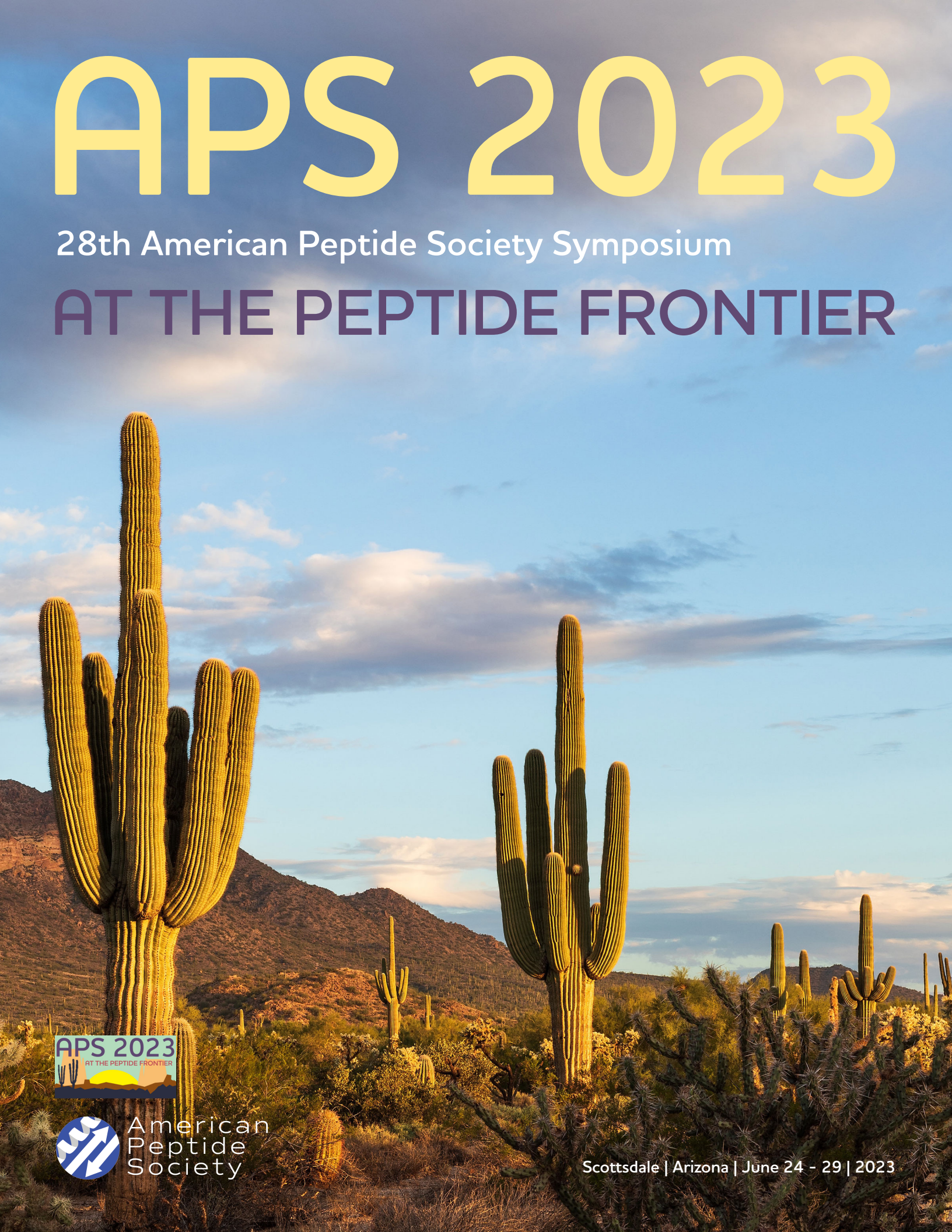
APS 2023

28th American Peptide Society Symposium

AT THE PEPTIDE FRONTIER



American Peptide Society

Scottsdale | Arizona | June 24 - 29 | 2023

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Welcome from Co-Chairs

Welcome to Scottsdale and the 28th American Peptide Symposium, APS2023! The American Peptide Society has decided to bring our peptide community together just one year after a successful APS2022 to capitalize on the momentum we see in the field of peptide science spanning academic, government and industry research. Given the exciting moment we are in, we decided to commune here in the Valley of the Sun at the frontier of peptide science. Our goal for this week is to discover new insights, explore potential collaborations and build relationships.

The agenda for APS2023 focuses on our frontiers for their scientific excellence, breadth and impact to society. We selected sessions that represented the following areas of peptide science: peptide

David Chenoweth

Robert Garbaccio

tools and modulators of biology, protein modifications, peptide architectures with designed structure and function, peptide materials and delivery, peptide drug discovery and natural products, peptide synthesis and green chemistry and the rapidly advancing field of peptide computational tools. Within these fields, we invited a diverse slate of speakers that will explore the breadth of our field and share their progress in an interactive, in-person forum. Our speakers come from academic, industry and government research institutions across the globe, span early to late career stages and represent the diversity of communities that are at the core of our peptide society.

APS2023 is bookended by two stellar keynote presentations. After the official welcome by APS president Joel Schneider, the opening keynote lecture "Peptide-Glycan Interactions in Immunity" will be delivered by Professor Laura L. Kiessling from the Massachusetts Institute of Technology at 6:00pm on Saturday, June 24th. To close the meeting, we have Professor James Wells from the University of California at San Francisco giving his keynote at 4:25pm on Thursday, June 29th. In between these exciting keynotes and sessions, we will celebrate this years' APS awards with presentations from each of our awardees. The Merrifield Award Lecture will be presented by Professor Sam Gellman from the University of Wisconsin at Madison on Sunday. On Monday, Professor César de la Fuente from the University of Pennsylvania will give the Makineni Lecture and Professor James Tam from Nanyang Tehcnological University will give the Goodman Lecture. Professor Helma Wennemers from ETH Zurich and Professor Marcey Waters from the University of North Carolina, Chapel Hill will deliver our two du Vigneaud Award Lectures on Wednesday. We will also feature two Early Career Award Lectureships presented by Professor Danny Chou from Stanford University on Sunday and Professor Lara Malins from the Research School of Chemistry at the Australian National University on Thursday. Throughout the week, we will have eight speakers competing in the Dr. Elizabeth Schram Young Investigator oral competition. Finally, a key part of the scientific program are the two poster sessions, which will be represented in our agenda through sixteen "flash talks".

In addition to our scientific program, this year's meeting has additional activities to further strengthen our community. On Saturday, we are pleased to again host a Workshop on Career Development that will feature a panel of academic and industry leaders to share their insight and experience from their careers. On Sunday, Dr. Shauna Clark, Scientific Diversity Advisor to the Director, Center for Cancer Research at NCI will host our second workshop titled 'Great Minds Think Differently: Inspiring Equity & Inclusion'. Opportunities for networking include the opening reception on Saturday night, a student mixer on Sunday night, the Dr. Elizabeth Schram Young Scientists' Lunch & Mixer on Tuesday and, of course, the closing banquet on Thursday night. To further enhance networking opportunities and information exchange, we will be using a mobile phone app, Whova, in lieu of a printed program for environmental sustainability.

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 industry-leading products, services, and equipment they have available. Without their continued support, the exciting scientific program as well as the social events would not be possible. We also wish to thank our APS2023 team for their commitment and support in planning this event: Lauren Cline, Stephen Miller, Wendy Harstock, Heba Salim, Lars Sahl, Tami Everdene and the IU conference team.

Finally, our ask is for everyone's engagement throughout this meeting so that we make the most of the time together as a community. Your questions during the scientific agenda, participation in our workshops and social events and engagement with our sponsors will elevate the symposium and help the APS to build momentum towards greater impact as a scientific society. Thank you for joining us this week in Scottsdale and we look forward to enjoying an exciting week at the peptide frontier.

Sincerely - Rob and Dave

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Welcome from the APS President

On behalf of the American Peptide Society, I would like to welcome you to the 28th American Peptide Symposium in beautiful Scottsdale Arizona. The theme of the meeting, "At the Peptide Frontier", captures the spirit of peptide science at this year's gathering. Our symposium co-chairs, Rob Garbaccio and Dave Chenoweth, have put together a fantastic program of cutting-edge science that will educate, excite and inspire. The symposium also showcases outstanding companies who will be exhibiting their services and technologies that help make the science possible. Take a break from the talks and go and see what they have to offer.

The impact of peptide science as a scientific discipline continues to be high. Peptides comprise new block buster drugs that directly impact our health. They are augmenters of immunity and building blocks for new materials, just to name a few of their functions. Synthetic chemistry and genetic methods continue to be developed that enable their production, and their physical properties still intrigue us, as we further tease out the molecular-level interactions that define their folding and function.

Our Society brings together the people that make this all possible. We are a community that have found common ground in a class of molecules we have all come to love. Our members are faculty and students from the best academic institutions across the world; we are researchers and leaders at top companies and government organizations; we are editors, policy makers and entrepreneurs starting companies. Our members make seminal discoveries that impact, build, and drive science. You will be reminded of this as you listen to the lectures, view the posters, and take in the talks of this year's award winners — congratulations are in order, their work has significantly impacted the field.

It has been a pleasure and honor to serve the society as president. I have had the pleasure to work with an outstanding group of officers, councilors, and administrators. Thank you all for your hard work. I wish the continuing officers and newly elected officials good luck in their ftuture endeavors. They are an outstanding crew; the Society is in very good hands.

I have been a member of the APS for my entire career and have, and continue, to thoroughly enjoy the experience. I have met some of my best friends amongst its members and continue to draw scientific inspiration from your discoveries. I encourage all of you to introduce the APS to your students, friends and colleagues so that they may experience the same. Importantly, please get involved. Run for office/council or join one of the committees. It will be rewarding.

This year's symposium was the result of a tremendous amount of hard work and dedication. Our sponsorship committee went above and beyond in their efforts, kudos to Steve Miller, Heba Salim and Wendy Hartsock. Our colleagues at Indiana University Conferences and Lars Sahl who make it all happen deserve a special thanks. If you happen to see the symposium co-chairs Rob and Dave strolling around the resort, give them a special shout-out, thanking them for their hard work. Importantly, the symposium, and quite frankly, our Peptide Society, would be lost without the dedication, perseverance, and diligent work of Lauren Cline. Thank you, Lauren!

Lastly, I would like to thank all of you for attending the symposium. Please engage, learn, network and enjoy the science and venue.

Best regards,
Joel Schneider

APS Symposia Chronology

SYMPOSIUM	YEAR	CHAIRS	VENUE
1st	1968	**Saul Landa** - Yale University **Boris Weinstein** - University of Washington at Seattle	Yale University New Haven, CT
2nd	1970	**F. Merlin Bumpus** - Cleveland Clinic	Cleveland Clinic Cleveland, OH
3rd	1972	**Johannes Meinhofer** - Harvard Medical School	Children's Cancer Research Foundation Boston, MA
4th	1975	**Roderich Walter** - University of Illinois Medical Center, Chicago	The Rockefeller University and Barbizon Plaza Hotel New York, NY
5th	1977	**Murray Goodman** - University of California at San Diego	UCLA at San Diego San Diego, CA
6th	1979	**Erhard Gross** - National Institute of Health	Georgetown University Washington, DC
7th	1981	**Daniel H. Rich** - University of Wisconsin at Madison	University of Wisconsin Madison, WI
8th	1983	**Victor J. Hruby** - University of Arizona	University of Arizona Tucson, AZ
9th	1985	**Kenneth D. Kopple** - Illinois Institute of Technology **Charles M. Deber** - University of Toronto	University of Toronto Toronto, Ontario Canada
10th	1987	**Garland R. Marshall** - Washington University School of Medicine	Washington University St. Louis, MO
11th	1989	**Jean E. Rivier** - The Salk Institute of Biological Studies, La Jolla	UCLA at San Diego San Diego, CA
12th	1991	**John A. Smith** - Massachusetts General Hospital	Massachusetts Institute of Technology, Cambridge, MA
13th	1993	**Robert S. Hodges** - University of Alberta at Edmonton	Edmonton Convention Ctr. Alberta, Canada
14th	1995	**Pravin T.P. Kaumaya** - The Ohio State University	The Ohio State University Columbus, OH
15th	1997	**James P. Tam** - Vanderbilt University	Nashville Convention Center Nashville, TN

APS Symposia Chronology, continued

SYMPOSIUM	YEAR	CHAIRS	VENUE
16th	1999	**George Barany** - University of Minnesota **Gregg B. Fields** - Florida Atlantic University	Minneapolis Convention Ctr. Minneapolis, MN
17th	2001	**Richard A. Houghten** - Torrey Pines Inst. for Molecular Studies **Michael Lebl** - Illumina Inc., CA	Town and Country Resort San Diego, CA
18th	2003	**Michael Chorev** - Beth Israel Medical & Harvard Medical School **Tomi K. Sawyer** - ARIAD Pharmaceuticals, Inc.	Marriott Copley Place Boston, MA
19th	2005	**Jeffrey W. Kelly** - The Scripps Research Institute **Tom W. Muir** - Rockefeller University, NY	Town and Country Resort San Diego, CA
20th	2007	**William D. Lubell** - University of Montreal **Emanuel H.F. Escher** - Univeristy of Sherbrooke	Palais des Congres Montreal, Canada
21st	2009	**Richard diMarchi** - Indiana University **Hank Mosberg** - University of MIchigan	Indiana University Bloomington, IN
22nd	2011	**Philip Dawson** - The Scripps Research Institute **Joel Schneider** - National Cancer Institute	Sheraton San Diego, CA
23rd	2013	**David Lawrence** - University of North Carolina at Chapel Hill **Marcey Waters** - University of North Carolina at Chapel Hill	Hilton Waikoloa Village Waikoloa, HI
24th	2015	**Ved Srivastava** - GlaxoSmithKline **Andrei Yudin** - University of Toronto	Hyatt Regency Grand Cypress, Orlando, FL
25th	2017	**Jonathan Lai** - Albert Einstein College of Medicine **John Vederas** - University of Alberta	Whistler Conference Center Whistler, BC, Canada
26th	2019	**Paramjit Arora** - New York University **Anna Mapp** - University of Michigan	Portola Hotel and Monterey Conference Center
27th	2022	**Mark D. Distefano** - University of Minnesota **Les Miranda** - Amgen, Inc.	Whistler Conference Center Whistler, BC, Canada
28th	2023	**David Chenoweth** - University of Pennsylvania **Robert Garbaccio** - Merck	Westin Kierland Resort Scottsdale, AZ

Schedule of Events - Overview

Saturday, June 24th

01:00pm - 06:00pm	**Registration**	Culture Keepers Registration
02:00pm - 05:30pm	APS Council Meeting	Powell Boardroom
03:30pm - 05:00pm	Workshop on Career Development	Cushing
06:00pm - 06:10pm	PRESIDENT'S WELCOME	Kierland Ballroom 3 & 4
06:10pm - 07:00pm	OPENING PLENARY KEYNOTE LECTURE	Kierland Ballroom 3 & 4
07:00pm - 09:00pm	Opening Reception With Exhibitors	Kierland Ballroom1 & 2

Sunday, June 25th

08:00am - 04:30pm	**Registration**	Culture Keepers Registration
08:00am	Group 1 - Poster Set-up	Hall of State
08:30am - 08:35am	OPENING REMARKS - APS 2023 CO-CHAIRS	Kierland Ballroom 3 & 4
08:35am - 10:25am	SESSION 1: PEPTIDE TOOLS & PROBES	Kierland Ballroom 3 & 4
10:25am - 10:50am	Coffee with Exhibitors & Posters	Hall of State, Kierland 1 & 2
10:50am - 12:00pm	SESSION 2: PROTEIN MODIFICATIONS FOR ENABLING BIOLOGY	Kierland Ballroom 3 & 4
12:00pm - 12:15pm	DR. ELIZABETH SCHRAM YI ORAL COMPETITION	Kierland Ballroom 3 & 4
12:15pm - 12:45pm	EARLY CAREER LECTURESHIP AWARD	Kierland Ballroom 3 & 4
12:45pm - 02:15pm	Lunch with Exhibitors – Provided	Kierland Ballroom 1 & 2
02:15pm - 03:45pm	SESSION 3: NOVEL MODULATORS OF BIOLOGY	Kierland Ballroom 3 & 4
03:45pm - 04:00pm	DR. ELIZABETH SCHRAM YI ORAL COMPETITION	Kierland Ballroom 3 & 4
04:00pm - 04:15pm	Afternoon Break	
04:15pm - 05:25pm	MERRIFIELD AWARD LECTURE	Kierland Ballroom 3 & 4
05:30pm - 06:30pm	Workshop: Great Minds Think Differently - Inspiring Equity & Inclusion	Cushing
07:00pm - 09:00pm	Student Mixer	Culture Keeper's Hall W

Monday, June 26th

08:00am - 04:30pm	**Registration**	Culture Keepers Registration
08:30am - 10:20am	SESSION 4: PROTEIN ARCHITECTURES – STRUCTURE AND FUNCTION	Kierland Ballroom 3 & 4
10:20am - 10:45am	Coffee with Exhibitors & Posters	Kierland Ballroom 1 & 2
10:45am - 12:15am	SESSION 5: PEPTIDE MATERIALS AND DELIVERY	Kierland Ballroom 3 & 4
12:15pm - 12:45pm	MAKINENI LECTURE	Kierland Ballroom 3 & 4
12:45pm - 02:30pm	Lunch with Exhibitors – Provided	Kierland Ballroom 1 & 2
01:00pm - 02:00pm	International Liaison Meeting	Powell Boardroom
02:30pm - 04:00pm	SESSION 6: PEPTIDE DRUG DISCOVERY	Kierland Ballroom 3 & 4
04:00pm - 04:30pm	DR. ELIZABETH SCHRAM YI ORAL COMPETITION	Kierland Ballroom 3 & 4
04:30pm - 04:45pm	Afternoon Break	
04:45pm - 05:25pm	POSTER FLASH TALKS	Kierland Ballroom 3 & 4
05:25pm - 05:55pm	GOODMAN LECTURE	Kierland Ballroom 3 & 4
06:00pm - 08:00pm	GROUP 1 POSTER SESSION & RECEPTION	Hall of State, Kierland 1 & 2

Tuesday, June 27th

08:00am - 12:30pm	**Registration**	Culture Keepers Registration
08:30am - 10:25am	SESSION 7: NATURAL PRODUCTS TO UNNATURAL BIOACTIVE PEPTIDES	Kierland Ballroom 3 & 4
10:25am - 10:50am	Coffee with Exhibitors & Posters	Hall of State, Kierland 1 & 2
10:50am - 11:20am	DR. ELIZABETH SCHRAM YI ORAL COMPETITION	Kierland Ballroom 3 & 4
11:20am - 12:50pm	SESSION 8: NATURAL PROD. BIOSYNTHESIS & NATURE INSPIRATION	Kierland Ballroom 3 & 4
12:50pm	Lunch on your own	

Schedule of Events - Overview, continued

Tuesday, June 27th, cont.

01:00pm - 02:30pm	Dr. Elizabeth Schram Young Scientists' Lunch & Mixer, by invitation	Deseo
01:00pm - 02:30pm	Exhibitor Workshop, hosted by Biotage	Cushing

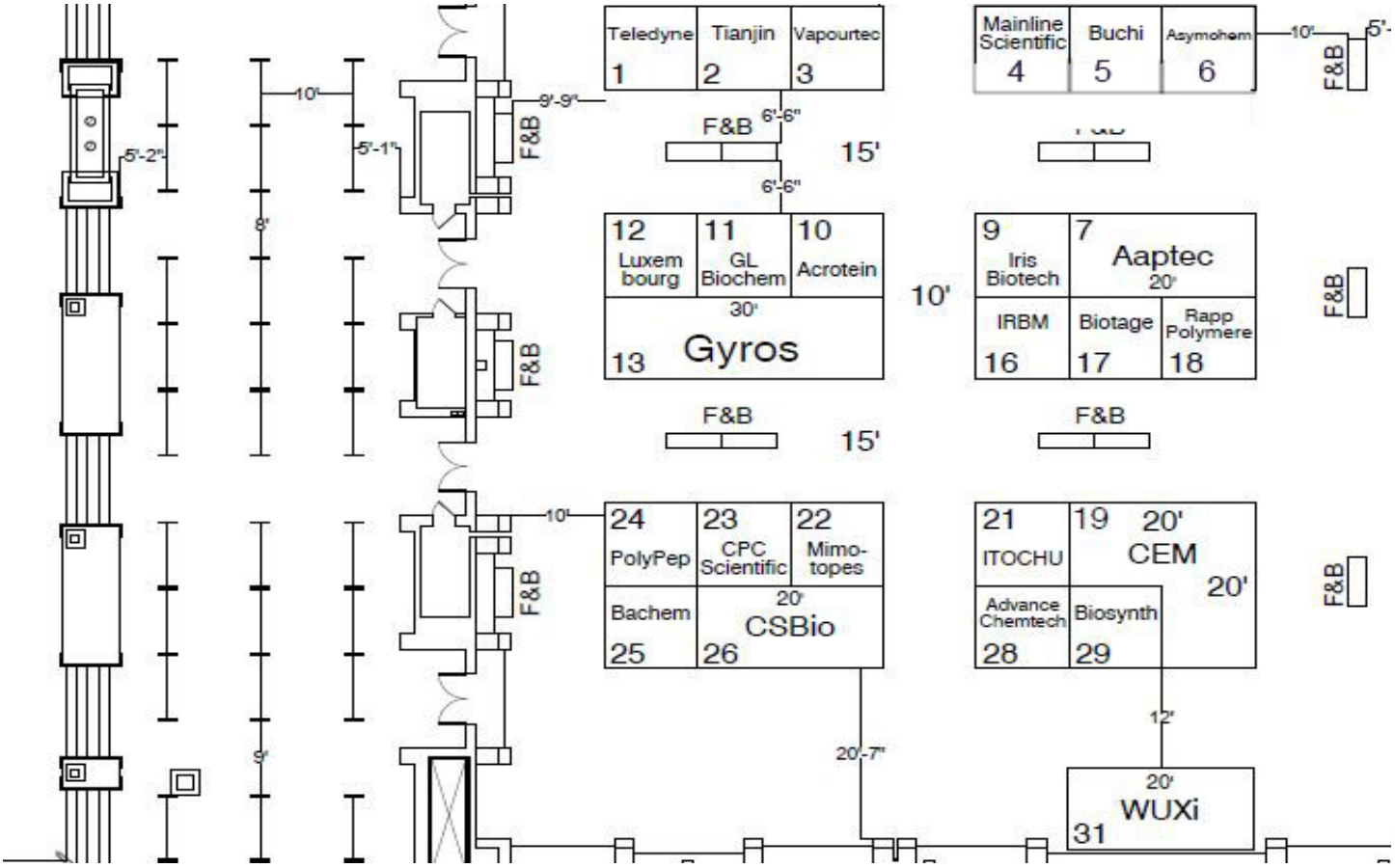
Wednesday, June 28th

08:00am - 04:30pm	**Registration**	Culture Keepers Registration
08:55am - 10:25am	SESSION 9: PEPTIDE SYNTHETIC METHODS & GREEN APPROACHES	Kierland Ballroom 3 & 4
10:25am - 10:50am	Coffee with Exhibitors & Posters	Hall of State, Kierland 1 & 2
10:50am - 11:05am	DR. ELIZABETH SCHRAM YI ORAL COMPETITION	Kierland Ballroom 3 & 4
11:05am - 12:15pm	SESSION 10: BIOINSPIRED & INTELLIGENT PEPTIDE MATERIALS	Kierland Ballroom 3 & 4
12:15pm - 12:45pm	DU VIGNEAUD LECTURE	Kierland Ballroom 3 & 4
12:45pm - 02:30pm	Lunch on your own	Kierland Ballroom 1 & 2
12:45pm - 02:15pm	Vincent du Vigneaud Award Lunch, by invitation	Tribal
02:30pm - 02:45pm	APS GENERAL ASSEMBLY	Kierland Ballroom 3 & 4
02:45pm - 04:15pm	SESSION 11: PEPTIDE DESIGN AND FUNCTION	Kierland Ballroom 3 & 4
04:15pm - 04:30pm	DR. ELIZABETH SCHRAM YI ORAL COMPETITION	Kierland Ballroom 3 & 4
04:30pm - 04:45pm	Afternoon Break	
04:45pm - 05:25pm	POSTER FLASH TALKS	Kierland Ballroom 3 & 4
05:25pm - 05:55pm	DU VIGNEAUD LECTURE	Kierland Ballroom 3 & 4
06:00pm - 08:00pm	GROUP 2 POSTER SESSION & RECEPTION	Hall of State, Kierland 1 & 2

Thursday, June 29th

08:00am - 10:30am	**Registration**	Culture Keepers Registration
08:30am - 10:05am	SESSION 12: NEW FRONTIERS IN COMPUTATIONAL PEPTIDE DESIGN 1	Kierland Ballroom 3 & 4
10:05am - 10:25am	Coffee Break	Hall of State
10:25am - 11:30am	SESSION 13: NEW FRONTIERS IN COMPUTATIONAL PEPTIDE DESIGN 2	Kierland Ballroom 3 & 4
11:30am - 12:00pm	EARLY CAREER LECTURESHIP AWARD	Kierland Ballroom 3 & 4
12:00pm - 02:00pm	Lunch on your own	
02:00pm - 03:05pm	SESSION 14: NOVEL ADVANCES IN PEPTIDE CHEMISTRY	Kierland Ballroom 3 & 4
03:05pm - 03:20pm	Coffee Break	
03:20pm - 04:25pm	SESSION 15: INNOVATIVE METHODS FOR PEPTIDE STRUCTURE	Kierland Ballroom 3 & 4
04:25pm - 05:15pm	CLOSING PLENARY KEYNOTE LECTURE	Kierland Ballroom 3 & 4
05:15pm - 05:20pm	CLOSING REMARKS	Kierland Ballroom 3 & 4
07:00pm - 10:00pm	CLOSING BANQUET	Kierland 1 & 2

Kierland Ballroom Floor Plan



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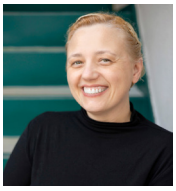

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Schedule of Events - Detail

Saturday, June 24th

01:00pm - 06:00pm	Registration	Culture Keepers Registration
02:00pm - 05:30pm	**APS Council Meeting**	Powell Boardroom
03:30pm - 05:00pm	**Workshop on Career Development** Join us in career development workshop to learn how to navigate your future career by using three key elements: Explore, Connect, Develop. **Panelists:** Rumit Maini, *Director, Business Development PepLib* Shauna Clark, *Senior Diversity Advisor, National Cancer Institute* Heba Salim, *Advisor, Eli Lilly and Company* Victor Outlaw, *Assistant Professor, University of Missouri* George Burslem, *Assistant Professor, University of Pennsylvania* Nat Goldberg, *Scientist, Aralez Bio*	Cushing
06:00pm - 06:10pm	**PRESIDENT'S WELCOME** Joel Schneider, National Cancer Institute, Center for Cancer Research	Kierland Ballroom 3 & 4
06:10pm - 07:00pm	**OPENING PLENARY KEYNOTE LECTURE** **Introduction:** Paramjit Arora New York University **L01 – Laura L. Kiessling**, Massachusetts Institute of Technology **Peptide-Glycan Interactions in Immunity**	Kierland Ballroom 3 & 4
01:00pm - 06:00pm	Registration	Culture Keepers Registration

Sunday, June 25th

08:00am - 04:30pm	Registration	Culture Keepers Registration
08:00am	**Group 1 - Poster Set-up**	Hall of State
08:30am - 08:35am	**OPENING REMARKS - APS 2023 CO-CHAIRS** Robert Garbaccio, Merck David Chenoweth, University of Pennsylvania	Kierland Ballroom 3 & 4
08:35am - 10:25am	**SESSION 1: PEPTIDE TOOLS & PROBES** **Session Chair:** Anna Mapp, University of Michigan	
08:35am - 09:00am	**L02 – Christian Becker,** University of Vienna **Zooming into Posttranslational Modifications by Protein Semisynthesis**	
09:00am - 09:25am	**L03 – Ross Cheloha,** National Institute of Health **Connecting Peptides and Antibodies to Probe Cell Surface Receptors**	
09:25am - 09:45am	**L04 – Nicholas Sawyer,** Fordham University **A Rational Crosslinking Approach for Peptide Polyproline II Helices**	
09:45am - 10:05am	**L05 – Dennis Bong,** The Ohio State University **Novel Fluorogenic Peptide Probes for Intracellular RNA and RNP Tracking**	
10:05am - 10:25am	**L06 – Amelia Fuller,** Santa Clara University **Aromatic Residue Placement Influences Helical Structure of Short Peptoid Oligomers in Aqueous Solutions**	
10:25am - 10:50am	**Coffee with Exhibitors & Posters**	Hall of State, Kierland 1 & 2
10:50am - 12:00pm	**SESSION 2: PROTEIN MODIFICATIONS FOR ENABLING BIOLOGY** **Session Chair:** James Checco, University of Nebraska-Lincoln	Kierland Ballroom 3 & 4
10:50am - 11:15am	**L07 – Champak Chatterjee,** University of Washington **SUMO: Wrestling with Gene Transcription**	

Schedule of Events, continued

Sunday, June 25th, cont.

11:15 am - 11:40am	**L08** – **Yael David,** Memorial Sloan Kettering Cancer Center **Uncovering Cancer-Associated Epigenetic Events using Novel Chemical Tools**	
11:40am - 12:00pm	**L09** – **Mark Distefano,** University of Minnesota **Protein Prenylation: New Tools Reveal New Biology**	
12:00pm – 12:15pm	**DR. ELIZABETH SCHRAM YI ORAL COMPETITION** **L10 - YI1** – **Zoë Adams,** The Scripps Research Institute **Stretching Peptides: Diyne-Braced Peptides as Beta-Strand Mimics**	Kierland Ballroom 3 & 4
12:15pm – 12:45pm	**EARLY CAREER LECTURESHIP AWARD** **Introduction:** Michael S. Kay, University of Utah School of Medicine **L11 - AW** – **Danny Chou,** Stanford University **Stimuli-Responsive Therapeutics through Peptide Chemistry**	Kierland Ballroom 3 & 4
12:45pm - 02:15pm	**Lunch with Exhibitors - Provided**	Kierland Ballroom 1 & 2
02:15pm - 03:45pm	**SESSION 3: NOVEL MODULATORS OF BIOLOGY** **Session Chair:** Yftah Tal-Gan, University of Nevada, Reno	Kierland Ballroom 3 & 4
02:15pm - 02:40pm	**L12** – **Tom N. Grossmann,** VU University Amsterdam **From Protein Structures to Functional Biomimetics**	
02:40pm - 03:05pm	**L13** – **Nir Qvit,** Bar-Ilan University **Engineered Novel Protein-Protein Interaction Regulators to Study Mitochondrial Function and Modulate Mitochondrial Homeostasis**	
03:05pm - 03:25pm	**L14** – **Vita Sereikaite,** University of Copenhagen **Towards Cognition Enhancing Therapeutics: Regiospecific Modulation of $GABA_B$ Receptors using Peptide-Based Inhibitors**	
03:25pm - 03:45pm	**L15** – **Eileen Kennedy,** University of Georgia **Novel Allosteric Targeting Strategies to Inhibit LRRK2 in Parkinson's Disease using Constrained Peptides**	
03:45pm – 04:00pm	**DR. ELIZABETH SCHRAM YI ORAL COMPETITION** **L16 -YI2** – **Benjamin Emenike,** Emory University **Chemical Tools for Profiling Lysine Dimethylation and N-Terminal Dimethylation of Proteins**	Kierland Ballroom 3 & 4
04:00pm - 04:15pm	**Afternoon Break**	
04:15pm - 05:25pm	**MERRIFIELD AWARD LECTURE** **Introduction:** James Checco, University of Nebraska-Lincoln **L17 - AW**– **Sam Gellman,** University of Wisconsin at Madison **Merrifield's Legacy: Transcending the Tyranny of the Ribosome**	Kierland Ballroom 3 & 4
05:30pm - 06:30pm	**Workshop: Great Minds Think Differently: Inspiring Equity & Inclusion** **Dr. Shauna Clark** **Scientific Diversity Advisor to the Director, Center for Cancer Research at NCI.** Attendees will learn about and discuss topics related to diversity, equity, inclusivity and accessibility, DEIA. First, Dr. Clark will address challenges to creating diverse and equitable workplaces. Then, Dr. Clark will provide examples of strategies and programs implemented at NCI that can accelerate research by improving climate and enhancing DEIA. The session will also include time for facilitated discussion. In these discussions, we will work collaboratively to identify actionable strategies that will elevate the value of the American Peptide Society to a diverse membership.	Cushing
07:00pm - 09:00pm	**Student Mixer**	Culture Keeper's Hall

Schedule of Events, continued

Monday, June 26th

08:00am - 04:30pm	Registration	Culture Keepers Registration
08:30am – 10:20am	**SESSION 4: PROTEIN ARCHITECTURES - STRUCTURE AND FUNCTION** **Session Chair:** Philip Dawson, Scripps Research Institute	Kierland Ballroom 3 & 4
08:30am - 08:55am	**L18** – **Ron Raines,** Massachusetts Institute of Technology **A New Role for Methionine Residues**	
08:55am - 09:20am	**L19** – **Emma Watson,** The University of Adelaide **New Selenium-Mediated Methods for Protein Synthesis and Modification**	
09:20am - 09:40am	**L20** – **Brett VanVeller,** Iowa State University **Thioimidates: An Obscure Functional Group Provides General Access to Peptide Bond Isosteres**	
09:40am - 10:00am	**L21** – **Marina Rubini,** VU University Amsterdam **4-Thiaproline Eliminates the Slow Folding Phase of Proteins With Cis Prolines in The Native State**	
09:00am - 09:25am	**L22** – **Jianfeng Cai,** University of South Florida **Sulfonyl-γ-AApeptides: Structure and Function**	
10:20am - 10:45am	**Coffee with Exhibitors & Posters**	Hall of State, Kierland 1 & 2
10:45am - 12:15pm	**SESSION 5: PEPTIDE MATERIALS AND DELIVERY** **Session Chair:** Stephen Miller, Genentech	Kierland Ballroom 3 & 4
10:45am - 11:10am	**L23** – **Christopher Alabi,** Cornell University **PROteolysis TArgeting Chimeras - PROTACs**	
11:10am - 11:35am	**L24** – **Scott Medina,** Penn State College of Engineering **Real-Time, In Situ Imaging of Macrophages via Phase-Change Peptide Nanoemulsionss**	
11:35am - 11:55am	**L25** – **Giovanna Ghirlanda,** Arizona State University **Membraneless Organelles by Design: The Carboxysome**	
11:55am - 12:15pm	**L26** – **Shiroh Futaki,** Kyoto University **Liquid Droplet Formation and Facile Cytosolic Translocation of IgG in the Presence of Attenuated Cationic Amphiphilic Lytic Peptides**	
12:15pm - 12:45pm	**MAKINENI LECTURE** **Introduction:** Anna Mapp, University of Michigan **L27 - AW** – **César de la Fuente,** University of Pennsylvania **Artificial Intelligence Approaches for Antibiotic Discovery**	Kierland Ballroom 3 & 4
12:45pm - 02:30pm	**Lunch with Exhibitors - Provided**	Kierland Ballroom 1 & 2
01:00pm - 02:00pm	**International Liaison Meeting**	Powell Boardroom
02:30pm - 04:00pm	**SESSION 6: PEPTIDE DRUG DISCOVERY** **Session Chair:** Ved Srivastava, Perpetual Medicines	Kierland Ballroom 3 & 4
02:30pm - 02:55pm	**L28** – **Ruchia Duggal,** Merck **DMPK Optimization of Macrocyclic Peptides: Targeting Intra vs. Extracellular Sites**	
02:55pm - 03:20pm	**L29** – **Miguel Castanho,** University of Lisbon **Developing Broad Spectrum Brain-Targeting Peptide-Drug Conjugates Against Flaviviruses and Other Envelope Viruses**	
03:20pm - 03:40pm	**L30** – **Hongchang Qu,** Eli Lilly **The Novel GIP, GLP-1, and Glucagon Triple Receptor Agonist LY3437943: From Discovery to Clinical Proof-of-Concept**	
03:40pm - 04:00pm	**L31** – **Florence Brunel,** Novo Nordisk **A High Potency Protein that Normalizes Body Weight in DIO Mice through Triple Agonism at the FGF21, Glp-1 and GIP Receptors**	

Schedule of Events, continued

Monday, June 26th, cont.

04:00pm – 04:30pm **DR. ELIZABETH SCHRAM YI ORAL COMPETITION** — Kierland Ballroom 3 & 4

04:00pm – 04:15pm **L32 - YI3** – **Carly Schissel,** University of California, Berkeley
In-Cell Penetration Selection — Mass Spectrometry Produces Noncanonical Peptides for Antisense Delivery

04:15pm – 04:30pm **L33 - YI4** – **Sheryl Sharma,** University of Nebraska at Lincoln
Identifying Peptide-Receptor Interactions using Aryl Diazonium-Labeled Peptide Ligands

04:30pm - 04:45pm **Afternoon Break**

04:45pm - 05:25pm **POSTER FLASH TALKS** — Kierland Ballroom 3 & 4

05:25pm - 05:55pm **GOODMAN LECTURE** — Kierland Ballroom 3 & 4
Introduction: Joel Schneider, National Cancer Institute, Center for Cancer Research

L34 - AW – **James P. Tam,** Nanyang Technological University
Finding from Nature a New Paradigm for Orally-Active and Cell-penetrating Bioactive Microproteins

06:00pm - 08:00pm **GROUP 1 POSTER SESSION & RECEPTION** — Hall of State, Kierland 1 & 2

Tuesday, June 27th

08:00am - 12:30pm Registration — Culture Keepers Registration

08:30am - 10:25am **SESSION 7: FROM NATURAL PRODUCTS TO UNNATURAL BIOACTIVE PEPTIDES** — Kierland Ballroom 3 & 4
Session Chair: Robin Polt, University of Arizona

08:30am - 08:55am **L35** – **Dale L. Boger,** The Scripps Research Institute
Maxamycins: Redesigned Vancomycins for Resistant Bacteria

08:55am - 09:20am **L36** – **Andrew Roberts,** University of Utah
Tyrosine-Selective Peptide Cyclization Methods Inspired by Cyclic Peptide Natural Products

09:20am - 09:40am **L37** – **Christoph Nitsche,** Australian National University
Biocompatible Peptide Cyclisation and Protein Modification

09:40am - 10:00am **L38** – **Christina Schroeder,** Genentech
Recifin A, a Novel and Selective Allosteric Inhibitor of Tyrosyl- DNA Phosphodiesterase I with a Unique Disulfide-bond Topology

10:00am - 10:25am **L39** – **Andrei Yudin,** University of Toronto
Development of Privileged Macrocyclic Motifs Using the Tools of Chemical Synthesis

10:25am - 10:50am **Coffee with Exhibitors & Posters** — Hall of State, Kierland 1 & 2

04:00pm – 04:30pm **DR. ELIZABETH SCHRAM YI ORAL COMPETITION** — Kierland Ballroom 3 & 4

10:50am - 11:05am **L40 - YI5** – **Simon Rössler,** Massachusetts Institute of Technology
Abiotic Peptides for the Encoding of Small Molecule Synthesis

11:05am - 11:20am **L41 - YI6** – **Estefania Martinez-Valdivia,** University of Michigan
A Lipopeptidomimetic Transcriptional Inhibitor

Schedule of Events, continued

Tuesday, June 27th, cont.

11:20am - 12:50pm	**SESSION 8: NATURAL PRODUCT BIOSYNTHESIS & INSPIRATION FROM NATURE** **Session Chair:** Stephanie Barros, Janssen R&D	Kierland Ballroom 3 & 4
11:20am - 11:45am	**L42** – **Wilfred A. van der Donk,** University of Illinois at Urbana-Champaign **Biosynthesis and Engineering of Cyclic Peptide Natural Products**	
11:45am - 12:10am	**L43** – **Betsy Parkinson,** Purdue University **Synthetic Natural Product Inspired Cyclic Peptides for Discovery of Bioactive Natural Products and Biocatalysts**	
12:10am - 12:30pm	**L44** – **Ines Neundorf,** University of Cologne **Peptides Interfering With TANGO1 Reduce Collagen Secretion and Prevent Skin Fibrosis**	
12:30pm - 12:50pm	**L45** – **Arundhati Nag,** Carlson School of Chemistry and Biochemistry, Clark University **Use of Proximity-Catalyzed IEDDA Reaction for Cyclic Library Screening to Target Point-Mutant Protein**	
12:50pm	**Lunch on Your Own**	
01:00pm - 02:30pm	**Dr. Elizabeth Schram Young Scientists' Lunch & Mixer - *By Invitation***	Deseo
01:00pm - 02:30pm	**Exhibitor Workshop, hosted by Biotage** **Streamlining Hit-to-Lead Optimization: Automating Highly Parallel Peptide Synthesis and Purification** Come join us for lunch and meet our scientific team to learn about new technologies enabling high throughput peptide library production – reducing the time required to deliver compounds for secondary assay evaluation!	Cushing

Wednesday, June 28th

08:00am - 4:00pm	Registration	Culture Keepers Registration
08:30am - 10:25am	**SESSION 9: PEPTIDE SYNTHETIC METHODS & GREEN CHEMISTRY APPROACHES** **Session Chair:** Krishna Kumar, Tufts University	Kierland Ballroom 3 & 4
08:55am - 09:20am	**L47** – **Jenn Stockdill,** Wayne State University **Waste Not, Want Not: Innovations in Complex Peptide Synthesis Fueled by a Focus on Green Chemistry**	
09:20am - 09:40am	**L48** – **Nicholas Mitchell,** University of Nottingham **A Radical Approach to the Site-Selective Modification of Peptides and Proteins**	
09:40am - 10:00am	**L49** – **Beatriz Garcia De La Torre,** University of KwaZulu-Natal **Refractive Index: A Process Analytical Tool for Real-Time Monitoring of SPPS. Beyond a Qualitative Application**	
10:00am - 10:25amt	**L50** – **Nina Hartrampf,** University of Zurich **A Versatile "Synthesis Tag" for Chemical Protein Synthesis**	
10:25am - 10:50am	**Coffee with Exhibitors & Posters**	Hall of State, Kierland 1 & 2
10:50am – 11:05am	**DR. ELIZABETH SCHRAM YI ORAL COMPETITION** **L51 - YI7** – **Troy Smith,** University of Arizona **Minimally Competent Lewis Acids: Effective and Efficient Catalysts for β-Glycosylation of Peptide Hormones**	Kierland Ballroom 3 & 4

Schedule of Events, continued

Wednesday, June 28th, cont.

