypertension and atherosclerosis. We hypothesize that combining the cardioprotective effects of adrenomedullin with the anti-obesity and glucoregulatory properties of amylin could be an effective treatment strategy. Here we show novel unimolecular peptide agonists with dual activity at both the

amylin and adrenomedullin receptors. Using SPPS, a library of hybrid peptides was rationally designed by substituting amino acids essential for amylin activity into the native adrenomedullin sequence. The pharmacokinetic profile of the analogues was optimized by N-terminal lipidation with a C20 diacid. The functional activities of the analogues were assessed using a cAMP accumulation assay in cells overexpressing the human amylin receptor subtype 3 (hAMY3-R) or human adrenomedullin receptor subtype 1 (hAM1-R). All peptides were measured to be full agonists on both receptors. The hAMY3R potencies were equivalent to human amylin while potencies on the hAM1-R showed a range from equipotent to 100-fold less potent as compared to human adrenomedullin. Ongoing studies are aimed at addressing the efficacy and potency in rodent models of metabolic diseases. In summary we have designed, synthesized, and evaluated novel hybrid amylin-adrenomedullin peptide agonists with dual activity at the hAMY3R and hAM1-R for the potential treatment of obesity patients with high cardiovascular risk.

YI-P186 Peptide-Based Drugs to Inhibit LDH5, a Potential Target for Cancer Therapy

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Lactate dehydrogenase 5 (LDH5) is overexpressed in many tumours, particularly in those with high metastatic potential[1]. Previous studies using chemical inhibition and RNA silencing technologies showed that LDH5 is involved in tumour initiation, maintenance and proliferation[2]. People carrying LDH5 deficiency do not show a severe phenotype and can have a healthy live, thus LDH5 is a very attractive target for anticancer therapy[3]. Great effort has been done to identify small-molecule drugs that inhibit LDH5 by competing with the substrate for its active site, but this strategy has not identified molecules able to target LDH5 inside cells[4]. Peptides are an attractive alternative to small-molecules to target intracellular protein-protein interactions (PPIs) as they can achieve high specificity due to their larger interface interaction (5), but they also have to be highly stable against proteases and reach the cytosol to inhibit LDH5. We aim to develop a stable peptide with ability to enter inside tumor cells and inhibit LDH5 oligomerization and consequently, its enzymatic activity. To do that, we use cyclic cell penetrating peptides and combine computer-based predictions, high-throughput screening, and rational design to identify sequences with ability to target and inhibit LDH5 inside cells. This approach is novel and will challenge the landscape of drug discovery programs exclusively dedicated to small-molecules that follow the Lipinski's rule of 5.

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P187 Utilizing Peptide Macrocycles for Screening, Sensing and Catalysis

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Chemical Epitope Targeting is a novel technology developed for designing peptide ligands with high affinity and specificity against specific regions of a protein that may be inaccessible to small molecules or antibodies. Operating on the same principles as antibody-antigen interactions, this technique involves chemically synthesizing the region of interest on the protein, called the epitope, as a polypeptide with a biotin detection tag and a strategically placed alkyne or azide presenting amino acid and screening it against a comprehensive library of azide or alkyne containing peptides. We applied this technology to develop a macrocyclic peptide that selectively binds, in vitro, to the phosphorylated Ser474 site of Akt2 (Protein Kinase B) with high affinity. Akt2 is an important oncoprotein that is hyperactivated via phosphorylation at Ser 474 in breast cancer, and so reagents for visualizing Akt2 in live cells would be useful. We discuss how we systematically iterated the chemical structure of the peptide to promote cell penetrating properties, while retaining its ability to selectively bind to phosphorylated Akt2.

In addition to further development of the Chemical Epitope Targeting strategy, the Nag laboratory is interested in design and synthesis of macrocyclic peptide libraries and in exploring sensing and catalytic properties of peptides. Our current research on determining optimal cyclization parameters for a macrocyclic peptide library cyclized on resin using copper (I) catalyzed alkyne-azide cycloaddition reaction has allowed us to determine optimal library sizes. We shall share insights obtained while exploring electrocatalytic properties of metal-peptide complexes and anion-sensing by a cyclic peptide.

P188 Cell-penetrating Peptide-conjugated Boron Therapeutic Agents for Intracellular Target Delivery in Boron Neutron Capture Therapy (BNCT)

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In BNCT, internalization of ^{10}B-boron atom by cancer cells leads to the induction of cell death by generation of alpha particles and recoiling ^{7}Li nuclei with high linear energy transfer and short range when irradiated with neutrons, leading to effective therapeutic benefits on intractable cancer, such as brain tumor[1]. However, insufficient accumulation and cellular uptake efficacy of second-generation boron compounds such as thiododecaborate (BSH) have been pointed out. In this research, we aim to develop BNCT technology using cell-penetrating peptides (CPPs)[2] for enhanced cellular uptake of boron compounds and their controlled locations inside cells.

We designed and synthesized organelle-targeted peptide-conjugated dodecaborate derivatives to increase their cellular uptake and to control the intracellular locations for induction of sophisticated cancer cell-killing activity (including efficacies and mechanisms) under BNCT. For example, boron compounds conjugated with mitochondria target CPP, *RLA* (amino acid sequence: $_D$[RLARLAR]$_2$) [3], showed significantly enhanced cellular uptake efficacy and mitochondrial accumulation of the boron clusters. Once the accumulation of the boron compounds in mitochondria (30 min treatment), the compounds were highly retained even after 24 hrs incubation. In BNCT experiments, we found that *RLA* peptide conjugated dodecaborate showed higher effects of cancer cell-killing activity than that of other CPPs (endosomes and cytosolic release) when irradiated with neutrons *in vitro* BNCT assay.

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YI-P189 pH-Reversible Assembly of Coiled-Coil Peptides into Nanostructures for Drug-Delivery Applications

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The successful application of synthetic biomaterials lies in their ability to mimic naturally occurring biological molecules and systems. The challenge, however, has been to generate materials with hierarchical assemblies down to the atomic level that have precisely tailored chemical heterogeneities and external stimuli-responsiveness. Self-assembling peptides have recently emerged as a potential avenue for the creation of novel biomaterials because they are materials based on natural building blocks. The knowledge about their sequence-structure relationship coupled with the ability to design and synthesize *de novo* peptides has sparked an interest in their use as biomaterials. Here we demonstrate the use of a GCN4 leucine zipper sequence-based coiled-coil trimer that has been radially functionalized with bipyridine moieties to build hierarchical assemblies. These higher-order assemblies, take the form of rectangular nanostructures, the shape and size of which can be controlled with the strategic placement and number of bipyridines along the backbone. More remarkably, their assembly was demonstrated to be reversible at acidic pH over several cycles, without any compromise of their structural integrity.[1] We discovered that the optimal size and pH-sensitivity of these materials render them favorable for reversible binding of therapeutic agents, such as doxorubicin, thus, providing scope for controlled therapy release in low pH environments. The ensuing results of the compatibility and efficacy of these drug transporter materials will be presented.

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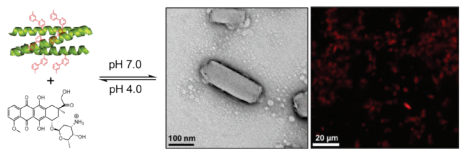

YI-P190 Development of Novel Ring-Opening Reaction of N-Terminal Thiazolidine for Chemical Protein Synthesis

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Native chemical ligation (NCL), which enable chemical protein synthesis, involves chemoselective condensation of N- terminal cysteinyl peptides with peptide thioesters.[1] Sequential NCL protocols have been widely used for the ligation of more than two fragments, allowing for synthesis of large proteins. Middle fragments in sequential protocol must have both the N- terminal cysteine and C-terminal thioester units. However, polymerization and/or cyclization of such middle fragments via intramolecular NCL occur. Therefore, several N-terminal protection strategies have been reported, among which N- terminal thiazolidine derivative has been used widely.[2] Ring- opening reaction of the thiazolidine has been generally performed by methoxylamine or palladium reagent,[3] but there is much room for improvement of the conditions. In this context, we attempted to reexamine the thiazolidine-ring- opening reaction which was already reported to proceed under copper catalyzed Huisgen cycloaddition reaction conditions.[4]

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P191 Lipophilic Permeability Efficiency Reconciles the Opposing Roles of Lipophilicity in Membrane Permeability and Aqueous Solubility

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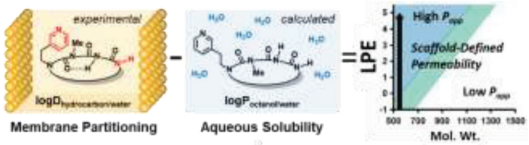

As drug discovery moves increasingly toward previously "undruggable" targets such as protein–protein interactions, lead compounds are becoming larger and more lipophilic. Although increasing lipophilicity can improve membrane permeability, it can also incur serious liabilities, including poor water solubility, increased toxicity, and faster metabolic clearance. Here we introduce a new efficiency metric derived from but not limited to "beyond rule of 5" cyclic peptides, that captures these opposing effects of lipophilicity on molecular properties. Lipophilic permeability efficiency (LPE) scales a simple hydrocarbon partition coefficient experiment to calculated octanol–water partition coefficients, providing a functional assessment of the efficiency with which a compound may achieve passive membrane permeability at a given lipophilicity. We expect this tool to serve the medicinal chemistry community via the following applications: (1) guiding property optimization within a series of related compounds, (2) prioritizing scaffolds in a lead development campaign, (3) defining structure- property

relationships (SPR), and (4) predicting properties in an SAR series or virtual library. Initial results will be reported regarding the application of this technique toward the discovery of cyclic peptide scaffolds capable of permeabilizing highly-polar sidechain elements.

YI-P192 Structure-self-assembly Relationships Study of Islet Amyloid Polypeptide

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The self-assembly of proteins and peptides into amyloid fibrils is associated with different diseases, including type II diabetes (DM-2). Compelling evidence has shown an array of toxic proteospecies spanning from oligomers to mature fibrils, which mediate cell and tissue degeneration. Recently, an increasing number of functional amyloids has also been found, indicating that not all amyloid assemblies are toxic to cells. Given these observations, it is important to better understand the relationship between amyloid structure, the self-assembly mechanism and cytotoxicity. Using islet amyloid polypeptide (IAPP), which deposition as insoluble aggregates correlates with the progression of DM-2, we performed a structure-self-assembly relationship study to delineate the molecular mechanisms of IAPP self-assembly and to better define the conformational nature of the cytotoxic species. Single mutations at asparagine 21, which plays a critical role in IAPP self-assembly, were performed (N21A, N21Aib, N21D, N21isoD, N21Dab, N21G, N21F, N21L, N21n and N21P). We initially observed by thioflavin T (ThT) fluorescence, circular dichroism spectroscopy and transmission electron microscopy that N21A and N21G have the same self-assembly kinetics as WT IAPP. Surprisingly, N21P promoted IAPP self-assembly. Successive substitutions of Asn-21 with hydrophobic residues led to the prompt formation of ThT-negative β-sheet-rich amorphous aggregates with high surface hydrophobicity. N21F worm-like aggregates induced apoptosis and significant cell death. Moreover, cellular assays revealed that the correlation between amyloidogenicity and cytotoxicity could not be easily rationalized according to *in vitro* biophysical studies. This study identifies position 21 as a hinge residue that controls both the amyloidogenicity and the cytotoxicity of IAPP.

PYI-193 A Photolabile Backbone Amide Linker for the Solid-Phase Synthesis of Cyclic and C-Terminally Modified Peptides

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A new photolabile backbone amide linker has been developed for the on-resin synthesis of cyclic and C- terminally modified peptides. The linker, 2-hydroxyl-4-carboxy-6-nitrobenzyl (Hcnb) is stable to strongly acidic conditions and instead releases the completed peptide through photolytic cleavage at 350 nm. Hcnb possesses four degrees of orthogonality and is amenable to the preparation of cyclic peptides, C-terminally modified peptides, and fully protected peptides due to its photolabile backbone amide linkage (**Figure 1**). The Hcnb precursor 2-hydroxyl-4-carboxy-6-nitrobenzaldehyde (Hcna) can be conveniently synthesized in 4 steps from commercially available 4-methyl-3,5-dinitrobenzoic acid. The C-terminal amino acid residue is loaded via reductive amination of Hcna followed by an O→N transacylation for the addition of the second residue in quantitative yields, even when employing sterically bulky residues. Standard Fmoc- or Boc-based synthesis can then be utilized to complete the desired peptide. We have shown that Hcnb can be used for the linear synthesis and subsequent on-resin cyclization of various cyclic peptides of interest, as well as synthesis of C-terminal thioesters on-resin.

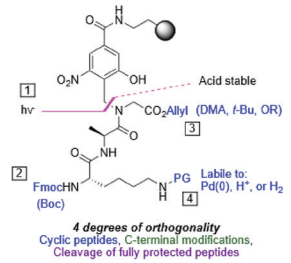

Figure 1: 4 degrees of orthogonality for Hcnb linker

YI-P194 Tailoring the Self-assembly of a Tripeptide for the Formation of Antimicrobial Surfaces

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Bacteria settling on surfaces are currently one of the greatest concerns for the supply of proper health, water and energy. Bacterial accumulation on medical devices and implants, impair their function and can lead to severe infections and even death. Materials addressing this phenomenon are called antifouling materials. Different materials have been developed in the last 50 years, however, no optimal solution has yet to be found.

Here, we describe the self-assembly of a short peptide into two different types of supramolecular structures, depending on the pH of the solution. These particles are designed to reduce bacterial adhesion and at the same time release biocidal compounds. By using NMR and molecular dynamics (MD), we determined the structures of the peptide monomers and showed the forces directing the self-assembly of each structure under different conditions.

When adhered to a surface, the peptide particles modify its chemical and physical characteristics and confer it with the ability to resist biofouling. The inclusion of biocidal compounds (e.g. antibiotic, enzyme and anticancer drug) in the particles resulted in an improved antimicrobial activity of the surface. This approach and the detailed understanding of the processes are relevant for developing new sterile surfaces for health-care systems, water purification devices, food packaging or any environment that suffers from biocontamination.

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YI-P195 The Development of Conformationally Specific Peptides Targeting Huntigtin Exon-1 Fibrils

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease with multiple symptoms including abnormal involuntary movements and psychiatric disturbances. This orphan disease can be fatal and currently there is no effective treatment. HD is caused by aggregation of mutant huntigtin proteins containing polyglutamine (polyQ) expansions in exon 1. It has been shown that the aggregation process occurs in a step-wise manner resulting in several misfolded species. However, there is a limitation in current biomarker tools to detect these different misfolded species. We used mRNA display to develop peptides selectively binding to fibrils of the exon-1fragment of huntingtin (Httex1), which could be used as a diagnostic tool by monitoring Httex1 fibrils in cells or brain tissues. Several peptide binders were identified from a library of over one trillion unique ligands through numerous rounds of *in vitro* selection. Among them, we characterized two peptide binders in detail. We hope that these peptides will help us better understand the mechanism of the disease. Further development of these peptides could potentially lead to therapeutic molecules that prevent the misfolding and aggregation of proteins.

P196 Development of Peptide Foldamers Changing their Secondary Structures in Response to the Environmental Milieu

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α,α-Disubstituted α-amino acids (dAAs) are promising tools for design of peptide foldamers. Peptides containing cyclic dAAs are likely to adopt a helical structure. In contrast, peptides composed of acyclic dAAs with two bulky substituents equal or larger than ethyl groups are likely to form an extended planar C5 conformation. Accordingly, changes in the side chain structure of dAAs from a cyclic to an acyclic structure in peptides may lead to changes in the peptide secondary structures from a helical to a planar or random structure. In this study, we designed cyclic dAAs possessing a cyclic acetal or a disulfide bond in the side chain. A cyclic acetal is hydrolyzed by an acidic treatment and gives an acyclic diol. A disulfide bond is cleaved in response to the reductive milieu and generates two thiol groups. We incorporated the cyclic dAAs into L-leucine sequences and studied their secondary structural changes in response to the acidic or reductive milieu.

YI-P197 Inhibition of Myostatin Activity by Peptide-photooxygenation Catalyst Conjugate

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Myostatin belongs to the transforming growth factor-β (TGF-β) superfamily and negatively regulates skeletal muscle growth. Therefore, inhibition of the myostatin activity can be a treatment for amyotrophic diseases including muscular dystrophy, cancer cachexia, sarcopenia and miscarriage muscular atrophy.

Oxygenation is one of promising approaches to the inactivation of protein / peptide function because it could alter the conformation of proteins and peptides.

In the present study, a conjugate of a photo-oxidation catalyst[1] to a myostatin-binding peptide[2] was synthesized and evaluated its oxygenation activity. As a result, the conjugate selectively oxygenated amino acid residues of myostatin under light irradiation, resulting in the dysfunction of myostatin.

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P198 Cost Efficient Peptide Purification via ZEOsphere DRP Mixed-Mode Chromatography

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ZEOsphere DRP are novel ion exchange / Reversed Phase Mixed-Mode (HP(LC) stationary phases for the purification of especial peptides, insulins (analogues), oligonucleotides and other charged molecules. Utilizing both ion exchange and reversed phase ligands, a substantial increase in selectivity can be observed. Retention can easily be adjusted by changing organic modifier (changing thedielectric constant) or buffer salt concentrations. As a replacement for standard silica based reversed phase and or ion-exchange materials, this novel type of Mixed-Mode material has shown clear yield increases and substantial decreases in downstream production process costs.

YI-P199 SAR Study of Negamycin Derivative Focused on the 3-amino Position for the Potent Readthrough Activity

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10-20% of hereditary diseases such as Duchenne muscular dystrophy (DMD) are attributed to nonsense mutations, which encode premature termination codons (PTCs). One promising approach against these diseases is a readthrough strategy, which is comprised of drugs that allow the translational machinery to skip PTCs, resulting in production of functional protein. (+)-Negamycin (1), a dipeptide-like antibiotic containing a hydrazide structure, exhibits readthrough activity and restores dystrophin expression in DMD model mice[1]. We performed the structure-activity relationship (SAR) study based on 1 and discovered TCP-112 (2) with a higher cell-based readthrough activity[2-3] than 1. In this study, we demonstrated a new SAR study focused on its 3-amino group to develop more potent compounds. As a result, by conducting the introduction of several acyl units onto the 3-amino group, we successfully obtained more potent derivative TCP-1109 (3). The readthrough activity of 3 was about ten times stronger than that of 1 and also stronger than that of G418, a reported most potent natural readthrough product. Moreover, 3 showed a significant cell-based readthrough activity against several PTC-containing DNA sequences derived from the nonsense diseases. These results suggest that 3 would be a useful drug candidate for the treatment of a variety of diseases caused by nonsense mutation.

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YI-P200 Development of Broad Spectrum α/β-Peptide Inhibitors of Viral Fusion

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Human parainfluenza virus type 3 (HPIV3) and respiratory syncytial virus (RSV) are the major causes of lower respiratory infections in children worldwide, leading to millions of hospital admissions and tens of thousands of deaths each year. Despite the demand for therapeutics to combat these pathogens, few effective treatment options are clinically available. Viral fusion has emerged as a compelling target for the development of novel antiviral therapeutics. Fusion by each of these viruses is mediated by a fusion (F) glycoprotein. We have previously developed a peptide (VIQKI) derived from the C-terminal heptad repeat (HRC) domain of HPIV3 F that potently inhibits infection by both HPIV3 and RSV. Despite the conserved fusion mechanism, F proteins possess distinct primary sequences; therefore, the ability of a single peptide to disrupt the fusion machinery within multiple viruses is highly intriguing. We have adopted a three-pronged approach to develop viral fusion inhibitors with increased efficacy and enhanced resistance to enzymatic proteolysis. First, to identify the interactions that underlie its broad-spectrum activity, we co-crystallized VIQKI bound to its viral targets within HPIV3 and RSV F. Both structures possess six-helix bundles that reveal the overall peptide secondary structure as well as the important sidechain and backbone interactions that make up the binding interface. Second, we used structure-guided design principles to systematically incorporate β-amino acid residues into the inhibitor sequence to create foldamers that structurally and functionally mimic VIQKI. These peptidomimetics maintain the potent activity against viral fusion but exhibit increased resistance to enzymatic proteolysis. Finally, to increase the potency of the inhibitors, we created peptide–cholesterol conjugates. The lipid functions by targeting the inhibitors to their site of action at the outer membrane. The resulting cholesterol-conjugated α/β-peptides are highly effective, protease resistant inhibitors of HPIV3 and RSV infection.