11:05am - 12:15pm	**SESSION 10: BIOINSPIRED & INTELLIGENT PEPTIDE MATERIALS** **Session Chair:** Ana Salome Veiga, Instituto de Medicina Molecular	Kierland Ballroom 3 & 4
11:05am - 11:30am	**L52** – **Matthew Tirrell,** University of Chicago , Pritzker School of Molecular Engineering **Peptide-Conjugated Polyelectrolyte Complex Micelles**	
11:30am - 11:55am	**L53** – **Abigail Knight,** University of North Carolina at Chapel Hill **Self-Assembly of Peptide-Polymer Hybrid Materials**	
11:55am - 12:15pm	**L54** – **Paula Gomes,** University of Porto **"Clicking" Peptides to Biomaterials and to Ionic Liquids: Towards Dual-Action Topical Formulations for Skin and Soft Tissue Infections**	
12:15pm – 12:45pm	**DU VIGNEAUD LECTURE** **Introduction:** Ron Raines, Massachusetts Institute of Technology	Kierland Ballroom 3 & 4
	L55 - AW – **Helma Wennemers,** ETH Zurich **Synthetic Collagen Peptides – From Structure to Function**	
12:45pm - 02:30pm	**Lunch on Your Own**	
12:45pm - 02:15pm	**Vincent du Vigneaud Award Lunch - *By Invitation***	Hall of State, Kierland 1 & 2
02:30pm - 02:45pm	**APS GENERAL ASSEMBLY**	Kierland Ballroom 3 & 4
02:45pm - 04:15pm	**SESSION 11: PEPTIDE DESIGN AND FUNCTION** **Session Chair:** Elisabetta Bianchi, IRBM	Kierland Ballroom 3 & 4
02:45pm - 03:10pm	**L56** – **Sidney M. Hecht,** Arizona State University/Biodesign Institute **Ribosomal Synthesis of Peptides and Proteins Containing Non-Canonical Amino Acids**	
03:10pm - 03:35pm	**L57** – **Maria Soloveychik,** SyntheX **Cell-Based Function Selection of Peptides for Protein-Protein Interaction Disruption and Targeted Protein Degradation**	
03:35pm - 03:55pm	**L58** – **Thomas Tucker,** University of Porto **Cyclic Peptide PCSK9 Inhibitors : The Design and Optimization of Highly Potent, Orally Bioavailable, and Clinically Viable Molecules as LDL-Cholesterol Lowering Therapeutics**	
03:55pm - 04:15pm	**L59** – **Ping Ye,** UCB **Discovery of Zilucoplan: A Potent Macrocyclic Peptide Complement Component 5, C5, Inhibitor in Acetylcholine Receptor Antibody-positive Generalized Myasthenia Gravis**	
04:15pm - 04:30pm	**DR. ELIZABETH SCHRAM YI ORAL COMPETITION**	Kierland Ballroom 3 & 4
	L60 - YI8 – **Alexander Lund Nielsen,** Ecole Polytechnique Fédérale de Lausanne **Assessing the Cellular Permeability of Peptidic Macrocycles in High-Throughput**	
04:30pm - 04:45pm	**Afternoon Break**	
04:45pm - 05:25pm	**POSTER FLASH TALK**	Kierland Ballroom 3 & 4
05:25pm - 05:55pm	**DU VIGNEAUD LECTURE** **Introduction:** Mark Distefano, University of Minnesota	Kierland Ballroom 3 & 4
	L61 - AW – **Marcey Waters,** University of North Carolina at Chapel Hill **How Do Post-Translational Modifications Turn on Protein-Peptide Interactions and how can We Inhibit Them?**	
06:00pm – 08:00pm	**GROUP 2 POSTER SESSION & RECEPTION**	Kierland Ballroom 3 & 4

Schedule of Events, continued

Thursday, June 29th

08:00am - 4:00pm	Registration	Culture Keepers Registration
08:30am - 10:05am	**SESSION 12: NEW FRONTIERS IN COMPUTATIONAL PEPTIDE DESIGN PART 1** **Session Chair:** E. James Petersson, University of Pennsylvania	Kierland Ballroom 3 & 4
08:30am - 08:55am	**L62** – **William "Bill" DeGrado,** University of California at San Francisco **De Novo Protein Design of Functional Proteins**	
08:55am - 09:15am	**L63** – **Fei Cai,** Genentech **Improved Naive Peptide Library Designs Guided by AI-prediction**	
09:15am - 09:40am	**L64** – **John Jumper,** DeepMind **Understanding Peptides and Their Interactions via Deep Learning Structure Prediction**	
09:40am - 10:05am	**L65** – **Carol K. Hall,** North Carolina State University at Raleigh **Computational Design of Peptides as Detectors, Sensors, and Drugs**	
10:05am - 10:25am	**Coffee Break**	Hall of State
10:25am - 11:30am	**SESSION 13: NEW FRONTIERS IN COMPUTATIONAL PEPTIDE DESIGN PART 2** **Session Chair:** Rumit Maini, PepLib	Kierland Ballroom 3 & 4
10:25am - 10:50am	**L66** – **Karen Akinsanya,** Schrödinger **Computational Peptide Design Advances: Past, Present, and Future**	
10:50am - 11:10am	**L67** – **Joshua Price,** Brigham Young University **Peptide/Protein Redesign Using Fine-Tuned ProtBert Masked Language Models**	
11:10am - 11:30am	**L68** – **Gaurav Bhardwaj,** University of Washington, Seattle **Machine Learning Guided Structure Prediction and Design of Macrocycles**	
11:30pm - 12:00pm	**EARLY CAREER LECTURESHIP AWARD** **Introduction:** Philip Dawson, Scripps Research Institute	Kierland Ballroom 3 & 4
	L69 - AW – **Lara Malins,** Research School of Chemistry at the Australian National University **Dialing in the Potential: Tunable Electrochemical Peptide Modifications**	
12:00pm - 02:00pm	**Lunch on Your Own**	
02:00pm - 03:05pm	**SESSION 14: NOVEL ADVANCES IN PEPTIDE CHEMISTRY** **Session Chair:** Anna Maria Papini, University of Florence	Kierland Ballroom 3 & 4
02:00pm - 02:25pm	**L70** – **Michael S. Kay,** University of Utah School of Medicine **D-Peptide Therapeutics**	
02:25pm - 02:45pm	**L71** – **Seino Jongkees,** Vrije Universiteit Amsterdam **Antiviral Peptides from mRNA Display Antiviral Peptides from mRNA Display**	
02:45pm - 03:05pm	**L72** – **Bobo Dang,** Westlake University, China **Copper-Assisted Sequence-Specific Chemical Protein Conjugation**	
03:05pm - 03:20pm	**Afternoon Break**	
03:20pm - 04:25pm	**SESSION 15: INNOVATIVE METHODS FOR PEPTIDE STRUCTURE** **Session Chair:** Wendy Hartsock, Aralez Bio	Kierland Ballroom 3 & 4
03:20pm - 03:45pm	**L73** – **Jose Rodriguez,** University of California at Los Angeles **Solving Peptide Structures and More: Frontier Advances in Electron Diffraction**	
03:45pm - 04:05pm	**L74** – **George Burslem,** University of Pennsylvania **Beta-Peptides as Conditional Ligands for Peptide Exchange in MHC-I**	
04:05pm - 04:25pm	**L75** – **Matthew Tucker,** University of Nevada at Reno **Mapping Structure and Dynamics with Site-Specific Vibrational Probe Pairs via 2D IR Spectroscopy**	

Schedule of Events, continued

Thursday, June 29th, cont.

04:25pm – 05:15pm	**CLOSING PLENARY KEYNOTE LECTURE** **Introduction:** William, "Bill" DeGrado, University of California, San Francisco **L76** – **Jim Wells,** University of California at San Francisco **Pirating Biology to Probe and Modulate the cell Surfaceome**	Kierland Ballroom 3 & 4
05:15pm - 05:20pm	**CLOSING REMARKS**	Kierland Ballroom 3 & 4
07:00pm - 10:00pm	**CLOSING BANQUET**	Kierland Ballroom 1 & 2

The Merrifield Award

Robert Bruce Merrifield, July 15, 1921 — May 14, 2006, was an American biochemist who won the Nobel Prize in Chemistry in 1984 for the invention of solid phase peptide synthesis. His wife Elizabeth, Libby, a biologist by training, joined the Merrifield laboratory at Rockefeller University where she worked for over 23 years. The Merrifield Award recognizes the lifetime achievement of a peptide scientist, whose work exemplifies the highest level of scientific creativity.

The R. Bruce Merrifield Award Recipients

2023	Sam Gellman	University of Wisconsin - Madison
2021	Padmanabhan Balaram	Indian Institute of Science, Bangalore, India
2019	Lila Gierasch	University of Massachusetts at Amherst
2017	Charles Deber	University of Toronto, Hospital for Sick Children
2017	Robert Hodges	University of Colorado at Denver
2015	Horst Kessler	TU München Institute for Advanced Study
2013	James P. Tam	Nanyang Technological University, Singapore
2011	Richard DiMarchi	Indiana University
2009	Stephen B.H. Kent	University of Chicago
2007	Isabella Karle	Naval Research Laboratory
2005	Richard A. Houghten	Torrey Pines Institute for Molecular Studies
2005	William F. DeGrado	University of Pennsylvania
2001	Garland R. Marshall	Washington University Medical School, St. Louis
1999	Daniel H. Rich	University of Wisconsin, Madison
1997	Shumpei Sakakibara	Peptide Institute, Inc.
1995	John M. Stewart	University of Colorado at Denver
1993	Victor J. Hruby	University of Arizona
1991	Daniel F. Veber	Merck, Sharp & Dohme
1989	Murray Goodman	University of California at San Diego
1987	Cho Hao Li	University of California at San Francisco
1985	Robert Schwyzer	Swiss Federal Institute of Technology
1983	Ralph F. Hirschmann	Merck, Sharp & Dohme
1981	Klaus Hofmann	University of Pittsburgh - School of Medicine
1979	Bruce Merrifield	The Rockefeller University
1977	Miklos Bodanszky	Case Western Reserve University

The 2023 Merrifield Award

Sam Gellman

Sam Gellman is the Ralph F. Hirschmann Professor of Chemistry and a Vilas Research Professor at the University of Wisconsin - Madison. He earned his A.B. from Harvard University in 1981 and his Ph.D. from Columbia University, under Ronald Breslow, in 1986. After a post-doctoral fellowship at the California Institute of Technology with Peter Dervan, Gellman joined the faculty at the University of Wisconsin - Madison in 1987. Major interests in Gellman's research program are focused on the structural and biological properties of peptides and proteins. Specific topics include fundamental studies of non-covalent interactions that control polypeptide folding and association, development and application of unnatural oligomers that display protein-like conformational behavior, "foldamers", creation of new amphiphiles for membrane protein manipulation, and development of biologically active polymers.

The accomplishments recognized by this award are those of the many talented and committed young scientists with whom Gellman has had the privilege to work. So far, this group includes 79 who have earned the Ph.D. and 32 who have completed post-doctoral studies in his laboratory.

Gellman was co-chair of the Peptides Gordon Research Conference in 2010. He served on the NIH Medicinal Chemistry Study Section 1999-2002 and on the NIGMS Advisory Council 2015-2018. Other service roles include the Board on Chemical Sciences and Technology, 2015-2021, and the Board of Trustees of the Gordon Research Conferences, 2020-2028. The work from Gellman's laboratory has been recognized by honors including the Vincent du Vigneaud Award, 2006, and the Makineni Lecture Award, 2013, from the American Peptide Society, the Ralph F. Hirschmann Award in Peptide Chemistry, 2007, and the Ronald Breslow Award in Biomimetic Chemistry, 2014, from the American Chemical Society, and the Meienhofer Award, 2019, from the Boulder Peptide Society. Gellman is a Member of the US National Academy of Sciences, a Foreign Member of the Hungarian Academy of Sciences, a Fellow of the American Academy of Arts & Sciences and a Fellow of the US National Academy of Inventors.

MERRIFIELD AWARD LECTURE

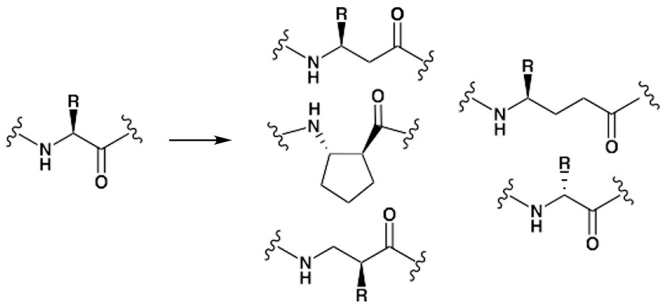
L17 - AW Sam Gellman

University of Wisconsin at Madison

Merrifield's Legacy: Transcending the Tyranny of the Ribosome

Samuel H. Gellman

The ribosome, aided by supporting actors such as tRNA synthetases, is the star of an extraordinary molecular spectacle. However, the chemist can imagine many constituents to be introduced into a polypeptide that the ribosome has not evolved to accommodate. Solid- phase synthesis circumvents this limitation. The creativity evident in contemporary peptide science reflects this capability.



Our group has participated in the extrapolation from conventional polypeptides by designing and evaluating examples containing β-, γ- and/or D-α-amino acid residues in addition to L-α-amino acid residues. Specific examples will be presented.

The Vincent du Vigneaud Award

The Vincent du Vigneaud Awards recognize outstanding achievement in peptide research at mid-career. The du Vigneaud Awards are sponsored by Bachem, and are awarded to two deserving recipients at the biennial American Peptide Symposia.

The Vincent du Vigneaud Award Recipients

2023	Helma Wennemers	ETH Zurich
2023	Marcey Waters	University of North Carolina at Chapel Hill
2021	Alanna Schepartz	University of California, Berkeley
2021	Joel Schneider	Center for Cancer Research, National Cancer Institute
2019	Annette Beck-Sickinger	Leipzig University
2019	Hiroaki Suga	The University of Tokyo
2017	Ronald Raines	University of Wisconsin at Madison
2017	Wilfred van der Donk	University of Illinois at Urbana-Champaign
2015	Jean Chmielewski	Purdue University
2015	David Craik	University of Queensland
2013	Michael Chorev	Harvard Medical Scool
2013	Kit Sang Lam	University of California, Davis School of Medicine
2011	Fernando Albericio	University of Barcelona
2011	Morten Meldal	Carlsberg Laboratories, Copenhagen
2010	Philip Dawson	Scripps Research
2010	Reza Ghadiri	Scripps Research
2008	Jeffery W. Kelly	Scripps Research
2008	Thomas W. Muir	Rockefeller University
2006	Samuel H. Gellman	University of Wisconsin
2006	Barbara Imperiali	Massachusetts Institute of Technology
2004	Stephen B. H. Kent	University of Chicago
2004	Dieter Seebach	Swiss Federal Institute of Technology at Zurich
2002	Robert Hodges	University of Colorado, School of Medicine
2002	Horst Kessler	Technical University of München
2000	Charles M. Deber	University of Toronto
2000	Richard A. Houghten	Torrey Pines Institute for Molecular Studies
1998	Peter W. Schiller	Clinical Research Institute of Montreal
1998	James A. Wells	Genentech, Inc.
1996	Arthur M. Felix	Hoffmann-La Roche, Inc.
1996	Richard G. Hiskey	University of North Carolina at Chapel Hill
1994	George Barany	University of Minnesota at Minneapolis
1994	Garland R. Marshall	Washington University Medical School at St. Louis
1992	Isabella Lugoski Karle	Naval Research Laboratory
1992	Wylie W. Vale	The Salk Institute for Biological Studies
1990	Daniel H. Rich	University of Wisconsin at Madison
1990	Jean E. Rivier	The Salk Institute for Biological Studies
1988	William F. DeGrado	DuPont Central Research
1988	Tomi K. Sawyer	The Upjohn Company
1986	Roger M. Freidinger	Merck, Sharp & Dohme
1986	Michael Rosenblatt	Massachusetts General Hospital
1986	James P. Tam	The Rockefeller University
1984	Betty Sue Eipper	The Johns Hopkins University
1984	Lila M. Gierash	University of Delaware
1984	Richard E. Mains	The Johns Hopkins University

The 2023 Vincent du Vigneaud Award

Marcey Waters

Professor Marcey Waters earned a bachelor's degree in chemistry at UC San Diego in 1992 after beginning her scientific career in the laboratory of Professor Charles Perrin in physical organic chemistry. She then completed a Ph.D. in chemistry from the University Chicago for her research in mechanistic organometallic chemistry in the group of Bill Wulff in 1997. She subsequently pursued research in bioorganic chemistry as an NIH postdoctoral fellow with Dr. Ronald Breslow at Columbia University. She joined the faculty of UNC Chapel Hill in 1999 where she is now the Glen Elder, Jr., Distinguished Professor of Chemistry.

Waters began work in peptides when she started her independent career and her early work focused on the study of beta-hairpin folding and function. Her current work focuses on the study of the molecular recognition of post-translational modifications and their isosteres in histone tail peptides in their role in the epigenetic regulation of gene expression. She served as the President of the American Peptide Society from 2017 to 2019. She is an AAAS fellow and has been recognized by UNC and the Association for Women Faculty and Professionals as a leader in mentorship for women and students from disadvantaged backgrounds. She has also received numerous teaching awards.

DU VIGNEAUD AWARD LECTURE

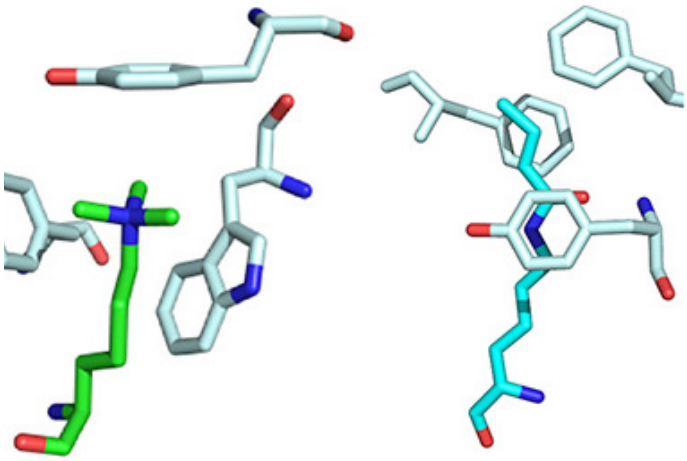
L61 - AW Marcey Waters

University of North Carolina at Chapel Hill

How Do Post-Translational Modifications Turn on Protein-Peptide Interactions and how can We Inhibit Them?

Marcey Waters

Post-translational modifications, PTMs, in histone proteins, including lysine methylation and acylation, regulate gene expression through recruitment of reader proteins to the nucleosome. Dysregulation of these events is prevalent in a wide range of diseases, such that there is much interest in characterizing these modifications and their binding partners as well as developing inhibitors for these protein-protein interactions.



I will discuss mechanistic studies of the factors that contribute to these PTM-mediated protein-protein interactions using a combination of high-throughput mechanistic screening, genetic code expansion, and investigation of PTM-isosteres, and how this has led to novel approaches to inhibit them.

Award Sponsor

BACHEM

The 2023 Vincent du Vigneaud Award

Helma Wennemers

Helma Wennemers studied chemistry at the Johann-Wolfgang-Goethe University in Frankfurt before moving to Columbia University, New York where she received her PhD degree for graduate studies with W. Clark Still in 1996. Following two years as a JSPS postdoctoral fellow at Nagoya University with Hisashi Yamamoto, she joined the faculty of Basel University as the Bachem-endowed assistant professor in 1999 where she was promoted to associate professor, 2004, and full professor, 2011. Since then she has been professor of chemistry at the ETH Zurich in the Laboratory of Organic Chemistry.

Helma Wennemers research group focuses on the development of peptides with functions that are fulfilled in nature by large macromolecules. She utilizes the power of synthesis to access functionalities that nature might not have had in the repertoire of building blocks. The focus is both on practical applications and an understanding of the properties of the peptides at the molecular level. This scope includes the development of peptides as asymmetric catalysts, functional collagen peptides, and peptidic scaffolds for applications in supramolecular and biological chemistry, for example cell-penetrating peptides, RNA recognition, and tumor targeting, and the controlled formation of metal nanoparticles.

Her work has been recognized by a number of awards, including the Leonidas Zervas Award from the European Peptide Society, 2010, the Inhoffen Medal from the Helmholtz Center, 2017, the Pedler Award from the Royal Society of Chemistry, 2016, the Netherlands Scholar Award for Supramolecular Chemistry, 2019, an Arthur C. Cope Scholar Award from the American Chemical Society, 2021, and the Scoffone Prize from the Italian Peptide Society, 2022.

DU VIGNEAUD AWARD LECTURE

L55 - AW Helma Wennemers

ETH Zurich

Synthetic Collagen Peptides – From Structure to Function

Helma Wennemers

Collagen, the most abundant protein in mammals, is a key contributor to the strength and stability of skin, bones, and connective tissue. Collagen formation is thus vital for the integrity of skin, tendons, and the tissue in essentially any organ. Excessive collagen formation is, however, characteristic of fibrotic and malignant diseases, which include major global health issues.

The Wennemers group has used collagen model peptides, CMPs, to understand the stability of collagen at the molecular level and to establish functional synthetic collagen triple helices. These include pH-responsive synthetic collagen, hyperstable triple helices, and heterotrimeric collagen. Building on these data, we designed and synthetized a chemical probe for the simultaneous monitoring and targeting of lysyl oxidase LOX-mediated collagen cross-linking. The probe allows for the detection of LOX activity in vivo and in tissue sections.

The Murray Goodman Scientific Excellence & Mentorship Award

The Murray Goodman Scientific Excellence & Mentorship Award was established in 2007 by an endowment from Zelda Goodman. 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. This award is presented at the biennial American Peptide Symposia.

The Murray Goodman Scientific Excellence & Mentorship Award Recipients

2023	James P. Tam	Nanyang Technological University
2021	Jean Chmielewski	Purdue University
2019	Fernando Albericio	University of KwaZulu-Natal
2017	Paul Alewood	The University of Queensland
2015	George Barany	University of Minnesota at Minneapolis
2013	Robert S. Hodges	University of Colorado at Denver
2011	Victor J. Hruby	University of Arizona
2009	Charles M. Deber	University of Toronto, Hospital for Sick Children

The 2023 Murray Goodman Scientific Excellence & Mentorship Award

James P. Tam

James P. Tam got his Ph.D. in Medicinal Chemistry with Daniel Rich at the University of Wisconsin, Madison and his postdoctoral training with Bruce Merrifield at The Rockefeller University. He held appointments as Associate Professor at The Rockefeller University, 1982-1991, Professor at Vanderbilt University, 1991-2004, and The Scripps Research Institute, 2004-2008. He is currently the Lee Wee Nam Professor and the Director of the Synzymes and Natural Products Center in School of Biological Sciences, Nanyang Technological University which he served as the Founding Dean of the School of Biological Sciences, the Founding Director of Biological Research Center and the Founding Director of the double-degree program in Biomedical Science and Chinese Medicine at Nanyang Technological University, Singapore.

His research interests cover peptide chemistry, discovery and applications for developing peptide biologics. He invented peptide dendrimers as protein mimetics in the 1980s for immunologics and synthetic vaccines, chemical ligations in the 1990s, and enzymatic Asx-ligation in 2010s. His recent research work focuses on the discovery, design and development of therapeutics for health extension, particularly ultra-stable, orally-active peptide biologics from medicinal plants. He has published >420 papers in these areas of research.

He has won major awards for his peptide research, including Vincent du Vigneaud Award, Bruce Merrifield award, Rao Makineni Lecture, all from the American Peptide Society, Akabori Memorial Award from the Japanese Peptide Society, Josef Rudinger Memorial Lecture Award, from the European Peptide Society, Cathay Award from the Chinese Peptide Society, and the Ralph Hirschman award from the American Chemical Society. In addition to mentor 31 PhD students, he has mentored >400 undergraduate students for their research and has been honored many times for his teaching, including Inspirational Mentorship of Koh Boon Hwee Scholars in 2012, 2013 and 2015, and International Traditional Chinese Medicine Contribution Award by the World Foundation of Chinese Medicine Society. He was elected as a Fellow by Singapore National Academy of Science in 2018.

GOODMAN AWARD LECTURE

L34 – AW James P. Tam

Nanyang Technological University

Finding from Nature a New Paradigm for Orally-Active and Cell-penetrating Bioactive Microproteins

James P. Tam, Shining Loo, Antony Kam, and Bamaprasad Dutta

A continuing challenge in discovery of peptide therapeutics from natural products is to find a new paradigm that target intracellular protein-protein interactions. An under-explored group of natural products that could fit such a paradigm is the family of super cystine-dense peptides or microproteins with 6-10 cysteine and 2-4 kDa in MW. They have sufficiently large footprints for on-target specificity and highly compact structures to resist proteolytic degradation.

Although cysteine-rich peptides of 2-7 kDa are found in hormones, growth factors, antimicrobials and toxins, few are orally active or cell- penetrating. Guided by traditional medicines, our laboratory has a long-standing research program in the discovery of orally-active super cystine-dense microproteins from medicinal plants.

Here, I will present our 25-year venture into their discovery, target identification, chemical synthesis of bioactive super cystine-dense microproteins. I will also present a new method for their chemical synthesis through an ultrafast oxidative folding that completes within a second. Our discoveries of "first-in-class" bioactive microproteins could provide therapeutic leads to address several prevalent health issues: cardiovascular diseases, stress and frailty, the underlying causes of numerous chronic and old-age-related diseases.

The Rao Makineni Lectureship

The Rao Makineni Lectureship was established in 2003 by an endowment by PolyPeptide Laboratories and Murray and Zelda Goodman. The Lectureship honors Rao Makineni, a long-time supporter of peptide science, peptide scientists, and the American Peptide Society. The Makineni Lectureship recognizes an individual who has made a recent contribution of unusual merit to research in the field of peptide science. The award is intended to recognize original and singular discoveries rather than cumulative or lifetime contributions. The award is presented at the biennial American Peptide Symposia.

The Rao Makineni Lectureship Award Recipients

2023	César de la Fuente	University of Pennsylvania
2021	Bradley L. Pentelute	Massachusetts Institute of Technology
2019	Xuechen Li	The University of Hong Kong
2017	Thomas Kodadek	Scripps Research Institute
2015	Paramjit Arora	New York University
2013	Samuel H. Gellman	University of Wisconsin at Madison
2011	Jeffery W. Kelly	Scripps Research
2009	William DeGrado	University of Pennsylvania
2007	Ronald T. Raines	University of Wisconsin at Madison
2005	Robin E. Offord	Centre Medical Universitaire, Switzerland
2003	James P. Tam	Vanderbilt University

The 2023 Rao Makineni Lectureship

César de la Fuente

César de la Fuente is a Presidential Assistant Professor at the University of Pennsylvania. De la Fuente is an NIH MIRA investigator and has received broad recognition and research funding. Prof. de la Fuente has received over 50 awards. He was recognized by MIT Technology Review as one of the world's top innovators for "digitizing evolution to make better antibiotics." He was selected as the inaugural recipient of the Langer Prize, an ACS Kavli Emerging Leader in Chemistry, and received the AIChE's 35 Under 35 Award and the ACS Infectious Diseases Young Investigator Award. In 2021, he received the Thermo Fisher Award, and the EMBS Academic Early Career Achievement Award "For the pioneering development of novel antibiotics designed using principles from computation, engineering, and biology."

Recently, Prof. de la Fuente was awarded the prestigious Princess of Girona Prize for Scientific Research, the ASM Award for Early Career Applied and Biotechnological Research and has been named a Highly Cited Researcher by Clarivate several times. Prof. de la Fuente has given over 200 invited lectures and his scientific discoveries have yielded over 110 publications, including papers in *Nature Biomedical Engineering, Nature Communications, PNAS, ACS Nano, Cell, Nature Chemical Biology, Advanced Materials,* and multiple patents.

RAO MAKINENI AWARD LECTURE

L27 - AW César de la Fuente

University of Pennsylvania

AI for Antibiotic Discovery

César de la Fuente

Artificial intelligence, AI, has the potential to outperform humans and revolutionize our world. In this talk, I will describe our efforts using AI to develop computational approaches for antibiotic design and discovery.

Computers can already be programmed for superhuman pattern recognition of images and text. In order for machines to discover novel antibiotics, they have to first be trained to sort through the many characteristics of molecules and determine which properties should be retained, suppressed, or enhanced to optimize antimicrobial activity. Said differently, machines need to be able to understand, read, write, and eventually create new molecules.

I will discuss how we trained a computer to execute a fitness function following a Darwinian algorithm of evolution to select for molecular structures that interact with bacterial membranes, yielding the first artificial antimicrobials that kill bacteria both in vitro and in relevant animal models.

My lab has also developed pattern recognition algorithms to mine the human proteome, identifying throughout the body thousands of antibiotics encoded in proteins with unrelated biological function, and has applied computational tools to successfully reprogram venoms into novel antimicrobials.

Computer-generated designs and innovations at the intersection between machine and human intelligence may help to replenish our arsenal of effective drugs, providing much-needed solutions to global health problems caused by infectious diseases.

Award Sponsor

PolyPeptide GROUP

The APS Early Career Lectureship

Established in 2019 and sponsored by the American Peptide Society, the Early Career Lectureship recognizes outstanding early career investigators who have demonstrated innovative research in peptide science. Two recipients will be chosen biennially and each will deliver a talk at the Symposium in a session appropriate to their work. The APS will support the registration, lodging at the conference hotel and up to $1000 in travel expenses of the awardees.

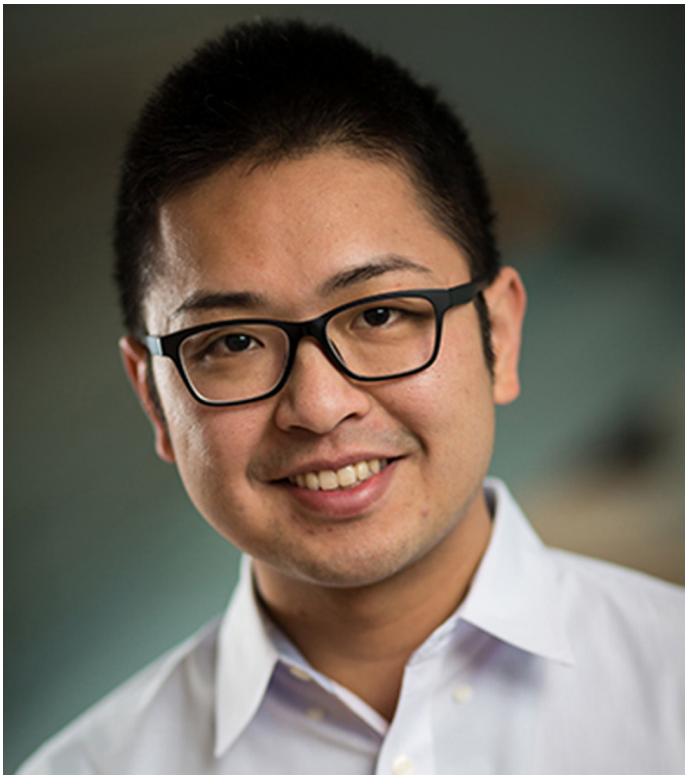
The APS Early Career Lectureship Award Recipients

2023	Lara Malins	Australian National University
2022	Danny Chou	Stanford University
2021	Caroline Proulx	North Carolina State University
2020	Yftah Tal-Gan	University of Nevada, Reno
2019	Monika Raj	Auburn University
2019	Jevgenij Raskatov	University of California at Santa Cruz

The 2022 APS Early Career Lectureship

Danny Chou

Danny Chou is an Assistant Professor of Pediatrics (Endocrinology and Diabetes) at Stanford University. He received his Ph.D. from Harvard University, working in the lab of Prof. Stuart Schreiber in 2011. His Ph.D. research involved the identification of suppressors of cytokine-induced apoptosis in pancreatic beta cells. He then moved to MIT, where he was a JDRF Postdoctoral Fellow in the Department of Chemical Engineering. He worked under the guidance of Profs. Robert Langer and Daniel Anderson, focusing on the development of glucose-responsive insulin derivatives.



Danny started his independent career in the Department of Biochemistry at the University of Utah in August 2014. At Utah, Danny's research focused on protein and peptide therapeutics for treating human diseases. In 2020, Danny moved his research lab to Stanford University to continue their efforts in developing novel peptide therapeutics.

The main research direction of the Chou lab is to use synthetic and enzymatic methods to engineer peptides with enhanced therapeutic potential focusing on insulin, interleukins and natural products from cone snail venom. He has received recognitions including a BPS Young Investigator Award, JDRF Career Development Award, Vertex Scholar, JDRF Postdoctoral Fellow and ADA Junior Faculty Award.

APS EARLY CAREER LECTURESHIP

L11-AW **Danny Chou**
Stanford

Stretching Peptides: Diyne-Braced Peptides as Beta-Strand Mimics

Danny Chou

Side effects from medicine often limit the therapeutic window and reduce overall clinical benefits. Approaches that can minimize side effects while maintaining therapeutic efficacies represent promising directions for therapeutic developments. Our lab develops stimuli-responsive systems where a physiological stimulus regulates the therapeutic efficacy of a biological drug. Such systems can shut down therapeutic effects in off-target situations and be activated upon stimulation, and therefore, increase the therapeutic window.

In this presentation, I will discuss our efforts in two disease areas. First, insulin analogs have markedly improved glycemic control in diabetics, but glycemic excursions still cause major health complications. The narrow therapeutic window of current insulin therapy makes it extremely difficult to maintain normoglycemia without risking severe hypoglycemia. We will discuss our efforts in glucose-responsive insulin development, which provides improved glycemic control in vivo.

Second, anticancer therapies often lead to serious side effects due to off-target effects. I will discuss our efforts to develop stimuli-responsive immunotherapies for cancer treatments. Such systems can reduce the off-target toxicity of anticancer drugs while maintaining therapeutic efficacy. Overall, our research aims to provide new therapeutic options that are more effective and safer for patients by exploiting the principles of peptide chemistry and stimuli-responsiveness.

The 2023 APS Early Career Lectureship

Lara Malins

Dr. Lara Malins is an Associate Professor at the Research School of Chemistry at the Australian National University, ANU. She completed her B.A. in chemistry at Boston University in 2009 before relocating to The University of Sydney to undertake her PhD with Professor Richard Payne on the development of chemoselective peptide ligation strategies. In 2015, Lara joined the laboratory of Professor Phil Baran at The Scripps Research Institute in La Jolla as a National Institutes of Health Ruth L. Kirschstein postdoctoral research fellow, where she worked on methods for the late-stage synthetic modification of peptides.

Dr. Malins returned to Australia in November 2017 to begin her independent career at the ANU, where she joined the Research School of Chemistry as a Lecturer and Australian Research Council, ARC, Discovery Early Career Research Fellow. She is currently a Westpac Research Fellow and Chief Investigator in the ARC Centre of Excellence for Innovations in Peptide and Protein Science, CIPPS.

The Malins lab focuses on the development of new synthetic methods for chemical biology, drug discovery, and natural product synthesis. The group's interest in leveraging underutilized modes of chemical activation, including electricity, light, and molecular strain, has led to the development of new strategies for the targeted functionalization of peptides, including highly tunable electrochemical oxidations and strain-promoted cycloaddition reactions. Dr. Malins has been recognized as an outstanding early career researcher as a recipient of the Australian Peptide Association's John Wade Early Career Researcher Award and the Royal Australian Chemical Institute's Rennie Medal.

APS EARLY CAREER LECTURESHIP

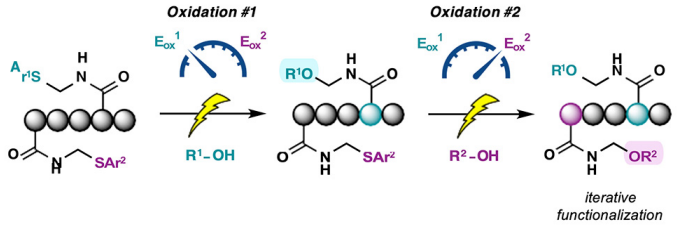
L69 – AW Lara Malins

Research School of Chemistry at the Australian National University

Dialing in the Potential: Tunable Electrochemical Peptide Modifications

Lara Malins

The use of electricity to drive chemical reactions is an appealing approach to green and sustainable synthesis. Moreover, electrochemistry provides powerful opportunities for the precise control of chemical reactivity by enabling practitioners to "dial in" the potential or current at which a reaction is performed. For highly functionalized molecules, this tunability provides unprecedented opportunities for targeted chemical modifications.



Despite these appealing characteristics, there are remarkably few examples in which electrochemistry is applied to the tunable functionalization of peptides. In this presentation, a method for the selective, iterative electrochemical modification of peptides is presented. Designer glutamine residues adorned with discrete "electroauxiliaries" — functional groups electronically-predisposed to anodic oxidation — are incorporated into peptides and exploited for iterative modifications to afford a library of high-value peptide N,O-acetals. The utility of the method is demonstrated on unprotected peptides and bioactive substrates and is applied in a novel approach to peptide macrocyclization.

This targeted approach to electrochemical activation serves to unlock a new level of orthogonality in peptide synthesis, and the strategy has promising applications for the preparation of peptide libraries, including for therapeutic development.

Dr. Elizabeth Schram Young Investigator Oral Competition

At each symposium, the ESCOM Foundation sponsors the Dr. Elizabeth Schram Young Investigator, YI, Oral Presentation Competition. The selected YIs are graduate students or postdoctoral scholars whose research has been recognized as cutting edge or work of high interest to the greater peptide community. Consequently, they have been invited to give an oral presentation on their research at the 2023 American Peptide Symposium this June. Their presentations will be judged by academic and industry professionals and winners of the competition will be declared at the end of the Symposium.

The 2023 Dr. Elizabeth Schram Young Investigator Oral Competition

L10-YI1
Zoë Adams
The Scripps Research Institute

L16 – YI2
Benjamin Emenike
Emory University

L41 – YI6
Estefania Martinez-Valdivia
University of Michigan

L60 – YI8
Alexander Lund Nielsen
The Scripps Research Institute

L40 – YI5
Simon Rössler
Massachusetts Institute of Technology

L32 – YI3
Carly Schissel
University of California, Berkeley

L33 – YI4
Sheryl Sharma
University of Nebraska at Lincoln

L51 – YI7
Troy Smith
University of Arizona

Young Investigator Poster Competition

At the biennial American Peptide Symposium, young investigators may elect to enter the Dr. Elizabeth Schram Young Investigator Poster Competition sponsored by the ESCOM Foundation. Young Investigators are defined as undergraduates, graduates and post docs. Cash prizes are awarded to the winners. These are this year's competitors.

2023 Dr. Elizabeth Schram Young Investigator Poster Competition

Aaghaz, Shams	National Institute of Pharmaceutical Education and Research
Abboud, Skander	The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology
Abid, Md Shadman Ridwan	University of Nebraska-Lincoln
Agrahari, Ashutosh	Purdue University
Akinwale, Ajayi	Chapman University School of Pharmacy
Al-Amin, Md	Arizona State University
Allen, A'Lester	University of Illinois Chicago
Almusaimi, Othman	Imperial College London
Anderson, Caleb	NCI, NIH
Ankrom, Emily	Lehigh University
Aoki, Keisuke	Kyoto University
Aróstica, Mónica	USM/PUCV
Austin, Maxwell	University of Utah
Babcock, Alana	University of Victoria
Beasley, Maryssa	US Naval Research Laboratory
Bhakta, Khushbu	Chapman University
Bhat, Prabhat	The Ohio State University
Bhattacharya, Sagar	Syracuse University
Biswas, Anamika	Tata Institute of Fundamental Research Hyderabad
Brennan, Alec	University of Nevada at Reno
Cardoso, Marlon	Universidade Católica Dom Bosco, UCDB
Carter, Molly	North Carolina State University
Chakraborty, Amit	University of Kwazulu-Natal
Chandra, Shambhu	University of British Columbia
Chang, Yanan	University of Pennsylvania
Chen, Chia-Yuan	University of Texas at Dallas
Cheng, Changfeng	University of Illinois at Chicago
Chingle, Ramesh	National Institutes of Helath
Choi, Yoonhwa	Seoul National University
Ciesiolkiewicz, Agnieszka	Wroclaw University of Science and Technology
Costantino, Michele	Arizona State University
Dahmen, Janina Kristin	University of Copenhagen
Deb, Rahul	CEITEC, Masaryk University
Diamandas, Matthew	University of Toronto
Ding, Yaoyu	King's College London
Draper, Steven	University of Utah
Dubey, Gopal	Texas A&M University
Durukan, Canan	Vrije Universiteit Amsterdam
Dutta, Chiranjit	George Mason University
Evenson, Garrett	University of Colorado at Boulder
Fredman, Jacob Dylan	University of Arizona

2023 Young Investigator Poster Competitors

Gauna, Adriana	ACCDIS, Universidad de Chile
Gimenez-Ibanez, Diana	University of Leeds
Gong, Xiaotian	Lafayette College
Gray, Mikaela	Georgia Tech
Grob, Nathalie	Massachusetts Institute of Technology
Hamdane, Yousra	University of Montreal
Han, Ingyu	Yonsei University
Harrelson, Skylar	NC State University
Hayward, Dee	University of East Anglia
Hink, Fabian	University of Copenhagen
Hintzen, Jordi	University of Gothenburg
Hornsby, Braxten	University of Utah
Ibukun, Olamilekan	Indian Institute of Science Education and Research - Kolkata
Islam, Majedul	Florida Atlantic University
Iwamoto, Naoya	Kyoto University
Jackson, Thomas	Imperial College London/ The Francis Crick Institute
Javed, Afraah	University of Southern California
Jedlinska, Zuzanna	University of Pennsylvania
Jin, Pengfei	University of Pennsylvania
Jing, Ruiheng	University of Colorado Boulder
Jones, Christopher	University of Rochester
Jones, Chelsea	University of California - Irvine
Kamayirese, Seraphine	Creighton University
Kelly, Joseph	University of Virginia
Kim, Chan Wook	Yonsei University
Knudson, Isaac	UC Berkeley
Lamer, Tess	University of Alberta
Lampkin, Bryan	Tufts University
Lepper, Hendrik	FAU Erlangen-Nuremberg
List, Moritz	Universität Leipzig
Liu, Zichen	University of Virgina
Lizandra Perez, Juan	Wroclaw University of Science and Technology
Lombardi, Lucia	Imperial College London
Maarouf, Nassim	University of Montreal
Macyszyn, Julia	University of Warsaw
Maloney, Robert	Temple University
Manne, Srinivasa Rao	University of KwaZulu-Natal
Marmorstein, Jason	University of Pennsylvania
Marshall, Liam	Syracuse University
Mehrani, Mona	University of Nevada Reno
Mifflin, Marcus	University of Utah
Mitrevski, Adam	Purdue University
Mondal, Sahabaj	IISER Kolkata
Moreno, Jonathan	Chapman University
Mousavi, Somayeh	University of Nebraska-Lincoln
Nadeau, Kyle	University of Rhode Island
Nagahara, Shingo	Tokyo University of Agriculture and Technology
Nakatsu, Koki	Nagoya University
Nambiar, Monessha	National Cancer Institute, NIH
O'Brien, Emily	Iowa State University

2023 Young Investigator Poster Competitors

Ocius, Karl	University of Virginia
Omura, Noriko	Tokyo University of Pharmacy and Life Sciences
Oppewal, Titia Rixt	University of Groningen
Panigrahi, Nihar	New York University
Paulussen, Felix	VU Amsterdam
Piast, Radoslaw	Warsaw University
Pifferi, Carlo	Center for Molecular Biophysics
Pinto, Denise	Philadelphia College of Osteopathic Medicine
Powell, Wyatt	University of Colorado Boulder
Prent, Luis	Universidad Andrés Bello
Qiu, Ryan	University of Alberta
Rafizadeh, Diane	University of Pennsylvania
Rahman, Mohammad Imtiazur	Arizona State University
Rai, Mrigank	Chapman University School of Pharmacy
Reja, Rahi Masoom	Boston College
Rezende, Samilla	Universidade Católica Dom Bosco
Robins, Marcy	University of Utah
Rodriguez, Jael	University of Michigan at Ann Arbor
Russell, Alexander	University of Washington
Samdin, Tuan	National Cancer Institute, NIH
Samiha, Rasheda	Clark University
Sarkar, Srijani	National Institute of Health
Scherer, Samuel	University of Utah
Sendanayake, Lasantha	Ohio University
Shankar, Sudha	Tel Aviv University
Sharma, Sachi	Imperial College London
Shida, Hayate	Tokyo University of Pharmacy and Life Sciences
Shimogawa, Marie	University of Pennsylvania
So, Regina	Brigham Young University
Spaltenstein, Paul	University of Utah
Srirangan, Showmika	Friedrich-Alexander-Universität, FAU
Stanford, Kevon	National Cancer Institute. NIH
Talukder, Zinya	Philadelphia College of Osteopathic Medicine
Tennett, Jessica	Fordham University
Tolulope Adebomi, Victor	University of Washington
Travis, Christopher	UNC - Chapel Hill
van Wier, Suzanne	University of East Anglia
Venneti, Naresh Murty	Wayne State University
Victorio, Clara	Fordham University
Voss, Saan	The Australian National University
Wang, Jing	Tufts University
Wani, Naiem	Weizmann Institute of Science
Warner, Ellen	North Carolina State University
Watari, Soei	Tottori University
Wei, Xiaozheng	University of Montreal
Xie, Yixin	National Cancer Institute
Yanagawa, Evan	University of Pennsylvania
Yang, Hyunjun	University of California San Francisco
Yeste Vazquez, Alejandro	VU Amsterdam
Yongo-Luwawa, Charity D.	Université de Montréal
Yoon, Leonard	The Scripps Research Institute

2023 Young Investigator Poster Competitors

Yoshida, Shuhei	Konan University
Yu, Lei	Janssen Research & Development
Yussif, Baba M.	University of Nebraska-Lincoln
Zhang, Yi	Stanford University
Zhao, Junwei	University of California - Davis
Zhou, Yan	Institute for Molecular Bioscience

2023 Travel Grant Recipients

The mission of the APS Travel Award Committee is to administer financial support to offset a portion of housing expenses in order to provide a broad opportunity for young investigators to participate in a major scientific event, meet leaders and colleagues in the field, and present their research projects to the scientific community.

2023 Travel Grant Recipients

Skander Abboud, The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology
Md Shadman Ridwan Abid, University of Nebraska Lincoln
Ashutosh Agrahari, Purdue University
A'Lester Allen, University of Illinois Chicago
Othman Almusaimi, Imperial College London
Emily Ankrom, Lehigh University
Khushbu Bhakta, Chapman University
Prabhat Bhat, The Ohio State University
Sagar Bhattacharya, Syracuse University
Amit Chakraborty, University of KwaZulu-Natal
Shambhu Chandra, University of British Columbia Vancouver
Agnieszka Ciesiolkiewicz, Wroclaw University of Science and Technology
Janina Kristin Dahmen, University of Copenhagen
Matthew Diamandas, University of Toronto
Hannah Distaffen, University of Rochester
Steven Draper, University of Utah
Gopal Krishan Dubey, Texas A&M University
Benjamin Emenike, Emory University
Diana Gimenez-Ibanez, University of Leeds
Xiaotian Gong, Lafayette College
Yousra Hamdane, Université de Montréal
Seraphine Kamayirese, Creighton University
Estefania Martinez Valdivia, University of Michigan Ann Arbor
Mona Mehrani, University of Nevada Reno
Adam Mitrevski, Purdue University
Shingo Nagahara, Tokyo University of Agriculture and Technology
Titia Rixt Oppewal, University of Groningen
Nihar Panigrahi, New York University
Wyatt Powell, University of Colorado Boulder
Tanya Román, Pontificia Universidad Católica de Valparaíso and Universidad Técnico Federico Santa María
Rasheda Samiha, Clark University
Sachi Sharma, Imperial College London
Marie Shimogawa, University of Pennsylvania
Christopher Travis, UNC- Chapel Hill
Naresh Murty Venneti, Wayne State University
Clara Victorio, Fordham University
Ellen Warner, North Carolina State University
Leonard Yoon, The Scripps Research Institute

Session Lecture Abstracts

OPENING PLENARY KEYNOTE LECTURE

L01 Laura Kiessling

Massachusetts Institute of Technology

Peptide-Glycan Interactions in Immunity

Zoë C. Adams, Anthony P. Silvestri, Sorina Chiorean, Dillon T. Flood, Brian P. Balo, Yifan Shi, Matthew Holcomb, Shawn I. Walsh, Colleen, A. Maillie, Gregory K. Pierens, Stefano Forli, K. Johan Rosengren, and Philip E. Dawson

Blueprints are needed to generate agents that alter immune responses, vaccines or tolerizing agents. The design principles have been elusive because many immune system receptors can transmit signals that lead to either immunity or tolerance, complicating a molecular understanding.

Our group is probing how cell surface glycans of foreign cells, pathogens, cancer cells, can be combined with antigenic peptides to influence immune responses. We are generating defined peptide-glycan conjugates that can co-opt the immune system to combat disease. Specifically, we found that glycan conjugates can elicit potent signals to mediate anti-cancer immunity. This seminar will discuss the relevant design features of these conjugates and the mechanisms underlying their activity against tumors.

SESSION 1: PEPTIDE TOOLS & PROBES

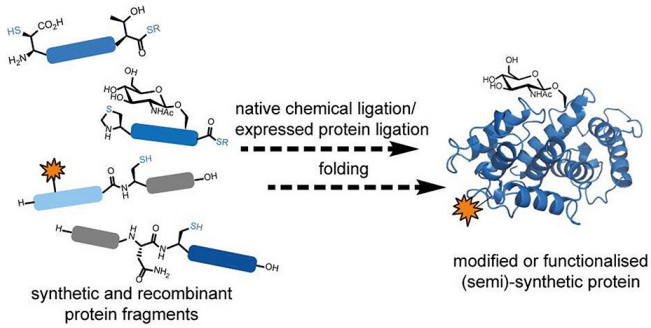
L02 Christian Becker

University of Vienna

Zooming into Posttranslational Modifications by Protein Semisynthesis

Christian F.W. Becker

At least 50% of all human proteins are predicted to experience one or more posttranslational modification, PTM, during their life cycle. These PTMs can result from enzymatic or non- enzymatic reactions. Enzymatic PTMs are well-known for being involved in regulating many cellular events such as gene expression, intracellular and extracellular signal transduction, protein- protein as well as cell-cell interactions. Non-enzymatic posttranslational modifications, nPTMs, are increasingly recognized to affect such events as well, with a special emphasis on age-related, metabolic and neurodegenerative diseases.



Here examples will be discussed that involve different semi-synthesis routes to access site-specific PTMs within proteins such as Hsp27, G-CSF and Tau. For the small heat shock protein Hsp27, which has been shown to carry non-enzymatic argpyrimidine, Apy, modifications, we can demonstrate that such Apy modifications significantly influence chaperon function for a variety of client proteins. Furthermore, new tools to access challenging protein targets via semi-synthesis will be discussed.

L03 Yong-Xiang Chen

Tsinghua University, Beijing

Synthesis and Functional Study on Diverse Phosphorus-Containing Proteins and Peptides

H.-Z. Duan, R. Chang, S.-H. Wang, Y. Li and Y.-X. Chen

Beyond canonical amino acids, natural and artificial modifications endow proteins and peptides with largely expanded properties and biofunctions. We have particular interests in various phosphorus- containing protein modifications, like natural phosphate, its phosphonate memetics and phosphine at low-oxidation state. The structure-defined homogeneous modified proteins and peptides are demanding for both mechanistic investigation and biomedical application.

In previous work, we have developed chemo- and bio- synthetic strategies for accessing proteins and peptides modified with phosphate or phosphatase-inert phosphonate, which have been applied to uncover the cross-talk of phosphorylation and lipidation on signaling protein K-Ras and Rnd3, as well as modulation of bioinorganic interface.3 Besides, we noticed that phosphorus at low oxidation state, as the privileged ligand of many renowned transitional metal catalysts, never appears in living organisms to function probably due to its oxygen sensitivity, while natural metalloproteins usually rely on nitrogen, sulfur and oxygen ligands.

We recently realized the first genetic incorporation of phosphine ligand into proteins by using a designed borane protected non-canonical amino acid, that overcomes the oxygen sensitivity of phosphine. Moreover, we disclosed a novel one- pot deprotection and metal coordination strategy to

allow for a facile synthesis of palladium-bound protein phosphine under aerobic conditions. This platform can enable the rational design of novel artificial metalloenzymes or functional metalloproteins.

L04 Nicholas Sawyer

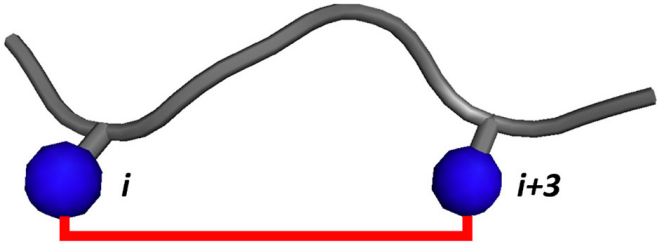
Fordham University

A Rational Crosslinking Approach for Peptide Polyproline II Helices

N. Sawyer

Polyproline II helices are ubiquitous structures in proteins with significant enrichment in both structural proteins, for example, collagen, and as ligands for modular protein-protein interaction domains, for example, SH3, WW, and EVH1. Thus, approaches to stabilize peptide polyproline II helices offer opportunities for advanced materials and enhanced modulators of protein-protein interactions. Here we describe a rational approach to stabilize peptide polyproline II helices using site-specific crosslinking.

Initial crosslinker design was inspired by a correlation between the number of crosslinker atoms and crosslinked distance across a variety of crosslinkers used to stabilize α-helices. Our model system was the EphB2 receptor-specific SNEW peptide, which adopts a polyproline II helix within its C-terminal half when bound to the receptor. We identified several crosslinked SNEW variants with enhanced inhibitory potency against the EphB2-ephrin B2 interaction, as expected for crosslinker stabilization of the bound peptide structure. These variants also displayed enhanced resistance to protease digestion and retained their specificity for inhibition of the EphB2 receptor over other Eph receptors. Structural analysis of the crosslinked peptide region was consistent with a polyproline II helix conformation.



We envision that this crosslinking approach will be generalizable to stabilize peptides in the polyproline II helical conformation for various biomedical and materials applications.

L05 Dennis Bong

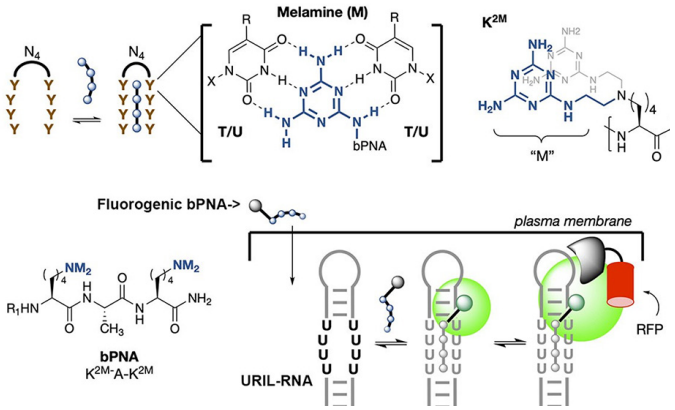
The Ohio State University

Novel Fluorogenic Peptide Probes for Intracellular RNA and RNP Tracking

Dennis Bong

We introduce herein a new peptide-based strategy for intracellular RNA and DNA tracking that is robust, orthogonal, and complementary to existing methods: Fluorogenic U-Rich Internal Loop, FLURIL, tagging with cell-permeable bifacial Peptide Nucleic Acids, bPNAs.

Bifacial bPNAs utilize synthetic triazine bases to form triplex hybrid stems with oligo T/U domains that are biomimetic of native triple stranded structures formed from UAU base-triples. Our approach uses an 8-nt (U_4xU_4) U-rich internal loop, URIL, in the RNA of interest, ROI, as a compact labeling site for fluorogenic triplex hybridization with a bPNA probe, ~ 1 kD, that derives from a tripeptide.



FLURIL tagging thus replaces a 4 bp duplex stem with a labeled 4-base-triple hybrid stem of similar structure and mass. In contrast to existing strategies for RNA tracking, FLURIL tagging can be applied to internal, genetically encoded URIL RNA sites *with minimal structural perturbation*, co-expression of protein-fusion labels, or significant increase in molecular weight and steric bulk. Together, these experiments show that FLURIL tagging can simply and reliably track intracellular RNA, RNPs, and DNA, with a streamlined molecular footprint relative to other methods. Our data also indicate that FLURIL tagging is fully compatible with existing labeling methods and thus may be used to broaden the scope of intracellular RNA and DNA tracking.

We further discuss structure-function studies that examine the impact of peptide structure on DNA/RNA binding by bPNAs. We find that there are two bPNA backbone modifications that significantly improve hybridization: alternating (D, L) configuration in open chain dipeptides and homochiral dipeptide cyclization to the diketopiperazine. The resulting family of di and tripeptides that efficiently bind T/U loops in nucleic acids is remarkably compact. We anticipate that the chemical tunability of this simple peptide probe scaffold will

enable a broad range of applications for URIL-tagged intracellular RNA and RNP interactions.

L06 Amelia Fuller

Santa Clara University

Aromatic Residue Placement Influences Helical Structure of Short Peptoid Oligomers in Aqueous Solutions

J. Javed, K. Scukas, and A. A. Fuller

Sequence-specific, monodisperse peptoids, N-substituted glycine oligomers, have found diverse utility in biological and materials applications. A foundational understanding of residues that promote peptoid structure by influencing *cis-trans* amide bond equilibria has emerged. Nonetheless, few studies of water-soluble peptoid structures are available. Access to short, water-soluble peptoids with well- defined three-dimensional structures will advance the functional repertoire of peptoids.

In this work, we will present our systematic study of the structures of water soluble 6-mer peptoids. These peptoids include both aromatic and polar, charged residues sequenced in a putative amphiphilic ordering. Residues include both aromatic and polar groups that exhibit an energetic preference for the cis-amide conformation and are thus expected to promote a helical peptoid structure. Structures of the synthesized peptoid oligomers have been studied by CD spectroscopy and NMR spectroscopy in a range of aqueous solution conditions to evaluate both sequence and environmental contributions to structural ordering.

We have observed that the positioning of the aromatic residues in the peptoid sequence has a pronounced influence on peptoid structure; when residues are at the N-terminal and fourth position in the sequence, the structural ordering is different than when these residues are placed at other *i*, *i* + 3 positions in the sequence. The effects of aromatic residue identity and the identity of polar residues on peptoid structures will also be compared and presented.

This work will build a foundation of understanding about peptoid sequence-structure relationships; we and others will build on this to design and study water-soluble peptoids with varied functions.

SESSION 2: PROTEIN MODIFICATIONS FOR ENABLING BIOLOGY

L07 Champak Chatterjee

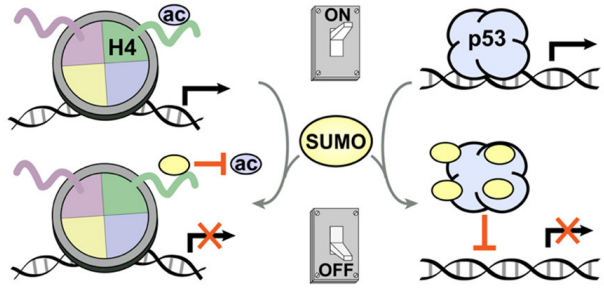
University of Washington

SUMO: Wrestling with Gene Transcription

K. Champak Chatterjee

The small ubiquitin-like modifier protein, SUMO, is found in all eukaryotic organisms where it plays key roles in regulating protein conformation, localization, and function. Enzymatic conjugation of SUMO with lysine side-chains in protein substrates, called sumoylation, is a tightly regulated and highly dynamic process in our cells.

Two biomedically important families of nuclear proteins modified by SUMO are transcription factors and histones. The mechanistic contribution of SUMO to their critical roles in gene regulation, however, are largely unknown due to the low abundance and dynamic nature of these sumoylated proteins.



We addressed this challenge by developing novel synthetic, semisynthetic, and molecular biological strategies to access site-specifically sumoylated human histone H4 and the transcription factor p53. Biochemical studies with the uniformly modified proteins revealed that SUMO inhibits RNA polymerase II mediated gene transcription in both contexts, but through distinct mechanisms.

Results from our mechanistic studies, including the discovery of novel biochemical crosstalk between sumoylation, acetylation, and methylation, will be discussed in the context of human gene regulation by SUMO.

L08 Yael David

Memorial Sloan Kettering Cancer Center

Uncovering Cancer-Associated Epigenetic Events using Novel Chemical Tools

Yael David

Cellular proteins continuously undergo non-enzymatic covalent modifications, NECMs, which alter protein structure, function, stability and binding-partner affinity. These chemical modifications accumulate under normal physiological conditions but can also be stimulated by various changes in the cellular environment, such as redox state and metabolite concentration. The half-life of histones is among the longest in the cellular proteome, making them prime targets for NECMs. In addition, histones have emerged as key regulators of transcription, acting primarily through post-translational modification to their disordered N-terminal regions, which are rich in lysines and arginines.

Indeed, we have recently shown that non-enzymatic glycation is a pathophysiological modification that specifically accumulates on histones in metabolically abrogated cells such as cancer tumors. We further showed that histone glycation disrupts regulatory post-translational modifications, PTMs, acetylation, methylation, et cetera, as well as chromatin architecture.

Finally, we identified several potent regulators of histone glycation *in vitro* and in cells and defined them as new therapeutic targets. In my talk I will describe how we used both small-molecule probes and novel peptides to characterize histones glycation, their effect on the epigenetic landscape as well as the enzymes we revealed to regulate them, presenting a new and direct link between metabolism and cell fate.

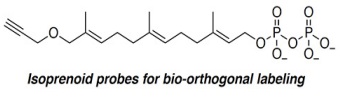
L09 Mark Distefano

University of Minnesota

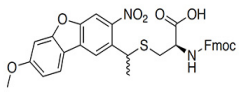
Protein Prenylation: New Tools Reveal New Biology

Mark Distefano

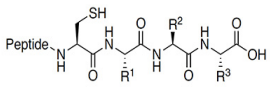
Protein lipid-modification involves the attachment of hydrophobic groups to polypeptides within cells after they are synthesized by ribosomes. The purpose of these modifications is to anchor specific proteins to the cell membrane where they can relay chemical messages from the exterior to the cellular interior.



Isoprenoid probes for bio-orthogonal labeling



Photoremovable protecting groups for thiol release



Peptide libraries to interogate enzyme specificity

Protein prenylation is one example of lipid modification and consists of the addition of either C_{15} or C_{20} isoprenoid groups to a variety of proteins; such proteins play key roles in regulating processes within cells including cell division, shape, differentiation and memory. A number of inhibitors of this enzyme and others in the protein prenylation pathway are currently in clinical trials for cancer therapy and other diseases.

This presentation will describe the development of new chemical tools and how they have been used to probe the biology of protein prenylation as well as streamline the development of new protein-based therapeutics. New methodology for the synthesis of peptide libraries has enabled the specificity of prenyltransferases to be probed in detail and illuminated new types of proteins that may carry this modification. Synthetic isoprenoid probes have been used to reveal dysregulated prenylation in a range of systems ranging from human cancer cells to Alzheimer's disease. New photoremovable protecting groups for thiols, that can be activated via two photon-excitation, have been used to trigger or inhibit protein lipid modification in living cells.

DR. ELIZABETH SCHRAM YOUNG INVESTIGATORS ORAL COMPETITION

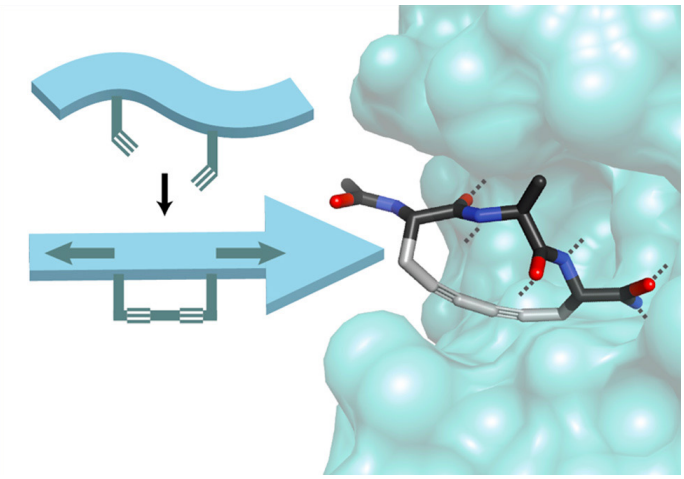
L10-YI1 Zoë Adams

The Scripps Research Institute

Stretching Peptides: Diyne-Braced Peptides as Beta-Strand Mimics

Zoë C. Adams, Anthony P. Silvestri, Sorina Chiorean, Dillon T. Flood, Brian P. Balo, Yifan Shi, Matthew Holcomb, Shawn I. Walsh, Colleen A. Maillie, Gregory K. Pierens, Stefano Forli, K. Johan Rosengren, and Philip E. Dawson

Extended amino acid backbone conformations are an abundant structural motif responsible for mediating a myriad of protein-protein interactions, PPIs. Yet, the shallow protein surfaces that dominate PPIs are challenging to target using standard methods and approaches for accessing extended backbone structures are limited. Often these types of interfaces include extended β-strand regions that define ordered backbone and side chain orientations that contribute to specific recognition of protein targets.



We explored the incorporation of a rigid, linear, diyne brace between side chains at the *i* to *i*+2 positions to generate a family of low-molecular-weight extended-backbone peptide macrocycles. NMR and DFT studies show that these stretched peptides adopt stable rigid conformations in solution and can be tuned to explore extended peptide conformational space.

This class of molecules is amenable to mimicking the backbone structure of natural β-strand motifs such as peptide ligands, inhibitors, and natural products by tuning the identity of the diyne macrocycle. The formation of the diyne brace is accomplished in excellent conversions, >95%, is amenable to high throughput synthesis, and thelow-molecular weight structure-inducing tripeptide core, <300 Da, is compatible with further synthetic elaboration. We showcase the utility of diyne-braced peptides with the synthesis of macrocyclic inhibitors of bacterial signal 1 peptidase.

EARLY CAREER LECTURESHIP AWARD

L11-AW Danny Chou

Stanford

Stimuli-Responsive Therapeutics through Peptide Chemistry

Danny Chou

Side effects from medicine often limit the therapeutic window and reduce overall clinical benefits. Approaches that can minimize side effects while maintaining therapeutic efficacies represent promising directions for therapeutic developments. Our lab develops stimuli-responsive systems where a physiological stimulus regulates the therapeutic efficacy of a biological drug. Such systems can shut down therapeutic effects in off-target situations and be activated upon stimulation, and therefore, increase the therapeutic window.

In this presentation, I will discuss our efforts in two disease areas. First, insulin analogs have markedly improved glycemic control in diabetics, but glycemic excursions still cause major health complications. The narrow therapeutic window of current insulin therapy makes it extremely difficult to maintain normoglycemia without risking severe hypoglycemia. We will discuss our efforts in glucose-responsive insulin development, which provides improved glycemic control in vivo.

Second, anticancer therapies often lead to serious side effects due to off-target effects. I will discuss our efforts to develop stimuli-responsive immunotherapies for cancer treatments. Such systems can reduce the off-target toxicity of anticancer drugs while maintaining therapeutic efficacy. Overall, our research aims to provide new therapeutic options that are more effective and safer for patients by exploiting the principles of peptide chemistry and stimuli-responsiveness.

SESSION 3: NOVEL MODULATORS OF BIOLOGY

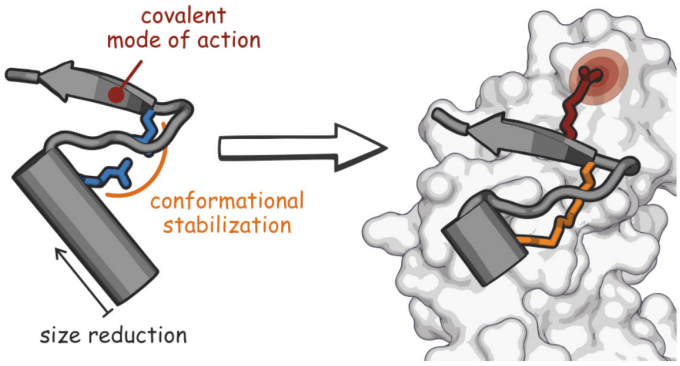
L12 Tom N. Grossmann

VU University Amsterdam

From Protein Structures to Functional Biomimetics

Tom N. Grossman

The inhibition of disease-relevant protein-protein interactions, PPI, represents an appealing strategy towards the development of novel therapeutics. Due to the extended nature of involved interaction areas, conventional ligand-discovery approaches that rely on small molecular scaffolds often fail to provide potent PPI inhibitors. Due to their excellent surface recognition properties, peptido- and proteomimetic molecules provide appealing alternatives.



Testing a new anti-microbial strategy, we designed a proteomimetic derived from the peri- plasmic region of a bacterial protein, FtsB, that is part of the divisome complex. The bioactive conformation of this peptide was stabilized by a customized cross-link resulting in a tertiary structure mimetic with increased affinity for a FtsB binding partner, FtsQ. To increase activity, a covalent handle was incorporated, providing an inhibitor that impedes the interaction between FtsQ and FtsB irreversibly. The covalent inhibitor reduced the growth of an outer membrane-permeable *E. coli* strain, and affected the infection of zebrafish larvae. This first-in-class inhibitor of a divisome PPI highlights the potential of proteomimetics as inhibitors of challenging therapeutic targets.

L13 Nir Qvit

Bar-Ilan University

Engineered Novel Protein-Protein Interaction Regulators to Study Mitochondrial Function and Modulate Mitochondrial Homeostasis

Nir Qvit

Mitochondria are maternally inherited, cytoplasmic organelles that play central roles in maintaining cellular metabolic

homeostasis, cell survival, and cell death. They are also the so-called "powerhouses" of cells as they are primarily responsible for providing the supply of adenosine triphosphate, ATP, the energy "currency" of the cell by converting oxygen and nutrients into ATP via oxidative phosphorylation in the inner mitochondrial membrane. The major mechanisms by which mitochondria maintain their homeostasis are mitochondrial quality control mechanisms such as mitophagy and mitochondrial dynamics including both fission and fusion.

Protein-protein interactions, PPIs, are central to most biological processes and are often dysregulated in many diseases, making them important therapeutic targets. Compared with the highly conserved nature of binding pockets, for example, substrate in enzyme, PPI interfaces are inherently more diverse. Hence, they offer the potential of differentiating between many proteins and even between homologous enzymes, since the sequence and/or structure of their PPI sites are usually unique. Peptides and peptidomimetics, modified peptide, are ideal candidates to target PPIs, as they demonstrate many advantages, such as conformational flexibility, high selectivity and potency.

Using a rational design approach, we developed modulators of selective PPI sites that target mitochondrial homeostasis in a highly specific and effective manner. These modulators modulate individual proteins and pivotal signaling pathways of the mitochondrial quality control mechanisms and reveal undiscovered pathways in mitochondrial homeostasis and may result in novel therapeutics that are of great clinical importance.

L14 Vita Sereikaite

University of Copenhagen

Towards Cognition Enhancing Therapeutics: Regiospecific Modulation of $GABA_B$ Receptors using Peptide-Based Inhibitors

Ziyang Chen, Pascal D. Rem, Jochen Schwenk, Søren Østergaard, Maria Vistrup-Parry, Christian R. O. Bartling, Mads M. Nygaard, David Gloriam, Bernhard Bettler, Kristian Strømgaard, Vita Sereikaite

Neuronal function is dependent on tightly regulated processes and a fine-tuned balance of synaptic excitation and inhibition. γ-Amino butyric acid, GABA, is the primary inhibitory neurotransmitter in the central nervous system, CNS, and activates ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors, GBRs, to modulate neuronal inhibition. The GBR is recognized as a major component in learning and memory, and dysfunction has been implicated in severe neurological disorders such as depression, anxiety, schizophrenia, and cognitive deficits.

Recent proteomic studies identified three transmembrane proteins interacting specifically with the GB1a/2 receptor through the sushi domains, SDs: the amyloid precursor protein, APP, the adherence-junction associated protein 1, AJAP1, and the PILRa-associated neural protein, PIANP.

Functionally, APP mediates the trafficking of GB1a/2 receptors to the presynaptic site, where GB1a/2 receptors are likely transferred to AJAP1 or PIANP that localize the receptor at the cell surface. Activated presynaptic GBRs at glutamatergic terminals inhibit the release of neurotransmitters and thereby inhibit excitatory neuronal transmission.

In this study, we aim to specifically target presynaptic GBRs, as a novel principle for a regulated increase in glutamate release and thereby providing novel means to potentially treat diseases related to cognitive impairment. We first employed a microarray peptide technology, μSPOT, to determine binding epitopes of SD-specific protein-protein interactions, PPIs. We used our newly developed array screening method to perform positional scans of the defined binding epitopes and deciphered key residues facilitating APP, AJAP1 and PIANP interactions with SD1. Subsequently, this information was combined with *in silico* screening resulting in highly potent peptide-based modulators of presynaptic GBR, providing promising lead compounds towards development of cognition enhancing therapeutics.

L15 Eileen Kennedy

University of Georgia

Novel Allosteric Targeting Strategies to Inhibit LRRK2 in Parkinson's Disease using Constrained Peptides

Eileen Kennedy

The Leucine-Rich-Repeat-Kinase-2, LRRK2, protein is a multi-domain protein and missense mutations along the different domains of LRRK2 are the most common cause of genetically associated Parkinson's Disease, PD. LRRK2 has a complex activation mechanism involving intra-molecular signaling, dimerization and protein-protein interactions.

It is not well understood how LRRK2 activity is regulated and how mutations in several domains of the protein alter its activity and function. Although symptomatic therapies are available for PD patients, there is currently no curative treatment for the disease. Several ATP-competitive kinase catalytic inhibitors have been developed for LRRK2, but many have side effects including kidney and lung toxicity and none of the inhibitors have yet received clinical approval for the treatment of PD.

As an alternative approach, we developed constrained peptides that either allosterically target LRRK2 itself or target a key downstream signaling pathway that is upregulated by pathogenic LRRK2. These are entirely new strategies to regulate LRRK2. As a strategy to disrupt LRRK2 activity, hydrocarbon-constrained "stapled" peptides were developed to target key intra- and intermolecular surfaces of the LRRK2 dimer as a novel strategy to downregulate LRRK2 function. These cell-permeable compounds were found to inhibit LRRK2 activation and disease-related signaling in both wild-type and mutant forms of LRRK2. Further, unlike many

ATP-competitive LRRK2 inhibitors, these peptide inhibitors did not induce LRRK2 mislocalization in cells. Overall, this work may demonstrate alternate, innovative approaches to allosterically inhibit/regulate altered LRRK2 activity.

DR. ELIZABETH SCHRAM YOUNG INVESTIGATORS ORAL COMPETITION

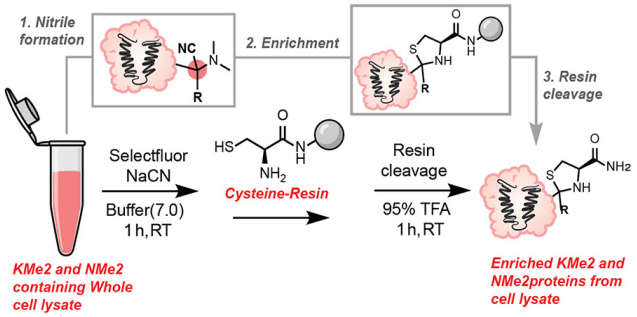
L16-YI2 Benjamin Emenike

Emory University

Chemical Tools for Profiling Lysine Dimethylation and N-Terminal Dimethylation of Proteins

Benjamin Emenike

One of the ways through which nature increases the functional diversity of the proteome is through a process known as posttranslational modifications, PTMs. Dimethylation of lysine, Kme2, and N-termini, NMe2, of proteins is a post-translational modification which involves the catalytic attachment of two methyl groups onto lysine side chain or the N-termini amines.



Despite the surging interest towards this modification and its emergence as a key component of diverse set of biological events, its global identification has remained an unachieved goal. This is due to the lack of pan specific chemical tools for tagging of Kme2 and NMe2.

Our approach towards the global profiling of Kme2 and NMe2 involves the use of two independent tertiary amine trapping reactions: tertiary amine coupling via oxidation, TACO, and tertiary amine nucleophilic substitution, TANS.

Preliminary data highlights the efficiency of both techniques in tagging KMe2 and NMe2 in a pan-specific manner, with the added advantage of faster reaction kinetics and near-quantitative conversions. Our long-term goal is to utilize these technologies for the global profiling of KMe2 and NMe2 sites, and identification of disease related KMe2 and NMe2 biomarkers in the human proteome. To the best of our knowledge, this represents the first attempt towards labeling of KMe2 and NMe2 using a chemical strategy, thus the innovation of this work.

MERRIFIELD AWARD LECTURE

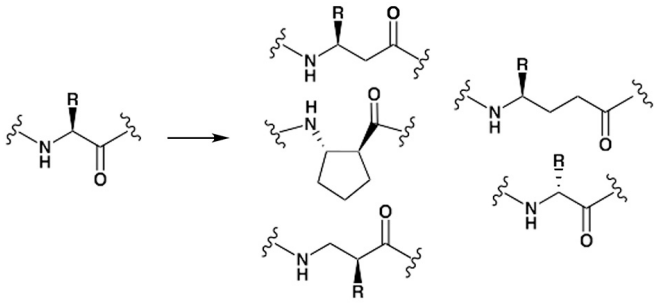
L17-AW Sam Gellman

University of Wisconsin at Madison

Merrifield's Legacy: Transcending the Tyranny of the Ribosome

Samuel H. Gellman

The ribosome, aided by supporting actors such as tRNA synthetases, is the star of an extraordinary molecular spectacle. However, the chemist can imagine many constituents to be introduced into a polypeptide that the ribosome has not evolved to accommodate. Solid- phase synthesis circumvents this limitation. The creativity evident in contemporary peptide science reflects this capability.



Our group has participated in the extrapolation from conventional polypeptides by designing and evaluating examples containing β-, γ- and/or D-α-amino acid residues in addition to L-α-amino acid residues. Specific examples will be presented.

SESSION 4: PROTEIN ARCHITECTURES – STRUCTURE AND FUNCTION

L18 Ron Raines

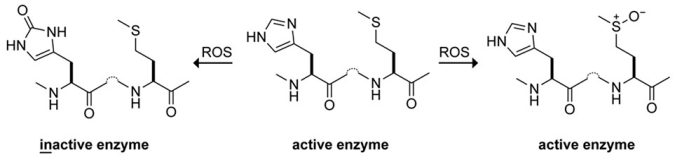
Massachusetts Institute of Technology

A New Role for Methionine Residues

Ronald T, Raines

Cysteine and methionine are relatively rare residues that are well known for their susceptibility to oxidation by reactive oxygen species, ROS. Histidine is the most common residue in enzymic active sites and also reacts with ROS, forming

2-oxo-histidine. The active site of RNase 1, which is a human homologue of RNase A, contains two histidine residues that perform acid–base catalysis to effect RNA cleavage in the oxygen-rich extracellular environment.



We observed that RNase 1 contains five methionine residues, each proximal to the active-site histidines. We reasoned that these methionine residues could serve as sacrificial "anti-oxidants" that preserve the integrity of the histidines. We found that the susceptibility of RNase 1 to oxidation does indeed increase upon treatment with an oxaziridine –which forms an S=N bond– or replacement of methionine residues with norleucine. Moreover, we find that S–aryl interactions, which can make methionine residues more, or less, susceptible to oxidation, conspire in the anti-oxidant activity.

Acknowledgements
This work was supported by NIH grants R35 GM148220 and R01 CA07308.

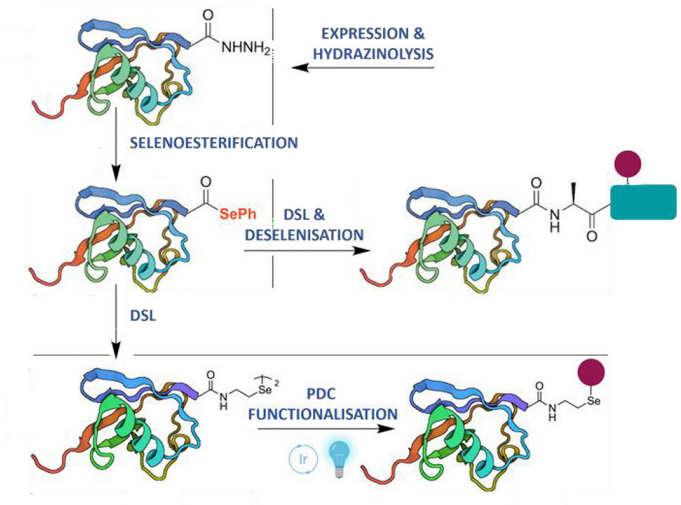
L19 Emma Watson

The University of Adelaide

New Selenium-Mediated Methods for Protein Synthesis and Modification

E. E. Watson, S. S. Kulkarni, J. W. C. Maxwell, J. Johansen- Leete, L. J. Dowman, A. M. Giltrap, A. R. Norman, C. F. W. Becker and R. J. Payne

Selenium provides unique opportunities in the chemical synthesis and modification of proteins owing to its enhanced reactivity compared to corresponding sulfur-containing components. We have extended the application of such reactivity to expressed protein fragments through the conversion of C-terminal acyl hydrazides to the corresponding selenoesters which provide valuable intermediates for the synthesis of proteins with modifications near their C-terminus via the Diselenide-Selenoester Ligation, DSL.



These selenoesters can also be used for the incorporation of diselenides into expressed proteins to serve as substrates for late-stage modification via Photocatalytic Diselenide Contraction, PDC. Taken together, these technologies allow us to access pure samples of homogenously modified proteins for downstream evaluation to elucidate the functional roles of natural and designer modifications, alike.

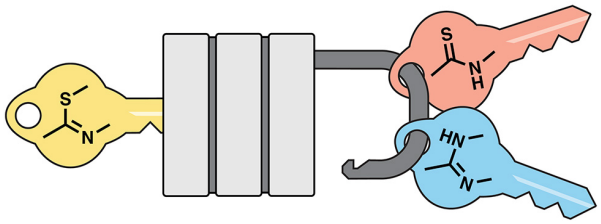
L20 Brett VanVeller

Iowa State University

Thioimidates: An Obscure Functional Group Provides General Access to Peptide Bond Isosteres

Brett VanVeller

The prevalence of post-translational modification in nature suggests that expanding beyond the suite of functional groups found in the 20 canonical amino acids can impart specialized utility to peptide-based biomolecules. Thioamides and amidines are two such modifications that can act as peptide bond isosteres with unique properties. Amidines, in particular, have received considerably little attention in peptides due to limitations in methods to access them. We developed the first robust and general procedure for the introduction of amidines into peptide backbones.



We exploited the utility of thioimidate protecting groups as a means to side-step reactivity that ultimately renders existing methods unsuitable for the installation of amidines along the main-chain of peptides. We also further demonstrate how thioimidate protecting groups can address many of the problematic synthetic issues surrounding thioamide backbone modifications as well.

This work is significant because it describes a generally applicable path to access unexplored peptide designs and architectures for new therapeutics made possible by the unique properties of thioamides and amidines.

L21 Marina Rubini

University College of Dublin

4-Thiaproline Eliminates the Slow Folding Phase of Proteins With Cis Prolines in The Native State

O' Loughlin J. and Rubini M

The *cis/trans* isomerization of peptidyl-prolyl peptide bonds

is an intrinsically slow reaction that significantly slows down the folding process in proteins that contain cis proline residues in the native state. To date, several proline analogues have been used in peptide/protein engineering 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.

We have analyzed the influence of 4,4-difluoroproline, Dfp, 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. Our results showed that the replacement of *cis*-Pro76 with Dfp in Trx1P causes a significant destabilization of the oxidised form of the fluorinated protein, while it did not eliminate the bottleneck of the Trx refolding reaction nor had an impact on the attainment of the *cis/trans* equilibrium in the unfolded state. Although Dfp displays the lowest energy barrier for *cis/trans* isomerization in model peptides in comparison to Pro and its 4-monofluorinated analogues, our results suggest that the pucker effect in the context of tertiary structures might prevail over *cis/trans* isomerization rates measured in the context of short model peptides.

Next, we investigated the effect of 4-thiaproline, Thp, on the refolding kinetics of Trx1P and pseudo-wild-type barstar, *cis*Pro48, as in model peptides Thp has been shown to decrease the free energy of activation for peptide isomerization by 10 kJ/mol in comparison to Pro. To date, no data have been reported concerning the impact of Thp on protein folding. We found that in the context of the tertiary structure of our model proteins, the kinetics of the rate-limiting step of the refolding reaction was accelerated by 2 orders of magnitude with a similar effect in the unfolded state, while thermodynamic stability and protein bioactivity was completely retained, unpublished results.

Our results indicate that Thp can eliminate the slow folding phase of proteins that display a *cis* peptide bond in the native state.

L22 Jianfeng Cai

University of South Florida

Connecting Peptides and Antibodies to Probe Cell Surface Receptors

Ross Cheloha

Peptides and antibodies represent two leading modalities for targeting protein receptors expressed on the surface of mammalian cells. Endogenous peptides often serve as leads for developing tools and therapeutic candidates for probing receptor function, but they often suffer from insufficient selectivity, especially when targeting members of large receptor families such as G protein-coupled receptors, GPCRs. Alternatively, antibodies are prized in biomedical research for their high specificity but typically lack any biological effect - inhibition or activation - when targeting receptors with orthosteric sites embedded within transmembrane domains. To leverage and expand the favorable properties of peptides and antibodies we have developed methods to create conjugates comprised of peptidic GPCR ligands and antibody fragments, nanobodies. Synthesis is performed through a combination of solid-phase peptide synthesis, site-specific labeling of recombinantly expressed nanobodies, and click chemistry. These conjugates show properties superior to those of antibodies or peptides alone, including receptor specificity and in vivo biological activity. We have further elaborated on this approach by developing chemistry that facilitates the covalent linkage between a nanobody and its binding partner through templated amide bond formation. This approach showed that covalent linkage of an agonist to its receptor resulted in enduring signaling. These tools should prove useful for mechanistic investigations of peptide-binding receptors and provide a new class of molecules with useful properties for biomedical investigation.

SESSION 5: PEPTIDE MATERIALS AND DELIVERY

L23 Christopher Alabi

Cornell University

PROteolysis TArgeting Chimeras – PROTACs

Christopher Alabi

PROTACs have been shown to selectively induce the degradation of a wide range of intracellular proteins. By employing a ligand that recruits an E3 ubiquitin ligase and another ligand that binds a protein of interest, POI, PROTACs induce an artificial interaction between an E3 and a POI that catalyzes selective ubiquitin-tagging of the POI, leading to its proteasome-mediated degradation.

As an alternative to small molecule- based approaches, peptide-based degraders have been developed alongside PROTACs for proteome editing in eukaryotic cells. Peptide-based PROTACs, otherwise herein referred to as PROteolysis TArgeting Peptides, PROTAP, share similar advantages to small molecule- based PROTACs with added significant advantages; they can be designed from protein structural data against any POI, and they possess large protein-protein interaction surfaces for targeting POIs for which no known small molecule ligand exists. One limitation, however, is that PROTAPs suffer from limited stability in biological fluids and have poor cellular permeability.

Given the advantages of peptide-based ligands over small

molecules, several PROTAPs targeting highly relevant oncogenes and transcription factors have made use of a cationic cell- penetrating peptide or stapling to facilitate cellular uptake. While promising, the doses required for activity are very high, 10-100 μM, relative to small molecule PROTACs that induce degradation at doses under 200 nM.

In this presentation, I will discuss strategies to enhance the delivery PROTPs, specifically those designed against onco-targets overexpressed in various Wnt-signaling pathway-driven cancer cell lines. Collectively, I will highlight how peptide composition and formulation conditions can be precisely tuned to enhance intracellular delivery efficiency.

L24 Scott Medina

Penn State College of Engineering

Real-Time, In Situ Imaging of Macrophages via Phase-Change Peptide Nanoemulsions

PLEASE SEE PAGE 161 FOR THIS ABSTRACT

L25 Giovanna Ghirlanda

Arizona State University

Membraneless Organelles by Design: **The Carboxysome**

Abesh Banerjee, Giovanna Ghirlanda

Membraneless organelles are widespread in biology, and their role in supporting cellular function is increasingly recognized. They form by liquid-liquid phase separation, LLPS, or coacervation of proteins and/or protein-nucleic acid mixtures, for example, by complex coacervation or associative LLPS of a positively charged macromolecule, for example, histone proteins, and a negatively charged macromolecule, for example, DNA, via electrostatic interactions. This mechanism leads to compartmentalization of the components within liquid droplets that can sequester solutes ranging from small molecules to proteins by partitioning, leading to dynamic control of function such as enzyme and ribozyme activity.