YI-P201 Nanostructure and Elemental Composition Control for Synthesis of Gold-Titania Photocatalysts Using DNAs and Designed Peptides

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Bio-mineralization, the precipitation of various inorganic compounds in biological systems, can be regulated in size, morphology and crystal structure by proteins and peptides. The mineralization control at nanometer level would offer manufacturing more complicated inorganic hybrid nanostructures. We partially succeeded in control of mineralization in nano meter level using DNAs and designed peptides in the previous study[1,2]. In this study, we demonstrated a more complicated site-specific precipitation of titania and gold using DNAs and two designed peptides in order to obtain gold-titania photocatalyst with visible light excitation. We performed micro-scale observation such as AFM and TEM for the photocatalyst. In addition, we conducted macro-scale observation such as DLS, ICP-AES and UV-VIS-IR DRS. These results suggested that the site-specific precipitation of titania and gold on DNA was successfully demonstrated. Additionally, we attempted to control the nanostructure and elemental composition of gold-titania photocatalysts by changing lengths of double strand DNA, which were binding region of gold precipitating peptide with acridine. TEM images and ICP-AES measurements showed that gold nanostructure and its elemental composition of long DNA (80 bp) were larger than short DNA (30 bp). Furthermore, we evaluated their photocatalytic activity under visible light irradiation using methylene blue. As a result, their nanocatalysts had visible light excitation characteristic. Our multiple site-specific precipitation method thus represents a powerful and fundamental tool for use in nanotechnology and biotechnology.

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YI-P202 Investigating the Effect of Alpha-synuclein Post-translational Modifications on Synaptic Vesicle Trafficking

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Alpha-synuclein (α-syn) is an intrinsically disordered protein that mediates synaptic vesicle trafficking. Abnormalities in α-syn's interactions with lipid vesicles and membranes lead to the formation of non-functional, misfolded fibrils, which are implicated in Parkinson's disease. A variety of post-translational modifications (PTMs) – covalent modifications made to proteins mainly through enzymatic processes – regulate protein activity, structure, and cellular localization. Many PTMs have been identified and studied in α-syn, the most common of which

is phosphorylation. A variety of other modifications such as nitration, arginylation, and N-terminal acetylation also affect the structure and behavior of α-syn and its interactions with other biomolecules. For example, the phosphorylation of a tyrosine in α-syn is throught to alter α-syn's helical conformation state when bound to lipid vesicles, while arginylation of glutamate residues in α-syn may protect against fibril formation. We combine protein semi-synthesis and biophysical techniques to determine the effect of various PTMs on the ability of α-syn to mediate vesicle fusion. Through peptide synthesis, recombinant protein expression with unnatural amino acids, and native chemical ligation, we introduce PTMs of interest and fluorophores into α-syn. We then determine changes in its binding affinity for lipid vesicles through Fluorescence Correlation Spectroscopy and validate the structural changes induced by the PTMs via single molecule FRET. We examine the effects of these structural and interactional changes on protein function by assaying the fusion of fluorescently labeled vesicles mediated by modified α-syn. Our study provides insight into the impact of PTMs on protein structure, function, and potential contribution to disease.

YI-P203 Physical and Chemical Perturbants of Aggregation in Amyloidogenic Proteins

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder with no clinically approved therapy to treat, arrest or delay its progression. Toxic aggregation of amyloid-β (Aβ) peptide and tau protein into plaques and neurofibrillary tangles (NFTs), respectively, have been identified as the key pathological hallmarks of AD. Aβ and tau thus stand as direct therapeutic targets to combat the disease. This present study reports a hybrid approach utilizing physical (electric/magnetic fields) as well as chemical (peptides) perturbants for impeding the aggregation of core peptide segments of Aβ peptide (Ac-KLVFFAE-am) and tau protein (Ac-VQIVYK-am and Ac-VQIINK-am).

In this study, we employ a series of biophysical assays like thioflavin T fluorescence assay, static and dynamic light scattering, tyrosine fluorescence assay, electron microscopy, infrared spectroscopy as well as cell-viability assays, to confirm the efficacy of our hybrid aggregation modulators. Our experimental results show that these physical as well as chemical perturbants could not only decelerate the self-assembly of the chosen aggregating peptide systems but also lower the cellular toxicity instigated by them in neuronal cells. The results obtained using field as an aggregation modulator corroborates well with the previously reported experimental (References 1-3 and MD (References 3,5) results. We propose further assessment of our physical and chemical modulators as therapeutic option for AD treatment.
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YI-P204 Insights into the Mechanism of Antimicrobial Activity of Seven-Residue Peptides

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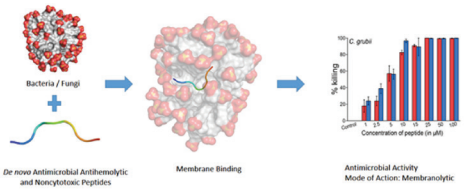
Antimicrobial peptides have gained widespread attention as an alternative to the conventional antibiotics for combating microbial infections. Here, we report a detailed structure–function correlation of two nontoxic, nonhemolytic, and salt-tolerant de novo designed seven-residue leucine–lysine-based peptides, $NH_2LKWLKKLCONH_2$ (P4) and $NH_2LRWLRRLCONH_2$ (P5), with strong antimicrobial and antifungal activity. Biological experiments, low- and high resolution spectroscopic techniques in conjunction with molecular dynamics simulation studies, could establish the structure–function correlation. The peptides are unstructured both in water and in bacterial membrane mimicking environment, suggesting that the secondary structure does not play a major role in their activity. Our studies could justify the probable membranolytic mode of action for killing the pathogens. Attempts to understand the mode of action of these small AMPs is fundamental in the rational design of more potential therapeutic molecules beyond serendipity in the future.



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YI-P205 Novel Linker for Fmoc Solid-phase Synthesis of Peptide Thioesters, Hydrazines and Phenyl Esters by Amide to Imide Intramolecular Activation

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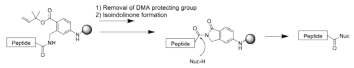
Facile formation of different C-terminal peptides like for e.g. peptide thioesters, hydrazines, phenyl esters, aldehydes etc. by Fmoc solid phase peptide synthesis is a prominent field of peptide chemistry. Especially peptide thioesters and hydrazides are of great interest, because of their direct involvement in native chemical ligation (NCL) that allows for a formation of proteins or long peptide sequence by assembling two or more peptide fragments. Unfortunately, the thioesters are not compatible with Fmoc chemistry due to iterative use of piperidine during deprotection. In order to create those peptide

thioesters using Fmoc SPPS one needs a special strategy to either mask the thioester moiety during synthesis or introduce it after peptide chain elongation steps. One of the examples of a successful linkers that allow for peptide thioesters formation is the Dawson's Nbz linker, which follows similar activation pattern as our linker[1]. On the other hand peptide hydrazides and phenyl esters are utilized as methods for chemical synthesis of proteins that are complementary to NCL[2,3]. Moreover, peptide phenyl esters are essential in His-Tag acylation[4].

Herein we report development of novel linker that enables for selective amide to imide intramolecular activation that follows with formation of isoindolinone moiety. This moiety can be displaced with wide array of nucleophiles to form corresponding peptide derivatives.



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YI-P206 Complete Synthesis of Mutacin 1140 Analog on Solid Phase Peptide Synthesis

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Lantibiotics are a unique class of antibiotics that are derived from ribosomal synthesis, followed by post-translational modifications, and which contain unusual amino acids such as 2,3-didehydroalanine (Dha), 2,3-didehydrobutyrine (Dhb), lanthionine and aminovinyl-D-cysteine (AviCys). These compounds have attracted numerous scientists' attention for their broad spectrum of antimicrobial activity and unique chemical functionalities. Mutacin 1140 (MU1140) is a naturally occurring lantibiotic from the Gram-positive bacterium *Streptococcus mutans*. Here, we report the complete solid phase peptide synthesis of a MU1140 analog, MU1140[Dha5Ala, AvisCys22βMEA], using orthogonally protected lanthionine building blocks. The building blocks were synthesized via lactone and aziridine ring openings with cysteines. The peptide building blocks were cyclized on-resin to form macro-rings followed by their coupling to achieve the synthetic analog. The antimicrobial activity of the analog was confirmed by minimum inhibitory concentration (MIC) testing on *Staphylococcus aureus*. It is anticipated that this synthetic approach can be used to synthesize a wide array of MU1140 analogs, and will open up new pathways to diverse therapeutic targets.

P207 Fibronectin and Integrin-Targeted Peptide Anticancer Drug Conjugates for Prostate Cancer

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Prostate Cancer (PCa) is the most common malignancy in men and is the leading cause of cancer-related male mortality. A ligand CTVRTSADC was found to target extra domain-B of fibronectin (EDB-Fn) in the extracellular matrix that can differentiate aggressive PCa from benign prostatic hyperplasia. Therefore, this ligand could be used for selective targeting of anticancer drug to PCa. Hence, we hypothesized that the EDB-Fn targeted peptide conjugates with anticancer drug via a hydrolyzable linker will provide selective cytotoxicity to the cancer cells. To test our hypothesis, we selected both the normal prostate cell lines RWPE-1 and cancerous forms of prostate cell lines PC-3, DU-145, Ln-Cap, and C4-2 to evaluate anticancer activity of synthesized peptide-drug conjugates and their corresponding physical mixtures. The peptide-drug conjugate (5 and 10) was found non-toxic to the RWPE-1 as compared to the drug. The cytotoxicity of synthesized peptide-drug conjugate and drug on normal and prostate cancer cells were found less cytotoxic on RWPE-1 as compared to drug alone while it is a little more cytotoxic on cancerous forms. For example, peptide-doxorubicin (Dox) conjugate were less cytotoxic (32% viability) as compared to Dox alone (20% viability at 72 h) on RWPE-1. The physical mixture of Dox and peptide also showed decrease in toxicity on normal cell by 8% without TGF-ß and 27% with overexpression, and enhancement of toxicity on PC3, Ln-Cap, and C4-2 cancer cells as compared to Dox in 72 h by 4.21~8.58% without TGF-ß, 5.55~11.45% with overexpression of TGF-ß. Similarly, the physical mixture of Docetaxel (Doce) and peptide physical mixture showed decrease in toxicity by 10% for RWPE-1, and increased in toxicity on both the PC3 and LNCaP cells by 2.9% and 12.8%, respectively. However, Doce conjugate does not shows improved cytotoxicity in the above-mentioned cancer cells with and without TGF-β treatment, after 72 h incubation.

P208 Lipidated, N-Terminal-to-Side-Chain Cyclic Analogues of PYY: An Approach Towards Half-Life Extension

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Peptide Tyrosine-Tyrosine (PYY) is a satiety-inducing peptide that is released from L-cells in the distal small intestine and colon in response to feeding. Initially released as a 36-mer peptide, PYY_{1-36} is rapidly cleaved in vivo by DPP4 to PYY_{3-36}, which is the main circulating form of the peptide. Whereas PYY_{1-36} interacts with various NPY receptor subtypes (Y1, Y2, Y5) with little selectivity; PYY_{3-36} is more selective for the Y2 receptor, activation of which leads to appetite suppression and inhibition of gastric motility. However, PYY_{3-36} undergoes rapid systemic elimination, making it unsuitable as a therapeutic agent.

In an effort to develop a selective Y2 receptor agonist with a therapeutically useful pharmacokinetic profile, we have investigated a series of N-terminus-to-side-chain-cyclic PYY_{3-36} analogues ("NTSC-PYY_{3-36}"), incorporating a cyclization motif designed to confer increased tertiary structural stability. A reduced amide linkage was also introduced at a key C-terminal proteolytic site to enhance metabolic stability, while lipidation was explored as a half-life extension strategy. The results of these investigations will be presented

P209 Benzylthiols as Scavengers in TFA Cleavages of Peptide Resins

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Aliphatic thiols such as EDT,[1] DTT[2] and DODT[3] are used as efficient scavengers in TFA cleavages of peptide resins. Nevertheless, these thiols are often malodorous, can form byproducts by reacting with peptides[4] and, due to the lack of chromophores, these scavengers can form impurities that can be difficult to detect by UV during downstream processing. On the other hand, aromatic thiols[5] are often less odorous and easier to detect. However, they are also less nucleophilic than aliphatic thiols and thereby less effective as scavengers. Herein we introduce benzylthiols as scavengers in peptide resin cleavages. These combine the high reactivity of aliphatic thiols with the UV visibility of aromatic compounds. We assess a series of benzylthiols in model peptide resin cleavages, evaluate their physicochemical properties and identify efficient scavengers suitable for manufacturing of therapeutic peptides fulfilling the increasing impurity requirements,[6] an example of which is 1,4-benzenedimethanethiol (1,4-BDMT), cas nr. 105-09-9.

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YI-P210 Selenoprotein Expression in Mammalian Cells through Genetic Code Expansion

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Proteins that contain the amino acid selenocysteine are members of the selenoprotein family. The 25-member human selenoprotein family includes redox enzymes such as glutathione peroxidases and thioredoxin reductases. Unlike cysteine, selenocysteine is less likely to be irreversibly oxidized, thereby protecting selenoproteins from oxidative damage. The mechanisms and functions of a number of selenoproteins are poorly understood, in part due to the difficulty in producing recombinant selenoproteins for biochemical and cell biological assays. Endogenous mammalian selenoproteins are produced through a non-canonical protein translation mechanism that requires suppression of the UGA stop codon and a selenocysteine insertion sequence element (SECIS) in the 3' untranslated region of the mRNA. Attempts to overexpress or produce mutated selenoproteins suffer from low efficiency of the endogenous selenocysteine incorporation process. We have utilized genetic code expansion technology in order to site-specifically incorporate a photocaged selenocysteine into proteins expressed in mammalian cells, bypassing the endogenous selenocysteine incorporation mechanism. Proteins containing the photocaged selenocysteine can then be uncaged via UV irradiation, in cells or in vitro, generating a selenoprotein. We have utilized this technology to produce both selenocysteine-containing GFP and the native selenoprotein methionine-R-sulfoxide reductase B1 (MsrB1). In addition to the expression of selenoproteins, this technology will allow for temporal control of selenoprotein function via UV-dependent uncaging. We anticipate that this technology will enable us to further interrogate the catalytic mechanisms and cellular functions of a number of eukaryotic selenoproteins as well as generate non-natural selenoproteins.

P211 Toward Theranostic Imaging Agents: One-step 18F-labeling of Peptides that are Modified with DOTA Conjugates for Chelating Radiotherapeutic Nucleides

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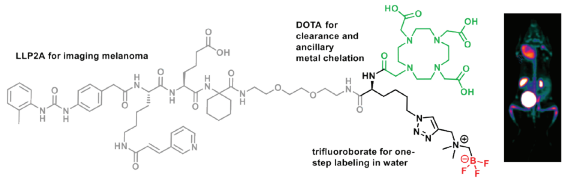
Molecular imaging is at the forefront of translational medicine with new developments in theranostic and multi-modal imaging applications on the horizon. Of the various imaging modalities, positron emission tomography (PET) is increasingly used for pre-clinical target validation and clinical cancer diagnosis due to its high sensitivity and dynamic spatio-temporal resolution. DOTA is a mainstay radioprosthetic group for use in radiometal chelation. However, in the absence of a *radiometal*, DOTA's role in favoring renal clearance represents an unexplored approach for ^{18}F-labeled tracers. To show the benefits of using a DOTA moiety to favor renal clearance, we chose to image the peptide LLP2A[1,2] that recognizes the transmembrane protein very-late antigen 4 (VLA-4) that is overexpressed by many cancers. Our work on kinetically stable organotrifluoroborates now enables a breakthrough one-step ^{18}F-labeling method for labeling nearly any peptide[3,4]. Interestingly, in the first-ever ^{18}F-labeling of the LLP2A peptide, [^{18}F]RBF_3. PEG_2-LLP2A derivatives gave low tumor uptake values and significant GI tract accumulation5. Hence, we designed a new RBF3-LLP2A bioconjugate with an appended DOTA moiety, which increased tumor uptake nearly 3-fold and dramatically reduced GI accumulation by more than 10-fold. [^{18}F]DOTA-$AMBF_3$-PEG2-LLP2A represents a promising VLA-4 radiotracer and provides key evidence as to how a DOTA appendage can significantly reduce GI-uptake in favor of urinary excretion. Moreover, this work portends the

development of the ultimate in theranostic agents based on dual-isotope "hot-cold/cold-hot" theranostics (e.g. ^{18}F/natLu and ^{nat}F/^{177}Lu), which could be labeled in a manner such that ^{18}F is used for PET imaging with a nonradioactive metal, whilst in a second application, the radiometal are used for targeted therapy. Time permitting, a summary of these methods as applied to numerous other peptides, dendrimer cores, and urea-based ligands for prostate cancer, along with correlated images, will be presented.



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YI-P212 Uncovering the Effects of Macrocyclic Tetrapeptides on the c- Myc Degradation Pathway in Prostate Cancer Cells

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Prostate cancer is the second leading cause of cancer-related deaths among men,[1] and most patients who receive the standard androgen deprivation therapy will develop recurrent castration resistant tumors within 12-33 months after this treatment.[2] The oncoprotein c-Myc is a transcription factor found to be overexpressed in prostate cancer that works independently from the androgen receptor.[3] We have demonstrated that the macrocyclic tetrapeptide natural product CJ- 15,208 (*cyclo*[Phe-D-Pro-Phe-Trp]) and its D-Trp isomer decrease the levels of c-Myc protein and inhibit cell proliferation in PC-3 prostate cancer cells.[4] We have screened over 50 analogues of our lead compounds for enhanced anti-proliferative activity and have identified compounds with higher activity than the leads. The effects of these compounds on proteins in the c-Myc degradation pathway will be presented. These compounds are promising leads for prostate cancer since their mechanism of action appears to be independent of the androgen receptor pathway. This work was supported by the Defense Health Program, through the Prostate Cancer Research program under award. W81XWH-14-1-0330.

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YI-P213 Gramicidin S as a Multipurpose Ligation Platform

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As it becomes more apparent that tumor cells downregulate receptors during treatment, the need to target multiple receptors increases.[1] However, creating such constructs is a difficult task. Here, we create a platform to which different VHH antibodies are attached with relative ease using bio-orthogonal ligation methods. VHH antibodies are single-domain antibodies engineered from camelids with a molecular weight of only 12-15 kDa. The VHH antibodies are engineered with a C-terminal cysteine to install a ligation handle complementary to that installed on the platform. Bio-orthogonal ligation methods are selected to install the VHH antibodies to the platform as it minimizes work-up and purification. Gramicidin S was chosen as the platform because it has a rigid secondary structure, is proteolytically stable and amenable to the chemical synthesis and modification. Using an orthogonal protection strategy and a novel side-chain anchoring method, Gramicidin S is synthesized on resin to allow for a facile functionalization with various ligation handles. Attachment of different antibodies to the Gramicidin S platform via bio-orthogonal ligation proved feasible.

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YI-P214 Thiol-ene Click Ligation as an Efficient Approach for Peptide Macrocyclization

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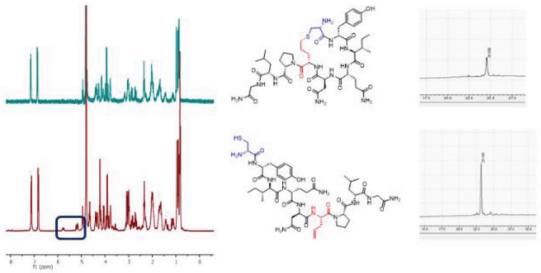

Figure 1. Schematic overview of Oxytocin macrocyclazation.

Interest in developing stable peptide analogues as therapeutics has grown tremendously in the pharmaceutical sector over the last decade. To date, peptide stapling has been demonstrated to be an efficient synthetic approach in addressing the limitations of linear peptides resulting in more stable molecules.[1] Peptide stapling techniques are based on different macrocyclazation chemistries, such as disulfide and thioester formation, lactamization, ring-closing metathesis and

cycloadditions, which were extensively investigated and applied to the synthesis of several cyclic peptides.[2,3] With the aim of promoting rapid and efficient synthesis of peptide macrocycles, a number of "*click reactions*" were also taken into account.[4] Thiol-ene 'click' (TEC) chemistry is a radical mediated addition of a thiol to an alkene. Herein we report a novel, fast and high-yielding synthetic method based on radical TEC ligation to generate Oxytocin thio- ether analogues of the natural substrate.