Here, we develop a bottom-up approach for creating functional designed organelles using complex coacervation between proteins with charged surfaces and a counter charged polypeptide. We started with a negative supercharged mutant of apo cytochrome b_{562}: the electrostatic repulsion due to close packing of the surface charges leads to the destabilization of the molten globule state resulting in an intrinsically disordered protein, IDP. Folding is rescued by counterions - for example by high concentrations of NaCl or low concentration of $CaCl_2$; binding to heme further restores a native-like structure.

We previously showed that cyt b_{562} catalyzes hydrogen production and carbon dioxide reduction when the native heme is replaced with cobalt protoporphyrin IX, CoPPIX. Supercharged CoPPIX-cyt b_{562} conserves folding and function of the artificial enzyme in the presence of salts. Mixing with poly-Arg results in complex coacervation as observed by turbidimetric assays and optical microscopy. The droplets contain folded CoPPIX-cyt b_{562}(-22) that display circular dichroism and UV-vis signatures identical to WT. The droplets catalyse production of molecular hydrogen and reduction of carbon dioxide with efficiency higher than WT. Surprisingly, the photosensitizer used in the reaction, $Ru(bpy)_3$, partitions spontaneously into the droplets and may contribute to the observed increase in activity.

Our results show that the supercharging of a protein creates a viable avenue for complex coacervation with an oppositely charged polymer, without interfering with function. Our ultimate goal is to create a functional membraneless organelle analogous to a carboxysome.

L26 Shiroh Futaki

Kyoto University

Liquid Droplet Formation and Facile Cytosolic Translocation of IgG in the Presence of Attenuated Cationic Amphiphilic Lytic Peptides

Takahiro Iwata, Hisaaki Hirose, Junya Michibata, Kentarou Sakamoto, and Shiroh Futaki

We previously reported an attenuated cationic amphiphilic lytic, ACAL, peptide, engineered from a natural hemolytic peptide, named L17E. Substantial cytosolic delivery of biomacromolecules, including immunoglobulin G (IgG), was attained in the presence of this peptide. Fc region binding peptide conjugated with L17E trimer [FcB(L17E)$_3$] was designed for more efficient IgG delivery into cells, see figure.

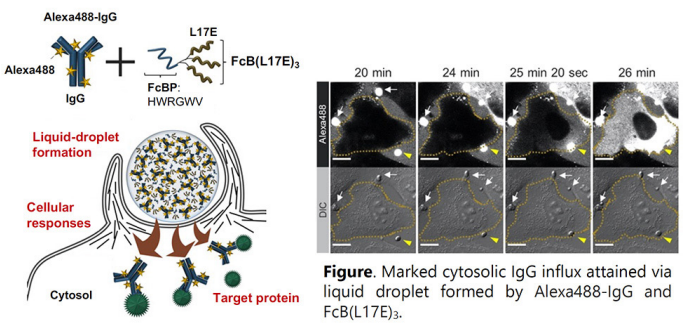


Figure. Marked cytosolic IgG influx attained via liquid droplet formed by Alexa488-IgG and FcB(L17E)$_3$.

Particle-like liquid droplets were generated by mixing Alexa Fluor 488 labeled IgG, Alexa488-IgG, with $FcB(L17E)_3$. Droplet contact with the cellular membrane led to spontaneous influx and distribution of Alexa488-IgG throughout cells in serum containing medium. Involvement of cellular machinery accompanied by actin polymerization and membrane ruffling was suggested for the translocation. Alexa488-IgG negative charges were crucial in liquid droplet formation with positively charged $FcB(L17E)_3$.

Successful intracellular delivery of Alexa Fluor 594-labeled anti-nuclear pore complex antibody allowed binding to cellular targets in the presence of $FcB(L17E)_3$. Conjugation of L17E to hydrophilic polymers was also found to provide a similar mode of cytosolic IgG delivery, suggesting design flexibility for intracellular delivery via liquid droplet, or coacervate.

MAKINENI AWARD LECTURE

L27-AW César de la Fuente

University of Pennsylvania

AI for Antibiotic Discovery

César de la Fuente

Artificial intelligence, AI, has the potential to outperform humans and revolutionize our world. In this talk, I will describe our efforts using AI to develop computational approaches for antibiotic design and discovery.

Computers can already be programmed for superhuman pattern recognition of images and text. In order for machines to discover novel antibiotics, they have to first be trained to sort through the many characteristics of molecules and determine which properties should be retained, suppressed, or enhanced to optimize antimicrobial activity. Said differently, machines need to be able to understand, read, write, and eventually create new molecules.

I will discuss how we trained a computer to execute a fitness function following a Darwinian algorithm of evolution to select for molecular structures that interact with bacterial membranes, yielding the first artificial antimicrobials that kill bacteria both in vitro and in relevant animal models.

My lab has also developed pattern recognition algorithms to mine the human proteome, identifying throughout the body thousands of antibiotics encoded in proteins with unrelated biological function, and has applied computational tools to successfully reprogram venoms into novel antimicrobials.

Computer-generated designs and innovations at the intersection between machine and human intelligence may help to replenish our arsenal of effective drugs, providing much-needed solutions to global health problems caused by infectious diseases.

SESSION 6: PEPTIDE DRUG DISCOVERY

L28 Ruchia Duggal

Merck

DMPK Optimization of Macrocyclic Peptides: Targeting Intra vs. Extracellular Sites

Rucchia Duggal

With advances in peptide screening technologies and strides in medicinal chemistry synthetic technologies, the ability of peptides to expand the druggable proteome is apparent. The path from validated hits to development of a peptide therapeutic requires DMPK optimization to enable optimal exposures near the pharmacological target. DMPK optimization approaches for peptides need to consider the target location and intended route of clinical administration to enable optimal target engagement and resulting pharmacological efficacy.

We will describe these considerations and present case studies from our peptide drug discovery pipeline demonstrating the divergence in peptide DMPK properties and how optimization strategies must be modified based on these considerations.

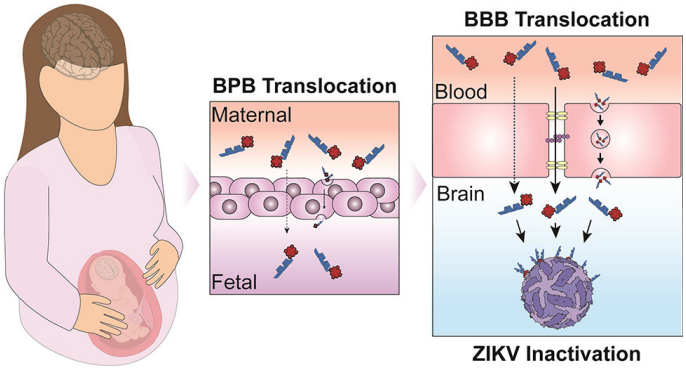
L29 Miguel Castanho

University of Lisbon

Developing Broad Spectrum Brain-Targeting Peptide-Drug Conjugates Against Flaviviruses And Other Envelope Viruses

Miguel Castanho

We are developing drugs able to inactivate Zika virus, Dengue virus, HIV, and SARS-CoV-2, among others, while having the ability to traverse the blood-brain and blood-placenta barriers, BBB and BPB, respectively. Zika is of particular interest because it combines high pandemic potential with severe neurological impairment in newborns when the infection takes place in pregnant women. So far, there is no effective therapy for infection with this virus due to the limited ability of current antiviral drugs to cross the BBB and/or the BPB.



Chemically, the drugs underdevelopment consist on the conjugation of an antiviral porphyrin to a trans-BBB carrier peptide. Proprietary trans-BBB peptides were obtained from templates based on domains of the capsid protein of Dengue virus.

The activity, toxicology, and brain-targeting efficacy of a panel of conjugates were evaluated both *in vitro* and *in vivo*. One of the conjugates is able to perform transcytosis across both the BPB and the BBB, has shown to be effective against Zika Virus, IC50 1.08 μM, and has high serum stability, t1/2 ca. 22 h, without altering cell viability at all tested concentrations. *In vivo* tests in animals confirm their high druggability.

Acknowledgements
This work is supported by the European Union, H2020-FETOPEN-2018-2019-2020-01 grant no 828774.

L30 Hongchang Qu

Eli Lilly

The Novel GIP, GLP-1, and Glucagon Triple Receptor Agonist LY3437943: From Discovery to Clinical Proof-of-Concept

Tamer Coskun, Hongchang Qu, Mei Teng Loh, Zvonko Milicevic, Axel Haupt, Charles Benson, and Ruth Gimeno

With an increasing prevalence of obesity, there is a need for new therapies to improve body weight management and metabolic health. Multi-receptor agonists in development may provide approaches to fulfill this unmet medical need. LY3437943 is a novel, unimolecular triple agonist peptide at the glucagon receptor, GCGR, glucose-dependent insulinotropic polypeptide receptor, GIPR, and glucagon-like peptide-1 receptor, GLP-1R. In *vitro*, LY3437943 shows balanced GCGR and GLP-1R activity, but more GIPR activity. In obese mice, administration of LY3437943 decreased body weight and improved glycemic control.

Body weight loss could be attributed to a combination of energy expenditure, primarily mediated by GCGR, and food intake, driven primarily by GLP-1R and GIPR. In a randomized, double-blind, placebo- controlled, Phase 1 proof-of-concept study, we assessed the safety and tolerability of multiple ascending doses of LY in patients with type 2 diabetes, T2D. Vital signs, laboratory data and adverse events, AEs, were monitored to assess safety and tolerability. Efficacy was assessed by monitoring change in glycated hemoglobin, HbA_{1c}, and body weight at week 12.

The most common treatment-emergent AEs were gastrointestinal, nausea and diarrhea, which were mostly mild in severity. By week 12, mean HbA_{1c} decreased from baseline in all groups, with higher doses of LY showing statistically significant baseline-adjusted decreases of up to 1.90%. Dose-dependent decreases in mean baseline-adjusted body weight of up to 8.65 kg were observed with LY.

In conclusion, LY3437943 showed a safety and tolerability profile similar to other incretins. Its pharmacokinetic profile supported once-weekly dosing. Promising glycemic and body weight loss efficacy within these studies highlights the potential for LY to provide additional benefit versus existing therapies in treatment of T2D and obesity.

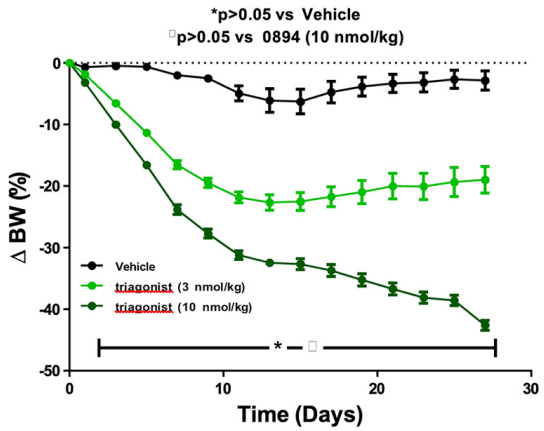
L31 Florence Brunel

Novo Nordisk

A High Potency Protein that Normalizes Body Weight in DIO Mice through Triple Agonism at the FGF21, Glp-1 and GIP Receptors

Florence Brunel

Obesity is endemic throughout much of the world and obesity-related morbidities include heart disease, stroke, non-alcoholic steatohepatitis, NASH, and type 2 diabetes, T2D. Glucagon-Like Peptide 1, GLP-1, agonists have emerged as highly effective treatment for T2D and more recently for management of obesity. Glucose-dependent Insulinotropic Polypeptide, GIP, agonists when combined with GLP-1 agonism strengthen the pharmacology and yield further decreases in hyperglycemia, body weight and adiposity.



The unimolecular GLP-1/GIP coagonist peptide named Tirzepatide recently received regulatory approval for the treatment of T2D, with significant associated loss of body weight. Fibroblast Growth Factor 21, FGF21, is also an endocrine hormone that has received appreciable attention for treatment of the metabolic syndrome. It has demonstrated profound reductions in serum lipids including triglycerides, LDL, total cholesterol, and hepatic fat fraction, but less impressive relative to incretin-based drugs in control of hyperglycemia.

Efruxifermin, an Fc-FGF21 agonist is being studied in the clinic for NASH. It has shown regression in hepatic fibrosis and normalization of liver fat in 12 weeks of treatment. Collectively the clinical studies with incretin and FGF-21 analogs have demonstrated the complimentary nature of these mechanisms to address the full spectrum of diseases associated with the metabolic syndrome.

As such we designed, synthesized, and biologically characterized a long-acting protein that is highly potent and balanced in activity at the GLP-1, GIP and FGF21 receptors. This first-in-class triple agonist showed exquisite efficacy at reversing diet-induced obesity in mice and simultaneously provides the precedent-setting glycemic and weight benefits of GLP-1 and GIP coagonism with the enhanced lipid lowering properties of FGF21 agonism.

DR. ELIZABETH SCHRAM YOUNG INVESTIGATORS ORAL COMPETITION

L32–YI3 Carly Schissel

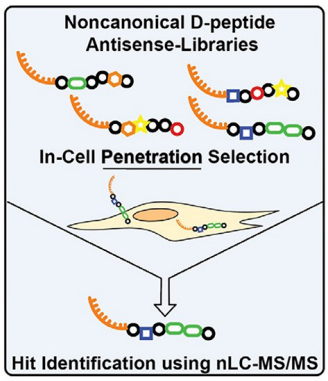
University of California, Berkeley

In-Cell Penetration Selection — Mass Spectrometry Produces Noncanonical Peptides for Antisense Delivery

Carly Schissel

Peptide-mediated delivery of macromolecules in cells has significant potential therapeutic benefits, but no therapy employing cell-penetrating peptides, CPPs, has reached the market after 30 years of investigation due to challenges in the discovery of new, more efficient sequences.

Here we demonstrate a method for in-cell penetration selection-mass spectrometry, in-cell PS-MS, to discover peptides from a synthetic library capable of delivering macromolecule cargo to the cytosol. This method was inspired by recent *in vivo* selection approaches for cell-surface screening, with an added spatial dimension resulting from subcellular fractionation.



A representative peptide discovered in the cytosolic extract, Cyto1a, is nearly 100-fold more active toward antisense phosphorodiamidate morpholino oligomer, PMO, delivery compared to a sequence identified from a whole cell extract, which includes endosomes. Cyto1a is composed of D-residues and two non-α-amino acids, is more stable than its all-L isoform, and is less toxic than known CPPs with comparable activity. Pulse-chase and microscopy experiments revealed that while the PMO-Cyto1a conjugate is likely taken up by endosomes, it can escape to localize to the nucleus without nonspecifically releasing other endosomal components. In-cell PS-MS introduces a means to empirically discover unnatural synthetic peptides for subcellular delivery of therapeutically relevant cargo.

L33 – YI4 Sheryl Sharma

University of Nebraska at Lincoln

Identifying Peptide-Receptor Interactions using Aryl Diazonium-Labeled Peptide Ligands

Sheryl Sharma, Makayla Gill, and James W. Checco

Endogenous bioactive peptides, for example, neuropeptides and peptide hormones, are a class of signaling molecules that play pivotal roles in maintaining cardiovascular, reproductive, gastrointestinal, and metabolic health. Dysregulation of peptide signaling is associated with diseases such as cancer, multiple sclerosis, Alzheimer's, diabetic nephropathy, and many more. However, despite the growing interest, the receptor proteins for many disease-relevant bioactive peptides are currently unknown, severely limiting their potential as therapeutic targets.

In a quest to discover novel peptide-protein interactions, and unveil molecular mechanisms underlying diseases, we report a proximity induced labeling strategy using aryl diazonium-modified bioactive peptides to covalently label corresponding membrane-bound receptors.

We describe the design principles needed for the synthesis of aryl-diazonium modified bioactive peptides to achieve efficient receptor labeling and demonstrate that a peptide's affinity to a given receptor can be harnessed to specifically label interacting proteins on living cells under physiological conditions. We hypothesize that the developed protein labeling strategy can be used for the enrichment and isolation of membrane proteins in an unbiased fashion. Long-term, this research may aid in identifying new therapeutic targets for a number of diseases caused by dysregulated cell-cell signaling peptides.

GOODMAN AWARD LECTURE

L34–AW James P. Tam

Nanyang Technological University

Finding from Nature a New Paradigm for Orally-Active and Cell-penetrating Bioactive Microproteins

James P. Tam, Shining Loo, Antony Kam, and Bamaprasad Dutta

A continuing challenge in discovery of peptide therapeutics from natural products is to find a new paradigm that target

intracellular protein-protein interactions. An under-explored group of natural products that could fit such a paradigm is the family of super cystine-dense peptides or microproteins with 6-10 cysteine and 2-4 kDa in MW. They have sufficiently large footprints for on-target specificity and highly compact structures to resist proteolytic degradation.
Although cysteine-rich peptides of 2-7 kDa are found in hormones, growth factors, antimicrobials and toxins, few are orally active or cell- penetrating. Guided by traditional medicines, our laboratory has a long-standing research program in the discovery of orally-active super cystine-dense microproteins from medicinal plants.

Here, I will present our 25-year venture into their discovery, target identification, chemical synthesis of bioactive super cystine-dense microproteins. I will also present a new method for their chemical synthesis through an ultrafast oxidative folding that completes within a second. Our discoveries of "first-in-class" bioactive microproteins could provide therapeutic leads to address several prevalent health issues: cardiovascular diseases, stress and frailty, the underlying causes of numerous chronic and old-age-related diseases.

Acknowledgment
This research was supported in part by a Competitive Research Grant by the Nanyang Technological University Internal Funding - Synzyme and Natural Products, SYNC.

SESSION 7: FROM NATURAL PRODUCTS TO UNNATURAL BIOACTIVE PEPTIDES

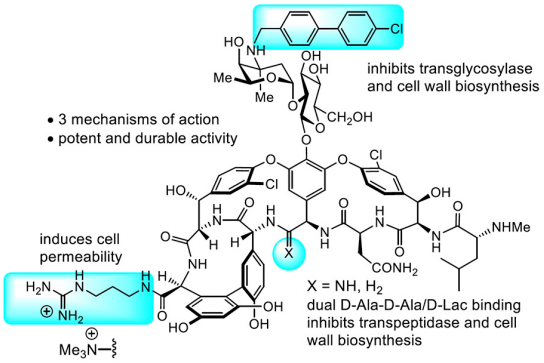
L35 Dale L. Boger

The Scripps Research Institute

Maxamycins: Redesigned Vancomycins for Resistant Bacteria

Dale L. Boger

Studies on the total synthesis and evaluation of the vancomycin family of glycopeptide antibiotics, their ligand binding pocket redesign that addresses the underlying molecular basis of resistance, and their subsequent peripheral tailoring to incorporate added new mechanisms of action will be presented.



The efforts have provided potent and especially durable antibiotics in efforts to address the emerging public health problem of antibiotic resistance.

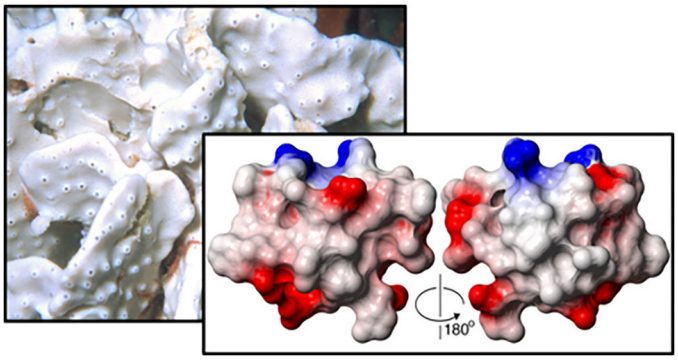
L38 Christina Schroeder

Genentech

Recifin A, a Novel and Selective Allosteric Inhibitor of Tyrosyl-DNA Phosphodiesterase I with a Unique tDisulfide-bond Topology

C.I. Schroeder

Tyrosyl-DNA phosphodiesterase 1, TDP1, is a molecular target for the sensitization of cancer cells to the FDA-approved topoisomerase inhibitors topotecan and irinotecan. However, current TDP1 inhibitors have low binding affinity or are substrate mimics with low specificity. Through high-throughput screening of natural products and extracts library in the search for novel TDP1 inhibitors, we identified a new class of complex knotted peptides with a unique disulfide-bond topology from the marine sponge *Axinella* sp.



The active component was a 42-residue peptide named recifin A. Unlike previously described TDP1 inhibitors which bind to the C-terminal catalytic domain of TDP1, recifin A acts as an allosteric inhibitor and binds to the N-terminal regulatory domain. The three-dimensional NMR structure revealed a novel fold comprising a four-strand antiparallel }-sheet and two helical turns stabilized by a complex disulfide-bond network that creates an embedded ring around one of the strands. The structure is locked in place by a centrally located tyrosine residue, resulting in the Tyr-lock family name.

Recifin A represents both the first of a unique structural class of knotted disulfide-rich peptides and defines a previously unseen mechanism of TDP1 inhibition that could lead to the development of a new class of TDP1 inhibitors with improved specificity that could be exploited for potential anticancer applications.

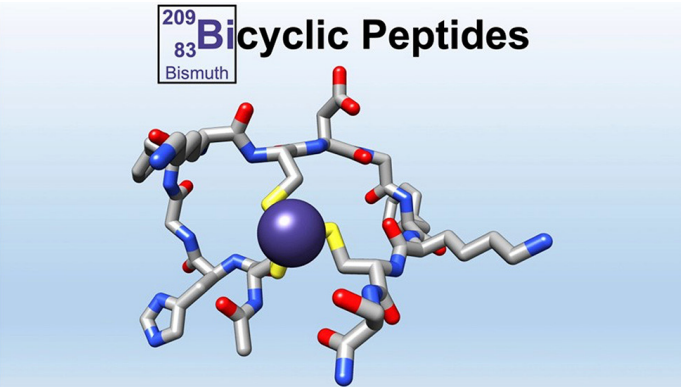
L37 Christoph Nitsche

Australian National University

Biocompatible Peptide Cyclisation and Protein Modification

C. Nitshce

Constrained and modified peptides fill an underexplored area of chemical space between small molecule therapies and larger antibodies. Noncanonical modifications, such as cyclisation or stapling, can **i** enhance metabolic stability by greater resistance towards proteolysis, **ii** promote biological uptake across cell membranes, and **iii** decrease the entropic penalty of binding by locking the peptide in the active conformation.



We developed various unnatural amino acids functionalized with cyanopyridine and 1,2-aminothiol groups which can be directly incorporated into peptides. Cyclisation and stapling reactions proceed under biocompatible conditions in presence of protein drug targets. Using these approaches in small screening campaigns, we were able to identify various macrocyclic peptide inhibitors of viral proteases. In addition, these amino acids can be incorporated into proteins enabling selective protein modification.

Bicyclic peptides offer even greater conformational rigidity, metabolic stability, and antibody-like affinity and specificity. We explored the reaction between 1,2-aminothiols and 2,6-dicyanopyridine to establish a biocompatible, selective, and catalyst-free pathway to access bicyclic peptides, which displayed plasma stability, conformational preorganization, and high target affinity. We also introduced bismuth as a selective, stable, rigid, and green reagent for peptide modification. Bismuth represents the smallest "scaffold" ever explored and allows in situ access to bicyclic peptides for biochemical screening assays.

L36 Andrew Roberts

University of Utah

Tyrosine-Selective Peptide Cyclization Methods Inspired by Cyclic Peptide Natural Products

E. Dalles Keyes, Marcus C. Mifflin, Maxwell J. Austin, Jesus Sandres, Georgia Brach, Michael Sherwood, and Andrew G. Roberts

Residue-selective methods for the modification and cyclization of peptides are useful for the discovery and development of therapeutic peptide leads with metabolically stable structures. To develop new methods, the Roberts laboratory draws inspiration from biologically active cyclic and polycyclic peptide natural products. Examples include the arylomycin and vancomycin families of antimicrobial peptides that scaffold tyrosine- and hydroxyphenylglycine-derived biaryl-linkages.

Accordingly, we have developed methods that leverage the in situ formation of 1,2,4-triazoline-3,5-dione, TAD, moieties, also known as urazoles, on native peptides to achieve tyrosine-selective peptide cyclizations and polycyclizations. Detailed accounts of these methods, and their applications toward biologically active cyclic peptides will be presented.

L39 Andrei Yudin

University of Toronto

Development of Privileged Macrocyclic Motifs Using the Tools of Chemical Synthesis

Andrei K. Yudin

Favorable drug-like properties is a fundamental challenge in the development of bioactive macrocycles. To achieve this objective, chemists have resorted to various strategies, the most common of which is deployment of N-methylated amino acids and/or unnatural amino acids. We recently explored a different approach and investigated the effect of heterocyclic grafts on the passive membrane permeability of macrocycles.

Through stepwise substitution of amino acid residues for azole rings, we have shown that lipophilicity and PAMPA permeability of a macrocycle can be vastly improved. Overall, changes in permeability do not scale linearly as more heterocycles are incorporated, underscoring the subtleties of conformation-property relationships in this class of molecule. NMR analysis and molecular dynamics simulations provide insights into the structural consequences of the added heterocycles. This study paves a way to the discovery of privileged macrocyclic scaffolds for drug discovery.

DR. ELIZABETH SCHRAM YOUNG INVESTIGATORS ORAL COMPETITION

L40–YI5 Simon Rössler

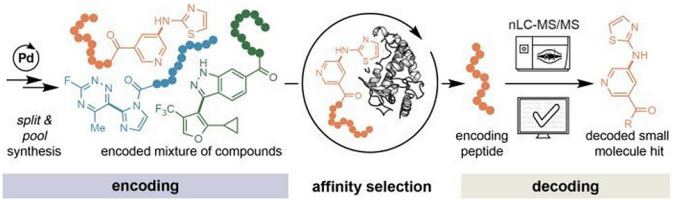
Massachusetts Institute of Technology

Abiotic Peptides for the Encoding of Small Molecule Synthesis

S. L. Rössler, N. M. Grob, S. L. Buchwald, and B. L. Pentelute

Encoded libraries of small molecules have vastly accelerat-

ed drug discovery. Most prominently, DNA-encoded libraries have found widespread success and adaption. However, oligonucleotides as carriers of information are hampered by inherent limitations relating to stability, which has limited the synthetic toolbox accessible for small molecule synthesis. Alternative biomolecules may offer transformative potential for encoding technologies, but lack the efficient recording and retrieval of information offered by DNA.



We have established abiotic peptides as a next-generation information storage system suited for the encoding of diverse small molecule synthesis. Therein, an encoding alphabet of non-isobaric amino acids in an optimized sequence provided broad chemical stability and high encoding density for sequencing by liquid chromatography-tandem mass spectrometry enabling high-fidelity decoding. Solid- phase combinatorial synthesis facilitated the implementation of peptide-encoded libraries, PELs, wherein the structural information of a small molecule is stored in a covalently linked peptide.

The chemical stability of the peptide-based tag allowed the use of palladium-mediated C–C and C–N bond-forming reactions for the efficient synthesis of PELs with large chemical diversity and excellent purity. Affinity selection against oncogenic proteins afforded an array of small molecules with affinity for the target proteins.

L41–YI6 Estefania Martinez-Valdivia

University of Michigan

A Lipopeptidomimetic Transcriptional Inhibitor

Estefania Martinez Valdivia, Olivia Pattelli, Matthew Beyersdorf, Clint S. Regan, Mónica Rivas, Tomasz Cierpicki, David H. Sherman, and Anna K. Mapp

Dysregulation in the protein-protein interactions, PPIs, of transcriptional components, such as coactivators and activators, profoundly affects gene expression and is prominent in disease. Coactivators act as regulators of gene expression by bridging activators engaged at enhancer regions of genes to the general transcription machinery. Thus, modulation of gene expression through the inhibition of these PPIs is an avenue to define the role that dysregulated networks play in the development of disease. Due to the intricacy in targeting these complexes, peptidomimetic molecules have the potential to be developed into selective and potent inhibitors.

Here, we report the development of a target-adjustable lipopeptide inhibitor of activator-coactivator complexes based on transcriptional activation domain, TAD, sequences of activators. Structure-activity relationship studies demonstrate adjustability in the selectivity of lipopeptide analogs for various coactivators, including Med25 and CBP/p300. We identified specific moieties on the lipopeptide that dictate the selective disruption of coactivator-activator PPIs. Additionally, the binding mode of potent inhibitors to their main target was determined using 2D NMR.

Our results suggest the occurrence of specific interactions between lipopeptide residues and coactivators, which are crucial for the activity and selectivity of these lipopeptides. Our data positions this molecule as a platform with modifiable selectivity, and it proposes lipopeptides as a novel scaffold for the selective inhibition and characterization of coactivator PPI networks.

SESSION 8: NATURAL PRODUCT BIOSYNTHESIS & INSPIRATION FROM NATURE

L42 Wilfred A. van der Donk

University of Illinois

Biosynthesis and Engineering of Cyclic Peptide Natural Products

Vilfred van der Donk

The genome sequencing efforts of the past 20 years have revealed that ribosomally synthesized and post-translationally modified peptides, RiPPs, constitute a large class of peptide natural products. These molecules are produced in all three domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast. Furthermore, they are increasingly recognized for their involvement in fighting or causing human disease.

This presentation will discuss the use of genome mining and synthetic biology for the discovery of new cyclic RiPPs via an automated platform and the unusual chemistry involved in their biosynthesis.

L43 Betsy Parkinson

Purdue University

Synthetic Natural Product Inspired Cyclic Peptides for Discovery of Bioactive Natural Products and Biocatalysts

Elizabeth Parkinson

Natural products, NPs, are a bountiful source of medicines, agricultural products, and chemical tools. Bioinformatics data suggest hundreds-of-thousands of novel NPs remain to be discovered from *Streptomyces*. Unfortunately, many NPs are not produced under standard laboratory conditions. We are

developing methods to access NPs from cryptic biosynthetic gene clusters, BGCs, utilizing a combination of bioinformatics, synthetic chemistry, and biocatalysis.

We recently developed SNaPP, Synthetic Natural Product Inspired Cyclic Peptides. SNaPP expedites bioactive molecule discovery by combining bioinformatics predictions of non-ribosomal peptide synthetases with chemical synthesis of the predicted natural products. SNaPP has enabled us to discover several interesting bioactive cyclic peptides. However, it also opened our eyes to the challenge of synthesizing small cyclic peptides.

Using the results from SNaPP, we identified PBP-like cyclases potentially capable of performing challenging cyclizations, such as for tetrapeptides. We have experimentally confirmed these activities and found one enzyme with a greatly expanded substrate scope that could have great utility as a biocatalyst.

L44 Ines Neundorf

University of Cologne

Peptides Interfering With TANGO1 Reduce Collagen Secretion and Prevent Skin Fibrosis

Ishier Raote, Ann-Helen Rosendahl, Pia Frommelt, Beate Eckes, Thomas Krieg, Vivek Malhotra, Ines Neundorf

Collagen is the most abundant protein in mammals and not surprisingly, dysregulated collagen homeostasis is related to different pathologic conditions. Extensive fibrosis is characterized by the overproduction of collagen and a feature of systemic sclerosis which can affect most organs. Since first symptoms are usually observed in the skin, and due to the accessibility of skin samples, it displays a useful model system for fibrosis.

Despite many efforts to characterize fibrosis, the underlying disease mechanisms are still only poorly understood. Proteins that play essential roles in collagen secretion belong to the TANGO1 family. TANGO1 is a transmembrane protein that is resident at the ER exit site, ERES. The ER luminal part recruits collagen to the ERES, while cytoplasmic regions specifically interact with its paralog cTAGE5 initiating the formation of transient inter-organelle tunnels to increase the secretory capacity for collagen.
To control and regulate collagen secretion we have developed membrane-permeant peptides that act as competitive inhibitors of TANGO1/cTAGE5 function. These peptide inhibitors are composed of regions of TANGO1 and cTAGE5 domains, conjugated to cell-penetrating peptides. We demonstrate that they are active in various systems including zebrafish and display therapeutic efficacy in primary fibroblasts from scleroderma patients. Our results let conclude that precise targeting of ERES proteins makes it possible to specifically control ER export. Moreover, this innovative approach might be a promising avenue for therapeutic intervention for currently intractable fibrotic disorders.

L45 Arundhati Nag

Carlson School of Chemistry and Biochemistry, Clark University

Use of Proximity-Catalyzed IEDDA Reaction for Cyclic Library Screening to Target Point-Mutant Proteins

Arundhati Nag

Antibodies, due to their high affinity and selectivity for epitopes on antigens have extensive use as therapeutics reagents, but their large sizes preclude them from being effective therapeutic agents inside cells. There is a gap in the technologies yielding smaller antibody alternatives of smaller size that target a specific protein region like antibodies. In fact, there is a distinct lack of technology to distinguish mutant protein-protein interactions, PPI, from wildtype PPI and selectively inhibit the mutant PPI. Our objective is to develop a technology that allows the creation of antibody alternatives targeted to a specific region of interest, ROI, irrespective of the structure and whether the region is a hot spot.

We are extending a previously developed strategy, Chemical Epitope Targeting, CET, that worked for targeting unstructured ROI with macrocyclic peptides for targeting highly structured ROI. We hypothesize that one can bias the protein screening process by adding biorthogonal reactive partners to the protein of interest, POI, at the ROI and to the library being screened so that the covalent interaction between the reactive partners reinforces the non-covalent interactions between a POI and its potential binder. We are adding a biorthogonal reaction handle like an alkene or alkyne close to the ROI on the full-length protein through unnatural amino acid incorporation.

We plan to screen the modified protein against an OBOC cyclic peptide library containing the second bio-orthogonal functionality, 1,2,4,5-tetrazine. The tetrazine on the peptide library acts both as the cyclization linker and as a biorthogonal reactive partner for the protein. As a proof-of-concept of the IEDDA proximity-catalyzed screening technology, we shall develop an inhibitor that inhibits the interaction of KRAS(G12V) protein, an oncogenic point-mutant protein, with its activating G-factor, SOS.

SESSION 9: PEPTIDE SYNTHETIC METHODS & GREEN CHEMISTRY APPROACHES

L46 Scott J. Miller

Yale University

New Catalytic Modalities for Peptide-Based Asymmetric Catalysis

Scott J. Miller

This lecture will describe recent developments in our efforts to develop catalysts for asymmetric reactions, in particular

for the preparation of densely functionalized, stereochemically complex structures. Over time, our foci have been on enantioselectivity, site-selectivity and chemoselectivity. In much of our current work, we are studying issues of enantioselectivity as a prelude to the extrapolation of catalysis concepts to more complex molecular settings where multiple issues are presented in a singular substrate.

A particular focus of this lecture will be the incorporation of new catalytic functionality that enables an expansion of the reaction types and mechanistic paradigms that may be applied amidst selectivity-defining noncovalent interactions that are signatures of peptide-catalyzed processes.

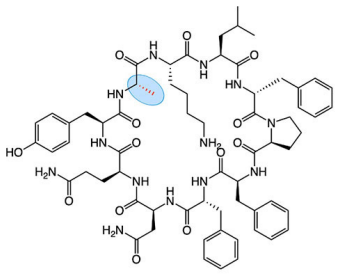
L47 Jenn Stockdill

Wayne State University

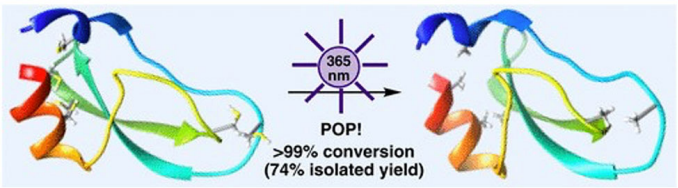
Waste Not, Want Not: Innovations in Complex Peptide Synthesis Fueled by a Focus on Green Chemistry

Naresh M. Venneti, Lawrence G. Mendoza, Rana M. I. Morsy, Ganesh Samala, Christine A. Arbour, and Jennifer L. Stockdill

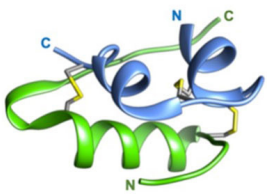
Peptides are important lead targets for pharmaceutical and vaccine development, but traditional methods for their synthesis, especially on an industrial scale, are problematic from an environmental and cost perspective. In particular, peptide synthesis is plagued by excess reagents, reaction by-products, harmful solvents, and large levels of solvent consumption during purification.



Macrocyclization



Photodesulfurization



N-to-C SPPS

This seminar will describe our recent efforts to apply green chemistry principles to the synthesis of synthetically challenging peptide targets. Highlighted methods will include self-cleaving macrocyclization, phosphine-only photodesulfurization, POP, and N to C SPPS.

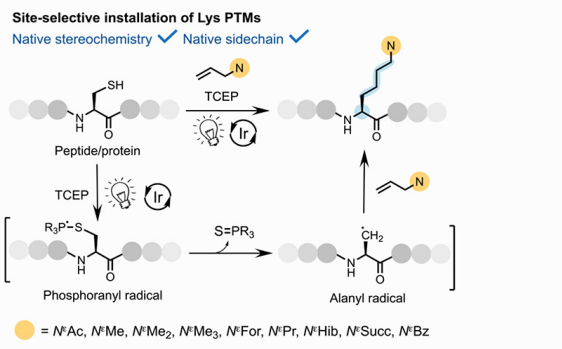
L48 Nicholas Mitchell

University of Nottingham

A Radical Approach to the Site-Selective Modification of Peptides and Proteins

Griffiths, R. C., Smith, F. R., Li, D., and Mitchell, N. J.

The diverse array of chemical functionality displayed by the 20 canonical amino acids presents both challenges and opportunities for the site-selective modification of peptides and proteins. An extensive range of reactions have been reported to modify the majority of the proteinogenic residues, providing tools to enable the study and manipulation of biological systems, and the preparation of therapeutic/diagnostic agents. To be effective, bioconjugation techniques must be rapid, high-yielding under mild conditions, and chemoselective. Owing to the superior nucleophilicity of the thiol group of cysteine, Cys, and its relatively low abundance across eukaryotic proteomes, circa 2%, many reported techniques target this residue to selectively install groups of interest.



Site-selective installation of N^{ε}-modified sidechains, Lys PTMs, via visible-light-mediated desulfurative C-C bond formation.

To further contribute to this synthetic tool kit, we are exploring the site-selective modification of peptides and proteins via interception of free-radical-mediated desulfurization. By exploiting the homolytic lability of the C-S bond of Cys, we have developed a visible-light-mediated desulfurative $C(sp^3)$–$C(sp^3)$ bond forming reaction that enables the site-selective installation of N^{ε}-modified sidechains into peptides and proteins of interest, see figure. Rapid, operationally simple, and tolerant to ambient atmosphere, we demonstrate the installation of a range of lysine, Lys, post-translational modifications, PTMs, and PTM mimics into model peptides and proteins.

Furthermore, by utilizing persistent radical traps, we have developed a broadly applicable bioconjugation technique to

install groups of interest via formation of an amino-oxy linkage. The reaction is rapid, high yielding and chemoselective; the resulting bioconjugate is stable at varying pH and high temperature, and selectively cleavable under mildly acidic conditions with the addition of a low oxidation state transition metal. This method translates well onto larger and more complex peptides and proteins carrying a wealth of chemical diversity.

L49 Beatriz Garcia De La Torre

University of KwaZulu-Natal

Refractive Index: A Process Analytical Tool for Real-Time Monitoring of SPPS. Beyond a Qualitative Application

B.G. de la Torre, S. Ramkisson, S.R. Manne, and J. Lopez, F. Albericio

Although SPPS is the method of choice for synthesis of peptides in both research and industrial modes, it could be interpreted as a black box. This creates an important challenge in the knowledge about the process, such as the reaction kinetics and reaction efficiency. There are indirect methods to analyze some of the steps involved in, but they led very often to false positive/negative results and require in all cases the interruption of the process.

Having this in mind, we have developed for the first time a process analytical tool, PAT, based on the refractive index, RI, that allows real-time monitoring of the three SPPS steps, coupling, deprotection, and washing.

The RI is a very sensitive parameter that varies in the function of the amount of mass dissolved in the reaction solution. Thus, by RI measurements we can detect: i decrease of protected amino acid in the solution during coupling, ii the dibenzofulvene concentration increases during Fmoc removal and iii the remaining compounds in the solution during the wash-steps. Moreover, the analysis of RI plots over time provide valuable information regarding the reaction kinetics, failures on the coupling or deprotection, unexpected reactivity of some protected amino acids, and even the presence of some side-reactions.

Using RI, we were able to determine the initial loading of the resin, optimize the synthesis of some peptides and, very importantly, evaluate new technologies such as coupling reagents and green solvents. Definitely, RI is an excellent PAT for Greening the SPPS process.

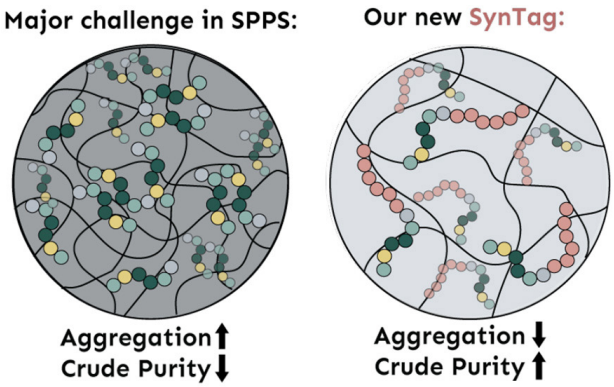
L50 Nina Hartrampf

University of Zurich

A Versatile "Synthesis Tag" for Chemical Protein Synthesis

Nina Hartrampf

Solid-phase peptide synthesis, SPPS, has been a standard method for chemical protein production for the past 60 years, but its outcome can be highly dependent on the peptide sequence synthesized. One issue that often arises is the aggregation of growing peptide chains, which can lead to incomplete couplings, or "difficult sequences". Previous research into this sequence-dependent phenomenon was limited by the lack of high-throughput analytical methods, thus impeding systematic analysis. While flow-based SPPS allows for aggregation detection, it has so far not led to the development of tools for its suppression.



Analogous to existing "solubility tags," which assist with the often-observed solubility issues of peptides, we set out to develop a "synthesis tag." Analysis of various parameters affecting aggregation, as well as the development of a synthesis tag, will be presented in the context of chemical protein synthesis. The tag significantly improves synthesis outcomes in flow- and batch-SPPS for various aggregating peptide and protein sequences.

DR. ELIZABETH SCHRAM YOUNG INVESTIGATORS ORAL COMPETITION

L51–YI7 Troy Smith

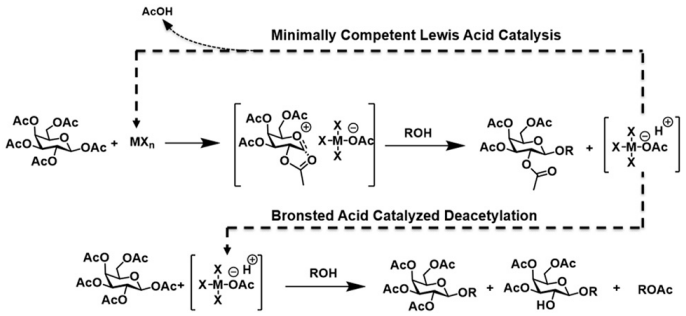
University of Arizona

Minimally Competent Lewis Acids: Effective and Efficient Catalysts for β-Glycosylation of Peptide Hormones

T.E. Smith, A, Myloserdnyy, and R. Polt

The synthesis of amino acid glycosides has been an ongoing synthetic challenge. Glycosylation of peptides has been shown to be an important way to enhance stability and transport of peptide-based drugs. Unfortunately, there are no universal conditions for the variety of glycosyl donors and acceptors commonly used, and the donors typically used are unstable.