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YI-P215 The Importance of Cell Membrane Composition in the Activity of Cell Penetrating Peptides Designed to Target Intracellular Protein-protein Interactions in Cancer Cells

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Inhibition of cytosolic protein-protein interactions (PPIs) involved in tumor development is an interesting strategy to develop specific anticancer drugs. Notably, MDM2:p53 and MDMX:p53 interactions have obtained lots of attention as potential target due to their role in different cancers, including some forms of sarcoma, breast and skin cancer. In healthy cells, MDM2 and MDMX inhibit and regulate the activity of the tumor supressor p53, but in some cancers these inhibitors are overexpressed and inactivate p53. cHLH-p53-R, is a promising cyclic helix-loop-helix peptide targeting p53:MDM2 interactions previously reported to bind to MDM2 in cancer cells and trigger apoptosis. The aim of the current study is to elucidate the mode of action of cHLH-p53-R to rationally design improved peptides. We developed a panel of analogues and examined their activity using bioassays, microscopy and biophysical methodologies. Our results show that cHLH-p53-R and analogues have high ability to inhibit p53:MDM2/X interactions in vitro, but the p53 pathway was not reactivated in reporter cell lines. The peptides demonstrated toxicity to a broad range of cells, including healthy cells and mutant p53 cells. Nevertheless, the peptides are more toxic towards cancer cells compared to healthy cells. Studies with model membrane suggested that the lipid composition of cell membranes modulate the toxicity and preference for cancer cells. Atomic force microscopy studies demonstrate that cHLH-p53-R disturb the cell membrane, possibly leading cells to necrosis. These results will guide rational design to improve this scaffold, decrease its unwanted toxicity to healthy cells and improve its intracellular activity.

YI-P216 Short Cyclic Peptide with the Ability to Perform a Synthetase-like Reaction

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We think of enzymes as about long, complex amino acid chains whose scaffold supports the formation of an active site able to perform a very specific action on a very specific substrate. The secondary, tertiary and quaternary structures play roles in activity regulation, specificity and enzymes destination. In light of evolution, however, these features of nowadays enzymes must have been developed from prototypical, less complex and probably much shorter structures such as peptides. Such a reductionist approach assumes the existence of so-called minimal enzymes — catalytic molecules made of short peptides (and plausibly short nucleic acids as well) reduced to bare enzymatic/catalytic activity.

Short peptidic catalysts are already known, Serine-Histidine dipeptide, for instance, is probably the most famous minimal enzyme. It performs reaction characteristic to serine hydrolases like trypsin, chymotrypsin and in the right environment is able to produce peptides out of amino acid. Serine-Histidine is a minimal hydrolase.

We have designed magnesium depended, six amino acids long, head to tail cyclic peptide able to phosphorylate itself using energy delivered from pyrophosphate hydrolysis and subsequently extend its own structure using nucleophile — leucine amide, from the environment to substitute for phosphoryl moiety. This catalytic, cyclic hexapeptide in light of this approach would be a minimal synthetase.

The existence of such nonspecific, short catalysts in early Earth conditions could provide a missing link between living structures and non-living matter by securing the production of molecules, which till now were assumed to be produced only by living organisms.

P217 Synthetic Immunotherapeutics against Bacterial Pathogens

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While traditional drug discovery continues to be an important platform for the search of new antibiotics, alternative approaches should also be pursued to complement these efforts. Towards these aims, the Pires laboratory has developed various strategies that use synthetic immunotherapeutics against bacterial pathogens. We designed a class of molecules that decorate bacterial cell surfaces with the goal of re-engaging components of the immune system towards pathogenic bacteria. By enlisting the immune system, these agents have the potential to pave the way for a novel antimicrobial modality by directly targeting pathogenic bacteria and potentiating existing FDA-approved antibiotics.

YI-P218 Peptide Mimicry with 4- and 5-substituted *N*-aminoimidazol-2-one Turn Surrogates

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Peptide turn mimicry is extensively studied due to their importance in biological recognition.[1] N-Aminoimidazolone (Nai) residues have been shown by X-ray analyses to adopt the central residues of type II β and γ turns in model peptides (Figure).[2,3]

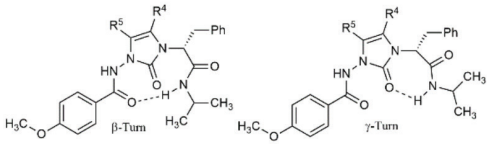

Figure 1: Nai residue at *i* + 1 and i positions of β- and γ-turn models

Synthetic strategies will be reported to make Nai residues with various 5-position substituents. For example, 5-aryl groups were introduced on the Nai ring by Pd-catalyzed cross-coupling reactions, and shown by computational analysis to adopt constrained χ dihedral angle geometry characteristic of the natural *gauche*-(-) conformation.[4] Alternative approaches will be revealed for accessing a wide variety of 5-position substituents to mimic various natural amino acids in turn structures.

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P219 How Much is that Salt Bridge Worth?: The Context-dependence of Non-covalent Interaction Strength in Peptides and Proteins

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Non-covalent interactions play a critical role in protein folding and conformational stability. Understanding these interactions is crucial to developing methods for predicting protein secondary, tertiary and quaternary structure, especially for proteins with limited sequence homology to well-characterized proteins. Efforts to assess the strength of a non-covalent interaction between two amino acid residues typically rely on double mutant cycle analysis, which depends on the assumption that the two residues of interest interact exclusively with each other and not with any other nearby amino acids. This assumption is useful to a first approximation but seems unlikely to be generally true given the structural complexity of most proteins. Instead, one might expect the strength of some binary non-covalent interactions to be substantially influenced by one or more additional nearby amino acid side chains. Computational predictions, bioinformatic studies, and triple mutant cycle analyses suggest that the synergistic coupling of three groups (i.e., a ternary interaction; sometimes called cooperativity) is feasible within the complicated architecture of proteins. Here we show that placing a non-polar or aromatic side chain between two oppositely charged residues along the solvent-exposed face of an α-helix facilitates a synergistic three-way interaction that would not otherwise be possible. We also discuss recent progress toward understanding how site-specific protein PEGylation influences the strength of a nearby salt bridge.

YI-P220 Synthesis, Characterization, and in *vitro* Evaluation of Synthetic Amatoxins and Corresponding Bioconjugates

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Cancer treatment using non-specific chemotherapeutics suffers from poor selectivity and severe toxicity. Targeted drug delivery aims to lower the overall systemic toxicity of chemotherapeutics by employing antibodies, aptamers, small protein scaffolds, and peptides to selectively deliver anticancer drugs to malignant cells that bind tumor-specific biomarkers.[1] α-Amanitin, a bicyclic toxic octapeptide, derived from the death cap mushroom, Amanita phalloides, is a potent inhibitor of RNA Pol II. Due to its unique mode of toxicity, α-amanitin kills both, actively dividing and dormant cells, giving it an advantage over current chemotherapeutics.[2] To date, several antibody drug conjugates of α-amanitin have been successfully developed and are advancing to clinical trials.[3] Although applications of small peptide α-amanitin bioconjugates have been largely unsuccessful, they remain an underexplored facet in the bioconjugate field.[4,5] Recently, the first synthesis ofα-amanitin was reported,[6] opening the doors for synthetic amatoxins with improved stability and diverse handles for bioconjugation. Taking this major advancement into account and given the urgent need for cancer therapeutics with novel modes of action, amanitin-based bioconjugates composed of synthetic amatoxins linked to small peptidic targeting agents were synthesized and evaluated *in vitro*. In addition, cleavable and non-cleavable linkers with varied mechanisms of toxin release were assessed. Overall, this study highlights important considerations for development of peptide-based targeted α-amanitin bioconjugates for a variety of cancer models.

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P221 Fast Solid Phase Peptide Synthesis Method Development for the Aynthesis of Aterically Hindered Peptides

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With the advancement of peptides into the clinic, the complexity of peptide sequences has continued to increase. With more complex sequences, synthesis difficulties arise,

for example, sterically hindered coupling reactions and increased hydrophobicity, among others. Parallel synthesis optimization with induction heating was performed on the solid phase peptide synthesis (SPPS) of several sterically hindered peptides, like Aib-ACP (H-VQ-Aib-Aib-IDYING-NH_2) and N-methylated peptides. Methods for SPPS of linear peptides have seen improvements with increased temperature protocols (>50 °C), resulting in shorter coupling cycles and higher crude purity[1-2]. In the synthesis of Aib-ACP an increase in peptide purity of 30% was observed just by increasing the temperature during synthesis. Here we describe the use of different coupling reagents, different temperatures and reaction times in parallel searching for improvements in crude peptide purity using fast reaction times.

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P222 Efficient Methods for Rapid Development of Therapeutic Peptides with Higher Structural Complexity

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Method development for the manufacture of therapeutic peptides remains a vital piece of the commercial peptide production process. An optimized solid-phase synthesis protocol can be challenging to develop. Recent advances in peptide therapeutics focus on greater structural complexity making peptides that are more physiologically stable products with increased target specificity and membrane permeability[1]. Peptides are also key players in the personalized medicine sector, which relies on faster peptide synthesis protocols and reduced processing times to reach patients effectively.

Here we show the complete parallel synthesis, from swelling to cleavage, of several biologically relevant peptides under different conditions, including increased temperatures (50-90°C), on an automated peptide synthesizer in high purity. In some examples, real-time UV deprotection monitoring has been used to assist in the synthesis optimization process. Optimization has also allowed a reduction in total synthesis times for preparation of the pure peptides.

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P223 Synthesis Strategy for Difficult Peptides to Improve Crude Purity – Optimization of PTH-1 Receptor Ligand, Abaloparatide

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Peptide based drug discovery has seen a revival in recent years with many peptide drugs reaching approval[1]. Peptides can address new therapeutic challenges due to their high target selectivity and potency with low toxicity. Automated solid phase peptide synthesis (SPPS) increases reliability, efficiency, and speed of synthesis of peptides in development and facilitates parallel synthesis of different conditions for faster optimization.

Increased development of neoantigens and personalized medicine further requires faster peptide synthesis protocols and reduced processing times for improved manufacturability. Abaloparatide (ABL; H-AVSEHQLLHDKGKSIQDLRRRELLE KLLXKLHTA-NH_2) is a 34 amino acid peptide with selective activity on the parathyroid hormone receptor type 1 (PTHR1) signaling pathway to treat osteoporosis[2]. ABL was shown to be more effective at inducing net bone-anabolic responses in vivo compared to PTH (1–34), thus favoring bone-formation rather than bone-resorption/calcemic effects. These effects are associated with intermittent as opposed to continuous PTH ligand exposure[3]. In this study, different conditions including multiple resins and coupling reagents were examined for the synthesis of ABL using parallel synthesis searching for optimal crude purity that may translate into easier purification during the manufacturing process.

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P224 Strategies for Efficient, High throughput Optimization of the Synthesis of GPCR Targeting Peptides

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Peptide therapeutics are at the forefront of drug development programs for personalized medicine, cancer therapeutics, and metabolic diseases among others. This has driven the search for faster and more efficient solid phase synthesis (SPS) protocols, making method development crucial in the discovery process. Advances in coupling chemistries and the use of increased temperatures (> 60°C) during coupling reactions in combination with automated synthesizers have expanded the SPS toolbox, allowing simultaneous optimization and high-throughput synthesis via parallel synthesis[1-2].

Here we describe the process development and parallel SPS optimization of G-protein coupled receptor targeting peptides. Solid-support and reagent screening are demonstrated using automated peptide synthesis as part of the optimization process for peptides and peptidomimetics. For example, parallel synthesis condition scanning for the fast synthesis of GLP-1 receptor agonists, Pramlintide (H-KCNTAT CATQ RLANFLVHSSNNFGPILPPTNVGSNTY-NH_2) and Lixisenatide (H-HGEGTFTSDLSKQMEEEAVRLFIEWLK NGGPSSGAPPSKKKKKK-NH_2), led to an increase of 27% in crude purity for both peptides by changing the resin and the coupling reagent used for the synthesis[3-4]. Coupling reagents, resins and different reaction time combinations for increased crude purity results will be discussed for peptides and peptidomimetic compounds.

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YI-P225 Development of Mirror-Image Monobodies for Targeted Cancer Therapies

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Monoclonal antibody therapies for targeted cancer treatment are a tremendous clinical success. CD20 alone is the target of six different monoclonal antibodies (mAbs) approved for clinical use, across a variety of therapeutic indications including chronic lymphocytic leukemia, Non-Hodgkin's lymphoma, rheumatoid arthritis, and multiple sclerosis. However, these mAbs possess significant downsides in common with other mAb therapies, including serious potential side effects and the possibility of severe immune responses.

One strategy to ameliorate these disadvantages is to use mirror-image protein therapies. Since mirror-image proteins use unnatural D-amino acids, these proteins are resistant to proteases and are non-immunogenic. However, since proteins consisting entirely of D-amino acids cannot be produced in biological systems, mirror image proteins cannot be directly used in protein libraries in order to select synthetic binding proteins. Instead, development of mirror-image protein therapies requires chemical synthesis of the mirror-image protein target, a selection step to identify or produce a protein therapeutic effective against the D target, then chemical synthesis of the D-protein therapeutic effective against the natural target.

Our protein scaffold of choice is the monobody, an attractive class of molecules that retain the essential features of antibodies while ameliorating their disadvantages. Monobodies are based on the fibronectin type 3 domain with a highly stable beta-sandwich core structure and have three randomizable surface loops and one randomizable beta-strand, presenting a large surface for potential protein-protein interactions. Crucially, at ~100 amino acids in length they are obtainable by chemical synthesis. We report here the development and evaluation of a mirror-image monobody targeting the membrane protein CD20, including the synthesis of the mirror image of the target, the selection of a monobody that binds the target using both phage and yeast display technologies, the total chemical synthesis of both enantiomers of the selected monobody, and *in vitro* characterization of the synthetic mirror-image monobody. We will also report on the synthesis and evaluation of a bivalent mirror-image monobody designed to engage multiple copies of CD20 on the cell surface.

YI-P226 Effect of Antifreeze Activity and Lipid Interaction of Proline Scanning of 11-residue Lysine Homopeptide

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Antifreeze peptides (AFPs) are present in fish that inhabit the polar zones, adapting to subzero temperatures[1]. This property is highly important in cryopreservation. There are different types of secondary structures for AFPs. In the present study, peptide of 11 lysine residues (K11) was synthesized and characterized, as well as two series in which each position of the peptide chain was replaced by a proline residue (Scan-Pro), used as models to examine point changes in homopeptides. The peptides were synthesized by solid phase using Fmoc methodology[2], purified and characterized by mass spectroscopy and HPLC. The antifreeze activity, whose effect was measured by differential scanning calorimetry (DSC), was dissimilar for both substitution series, being lower for the K11 homopeptide. The antifreeze activity was quantified through the inhibition of ice recrystallization in a non-colligative way, a phenomenon called thermal hysteresis (THA), showing that, independent of the peptide analyzed, the antifreeze activity increased with the decrease in the number of remaining nucleation points. The substitution of internal residues favored the inhibition of ice recrystallization, regardless of whether they were at the carboxy or amino terminal end of the peptide. Disruption of the secondary structure of peptide chain provoked by proline substitution showed a lesser effect on antifreeze activity than other substitutions; however, slightly increased the phase transition temperature of the lipid model of 1,2-dimyristoyl-sn-glycero-3-phosphocholine. This work will allow raising the potential effect on the inhibition of ice crystal growth during freezing by making point substitutions in heteropeptides.

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YI-P227 Antifreeze Activity of Peptides and Synthetic Dimers of Sequential Motives of Aantifreeze Proteins

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Antifreeze proteins (AFP) and antifreeze glycoproteins (AFGP) are molecules with a known anti-freeze effect[1]. Peptides with the alanine-alanine-threonine sequence (AATAATAATAA, ATAATAATAAT, TAATAATAATA and CAATAATAATAA) that belongs to the AFGP are good models for studying the importance of

both the order and the key extension in an antifreeze process. Another modification for appreciating possible changes in the properties of the peptide was the substitution of threonine for lysine and proline residues (AATAATAATAA, AAKAAKAAKAA and AAPAAPAAPAA). Additionally, a comparison was made with peptides of 12 residues with cysteine at its amino terminal (CAAKAAKAAKAA and CAAPAAPAAPAA).

The secondary structure determined by circular dichroism (CD) between 5°C and 50°C, showed that the peptides had a random coil structure, but the addition of cysteine increased the propensity to alpha helix structure. The secondary structure of each peptide increased its structure by decreasing the temperature of measurement.

All the peptides were synthesized by solid phase using Fmoc methodology[2], purified and characterized by mass spectroscopy and HPLC. The secondary structure was determined by CD Jasco J-815. The antifreeze activity was measured by differential scanning calorimetry (DSC).

Peptides without cysteine have a low thermal hysteresis in DSC, whereas peptides with cysteine have a higher thermal hysteresis at the same molar concentration; additionally, the latter showed a strong increase in their antifreezing activity as their concentration increases.

This work generates guidelines to create structures analogous to AFGP using a simple and low cost procedure of synthesis.

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P228 A Chameleonic Macrocyclic Seed Peptide with an Adaptable Fold

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Macrocyclic peptides with a head-to-tail cyclized backbone are intriguing natural products with interesting properties. A family of macrocyclic peptides named PawS-Derived Peptides (PDPs) is produced from precursors of seed storage albumins in species of the sunflower family. The prototypic member is the potent sunflower trypsin inhibitor-1 (SFTI-1). Here we report a novel PDP from the seeds of *Zinnia elegans* identified by *de novo* transcriptomics and liquid chromatography-mass spectrometry. PDP-23 is at 28 amino acids and with two disulfide bonds twice size of typical PDPs. PDP-23 was produced by chemical synthesis and two-dimensional solution NMR spectroscopy was used to elucidate its structural features. It adopts a unique structure in which two β-hairpins, each stabilized by one of the disulfide bonds, fold on top of each other enclosing a hydrophobic core. The clam-shaped tertiary structure is remarkably thermally stable. However, unlike other disulfide stabilized peptides the fold is not restrained by the disulfides allowing the peptide to open and adopt a different structure upon interactions with membranes, suggesting it might have advantages over more rigid scaffolds and represent a versatile template for drug development.

YI-P229 The Synthesis and Application of a Dual Tobramycin and Antibacterial Cell Penetrating Peptide for the Clearance of Intracellular Pathogenic Bacteria

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There are many classes of pathogenic bacteria which are able to hide within mammalian cells and promote bacterial genesis. However, traditional antibacterial agents, such aminoglycosides and β- lactams, are unable to penetrate the mammalian cell membrane. Therefore, there is an urgent need for therapeutics capable of accumulating inside cells at therapeutic concentrations. Herein a novel synthetic route is described to prepare a new cell penetrating dual antibiotic conjugate consisting of tobramycin, an aminoglycoside, and the Cationic Amphiphilic Polyproline Helix (CAPHs) peptide, P14LRR- -a broad-spectrum antibiotic and cell penetrating peptide (TobP14S). Using this methodology, a UV active tobramycin intermediate circumvents previous purification issues and allows for the synthesis of two distinct isomers of the conjugate. Each TobP14S conjugate isomer allows for tobramycin to enter mammalian cells as part of this prodrug conjugate, that reverts to tobramycin and antibacterial peptide once inside the reducing environment of the cell. Both TobP14S conjugates have been demonstrated to regenerate monomeric tobramycin and significantly clear intracellular bacteria.

YI-P230 Chemoproteomic Exploration of the Cellular Citrullinome

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There is growing evidence linking cancers and other diseases to the dysregulation of protein citrullination. We hypothesize that citrulline-containing peptides represent potential neo-antigens that can be used to stimulate an immune response towards cancerous tissue. Thus, our long-term goal is to identify the sites of citrullination in breast cancer tissues and produce novel citrullinated-peptide-antigen-based vaccines that can be used to stimulate the immune system to attack tumors. The identification of citrullinated antigens is significant in that it would add to the growing field of immune-response based treatments for devastating cancers that have, up until recently, been therapeutically intractable for patients. Herein, we report the synthesis and use of citrulline-specific affinity probes that can visualize, isolate, and quantify citrullinated proteins. We also report for the first time numerous proteins that are uniquely citrullinated in breast cancer.

P231 Fmoc-SPPS Chain Termination due to Formation of N-terminal Piperazine-2,5-diones: Structure Elucidation of a By- product by NMR, Proposal for the Mechanism of Formation, and Prevention Strategies

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We observed significant amounts of chain termination in Fmoc-SPPS while synthesizing the N-terminal Xaa-Asp-Yaa motif. This termination was caused by the formation of piperazine-2,5-diones. We investigated this side reaction using a linear model peptide and independently synthesizing its piperazine-2,5-dione derivative. NMR data of the side product proves that exclusively the 6-membered ring is formed whereas the theoretically conceivable 7-membered 1,4-diazepine-2,5-dione is not found. We propose a mechanism where nucleophilic attack of the N-terminal amino function takes place at the α-carbon of the carbonyl group of the corresponding Aspartimide intermediate. Hence, hindering or avoiding Aspartimide formation during SPPS reduces or completely suppresses this side reaction.

P232 Minimizing Apartimide Formation in Fmoc Microwave Assisted Solid Phase Assembly

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In the last decade microwave solid phase peptide synthesis has been establishing itself as one of the preferred technology for the synthesis of long and difficult peptides. The advantage of this approach is the short coupling reaction time due to the increased coupling efficiency reached by microwave irradiation.

During our routine microwave synthetic work, we realized that in sequences containing aspartic acid residues, the aspartimide side reaction is further exacerbated during the microwave assisted basic deprotection step despite the use of protecting groups such as OMpe,[1] and OBno,[2].

In order to minimize this side reaction caused by repeated exposure of aspartic acid containing sequences to piperidine, we implemented a series of protocols on control peptides by exploring microwave technology with the use of different protecting groups in combination with various deprotection temperatures and timing, washing cycles and bases.

Here we demonstrate that it is possible to prevent aspartimide formation by using optimized protocols that have been implemented in our state-of-the art peptide synthetic procedures in our laboratory.

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P233 Exploring the Impact of Peptide Backbone N-heteroatom Substitution on Secondary Structure

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The introduction of backbone amide substituents has a profound impact of the conformation and stability of host peptides. Recently, we reported the synthesis of diverse chiral α-hydrazino acids that can be chemoselectively incorporated into a growing peptide chain on solid support to afford *N*-amino peptides (NAPs). Our results suggest that NAPs are capable of adopting β-sheet like folds through cooperative steric, electrostatic, and hydrogen-bonding interactions. Here, we have expanded our investigation to include *N*-hydroxy and *N*-alkoxy residues and demonstrate their synthesis and incorporation on solid support to afford N-hydroxy peptides (NHPs). The synthesis and conformational analysis of β-hairpin model peptides featuring various N-hydroxylated residues was investigated by NMR and CD. Our results demonstrate that *N*-hydroxylated residues are accommodated into the strand regions of β-hairpin peptides without energetic penalty. Additionally, we are now investigating how *N*-hydroxylated residues impact α-helical stability in model systems. Our findings thus expand the repertoire of non-canonical amino acid derivatives that can be incorporated into well-folded secondary structures.

P234 A Novel Peptidomimetic β-turn that Readily Forms 13-membered Cyclic Tetrapeptides and Provides Synthetic Analogues of β-hairpin Antimicrobials with Enhanced Therapeutic Window

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Naturally occurring β-turns are important in mediating protein folding, receptor binding interactions as well as in serving as sites for post-translational modification in proteins.[1-3] Therefore, the development of novel β-turn mimetics is of fundamental interest as well as aid in providing novel frameworks for drug design. Non-protein amino acids bearing *N*-methylation, α,α-dialkylation or D-configurations have been found to induce β-turns and stabilize β-hairpin conformations in synthetic peptides.[4-6] Despite these literature efforts, the realization of designed β-hairpin peptides with biological activities and the use of novelβ-turns to facilitate the syntheses of cyclic tetrapeptides remains relatively less documented and present several challenges. Our recent research developed a novel β-turn scaffold, that permits the pre-organization of designed linear tetrapeptides and facilitate their cyclization to yield synthetically challenging cyclic tetrapeptides (CTPs) in quantitative yields.[7] Parameters such as solvent polarity and ring dissection were investigated during the syntheses of the CTPs resulting in optimized synthetic strategies with significant improvements to product yields than previously reported.[7,8] The designed β-turn was also incorporated in analogues of naturally occurring β-hairpin antimicrobials and de novo designed peptides resulting in synthetic analogues with altered molecular architecture and providing broad-spectrum β-hairpin antimicrobial peptides with enhanced therapeutic window.[9] Further investigations have now allowed us to fine-tune the amino acid sequences for engineering acyclic and cyclic antimicrobial peptides that adopt β-hairpin structures. A summary of these findings that include chemical syntheses, structural analyses and bioactivity studies will be presented.

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YI-P235 Macrocyclization of Small Peptides Enabled by Oxetane Incorporation

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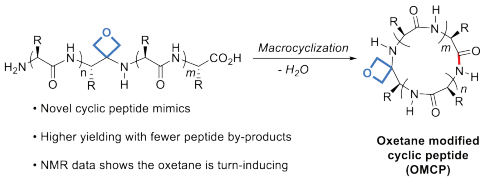
Cyclic peptides are privileged structures with broad biological activity, but a major obstacle to their development relates to difficulties associated with their synthesis.[1] We have developed a methodology that replaces one amide bond in the linear peptide backbone with the 3- aminooxetane moiety, which enhances macrocyclizations to form cyclic peptides. The oxetane is turn inducing and leads to significant improvements in isolated yield, product distribution and reaction rate.[2] Careful LC-MS analysis shows the oxetane incorporation produces cleaner macrocyclisations with fewer peptide by-products arising from epimerization, dimerization and oligomerization. The scope of the macrocyclization is broad, with up to 4-fold improvement in yield over a range of challenging ring sizes (tetra-, penta- and hexapeptides). Furthermore, oxetane modification is superior to other commonly used peptide modifications such as N-methylation. In a model peptide c(LAGAY), molecular dynamics simulations offer insights into the structural differences of the modified and unmodified cyclic peptides, with evidence for a new intramolecular H-bond established as well as reduced flexibility of the macrocycle. Importantly, incorporation of an oxetane into a cyclic peptide inhibitor of Aminopeptidase N, expressed on many cancer cells, shows similar IC50 values to the parent peptide indicating that bioactivity is retained upon replacement of the amide bond.



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YI-P236 Hydrogen Bond Surrogate β-Hairpins for Inhibiting Protein-Protein Interactions

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Protein-protein interactions (PPIs) play a key role in biological systems and provide specific targets for clinical intervention in a wide array of diseases, including cancer, neurodegeneration, and host-microbe interactions. Specific inhibition of PPIs depends on the use of synthetic molecules that occupy one or more "hotspots" within large, flat target protein surfaces to effectively compete with a protein's native binding partner(s). Here we present the design and evaluation of synthetic inhibitors for challenging protein-protein interaction (PPI) targets of biomedical interest, namely those involving β-sheets. First, we adapted the hydrogen bond surrogate (HBS) approach to address a longstanding challenge in specific inhibition of β-sheet-mediated PPIs: the introduction of a moiety that stabilizes the β-sheet without replacing β-strand or turn residues that play a key role in PPI binding. The HBS approach replaces a main-chain hydrogen bond with an isosteric covalent bond and was originally developed to nucleate helical structures. We found that strategic placement of the HBS linker stabilizes the desired β-sheet structure in the context of several different peptide sequences, including designs that mimic PPI interfacial β-sheets. We envision that HBS-stabilized β-sheets will provide valuable tools to examine PPI function and may provide new strategies for clinical intervention in PPI-associated diseases.

YI-P237 The Isonitrile-Chlorooxime Bioorthogonal Ligation

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The development of chemical reactions suitable for application in biological environments is important for the study of biomolecules in living systems and thus the elucidation of complex biological processes.[1] The "chemical reporter strategy" represents one of the most powerful methods for the labeling of biomolecules in their native environment.[2] This strategy utilizes the cell's own biosynthetic machinery to incorporate non-natural metabolites with unique functional groups into specific cellular components. In the second step these metabolites, 'reporters', are selectively targeted with a complementary functional group that is bound to a detectable probe. Thus, this method allows for the visualization and isolation of specifically

targeted biomolecules such as glycans, lipids and proteins.[3] Despite recent improvements in reactivity and selectivity of bioorthogonal reactions, limitations remain such as insufficient biocompatibility, slow kinetics, structurally invasive ligation products or cross reactivity with other bioorthogonal ligation methods, precluding multicomponent imaging experiments. Here we present a new type of bioorthogonal ligation based on an isonitrile as chemical reporter and chlorooximes as probe (Fig.).[4] This ligation proceeds with high reactivity and selectivity under physiological conditions and is orthogonal to most commonly used bioorthogonal ligation methods, including strain-promoted azide alkyne cycloaddition (SPAAC) and the Staudinger ligation.[3a,b,e] Furthermore, the selectivity and applicability of this new ligation method was demonstrated in vitro by imaging metabolically labelled glycans on cell membranes.

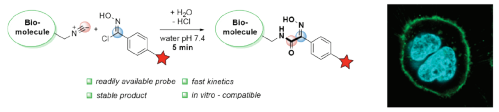

Figure left: The Isonitrile-Chlorooxime Ligation. right: Glycan-labeling of cell-membranes using Isonitrile-Chlorooxime Ligation visualized with confocal microscopy.

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YI-P238 Peptiligases as Tools for the Efficient Assembly of Cyclotides

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In the last decade, macrocyclic peptides have gained increased traction as a promising class of therapeutics, with many presently under clinical investigation. In particular, disulfide-rich macrocycles such as cyclotides or θ-defensins, characterized by their distinctive knotted disulfide pattern and backbone cyclization, are an exceptionally stable class of molecules with diverse bioactivities that are currently being pursued in drug discovery. However, both the synthesis of comprehensive libraries and assembly at large scale still pose a significant challenge and novel tools are highly desired. Here, we present the use of peptiligase variants such as omniligase-1 for the modular, scalable and traceless chemo-enzymatic assembly of several prototypical disulfide-rich macrocycles, including MCoTI-II, Kalata B1, RTD-1 and variants thereof. For example, omniligase-1 not only efficiently catalyzes head-to-tail cyclization in high yields, but also allows cyclization and oxidative folding in a one-pot procedure, as can be demonstrated for all the aforementioned cyclotides. In addition, multiple ligation sites enable the modular assembly of cyclotides from two or more fragments and the generation of cyclotide conjugates (*e.g.* fluorophores, payloads) using specific peptiligase variants. Moreover, this approach can significantly facilitate grafting studies. We demonstrate that peptiligases represent a powerful tool for the engineering and manufacture of several native disulfide-rich macrocycles without requiring any mandatory recognition sequence.

YI-P239 Influence of the trans/cis Conformer Ratio on the Stereoselectivity of Peptidic Catalysts

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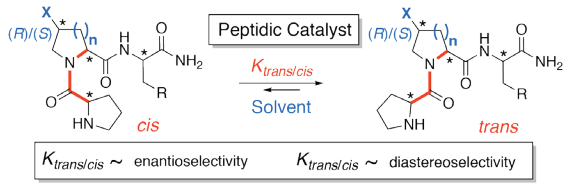
The trans/cis isomerization of Xaa-Pro amide bonds (Xaa: any amino acid) is key for the structure and function of several enzymes. In recent years, numerous versatile peptidic catalysts have been developed that bear Xaa-Pro amide bonds. We envisioned that control over the trans/cis amide bond ratio might provide a tool to optimize the catalytic performance of peptidic catalysts.[1,2]

Our group developed peptides of the H-Pro-Pro-Xaa type that are highly reactive and stereoselective catalysts for organocatalytic C-C bond formations, such as aldol reactions and conjugate addition reactions of aldehydes to nitroolefins and unprotected maleimide.[2] Here, we shed light on the influence of the amide bond conformation on the stereoselectivity and reactivity of H-DPro-Pro-Xaa-NH type catalysts in conjugate addition reactions.[3] The middle Pro residue within the tripeptides was replaced by analogues of varying ring sizes or Cγ-substituted Pro derivatives to produce different *trans/cis* ratios in different solvents. Our investigations revealed a direct correlation between the *trans/cis* amide bond ratio and the enantio- and diastereoselectivity of the tripeptidic catalysts, which led to the identification of H-DPro-Pip-Glu-NH_2, a catalyst that allows C–C bond formations in the presence of as little as 0.05 mol %, which is the lowest catalyst loading yet achieved for organocatalyzed reactions that rely on an enamine-based mechanism.



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YI-P240 Tripeptide-induced Mechanical Property Modulation of Mesenchymal Stem Cells Stimulates Proliferation and Wound Healing

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Mechanical properties of cells and extracellular matrix plays important role in many biological processes like cell proliferation, differentiation, and wound healing. Mesenchymal stem cells (MSCs) are multipotent cells, capable of differentiating into many cell-types such as cartilage, osteoblast, adipocytes, and endothelial cells. In the present study we evaluated the effect of a tripeptide on hMSC proliferation, differentiation, wound healing, and cellular elasticity as an indicator mechanobiological modulation. Atomic Force Microscopy (AFM) was employed to investigate the elastic modulus of tripeptide molecules treated hMSCs. Treated hMSCs showed Young's modulus of 9.8± 0.09 kP which is about 2.5- fold higher than the control group (3.9±0.47 kP). Interestingly, peptide was also found to promote proliferation, adhesion, and migration of hMSCs and ameliorated wound healing. Collectively, the present study provides a quantitative description of peptide treated hMSCs mechanical behavior, which supports the process of growth and proliferation.

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P241 Peptide Engineering Strategies for Long-acting Peptide Hormones

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As a drug class, peptides offer exquisite specificity and potency, but also present challenges associated with poor stability and short half-life, manifesting in the need for frequent injections, poor patient compliance, and overall compromised efficacy. We have developed a novel peptide engineering strategy that incorporates a serum protein binding motif onto a covalent side-chain stapling and applied to multiple peptide hormones to enhance their helicity and stability, and therefore, to enhance their *in vitro* potencies and *in vivo* half-lives and efficacies. Detailed rational design and its application into GLP1R single agonist, GLP2R agonist, NPY2R agonist, GPR10 agonist and GLP1R/GCGR dual agonist will be covered. Rodent and cyno PK indicate once weekly projected half-life in human.

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4 Avinash Muppidi et al "Design of potent, proteolytically stable oxyntomodulin analogs" *ACS Chem Biol* **2016**,*11*(2):324-8

YI-P242 Enzyme-dependent Modulation of de novo Designed Membrane-active Peptides

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The capability to cross the biological membrane and access the cell interior is still a major challenge for current drug development. Cell penetrating peptides (CPP) represent an efficient vehicle to carry various therapeutic agents (e.g. Protein, RNA) into cells or tissues. However, several limitations are inherent to CPPs before clinical use comes to fruition including: 1) lack of cell specificity and 2) hemolytic potential at high peptide concentrations. We are developing a membrane active peptide that can selectively target cancer cells, and is characterized by a significant reduction in hemolytic potential. We determined that the phosphorylation state of tyrosine can be used to modulate the ability of peptides to enter cancer cells. Designed peptides containing tyrosine phosphate are unable to enter nearly all cell types. However, certain cancer cells overexpress alkaline phosphatase (ALP) relative to non-cancer cells. Thus, dephosphorylation, at the surface of cancer cells, transforms the peptide into a molecule capable of rapidly enter the cells. This allows peptide penetration preferentially into cancer cells. Confocal images show that fluorescein-tagged peptide accumulated in the cytosol of high ALP expressing cells (e.g. SaOs2 cell), and little peptide enters low ALP expressing cells (e.g. A549 cell and HDF cell). Flow cytometry also shows that the peptide preferentially enters cells with higher ALP expression. To Investigate the potential of the CPP to deliver drugs, doxorubicin, an extensively used anti-cancer drug, was ligated to the designed CPP. Comparing the toxicity of this drug conjugate toward SaOs2 cells and A549 cells, the ratio of IC50 values is around 53, strongly suggesting that the designed peptide can target the delivery of doxorubicin to cells with high ALP expression. Taken together, this work provides a novel approach for targeting drug delivery.

YI-P243 Stress Responsive Pharmacological Modulation of PERK Signaling Showed Neuroprotective Effects on Tauopathies

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A number of neurodegenerative diseases including tauopathies are caused by an abnormal proteostasis and accumulation of aggregation-prone proteins in neurons. In order to find small molecules suppressing Tau protein aggregation in cells, we performed a phenotype-based screening using HEK293 Tau BiFC (Bimolecular Fluorescence Complementation)-Venus cells. SB1617 was selected as a leading compound after the structure activity relationship study with its high potency in reducing tau protein oligomerization and low cytotoxicity. We obtained a possible target protein list of SB1617 by applying FITGE (Fluorescence difference in two-dimensional gel electrophoresis) and TS-FITGE (thermal shift FITGE) methods. Further gene knockdown experiments for those proteins, biophysical tests and in vitro bio-functional tests revealed that SB1617 activates PERK signaling under the cell stressed conditions. Stimulated PERK signaling by SB1617 caused sustained EIF2alpha phosphorylation, which suppressed global

protein synthesis and relieved ER workload. The downstream of PERK signaling, ATF4 level was transiently enhanced by SB1617 that is a transcription factor regulating autophagic genes. When Traumatic Brain Injury model mice were treated with SB1617, hippocampal and cortical region showed enhanced autophagy and increased level of Brain-derived neurotrophic factor (BDNF) compared to the control group. SB1617 was effective in ameliorating motor neuron behavior in TBI mice. Further elucidation of mechanism regarding conditional PERK activation and proteostasis regulation will accelerate developing therapeutics for neurodegenerative diseases.

YI-P244 Identification of Novel Site 2 Targeting Peptides Using One-Bead One-Compound Technology

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Integrins are a family of transmembrane proteins viewed as potential therapeutic targets because of their integral function in various physiological and pathological pathways. A common binding site associated with the integrin avß3, as well as other related integrins, is known as the RGD binding site. However, a recent study on Fractalkine has shown that there is an additional binding site (site 2) located on the integrin closed headpiece that is distinct from the classical RGD (site 1) site. This theory was shown to apply to other proteins when solubilized avß3, in its inactive state, was tested against human secreted Phospholipase A2 type IIA (sPLA2-IIA), which is known to bind this integrin as well. In this study we have generated One-Bead One-Compound (OBOC) combinatorial peptide libraries of varying lengths and screened against a 20-mer peptide from site 2, to discover novel interacting peptides. Positive hits from these libraries were sequenced and checked for sequence homology and used to generate homolog libraries. Concurrently a lead peptide was isolated using flow cytometry to eliminate peptides that bound weakly to cells. This lead peptide was tested using Bio-Layer Interferometry (BLI) to determine its binding affinity to our primary protein as well as related proteins. These peptides will be further optimized with focused libraries and libraries containing molecular rotor dye, such that fluorescent activated ligands (FAL) against site 2 can be developed as tools to study integrin biology and biochemistry, and as lead compounds to discover integrin therapeutics

YI-P245 GESI Project (The Genetically Encoded Small Illuminant): A Novel Technology for Functional Imaging in Living Cells

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Membrane receptors, including GPCRs, receptor kinases, adhesion molecules, represent popular cancer drug targets. However, tracking the function of these receptors in living cells, especially their activation status, such as ligand-binding and post-translational modification (PTM), has been difficult. The molecular imaging system proposed here consists of using an one-bead-one-compound (OBOC) combinatorial library screen to identify short peptide(s) that activate fluorophores/dyes, which can be coupled to alterations of their chemical environment including conformational change upon ligand binding and phosphorylation of the peptide. These peptides can then be genetically fused to a target protein to enable functional cellular imaging *in vitro* and *in vivo*. As proof of concept, we will fuse the identified peptide to integrin and use the exogenously added GESI-peptide-activating dye to demonstrate the utility of the proposed genetically encoded functional imaging system in living cells. We will test their ability to measure spatiotemporal dynamics, interactions with ligands and phosphorylation of intracellular domain. Integration of these distinct GESIs into one integrin protein will allow concurrent probing of multiple integrin functions in real time in living cell.