Previous methods often require lengthy synthetic routes, unstable reagents, and low temperatures to provide the required glycosides.



This study examines the use of minimally competent Lewis acids, MCLAs, as robust catalysts for β-glycosylation of serine using stable sugar peracetates with the goal of developing more finely tuned catalysts for glycopeptides and other types of glycosides.

SESSION 10: BIOINSPIRED & INTELLIGENT PEPTIDE MATERIALS

L52 Matthew Tirrell

University of Chicago, Pritzker School of Molecular Engineering

Peptide-Conjugated Polyelectrolyte Complex Micelles

Matthew Tirrell

Vascular disease is a leading cause of morbidity and mortality in the United States and globally. Pathological vascular remodeling, such as atherosclerosis and stenosis, largely develop at arterial sites of curvature, branching, and bifurcation, where disturbed blood flow activates vascular endothelium. Current pharmacological treatments of vascular complications principally target systemic risk factors. Improvements are needed.

We have devised a peptide targeted polyelectrolyte complex micelle to deliver therapeutic nucleotides to inflamed endothelium in vivo by displaying the peptide VHPKQHR targeting vascular cell adhesion molecule 1, VCAM-1, on the periphery of the micelle. This platform, with a different targeting peptide, also works well for delivering therapeutic agents to inflamed lung tissue.

Acknowledgment
In collaboration with Professor Yun Fang, Department of Medicine, University of Chicago

L53 Abigail Knight

University of North Carolina at Chapel Hill

Self-Assembly of Peptide-Polymer Hybrid Materials

Abigail Knight

Composed of only twenty amino acids, proteins offer complex functions and impressive binding capabilities. Synthetic polymers offer an expansive monomer scope yielding tunable chemical and physical properties. Building on the strengths of both classes of macromolecules, we have designed several hybrid architectures.

We have leveraged small synthetic oligomers to control the assembly of peptides using peptide-polymer amphiphiles. The morphology and dynamics of the assembled nanomaterial can be tuned using properties of the synthetic tail. Complementarily, we have leveraged peptides as a pendent group on synthetic polymers to generate local rigidity analogous to secondary structure in synthetic random copolymers. As secondary structure is critical for protein function, this local rigidity impacts the function the synthetic polymer.

These efforts are motivated by both developing a deeper understanding of the impact of secondary structure on protein properties and developing functional scalable materials that can target global challenges in sustainability such as industrial separations and water purification.

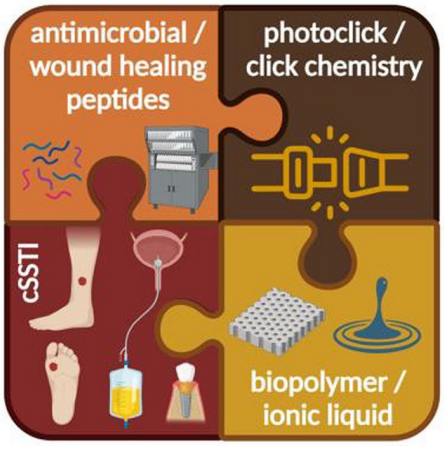
L54 Paula Gomes

University of Porto

"Clicking" Peptides to Biomaterials and to Ionic Liquids: Towards Dual-Action Topical Formulations for Skin and Soft Tissue Infections

P. Gomes

"Clicking" Peptides to Biomaterials and to Ionic Liquids: Towards Dual-Action Topical Formulations for Skin and Soft Tissue Infections



Multidrug-resistance, MDR, is spiraling worldwide, and the currently available antibiotics are no longer able to provide an efficient response. Alongside the growing life expectancy and aging-associated diseases, MDR underlies the concerning rise of the incidence, the severity, and the chronicity of both medical device associated infections in soft tissues, for example, implant- and catheter-associated infections, and

non- healing wounds, for example, venous leg ulcers, diabetic foot ulcers, and pressure ulcers.

Such complicated skin and soft tissue infections, cSSTI, typically have a polymicrobial nature, often associated to mixed species biofilms involving some of the most concerning MDR bacterial pathogens of the so-called ESKAPE group as well as pathogenic fungi. Host defense peptides, HDPs, encompassing antimicrobial and/or immunomodulatory molecules, are increasingly regarded as key players towards the post-antibiotic era, including for topical use, where the ability to promote fast recovery of a healthy skin is equally important.

In this regard, our group has been using [photo]click chemistry as a tool to produce peptide-based constructs, including peptide-grafted biomaterials and peptide-ionic liquid conjugates, aiming at concomitant antimicrobial, antibiofilm, and collagen-boosting actions. Latest findings will be highlighted herein.

Funding
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Acknowledgements
Thanks are due to FCT for funding LAQV-REQUIMTE through project UIDB/50006/2020. Thanks are also due to all present and past members of the Paula Gomes Lab, and to the teams of Prof. Drs. Paula Gameiro, LAQV-REQUIMTE, FCUP, M. Cristina L. Martins, i3S-UP, Eugénia Carvalho and Teresa Gonçalves, CNC-UC, for their active and deep involvement in the research projects covered in this communication.

DU VIGNEAUD LECTURE

L55-AW Helma Wennemers

ETH Zurich

Synthetic Collagen Peptides – From Structure to Function

Helma Wennemers

Collagen, the most abundant protein in mammals, is a key contributor to the strength and stability of skin, bones, and connective tissue. Collagen formation is thus vital for the integrity of skin, tendons, and the tissue in essentially any organ. Excessive collagen formation is, however, characteristic of fibrotic and malignant diseases, which include major global health issues.

The Wennemers group has used collagen model peptides, CMPs, to understand the stability of collagen at the molecular level and to establish functional synthetic collagen triple helices. These include pH-responsive synthetic collagen, hyperstable triple helices, and heterotrimeric collagen. Building on these data, we designed and synthetized a chemical probe for the simultaneous monitoring and targeting of lysyl oxidase LOX-mediated collagen cross-linking. The probe allows for the detection of LOX activity *in vivo* and in tissue sections.

SESSION 11: PEPTIDE DESIGN AND FUNCTION

L56 Sidney M. Hecht

Arizona State University

Ribosomal Synthesis of Peptides and Proteins Containing Non-Canonical Amino Acids

Sidney M. Hecht

Several strategies now exist for the ribosomal synthesis of peptides and proteins containing non-proteinogenic amino acids. These enable the incorporation of one or more modified amino acids into predetermined positions in a peptide or protein. While a wide variety of amino acid side chains not found in natural proteins can be incorporated, bacterial ribosomes do not incorporate amino acid analogues such as D-amino acids or β-amino acids to a reasonable extent.

In the past several years, we have developed a strategy for modifying the 23S ribosomal RNA in *E. coli* ribosomes; this is the ribosomal constituent that mediates the peptide bond formation. By the use of structurally modified puromycin analogues, libraries of clones harboring plasmids with modified 23S rRNAs can be screened to identify clones capable of incorporating modified amino acids not normally incorporated by bacterial ribosomes.

Presently, I will discuss the incorporation into peptides and proteins of D-amino acids, β-amino acids, dipeptides and dipeptidomimetics, as well as nucleobase amino acids. Unique properties of the derived proteins will be described, including their ability to alter bacterial phenotype, and modulate gene expression in mammalian cells.

L57 Maria Soloveychik

SyntheX Labs

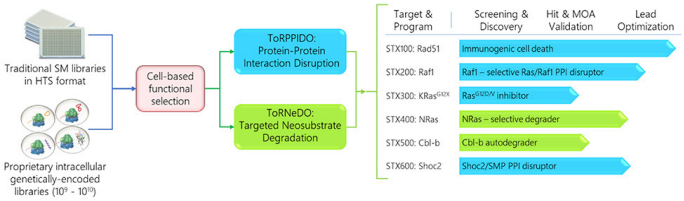
Cell-Based Function Selection of Peptides for Protein-Protein Interaction Disruption and Targeted Protein Degradation

R. Bond, S. Lin, D. Nielsen, C. Chahwan, M. Soloveychik

SyntheX created empirical drug discovery platforms to discover protein interaction modulators. ToRPPIDO to allow

for the discovery of compounds that can disrupt a specific protein-protein interaction, PPI. ToRNeDO achieves the inverse and discovers compounds that bring an E3 ubiquitin ligase and a neosubstrate of interest together to achieve targeted protein degradation. Using genetically engineered circuits, the platforms rely on intracellular drug selection.

This allows for the unbiased discovery of compounds that can engage targets in either competitive or allosteric ways to achieve a desired mechanism of action. This intracellular approach bypasses many bottlenecks that exist with canonical in-vitro screening assays.



The platforms are modality agnostic and can be used to discover small molecules or biologics. Taking additional advantage of intracellular screening, the cells are further engineered to ribosomally produce libraries of peptides or diversified biologics. This allows for the simultaneous production and selection of potent compounds in an efficient manner while sampling billions of sequences for very specific functions.

This approach results in the concurrent generation of a tool compound and the mapping of an exploitable pocket for protein interaction modulation. The platforms have generated an internal oncology pipeline focused on unique mechanisms of action around genetically validated drug targets, largely targeting crucial nodes in the Ras-MAPK pathway in novel ways.

L58 Thomas Tucker

Merck Research Laboratories

Cyclic Peptide PCSK9 Inhibitors : The Design and Optimization of Highly Potent, Orally Bioavailable, and Clinically Viable Molecules as LDL-Cholesterol Lowering Therapeutics

T. J. Tucker

Proprotein convertase subtilisin-like/Kexin type 9, PCSK9, is a clinically well-validated and critically important target for treating high LDL-cholesterol and potential coronary artery disease. Two antibody-based and one siRNA based anti-PCSK9 therapeutics have been approved by the FDA for treating high LDL-cholesterol levels and have demonstrated excellent clinical efficacy for lowering LDL levels and preventing adverse cardiac events. However, all of these therapies are parenterally delivered and to date an efficacious, orally dosed anti-PCSK9 therapeutic has not been identified. We focused our efforts on discovering and optimizing novel, orally bioavailable cyclic peptide agents based on leads derived from an mRNA display screening campaign. From the mRNA display screening, we were able to identify moderately potent inhibitor leads. Guided by structural data, we were able to optimize our early leads to enhance metabolic stability, potency, and engineer out several unfavorable off-target activities to provide advanced next generation development candidates.

Using an enabled formulation-based approach, we demonstrated acceptable oral bioavailability and good overall pharmacokinetics for these molecules and using a Target Engagement assay were able to build clear PK/PD relationships in primates. Final optimization of candidate molecules to address formulation-related issues led to the discovery of MK-0616, which is currently undergoing clinical investigation as an LDL-cholesterol-lowering agent.

In this talk, we will detail the systematic optimization of these molecules guided by structural data, leading to the discovery of the clinical compound.

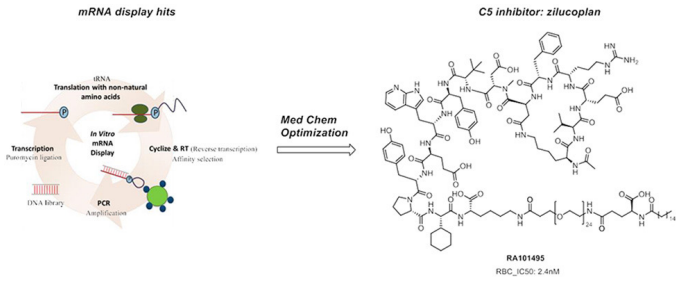
L59 Ping Ye

UCB

Discovery of Zilucoplan: A Potent Macrocyclic Peptide Complement Component 5, C5, Inhibitor in Acetylcholine Receptor Antibody-positive Generalized Myasthenia Gravis

Ping Ye

Cyclic peptides are diverse molecules that are now a focus in drug discovery efforts. Their molecular size, between small molecules and biologics, provides attractive scaffolds to screen against some challenging targets, including protein-protein interactions and those considered to be "undruggable" proteins. With messenger ribonucleic acid (mRNA) display screening technology now able to produce trillions of peptide molecules for screening and quickly identify tight binders against targeting proteins, an exciting time of cyclic peptide drug discovery has come.



We have been working on cyclic peptide drug discovery since 2010, and have successfully identified two compounds derived from mRNA display that have entered clinical trials. One of them is a complement C5 inhibitor, zilucoplan, that has completed a phase III study with positive results in adult patients with AChR+ myasthenia gravis.

Here we present the discovery of zilucoplan, starting from hits identification via mRNA display screening against C5, followed by medicinal chemistry modifications to improve the potency, plasma stability and PK properties, leading to the clinical candidate.

DR. ELIZABETH SCHRAM YOUNG INVESTIGATORS ORAL COMPETITION

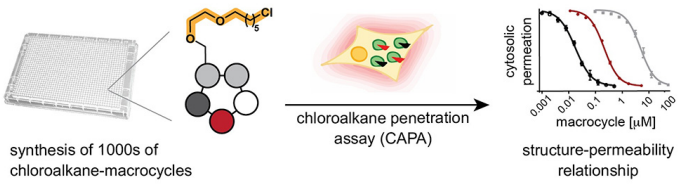
L60-YI8 Alexander Lund Nielsen

Ecole Polytechnique Fédérale de Lausanne

Assessing the Cellular Permeability of Peptidic Macrocycles in High-Throughput

Alexander L. Nielsen, Christian R.O. Bartling, Kristian Strømgaard, and Christian Heinis

Small cyclic peptides provide an attractive modality for drug development due to their ability to bind challenging targets and their potential to cross membranes for reaching intracellular proteins. In our laboratory, we have recently developed methods to synthesize and screen large combinatorial libraries of small cyclic peptides. For example, *"m"* short linear peptides containing thiol groups at both ends were combinatorially cyclized with *"n"* bis-electrophilic linker reagents to obtain *m* × *n* cyclic peptides that were screened in microwell plates as crude products. While the approaches yielded ligands to several disease targets, not all of them were membrane permeable. A full picture of the membrane permeability of the newly developed format of peptidic macrocycles, and factors that determine their permeability, was lacking.



In this work in progress, we have taken advantage of the chloroalkane permeation assay, CAPA, that has recently emerged as a robust method to determine cytosolic permeability of chloroalkane-tagged biomolecules. We have established a method to synthesize thousands of diverse chloroalkane-tagged peptidic macrocycles to determine their cytosolic permeability using CAPA. This has given us a new insight into the structure-permeability relationships of an unprecedented number of macrocycles and provides a clearer picture of what features govern permeability of macrocyclic compounds in cellular systems.

DU VIGNEAUD LECTURE

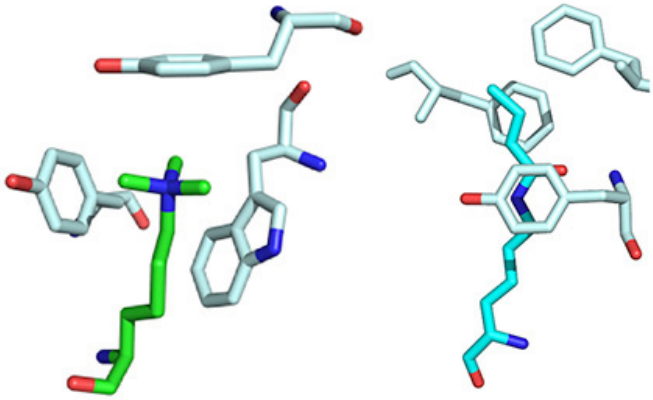
L61-AW Marcey Waters

University of North Carolina at Chapel Hill

How Do Post-Translational Modifications Turn on Protein-Peptide Interactions and how can We Inhibit Them?

Marcey Waters

Post-translational modifications, PTMs, in histone proteins, including lysine methylation and acylation, regulate gene expression through recruitment of reader proteins to the nucleosome. Dysregulation of these events is prevalent in a wide range of diseases, such that there is much interest in characterizing these modifications and their binding partners as well as developing inhibitors for these protein-protein interactions.



I will discuss mechanistic studies of the factors that contribute to these PTM-mediated protein-protein interactions using a combination of high-throughput mechanistic screening, genetic code expansion, and investigation of PTM-isosteres, and how this has led to novel approaches to inhibit them.

SESSION 12: NEW FRONTIERS IN COMPUTATIONAL PEPTIDE DESIGN PART 1

L62 Bill DeGrado

University of California at San Francisco

***De Novo* Protein Design of Functional Proteins**

Bill DeGrado

Not too long ago, the design of proteins from scratch that fold into predictable structure was considered an impossible task, but it is now increasing routine. Given our ability to design proteins structures the next challenge has been to design function. The success or failures of the designs informs our understanding of the principles underlying the

desired function and additionally provides the first step towards design of proteins with useful functions not available to natural proteins.

Some functions, such as binding to protein interfaces have been relatively easy, because the interactions that stabilize interfaces are also used to stabilize the folded structures of proteins, and available computational methods have been well calibrated to this task. It has been more difficult to design functions that involve molecular recognition of small, polar molecules or dynamic processes. I will describe methods for the design of proteins that bind small molecules, and the use of these proteins in potential biomedical applications such as drug delivery and as drug reversal agents.

A second challenging area of interest in de novo protein design has been the construction of useful membrane proteins. I will describe the design of semisynthetic membrane protein that function as ion channels, and their application to DNA and RNA sequencing. The talk will also how de novo design can be used to test the mechanisms by which protons are conducted with high specificity and efficiency through transmembrane proton channels.

L63 Fei Cai

Genentech

Improved Naive Peptide Library Designs Guided by AI-prediction

F. Cai, A. Chang, and Y. Zhang

It has been widely accepted that using peptide-based molecules to disrupt protein-protein interactions is a promising strategy to address some disease targets, especially those that are difficult to be treated with small molecules. Many successful examples in the pharmaceutical field show that high-affinity therapeutic peptides can be discovered by performing peptide library screening followed by affinity maturation and optimization. However, sometimes one found that peptides' stability may be sacrificed while binding affinity is improved. In that case, optimization of lead molecules usually requires a lot of peptide synthesis and function assays, which are both cost- and time-consuming. To address this problem, we proposed an AI-assisted library design strategy to make sure screening can start from functional libraries with better stability and folding property.

We performed Alanine scanning and NGS analysis on hyper- stable constrained peptides, HCPs. Subsequently, feeding NGS data into the DeepSeq.AI algorithm resulted in a tailored prediction model for peptide stability and folding. Based on predictions, we designed naïve libraries and performed test panning. The panning success rate is encouraging, and selected peptide binders were synthesized and present good folding properties.

L64 John Jumper

DeepMind

Understanding Peptides and Their Interactions via Deep Learning Structure Prediction

John Jumper

Modern protein structure prediction via AlphaFold and related methods has shown a remarkable ability of machine learning methods to learn chemical and evolutionary principles from experimental biology data. Because of this understanding, these models are able to accurately model the structure of protein-peptide interactions and in some cases even identify the relative strength of different binders.

In this talk, I will discuss what ideas in machine learning enabled the high accuracy of these new models, how the community is applying them to understand peptides and their interactions, and how these models may improve in the future.

L65 Carol K. Hall

North Carolina State University at Raleigh

Computational Design of Peptides as Detectors, Sensors, and Drugs

Carol K. Hall

We describe our efforts to develop an efficient computational algorithm that searches for peptides that bind strongly and selectively to specific biomolecular targets, and to use that algorithm in the design of peptide-based sensors, drugs, and affinity chromatography ligands. The algorithm is an iterative procedure that involves as many as 50,000 sequence mutation moves and/or peptide backbone conformation moves to arrive at the peptide sequence and conformation that has the lowest binding energy to the target. The top scoring peptides are then further evaluated by performing explicit-solvent atomistic simulations of the peptide–target complex to determine their binding free energies.

The biomarker chosen for initial study was Cardiac Troponin I, a 210-amino acid protein that is elevated in the blood of heart attack victims. The top-scoring peptides were synthesized by collaborators at the Air Force Research Laboratory who then used a variety of experimental techniques to assess the binding capabilities of the top candidate peptides. The measured binding affinity of the designed variant, P2, for cTnI was extremely strong, KD = 0.27 nM, and comparable to that of the natural antibody, KD = 0.12 nM.

We further describe two other projects: **1**, design of peptide affinity ligands for bioseparating monoclonal antibody, IgG, from the cell cultures in which they are synthesized, and **2**, design of peptides to block the action of the toxins secreted by C- difficile bacteria in the large intestine.

SESSION 13: NEW FRONTIERS IN COMPUTATIONAL PEPTIDE DESIGN PART 2

L66 Karen Akinsanya

Schrödinger

Computational Peptide Design Advances: Past, Present, and Future

Karen Akinsaya

Designing therapeutic peptides that adhere to a range of target product profile criteria is a multiparameter optimization, MPO, problem that is challenging to solve when programs are restricted to a relatively limited number of design ideas.

This talk will address advances in the development and cloud-based deployment of atomistic physics-based computational approaches, which are having an increasing impact on drug discovery in the biopharmaceutical industry. Combining these methods with broader access to experimental ligand-bound structures and computational protein structure models has enabled advances in understanding structure-function relationships and the design of peptide therapeutics for a range of molecular targets from GPCRs to protein-protein interactions. Small-molecule therapeutics have benefited from the availability of accurate computational methods that predict the binding mode and affinity of ligands to their protein targets and other physics-based properties.

With increasing focus on the development of peptide-based therapeutics, including peptidomimetics and macrocycles, which are usually larger and more conformationally flexible than traditional drug-like molecules, atomistic physics-based and structure-based approaches can be applied or adapted for use in the identification and optimization of peptide ligands for proteins. These include molecular docking, relative binding free energy predictions, conformational searches, and structural refinement. For example, refined high- resolution cryogenic-electron microscopy, cryoEM, structures of active-state GPCRs in complex with G-proteins and ligands complemented by molecular dynamics simulations and binding or functional assay data provide key insights into ligand recognition and receptor activation to inform structure-based peptide design.

L67 Joshua Price

Brigham Young University

Peptide/Protein Redesign Using Fine-Tuned ProtBert Masked Language Models

Joshua Price

Machine learning language models have mastered human dialect; models like GPT-2, GPT-3, and the ChatGPT can write sophisticated realistic responses to simple prompts. Other models like BERT can classify text in useful ways for computational and machine-learning work.

Based on the hypothesis that a sequence of amino acids, AAs, can be interpreted like written language, specialized models have been developed for protein-specific tasks. For example, the ProtBERT masked language model, MLM, was trained on protein sequences in the UniProt and BFD databases for "fill-in-the-blank" tasks. Given an input AA sequence, ProtBERT will deterministically replace one AA with one of other canonical AAs based on features extracted from its training data and on context clues from the surrounding sequence. Its current strength is the ad lib "hallucination" of new protein sequences, which are predicted to share many features of known proteins and to explore new areas of sequence space. However, ProtBERT is not currently able to generate a protein with a specified secondary/tertiary structure or function.

We have fine-tuned ProtBERT by training it on selected subsections of the PDB with a characteristic secondary structure. The resulting specialized models, αProtBERT and βProtBERT, reliably generate α-helices and β-sheets, respectively, and should be useful for redesigning selected regions of a protein while accounting for specific context-clues within the surrounding constant sequences, a skill that would be essential for redesigning proteins that have active sites, binding interfaces, or consensus sequences a user does not wish to disturb.

L68 Gaurav Bhardwaj

University of Washington, Seattle

Machine Learning Guided Structure Prediction and Design of Macrocycles

Gaurav Bhardwaj

Machine learning, ML, methods have demonstrated tremendous success in the structure prediction and design of larger proteins. However, these established methods do not translate well to small peptides with non-canonical amino acids and crosslinks. We have previously described Rosetta-based computational methods for accurately designing constrained peptides and macrocycles. We have recently extended these approaches to design peptide binders against therapeutic targets and macrocycles with enhanced membrane permeability and oral bioavailability.

Here, we present our recent breakthroughs in machine learning-guided methods for structure prediction and *de novo* design of macrocycles for diverse structures and functions. In our benchmarking studies, these methods accurately predict the structure of macrocycles available in the Protein Data Bank.

Next, we extended these methods for redesigning and hallucinating structurally and chemically diverse macrocycles. Experimentally determined structures of these hallucinated macrocycles match very closely with the design models. In parallel, we have also developed generative models for macrocycles, which are based on variational autoencoders and denoising diffusion probabilistic models and show substantial improvements in time and accuracy.

These generative macrocycle models enable "unconstrained hallucination" for sampling diversity and "constrained hallucination" to incorporate arbitrary chemical and structural requirements in the de novo-designed macrocycles. Overall, these new ML-guided methods offer opportunities for accurate and custom design of peptides for various functional properties.

EARLY CAREER LECTURESHIP AWARD

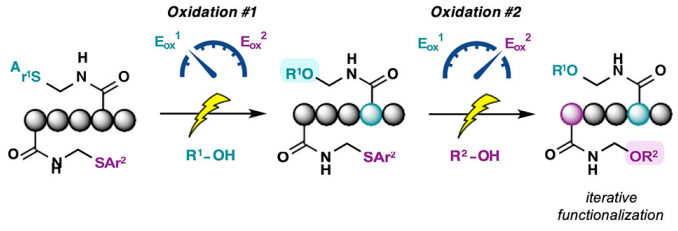
L69–AW Lara Malins

Research School of Chemistry at the Australian National University

Dialing in the Potential: Tunable Electrochemical Peptide Modifications

Lara Malins

The use of electricity to drive chemical reactions is an appealing approach to green and sustainable synthesis. Moreover, electrochemistry provides powerful opportunities for the precise control of chemical reactivity by enabling practitioners to "dial in" the potential or current at which a reaction is performed. For highly functionalized molecules, this tunability provides unprecedented opportunities for targeted chemical modifications.



Despite these appealing characteristics, there are remarkably few examples in which electrochemistry is applied to the tunable functionalization of peptides. In this presentation, a method for the selective, iterative electrochemical modification of peptides is presented. Designer glutamine residues adorned with discrete "electroauxiliaries" — functional groups electronically-predisposed to anodic oxidation — are incorporated into peptides and exploited for iterative modifications to afford a library of high-value peptide *N,O*-acetals. The utility of the method is demonstrated on unprotected peptides and bioactive substrates and is applied in a novel approach to peptide macrocyclization.

This targeted approach to electrochemical activation serves to unlock a new level of orthogonality in peptide synthesis, and the strategy has promising applications for the preparation of peptide libraries, including for therapeutic development.

SESSION 14: NOVEL ADVANCES IN PEPTIDE CHEMISTRY

L70 Michael S. Kay

University of Utah School of Medicine

D-Peptide Therapeutics

Michael S. Kay

D-peptides are promising therapeutic agents because of their resistance to proteases and low immunogenicity. However, D-peptide discovery using high-throughput screening methods like mirror-image phage display requires chemical protein synthesis, CPS, of mirror-image target proteins, which previously limited such efforts to relatively small proteins.

Recent CPS advances like solubilizing "helping hands" and computational evaluation of potential synthesis pathways have greatly improved CPS efficiency and expanded its reach to larger target proteins.

Here we describe our progress in developing antiviral D-peptides, including an HIV entry inhibitor in clinical trials, as well as earlier-stage efforts to develop D-peptides targeting larger bacterial targets.

L71 Seino Jongkees

Vrije Universiteit Amsterdam

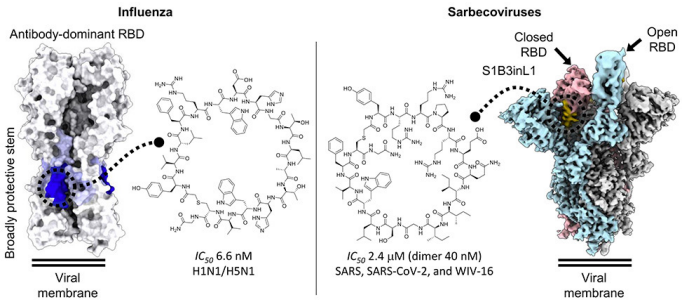
Antiviral Peptides from mRNA Display Antiviral Peptides from mRNA Display

S. A. K. Jongkees

The current Covid-19 pandemic dramatically illustrates the need to find new anti-viral agents, and underscores the importance of finding these agents before they are needed. Influenza remains a pandemic threat, and resistance is building to many of the current anti-influenza treatments on the market. While antibodies are being explored for treatment for both of these viruses, they are not practical to deploy at a population level.

As an alternative approach that we believe captures many of the advantages of antibodies in a synthetically tractable mol-

ecule, we have used mRNA display under a reprogrammed genetic code, RaPID system, to find antiviral peptides with broad protective effects against infection by both influenza, H1 and H5 cross-reactivity, and sarbecoviruses, SARS and SARS-CoV-2 cross-reactivity.



Hydrogen-Deuterium exchange footprinting of our anti-influenza peptides revealed a surprising preference for these to bind to the more conserved "stem" region of the protein, where the "head" region is typically dominant for antibody binding. Passaging the virus with one of these inhibitors does lead to resistance, but raising new peptides against the resistant variant gives new hits that force the virus to compromise fitness to escape when administered together with the original hits.

A cryo-EM structure of our anti-coronavirus peptide reveals that it is binding to a highly conserved and previously unexploited ternary site between the S1A, S1B, and S2 domains. Overall these molecules emphasize the promise of small macrocyclic peptides in combating new viral outbreaks by targeting viral proteins on the particle surface.

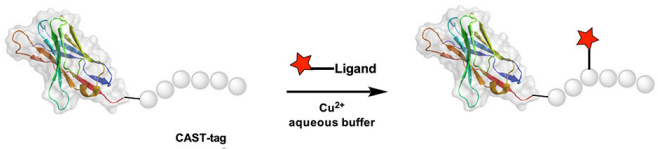
L72 Bobo Dang

Westlake University, China

Copper-Assisted Sequence-Specific Chemical Protein Conjugation

Mengzhun Guo, Kai Zhao, Liang Guo, Qiuju He, Rui Zhou, Kuan Lu, Tian Li, Dandan Liu, Xin Fu, Samuel I. Mann, Jing Huang, Bing Yang, William F. DeGrado, Ting Zhou, Yingjie Lei, and Bobo Dang

Direct site-specific methods of chemical protein functionalization are highly desirable. However, such methods are particularly challenging to develop due to the tremendous difficulty of chemically differentiating the same amino acid type at different protein sites.



Herein, we proposed "metal binding targeting" strategy and developed Copper Assisted Sequence-specific conjugation Tag, CAST to achieve single site-specific protein conjugations. CAST possesses superior reaction kinetics with a rate constant of 8.1 M^{-1} s^{-1} in aqueous buffer.
Importantly, CAST conjugation can be employed as a universal method for efficient and quantitative payload attachment on different proteins. We also highlight that an antibody drug conjugate prepared using CAST is highly stable in plasma and exhibits potent efficacy *in vitro* and *in vivo*.

SESSION 15: INNOVATIVE METHODS FOR PEPTIDE STRUCTURE

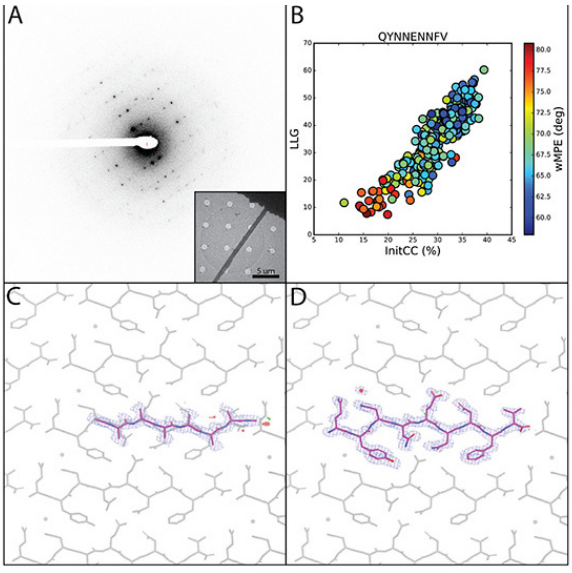
L73 Jose Rodriguez

University of California at Los Angeles

Solving Peptide Structures and More: Frontier Advances in Electron Diffraction

Jose Rodriguez

Electron diffraction has historically played an important role in the advancement of crystallographic approaches for the determination of complex small molecule structures. A broad array of atomic structures has now been determined by micro crystal electron diffraction, MicroED. They include naturally occurring peptides, synthetic protein fragments and peptide-based natural products. However, *de novo* structure determination by MicroED remains problematic for all but ideal crystals. Automated, fragment-based approaches to structure determination eliminate the need for atomic resolution diffraction, instead enforcing stereochemical constraints through libraries of small model fragments.



Determination of atomic resolution peptide and small molecule structures by frontier electron diffraction methods. Electron diffraction patterns, A, can be collected from nanoscale peptide crystals as inset, phased by fragment-based methods, B, and lead to accurate atomic structures, C and D.

We have demonstrated the application of fragment-based phasing on various macromolecular structures including some for which all traditional phasing methods have failed, see image below. Most recently, we have endeavored to determine structures where resolution is far beyond the reach of direct methods, yielding novel solutions with 1.4-2.2Å data.

The determined structures are accurate and in test cases, reflect structures determined by traditional X-ray crystallographic approaches. New nanobeam electron diffraction methods are further advancing our ability to determine novel structures, by yielding atomic insights from regions of lattices containing as few as 1000 molecules.

Together, these approaches advance the frontier of electron diffraction-based structure determination methods, and are increasingly accessible to the broader field of structural science.

L74 George Burslem

University of Pennsylvania

Beta-Peptides as Conditional Ligands for Peptide Exchange in MHC-I

Trenton J. Winters, Yi Sun, Nikolaos G. Sgourakis, and George M. Burslem

The proteins of class I major histocompatibility complex, MHC-I, display epitopic peptides on the cell surface, providing the foundation for immune surveillance of intracellular threats. MHC-I folding and peptide loading are subject to intricate cellular quality control. The peptide loading complex, PLC, comprising the TAP transporter, the molecular chaperones tapasin, ERp57, and calreticulin, assemble peptide-MHC-I, pMHC-I, molecules with high-affinity peptides in the endoplasmic reticulum, ER, in a process termed peptide editing. However, the recapitulation of loaded MHC-1 complexes *in vitro* has been a long-standing challenge.

Here we report on the use of beta-peptides as conditional ligands for refolding of MHC and their facile exchange to desired peptide sequences via the use of molecular chaperones. This technology can be utilized in various cancer immunotherapeutic settings to narrow the peptide repertoire, thereby increasing neoepitope immunogenicity.

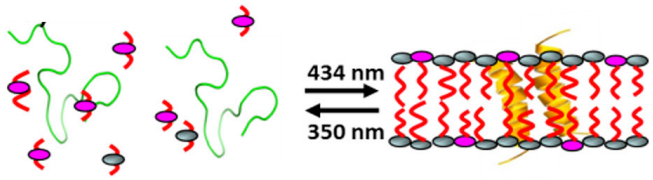
L75 Matthew Tucker

University of Nevada at Reno

Mapping Structure and Dynamics with Site-Specific Vibrational Probe Pairs via 2D IR Spectroscopy

Matthew Tucker

2D IR vibrational probe pairs provide structural maps for uncovering dynamics associated with active biomolecules, providing insights into the molecular movements leading to their functionality along equilibrium and non-equilibrium pathways. Well-positioned probe pairs can simultaneously detect the dynamics within two different regions and measure distances in places where biological function takes place. Up until this point, a systematic study of a variety of 2D IR probe pairs, including labelled amides, cyano- modes, ring modes, and azido-modes have provided structural tools with many different metrics.



Now, we are moving toward transient 2DIR measurements to track non-equilibrium structural dynamics along the antimicrobial peptide pathway. Applications of these measurements to different chemical ecology problems will also be highlighted.

CLOSING PLENARY KEYNOTE LECTURE

L76 Jim Wells

University of California at San Francisco,
Dept of Pharmaceutical Chemistry

Pirating Biology to Probe and Attack the Cell Surface Proteome

Matthew Tucker

The cell surface proteome, the surfaceome, is a major hub for cellular communication and a primary source of drug targets, especially for biologics. Identifying how the surfaceome changes in cancer is a central challenge for identifying and targeting new disease associated proteins.

We have used chemical methods and engineered proteins to facilitate identification of membrane proteins, both native and post-translationally modified versions, that change with oncogene transformation and or hypoxia a characteristic of the tumor micro-environment.

We then target proteins either upregulated, proteolyzed or both with recombinant antibodies derived by phage display to be used as validation tools and potential therapeutic leads.

We also have developed degraders for extracelluar targeted protein degradation called AbTACs and KineTACS. I'll discuss some of our latest results.

Poster Abstracts

P001 Toshiya Hatekanaka

Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology

Predicting Proline-Containing Tripeptides with Angiotensin I-Converting Enzyme Inhibitory Activity Using Machine Learning Models

T. Hatakenaka, Y. Fujimoto, K. Okamoto, and T. Kato

Hypertension is a major risk factor for brain and cardiovascular diseases, and angiotensin I-converting enzyme, ACE, is a target for antihypertensive drugs due to its role in generating angiotensin II, which raises blood pressure. Previous studies have found a number of peptides that exhibit ACE inhibitory activity.

The aim of this study is to search for novel peptides with ACE inhibitory activity. To this end, since ACE inhibitory peptides often contain proline residues, we collected the IC_{50} values, a standard index of ACE inhibitory activity, for tripeptides containing proline from a database.

Next, docking simulations were performed for each tripeptide and ACE, and docking scores and their descriptors were calculated. Using the known IC_{50} values, docking scores, and descriptors for each peptide as training data, the Extra-Tree Regressor model demonstrated the highest coefficient of determination, R^2, when evaluated using PyCaret. The machine learning models were then utilized to identify candidate peptides with potent inhibitory activity against ACE. Candidate peptides were synthesized using solid-phase methods and evaluated for ACE inhibition *in vitro*.

The results demonstrate the potential of employing machine learning, such as PyCaret, to identify novel and effective ACE inhibitors. The screening techniques employed in this study could also be applied to the development of new antihypertensive agents with high efficacy, safety, and minimal side effects.

YI-P002 Kyle Nadeau

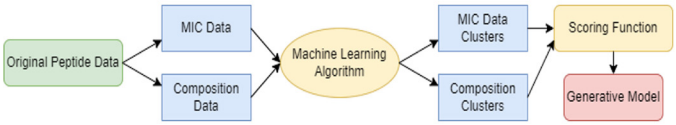
University of Rhode Island

Sequential Analysis of Antimicrobial Peptides with Machine Learning Available on AMPed

Kyle Nadeau, Sarah M. Brown, Anusha Singmaneni, Joan Peckham, Nicholas Alessandri, and Lenore M. Martin

Machine learning offers promising solutions drawing on peptides with antimicrobial activity that are generated naturally by organisms as a part of their innate immune response. Evolution of resistance within pathogens detrimental to human health has rendered many treatments ineffective.

The publicly-available AMPed.uri.edu-AntiMicrobial Peptide EDitable database-is an interdisciplinary online research platform that allows us to easily incorporate new software tools of interest to the peptide community. We report that application of Machine Learning algorithms, to existing datasets on the antimicrobial activity of synthetic and naturally occurring peptides, allows for large amounts of previously published data to be analyzed with increased efficiency. This generates new peptide sequences for evaluation as novel therapeutics, incorporating multiple aspects of known highly effective antimicrobial peptides. Initially, we demonstrated proof-of- concept on a small set of peptides, using SciKitLearn to generate algorithms. Clustering allows us to group "similar" peptides based on their amino acid composition and inhibitory activity. Resulting data shows over an 83% correlation between composition and inhibitory activity; highly active peptides were often grouped together based on percent amino acid composition.



Analysis of overlap between clusters allowed for further development of a predictive algorithm that trains on larger sets of peptide activity data with the potential to generate novel active peptide sequences. Expansion of this research using invertible neural networks will allow for less ambiguity when studying parameterization of our algorithms for more accurate generative modeling.

We will chemically synthesize and evaluate novel AI-generated sequences within our lab for further antimicrobial testing.

P003 Daniela Kalafatovic

University of Rijeka

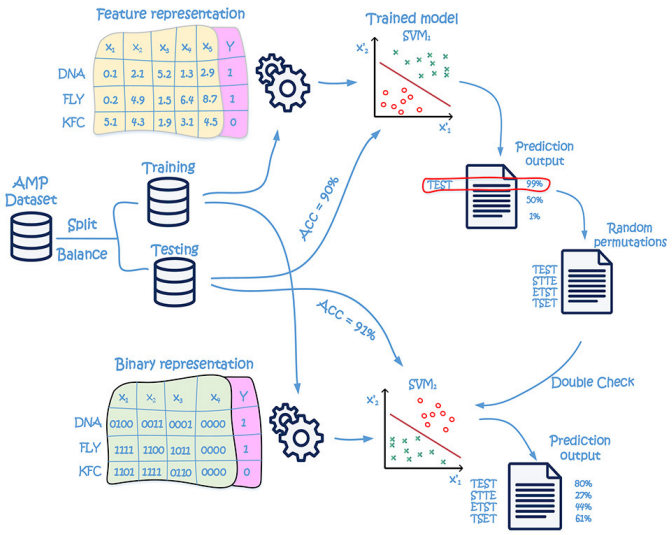
Soft Computing Guided Discovery of Active Peptides

D. Kalafatovic

The discovery of new active peptides, that is, antimicrobial, antiviral, catalytic, is challenging, as they are part of a very large search space and the correlation between the peptide sequence and the desired activities and/or functions is not yet fully understood. To avoid expensive and time-consuming guesswork and experimental failure, our strategy is to apply soft computing techniques to accelerate peptide discovery.

Soft computing is a set of probabilistic algorithms, which are robust to imprecision and tolerant to uncertainty, that enable us to grapple with analytically intractable problems

and make up for the lack of theoretical knowledge. We applied a wide range of soft computing models to predict peptide activity, construct novel peptides and cover the chemical search space. More in detail, we tackle the problems of **i** sensitivity of highly accurate predictive models, **ii** building predictive models with low amount of available data, **iii** interpretability of neural network-based classifiers, **iv** ability to generate new peptide sequences, and **v** coverage-based parallel exploration of chemical space.



Focusing on therapeutic peptides, we addressed the issue of sensitivity of highly accurate predictive models and proposed the sequential properties representation scheme to improve their predictive power. This provided the foundations to employ deep learning models improved by transfer learning for the prediction of underrepresented categories or poorly researched peptide functions, such as their predisposition towards self-assembly or catalysis.

To gain insight into the decision process of black-box neural network models we employ the Grad-Cam technique, which enables us to pinpoint the properties and residues important for the prediction results and analyze their behavior.

We envision that these strategies will maximize the chance of successful identification of functional peptides, partly reducing the environmental impact of failed experimental attempts.

YI-P004 Samuel Scherer

University of Utah

Computational Design and Synthesis of FmlH for Mirror-Image Phage Display

Samuel Scherer

Chemical protein synthesis, CPS, enables access to non-natural protein structures, including those in mirror-image. CPS limitations such as segment insolubility, aggregation, and slow ligation kinetics have necessitated the creation of a variety of chemical tools such as traceless solubility tags, kinked building blocks to prevent on-resin aggregation, and desulfurization to increase the number of suitable native chemical ligation junctions. Still, residue-specific constraints of CPS limit its reach. Some proteins, particularly those with large regions of predicted insolubility or a dearth of suitable ligation junctions, are likely to have increased synthesizability with mutations of their native sequence.

Building off previous lab tools that identify CPS strategies, this work will develop a computational workflow to discover tolerable mutations that enhance CPS and provide enhanced strategies for peptide synthesis and ligation. The predictive portion of this pipeline will consist of a combination of a deep learning algorithm to predict residue identity based on its local protein environment, Protein MPNN, and a simulation-based model in Rosetta. Our first target of this generalizable computational workflow is the design and synthesis of the FmlH lectin domain, a 160-amino acid adhesion protein that mediates severe upper urinary tract infections and is predicted to have a challenging synthesis. D-FmlH will be synthesized and carried forward as a target for mirror-image phage display with the goal of identifying an inhibitory D-peptide therapeutic.