P246 On-Column Dilution: A Technique to Load Samples Dissolved in DMSO

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Peptide purification efficiency is often limited by resolution. Resolution from sample loading is decreased if samples need to be dissolved in solvents such as DMSO or DMF, particularly for water-soluble peptides. On-column dilution is a technique that allows samples to be dissolved in strong solvents such as dimethyl sulfoxide (DMSO) or dimethyl foramide (DMF) while maintaining resolution during preparative chromatography. The sample is "bracketed" between slugs of weak solvent. Air-gaps between the sample and weak solvent prevent precipitation of the sample during injection. A 300% increase in peptide sample loading is demonstrated.

YI-P247 De novo Designed Tryptophan-zippered Peptides with Pathogen-specific Antibacterial Activity

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Antibiotics are our best weapons against bacterial infections. However, their excessive use has accelerated the emergence of multidrug resistance and we find ourselves quickly running out of useful drugs. A critical, yet often overlooked, contributor to resistance is the human microbiota, which, when overexposed to antibiotics, serves as a reservoir of resistance genes that can be horizontally transferred to invading pathogens. This urges the development of narrow-spectrum therapies that can precisely kill microbes without disrupting protective native flora. Here, we report the *de novo* design of a tryptophan-rich peptide capable of potently killing *Mycobacterium tuberculosis* pathogens, the eponymous causative agent of tuberculosis, while being remarkably biocompatible towards model commensals and human cells. Circular dichroism spectroscopy and electron microscopy reveal that this peptide, named myco-membrane associated disruption 1 sequence (MAD1), assembles into monomorphic fibrils under acidic conditions that are stabilized by an intermolecular tryptophan zipper. Parallel studies using model membranes and mycobacterial cultures demonstrate that MAD1 preferentially interacts with the unique mycolic-acid rich surface of mycobacteria to elicit membrane depolarization. Conversely, MAD1 is unable to intercalate into other gram-positive and gram-negative microbial membranes to elicit bacteriolysis. Together with the unusually tryptophan-rich nature of many mycobacterial transmembrane proteins, this suggests that tryptophan-zippered MAD1 nano-assemblies may mimic myco-membrane proteins to enable their selective integration into, and disruption of, the mycobacterial cell wall. This work may open new paradigms in the rational design of pathogen-specific AMPs through membrane-protein biomimicry, and advance development of narrow-spectrum biotherapeutics that synergize with conventional antibiotics to combat antimicrobial resistance.

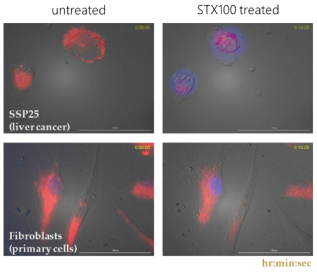
P248 Cell Based Production and Selection of PPI-Modulating Compounds, and their Applications in Oncology

Charly Chahwan and Maria Soloveychik. SyntheX Inc., 953 Indiana Street, San Francisco, CA, 94107.

At SyntheX, we are discovering and developing peptide-based compounds for targeting intracellular protein-protein interactions (PPIs). Using synthetic biology, we have engineered a cell-based drug selection platform, ToRPPIDO, that relies on a simple viability readout for scoring hits. The technology allows us to produce and screen libraries of peptides or macrocycles that can selectively either disrupt or bridge PPIs. The platform is broadly applicable, and our first area of focus is oncology.

We selected a PPI that is crucial in the DNA repair process of Homologous Recombination (HR). HR plays a crucial role in maintaining genomic stability in cancer cells and is highly induced by oncogenes such as Myc, CycE, and KRas. In accordance, overexpression of HR pathway components correlates with poor prognosis and chemo-resistance in many tumor types.

Application of ToRPPIDO towards a crucial PPI within the HR pathway led to the discovery of STX100. Derivatives of STX100 exhibiting low nanomolar affinity to target, cell permeability, and proteolytic stability were tested for activity against various cell lines. Results from a panel of over 30 cancer and primary lines demonstrated acute mono-agent activity of STX100 derivatives selective towards HR-overexpressing cancer cells. Interestingly, STX100-mediated cell killing is independent of canonical cell death mechanisms (apoptosis, necroptosis, pyroptosis, ferroptosis, etc.). Rather, the mechanism exploits the elevated abundance of the HR target protein in cancer cells relative to healthy tissues to elicit an acute calcium-dependent cytotoxicity upon STX100 target engagement. ADME/PK and in-vivo efficacy studies indicate favorable characteristics and further pre-clinical development is currently ongoing.



P249 Half-life Extenders Affect PYY3-36 Internalization

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Peptide YY$_{3-36}$ (PYY$_{3-36}$) is an endogenous ligand of the neuropeptide Y2 receptor (Y2R), through which it acts to reduce food intake. Accordingly, PYY$_{3-36}$ analogues are interesting as potential anti-obesity pharmaceuticals. However, native PYY$_{3-36}$ is rapidly cleared from circulation, and half-life extension is thus a prerequisite for PYY$_{3-36}$ based pharmaceuticals. This is typically achieved by covalent attachment of PYY$_{3-36}$ to macromolecules (e.g. PEG) or through lipidation which promotes non-covalent interactions to albumin.

Many peptide drugs, like PYY$_{3-36}$, require binding to a specific G protein coupled receptor (GPCR) to exert their effect. GPCRs are desensitized and internalized following intracellular binding of β-arrestins, which bind to the ligand-activated conformation of the receptor.[1]

Beck-Sickinger and co-workers recently reported how PEGylation and lipidation differentially directs arrestin 3 (Arr3) recruitment and receptor internalization of obinepitide, another peptide of the neuropeptide Y receptor system.[2] Hence, the half-life extender selected for PYY3-36 therapeutics may alter its efficacy. Accordingly, we aimed to investigate how three commonly applied half-life extenders, PEGylation (PEG20), hexadecanoic acid (C16), and octadecanedioic acid (C18-acid), directs Y2R-mediated internalization of PYY$_{3-36}$. Here we report how PEGylation leads to a G protein bias and reduced Y2R internalization. We further report how lipidation with both C16 and C18-acid did not bias Y2R signalling, whereas only C16 increased Y2R internalization. Finally, we demonstrate how binding kinetics underlay some of these differences.

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YI-P250 Pyridyl Diselenide: A Chemoselective Tool for Peptide Synthesis

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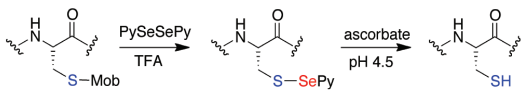
Many disulfide rich peptides and proteins have biological activity that relies heavily on correct disulfide pairing. When synthesizing these Cys-rich peptides/proteins, care must be taken to ensure proper disulfide connectivity at each step, otherwise the overall yield of correctly folded peptide will be low. One option is to use a regioselective strategy involving stepwise formation of disulfide bonds. This can be accomplished by using various orthogonal protection schemes of the side-chain protecting groups for Cys. Many methods for regioselective disulfide pairing suffer from a lack of chemoselectivity, efficiency, and high yields. Here, we show the utilization of pyridyl diselenide as a chemical tool for the regioselective/chemoselective formation of disulfide bonds in peptides. When we treated peptides containing Cys(Mob), Cys(Acm), or Cys(Bu^t) residues with pyridyl diselenide in TFA, deprotection occurred with formation of a cysteine-S-selenopyridyl adduct, which contains a unique S–Se bond. The advantage of this S–Se pyridyl bond is that it can be reduced chemoselectively with ascorbate, without concomitant reduction of other disulfide bonds. This chemoselective reduction by ascorbate will undoubtedly find utility in other biotechnological applications. We show here that pyridyl diselenide, in combination with chemoselective reduction by ascorbate, can be utilized for the regioselective formation of disulfide bonds through a step-wise, iodine-free synthesis of the human intestinal hormone, guanylin.



YI-P251 Unitization of Alpha-methyl Selenocysteine as a Glutathione Peroxidase Mimic

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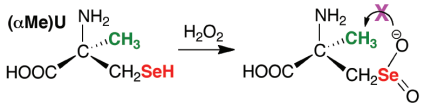
Selenocysteine (Sec) has found wide utility in peptides largely as a replacement for cysteine (Cys). Sec residues in peptides are rapidly oxidized in aqueous buffer and can undergo β-syn elimination reactions resulting in the conversion of Sec to dehydroalanine, rendering the Sec-containing peptide inactive. β-syn elimination reactions rely upon the presence of an accessible Cα-H. To prevent β-syn elimination, we have substituted Cβ-H with a methyl group. The resulting amino acid is a derivative of Sec called β- methylselenocysteine ((βMe) Sec). Here, we incorporate αMe)Sec into a peptide that mimics the active site of Sec-containing glutathione peroxidase (GPX) and monitored the peptide's ability to reduce H2O2 and found it to be a useful GPX mimic. We next compared our (αMe)Sec-containing peptide to a Sec-peptide control and found that the (αMe)Sec-peptide showed superior stability when both peptides were incubated for prolonged periods of time at basic pH. While β-syn elimination was apparent for the Sec-peptide, no β- syn elimination occurred with the (αMe)Sec-containing peptide as this peptide retained a significant level of GPX activity. This is the first reported incorporation of (αMe)Sec into a peptide as well as the first reported biochemical application of this unique amino acid.



YI-P252 Targeting Protein Kinases with DNA-encoded Chemistry for Peptide Substrate Competitive Inhibitors and Activity Profiling

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Protein kinases are essential in cell signaling pathways and are well-validated targets for cancer therapeutics. We present two approaches to facilitate the study of protein kinases using DNA-encoded chemistry. First, a combinatorial library of peptidomimetic small molecules was prepared and subjected to a selection for detection of molecules that can serve as tyrosine kinase substrates. Potential substrate-DNA conjugates were phosphorylated by Src kinase and then captured using anti-phosphotyrosine immobilized on magnetic beads. Eluted substrates were PCR amplified and sequenced. The resulting hits were investigated as novel peptide-substrate competitive inhibitors. In the second area, we used the same substrate selection strategy with a collection of DNA conjugates of known tyrosine kinase peptide substrates to profile kinase activities within samples. This DNA-based approach was validated by comparative analysis of activities in cell lysates to a commercially available kinase substrate microarray approach. We observed comparable results with dramatically increased throughput and cost-effectiveness. We identified peptide substrates that are differentially phosphorylated among Src, Lyn, and Syk kinases, and profiled activity in lapatinib resistant and sensitive breast cancer cell lines. Kinome activity profiling with DNA-encoded substrate probes is a promising approach to assist drug target validation, unveil drug mechanisms of action, and understand the role of kinases in biological processes.

YI-P253 The Metal Mediated Self-Assembly of a Cell Adhesive Collagen-Mimetic Peptide

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Over the past two decades, peptide self-assembly has been studied extensively towards constructing mimetics of natural biomaterials such as collagen, amyloids, and viral capsids.[1] Recently, a class of collagen mimetic peptides that undergo metal dependent self-assembly has been described.[2] Here, we report the properties of NCoH-FOGER, a collagen mimetic peptide incorporating an integrin-binding cell adhesive sequence and metal-binding ligands. This peptide demonstrated the ability to form fibrillar particles upon addition of divalent metal cations, as observed by electron microscopy. Upon negative staining, a regular banding pattern is observed on the surface of these particles, a property exhibited by natural collagen. Based on our observations, we propose a structure and a mechanism for the formation of these collagen-peptide materials. With these observations and structural proposals in hand, we hypothesized that these particles were poised for translation as topical nanopatterned carriers for wound healing factors. As such, we present initial data towards this, demonstrating the ability of the particles to bind cells while carrying histidine-tagged cargos.

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P254 Analytical Characterization of a Dual Action Glucagon/GLP-1 Agonist and a Selective Glucagon Agonist

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To improve the design and successfully progress SAR (Structure-Activity Relationship) of a dual action glucagon/GLP-1 agonist (ICA6160499A) and a glucagon selective analog (ICA6160102A) as tool molecules, it is critical to understand the chemical characteristics of native glucagon (HSQGTFTSDYSKYLDSRRAQDFVQWLMNT) and other glucagon analogs. Impurity profiling via stressed degradation (at various tempera-tures, pH's, formulations) was performed on the above molecules to identify cleavage sites, which aid in interpretation of the chemical be-havior of these molecules. This was achieved by identifying degradants from stress-induced ther-apeutic peptides using RP-HPLC, SCX-HPLC, LC-MS/MS and DLS. The information learned from LC-MS/MS fragmentation patterns, degra-dation products, and characterized impurities of the above molecules will be presented. These data, in turn, were utilized to further modify ICA6160102A to improve shelf-life, in-vivo stability, and solubility, leading to the selection of a clinical candidate ICA6150349.

P255 Mitochondria-targeting and ATP-boosting Hyperstable Peptide Bioenergetics

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Mitochondria are the main organelles which regulate energy production in living cells. Accumulating evidence suggests that dysfunctional mitochondria contribute to degenerative diseases, aging, and attendant aging issues, such as frailty and chronic inflammatory diseases[1-3]. Strategies to slow the decline of mitochondrial functions show promise in delaying the onset, reducing the severity of age-related diseases, and increasing the health span of aging population4. Here, we report the discovery of a mitochondria-targeting and ATP-boosting peptide, roseltide rT1 which is a 27-residue cysteine-rich peptide isolated from the medicinal herb *Hibiscus sabdariffa*[5]. Results from flow cytometry, live-cell imaging, pull-down assays, and specific genetically-modified cell lines showed that roseltide rT1 is a cell-penetrating peptide, targeting the mitochondria to increase ATP production by entering cells via glycosaminoglycan-dependent endocytosis and then mito-chondria through TOM20, a mitochondrial protein import receptor. We also showed that roseltide rT1 increases cellular ATP production via mitochondrial membrane hyperpolarization and reactive oxygen species generation. Using a combina-tion of biotinylated roseltide rT1 for target identification and proteomic analysis, we further showed that human mito-chondrial membrane ATP synthase subunit O is an intra-mitochondrial target. Chemical synthesis together with structure-activity studies showed that roseltide rT1 contains a non-canonical mitochondria-targeting motif and structure, devoid of helical structure and resistant to heat denaturation and proteolytic degradation. Collectively, these data support our dis-covery that roseltide rT1 is a first-in-class mitochondria-targeting hyperstable peptide which increases cellular bioener-getics with potentials to extend health span of aging populations.

Acknowledgment
This work was financially supported by a Competitive Research Grant from the AcRF Tier3 funding (MOE2016- T3-1-003) and the Nanyang Technological University (NTU) internal funding–Synzymes and Natural Products (SYNC),

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P256 Comparative Molecular Transporter Properties of Cyclic Peptides Containing Tryptophan and Arginine Residues Formed through Disulfide Cyclization

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We have previously reported a cyclic cell-penetrating peptide $[WR]_5$ containing alternate tryptophan and arginine amino acids, which was able to transport a variety of cargos across the cellular membrane, such as anti-HIV drugs and phospho-peptides. Cyclic peptide-doxorubicin conjugate demonstrated higher retention and antiproliferative activities doxorubi-cin in specific cancer cell lines. To optimize further the utility of our developed peptide for targeted therapy in cancer cells using redox condition, we designed a new generation of peptides and evaluated their cytotoxicity as well as uptake behav-ior against different cancer cell lines. The rationale of current studies was that cancer cells have a higher percentage of glu-tathione compared to normal cells. Thus, disulfide bonds can be reduced in cancer cells significantly higher than normal cells, providing selective delivery of anticancer drugs to cancer cells. Therefore, $[C(RW)_4C]$ and $[C(RW)_5C]$ peptides and their linear counterparts were synthesized using Fmoc/tBu solid-phase peptide synthesis, purified using high-pressure liquid chromatography (RP-HPLC), and characterized with matrix-assisted laser desorption/ionization (MALDI). The com-pounds did not show any significant cytotoxicity at a concentration of 25 μM against ovarian (SK-OV-3), leukemia (CCRF-CEM), and kidney (LLCPK) cells. Both cyclic [C(RW)5C] and linear $(C(RW)_5C)$ demonstrated comparable molecular transporter properties versus $[WR]_5$ in the delivery of a cell-impermeable negatively-charged phosphopeptide in CCRF-CEM cells.

P257 Evaluation of Structure-Activity Relationship of Cyclic Peptides $[W_4R_4]$ and Amphipathic Fatty Acyl-Cyclic $[W_4R_4K]$ as Potential Antibacterial Agents Against Pathogenic Bacteria

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Cyclic peptide $[W_4R_4]$ containing positively-charged arginine (R) and hydrophobic tryptophan (W) residues had antibacterial activity with a minimum inhibitory concentration (MIC) value of 4 μg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA) and 42 μg/mL against *Pseudomonas aeruginosa* (PSA). The purpose of this study was to optimize the antibacterial activity of the cyclic peptide by increasing the number of residues and or conjugation with fatty acids. The antimicrobial effects were determined against MRSA, PSA, *Klebsiella pneumoniae* (KPC), and Escherichia coli using meropenem and vancomycin as controls and compared with $[W_4R_4]$. All the peptide were synthesized using Fmoc-based solid-phase chemistry and was characterized by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectroscopy (MALDI-TOF) and purified with high-performance liquid chromatography (HPLC). The cyclic peptide fatty acyl anhydrides (RCO-O-COR) where R = CH_3, $CH_3(CH_2)_{2n-2}$) were conjugated with purified peptides to obtain $[W_4R_4K(C_2)]$, $[W_4R_4K(C_4)]$, $[W_4R_4K(C_6)]$, $[W_4R_4K(C_8)]$, $[W_4R_4K(C_{10})]$, $[W_4R_4K(C_{12})]$, and $[W_4R_4K(C_{14})]$. All the synthesized peptide were evaluated for their MIC values against MRSA, PSA, KPC and *E. coli*. $[W_4R_4K(C_{10})]$ peptide had antibacterial activity against Gram-positive strains like MRSA with MIC value 4 μg/mL but less activity against Gram-negative bacteria like KPC, PSA, with MIC values of 32 μg/mL and 64 μg/mL, respectively. Variation in the number of arginine and tryptophan residues in cyclic peptide did not improve potency. [W4R4K(C10)] demonstrated 6.4% hemolytic effect on human red blood cells

while cyclic peptide [W4R4] showed a 10.2% hemolytic effect at a concentration of 128 μg/mL.

YI-P258 Fluorescent Isoindole Crosslinking of Biologically Active β-Turn Peptides

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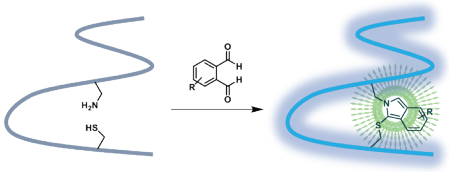

The stabilization of secondary structure is a ubiquitous pursuit in peptide chemistry. Inspired by the natural heterocyclic crosslinks found in the amatoxins, phallotoxins, celogentins and moroidins, we employed modified o-phthalaldehyde derivatives to staple linear and cyclic peptides to produce fluorescent, rigid cyclic and bicyclic peptides. The mild conditions and rapid nature of the reaction conditions empowered us to construct a library of α-MSH derivatives. This allowed us facile access to many derivatives from a smaller pool of linear scaffolds. We then tested their affinity for melanocortin receptor 1 (MCR1) and were pleased to discover that many of our compounds were more potent than the native ligand and had a Ki ~1nM. With this result in hand, we turned our attention to the possibility of mimicking a complex peptide natural product. With our group's expertise in the synthesis of α-amanitin and its analogues, it seemed a logical proving group for our stapling method. We adapted our total synthesis strategy for α-amanitin to allow for an ultimate intra-annular cyclization step that gave us access to a fluorescent isoindole amatoxin. We were pleased to find that it retained some toxicity (IC50 against CHO cells = 50μM) believe it may find utility as a fluorescent transcription probe.

YI-P259 Design and Synthesis of Cationic NucleoPeptides (CNPs) as Noncovalent Carriers for PNAs and Oligonucleotides

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Peptidonucleic acids (PNAs) and oligonucleotides (ONs) open the way for a fine modulation of non-coding RNAs function. Despite the efforts carried out to enhance their physical-chemical properties, cell penetration still represents a matter and carrier-cargo approach is often required. In this respect, herein we present a class of molecules, namely Cationic NucleoPeptides (CNPs), conceived reverting the paradigma of nestling Cell Penetrating Peptides (CPPs) sequences/components on nucleic acid scaffolds. In NCPs, nucleobases protrude from a peptide backbone consisting in a pattern of lysines, bearing nucleobases on side chains, and arginines, to assist cell penetration. We developed a switchable protective strategy to fully assemble homo- and eteronucleobase sequences on solid support with high purity.[1] CNPs binding with complementary nucleic acids was investigated by circular dichroism analysis and UV/CD melting curve and their stability compared with PNA/ON models. Upon ascertaining penetration in LN229 glioblastoma cells, we balanced NCPs recognition properties in complementary PNA and RNA hybrids to deliver these noncovalent assemblies in cell.[2]

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YI-P260 The Permeability Landscape of Cyclic Hexa- and Heptapeptides

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Broad exploration of the physicochemical properties of cyclic hexa- and heptapeptides was accomplished through OBOC synthesis of geometrically diverse libraries of 1800 and 3600 compounds respectively. Stereochemistry was encoded by isotopic labeling, enabling PAMPA (parallel artificial membrane permeability assay) and hydrocarbon partition coefficient determination on complex mixtures of 150 compounds each. Sequencing of individual peaks was performed by CycLS[1] (MSMS-based). Assay data was correlated to tandem mass spectrometry data via retention time matching and analyzed for general and motif-specific trends. Several compounds with high measured LPE[2] (lipophilic permeability efficiency) were resynthesized and assayed for permeability and hydrocarbon partition coefficient to confirm mixture-based physical property measurements. The predictive ability of LPE for changes in passive permeability provoked by side-chain variance in these backbone geometries was investigated by synthesis of OBOC libraries which underwent the same assays and sequencing procedure as the initial exploratory libraries.

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YI-P261 Unexpected Findings in the Development of Bivalent Polo-like Kinase 1-binding Ligands with Potentially Important Implications

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The polo-like kinase 1 (Plk1) is a Ser/Thr-specific kinase, which is an important mediator for cell cycle regulation and a recognized anti-cancer molecular target. In addition to its catalytic kinase domain (KD), Plk1 contains a polo-box domain (PBD), which engages in protein-protein interactions (PPIs) essential to proper Plk1 function. We have developed a number

of low nanomolar-affinity PBD-binding peptide inhibitors. However, we have reached an apparent limit to increasing the affinities of these monovalent ligands. Accordingly, we undertook an extensive investigation of bivalent ligands, designed to simultaneously engage both KD and PBD regions of Plk1. This has resulted in bivalent constructs exhibiting more than 100-fold Plk1 affinity enhancement relative to the best monovalent PBD-binding ligands. Startlingly, and in contradiction to widely accepted notions of KD – PBD interactions, we have found that full affinities can be retained even with minimal linkers between KD and PBD-binding components. In addition to significantly advancing the development of PBD-binding ligands, our findings may cause a rethinking of the structure – function of Plk1.

P262 Minimum Acquisition of Double Mutation in Human Calcitonin Enhances its Resistance to Fibrillization and its Use as Therapeutic Polypeptides

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The most common obstacles in the development of therapeutic polypeptides are peptide stability and aggregation. Human calcitonin (hCT) is a 32-residue hormone polypeptide secreted from the C-cells of the thyroid gland and responsible for calcium and phosphate regulation in the blood. hCT reduces calcium levels by inhibiting the activity of osteoclasts which are bond cells that break down bone tissue or decreasing the resorption of calcium form kidneys. Calcitonin injection has been used to treat osteoporosis and Paget's disease of bone. However, hCT is an aggregation-prone peptide with a high tendency to form amyloid fibrils. As a result, salmon calcitonin (sCT) which has 16 different residues to hCT and lower propensity to aggregate has been chosen as clinically substitute to hCT. However, significant side effects including immune reactions have been shown along with the use of sCT injection. To overcome this difficulty, we found that the two residues (Y10 and N17) would play key roles in inducing fibrillization of hCT. Double mutation of hCT could greatly enhance its resistance to aggregation and also can serve as peptide- based inhibitor to prevent the amyloid formation by hCT.

P263 The Effects of Bioactive PeptidesTM on Skin Wound Healing in Rats

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Wound healing is a delicate sequential event consisting of hemostasis, inflammation, proliferation, and remodeling. Any step delayed during wound healing can cause chronic wounds and threatens patients' health. In this study, we tested five compounds (A to E) with a unique blend of bioactive peptides and/or chemicals in each of them. We utilized a full-thickness skin excisional wound SD rat model to monitor weight gain and the rate of wound closure for 7 days post wound induction. Skin tissues were harvested at the end of the experiment and histological analyses (hematoxylin/eosin and Masson's trichrome staining) were conducted for wound healing process. There is no significant difference in weight change among the five treatments, although rats treated with compound C showed slightly higher weight gain from day 3 to the end of experiment. Compared to the non-treated side, compounds B and C enhanced rate of wound closure significantly at day 5 and 7 (all $p<0.05$). We found that the compound B- and C-treated side exhibited significantly thicker epithelium than its non-treated side (both $p<0.05$). We also found that compound A could promote wound healing by allowing faster progression from inflammation to proliferation and remodeling phases. In addition, compared to compound D, compound E exhibited slightly stronger effects on wound area reduction and early collagen formation. In summary, our data suggest that both compound B and C were capable of promoting wound reepithelization. We will further test if these compounds can help wound healing in a diabetic animal model.

The project collaborated with the Hymed Group Corporation, Bethlehem, PA 18015.

P264 A Strategy for Thioamide Incorporation during Fmoc SPPS with Robust Stereochemical Integrity and Applications to Peptide Chemistry

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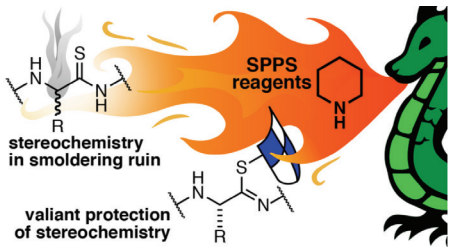
Thioamides are versatile chemical probes for studying the function, behavior and stability of protein structure. Because thioamides display differences in hydrogen bond- donating ability and Lewis basicity relative to amides, they have been used to probe the role of hydrogen bonding and $n\rightarrow\pi^*$ effects in protein secondary structure. Further, thioamides can also be employed in photochemical schemes as photoswitches or as fluorescent quenchers to study conformational changes within peptides.

Given the importance of thioamides in peptide chemistry, methods for their incorporation into peptides with robust stereochemical integrity are highly desirable. While approaches for site-selective insertion of a thioamide into the sequence have been developed, the α-C of the thioamide residue is prone to racemization under conditions required for Fmoc deprotection. Subsequent coupling and Fmoc-deprotection of additional residues following the thioamide further erodes the stereochemical integrity of the thioamide residue. For this reason, thioamides are often only incorporated within a few residues of the end of the sequence to mitigate the risk of racemization. Unfortunately, this restriction also limits the sequence space in which thioamide may be introduced to probe structure. We developed a strategy for robust synthesis of thioamide-containing peptides with high stereochemical integrity. We demonstrate that thioamides can be reversibly protected to preserve the α-C stereochemistry of the thioamide residue during SPPS. This capability enables thioamides to be incorporated into longer sequences (and near the C-terminus). Applications to folded peptide structures will also be discussed.



P265 Insights into the Mechanism of Action of pepR, a Viral-derived Peptide, against *Staphylococcus aureus* Biofilms

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Bacterial infections are a major human health threat given both the increasing incidence of drug-resistant bacteria and the ability of bacteria to form biofilms. Biofilm-related infections are particularly difficult to treat due to their reduced susceptibility to conventional antibiotics. Given the increasing interest in the use of antimicrobial peptides (AMPs) as alternatives against bacterial biofilms, our work is focused on the key factors that govern the antibiofilm action of a model AMP at the molecular level. pepR, a peptide derived from the Dengue virus capsid protein, was selected as an AMP model because it abrogates biofilm formation and kills bacteria in preformed *S. aureus* biofilms. Using a combination of flow cytometry and confocal fluorescence microscopy assays, with quantitative imaging data treatment, we showed that the ability of pepR to prevent biofilm formation and act on preformed biofilms is directly related to bacterial membrane permeabilization. The effect of the peptide on biofilm-associated bacteria is dose and depth-dependent, and is controlled by its diffusion along the biofilm layers. Overall, our study contributes to shed light on the antibiofilm mechanism of action of AMPs, particularly regarding the importance of their diffusion through the biofilm matrix on their activity.

YI-P266 A Light-Responsive Platform Utilizing a Photoactivatable Hemolytic Peptide Derivative for Therapeutic Protein Delivery

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Peptides and proteins often serve as desirable therapeutic alternatives to small molecules due to their specificity and potency. However, the beneficial properties of biotherapeutics are often overshadowed by their poor in vivo stability and/or rapid clearance. Although the latter undesirable traits can be partially overcome by high therapeutic doses, this can result in deleterious side effects. We have developed a red blood cell (RBC)-based biomolecule delivery system that is mediated by a photoactivatable hemolytic peptide derivative. This targeted delivery system offers several potential attributes: on-command delivery to a targeted site, applicability to an array of protein and peptide therapeutics, and dramatically reduced therapeutic dose.

We have developed a peptide derivative that is lytically inactive until exposed to long-wavelength light. This light-activatable peptide is externally loaded onto the outer membrane of RBCs internally loaded with therapeutic proteins, generating "light-responsive RBCs". Exposure to long-wavelength light triggers hemolysis, resulting in the release of therapeutic proteins from the RBCs with precise spatial and temporal control. We have also demonstrated that light-induced protein release can be tailored to different wavelengths using distinct fluorophore antennas. This property potentially allows different protein/peptide therapeutics to be sequentially delivered to a diseased site.

Targeted delivery systems of proteins and peptides have the potential to increase the stability of the therapeutic, deliver a more concentrated amount at the diseased site, and decrease side effects relative to systemically distributed therapeutics. In summary, our peptide-based photo-hemolytic release system potentially offers unprecedented control of the release of biomolecules.

YI-P267 Synthesis of Aryl-Thioglycopeptides through Chemoselective Pd- Catalyzed Conjugation

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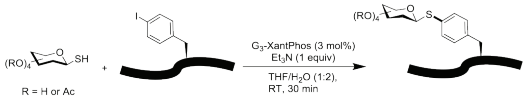
We describe herein a methodology for the thioglycoconjugation of iodoaryl peptides and aminoacids through palladium-catalyzed coupling with glycosyl thiols. Palladacycle pre-catalyst G3- XantPhos[1] was found to be highly efficient for the *C-S* bond formation under semi-aqueous conditions. The *S*-glycosylation is operationally simple and displays broad substrate scope. A wide range of thioglycosylated amino acids building blocks were synthesized with complete control of anomeric stereochemistry. We studied the suitability of the synthesized building blocks in standard Fmoc-based SPPS protocols. Thioglycoconjugation was carried out at Tyr1 of a lipo-triazolo-peptide analogue of the neuropeptide kisspeptin-10 (KP10).[2] The strategy was also successfully applied to the thioglycosylation of unprotected peptides: to demonstrate the broad potential of this technique for late stage functionalization, we incorporated challenging β-S-GlcNAc- and α-S-GalNAc-derivatives into very long unprotected peptides derived from the mucin MUC1. The generated thioglycoconjugates were used as biochemical tools to study their recognition by *O*-GalNAc-specific lectins, suggesting future in applications of this method in chemical (glyco)biology.[3]



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P68 From IUPAC Nomenclature to Nobel Prize

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In 2006, the field of peptide science lost one of its most distinguished contributors, Bruce Merrifield, 1984 Nobel Laureate in Chemistry. Hopefully, the Nobel Committees will, in the future, again recognize the importance of peptides in all facets of life. Toward that end, a project has been developed in the area of molecular literature to gain the attention of the Swedish Academy, the organization that selects the winners of the Nobel Prize in Literature. This project involves the design and study of two "name peptides".

The International Union of Pure and Applied Chemistry-International Union of Biochemistry, Joint Commission on Biochemical Nomenclature's (IUPAC-IUB, JCBN's) single letter symbols for the names of amino acids includes unambiguous assignments for 21 of the 26 letters of the English language. The only letters without such assignments are J and O (unassigned), and B, X, and Z (ambiguous assignments). The unambiguous symbols/letters can be used to form an enormous variety of names, words, and phrases that can be considered as potential "name peptides". Consequently, dictionaries and other literature sources can be used as sources of ideas for potential novel peptides. For example, the name of a well-known retailing company, Walmart, was used to design and create peptide WALMART, which was then tested for, and found to exhibit, anticancer and antimicrobial properties. A similar approach will be used for the names, Swedish Academy and its Swedish language equivalent, Svenska Akademien, to produce and study the name peptides, SWEDISHACADEMY and SVENSKAAKADEMIEN.

P269 Leveraging ADME Tools to Optimize Peptide Drugs for Intracellular Targets

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Poor intracellular exposure is a primary barrier to translating biochemically active peptides to pharmacologically active drug candidates. Intracellular exposure is primarily determined by two factors: metabolic stability and membrane permeability. This poster presents examples of application of ADME assays to inform SAR for permeability and stability, both required for intracellular exposure.

A Hela cell homogenate stability assay — measuring the rate of intact peptide disappearance over time — was used to assess metabolic stability as well as to identify the primary site of metabolism by high resolution mass spectrometry (HRMS). Within programs, good cell activity correlated with higher proteolytic stability measured in the cell homogenate assay. Only peptides with longer half-life (>1hr) showed strong potency in a cell activity assay despite strong binding to the target in a biochemical assay. Structural modifications around the primary soft spot improved metabolic stability and cell activity. Computational tools, model membrane systems and cell uptake were used to evaluate membrane permeability. The total drug partitioning constant (Kp), defined as the peptide concentration ratio of cells to media, was determined for metabolically stable peptides. Kp value aligned with cell activity, suggesting Kp could be a valid tool to differentiate peptides. The possible mechanism of peptide permeation will be discussed in the poster too.

In summary, approaches to characterize and optimize metabolic stability combined with membrane permeability have been applied to drive SAR of novel peptide drug candidates.

P270 Anchor Residues Guide Form and Function in Grafted Peptides

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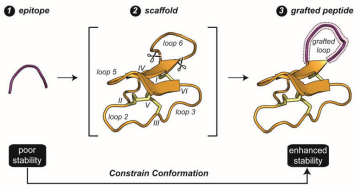
Loops at protein-protein interfaces are a rich source for peptide leads that have high specificity and low toxicity. Although such peptides typically need to be constrained to overcome thermodynamic and metabolic limitations, design guidelines to obtain a successfully constrained peptide, and thus facilitate transition from loop to drug, are relatively poorly formulated. Here, we survey the structures of interface loops and find the position of the terminal residues to be a key determinant of conformation. We use this knowledge to improve the process of molecular grafting, a valuable approach for constraining and stabilising peptides by fusing them to a suitable scaffold. We show that an informed choice of where a loop is 'anchored' to a scaffold improves its form and function. This knowledge can help guide the choice of loop and its matching scaffold, and thus increase the success rate of designing stable and potent peptide drug leads.



P271 A Fluorine-thiol Stapled Peptide to Probe Protein-protein Interactions

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Protein-protein interactions (PPIs) are responsible for important molecular processes and are involved in the development of many diseases including cancer. For instance, the Wnt pathway involved with the β-catenin-Axin interaction is crucial for the development of acute myeloid leukemia (AML). Thus, targeting PPIs has emerged as the central paradigm of medical chemistry since molecules inhibiting a single PPI is expected to be more specific to biological pathways than enzyme-inhibiting molecules. Since PPIs usually contain large and shallow surface areas, it is challenging to develop small molecule inhibitors except for a few successful ones. Alternatively, peptide mimics of the interacting proteins, usually an α-helix stabilized by covalent crosslinking, can maintain the desired tertiary structures, the associated binding specificities, and even improve the stability and cellular permeability in comparison to natural proteins. Hence, stapled peptides are becoming a major force in PPI modulation, and one lead p53 mimic (ALRN-6924) has entered clinical testing to treat a diverse set of tumors. We are hereby reporting a new stapling approach using our recently discovered fluorine-thiol chemistry, which is highly efficient in

solution. The α-fluoroamide containing building block can be incorporated into model and Axin-derived peptides by solid phase synthesis, followed by efficient macrocyclization with thiolated linkers that incur less membrane toxicity compared to the most commonly used hydrocarbon crosslinker for olefin metathesis. Circular dichroism studies further confirmed the stapling linker's effect on promoting helicity. Finally, enhanced cell penetration has been also observed with microscopic imaging of treated cancer cell lines.

YI-P272 Development of Selective and Cell-permeable CBX8 Chromodomain Ligands using DNA-encoded Libraries of Peptidomimetics

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Polycomb repressive complex 1 (PRC1) has been implicated critical for epigenetic regulations at the level of biochemical activity and cancer relevance. Polycomb group Chromobox protein homolog (**CBX**) proteins, CBX2, 4, 6, 7, 8, mediate specific targeting to genomic loci through binding to methylation sites on histone tails via the N-terminal chromodomain (ChD). High homologies in sequence and structure among the CBX chromodomains (ChD), however, obstruct the development of selective CBX ChD inhibitors, which would be chemical probes for determination of paralog specific function. The CBX ChDs bind target peptides in an extended conformation, with histone peptides completing a beta sheet structure with the ChD. The necessity of these hydrogen bonding interactions make development of cell permeable peptide ligands a challenge. We have utilized DNA-encoding and affinity selection with iterative, on-DNA medicinal chemistry optimization for the development of peptidic and peptidomimetic ligands for the CBX ChDs. This effort has led to the development of SW2_110A, a potent (800 nM), selective (minimally >20-fold selectivity compared to other paralogs), cell-permeable peptidomimetic inhibitor of the CBX8 ChD. Cellular binding validation and activity evaluation indicated the therapeutic potential of targeting the CBX8 ChD in MLL-AF9-translocated leukemogenesis. Cytosolic delivery efficiency of SW2_110A was evaluated and quantified using the chloroalkane penetration assay (CAPA). In addition to defining the role of CBX8 in oncogenesis, this chemical probe is used to decipher CBX paralog-specific roles in chromatin regulation. The success of SW2_110A provides great promise to the development of potent, selective and cell permeable probes for the full CBX family. This work highlights the advantages of low cost and ease of use of DNA-encoding for scanning positional peptide and peptidomimetic libraries.

P273 Short Arginine Analogs: Peptide Synthesis and Prediction of Biological Effects

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Arginine, a semi-essential amino acid, is involved in various important metabolic processes. Moreover, Arg plays an important role in signal transduction as substrate of mammalian NO synthases, which convert arginine to citrulline and the crucial cellular signaling molecule nitric oxide. In order to conduct SAR studies on enzymes that metabolize arginine, peptides containing short-chain analogues of Arg such as 2-amino-4-guanidino-butyric acid (Agb) and 2-amino-4-guanidino-propionic acid (Agp) are interesting tools.

The purpose of this work was to synthesize model peptides containing short-chain arginine analogs (Agb and Agp), to explore their proteolytic stability using docking simulations, and to computationally predict the impact of these analogs on enzymes of arginine metabolism.

For the synthesis of peptides with arginine analogs, different combinations of protecting groups were used. It turned out that for the analog with the shortest chain (Agp), best results were obtained with bis-Boc protection in the side chain, whereas for Agb, mixed Pbf,Boc protection of the guanidino group is favorable.

Docking results of those model peptides with trypsin correlate well with the experimentally obtained data for their hydrolysis. Our further computational docking studies suggest that short-chain analogs Agp and Agb would not interact with the following enzymes: ARG, iNOS, ADI and ADC. However, Agp and Agb should bind strongly to eNOS, AGAT and ASS, forming stable enzyme-substrate complexes, thereby potentially blocking these enzymes' actions. Therefore, shorter arginine analogs might be used in the treatment of certain disorders, as they might block important metabolic pathways.