P005 Hyeong Woon Choe

Seoul National University

Proline-Hinged A-Helical Peptides Sensitize Gram-Positive Antibiotics, Expanding Their Physicochemical Properties to be Used as Gram-Negative Antibiotics

Hyeong Woon Choe, Yunhwa Choi, Minsoo Kook, Seolah Choo, Tae Woo Park, Soeun Bae, Heeseung Kim, Yang Soo Kim, and Jaehoon Yu

The outer membrane, OM, of Gram-negative bacteria is the most difficult obstacle for small-molecule antibiotics to reach their targets in the cytosol. The molecular features of Gram-negative antibiotics required for passing through the OM are that they should be positively charged rather than neutral, flat rather than globular, less flexible or more increased amphiphilic moment. However, because of these specific molecular characteristics, the development of Gram-negative antibiotics is difficult. Consequently, the number of available drugs is limited.

We focused on an alternative approach to circumvent these issues, which involves using sensitizer peptides to facilitate the passage of hydrophobic Gram-positive antibiotics through the OM.

In this study, we explored ways of improving the sensitizing ability of proline-hinged α-helical peptides by adjusting their length, hydrophobicity, and N-terminal groups. We found that a novel peptide, 1403, improves the potentiation of rifampin *in vitro* and in animal models. Additionally, this novel

peptide can potentiate most Gram-positive antibiotics.

Taken together, the results of this investigation show that the 'sensitizer' approach for repositioning Gram-positive to Gram-negative antibiotics is more plausible than those that rely on conventional drug discovery methods concerning drug development costs and the development of drug resistance.

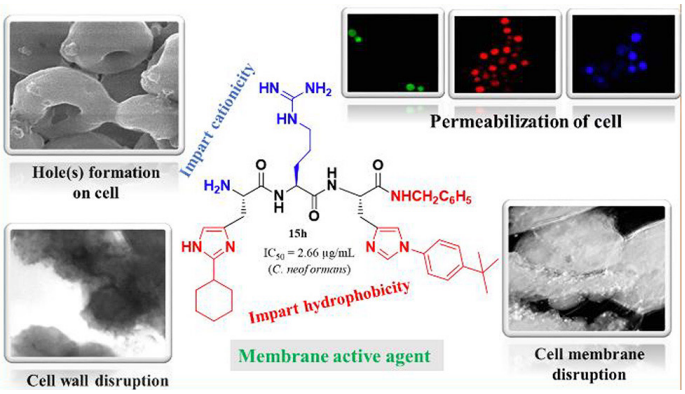
YI-P006 Shams Aaghaz

National Institute of Pharmaceutical Education and Research, India

Ultrashort Cationic Antimicrobial Peptides as Membrane Active Agent Against Cryptococcus neoformans

Shams Aaghaz, Komal Sharma, Indresh Kumar Maurya, and Rahul Jain

Global fungal infection rates are rising, which necessitates the need to find new antifungal agents. Furthermore, the emergence of resistance to current antimicrobial drugs mandates the discovery of new chemical entities. The available drugs also have drawbacks such adverse side effects, inadequate pharmacokinetics, undesirable drug-drug interactions and limited solubility. Therefore, we synthesize a new class of synthetic peptides by integrating 2-alkylated and 1-arylated histidine with arginine.



Arginine, a positively charged amino acid, interacts with the negatively charged microbial membranes, whereas arylated and alkylated histidine gives lipophilicity to the molecules that further enhances its penetration into the microbial membrane. The synthesized peptides demonstrated potency against *Cryptococcus neoformans*.

Plausible mechanistic pathways have been performed using flow cytometry, confocal laser scanning microscopy, scanning electron microscopy and transmission electron microscopy. Short sequence of peptides makes it more attractive due to ease of synthesis and low cost of production. It is noteworthy that the lipophilicity offered by the modification at the histidines ring system played a significant role in the effectiveness against fungal cells. At their MICs, these peptides did not exhibit any significant cytotoxicity and hemolysis.

Microscopic studies revealed that peptides are acting by disrupting cell membranes. This study supports the importance of suitably modified amino acids in peptide sequence towards the synthesis of amphipathic antifungal ultrashort peptides with high selectivity to fungal cells.

P007 Vera Neves

Instituto de Medicina Molecular, Lisbon

Blood-Brain Barrier Peptide Shuttles to Improve Therapeutics Action in the Central Nervous System

Marco Cavaco, Javier Valle, Patrícia Fraga, Silva R.D.M., Correia J.D.G., David Andreu, Miguel A. R. B. Castanho, Vera Neves

The delivery of therapeutic molecules to the central nervous system remains difficult to translate into improved clinical outcomes. This is largely due to the blood–brain barrier, BBB, the most tightly regulated interface in the human body, which can exclude most therapeutics. Therefore, for brain delivery of large drugs, for example, peptides, protein and nanoparticles, there is need to modify the drug to facilitate BBB crossing.

These can be achieved using physiological approaches, such as receptor-mediated transport, RMT, and adsorptive-mediated transport, AMT. To that end, cell-penetrating peptides, CPPs, have been successfully applied for drug delivery, owing to their capacity to internalize cells without membrane damage. However, is not clear if CPPs are able to traverse biological barriers, in particular the BBB.

Therefore, we have recently made a thorough examination of the main physicochemical properties for all peptides that are reported to cross the BBB, named BBB peptide shuttles, BBBpS, and found that CPPs and BBBpS belong to different families of peptides. Consequently, CPP can be explored to delivery payloads to the cytoplasm and nucleus of different cells, while BBBpS can be used for brain delivery, via trancytosis of an endothelial cell. Here, we compare 10 CPPs with BBBpS potential with three CPPs, non-BBBpS, to validate the method.

Through our experimental results, we were able to identify four new BBBpS having high translocation properties both *in vitro* and *in vivo*, displaying similar or improved brain penetration, when comparing with PepH3, a well-characterized and successful BBBpS . On the other hand, non-BBBpS displayed poor BBB permeability, thus corroborating the methodology adopted.

YI-P008 Emily Ankrom

Lehigh University

Selective Display of Immuno-Engagers on Tumor Cells via the pH-low Insertion Peptide

Emily Ankrom

The tendency of cancer therapies to be highly toxic and lack targeting features emphasizes the need for tumor-directed

delivery strategies. The lack of specific tumor biomarkers is a major hurdle for developing targeted therapeutics. However, one feature characteristic of nearly all solid tumors is their extracellular acidity. This inherent acidity provides the basis for drug delivery via pH-low insertion peptides, pHLIP, a family of peptides that insert in cell membranes as a transmembrane helix under acidic conditions and that are well-evidenced to selectively accumulate in tumors *in vivo*. The unidirectional membrane-insertion property of pHLIP allows cargoes linked to its N- terminus to be displayed on the extracellular face of the membrane.

Our strategy is to use pHLIP to decorate tumor cells with immune cell engagers and facilitate killing by engineered effector cells. We have previously established that we can use pHLIP to display antigens on tumor cells, recruit antibodies, and induce killing by engineered NK cells.

We have explored a novel strategy to use a covalent reactive handle on pHLIP to facilitate antibody recruitment to the cancer cell surface and subsequent killing by NK cells.

Lastly, we are also exploring the use of antigen-pHLIP conjugates to induce the killing of tumor cells by antigen-specific CAR-T cells.

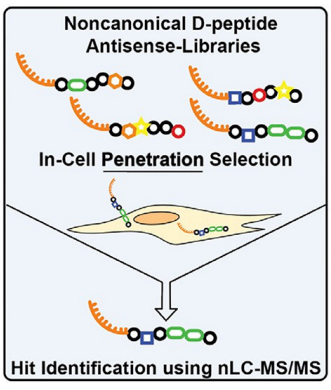
P009 Carly Schissel
L32-YI3

University of California, Berkeley

In-Cell Penetration Selection — Mass Spectrometry Produces Noncanonical Peptides for Antisense Delivery

Carly K. Schissel, Charlotte E. Farquhar, Andrei Loas, Annika B. Malmberg, Bradley L. Pentelute

Peptide-mediated delivery of macromolecules in cells has significant potential therapeutic benefits, but no therapy employing cell-penetrating peptides, CPPs, has reached the market after 30 years of investigation due to challenges in the discovery of new, more efficient sequences.



Here we demonstrate a method for in-cell penetration selection-mass spectrometry, in-cell PS-MS, to discover peptides from a synthetic library capable of delivering macromolecule cargo to the cytosol. This method was inspired by recent *in vivo* selection approaches for cell-surface screening, with an added spatial dimension resulting from subcellular fractionation.

A representative peptide discovered in the cytosolic extract, Cyto1a, is nearly 100-fold more active toward antisense phosphorodiamidate morpholino oligomer, PMO, delivery compared to a sequence identified from a whole cell extract, which includes endosomes. Cyto1a is composed of D-residues and two non-α-amino acids, is more stable than its all-L isoform, and is less toxic than known CPPs with comparable activity. Pulse-chase and microscopy experiments revealed that while the PMO-Cyto1a conjugate is likely taken up by endosomes, it can escape to localize to the nucleus without nonspecifically releasing other endosomal components. In-cell PS-MS introduces a means to empirically discover unnatural synthetic peptides for subcellular delivery of therapeutically relevant cargo.

P010 Austin Schlirf

University of California, Berkeley

Evaluation of On-Resin Head-Tail Cyclization of Peptides Using Automated Peptide Synthesizers

Austin Schlirf

Peptide-mediated delivery of macromolecules in cells Cyclic peptides are becoming increasingly relevant as a drug modality due to their propensity for cytoplasmic localization, proteolytic stability, and target specificity.

The ability to rapidly synthesize cyclic peptides for study has been aided by solid phase supports when utilizing disulfide bonds or side chain to side chain lactam bridges. However, head to tail cycles are difficult to synthesize on solid supports due to the inability to access the C-terminus while bound to resin. Some techniques have been described that perform cyclization and concomitant cleavage of peptides, however for improved recovery of the cyclic products these reactions typically run overnight.

Here we evaluate conditions to automate head-to-tail cyclization reactions while leaving the peptide on-resin to improve recovery and decrease synthesis time.

YI-P011 Hayate Shida

Department of Medicinal Chemistry, Tokyo University of Pharmacy and Life Sciences

Development of Methionine-Mediated Sulfenylation of Tryptophan-Containing Peptide

H. Shida, Y. Tokita, A. Taguchi, R. Kishi, S. Konno, A. Taniguchi, and Y. Hayashi

Chemical modification of peptides and proteins is an important fundamental technology for the preparation of new hybrid macromolecules which are useful as drugs and chemical tools with fluorescent and radioactive tags. Particularly, specific and selective chemical modification of the Trp residue would be a valuable method, because Trp is a unique amino acid that is present in most proteins, despite its very low abundance in eukaryote proteins, about 1%. To this end,

modifications of the indole ring of Trp using organic radical reaction and sulfenylation with *S*-protected cysteine sulfoxides have already been reported.

We recently reported that Npys-OPh(*p*F) is a stable surrogate with a similar reactivity to Npys-Cl as a disulfide-formation reagent. In the present study, we found the sulfenylation of the indole ring of Trp using Npys-OPh(*p*F) and we successfully obtained the desired sulfenylated Trp derivative in the presence of thioethers under acidic conditions at room temperature with high HPLC yield, 95%. This result gave us the idea that the Trp sulfenylation could proceed in Met-containing peptides without the addition of thioethers, because Met has a thioether structure.

To demonstrate this concept, a model peptide containing both Trp and Met was treated with Npys- OPh(*p*F) under acidic conditions. As a result, the desired sulfenylated- Trp peptide was efficiently obtained in 91% HPLC yield, while the reaction with no Met residue did not proceed. These findings could be useful in the selective chemical modification of peptides and proteins

P012 Kenji Usui

Faculty of Frontiers of Innovative Research in Science and Technology, FIRST, Konan University, Japan

Rapid Sensitization Assessment Using Peptidyl PEGA Resins as Alternatives to Animal Testing

Kenji Usuia, Masae Fukudaa, Aki Nishimuraa, Fumihiro Kayamoria, Hideto Ariumib, Yoshio Hamada

The direct peptide reactivity assay, DPRA, assesses the binding reaction of chemical substances to Lys or Cys of the peptides as an effective alternative to animal testing for skin sensitization. However, DPRA cannot be utilized in assay for poorly water-soluble chemicals, and HPLC in the protocol is time-consuming and difficult to handle compared to other general analyses.

Recently, we had developed a novel sensitization assay, chromophore solid phase peptide reaction assay, C-SPRA, to solve these problems. In this study, we further improved the C-SPRA system and optimized its protocol for assessing numerous types of samples in a short period of time, 10-30 minutes, using PEGA resins.

At first, we selected the resin for immobilization of the peptides for rapid assessment of numerous types of samples. PEGA, (Poly[acryloyl-bis(aminopropyl)polyethylene glycol]) resin was chosen as much more suitable for the rapid reaction of chemicals and peptides. Then we optimized the assessing protocol. Especially, the preparation protocol of the peptidyl resin before the assessment was simplified. In the preparation protocol we could skip the swelling step every time before the assay using PEGA resin.

Throughout these studies we successfully modified the conventional C-SPRA. Our method allows a next-generation high-throughput measurement for in vitro skin sensitization assessment with easy handling in 10-30 minutes.

YI-P013 Shuhei Yoshida

Graduate School of Frontiers of Innovative Research in Science and Technology, FIRST, Konan University, Japan

Development of Peptidyl PEGA Resins for Lead Ion Recovery

Shuhei Yoshidaa, Taichi Isozakia, Takaaki Tsuruokab, Kenji Usui

Pollution and poisoning by heavy metals are serious problems, especially in developing countries. Therefore, achievement of methods for selectively separation of trace amounts of target metals from mixture solutions would be necessary for environmental cleanup, medical treatment and recycle. Ion-binding using peptides would be one of the most powerful approaches to remove heavy metal ions. Peptides have various advantages such as high affinity for metal ions and immobilization onto solid phase.

From these points of view, we attempted to selectively separate heavy metal ion from various mixture solution using peptide-immobilized resin for applications such as cleanup of heavy metal pollution.

In this study, we optimized resins and peptide sequences. We focused on Pb^{2+}, a heavy metal ion, which cause serious health problems in developing countries even today. At first, we designed three kinds of Pb^{2+} binding peptides. These designed peptides were synthesized and immobilized on two different resins without a cleavage linker by SPPS. Using the peptidyl resins after the deprotection, the separation of Pb^{2+} was conducted. The removal amounts of Pb^{2+} were measured by UV-Vis measurement using EDTA. As a result, we successfully removed Pb^{2+} from 500 nM of $Pb(NO_3)_2$ solution.

Furthermore, removal of Pb^{2+} from the solution with serum was demonstrated. This approach with more improvement would contribute to the treatment of heavy metal poisoning or environmental cleanup of heavy metal pollution by changing peptide sequences.

P014 David Wade

Wade Research Foundation

Name Peptides

David Wade

The International Union of Pure and Applied Chemistry-International Union of Biochemistry and Molecular Biology, Joint Commission on Biochemical Nomenclature chose 22

letters of the 26-letter basic Latin alphabet , that is, all letters except B, J, X, and Z, as unambiguous 1-letter symbols for the names of amino acids, AAs.

This 1-letter nomenclature simplifies descriptions of the AA compositions and sequences of peptides and proteins, and is the method used to store the AA sequences of more than 500 million proteins in computer databases. It also enables the conversion of the letter sequences of names into sequences of AAs, that is, name peptides.

For example, the name of the largest retail company in the USA is Walmart, and the letters of the name could represent a sequence of 7 AAs:

Tryptophan (W)-Alanine (A)-Leucine (L)-Methionine
(M)-Alanine (A)-Arginine (R)-Threonine (T)

Once a name peptide has been designed, it can be subjected to theoretical analyses and/or synthesis and laboratory testing. A BLAST search revealed that peptide WALMART occurs in 833 proteins of the more than 500 million proteins of the N.C.B.I. nonredundant protein database.

Docking studies indicated that it can bind to AAs of the SARS-CoV-2 virus spike protein that are involved in interaction with the human ACE-2 receptor protein, and might prevent infection by the virus. *In vitro* studies with synthetic peptide WALMART also showed that it had anticancer and potential antimicrobial properties. Names represent a reservoir of potentially useful novel peptides.

YI-P015 Alec Brennan

University of Nevada, Reno

Elucidating the Role of the Competence Regulon Quorum Sensing Circuitry in Streptococcus cristatus

Brennan, A. B., and Tal-Gan Y.

In an era of continually emerging antibiotic resistant bacteria, it is essential to develop additional methods to effectively treat infections while circumventing the selection for antibiotic resistance. Investigation of bacterial communication has proven effective in unveiling mechanisms by which bacteria actively promote their survival, as well as regulate virulence and pathogenesis pathways. Gram-positive bacteria known as streptococci are of particular interest, as this group has shown a substantial rise in the prominence and spread of antibiotic resistance.

Many streptococci are considered opportunistic pathogens and possess natural competence for genetic transformation. This allows for the acquisition of antimicrobial resistance genes via the competence stimulating peptide, CSP-mediated QS pathway known as the *comABCDE* regulon. This study seeks to explore the *comABCDE* regulon and associated QS phenotypes of a reclassified species, *Streptococcus cristatus*. It is postulated that natural competence in this species allows it to act as a reservoir for antibiotic resistance genes, aiding in the development of antibiotic resistance in other species. *S. cristatus* has also been shown to have the ability to inhibit neighboring pathogenic species, like *S. mutans*, which suggests that *S. cristatus* has the potential to serve as a biotherapeutic.

In this work, we seek to develop CSP-based analogs derived from the native CSP to directly modulate QS and gain a comprehensive understanding of QS in *S. cristatus*. The developed analogs will be used to attenuate bacterial infectivity and virulence, explore beneficial proliferative phenotypes, and work towards the development of peptide-based therapeutics that circumvent the selection for antibiotic resistance.

P016 James Checco

University of Nebraska at Lincoln

Small- to medium-sized peptides can act as signaling molecules to carry out complex tasks in living systems. For example, neuropeptides and peptide hormones are cell-to-cell signaling molecules that facilitate communication between cells within the central nervous and endocrine systems, and dysregulation of these signaling events often leads to disease. Characterizing the active forms and molecular-level interactions of endogenous peptides represents a significant goal to understand how living systems carry out complex tasks, for example responding to stress, regulating bodily functions, learning, and may identify novel therapeutic targets. Our research combines approaches from chemical biology, bioanalytical chemistry, and synthetic chemistry to advance our understanding of specific cell-cell signaling pathways, identify new pathways for further exploration, and provide innovative starting points for future therapeutics. Two areas of research pursued in our group are described below.

Research Area 1:
Endogenous peptides are derived from larger precursor proteins that nearly always undergo extensive post-translational modifications. A given precursor protein often produces multiple peptides, and peptide abundance and processing can differ between tissues and physiological states. Because protein translation, peptide processing, and peptide degradation occur independently of mRNA transcription, mRNA levels do not necessarily correlate with protein levels. Several projects in our lab use mass spectrometry-based "peptidomics" approaches to characterize changes in endogenous peptides during disease or changes in physiology. Understanding these changes may lead to identification of novel disease biomarkers, or may identify biochemical pathways as new therapeutic targets.

Research Area 2:
Understanding cellular communication requires not only knowledge of the transmitter, that is, the peptide ligand, but also information regarding the cognate receptor that mediates signaling on the partner cell. However, there exist a relatively large number of biologically active peptides whose cell-surface receptor/s are not known, primarily because of a lack of techniques to reliably identify peptide receptors de novo. In addition to the peptidomics research

described above, our group is also working to develop new techniques to identify these receptors are needed to better understand the molecular mechanisms of cellular communication.

YI-P017 Thomas Jackson

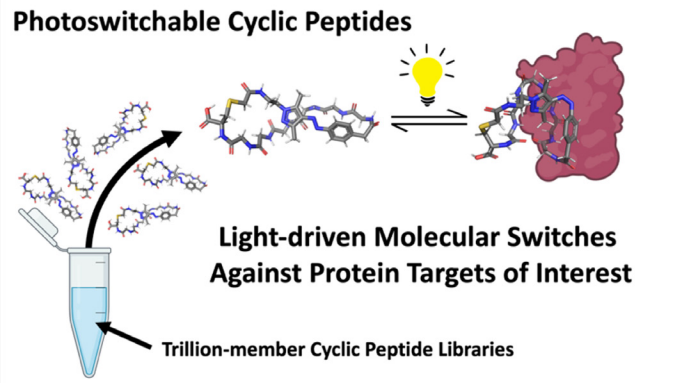
Imperial College London - The Francis Crick Institute

One Trillion Photoswitchable Cyclic Peptides: Shining Light on Questions in Chemical Biology

Thomas B. Jackson, Prof. Matthew J. Fuchter, Prof. Edward W. Tate and Dr Louise J. Walport

Cyclic peptides are an emerging class of exciting chemical tools, bridging the gap between small molecule therapeutics and larger biologics. They have recently gained traction probing classically undruggable protein targets of interest. Many powerful methodologies to identify novel cyclic peptides have been developed, including mRNA display-based RaPID, *Random Non-standard Peptide Integrated Discovery*. Such platforms enable the screening of >1 trillion-member cyclic peptide libraries incorporating non-proteogenic amino acids, against any protein of interest.

Despite the development of cyclic peptides as novel tools to answer questions in Chemical Biology, the fine-tuned druggability of complex biological pathways remains elusive. Light is an incredibly powerful non-invasive stimulus to introduce temporal control within a system. Photoswitches are small molecules that can undergo isomerisation via the stimulus of a specific wavelength of light. This project focuses upon the integration of light controllable 'photoswitches' within every macrocycle of the trillion-member library, via genetic code re-programming, inducing global conformational changes within the macrocyclic peptides structure to give switchable binding.



As a proof-of-concept, methodology for the identification of photoswitchable macrocyclic peptides has been developed against human Peptidyl Arginine Deiminase II, hPADI2. Identified peptides show up to 16-fold differential binding and inhibition of hPADI2 between each of the two light-dependent, E/Z, macrocyclic isomers. Further work to improve the binding differential is underway, honing the methodology to allow the robust identification of light-dependent photoswitchable macrocyclic peptides against any protein target of interest within complex biological systems.

P018 Matthew R. Pratt

University of Southern California, Los Angeles

Build It to Understand It: O-GlcNAc Modification Of the Small Heat Shock Protein Increases its Chaperone Activity in Multiple Contexts

Matthew R. Pratt

O-GlcNAc is a dynamic form of intracellular glycosylation found throughout the nucleus, cytosol, and mitochondria. This posttranslational modification is essential for development in mammals and changes in O-GlcNAc levels are associated with a variety of human diseases, including cancer, diabetes, and neurodegeneration. Proteomics experiments have identified thousands of O-GlcNAc modified proteins, but the consequences of the vast majority of these modification events, if any, are totally unknown.

To address this lack of understanding, my lab uses protein ligation to synthesize site- specifically O-GlcNAc modified proteins for subsequent biological experiments. HSP27 is a critical chaperone that functions by "holding" misfolded proteins and preventing their aggregation.

We published data showing that O-GlcNAc of HSP27 increases its chaperone activity against amyloid aggregation, potentially explaining one protective role for O-GlcNAc against neurodegenerative diseases. But, this is not the only function of HSP27. For example, it can inhibit apoptosis through interactions with proteins in the cell death cascade, including caspases. Additionally, it can participate in protein refolding by trafficking substrates to HSP70.

Here, I will describe how we used our synthetic proteins to discovery that O-GlcNAc also increases HSP27 activity in these contexts, with important implications in human biology and disease

YI-P019 Afraah Javed

University of Southern California, Los Angeles

Investigating the Effects of O-GlcNAc on sHSP Activity and Interactions

Afraah Javed, Aaron T. Balana, Andreas Langen, Matt R. Pratt

Small heat shock proteins, HSPs, are ubiquitous ATP-independent chaperone proteins involved in maintaining protein homeostasis in humans. Expression of these chaperones is upregulated under conditions of cellular stress, such as disease pathogenesis. Small HSPs are known to be post-translationally modified with O-GlcNAc in their C-terminal domains, which increases their ability to inhibit protein misfolding and aggregation.

Here, I will present how we prepared O-GlcNAc modified HSP27 to characterize its activity and interactions using a variety of techniques including *in vitro* biochemistry and proteomics. Using protein semi-synthesis, we constructed

site-specifically O-GlcNAc modified HSP27 to probe its differential protein-protein interactions and activity. O-GlcNAc modified HSP27 shows increased interaction with BAG3, a scaffolding protein that effectively bridges both the non-enzymatic and enzymatic heat shock protein systems to promote substrate refolding.

P020 Krista Wilson

Wingate University

Design, Synthesis, and Analysis of a Peptide Specific for the "WU1896" Receptor: An Undergraduate Biological Chemistry Laboratory Experiment to Teach Rational Drug Design and Solid Phase Peptide Synthesis

Krista Wilson

Peptide research performed at Primarily Undergraduate Institutions, PUIs, can be challenging. Nevertheless, there are a large number of Chemistry and Biology majors who are interested in the Health Sciences. Junior and senior students are deciding what they want to do next in their education, therefore exposure to the opportunities available in the world of Peptides is important for the recruitment of these future graduate students. To target these students, a Lab Experiment to be used in a Biological Chemistry course was designed as an introduction to the field of Medicinal Chemistry, specifically Rational Drug Design.

To help students connect with the lab, a fictional receptor system known as "WU1896"" was imagined that is activated by a peptide target. Students are presented with "SAR data" for this peptide and asked to use their knowledge of amino acid structure, intermolecular forces, and the secondary structure of proteins obtained during the lecture portion of the class to design a peptide consistent with the SAR data.

Microwave-assisted SPPS is then used to synthesize their peptides. Analytical characterization of the peptide is carried out by the student using HPLC and mass spectroscopy. The students then write a journal-style lab report which includes a discussion, in part, of how their peptide fulfills the structural requirements for a "WU1896" receptor ligand as compared with the SAR data, as well as "next steps" that would be taken in real life to test the biological efficacy of their peptide.

This lab introduces peptide science by using an interactive model to increase student engagement. Students leave the class with SPPS on their resumes as well as an excellent introduction to the fields of Medicinal Chemistry and Peptide Science.

YI-P021 Hendrik Lepper

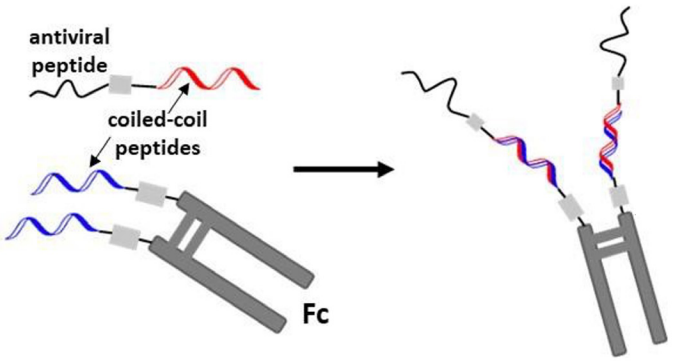
National Institue of Health

Site-Selective Fusion of Synthetic Antiviral Peptides to the Fc protein Using Chemo-Selective Ligation

H. Lepper, L. Weißenborn, J. Beutel, J. Eichler

The effectiveness of neutralizing antibodies relies on their high affinity to their pathogen targets, as well as on the Fc-mediated activation of effector cells through antibody-dependent cellular cytotoxicity, ADCC. Therefore, the fusion of SARS-CoV-2 neutralizing peptides developed in our laboratory to the Fc protein, is a promising strategy to endow the antiviral peptides with a cytotoxic component. Unlike in peptibodies, in which the peptide is grafted onto human Fc recombinantly, our approach is based on a chemo-selective ligation strategy using covalently stabilized coiled- coil peptides as adapter moieties.

This method enables the site-selective fusion of chemically synthesized peptides and other molecules to proteins in a chemo-selective fashion, extending the chemical diversity and flexibility compared to methods relying solely on recombinant protein synthesis. Moreover, the selected ligation method does not require the use of cysteine or enzymes to ensure chemo-selectivity.



P022 Ross Cheloha

National Institue of Health

Connecting Peptides and Antibodies to Probe Cell Surface Receptors

Ross Cheloha

Peptides and antibodies represent two leading modalities for targeting protein receptors expressed on the surface of mammalian cells. Endogenous peptides often serve as leads for developing tools and therapeutic candidates for probing receptor function, but they often suffer from insufficient selectivity, especially when targeting members of large receptor families such as G protein-coupled receptors, GPCRs. Alternatively, antibodies are prized in biomedical research for their high specificity but typically lack any biological effect - inhibition or activation - when targeting receptors with orthosteric sites embedded within transmembrane domains.

To leverage and expand the favorable properties of peptides and antibodies we have developed methods to create conjugates comprised of peptidic GPCR ligands and antibody

fragments, nanobodies. Synthesis is performed through a combination of solid-phase peptide synthesis, site-specific labeling of recombinantly expressed nanobodies, and click chemistry. These conjugates show properties superior to those of antibodies or peptides alone, including receptor specificity and in vivo biological activity.

We have further elaborated on this approach by developing chemistry that facilitates the covalent linkage between a nanobody and its binding partner through templated amide bond formation. This approach showed that covalent linkage of an agonist to its receptor resulted in enduring signaling. These tools should prove useful for mechanistic investigations of peptide-binding receptors and provide a new class of molecules with useful properties for biomedical investigation.

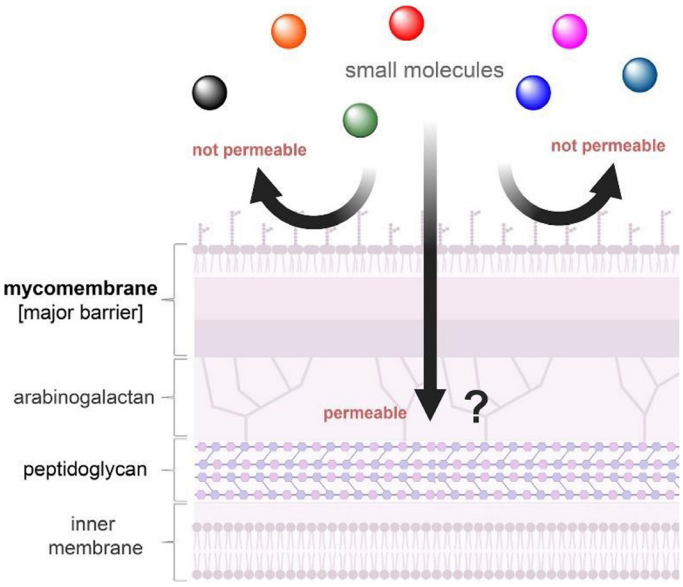
YI-P023 Zichen Liu

University of Virginia

Measurement of Permeation of Small Molecules into Mycobacteria with Stem Peptide Probes

Zichen Liu, M. Sloan Siegrist, Marcos M. Pires

Mycobacteria process an outer membrane like gram-negative bacteria but have a more complex cell wall envelope, consisting of a highly hydrophobic mycomembrane on the outside, and an arabinogalactan layer covalently attached to its peptidoglycan, PG. The presence of mycomembrane provides a drug penetration barrier for the antibiotics to reach their target, which has been long hypothesized to be one of the major reasons for the failure of antibiotics in mycobacteria. However, there is limited information about the structure scaffolds that can readily penetrate the mycomembrane.



In this work, we used a tetra peptide-based probe that mimics the sequence of stem peptides which can be metabolically incorporated on the PG of *Mycobacterium smegmatis*, *M. smegmatis*. An assay was developed to quantify the permeability of different small molecules to reach the PG. Tetra peptide probes with dibenzocyclooctyne, DBCO on the N-term was used in this work to graft bio-orthogonal click chemistry handle on the PG of *M. smegmatis*. The bacterial cells were then incubated with small molecules with azide handle to click with DBCO on the PG. The rest of DBCO sites on the PG were determined by incubation with fluorescein azide, which can give cellular fluorescent signal reversely related to small molecule permeability. Bacterial cells tested with small molecules with high permeability to the PG were expected to show low fluorescent intensity and vice versa.

We project that this assay would provide a high-throughput method for screening structure scaffolds that can have high permeability through the cell envelope for future therapeutic discovery against *Mycobacteria tuberculosis*.

P024 Keykavous Parang

Chapman University School of Pharmacy

Cyclic Peptide-Doxorubicin Conjugates to Overcome Multi-Drug Resistance and Reduce Toxicity

Keaykavous Parang

The use of doxorubicin, Dox, an anthracycline chemotherapeutic agent, is associated with cardiotoxicity and inherent acquired resistance. Thus, there is an immediate need for delivery systems to minimize toxicity and deliver Dox to sensitive and resistant cells. We have previously shown several cyclic peptides containing alternate tryptophan, W, and arginine, R, residues act as efficient molecular transporters. The peptides were conjugated with Dox via a glutarate linker to afford cyclic peptide-Dox conjugates.

While the LC_{50} values of free and conjugated Dox were comparable to those in wild-type MDA-MB-231 cells, 0.45 vs. 0.56 μM, respectively, peptide-conjugated Dox was significantly more effective in both Dox-resistant MDA231R cells, LC_{50} of 2.3 vs. 14 μM, respectively, and MES-SA/MX2 cells, LC_{50} of 4.3 vs. 20 μM, respectively.

Free Dox, 5 μM, reduced the viability of the kidney, LLCP-K1, ATCC CRL-1392, and rat myocardium, H9C2, ATCC CRL 1446, cells by 85% and 44%, respectively. $[R_5K]W_7A$-Dox and $[R_5K]W_7C$-S-S-Dox showed minimal toxicity to LLCP-K1, 5-7%, and H9C2, <9%, cells at similar or higher concentrations.

Fluorescence micrographs were consistent with cytotoxicity studies, indicating minimal uptake of $[R_5K]W_7A$-Dox in heart cells. The total concentration of Dox, conjugated and released, in the nucleus after 4 h exposure to $[W_9R_8K$-β-A]-Dox was higher than free Dox.

The mechanistic data indicated endocytosis independence and suggested direct trans-membrane localization. Our data

show that appropriate peptide-Dox conjugates can effectively internalize into resistant cells that pump out the free drug and do not cause similar toxicity in normal non-cancerous cells.

YI-P025 Maryssa Beasley

US Naval Research Laboratory

Biocatalysis in Extreme Environments: Chimeric Peptide Designs that Enhance Assembly and Catalysis Through Amyloid Structure

Maryssa A. Beasley, Cynthia Pyles, Adam D. Dunkelberger, Kenan P. Fears, Jeffrey C. Owrutsky, and Christopher R. So

Enzymes rely on their intricate backbone structure for survival in non-physiological conditions, often losing activity due to denaturation. We have demonstrated that catalytic amyloids assembled from a short amphiphilic septapeptide [Ile-His-Ile-His-Ile-Glu-Ile] can maintain both structure and activity in extreme synthetic conditions such as at high temperatures, high pressures, and in non-aqueous solvents. However, while amyloid structures confer stability, designing selectivity has been challenging. For example, residues far from the active site have been shown to have significant impact on metal center selectivity.

To elucidate the effect of active site structuring on catalytic activity, we have incorporated sequences into the peptides that template specific amyloid backbone structures and, as a result, subtly shift the structure of the catalytic active site. By combining advanced non-linear infrared spectroscopy techniques (2D IR) with molecular dynamics simulations, we characterize how each structural domain impacts the amyloid backbone organization and the resulting effect on the active site structure, which we then correlate to changes in catalytic activity. Through this, we have developed a modified metal center design that is uniquely structured to allow the catalysis of a wider range of substrates with high reaction rates. Additionally, we find that enhanced activity does not come at the cost of structural stability, as our new structures survive in temperatures exceeding 200 °C and in organic solvents.

By understanding how structure impacts activity, we have improved upon previous catalytic amyloid-forming peptide designs and have opened the door to using metal centers in amyloids for the breakdown of broader chemistries.

P026 Marlon Cardoso

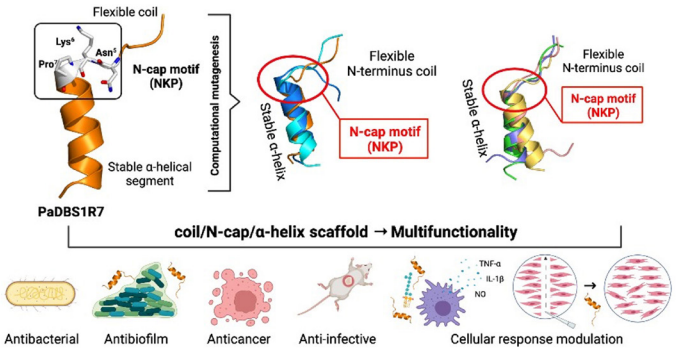
S-Inova Biotech, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco

An N-Capping Asparagine-Lysine-Proline, NKP, Motif Contributes to a Hybrid Flexible/Stable Multifunctional Peptide Scaffold

M.H. Cardoso, L.Y. Chan, E.S. Cândido, M.T. Torres, S.B. Rezende, K.G.N. Oshiro, I.C. Silva, S. Gonçalves, D.F. Buccini, T.K. Lu, N.C. Santos, C. de la Fuente, D.J. Craik, and O.L. Franco

Structural diversity is intrinsically related to biological activities in peptide drug candidates. Therefore, numerous peptide scaffolds have been reported to date and, in some cases, correlated with a given biological activity. Here, we describe an unusual N- capping asparagine-lysine-proline, NKP, motif that confers a hybrid flexible/stable multifunctional scaffold to a computationally designed peptide, PaDBS1R7. PaDBS1R7 has a shorter α-helix segment than two other computationally designed peptides of similar sequence but with key residue substitutions. Although this motif acts as an α-helix breaker in PaDBS1R7, the Asn5 presents exclusive N-capping effects, forming a belt to establish hydrogen bonds for an amphipathic α-helix stabilization from Pro7-Ile17. The solution nuclear magnetic resonance structures, amide proton temperature coefficient spectra and computational peptide mutants reinforced the role of the NKP motif for a coil/N-cap/α-helix scaffold.

Biological studies revealed that all PaDBS1 peptides presented antibacterial activities without damaging bacterial surfaces at their minimal inhibitory concentrations. However, only PaDBS1R7 displayed anticancer properties, eradicated *Pseudomonas aeruginosa* biofilms, decreased bacterial counts by 100–1000-fold *in vivo*, reduced lipopolysaccharide-induced macrophages stress, and stimulated fibroblast migration for wound healing, also modulating the expression of pro-inflammatory cytokines.



This study extends our understanding of an N-capping NKP motif to engineering hybrid multifunctional peptide drug candidates with potent anti-infective and immunomodulatory properties.

P027 Łukasz Berlicki

Wroclaw University of Science and Technology

Exploring Miniproteins — Structures and Functions

Łukasz Berlicki

Miniproteins can be defined as oligopeptides or proteins with molecular weight not exceeding 10 kDa and with stable tertiary structures. Although some natural as well as *de novo* designed miniproteins are known, it is evident that the world

of miniproteins is far from being fully explored. Furthermore, several examples of functional miniproteins were published, including molecules with antiviral, anticancer, and analgesic properties. However, the size and potential structural diversity of miniproteins provides possibilities for numerous new functions.

Here, new strategies for exploring the structures and functions of miniproteins will be presented. In particular, we have constructed several new miniproteins that incorporate constrained β-amino acid residues using computer-aided design. Furthermore, miniprotein scaffolds were applied to create biologically active structures and enzyme-like catalysts. First, miniprotein-based inhibitors of challenging protein-protein interaction, namely PD1-PDL1, were obtained and exhibited nanomolar activity. Second, a highly active artificial retro-aldolase was developed in a series of rounds of optimization that included grafting of the active motif in the cleft of the miniprotein, engineering the active site structure, and modifying the charge distribution on the surface of the miniprotein.

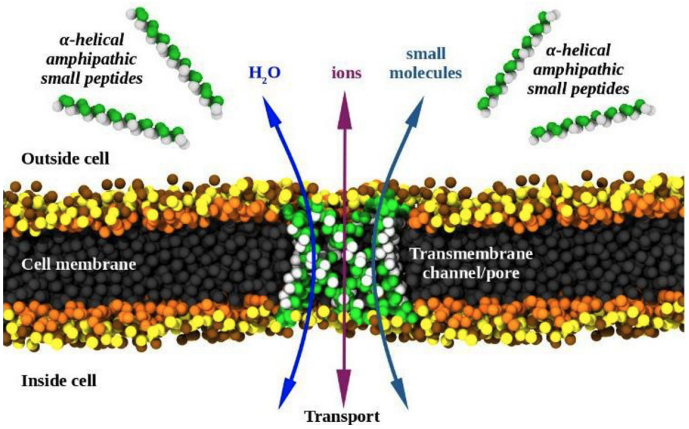
P028 Rahul Deb

Central European Institute of Technology, Masaryk University, Brno, Czech Republic

Design Guidelines for α-Helical Peptides that Self-Assemble into Transmembrane Barrel Pores Killing Antibiotic Resistant Bacteria

Rahul Deb, Ivo Kabelka, Jan Přibyl and Robert Vácha

De novo design of peptides that self-assemble into transmembrane barrel pores is challenging due to the complexity of several competing interactions involving peptides, lipids, water, and ions. Optimization of transmembrane barrel pores for specific functions is even more challenging because the role of individual residues remains mostly unknown. We have developed a computational approach using molecular dynamics simulations for the *de novo* design of α-helical peptides that self-assemble into stable transmembrane barrel pore structures with a central functional channel, i.e., capable of conducting water, ions, and small molecules across the lipid membranes.



We have formulated the missing design guidelines and reported 52 sequence patterns for the pore-forming peptides that can be tuned for specific applications using the identified role of each residue. Atomic force microscopy and fluorescent dye leakage experiments confirmed that the designed peptides form leaky membrane pores *in vitro*.

We have demonstrated fine-tuning of these pore-forming peptides into potent antimicrobial compounds able to kill even antibiotic-resistant ESKAPE bacteria at micromolar concentrations, while exhibiting low toxicity to human cells.

Designed peptides and their assembled structures can be similarly tuned for other medical and biotechnological applications, including anticancer agents, and for single molecule sensing and sequencing.

YI-P029 Diana Gimenez-Ibanez

University of Leeds

Design and Development of TACC3/AurA PPI Peptide Inhibitors

Diana Gimenez, Jennifer Miles, Claire Smith, James Holder, Colin A. Johnson, Fanni Gergely, Richard Bayliss, Megan Wright, and Andrew J. Wilson

Protein-protein interactions, PPIs, play a crucial role in regulating and controlling a broad variety of cellular processes and thus, constitute an important class of targets in drug discovery.

Aurora-A is a Ser/Thr protein kinase that regulates key mitotic events such as centrosome maturation, mitotic entry, and spindle assembly. Aurora-A exerts its activity by recruiting and phosphorylating a range of different binding partners. Among them, Aurora-A interaction and activation of TACC3, Transforming Acidic Coiled-Coil Containing Protein 3, is instrumental for spindle assembly and chromosome segregation. TACC3 has been found to be frequently overexpressed in many different cancer types, with its fusion products also exhibiting oncogenic activity.

This poster will describe how we used a combination of chemical, biophysical and *"in-silico"* tools and analyses, such as X-ray analysis, rational unnatural amino-acid incorporation, stereo-electronic effects, peptide stapling and MD simulations to design and develop a series of constrained TACC3 peptides variants that show up to 10-fold increased binding potencies, K_d, to Aur-A than the parent peptide and enhanced proteolytic stability.

Overall, this work reports on the first stages towards the development of a new class of therapeutically relevant TACC3/Aur-A PPI inhibitors with potential as future chemical probes.