P274 Impact of Aza-Amino Acid Substitution on β-Hairpin Stability

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Peptidomimetics are designed to alter the chemical characteristics of a natural peptide to increase resistance to proteolysis, often with the additional goal to stabilize specific secondary structures such as β-turns, helices, and β-sheets.[1] An aza-amino acid is defined by a Cα to N substitution, resulting in local conformational constraints alongside alterations in hydrogen bonding properties and loss of a defined chiral center.[2] This substitution has been shown to induce turn-like geometries when installed in the *i*+1 or *i*+2 positions of the turn region[2] and be tolerated within type II polyproline helices, leading to hyperstability in collagen peptide mimics.[3] Here, we envisioned the single carbon to nitrogen atom substitution could be used to reinforce cross-strand hydrogen bonding capabilities while simultaneously reducing the propensity for self-aggregation in β-hairpin peptidomimetics. Minimalistic, water

soluble, β-hairpin model systems have been created for the purpose of studying β-sheets. [4] Initial investigations into the introduction of an aza-amino acid within the extended β-strand region of an antiparallel β-sheet model were conducted. Folding propensity of the azapeptide β-hairpin analogs was assessed using NMR spectroscopy and subsequently compared with a negative (unfolded) and positive (cyclic) controls.

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YI-P275 Development of Protein Catalyzed Capture Agents for the Detection of *E. coli* O157:H7 in Food

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Foodborne pathogens represent a significant health concern worldwide with the World Health Organization estimating 582 million illnesses annually.* The enterohemorrhagic *E. coli* (EHEC) O157:H7 is of particular interest, attributing to more than 3,600 illnesses in the US each year.* Current culture based detection methods are slow and fail to detect contamination in real-time. Newer assays utilizing *E. coli* specific antibodies to capture and concentrate target bacteria have demonstrated improved detection times and limits. The use of antibodies as a target recognition element for bio-sensing represents the current gold standard as they exhibit target specificity and excellent binding affinities however, they are costly, labor intensive to produce, and have limited storage and shelf-life capabilities. Peptides provide an advantageous alternative to antibodies as they exhibit improved storage and shelf-life capabilities, are inexpensively generated by chemical synthesis, and can incorporate unnatural chemistries. This work seeks to identify *E. coli* O157:H7 specific peptide affinity ligands, developed through the protein catalyzed capture (PCC) agent method, for applications in detecting pathogen contaminated food and beverages. A One Bead-One Compound (OBOC) cyclic peptide library is screened against synthetic peptide epitopes (SynEps) derived from unstructured, solvent exposed domains of intimin, a protein target of interest. Library "hits" are generated *in situ* by uncatalyzed, entropically favored "click" reactions between alkyne and azide functional handles located on the library's cyclic peptide and SynEps respectively. Magnetic beads will then be functionalized with designed multi-valent peptide constructs to enable *E. coli* separation and subsequent detection from the bulk food sample.

P276 Short-Acting Peptidic V2 Receptor Agonists for the Treatment of Water Retention Disorders

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Arginine vasopressin (AVP) is the primary hormone controlling water homeostasis in animals and humans.[1] The vasopressin analogue desmopressin (dDAVP) is a potent V_2 receptor agonist and is approved in many countries for the treatment of diabetes insipidus, primary nocturnal enuresis and nocturia. In addition, dDAVP is marketed for the treatment of coagulation disorders such as haemophilia A and von Willebrand's disease. Hyponatremia, the most frequent plasma electrolyte abnormality in humans, is a known adverse effect of dDAVP. Since excretion is primarily via the kidneys, age-related decline in kidney function can lead to slower elimination and prolonged antidiuresis. To identify peptidic V_2R agonists with a low risk of hyponatremia, a series of [Val[4]]dDAVP analogues truncated at the C-terminus were synthesized and evaluated for *in vitro* potency at the hV_2 receptor, selectivity versus related receptors ($hV_{1a}R$, $hV_{1b}R$, hOTR) and their pharmacokinetic profiles in rodents and other species were assessed. In addition, non-renal clearance was determined in nephrectomized rats to identify compounds less reliant on glomerular filtration. Allometric scaling was used to predict pharmacokinetic parameters in humans. Analogues with predicted higher systemic clearance than dDAVP were selected to enable treatment of nocturia or other indications where shortened antidiuretic action might be beneficial. Several analogues with desirable profiles were identified and the following two candidates were nominated for clinical development in nocturia: (c(Bua-Cpa-Thi-Val-Asn-Cys)-Pro-Agm) and c(Bua-Cpa-Thi-Val-Asn-Cys)-Pro-D-Arg-NEt_2).

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P277 Evaluation of Hybrid Peptidomimetics for the Use in Neuropathic Pain

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Neuropathic pain (NP) is defined 'as pain caused by a lesion or disease of the somatosensory nervous system' (IASP 2011). NP can have a significant negative impact on a patient's quality of life. Data have indicated that 7 - 10% of the general population in the different countries are affected by NP. This kind of pain is difficult to manage because of the heterogeneity of its aetiologies, symptoms and underlying mechanisms, furthermore it responds poorly to standard treatments, including those used to treat acute pain. A new approach to the neuropathic pain treatment and the search for new, effective drugs and their combinations is needed.

In this work we present the synthesis, in vitro and in vivo assay results of novel hybrid peptidomimetics for the use in neuropathic pain, comprising two pharmacophores: an opioid

receptors agonist (OP) and an melanocortin receptor (MC4R) antagonist. The pharmacophores were connected by spacers (linkers) of different lengths. The results of affinity to different type of receptors (MOR, DOR and MC4R) and in vivo activity in the model of neuropathic pain will be shown.
Acknowledgment

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P278 Methods for the Study of Catalytic Peptides using a Colorimetric Assay

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Peptides and peptidomimetics are appealing classes of catalysts because they occupy a middle ground between enzymes and organocatalysts, retaining some of the complexity of the former and the synthetic accessibility and robustness of the latter. However, studies of lead catalytic peptides have shown that small chemical changes in the structure can result in unexpected differences in catalytic activity. Therefore, methods to quickly screen large numbers of catalytic peptides and explore analogues of lead sequences would be beneficial for the identification of novel catalysts and for the study of the effects of structure on catalytic activity for a given reaction. We have developed a colorimetric assay based on dye displacement to study minimal peptide catalysts for aldol reactions. Efforts towards using this type of assay to study a series of catalytic sequences will be presented.

P279 NanoClick Assay: A High-throughput, Target Agnostic Permeability Assay Combining NanoBRET Technology and Intracellular Cycloaddition Chemistry

Chengwei Wu, Jeffrey Schubert, Raymond Evers, Lan Ge, Anthony Partridge, Andrea Peier, Tomi Sawyer, David Tellers

Measuring cell permeability of peptides is critical for understanding and optimizing activity of ligands with intracellular targets and assessing potential for oral bioavailability. While small molecule permeability has been extensively studied, methods for assessing peptide permeability are often lower throughput, target-specific, or less robust. Herein we describe the Nanoclick assay, a high-throughput and target agnostic approach for assessing the permeability of azide-containing peptides. We examined p53/MDM2 model system to study macrocyclic peptide cell permeability and cellular activity against intracellular target. High correlation between NanoClick and p53 cell functional assays were found.

YI-P280 Enhanced Conformational Stability of WW and SH3 by PEG Staple

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Conformational stability of peptides is of particular interest in both industrial and academic research. A lot of effort has been put on the conformational stability of helixes whereas few cases of stabilizing a β-sheet peptide were reported. Herein, based on a β-sheet peptide, a new method of conformational constraint via stapling together with site specific PEGylation is reported, where the conformational stability is enhanced and aggregation propensity is reduced. Upon the optimization of the PEG (polyethylene glycol) length, we found an optimum length for stapling a β-turn at a distance of i and i+3, where the conformational stability can be improved to -1.15 kcal/mol. This PEG based stapling strategy can not only be applied to a β-turn, it can also improve the conformational stability through space, at position i and i+16. We further applied this long distance space crossing PEG stapling to a small protein SH3 domain, and it turned out the protein can be stabilized by -1.26 kcal/mol. To the best of our knowledge, it is the first time that polyethylene glycol is used in a stapling strategy to be reported. Overall, on the way of peptide drug discovery, our research provides another method of peptide and protein modification, with improved thermodynamic stability and reduced aggregation propensity.

YI-P281 Long-term Walnut Oligopeptides Prevents Memory loss in Aged SAMP8 Mice by Decreasing Oxidative Stress and Down-regulating the PI3K/Akt Signaling Pathway in Hippocampus

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Walnut Oligopeptides (WOPs), the effective component of walnut, has been reported to have a neuron protective effect, but the preventive effect on Alzheimer's disease (AD) related memory loss and the underlying mechanisms have not been well determined. The senescence-accelerated mouse (SAM) is a useful model of AD-related memory impairment. In the present study, SAMP8 mice aged 4 months were chronically treated with ginsenoside (3 dose groups were given WOPs in diet for 6 months). The three groups were treated with WOPs 110, 220 and 440 mg/kg•bw per day, respectively. Placebo-treated aged mice and young ones (4 months old) were used as controls. In addition, SAMR1 mice were used as "normal aging" control. The beneficial role of WOPs was manifested in the prevention of memory loss in aged SAMP8 mice. The optimal dose of WOPs is 220 or 440 mg/kg per day. WOPs as found to significantly improve the memory ability of AD rats and anti-oxidase level significantly increased in serum. WOPs also reduced the content of Aβ and p-tau and improved the expression of PI3K and p-Akt/Akt in the hippocampus. In conclusion, WOPs could improve the memory ability and reduce the content of Aβ and p-tau in SAMP8. The beneficial effects of WOPs were in part mediated by PI3K/Akt signaling pathway activation.

P282 Developing D-peptide Based Combination Immunotherapy to Attack Colorectal Cancer

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Colorectal cancer is the third most common cancer and the second leading cause of cancer death in the United States. With the recent rapid development of immunotherapy, immune checkpoint inhibitors, such as Keytruda (anti-PD-1 monoclonal antibody), have been FDA-approved to treat microsatellite instability-high (MSI-H) colorectal cancer. However, Keytruda therapy shows low efficacy in these tumors due to drug

resistance. Therefore, combination therapy with other immune checkpoint inhibitors is being pursued to increase therapeutic efficacy. A recently discovered immune checkpoint, VISTA, is reported to be highly expressed in colorectal cancer and may be responsible for tumor immune escape[1]. Another study reported that inhibition of the DKK2/LRP5 interaction reduces tumor immune evasion and enhances the effect of PD-1 blockade in colorectal cancer[2].

Here, we describe our efforts to develop mirror-image peptide (D-peptide) inhibitors against two immune checkpoints (PD-1 and VISTA) and one secreted protein (DKK2) for colorectal cancer. We will screen for such inhibitors using mirror-image phage display, which requires chemical synthesis of mirror-image versions of the functional domain in these protein targets (PD-1 IgV, 126 aa; VISTA IgV, 144 aa; DKK2C, 88 aa). The total chemical synthesis of these proteins (in L-) has been achieved using a combination of solid-phase peptide synthesis and native chemical ligation. Hydrophobic aggregation and poor solubility during synthesis and purification were severe challenges, which we overcame using isoacyl dipeptides and a "Helping Hand" traceless linker to introduce solubilizing Arg tags[3]. The mirror-image version of these proteins are now being synthesized using the same scheme. We will then use these mirror-image targets to screen for D-peptide inhibitors. Subsequently, combination D-peptide therapy will be tested using colorectal cancer tissue culture and animals models. Compared to monoclonal antibodies, D-peptides have several advantages, including lower manufacturing cost, lower immunogenicity, and improved diffusion into solid tumors[4]. Therefore, D-peptide immune checkpoint inhibitors may provide a promising alternative to antibodies for treating drug-resistant cancers.

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P283 A Cyclic Peptide as a Target Cell Selective Drug Delivery Vehicle

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Targeted drug delivery through drug-conjugates is an active area of research. In order to achieve target selectivity, researchers has been exploiting binding affinity of drug delivery vehicle such as antibodies and small molecules against a target protein on a cell. In this study, we have attempted to utilize a cyclic peptide as such a vehicle by the use of virtues of their high specificity and affinity to a target protein of interest. A major advantage of this approach is the ability to prepare a homogenous peptide-drug conjugate since the cyclic peptide can be synthesized by solid-phase peptide synthesis method, giving full control over regiospecificity. We report a cyclic peptide-drug conjugate (cPDC) using a cytotoxic drug and a previously selected cyclic peptide with a very high affinity for the target membrane protein. The cyclic peptide for cPDC was obtained by the RaPID (Random nonstandard Peptides Integrated Discovery) system. We conjugated the obtained peptide and a drug via a linker and evaluated the selective cytotoxic activity of the synthesized cPDCs whose activity was dependent on the target protein expression.

YI-P284 Using X-ray Crystallographic Structure of a Teixobactin Derivative to Probe the Mechanism of Action of Teixobactin

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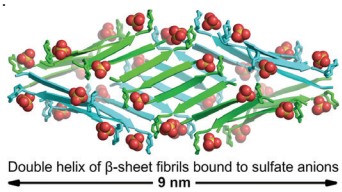
This presentation will describe our ongoing studies of the antibiotic teixobactin through X-ray crystallography and structure-activity relationships of teixobactin analogues. The X-ray crystallographic structure of a teixobactin analogue shows supramolecular assembly through the formation of antiparallel β-sheets and creates binding sites for oxyanions. β-Sheet dimers are key subunits of these assemblies. We are currently using hints from the structure to designe structure- activity relationship experiments to elucidate mechanism of action of teixobactin.



YI-P285 Development of Photoimmunotherapy Probe using a Small Antibody Mimetic with Structurally Constrained Anti-HER2 CDR Peptides

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Small proteins that have high affinity for cancer cell surface markers are promising cheap alternatives for antibody mimetics. These small proteins overcome some limitations of antibody such as high molecular weight, limited tissue penetration, and high production cost. In addition, small antibody mimetics may be applicable to an emerging photoimmunotherapy that uses infrared light to activate rapid and selective killing of cancer cells.

We recently designed 75 types of small antibody mimetics, called FLAPs, which are composed of a biocompatible small scaffold protein and structurally constrained 6-amino-acid CDR peptides derived from anti-HER2 monoclonal antibodies (mAbs). After evaluation of the FLAPs, we found that FLAP-113 has the highest affinity for HER2 with a KD value of 24 nM. By using FLAP-113, we are trying to develop an in vivo immunoprobe for imaging and targeting HER2-expressing tumors. The binding specificity of FLAP-113 was confirmed by competitive ELISA using parental mAb (trastuzumab) that recognizes the same HER2 epitope as FLAP-113. Evaluation with cultured cells revealed that FLAP-113 specifically bound to SK-BR-3, a human breast cancer cell line that overexpresses

HER2, but not to HeLa cells that hardly express HER2. Then, FLAP-113 was fused to c-tag, a tag sequence containing cysteine residue at the C-terminus, and covalently linked to a synthetic linker labelled with a near infrared fluorescent-dye through the c-tag. Currently, we are optimizing the construction to assess its selective killing of cancer cells overexpressing HER2. Our approach may open a new avenue to develop practical probes for targeting and imaging molecules for clinical use.

YI-P285 Generation of Cell Culture Substrate by Protease Digestion of Amyloid beta Peptide

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Amyloid bata peptide (Aβ) forms amyloid fibrils involved with Alzheimer's disease[1]. Recent studies about Aβ in nanomaterials field were undertaken since amyloid aggregates possess high regula1rity of molecular structure, thermodynamic stability and mechanical strength. Additionally, some studies showed that morphologies of amyloid fibrils changed by mixing Aβ with another peptide. In this study, focusing on development of cell culture substrate, we attempted to generate nanostructures composed of some Aβ fragments by following two processes; β fibrils were formed by assembly of Aβ; β) the fibrils were digested by protease to obtain nanostructure composed of some fragments (dAβs). Then, we identified fragments in dAβs and attempted to compare cell adhesion abilities of dAβs with those of the fragments.

We first generated amyloid fibrils by aggregation of Aβ(1-40) and performed digestion of the fibrils using three proteases to obtain dAβs. We conducted cell adhesion assay using these dAβs. One of dAβs, Tryp-dAβs (Aβ digested by trypsin), showed a better cell adhesion ability than gelatin. Then, we identified some Aβ fragments in Tryp-dAβs using HPLC and MALDI-TOF MS. Futhermore, we demonstrated cell adhesion assay using these Aβ fragment samples after synthesizing the fragments. Tryp-dAβs showed better cell adhesion ability than Aβ fragment alone samples. Throughout this study, nanostructures consisting of some Aβ fragments were successfully obtained by using this method. Funtermore, they were more useful than nanostructures consisting of one Aβ fragment for cell culture substrate.

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YI-P287 An Efficient Method for the Expression and Purification of Aβ(M1–42) and N-terminal Cysteine Aβ(1–42)

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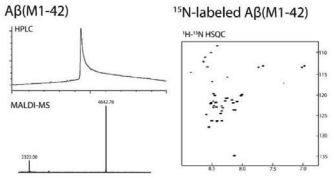
N-Terminal methionine-extended Aβ, Aβ(M1–42), is a readily expressed and widely used form of Aβ with properties comparable to those of the natural Aβ(1–42) peptide. Expression of Aβ(M1–42) is simple to execute and avoids an expensive and often difficult enzymatic cleavage step associated with expression and isolation of Aβ(1–42). This presentation reports an efficient method for the expression and purification of Aβ(M1–42) and 15N-labeled Aβ(M1– 42). This method affords the pure peptide at β19 mg/L of bacterial culture through simple and inexpensive steps in 3 days. A simple method for the construction of recombinant plasmids and the expression and purification of Aβ(M1–42) peptides containing familial mutations is developed. This presentation also reports an efficient expression of N-terminal cysteine Aβ by taking advantage of N-terminal methionine processing and a utility of N-terminal cysteine peptides through fluorescently labeling using cysteine-maleimide conjugation. We anticipate that these methods will enable experiments that would otherwise be hindered by insufficient access to Aβ.



P288 Antibacterial Activity of KU-2657 Peptide in In vivo Model

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Increasing resistance to conventional antibiotics in bacteria is a global obstacle to effective treatment of bacterial infections. To overcome this problem, a lot of peptide agents derived host defense peptides have been developed. In the present study, we identified the novel peptide, KU-2657, a 23-residue antimicrobial peptide, which was designed by bioinformatics. This peptide showed bactericidal activity against gram-positive bacteria as well as gram-negative bacteria. It also exhibited strong bactericidal activity against multi-drug resistant bacteria. To examine the molecular mechanism, electron microscopy (scanning and transmission) and flowcytometric analysis were applied. The peptide treated into bacteria efficiently binds to bacterial membrane. From the results obtained from this study, it seems that KU-2657 disrupts bacterial membranes to release bacterial cytoplasmic contents to the outside. The precise molecular mechanism of this activity remains to be studied. Taken together, the results of this study provide insight into the investigations to understand the action of peptides in human serum and KU-2657 is a promising agent to overcome multi-drug resistance.

YI-P289 Synthesis of Fyn SH2 Superbinder Domain

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Wild-type SH2 domains bind to the Stat3 protein at a phosphorylated tyrosine (pTyr) during JAK2/STAT3 signaling. This signaling is responsible for cell growth but is constitutively active in several human cancers. Efforts towards pTyr-containing peptide mimics targeting the SH2 domain of Stat3 have garnered preliminary success *in vitro*, validating the druggability of the pathway.[1] However, peptide drugs are limited by their innate immunogenicity and susceptibility to enzymatic hydrolysis. Non-peptidic small molecule inhibitors have also

been developed, but even optimized compounds bind with only micromolar affinity.[2] Looked at another way, the interaction of Stat3 with its receptor could be blocked by an SH2 superbinder domain with evolved sub-nanomolar affinity, as previously studied by Sidhu et al.[3] This approach harnesses the intrinsic selectivity of a protein for its receptor and limits off-target SH2-peptide interactions. We report the synthesis of the Fyn SH2 superbinder domain from Sidhu et al. using solid phase peptide synthesis and sequential native chemical ligation of three polypeptides.

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P290 *N*-Arylation of Amino Acid Esters to Expand Side Chain Siversity in Oxime Ligations

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While palladium-catalyzed Buchwald-Hartwig cross coupling reactions between amines and aryl halides/triflates have been demonstrated using a variety of substrates, efficient and mild methods for the *N*-arylation of amino acids without racemization have only recently emerged.[1] We previously demonstrated the utility of *N*-phenylglycinyl peptides in oxime ligation reactions, where electron-rich *N*-phenylglycine residues were found to readily oxidize into reactive α-imino amide intermediates under mild conditions.[2] Here, we expand the scope of amino acid ester *N*-arylations using electron-rich aryl bromides and Buchwald's t-BuBrettPhos Pd G3 precatalyst. Coupling of the *N*-aryl amino acid building blocks to a series of peptides and exploration of their reactivity in oxime ligation reactions will also be reported.