Acknowledgements
This work is financed by the Biotechnology and Biological Sciences Research Council, BBSRC, BB/V003577/1

P030 **Jason Heier**
University of Minnesota

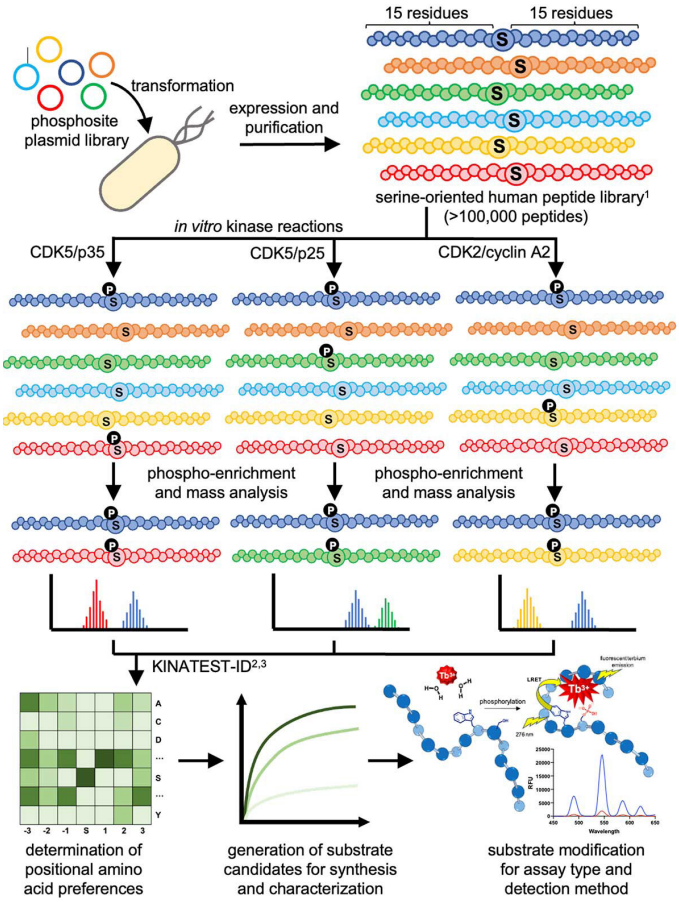
Peptide Substrates Designed to Specifically Assay Cyclic-Dependent Kinase 5, CDK5, for Alzheimer's Disease Drug Discovery

Jason Heier

Growing evidence shows cyclin-dependent kinase 5, CDK5, as a key mediator of Alzheimer's Disease, AD, and an attractive therapeutic target. CDK5 is a serine/threonine kinase which plays a crucial role in brain development and synaptic activity. However, when dysregulated, CDK5 contributes to all three histopathological hallmarks of AD: β-amyloid plaque deposition, formation of neurofibrillary tangles, and neuron death.

Unlike other CDKs, CDK5 activity is not dependent on a cyclin but is regulated by membrane-anchored protein p35. In response to neurotoxic signals, proteolytic calpain cleaves p35 into untethered, fully mobile p25. Whether aberrant CDK5 activity is the result of its mislocalized and prolonged activation or involves an altered substrate specificity when complexed by p25 rather than p35 has yet to be determined.

Although CDK5 is an obvious target, there are currently no specific inhibitors available and no tools to measure its activity in cell-based assays. The development of specific peptide substrates as reporters in a physiologically relevant environment is critical for screening and testing inhibitors targeting aberrant CDK5 activity.



In recent efforts to develop tools to better study kinases, the Parker Lab has discovered novel peptide substrates of CDK5 that when phosphorylated chelate terbium to produce easily detectable time-resolved luminescence. Not only do these peptides allow for affordable antibody-free CDK5 assays, but also show no cross-reactivity with close homolog CDK2. In additional research, we report the substrate specificity of CDK5/p25 and CDK5/p35 as determined from *in vitro* reactions with a genetically encoded peptide library based on more than 100,000 human serine phosphorylation sites. The combined results of these studies will be applied in further assay/inhibitor studies targeting pathological CDK5/p25 activity.

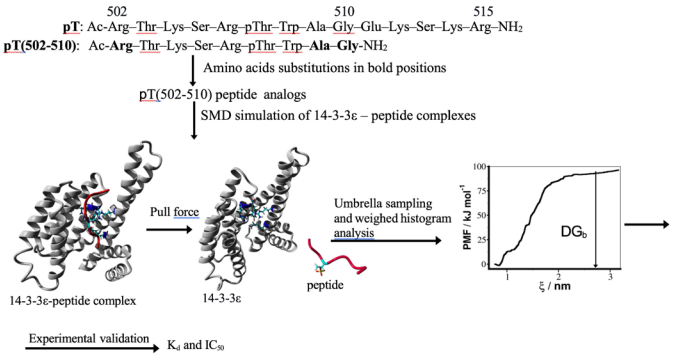
YI-P031 **Seraphine Kamayirese**
Creighto University

Optimizing Peptide Structures that target 14-3-3ε in Cutaneous Squamous Cell Carcinoma

Seraphine Kamayirese, Sibaprasad Maity, Laura A. Hansen and Sándor Lovas

Cytoplasmic mislocalization and overexpression of CDC25A in cutaneous squamous cell carcinoma (cSCC) is associated with resistance to apoptosis. This anti-apoptotic activity of CDC25A is reliant on its interaction with 14-3-3ε protein. Previously, this group developed a novel peptide; Ac- CDC25A(502-515)-NH_2 (pT), that binds 14-3-3ε, and induces cell death of cSCC cells.

Here, we modified pT to improve its binding affinity for 14-3-3ε, thus, promoting apoptosis in cSCC. The pT was truncated to pT(502-510). On the basis of computational Ala-scanning, Arg, Ala and Gly residues of pT(502-510) were substituted, bold face fonts in figure and free energies of binding (ΔG_b) of the peptide analogs to 14-3-3ε were determined in a multi-step molecular dynamics simulations. Using synthetic peptides, binding of the peptides to recombinant 14-3-3ε were confirmed by differential scanning fluorimetry, and surface plasmon resonance.



Proteolytic stability of pT(502-510) was determined by incubating it with pronase. Digestion products were identified by LC-MS. Shortening the pT peptide improved its binding to 14-3-3ε. Aromatic substitutions at the C-terminus of pT(502-510) significantly improved binding affinity (Kd) to

14-3-3ε. Proteolytic digestion showed that the peptide was cleaved at Lys504-Ser505 and Ser505-Arg506 bonds. Unnatural amino acids will be introduced at scissile bonds to improve proteolytic stability of the analogs. The ability of the analogs, with improved Kd and proteolytic stability, to induce apoptosis will be studied in cSCC cells.

Acknowledgment:
The research was supported by R01 CA253573-01 grant

YI-P032 Juan Lizandra Perez

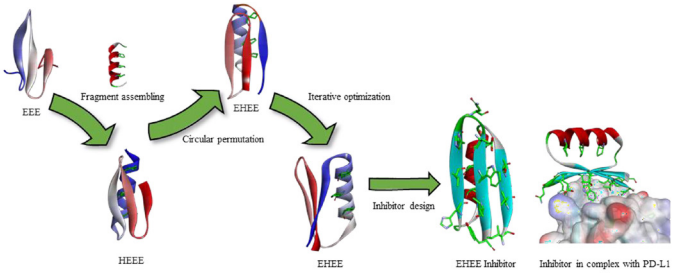
Wroclaw University of Science and Technology

β-Amino Acid Containing Miniproteins as Scaffolds for PD-1/PD-L1 Interaction Inhibitors

Juan Lizandra Pérez, Łukasz Berlicki

Miniproteins with partially artificial backbone, are excellent platforms for the study of protein folding due to their relatively small size and synthetic availability. In addition, the modified backbones and side chains, allows for the design of novel folds and functionalities. Such characteristics confers these systems with the potential to be scaffolds for the construction of bioactive compounds towards challenging targets with large interacting surfaces.

In the present work, we sought to design a miniprotein with a partially artificial backbone predicted to fold into a stable EHEE, E-extended, H-helix, topology. The resulting scaffold is expected to endure mutations, to attain high affinity towards PD-L1, without loss of its three-dimensional arrangement.



We report the successful design of a novel miniprotein with the desired topology and its application as scaffold for the construction of PD-1/PD-L1 immune checkpoint inhibitors. Initially, the WW-prototype, a triple stranded antiparallel beta-sheet, was fragment assembled to a (1*S*,2*S*)-aminocyclopentanecarboxylic acid containing (*trans*-ACPC) helix. The assembling was followed by circular permutation and an iterative optimization of the sequence. This way we have been able to explore in depth a series of topologies, from EEE, through HEEE, to a final EHEE. The optimized miniprotein showed Tm values of 72°C, and a free energy of folding (ΔG) of -4.2 kcal/mol. Modifications, of the outer beta-sheet residues, led to a set of inhibitors with nanomolar affinities towards PD-L1 as studied by BioLayer Interferometry (BLI) and nanomolar inhibition of PD-1/PD-L1 interaction as studied by Homogenous Time Resolved Fluorescence, HTRF.

P033 Taeju Park

Children's Mercy Kansas City

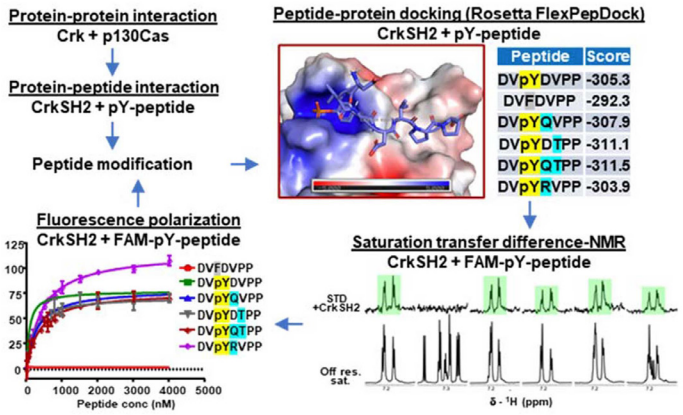
Structure-Function Relationship Study to Design High-Affinity Peptides for Targeting the Crk/CrkI-p130Cas Axis

Taeju Park

The expression levels of CT10 regulator of kinase, Crk, and Crk-like, CrkL, are elevated in many human cancers, including glioblastoma, and Crk and CrkL have been proposed as therapeutic targets. However, the lack of Crk and CrkL inhibitors makes it challenging to study how elevated Crk and CrkL protein levels enhance tumorigenesis. Our preliminary data suggest that overexpression of Crk and CrkL leads to tyrosine phosphorylation of p130Cas and the Crk/CrkL-p130Cas complex formation.

We hypothesize that the Crk/CrkL-p130Cas axis is crucial for the oncogenic effects induced by Crk/CrkL overexpression.

For the rational design of inhibitors targeting the Crk/CrkL-p130Cas axis, we designed a phospho-p130Cas-mimicking peptide, PCP. The PCP inhibited the binding between phospho-p130Cas protein and purified CrkSH2 protein. We created 3-dimensional models of CrkSH2 and CrkLSH2 for molecular modeling, carried out PCP-SH2 molecular docking using FlexPepDock, and calculated Rosetta energy scores to predict binding strength.



To validate the molecular modeling, we modified the core amino acids of the PCP between the conserved phosphotyrosine and proline and calculated binding scores. Then, the biochemical activities of the modified peptides were determined by *in vitro* peptide-protein binding assays using saturation transfer difference nuclear magnetic resonance, STD-NMR, and fluorescence polarization, FP.

The results from the molecular modeling, STD-NMR, and FP were compared to refine the modeling protocol and the interpretation of the peptide-protein docking models. We are modifying N- and C-terminal amino acids to improve the binding affinity. Our results demonstrate that the structure-function relationship study using the designer peptides

serves as a platform for structural understanding of the Crk/CrkL-p130Cas axis and designing inhibitors.

YI-P034 Liam Marshall

Syracuse University

Catalytic Diversity Within Minimal Amyloid Libraries

L. R. Marshall, O. Zozulia, Z. Lengyel, M. Jayachandran, I. Kim, E. Kohn, and I. V. Korendovych

Amyloids have traditionally been regarded as the rock bottom thermodynamic minimum of protein folding, a disease by-product with little utility, but their stability and ease of synthesis makes them an ideal starting point for catalyst design. Given that extremely minimalist sequences can form amyloid species, these small peptides can display remarkable efficiency, with some sequences displaying pNPA hydrolysis activity on a weight basis greater than carbonic anhydrase. They can also be combined to form mixtures with positive synergy.

In this work, we describe their use as both hydrolysis and oxidation catalysts, and how multicomponent systems can be formed from mixtures of peptides. We constructed an initial set of charge-charge design rules for positively synergistic mixtures and demonstrated positively synergistic residues can be installed in more complex β-sheet assemblies like hairpins and macrocycles. Small changes within the sequence alter the cofactor binding site, allowing access to heme-functionalised redox chemistry. Libraries were synthesised and screened for their catalytic behaviour in different model oxidation reactions. In the best example, catalytic efficiencies only an order of magnitude lower than the best designed species are observed ($k_{cat}/K_M = 3 \times 10^5\ M^{-1}\ s^{-1}$).

These findings demonstrate the diversity of catalytic assemblies from a minimal library of peptide species, simplifying synthesis while increasing the active site space explored.

YI-P035 Hyunjun Yang

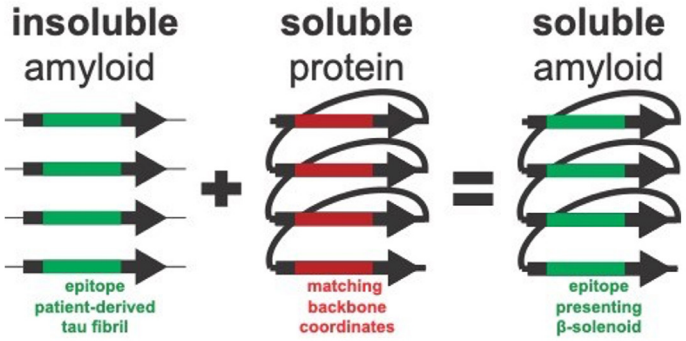
University of California San Francisco

Patching an Amyloid Surface onto a Soluble Protein

Hyunjun Yang, Dibyendu Mondal, Andrej Šali, and Bill DeGrado

Amyloids are hallmarks of neurodegenerative diseases. With cryo-EM advancement, high-resolution amyloid structures are on the rise, and its polymorphic nature is echoed at the molecular level. Interestingly, these patient-derived structures reveal clinicopathology correlation with the observed conformational strain. Herein, I report the *de novo* peptide design method to patch the surface of biologically relevant amyloid on a soluble protein motif.

In this approach, tertiary ensemble representatives of amyloid fragments are analyzed to derive a physical score function to quantify amyloido-structural property in all RCSB protein database. The quantified fragments are analyzed and further designed with Rosetta & deep learning-based sequence design to yield a β-solenoid structural motif presenting an epitope of amyloid surface observed in neurodegenerative disease patient samples.



This approach yields a uniform and soluble amyloid surface and enables downstream drug library screening and conformational strain specific antibody production.

P036 Dominik Sarma

Gyros Protein Technologies

High-throughput purification with orthogonal PurePep® EasyClean accelerates reliable peptide drug discovery

S. Lüdtke, R. Zitterbart, and D. Sarma

Speed and reliability are critical factors in peptide drug discovery to identify suitable lead targets. Typically, crude peptides are used because purification by conventional chromatography is prohibitively time-consuming and requires the extensive use of organic solvents. However, the use of crude peptides carries a significant risk of false-positive or false-negative results.

PurePep® EasyClean, PEC, technology provides a high-throughput, plate-format purification method that enables rapid and reliable production of 96 PEC-grade peptides in as little as one day using only one liter of organic solvent.

PEC technology is based on a catch-and-release, C&R, approach using a universal linker system with a reduction-triggered safety release. PEC is ideal for parallel purification because it is processed in fritted cartridges without high pressure. The use of the 96-well plate format allows rapid production of purified PEC-grade peptide libraries. The orthogonal nature of the purification process removes co-eluting impurities, reducing the risk of false-positive or false-negative results. For example, PEC-grade libraries are as reliable as HPLC-grade peptides in protein binding and immunological assays.

In this poster presentation, we will present the PEC protocol adapted to 96-well filter plate formats and highlight use cases of PEC-grade peptide libraries in academic and clinical settings.

YI-P037 Md Shadman Ridwan Abid

University of Nebraska

Peptidomics Analysis Reveals Changes in Small Urinary Peptides in Patients with Interstitial Cystitis/Bladder Pain Syndrome

Md Shadman Ridwan Abid, Haowen Qiu, Bridget A. Tripp, Aline de Lima Leite, Heidi E. Roth, Jiri Adamec, Robert Powers, and James W. Checco

Interstitial cystitis/bladder pain syndrome, IC/BPS, is a condition that causes persistent, debilitating discomfort in the bladder and urinary tract. Since many IC/BPS symptoms are also present in other urological conditions, a clear diagnosis of IC/BPS can be difficult to make. This study looks at the profile of endogenous peptides in IC/BPS patients' urine to find potential peptide biomarkers.

Here, we present a non-targeted peptidomics analysis of urine samples collected from IC/BPS patients and asymptomatic controls. In addition, we also assessed the relative abundance of a previously proposed biomarker "Antiproliferative factor, APF, peptide" from patient urine samples through our developed multiple reaction monitoring method.

Our non-targeted peptidomics analysis showed significant alteration of 71 peptides generated from 39 different proteins. All of the differential peptides were found in a higher abundance in IC/BPS relative to control samples. Prior studies of IC/BPS also showed increased protease abundance in urine/bladder cells which is consistent with the abundance of many small peptides found in our study.

In parallel, we developed an LC-MRM method to quantify APF by using synthetic APF on commercially available urine sample. The APF peptide was shown to be present in urine samples from both IC/BPS patients and healthy controls, suggested limited utility of this peptide as a sole biomarker of this disorder. Overall, our findings revealed variations in the profiles of small urine peptides from IC/BPS patients compared to age-matched controls.

These results may serve as a foundation for the development of novel potential biomarkers of this condition in the future.

YI-P038 Adriana Gauna

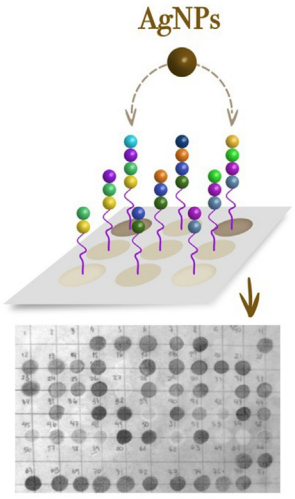
Advanced Center for Chronic Diseases, ACCDIS, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile

SPOTs Synthesis for the Screening of Silver Nanoparticles-Binding Peptides

A. Gauna , F. Sanchez, and M. Kogan

Peptide–nanoparticle conjugates have recently emerged as a versatile tool for biomedical applications. Synergism between the two promising classes of materials allows enhanced control over their biological behaviors, overcoming intrinsic limitations of the individual materials. For this reason, it is important to generate techniques that allow to determine the binding of nanoparticles to specific peptide sequences, that also could help to understand peptide-nanoparticle interactions.

Peptide synthesis on cellulose membranes using the SPOTS technology developed by Frank is a simple and flexible technique for the analysis of peptide libraries. In this opportunity, this technique was used to carry out a screening of silver-binding sequences on a cellulose paper, which was later exposed to a suspension of silver nanospheres, which binding was recorded as the appearance of an amber-gray coloration on the SPOTs.



In this way, a screening of silver-binding sequences Ag4, Ag5, AgP35, AGgBP1 and AgBP2 was carried out, including abbreviated peptides and the substitution of some amino acids. It was observed that the substitution of apolar aliphatic amino acids for basic amino acids such as arginine and histidine leads to a greater binding of silver nanoparticles, AgNPs. Additionally, it was observed that smaller sequences may have an uptake of AgNPs that is comparable to native peptides.

This methodology proved to be simple, cheap and versatile for the determination of binding sequences to AgNPs, and can be used to search for peptides capable of binding to other metal nanoparticles in order to optimize their functionalization, stabilization or immobilization.

YI-P039 Nathalie Grob

Massachusetts Institute of Technology

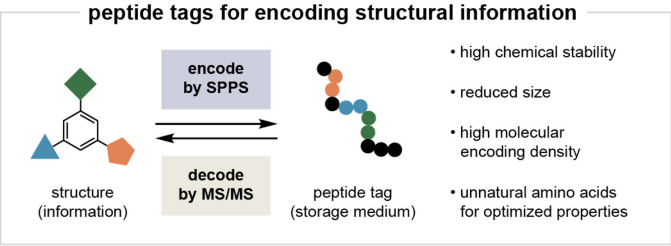
Peptide-Encoded Libraries of Small Molecules for Drug Discovery

Nathalie M. Grob, Simon L. Rössler, Stephen L. Buchwald, and Bradley L. Pentelute

The discovery of small molecule binders of proteins with therapeutic value can be facilitated by affinity selections using synthetic combinatorial libraries. However, traditional technologies used for hit identification, such as DNA-encoded libraries, can hinder binding to the target by steric hindrance of the encoding tag. Additionally, oligonucleotide tags, which constitute the encoding entity in DNA- encoded libraries, have limited stability in a broad range of reaction conditions thus restricting the chemical diversity of combinatorial libraries.

To address these limitations, we established an alternative encoding strategy relying on hit identification by tandem mass spectrometry and *de novo* sequencing of abiotic peptides. Our encoding strategy enabled the implementation of optimized chemical conditions for the preparation of high-diversity combinatorial libraries while reducing the size of the encoding entity and its potential for steric interference. We overcame technical challenges in the design and development of peptide tags for high-fidelity retrieval of synthetic information with as little as 10 fmol of material.

To demonstrate the potential of peptide-encoded libraries in drug discovery, we engaged in affinity selections with several protein targets relevant to oncology, which led to the discovery of novel small molecules with binding affinities in the nM to µM range.



We expect our peptide-based technology to enable the discovery of small molecule candidates for emerging targets of therapeutic interest.

YI-P040 Fabian Hink

University of Copenhagen

Peptides with Novel Helix-Inducing N-Cap Motif Disrupt the Mcl-1–BH3 Interaction

Fabian Hink, Hiroaki Suga, and Joseph M. Rogers

In over 60 % of the protein complexes in the protein database, α-helices are involved in protein- protein interactions (PPIs). Many of these PPIs are associated with disease, making them attractive drug targets. One example is myeloid cell leukemia-1 (Mcl-1) that binds an extended α-helix of BH3-only proteins preventing apoptosis. Mcl-1 is overexpressed in several types of cancer supporting the survival of cancer cells. Since peptides can form extensive molecular interfaces and are able to adopt secondary structures such as an α-helical conformation they represent a promising approach to inhibit PPIs. Specific macrocyclization strategies such as stapling or capping constraining the conformation can further improve affinity, stability, and cell permeability.

Here, we employed the RaPID system, a combination of flexizyme technology and mRNA display, to screen trillions of unique macrocyclic peptides for their ability to bind Mcl-1. We discovered Mcl-1-binding peptides able to outcompete BH3 peptides. These peptides contained a specific macrocycle connecting the acetylated N-terminus with the sidechain of cysteine in the 4th position via thioether formation. This motif is an N-cap stabilizing the peptide in a helical confirmation and improved binding affinity. The compatibility with solid-phase peptide synthesis and mRNA display makes it an attractive moiety for future peptide drug discovery efforts.

P041 Deborah Shalev

Azrieli College of Engineering Jerusalem

Exploring the Role of Copper Salts in the Self-Assembly of Short Peptides for Antibacterial Coating

Deborah E. Shalev

Bacterial infections can cause significant burden in many biological systems. Antimicrobial coatings were created by simply mixing a short peptide, containing a DOPA residue, with copper salt in water, which promoted self-assembly of the peptide and attachment to surfaces, resulting in an antibacterial coating.

Paramagnetic relaxation enhancement-nuclear magnetic resonance, PRE-NMR, was used to explore the binding mechanism of copper (II) to the peptide. Initial binding to the peptide was probably through the meta hydroxyl of the DOPA, which strongly reduced the intensities of adjacent hydrogen signals due to PRE. The gradual reduction of signal intensity together with changes in chemical shift as a function of titration with copper (II) were most evident in the DOPA beta hydrogens and on the aromatic ring, which corroborates this finding. Signals from the adjacent residues showed much less reduction in signal and change of chemical shift, indicating less involvement in copper-binding.

The PRE data and the structure of the peptide determined in equilibrium with copper (II) were combined to give a model for the copper (II)-bound complex and compared to the unbound peptide structure.

The coating reduced bacterial load by seven orders of magnitude, indicating that it has the potential to be used in coating systems to afford effective protection against bacterial infections in biological systems.

YI-P042 Ashutosh Agrahari

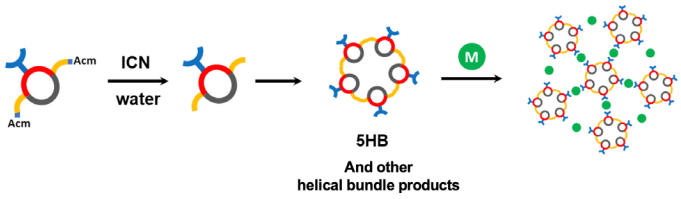
Purdue University

Metal-Promoted Higher-Order Assembly of Homocysteine-Stapled Helical Bundles

Ashutosh Agrahari, and Prof. Jean Chmielewski

Helical bundles have shown great promise as building blocks in the development of biomaterials. They contain four or more amphiphilic peptides having the secondary structure of alpha helix with a hydrophobic and hydrophilic face. These helices come in the form of bundles with inter-helix hydrophobic interactions. Here we present a disulfide-stapled, covalently stabilized helical bundle as a building block for mesh-like biomaterial.

The helices contain histidine in the center as a ligand to produce a 3D mesh-like assembly upon metal, Zn^{2+}, Cu^{2+}, and Fe^{2+} addition in a pH-dependent manner. The assembly has been found to be reversible upon the addition of a known strong metal chelator ethylenediaminetetraacetate, EDTA.



Along with the reversibility, we also show that such mesh can be decorated with his-tagged, His6, small fluorescent molecules and green fluorescent protein. Apart from the metal-triggered higher-order assembly, the helical bundle can accommodate linear aliphatic long-chain, C9 and C12, containing molecules in its hydrophobic cavity, making it a potential hydrophobic host. The hydrophobic cavity has also been validated with in silico modeling.

This work extends the application of helical bundles to make mesh-like assemblies that can be decorated with His6-tagged cargo and explores their ability to serve as potential hosts for aliphatic long-chain guest molecules.

YI-P043 Michele Costantino

Arizona State University

Functionalization of shell proteins with disordered regions promotes membrane formation around condensates

M. Costantino, E. Young, C. Kerfeld, and G. Ghirlanda

To carry out complex reactions, cells sequester enzymes and small molecules through the formation of compartments. These include organelles surrounded by lipid membranes, bacterial microcompartments, BMC, formed by shell proteins similar to viral capsids, and membraneless organelles made of dense droplets of intrinsically disordered proteins, IDP, through liquid-liquid phase separation, LLPS. The last two options are of interest to the ProteoCell project as their components are DNA encodable.

We used the RGG domain of the common IDP LAF-1 to observe LLPS. Like other IDPs, RGG droplets sequester small molecules and a wide array of proteins. The ability to restrict access has then become a recent interest. Research has shown that lipids will form a membrane around the droplets, providing both a barrier and stabilization of the dynamic system. To form a proteinaceous membrane, we expressed BMC-H shell protein 5815 with the RGG domain in varying lengths at the C-terminus.

It was observed that during LLPS, the shell proteins with RGG truncations will form a membrane at the droplet interface. Without the RGG domain, BMC proteins are sequestered entirely within the droplet, indicating that a disordered domain is necessary for shell formation in a phase separated system.

Additionally, the amount of partitioning is related to the length of the truncation. Longer disordered sequences were sequestered more within the droplets suggesting an optimal length of the disordered region for membrane formation at the interface.

P044 Jillian Smith-Carpenter

Fairfield University

Characterizing the Chemistry of Self-Assembling Nucleopeptides

S. O'Neill, J. Manson, B. Pineiro, R. Black and J. Smith-Carpenter

The Smith-Carpenter lab has developed short, self-assembling peptides that have been modified on the N-terminus with guanosine. These nucleopeptides combine the self-assembling properties of short peptides and hydrogen bonding recognition along the Hoogsteen face of guanosine to form nanostructures with different higher-order guanosine architectures dependent on their sequence and C-terminus chemistry.

We characterized these higher ordered guanosine-based structures, such as G-quartets or G-ribbons, by using infrared spectroscopy and circular dichroism. Additionally, we used ^{1}H-NMR techniques to track the early non-covalent interactions that drive nucleopeptide aggregation. Previous studies have shown that G- quadruplex containing structures can interact with a hemin cofactor in the presence of hydrogen peroxide and catalyze the oxidation of 2,2- azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS.

Our lab has used this ABTS assay to characterize the catalytic potential of the nucleopeptide hypothesized to form G-quartets and compare it to other G-quadruplex containing higher order assemblies. We have characterized the kinetics of this ABTS oxidation by the nucleopeptides in the

presence and absence of the hemin cofactor. Our work underscores the importance of supramolecular surfaces of nucleopeptides to facilitate chemical reactions.

YI-P045 Chiranjit Dutta

George Mason University

Modulating Heme Coordination and Activity with Self-Assembling Peptide Amphiphiles

C. Dutta, V. Lopez, A. M. Rogers, C. Preston, and L. A. Solomon

Heme-binding proteins were designed by nature to perform catalytic activities such as electron transfer and oxygen delivery. Hemoglobin is a globular protein that utilizes a bound heme group to transport oxygen from the lungs to the tissues. Heme proteins necessitate well-defined biological systems to maintain stability and functionality. Modifying these natural proteins to function in non-native environments presents a challenge. However, peptide amphiphiles are a promising tool for designing higher-order self-assembling biopolymers with natural heme- functions.

In this study, we describe amphipathic peptides that self-assemble and non- covalently bind to cofactor heme-B. The peptide consists of a fiber-forming β-sheet region, a pH-triggered cationic polylysine moiety, and a catalytic heme-binding site. Additionally, a hydrophobic palmitoyl moiety was conjugated to enhance hydrophobic collapse. We used atomic force microscopy to capture the self-assembled structures of the peptides. We investigated the effect of heme reduction potential by testing many sequences with altered hydrophobicity in the β-sheet region of peptide amphiphiles. Furthermore, we employed pH- triggered morphological changes, micelles versus fibers, in peptide self-assembly to study how the different structures affect the heme's reduction potential and material properties.

Our results show that both structures of peptide materials are accessible to heme for catalysis, and that simple modifications can raise the reduction potential by over 180 mV. We also show variation in reduction midpoint potentials based on structures and morphologies. Finally, we demonstrate how material properties can vary as a function of peptide sequence. These findings have implications for designing next-generation blood substitutes using functional self-assembled peptide biomaterials.

YI-P046 Mikaela Gray

Georgia Tech

Engineering Protein Vesicles with Unique Properties via Non-Alpha Amino Acid Itncorporation

Mikaela A. Gray, Cameron V. Swenson, Elise D. Ficaretta, Joshua A. Walker, Alanna Schepartz, and Julie A. Champion

Nanoparticle delivery systems aim to improve drug delivery by using targeting and protecting from proteolytic degradation, however, face the challenge of endosomal escape. Protein vesicles are advantageous because they don't require solvent and are biodegradable. Protein vesicles are made up of ZR-ELP and globule-ZE recombinant fusion proteins, where ZR- ELP contains a hydrophobic temperature sensitive elastin like polypeptide, ELP, fused to an arginine-rich leucine zipper, ZR. The globular protein domain is fused with a glutamic acid-rich leucine zipper, ZE, which binds strongly to ZR to create a protein complex that transitions to vesicles. By engineering the ELP, we have pH-responsive vesicles stable in physiological conditions that disassemble as the pH acidifies.

To improve release of vesicle cargo and reduce the necessity for very hydrophobic amino acids, we are formulating translational and post-translationally modified ELPs to endow them with properties not possible with canonical amino acid. By incorporating hydroxy boc lysine and hydroxy acid tyrosine residues, we examine effects on vesicle formation and vesicle disassembly. Hydroxy acid tyrosine containing ELPs form vesicles that disassemble at pH 5 over 2 hours.

Then, to examine post-translational effects on ELP behavior we inserted an enzyme recognition sequence SGMCAYDGS where MicD-F and ArtGox install heterocyclic backbones, which alter protein backbone flexibility. Enzyme modification resulted in the best conversion for ELPs with the most insertion sites indicating the reaction is most effective with multiple accessible insertion sites. Overall, this work examines how translational and post translational modifications influence ELP behavior and vesicle disassembly.

YI-P047 Ingyu Han

Yonsei University

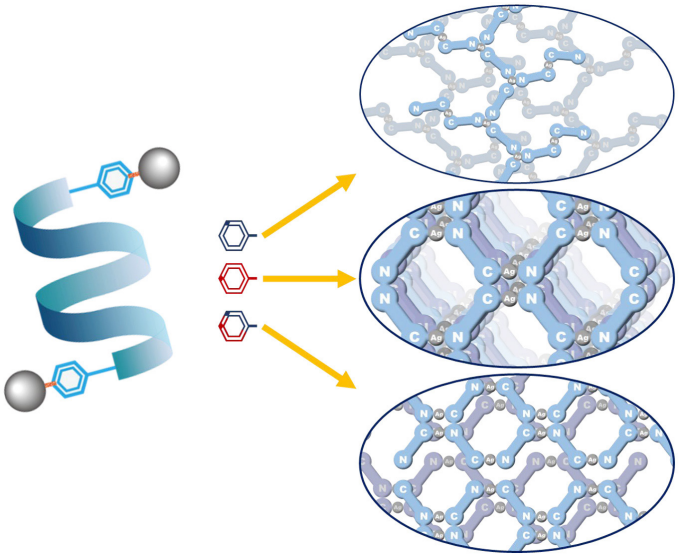
Utilization of 12/10-Helical β-Peptide Backbone as a Robust Pillar-Like Linker for the Metal-Peptide Networks

Ingyu Han, and Soo Hyuk Choi

With advantages in chirality and modularity, peptide-based organic linkers were broadly studied as novel building blocks for metal-coordinated networks. Among them, short peptides that adopt helical secondary structures received particular interest as potent, flexible organic linkers for building porous and/or channel-like materials. To study the functionality and applicability of Metal-Peptide Networks, MPNs, understanding and designing the three-dimensional structures of MPNs should be preceded. However, predicting the actual conformation is still challenging as little structural elucidation of MPNs has been made yet.

Herein, we demonstrate the crystal structures of the three unique silver(I)-coordinated MPNs with the same helical β-peptide backbone but with different terminal moieties. Although the three β-peptides adopt very similar, 12/10-helical conformations, the corresponding MPNs display different

three- dimensional structures arising from various angles between the silver ion and the terminal pyridine groups. Additionally, we characterized the crystal structure of a copper(II)-coordinated MPN with one of our β-peptide ligands used for silver(I) complexation.



These results suggest that the helical folding of short peptides can be retained even at the complexation with other transition metal ions if the ligand has sufficient conformational robustness. We believe these works can contribute to building more functional and utilizable metal-peptide complex materials.

P048 Sho Konno

Tokyo University of Pharmacy and Life Sciences

Substrate Peptide-Based Mixed Phosphonates as an Irreversible Inhibitor for Thioesterase Domain in Nonribosomal Peptide Synthetases

S. Konno, M. Tanaka, T. Mizuguchi, H. Toyokai, A. Taguchi, A. Taniguchi, and Y. Hayashi

Macrocyclic peptides biosynthesized by a nonribosomal peptide synthetase, NRPS, are medicinal resources that are important for development of new therapeutic agents. In NRPS assembly line, a thioesterase, TE, domain, a member of serine hydrolase superfamily, is responsible for the macrocyclization of linear peptide substrates. Because TEs can also cyclize synthetic peptide thioester, these enzymes can be utilized as biocatalysts for preparation of macrocyclic peptide natural product analogs.

Although the engineering of TEs to expand the substrate specificities is considerable interest, the substrate peptide-TE interactions in the macrocyclization step are less understood due to a lack of chemical tools to trap the intermediate state.

To facilitate the understanding of substrate-TE interactions, here we report peptide substrate-based inhibitors with a mixed phosphonate warhead, which can react irreversibly with Ser residue at the active site of TEs. We demonstrated that tyrocidine A linear peptide with p- nitrophenyl phosphonate can efficiently form stable covalent adducts with tyrocidine synthetase TycC-TE.

These results would be useful for the structural analysis of the substrate-bound complex using protein NMR and X-ray crystallography.

YI-P049 Olamilekan Ibukun

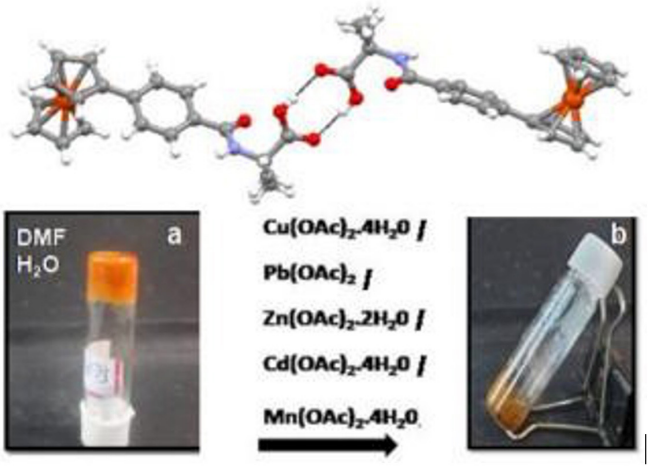
Indian Institute of Science Education and Research, Kolkata

Synthesis, Structure and Stimuli-responsive Metallogel of a Designer Ferrocene Appended Peptide Mimetic

Olamilekan Joseph Ibukun and Debasish Haldar

Engineering gelator molecule is highly important for the development of advance functional materials. Gels are semi solid materials that shows both solid- and liquid-like properties. These gels have been applied in petrochemical industry, tissue engineering, controlled drug release, and sensors.

This study reports a simple protocol for the synthesis of ferrocene substituted benzoic acid and an efficient synthetic approach towards ferrocene appended peptide mimetic with a high reaction rate, yields and purity.



From X-ray single crystal analysis, the ferrocene appended peptide mimetic adopts an extended conformation and self-aggregate to form dimer structure by intermolecular hydrogen bonds. The dimer formation is also supported by mass spectrometry. In higher order packing, the dimmeric subunits further self-assembled to form a complex sheet-like structure stabilized by multiple π-π stacking interactions between phenyl and cyclopentandienyl ring of ferrocene.

Moreover, the ferrocene appended peptide mimetic forms stimuli-responsive metallogel in DMF-water. The rheology experiments support the formation of strong gel. The metallogel burst on addition of other salt such as $Cu(OAc)_2.4H_2O$, $Pb(OAc)_2$, $Zn(OAc)_2.2H_2O$, $Cd(OAc)_2.4H_2O$, $Mn(OAc)_2.4H_2O$.

YI-P051 Pengfei Jin
University of Pennsylvania

Programming the Nanofibrous Self-Assembly of Collagen Mimetic Peptides

P. Jin and D.M. Chenoweth

As the most abundant protein in mammals, collagen provides stiffness and resilience to tissues and organs and plays a vital role in cell adhesion, cell migration, tissue scaffolding, and signaling. There is significant interest in developing peptide materials that mimic fibrillar collagen to emulate its role in the extracellular matrix, owing to the accessibility and modifiability of synthetic peptides. However, controlling the formation of collagen-mimicking filaments and hierarchical structures using synthetic peptide building blocks poses a challenge due to the limited specificity in the formation of collagen triple helices from individual peptides.

In this study, we developed a general strategy for designing peptides that form triple-helical collagen-mimicking filaments. Utilizing steric interaction in a "bump-gap" design, we achieved remarkable specificity by destabilizing undesired peptide binding registers, thus constructing interlocked peptide triple-helical filament structures. This exceptional specificity results in continuous single triple helices reaching micrometer scales, and a variety of higher-order structures emerge when the filaments aggregate, including peptide gel networks, filament bundles, and fibrils. The aggregation of the filaments is highly sensitive to peptide sequences and can be responsive to pH or temperature, which potentially allows fine-tuning of the higher-order structure and the material property of the filament aggregates.

These filament-forming peptides are highly programmable and functionalizable with the preservation of the filament-forming feature. Modifications like non-covalent protein binding, unnatural amino acid incorporation, and protein binding sequence incorporation have been investigated, and the peptides keep the filamentous secondary structure. The reported family of filament-forming collagen mimetic peptides presents the possibility of creating collagen surrogates and materials with novel functions, broadening the scope for applications of peptide materials in biological and biomedical studies.

P052 Katarzyna Slowinska
University of Pennsylvania

Non-covalent Peptide-Based Complexes for Delivery of Nucleic-Acid-Therapeutics

Katarzyna Slovinska

The effective delivery and release of nucleic acid therapeutics, NAT, is a key challenge in gene therapies. While there are several effective methods to deliver short NATs, siRNA, ASO, the transport of large vectors like plasmid DNA, pDNA, is very challenging.

Here we present the NAT carrier based on a hybrid peptide containing triple helical collagen, COL, and cell penetrating, CPP, domains. The COL domain facilitates the formation of high aspect ratio nanoparticle by folding into a triple helix. The positively charged CPP enables cellular uptake and complex formation with negatively charged NAT.

The peptide/siRNA complex has quaternary structure that forms 10 nm stoichiometric nanoparticles. The complex shows fast cellular uptake, <30 min, effective siRNA release, and gene silencing. Moreover, it also provides capsid-like protection for siRNA against nucleases without being immunostimulatory, or cytotoxic. The increase in NAT size to pDNA dramatically changes the complex formation and thus the delivery. The strategies for packing and delivery of pDNA as a peptide/pDNA complex will be discussed.

As proof of concept, we will also discuss the option of the charged CPP sequence replacement to charge-free CPP sequence: PFVYLI, known CPP, and modifications of COL domain to facilitate the non-covalent peptide/pDNA complex formation and delivery. The presented data suggests that our strategy for development of the NAT/peptide complexes can provide an avenue for effective delivery of NAT.

YI-P053 Caleb Anderson
NCI/NIH

High Scalability and Kinetic Control in Polyelectrolyte Complex Assembly of microRNA/peptide Nanoparticles

Caleb F. Anderson, Poulami Majumder, and Joel P. Schneider

Current treatment options for mesothelioma are not effective, as complete surgical tumor resection is nearly impossible due to the complicated sheet-like anatomy of these tumors. Therefore, we previously engineered a surface-fill hydrogel, SFH, that can be syringe- or spray-delivered to surface cancers during surgery or as a primary therapy to deliver loaded tumor-specific miRNA-peptide polyelectrolyte complex nanoparticles, NPs, that attenuate the oncogenic signature of cancer cells. Once applied, SFH can shape-change in response to alterations in tissue morphology and locally release NPs. However, the NPs exhibit broad size ranges and are metastable, where NPs will begin aggregating within ~2 h if not encapsulated within the gel phase, complicating their translation to large-scale, clinical settings.

Thus in this report, we utilize a scalable process called “flash nanocomplexation,” FNC, that facilitates shortening of the diffusion paths of the assembly components, yielding highly uniform and stable NPs. Using a confined impinging jet mixer, we achieve controlled turbulent micromixing of cationic peptide and miRNA, producing NPs with extremely narrow size distributions and enhanced stability.

We systematically vary FNC processing parameters - such as inlet flow rates, charge ratios, pH, and assembly component concentrations - and peptide composition to assess their influence on resulting NP size, polydispersity, surface charge, colloidal stability, and their ability to internalize into cells, escape endosomes, and silence target genes important in mesothelioma.