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YI-P291 The Effects of Protein Kinase C Beta II Peptide Modulation on Superoxide Release in Rat Polymorphonuclear Leukocytes

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Phorbol 12-myristate 13-acetate (PMA; a diacylglycerol mimetic) is known to augment polymorphonuclear leukocyte (PMN) superoxide (SO) release via protein kinase C (PKC) activation. However, the role of PKC beta II (βII) mediating this response is not known. It's known that myristic acid (myr-) conjugation facilitates intracellular delivery of the cargo sequence, and that putative PKCβII activator and inhibitor peptides work by augmenting or attenuating PKCβII translocation to cell membrane substrates (e.g. NOX-2). Therefore, we hypothesize that myr- conjugated PKCβII peptide-activator (N-myr-SVEIWD; myr-PKCβ+) would increase PMA-induced rat PMN SO release, whereas, myr-PKCβII peptide-inhibitor (*N*-myr-SLNPEWNET; myr-PKCβ-) would attenuate this response compared to non-drug treated controls. Rat PMNs ($5x10^6$) were incubated for 15min at 370C in the presence/absence of myr-PKCβ+/- (20 μM) or SO dismutase (SOD;10βg/mL; n=8) as positive control. PMA (100nM) induced PMN SO release was measured spectrophotometrically at 550nm via reduction of ferricytochrome c for 390 sec. PMN SO release increased absorbance to 0.39±0.04 in non-drug treated controls (n=28), and 0.49±0.05 in myr-PKCβ+(n=16). By contrast, myr-PKCβ- (0.26±0.03; n=14) significantly attenuated PMA-induced SO release compared to non-drug controls and myr-PKCβ+ ($p<0.05$). SOD-treated samples showed >90% reduction of PMA-induced SO release and was significantly different from all groups ($p<0.01$). Cell viability ranged between 94± to 98±2% in all groups as determined by 0.2% trypan blue exclusion. Preliminary results suggest that myr-PKCβ- significantly attenuates PMA-induced SO release, whereas myr-PKCβ+ shows a trend to augment PMA-induced SO release. Additional dose response and western blot experiments are planned with myr-PKCβ+/- in PMA-induced PMN SO release assays.

YI-P292 Site-specific Bioconjugation to Monoclonal Immunoglobulin Promoted by Proximity-Induced Chemistry

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Antibody Drug Conjugates (ADC) are gaining clinical importance for precision drug delivery in the treatment of cancers. There is great need for the development of novel site-specific bioconjugation methodology to assemble ADCs so that they are safer, more reliable, and with predictable and reproducible pharmacokinetics. Exploiting the modest affinity of indole towards nucleotide binding pocket (NBP) in Fab arms of immunoglobulin, our team previously reported a Fab-specific indole-directed conjugation strategy based on high-throughput synthesis and screening of one-bead one-compound (OBOC) combinatorial peptide libraries.[1] This successful precedence has encouraged us to further optimize the strategy by applying novel ligand design and improved screening protocols. We have replaced 1,5-difluoro-2,4-dinitrobenzene (DFDNB) electrophile with acrylamide and maleimide for better biocompatibility and synthetic accessibility, and placed them in different positions to generate greater library diversity. Using an optimized screening protocol, a number of affinity elements capable of site-specific ligation to rat anti-mouse PD1 monoclonal antibody at neutral pH were discovered. We were able to use some of these affinity elements to site-specifically biotinylate the Fab domain of immunoglobulins. These results have demonstrated the effectiveness of indole-directed conjugation strategy, and that the site-specificity is the interplay between indole directing group and chemical reactivity of electrophiles. Future revelation of mechanistic and structural details may provide important insights on how to further improve the design of the affinity elements such that precision site-specific ligation of immunoglobulins can be achieved.

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P293 Structure and Cellular Function of FAS1 Domains of Human Periostin, an Extracellular Matrix Protein Involved in Asthma and Cancer Metastasis

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Periostin, an extracellular matrix protein, is secreted by fibroblasts and is overexpressed in chronic allergic inflammation diseases and various types of cancers. Periostin is composed of a cysteine-rich EMI, four internal repeat FAS1, and a hydrophilic C-terminal domain. FAS1 domains of periostin play crucial roles in promoting tumor metastasis and progression via interaction with cell surface integrins. We systemically prepared various constructs of the FAS1 domains of the periostin and tried to express each domain in *E. coli*. Although all FAS1 domains were well expressed, only single FAS1-II and -IV domains were highly soluble. Circular dichroism (CD) and nuclear magnetic resonance (NMR) studies revealed that the FAS1-IV domain was suitable for three-dimensional structure determination using NMR spectroscopy. We almost completely assigned backbone and sidechain resonances using 3D NMR spectroscopy and conducted structure calculation of FAS1-IV domain using CYANA and Xplor-NIH programs. The structure of FAS1-IV domain consists of a mixture of two helix triangle module and two orthogonal β-sheets. We confirmed that FAS1-IV domain of periostin induce cell adhesion, migration, proliferation, and invasion in colon and breast cancer cells. These results suggest that FAS1-IV domain of periostin can be considered as a potential therapeutic target for various cancer.

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YI-P294 Peptide Dendrimers as Delivery Systems for Nucleic Acids

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Gene therapy is a powerful technique that allows the treatment of many diseases at a molecular level through the introduction of genetic materials into cells. However, the transfection procedure is often very challenging, due to the lack of efficient delivery systems.[1]

Peptide dendrimers/lipid hybrid systems were showed to be efficient transfection reagents for DNA[2] and siRNA into HeLa cells[3]. Recently, these compounds have also been successfully applied in oligonucleotides delivery[4].

We are now exploring a new library of third generation peptide dendrimers in order to perform transfection of plasmid DNA coding for CRISPR-Cas9. The optimization of hydrophilicity, hydrophobicity and the introduction of non-natural amino acids in the structure allowed us to obtain systems displaying high pDNA transfection efficiency in absence of helper lipid. In particular, biological experiments performed on fibroblast cell lines showed high transfection efficiency measured as GFP expression by FACS analysis.

Furthermore, pDNA transfection experiments performed on mouse intestinal organoids showed promising transfection efficiency and low cytotoxicity, properties necessary for potential *in vivo* applications.

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YI-P295 Structural Elucidation of Amyloid-β 20-34 and an Analogue with Alzheimer's-associated Isomerization of Asp23

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The influence of post-translational modifications on the structure of amyloid deposits requires an atomic understanding of amyloid-forming peptides harboring specific modifications in their aggregated state. Despite the availability of structures of the amyloid beta peptide in its fibrillar state, no atomic structure of a wild-type or post-translationally modified amyloid beta fibril exists. Use of micro-electron diffraction (MicroED), revealed the 1.1Å structure of an Aβ 20-34 fibril, the most complete crystalline structure of Aβ WT to date, as well as an 1.1Å structure of its isomerized form, Aβ 20-34 L-isoAsp23 - an Alzheimer's-associated modification. Our unprecedented resolution of this segment reveals a conserved protofibrillar arrangement for both peptide structures, with the isomerization at residue Asp23 producing an elongated backbone and inducing a more tightly packed, dry fibril interface compared to its analogous interface in the native fibril. These structures present atomic basis for how this modification may enhance amyloidogenicity through a more favorable secondary nucleation site and, therefore, reduce the concentration threshold necessary for nucleation. Amyloid seeding essays using chemically synthesized Aβ1-40 WT and Aβ1-40 isoAsp23, corroborate the relevance of this isomerization as evidenced by cross-seeding experiments between the isomers.

YI-P296 Dual-function Conjugates of Vancomycin with Cell-penetrating, Antimicrobial, Cationic Amphiphilic Polyproline Helices (CAPHs)

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An antibiotic-resistance crisis is progressively approaching as once-treatable pathogens are evading current therapeutics. Vancomycin is a glycopeptide antibiotic commonly referred to as the "antibiotic of last resort" for the treatment of methicillin-resistant *S. aureus* (MRSA) and similar bacterial infections. The therapeutic must be administered in high concentrations and frequently, which increases the risk of adverse effects, drug resistance and toxicity. In order to improve the efficacy, a vancomycin conjugate was synthesized by tethering a cationic amphiphilic polyproline helix (CAPH) known to have effective ability to transverse cellular membranes and effectively reduce bacteria within mammalian cell cultures. This vancomycin-CAPH conjugate has been strategically designed with a cleavable disulfide linkage, capable of reducing within the intracellular environment. Details of this dual-therapeutic strategy on cellular accumulation of the therapeutics, membrane interactions, and delivery of the two independently potent antibiotics to bacterial infections will be discussed.

YI-P297 Smart Peptide-based Nanoparticles Targets and Transforms for Inhibition of Her-2 Oligomerization

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We rationally constructed a smart supramolecular material based on a versatile peptide, which could assemble into nanoparticles (NPs), followed by in situ transformation into nanofibrous (NFs) structure upon binding to the Her2 receptors in tumor sites for significantly enhanced receptor-based therapy of Her-2 positive breast cancer by suppressing the dimerization of Her-2 receptors (Figure 1). Intriguingly, the vast majority of Her-2 extracellular receptor protein was bound by dense fibrous Her-2 ligands, which significantly suppressed the dimerization of Her-2 to great extent and further prevented downstream gene signals of proliferation and survive from being transmitted to the nucleus. Compared with simplistic binding behavior of spherical nanoparticles to Her-2 protein, the in situ constructed fibrous structure undoubtedly could better limit the internalization and dimerization of Her-2 receptor proteins. The structural transformation-based supramolecular material represents a novel strategy for the design of receptor-specific tumor therapy.

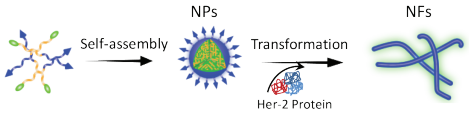

Figure 1. Assembly and transformation of the supramolecular peptide materials.

YI-P298 Stained Molecules Enabled Epimerization-free Peptidyl Couplings, a General Strategy for Challenging L-cyclo-tetrapeptide Synthesis

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The broad application of macrocycles have illustrated significant potential as therapeutic agents. 12-membered ring cyclo- tetrapeptides are particularly attractive entities among all the cyclic peptides. Compared with macrocycles of larger ring size, the characteristic head-to-tail or end-to-end peptidyl backbone provides intrusive structural motif of circular peptides grants distinct biological properties such as resistance to degradation, enhanced conformational stability, and increased epitope interactions with other biomolecules.[1] Ability to acquire a sufficient quantity of high purity of cyclo-tetrapeptide could significantly promote their chemical and biological studies.

It is worth noting that the traditional coupling methods to achieve chemical synthesis of macrocycles have been a formidable challenge, requiring fully protected linear precursors and harsh conditions to achieve activation of the C-terminal residue for the cyclization. The harsh activation conditions are required to overcome the entropy barrier during the coupling often lead to epimerization of the C-terminal amino acid residue and peptide oligomerization. The practical strategy to attain macrocycles with a consensus sequence of L-cyclo(Pro-Xxx-Pro-Xxx), where Xxx = Val, Tyr, Leu, Phe, are elusive.[2] There is the urgent need to develop a strategy to prepare circular tetrapeptides.

Based on our previously reported β-thiolactone mediated chemistry,[3] we were able to construct tetra-cyclic peptides in high yields. The strategy could be applied to produce a broad number of all-L-cyclo-tetrapeptides and the reactions were performed at room temperature in the aqueous buffer. The β-thiolactone furnished cyclization protocol prevented the amino acid epimerization during the cyclization and proved as a powerful general strategy for cyclic peptide synthesis.

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P299 Improving the Efficacy of PYY Analogs through Cyclization, Lipidation, and Bioconjugation

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Peptide tyrosine tyrosine (PYY) is released from endocrine L-cells in response to food consumption. After secretion, PYY(1-36) is readily degraded by the endothelial serine protease DPP-IV to PYY(3-36), which has been demonstrated to reduce food

intake through engagement of the neuropeptide Y2 receptor in mice, rats, rabbits, monkeys and humans. It has also been shown to improve insulin-mediated glucose disposal and insulin sensitivity in DIO rodents. However, the development of PYY(3-36) as a therapeutic agent is limited due to its rapid metabolism and resultant short in vivo half-life. We designed and synthesized novel PYY analogs with staples or macrocyles to stabilize helical structure or the PP-fold conformation, respectively. Selected potent analogs were lipidated with palmitic acid, or conjugated to an immunologically silent monoclonal antibody through a site-specific, stable thioether linkage. The lipidated analogs significantly reduced food intake over 12 hours in an acute mouse study. The mAb-cyclic PYY conjugate exhibited prolonged stability in mice. These design strategies show potential for addressing a critical liability of PYY as a therapeutic agent for treatment of obesity and type 2 diabetes.

P300 Utilization of Azide-Alkyne Cycloaddition Reactions to Develop Highly Selective Peptidic Polo-like Kinase 1 Polo-box Domain-binding Inhibitors

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Members of the polo-like kinase (Plk) family of serine/threonine protein kinases play crucial roles in cell cycle regulation and proliferation. Of five Plks (Plk1 – 5), Plk1 is recognized as an anticancer drug target. Plk1 contains multiple structural components that are important for its proper biological function. These include an N-terminal catalytic domain and a C-terminal non-catalytic polo-box domain (PBD). The PBD binds to phosphothreonine (pThr) and phosphoserine (pSer)-containing sequences. Blocking PBD-dependent interactions offers a potential means of down-regulating Plk1 function that is distinct from targeting its ATP-binding site. Previously, we demonstrated by tethering alkylphenyl chains from the N(π)-position of the His residue in a 5-mer PLHSpT peptide, that we were able to access a hydrophobic "cryptic" binding pocket on the surface of the PBD, which enhanced peptide-binding affinities by approximately 1000-fold. More recently, we optimized these PBD-ligand interactions using an oxime ligation-based strategy. Herein, we explore new PBD-binding antagonists using azide-alkyne cycloaddition reactions. This work has allowed us to identify new ligands that retain the high PBD-binding affinity of the parent peptide, while enhancing selectivity for Plk1 relative to the PBDs of Plk2 and Plk3. Our findings may be generally applicable in the further development of a range of Plk1 PBD-binding inhibitors.

P301 Apelin Analogues as Natural Cardio-Protective Drugs

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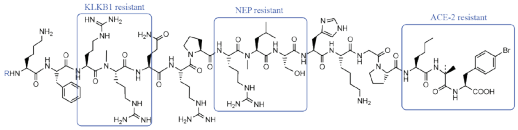
Cardiovascular diseases including heart attack, stroke and ischemic reperfusion injury are the top killers worldwide accounting for almost 30% of global deaths.[1] Apelin is the natural substrate for the apelinergic system (APJ receptor) – an endogenous peptide hormone system that mediates a cascade of other cardio-metabolic processes ultimately fine-regulating the cardiovascular output. Apelin gets rapidly degraded in plasma owing to the activity of various proteases including Angiotensin converting enzyme 2 (ACE-2) and Neprilysin (NEP) and Plasma kallikrein (KLKB1), limiting its half-life to under 2 minutes.[2,3] Apelin analogues including N-terminal extended isoforms and peptide isosteres reflect a promising alternative to minimize proteolytic degradation. The poster highlights the synthesis, metabolic stability and physiological activity of such Apelin analogues. These ACE2- and NEP-resistant drug candidates show potent blood pressure lowering and ischemic reperfusion recovery effects. They have considerable potential for their development as new agents for treatment of cardiovascular diseases.[4,5]



Metabolically stable apelin analogue with cardio-protective effects.

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P302 Reliable Data Sharing on Antimicrobial Peptide Efficacy

Lenore M. Martin[a], Katelyn Raimond[a], Kimberly Kluglein[a], Husam Bahra[a], Atalia Rodrigues[a], Matthew Daily[b], Emily Costello[c], Archana Chittoor[b], and Joseph Janiszewski[b]
Departments of Cell and Molecular Biologya, and Computer Science and Statisticsb, and Biomedical and Pharmaceutical Sciencesc at The University of Rhode Island, Kingston, RI 02881 USA

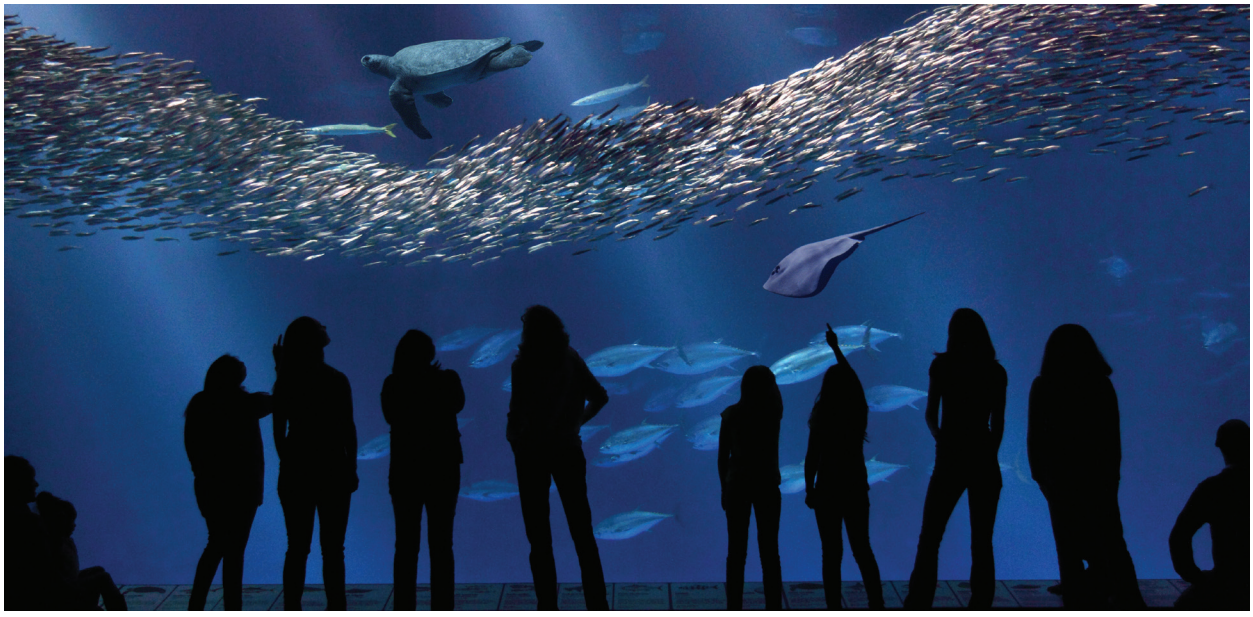
Reliable and consistent experimental data may be shared and compared describing the effectiveness of Antimicrobial Peptides (AMPs) against well-defined pathogens using software tools we are developing and sharing on the antimicrobial peptide editable (AMPed.uri.edu) database platform. Antimicrobial resistance is a growing problem in treatment of bacterial infections. To fight back against resistance, our team tested six synthetic antimicrobial peptides (AMPS) at various concentrations against 2 strains of bacteria known to cause infection, Escherichia coli and Staphylococcus aureus. In the 96-well plate format, we used two controls: NaOH and chloramphenicol in Müeller-Hinton broth assays. To analyze the growth or inhibition of the bacteria after treatment with AMPs, serial dilutions of each AMP were prepared using the “Hancock diluent”1 consisting of 0.01 % acetic acid and 0.2% BSA. We monitored growth in real time using the optical density at 630 nm but also determined the inhibition of bacterial growth more accurately by plating different dilutions of bacterial cultures and counting the colony forming units (CFUs). Based on these results, we concluded that among the six AMPs tested, peptides LM4-15 and CP-29A caused the most potent inhibition in the

growth of these 2 bacterial species, and verified the sequences of LM4-15 and CP-29A by comparing the theoretically calculated molecular masses to those obtained using matrix-assisted laser desorption mass spectrometry (MALDI). Future experiments will explore the activity of these peptides against clinical isolates of antibiotic-resistant Staphylococcus aureus at Rhode Island Hospital. http://cmdr.ubc.ca/bobh/method/modified-mic-method-for-cationic- antimicrobial-peptides/ last accessed on 2019-05-17

PHOTOS BELOW

Top Row: Monterey Harbor at Dawn | Otters
Middle Row: Cannery Row | Moss Landing
Bottom Row: Monterey Aquarium

Photos courtesy of SeeMonterey.com



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