YI-P054 Khushbu Bhakta

Chapman University

Cyclic and Linear Peptides Containing Tryptophan and Arginine Residues as Cell-penetrating Peptides

Khushbu Bhakta

The cell membrane is composed mainly of phospholipids, which separate cells' interior from the extracellular space. The properties of the cell membrane create a significant obstacle in intracellular cargo delivery. Cell-penetrating peptides, CPPs, have been introduced as a promising drug delivery system that can penetrate the cell membranes. Some of the CPPs can improve the cellular uptake of various cell impermeable compounds. Amphiphilic cyclic peptides containing alternative arginine and tryptophan such as [WR]5 have been shown to enhance the transport of cargo molecules across the cell membrane.

Herein, we report the synthesis and biological evaluation of a novel series of peptides containing alternative positive and hydrophobic amino acids, namely [(RW)5K] WR, [(RW)5K](WR)2, [(RW)5K](WR)3, [(RW)5K](WR)4, and [(RW)5K](WR)5. The peptides were synthesized through Fmoc solid-phase peptide synthesis to compare their molecular transporter efficiency. The chemical structures of the final products were confirmed by high-resolution MALDI-TOF. Final compounds were purified by a reversed-phase HPLC. The cytotoxicity of linear and cyclic peptides was evaluated in SK-OV-3, human, epithelial ovarian adenocarcinoma, CCRF-CEM, human, lymphoblast peripheral blood, and HEK-293, human, epithelial embryonic kidney healthy, cells using the MTS assay. The peptides did not exhibit any significant cytotoxicity at the concentration of 10 μM in CCRF-CEM, HEK-293, and SK-OV-3 cells after 3 h incubation. The cellular uptake of a fluorescence-labeled phosphopeptide (F′-GpYEEI) and anti-HIV drugs (lamivudine (F′-3TC), emtricitabine (F′-FTC), where F' is carboxyfluorescein, were measured in the presence of the peptide in CCRF-CEM and SK-OV-3 cells.

Among all peptides, [(RW)5K] (WR)5 (10 μM) was the most efficient and improved the cellular uptake of F'-GpYEEI (2 μM) by 13-fold when compared with F'-GpYEEI alone. These data suggest the potential of hybrid cyclic-linear peptides as molecular transporters.

P055 Roman Fabry

Arizona State University

Peptide-Based Coacervates as Model Hydrogenases

Roman Fabry, Abesh Banerjee, and Giovanna Ghirlanda

Membraneless organelles regulate countless processes within cells in a dynamic manner, by temporarily compartmentalizing biomolecules such as RNA and proteins. These organelles are formed through spontaneous liquid-liquid phase separation driven by complex coacervation of charged biomolecules acting as polyelectrolytes. The relative simplicity of this compartmentalization strategy, compared to the encapsulation of cellular elements within membrane bilayers, has led to the hypothesis that complex coacervation could have led to protocells in a prebiotic world.

Here, we investigate whether model coacervates formed by polycationic polypeptides and by ATP could compartmentalize simple reaction cascades. We use as proof of principle the light driven catalysis of proton reduction by cobalt porphyrins. We show that metalated porphyrins spontaneously partition into coacervates formed by poly-His and poly-Lys, together with ruthenium based photosensitizers. UV-vis spectroscopy indicates axial coordination of the metal porphyrin by the amino acid side chains.

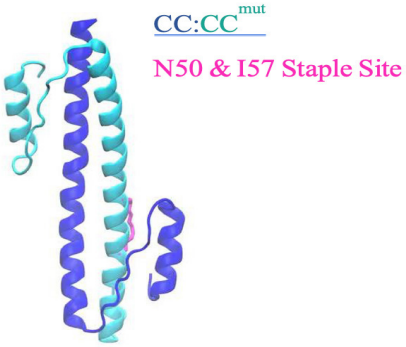
YI-P056 Braxten Hornsby

University of Utah

Stapled Coiled-Coil Inhibitors Against BCR-ABL for the Treatment of Chronic Myeloid Leukemia

Braxten Hornsby

Chronic myelogenous leukemia, CML, is a myeloproliferative neoplasm characterized by the presence of the Philadelphia Chromosome, a genetic abnormality which results in fusion of the Bcr-Abl oncogene. The expressed Bcr-Abl protein, a constitutively active tyrosine kinase, is the causative agent of CML and is oncogenically activated by homodimerization of the N-terminal Bcr coiled-coil, CC, domain. This mechanism remains untargeted by clinically approved agents, with the current standard of care comprising treatment with various generations of tyrosine kinase inhibitors which are subject to mutational evasion and adverse events.



Previously, the Lim Lab recombinantly developed a targeted Bcr-Abl mutant, herein referred to as CPP-CC^{mut}, capable of preferential heterodimerization and inhibition of Bcr-Abl *in vitro*. Translation of CPP-CC^{mut} is challenged by structural stability and proteolytic degradation however, which may be addressed through synthetic, α-helical crosslinking with hydrocarbon staples.

Herein, we explore synthetic strategies to produce CPP-CC^{mut} and stapled candidates, CPP-CC^{mut}-st, utilizing solid-phase peptide synthesis and ring-closing metathesis.

We demonstrate that CPP-CC^{mut} and substituted staple candidates, 82 aa, can be efficiently produced in a single, continuous reaction without the need for native chemical ligation. To our knowledge, this represents one of the largest sequences to have been successfully stapled. Successful synthesis of CPP-CCmut-st candidates allows for translational study of our constructs in animal models to evaluate the pharmacokinetic impact of peptide stapling technologies when applied to a small protein therapeutic.

YI-P057 Joseph Kelly

University of Virginia

Post-Translational Modification Impact on MHC Peptide Binding and TCR Engagement

Joseph Kelly, Nathaniel Bloodworth, Qianqian Shao, Jeffery Shabanowitz, Donald Hunt, Jens Meiler, Marcos M. Pires

The human major histocompatibility complex, MHC, plays a crucial role in the presentation of peptidic fragments from proteins in the cytosol; these peptides can be derived from self-proteins or from non-human antigens, such as those produced by viruses or bacteria. To prevent cytotoxicity against healthy cells, thymocytes expressing T-cell receptors, TCRs, that recognize self-peptides are removed from circulation in a process called negative selection. However, post-translational modifications, PTMs, are excluded from negative selection; this feature opens the door to the possibility that PTMs directly contribute to the development of autoreactive T-cells and subsequent autoimmune disease.

PTMs are carried out by a specific set of enzymes, and the resulting modified amino acids often alter protein activity, localization, and interaction of the protein with other cellular components. Because PTMs are covalent modifications, most are stable enough to persist through proteasomal processing; therefore, the resulting modified peptide fragments can potentially be presented in the context of MHC for immune surveillance.

Despite it being well-established that PTMs are prevalent in peptides presented on MHCs, the exact mechanisms by which PTMs influence the antigen presentation machinery remains poorly understood. In this study, we introduce chemical modifications mirroring PTMs onto peptides to systematically investigate their impact on MHC binding and TCR recognition. Our findings reveal the numerous ways PTMs alter antigen presentation, which could have implications for tumor neoantigen presentation.

P058 Jo Lohman

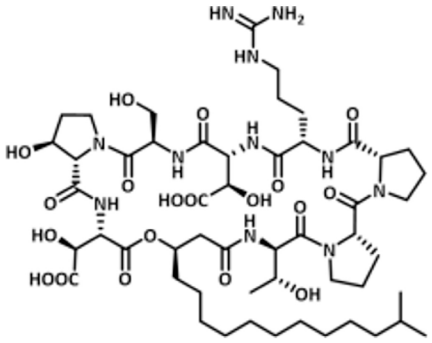
Indiana University

Progress Towards the Total Synthesis of Antibiotic Tripropeptin D and its Analogs

Jo Lohman, Michael S. VanNieuwenhze

Throughout the 1940s and well into the 1960s, antibiotic discovery and development was at its peak. However, since then, the number of antibiotics being pushed into the market has drastically decreased, so much so, that antibiotic resistance has caught up and has now been found for nearly every antibiotic on the market.

Bacteria have developed resistance in a number of different ways, so there is a demand for new antibiotics with novel mechanisms of action. The peptidoglycan biosynthetic pathway is a promising target for antibiotics as it is bio-orthogonal to mammalian cells, and easily accessible as it decorates the outside of the bacterial cell.



Tripropeptins, which belong to a family of antibiotics shown to have excellent antibacterial activity against Gram-positive bacteria, are hypothesized to target a lipid intermediate within the pathway thus inhibiting peptidoglycan biosynthesis. Specifically, tripropeptin D, see figure above, has been shown to have the best activity amongst all the tripropeptins.

Therefore, continued synthetic progress towards these compounds and biological studies of the tripropeptin antibiotics can provide a better understanding of their mechanisms of action and offer good candidates for the development of novel antibiotic drugs.

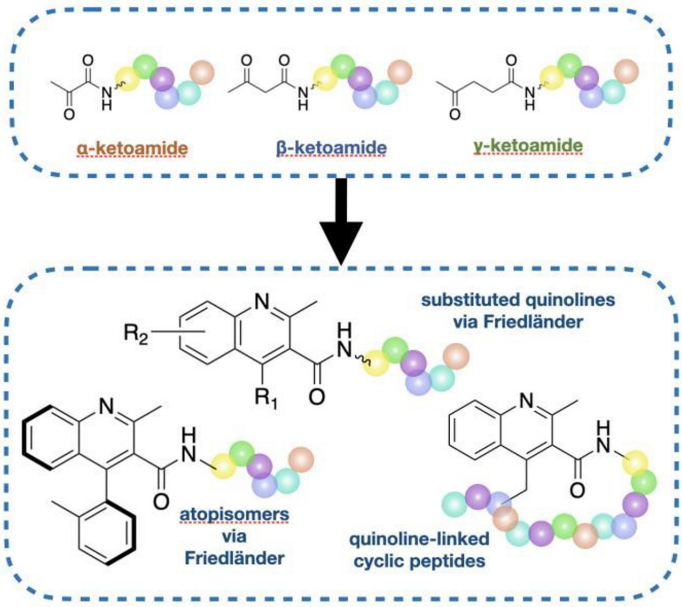
YI-P059 Isaac Knudson

University of California at Berkeley

Post-Translational Friedlander Reactions for Diversified, Atropisomeric, and Macrocyclic Peptides

I. J. Knudson, D. Dilworth, S. Miller and A. Schepartz

In nature, hybrid polyketide-peptides produced nonribosomally achieve vast chemical diversity and potent bioactivity. Our lab is interested in introducing the chemical elements of polyketides, namely simple methyl-ketone species, into genetically templated ribosomal synthesis. We have demonstrated that two different methyl ketone-containing monomers are able to acylate initiator tRNAs and initiate peptide synthesis. The products are novel keto-peptide hybrids bearing either a β-keto amide or a γ-keto amide at the N-terminus. In analogy to polyketide synthesis, these simple building blocks can be derivatized into complex heterocyclic species, such as quinolines.



The Friedlander reaction has been a preferred route of quinoline synthesis for over 130 years. We developed catalyst-free aqueous conditions that are performed in one-pot with the from β-keto amide peptide products of an *in vitro* translation reaction to access a variety of substituted quinoline-peptides. Unfortunately, the catalyst free-conditions proved unsatisfactory for more difficult substrates, such as atrop-axis inducing ortho-substituted amino-benzophenones or peptide cyclization via internally-incorporated kynurenine.

After screening a variety of Lewis and Bronstead acid catalysts, Yb(OTf)3 in ethanol was found to be an excellent catalyst of the Friedlander reaction to generate atropisomers and cyclic peptides. Further work aims to test the compatibility of this chemistry with mRNA display platforms for (cyclic)peptide screening and explore the functional outcomes of quinoline incorporation. It is hoped that the introduction of more complex, hydrophobic, and rigid topologies, such as atropisomeric quinoline cyclizations, should allow for more potent cyclic peptide lead compounds in drug discovery or improve cellular delivery.

P060 Luis Alberto Vallejo Castillo

Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional

The Peptides of Transferon Oral®, a Dialyzable Leukocyte Extract, are Absorbed into Cervical Nodes after Oral Administration in a Murine Model

Fragozo A, Trejo-Martínez I, Valencia-Martínez L, Domínguez-Bernal K, Pavón L, Pérez-Tapia SM, and Vallejo-Castillo L

Transferon Oral® is a complex mixture of peptides smaller than 10 kDa. It is used to treat infections, allergies, and autoimmune diseases. Transferon Oral® reduces the production of proinflammatory cytokines such as TNF-α and IL-6 and increases the IFN-α levels when administered by different routes, including oral. The complexity of oral Transferon Oral® limits the characterization of its Mechanism of Action, MOA, and pharmacokinetics, among other aspects.

This project aimed to determine the biodistribution of Transferon oral® peptides to shed light on its Pharmacokinetics and MOA. To achieve this goal, the peptides of Transferon oral® were covalently linked to a fluorophore, Alexa Fluor-488. RP-UPLC was employed to determine the extent of labeling and free fluorophore. In biodistribution assays, the labeled peptides must maintain their biological activity. In this sense, Transferon Oral® labeled and non-labeled peptides increase the survival in an HSV-1-infection murine model.

Labeled peptides were orally and subcutaneously administered in nude Foxn1nu mice, and the accumulation kinetic was determined using an in vivo fluorescence/luminescence IVIS equipment imaging system. Imaging analysis revealed that Transferon oral® peptides are absorbed in the gastrointestinal tract, biodistributed, and accumulate in the lymph nodes in both administration routes. In addition, it was observed that labeled peptides are eliminated by renal filtration within 3 hours after administration. In addition, our results suggest that the immune cells related to lymph nodes are relevant for characterizing the MOA of Transferon Oral®.

Acknowledgements:
This study was funded by Frontier Science 2023, Project number CF-2023-G-836. F A also thanks CONACYT for the postgraduate scholarship, 787378.

YI-P061 Deanne Hayward

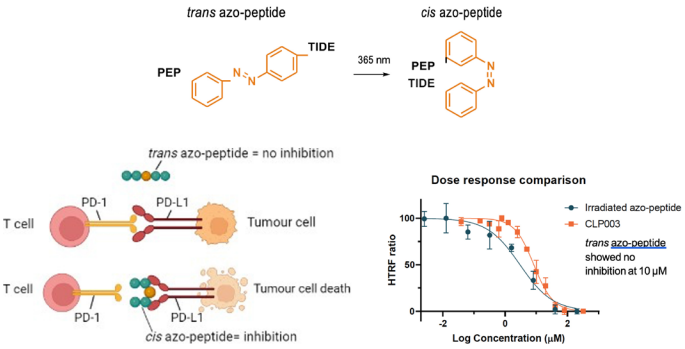
University of East Anglia

Using Light to Control the Immune System

Deanne Hayward, Mark Searcey, and Andrew Beekman

Immunotherapy provided a turning point in oncology, transforming treatment efficacy with a more targeted approach than conventional treatments. By blocking the interaction between the immune checkpoint molecule and its ligand, T

cells can be activated to eliminate tumour cells. Programmed cell death 1, PD-1, and programmed cell death ligand 1, PD-L1, are well described checkpoint proteins that have been successfully targeted with monoclonal antibodies. Recent research has identified cyclic and turn-motif peptides capable of inhibiting the PD-1/PD-L1 interaction, offering cheaper antibody alternatives with less adverse immune effects.



Incorporation of a photo-switchable compound into a peptide can provide a light controllable inhibitor of the PD-1/PD-L1 interaction. Azobenzenes have been extensively studied and are typically found in the *trans*-form to isomerize to the *cis*-form upon ultraviolent, UV, irradiation at 350 nm. In the *cis*-form, the azo-peptide has the ability to mimic the conformational turn of cyclic and turn- motif peptides to inhibit the interaction by binding to PD-L1.

The design and synthesis of light activatable azo-peptides that show the capability to inhibit the PD- 1/PD-L1 interaction will be reported. In addition, modifications to the azobenzene structure to tune wavelength sensitivity will be disclosed.

P062 Aswini Giri

Bicycle Therapeutics

Discovery and Optimization of Synthetic EphA2-Dependent CD137 Bicycle® Tumor-Targeted Immune Cell Agonists

Aswini Kumar Giri, Punit Upadhyaya, Julia Kristensson, Lia Luus, Anna Devlen, Gemma Mudd, Kristen Hurov, Johanna Lahdenranta, Kevin McDonnell, Phillip E. Brandish, Phil Jeffrey and Nicholas Keen

CD137 (4-1BB), a costimulatory receptor belonging to the tumor necrosis factor, TNF, receptor superfamily, has emerged as a promising target in immunotherapy based on robust preclinical proof of concept. After the first generation of monoclonal antibodies were limited by hepatotoxicity or lack of efficacy, a new generation of CD137 agonists are now in clinical development but they exclusively utilize large molecules derived from recombinant technology with long circulating half-lives. Intermittent target engagement which mimics the physiologic context of T-cell costimulation has not yet been explored by current modalities targeting CD137.

Bicycles are a class of small, MW ~2kDa, highly constrained peptides characterized by formation of two loops cyclized around a symmetric scaffold. EphA2/CD137 *Bicycle*® tumor-targeted immune cell agonists, *Bicycle*® TICAs, were synthesized by linking a *Bicycle*® binder to EphA2, a highly expressed tumor antigen expressed in several tumor types of high unmet medical need, to a *Bicycle*® binding CD137.

Systematic structure activity relationship, SAR, studies, biochemical binding assays as well as a suite of *in vitro* and *in vivo* assays, led to the discovery of novel EphA2-dependent CD137 agonists. The findings from these studies suggest a strong rationale to develop EphA2/CD137 *Bicycle*® TICAs to potentially treat EphA2 expressing cancers.

YI-P063 Bryan Lampkin

Tufts University

A Platform for HaloTag Evolution – Working Towards a Quantitative Endosomal Escape Kinetic Assay

B. J. Lampkin and J. A. Kritzer

HaloTag is a self-labeling enzyme that covalently reacts with a chloroalkane ligand. This enzyme is often used as a protein fusion and thus enables the colocalization of a synthetic molecule to a protein of interest with chemical and genetic specificity. Fluorogenic dyes have been employed as HaloTag ligands and will turn-on in fluorescence after covalent conjugation to this enzyme. However, fluorogenic dyes are synthetically optimized to turn-on in the presence of HaloTag and thus screened in a low-throughput manner. We have developed a molecular evolution platform for HaloTag using yeast surface display.

With this platform, we can evolve HaloTag by screening >107 multi-mutant HaloTag variants against a single fluorogenic dye to enhance its photophysical properties. While the evolution of HaloTag to introduce novel secondary function has many possible downstream applications, we are working towards employing an evolved HaloTag•Dye pair in a novel cellular assay that can rapidly quantitate the endosomal escape of biomolecular therapeutics in real-time. This will enable the systematic study of the cellular penetration and endosomal escape of biomolecular therapeutics and will further inform their design.

P064 Elisabetta Bianchi

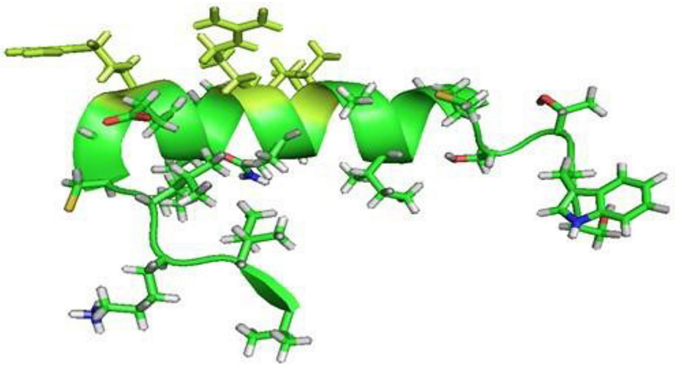
IRBM

Potent and Long-Acting Single Chain Peptide Mimetics of Relaxin-2, Profiling of Therapeutic Candidates for CV Diseases

E. Bianchi, S. Mallart, R. Ingenito, A. Bresciani, F. Caretti, S. Esposito, M.Gallo, P.Magotti, E.Monteagudo, L.Orsatti, D. Roversi, A.Santoprete, F. Tucci, M.Veneziano, R.Bartsch, C. Boehm, D. Brasseur, F. Bonche, P. Bruneau, A. Corbier, J. Froissant, L.Gauzy-Lazo, V.Gervat, A.Krick, F. Marguet, C. Mau-

riac, I.Menguy, C.Minoletti, M-F. Nicolas, O. Pasquier, B. Poirier, A. Raux, L. Riva, P.Janiak, H Strobel, C.Philippo, O. Duclos, P. Janiak, S. Illiano

Relaxin-2 is a peptide hormone of the insulin-like family with a complex two-chain structure. Its therapeutic potential in acute heart failure is hampered by a short half-life and the challenging chemistry. Recently we disclosed structurally simplified single-chain mimetics of Relaxin-2 as extremely potent sub-nanomolar agonist of RXFP1. Optimization of these candidates led to improved metabolic stability with extended half-lives, high subcutaneous bioavailability and activity in *in vivo* translational models.



During the early development process, pharmacokinetics studies revealed a relevant, unexpected oxidative metabolism occurring on the fatty acid chain primarily in preclinical species as dog and minipig. This presentation will address how the discovery team addressed these issues for the identification of improved therapeutic candidates for the clinic.

YI-P065 Moritz List

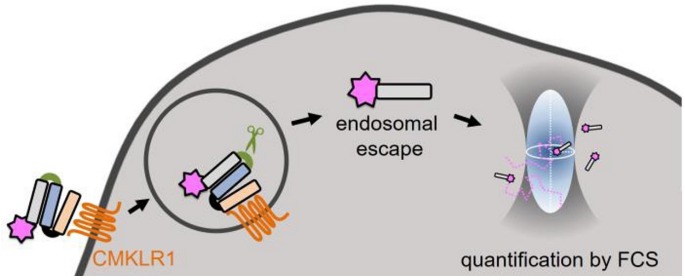
Universität Leipzig

A Cytosolic Delivery System for Therapeutic Peptides Based on CMKLR1-Mediated Etndocytosis

Moritz List, and Annette G. Beck-Sickinger

Therapeutic peptides are promising tools to address intracellular protein-protein interactions, PPIs, which are challenging targets for conventional drug moieties. However, effective cytosolic delivery still proves to be a major problem in the development of therapeutic peptides targeting intracellular PPIs. Additionally, some common strategies to improve membrane permeability of peptides can result in off-target effects or toxicity. Therefore, selective and efficient delivery systems are highly desirable.

Here we present a shuttle system for peptide cargos using the chemokine like receptor 1, CMKLR1. CMKLR1 is highly expressed in a variety of tissues and an efficiently internalizing nonapeptide ligand with nanomolar affinity, chemerin-9, has been identified. Further, cyclic variants of chemerin-9 have been shown to be metabolically stable and therefore are suitable for a therapeutic shuttle system.



Here we demonstrate that large peptide cargos can be N-terminally attached to chemerin-9 while still maintaining excellent internalization behavior. However, peptides internalized by receptor-mediated endocytosis usually become trapped in the endosome without reaching their site of action in the cytosol. To enable endosomal escape, we tested different approaches and quantified the cytosolic concentration of the delivered peptide cargo using fluorescence correlation spectroscopy, FCS. Overall, our results give an insight into the efficiency of different known endosomal escape strategies for a targeted application of therapeutic peptides.

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P066 Justin Holub

Ohio University

Developing Helical Peptide Antagonists of the Human Growth Hormone Receptor

K. Nahar, J. Pettis, R. Basu, J.J. Kopchick3 and J.M. Holub

Growth hormone, GH, is a single-chain polypeptide that acts as a key stimulator of cell growth and proliferation. GH exerts its physiological effects by binding to the extracellular domain of the growth hormone receptor, GHR; this binding event activates pleiotropic intracellular signaling cascades that lead to the expression of growth-promoting genes. Given the profound influence of GH on cell biology, there is considerable interest in developing antagonists of GH action to treat diseases such as cancer and acromegaly.

We recently reported the synthesis and characterization of a 16-residue peptide, S1H, designed to mimic the site 1-binding helix, residues 36-51, of human GH. Our studies indicated that S1H is stable in human serum and inhibits GH-mediated STAT5 phosphorylation in cultured cells co-treated with GH. Furthermore, structure-activity relationships showed a strong correlation between S1H helicity and GHR antagonism, indicating that helical propensity is required for biological activity.

To enhance the pharmacological properties of S1H, we have developed a series of S1H derivatives that fold into stable helical structures through hydrocarbon stapling.

The constrained S1H peptides proved to be more helical and protease-resistant compared to wild-type, linear, S1H. In addition, constrained S1H constructs showed enhanced efficacy over wild-type S1H for inhibiting GH-mediated STAT5 phosphorylation in bladder cancer cells.

Taken together, these studies have enhanced our understanding of the molecular mechanisms through which S1H, and its structured derivatives, targets GHR and have identified potential lead compounds that may be used as emerging therapeutics for treating endocrine and geriatric disorders associated with high GH activity.

YI-P067 Robert Maloney

Temple University

A New Class of Fluorine-Thiol Displacement Reaction, FTDR, Stapled Peptides Displaying Enhanced Cellular Uptake

R. Maloney, T.R. Lewis, C. Cheng, S.L. Junod, M.S. Islam, T.W. Moore, W. Yang, R.E. Wang

The development of bioactive peptides is rapidly gaining traction in the field to probe intracellular protein targets. However, the use of peptides as intracellular probes is hindered by the intrinsic limitations of proteolytic stability, affinity, and membrane permeability. Through synthetic modifications, progress has been made to address these shortcomings, using techniques such as peptide stapling and cell-penetrating motifs. Despite the advances achieved so far, current stapling methods still often result in peptides that suffer from poor cell permeability. Conjugation of cell-penetrating motifs can overcome this but requires the addition of excessive positive charge/s or hydrophobic groups which can detract from the native peptide's function, target affinity, and compromise the integrity of cell membranes.
We sought to overcome these challenges through a new class of stapled peptides using our fluorine-thiol displacement reaction, FTDR. FTDR allows for selective modifications in unprotected peptides of an incorporated orthogonal fluoroacetamide building block capable of sparing internal cysteines. Peptides stapled through FTDR appear to display universally enhanced cell permeability compared to those made using the traditional ring-closing metathesis, RCM, method.

Here, we report example peptides modeled to target classic protein-protein interactions such as Axin-Beta catenin and p53-MDM2/MDMX. We also wanted to share one unpublished follow-up story probing a coactivator interaction on a relevant breast cancer target, estrogen-receptor α (ERα). ERα-targeting FTDR-stapled peptides displayed more potent anti-cancer activity and markedly improved cell permeability even though the RCM construct possessed greater α-helicity. Surprisingly, the N→C terminal L/D chirality combination displayed better helicity and functional activities than the L/L chirality peptide; despite previously reported trends that the inclusion of D chirality is notorious for disrupting α-helical structure.

P068 Ruiwu Liu

University of California Davis

Spike Protein-Targeting Transformable Nanomedicine for COVID-19 Treatment

Riuwu Liu

The COVID-19 global outbreak demands the accelerated development of novel anti-viral therapies. The common treatment for COVID-19 includes polymerase inhibitor, protease inhibitors, and monoclonal antibodies. Realizing the pros and cons of these methods, we developed SARS-CoV-2 viral-targeting transformable nanoparticle, VTPN, as a novel COVID-19 therapy.

The VTPNs are self-assembled from a transformable peptide monomer, TPM, under aqueous conditions. TPM consists of a high-affinity peptide ligand against spike protein, S-protein, of the SARS-CoV-2 virus and a KLVFFK(Pa) peptide sequence wherein KLVFF is the β-sheet forming peptide and Pa stands for pheophorbide a, a hydrophobic porphyrin derivative. Upon binding with spike-protein displayed on the SARS-CoV-2 surface, the VTPNs can subsequently undergo *in situ* transformation into the fibrillar structural network to prevent viral attachment and fusion with target cells.

Several 8-10mer peptide ligands with nM binding affinity against SARS-CoV-2 S-protein as well as the delta and omicron variants, have been discovered from One-Bead One-Compound, OBOC, combinatorial peptide libraries.

TPMs were synthesized by solid phase peptide synthesis method and their physico-chemical properties were characterized. The formation of VTPNs was verified by dynamic light scattering, DLS, and transmission electron microscopy, TEM. Two VTPNs demonstrated potent in vitro anti-viral activities as evidenced by reduced viral RNA levels and titers in SARS-CoV-2-infected epithelial cells.

VTPN represents a new class of antiviral nanotherapeutics that directly target viral surface proteins. It is conceivable that concurrent targeting of two or more orthogonal sites of viral proteins would greatly lower the chance of viral resistance to VTPN therapy.

YI-P069 Adam Mitrewski

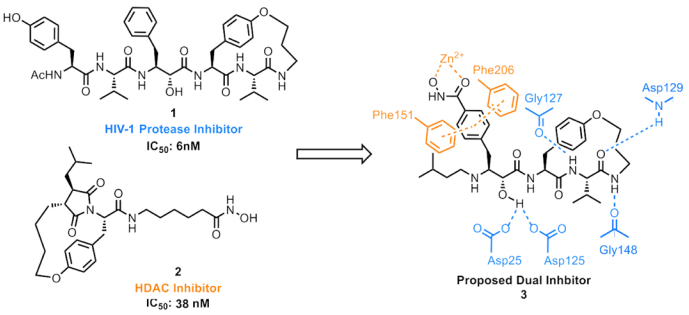
University of California Davis

Design and Synthesis of a Dual-Action Agent Capable of Simultaneously Activating HIV-1 Latency and Preventing New Infection

Adam Mitrewski

The complete eradication of human immunodeficiency virus type 1, HIV-1, from infected individuals is complicated by HIV latency, the ability of the virus to remain quiescent within certain immune cell lines. The "shock and kill" approach has been proposed to reactivate latent proviruses and eliminate

the virus *in vivo*. In principle this can be accomplished using a histone deacetylase, HDAC, inhibitor to reverse latency and an HIV-1 protease inhibitor to eradicate the virus.



Known macrocyclic inhibitors of HIV-1 protease and HDAC2, **1** and **2**, respectively, were synthesized and studied using 2D NMR techniques to establish their structures in solution. Those structures were used in computational docking experiments to design a hybrid HIV protease/HDAC inhibitor, **3**. The design, synthesis and evaluation of **3** will be presented.

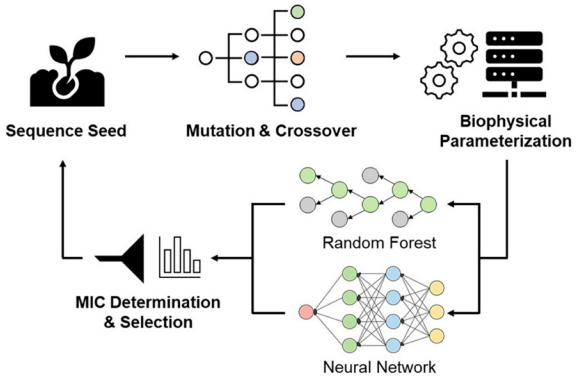
P070 Scott Medina

Penn State University

Artificial Intelligence Approaches Optimize Potency, Specificity, and Safety of Anti-Tubercular Peptides

Sara Benson, Aidan Matunis, Diptomit Biswas, Adam Wertz, Miki Gebretsadik, Michael Howe, Anthony D. Baughn, and Scott H. Medina

Precision antimicrobials that kill respiratory pathogens without damaging host commensals hold potential to cure disease without antibiotic-associated dysbiosis. We recently reported a *de novo* designed peptide, MAD1, that selectively kills *Mycobacterium tuberculosis*, the causative agent of Tuberculosis, TB, without harming lung commensals. *In vitro* and *in vivo* assays further demonstrated MAD1 could be paired with antibiotics to rapidly clear drug-resistant pulmonary infections, and efficiently delivered into animal models using aerosol carriers.



Here, we describe the development of AI machine-learning algorithms than predict second-generation MAD1 sequences with improved potency, specificity and safety. Random forest and neural network algorithms were built using discretized biophysical peptide properties and trained via MIC data from our lab, as well as literature, see figure. The predicted, potency-optimized, sequences showed a remarkable preference for thirteenmers, yielding peptides with lengths that match the ~8nm thickness of the mycobacterial outer membrane. This suggests sequence length may be a unique driver of anti-TB peptide activity, rather than the classical charge and hydrophobicity factors identified for other antimicrobial peptides.

Twenty predicted sequences were subsequently synthesized and screened against TB pathogens, non-mycobacterial lung commensals, and mammalian cell lines to evaluate activity, specificity, and toxicity, respectively. Although several candidates showed modest improvement in anti-TB potency and specificity, the most significant change was a >5-fold improvement in safety. In sum, we report previously underappreciated biophysical peptide properties that confer anti-mycobacterial activity and identify narrow-spectrum antimicrobials for TB therapy.

YI-P071 Noriko Omura

Tokyo University of Pharmacy and Life Sciences

Readthrough Activity of Negamycin Derivatives Against TP53 Nonsense Mutant

N. Omura, A. Ema, A. Taguchi, K. Hamada, S. Konno, K. Takayama, A. Taniguchi, and Y. Hayashi

The tumor suppressor p53, encoded by the TP53 gene, is the most frequently mutated gene in human cancers. Approximately 10% of these mutations are nonsense mutations that change a sense codon to a premature termination codon, PTC, resulting in the termination of the translation and the expression of truncated proteins. Recently, the "Readthrough compounds" that can skip PTCs and reproduce full-length proteins is focused on as one of the promising approaches to treat inherited diseases caused by nonsense mutations. We previously performed the structure-activity relationship study of negamycin which is reported as the readthrough compound and discovered several derivatives with potent readthrough activity against TGA-type PTC.

In this study, to evaluate the ability of these derivatives as readthrough anti-cancer agents, DMS-114 cells that are endogenous R213X (TGA) mutant *TP53*-containing human lung carcinoma were treated with these derivatives, and the expression of full-length p53 protein was analyzed by western blotting. Among several negamycin derivatives, the ester-type prodrug TCP-199 produced the highest amount of full-length p53 proteins. Furthermore, TCP-199 exhibited anti-proliferative activity against the same cancer cells. From these results, it is suggested that TCP-199 induced the PTC-readthrough of the *TP53* in cancer cells and would be a new chemotherapeutic agent to treat human cancers associated with nonsense mutations.

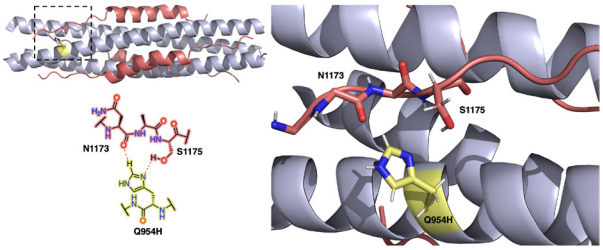
P072 Victor Outlaw

University of Missouri

SARS-CoV-2 Omicron Variant Spike Mutation Q954H Enhances Fusion Core Stability Relative to Previous Variants

R. H. Apurba, N. Vithanage, C. Bair, and V. K. Outlaw

The severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, Omicron variant of concern has rapidly spread across the globe to become the dominant form of COVID-19 infection. The Omicron spike, S, glycoprotein, which mediates viral entry into cells, possesses 34 mutations relative to the initial SARS-CoV-2 wild type variant. The Omicron S receptor- binding domain, RBD, and N-terminal domain, NTD, the targets of currently approved therapeutic antibodies, harbor 15 and 11 mutations, respectively, and the links between these mutations and decreased effectiveness of existing antibodies have been widely studied. The effects of mutations in other regions of Omicron S, however, remain underexplored.



Three mutations, Q954H, N969K, and L981F, occur within the N- terminal heptad repeat, HRN, domain. During viral entry, the HRN domain co-assembles with the C-terminal heptad repeat, HRC domain to form a stable six-helix bundle or "fusion core" structure, which brings the viral envelope and host membrane into proximity and thermodynamically drives membrane fusion.

Here, we demonstrate using synthetic peptides that the Q954H mutation in the Omicron variant HRN domain enhances interaction with the HRC domain, thereby stabilizing the fusion core assembly relative to prior variants. We also report the first X-ray crystal structure of the Omicron S fusion core, which reveals that the Q954H side chain forms a N•••H-O hydrogen bond with the side chain hydroxyl of S1175 within the HRC domain, as well as an unexpected C-H•••O hydrogen bonding interaction with the backbone carbonyl of N1173. These structural insights will be valuable for analyzing the factors that drive viral evolution, as well as for the development of inhibitors of SARS-CoV-2 entry.

YI-P073 Ryan Qiu

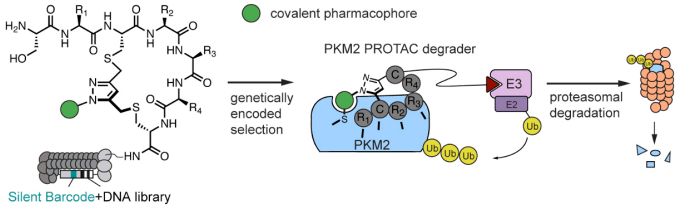
University of Alberta

Genetically Encoded Fragment-Based Discovery of Covalent Peptide Macrocycle PROTACs Degraders of PKM2

Arunika I. Ekanayake, Ryan Qiu, James Walker, and Ratmir Derda

Proteolysis-targeting chimeras, PROTACs, are an effective and promising endogenous degradation technique with multiple drug candidates in phase II trials. This strategy utilizes a heterobifunctional Cereblon, E3 ligase, ligand linked to a functionalized protein ligand for marking a protein of interest for proteasomal degradation.

Compared to small molecule chemotherapeutics, PROTACs ligands offer significant advantages: **i** higher potency and longer sustenance since they can be recycled, **ii** exceptional selectivity and affinity, **iii** potential to overcome active site mutations. Upregulation of pyruvate kinase muscle isozyme II, PKM2, is due to a switch to a glycolytic phenotype known as the Warburg effect, therefore it is a promising target for inhibiting tumorigenesis. PKM2 bears two nucleophilic cysteine residues in the active site targetable using electrophilic pharmacophores. Covalent interactions between cysteine residues and propiolamide electrophile-bearing fragments yields biorthogonal highly potent and specific permanent enzyme modifications.



We utilized a genetically encoded fragment-based discovery approach to identify covalent macrocyclic ligands towards PKM2. Genetically encoded macrocyclic peptide libraries with unnatural pharmacophores are valuable sources for discovering high-affinity covalent ligands to target proteins contributed by high-avidity side-chain interactions. Peptide library cysteine-thiol macrocyclization with a 1,3-diketone bearing linchpin generates shelf-stable precursors for late-state functionalization of terminal alkyne-bearing pharmacophores. Selection of this library against PKM2 yielded promising candidates for targeted degradation. These ligands will then be converted to PROTACs degraders by conjugating the electrophile-bearing macrocycle to a Cereblon targeting ligand that will ubiquitinate PKM2 for *in situ* targeted proteasomal degradation.

P074 Tanya Román

Pontificia Universidad Catolica de Valparaiso,
Universidad Tecnica Federico Santa María

Novel Antiparasitic Peptides with Potential Activity Against *Caligus rogercresseyi*

Tanya Román, Constanza Cárdenas, Claudio Álvarez, Paula Santana, and Fanny Guzmán

The Chilean salmon aquaculture industry is highly vulnerable to infections by ectoparasites such as *Caligus rogercresseyi* that greatly affect the fish's immune system making them susceptible to many diseases, and thus causing large eco-

nomic losses. Control of the sea louse relies on chemical treatments with organophosphates as Azamethiphos, able to inhibit the acethylcholinesterase, AChE, enzyme in the parasite, however in 2014 in Chile, a reduced sensitivity was detected. For this reason, in the search for new strategies for parasites elimination, the use of new molecules like peptides able to inhibit the AChE in this parasite is proposed.

Up to now, studies of peptides in fish have focused on their bactericidal or antiviral properties, with little evidence of their participation in the control of parasites, so the use of antiparasitic peptides, APPs, is proposed to control or eliminate this pest. For this reason, bioinformatics tools were used under the application of Quantitative Structure-Activity Relationship, QSAR, procedures. Those had as objectives, on the one hand, to identify the main properties and structural characteristics responsible for the antiparasitic activity. On the other, carrying out a simulation with active peptides described in the literature, followed by evaluation and construction of a model with good statistical quality for its validation. The results allowed us to obtain 21 sequences with a promising IC50. These sequences were synthesized, characterized and evaluated with the nauplius larval stage of *C. rogercresseyi*. The results showed 4 peptides with antiparasitic activity and that, at the concentrations tested, did not have hemolytic activity. This allows us to conclude that it is possible to obtain peptides to combat this sea louse.

Acknowledgment:
Supported by grant Fondecyt Regular N° 1210056

YI-P075 Mrigank Rai

Chapman University School of Pharmacy

Delivery of siRNA using Oleyl-(WRH)n Cell-Penetrating Peptides

Mrigank S. Rai, Keykavous Parang, Rakesh K. Tiwari

The delivery of nucleic acid therapeutics across various cell membranes has always been one of the major challenges. Cell-penetrating peptides, CPPs, have been found as promising agents for developing an efficient siRNA delivery system. A cyclic CPP, $[WR]_5$, was found to deliver siRNA intracellularly.

We hypothesize that the siRNA intracellular delivery could be enhanced using fatty acylation with the oleyl chain to the newly designed $(WRH)_n$ peptide. Previously, histidine has been found to enhance the endosomal release and modulate the cytotoxicity of CPPs. Therefore, the oleyl conjugated $(WRH)_n$ (n=1-4) peptides were synthesized using Fmoc/tBu solid-phase peptide chemistry, followed by purification using Reverse-Phase High-Performance Liquid Chromatography, RP-HPLC, and characterization using matrix-assisted laser desorption/ionization, MALDI, mass spectroscopy.

We will report the cytotoxicity studies of the peptides using a panel of breast cancer and normal cell lines, their binding affinity with siRNA at various N/P ratios, their zeta potential of peptide:siRNA complex, serum stability of peptide:siRNA complex, and cellular uptake of siRNA using flow cytometry and confocal microscopy. The most active peptide will be used to provide evidence of gene silencing with the successful delivery of their respective siRNA.

P076 Ashweta Sahni

Entrada Therapeutics, Inc., Boston, MA

Endosomal Escape Vehicle-Oligonucleotide Conjugates for the Targeted Upregulation and Downregulation of Gene Expression

Ziqing Leo Qian, Xiang Li, Xiulong Shen, Mahboubeh Kheirabadi, Amy N. Hicks, Mark Wysk, Haoming Liu, Ajay Kumar, Nelsa L. Estrella, Patrick G. Dougherty, Suresh Peddigari, Anushree Pathak, Kimberli J. Kamer, Wenlong Lian, Nanjun Liu, Matthew Streeter, Sara L. Blake, Christopher M. Brennan, Ning Li, Vlad Batagui, Keyede Oye, Ningguo Gao, Daniel Wang, Arianna Bonilla, Mohanraj Dhanabal, Mahasweta Girgenrath, and Natarajan Sethuraman

To overcome current limitations of oligonucleotide therapeutic delivery, we have designed a family of proprietary cyclic CPPs that form the core of our Endosomal Escape Vehicle, EEV™, technology covalently conjugated to oligonucleotides. Through screening a library of EEVs, we identified EEVs for functional delivery to target cell and tissue types.

We employed our EEV-PMO, phosphorodiamidate morpholino oligomer, technology in Duchenne muscular dystrophy, DMD, preclinical models and evaluated the ability of our EEV-PMO conjugates to upregulate specific gene expression via exon skipping. In DMD, mutations result in a reading frame shift causing a lack of functional dystrophin. In mice harboring a *DMD* exon 23 nonsense mutation, we showed that monthly doses of an exon 23-skipping EEV-PMO resulted in restoration of dystrophin protein in skeletal and cardiac muscle.

Next, we applied our EEV-PMO approach to down-regulate interferon regulatory factor 5, IRF5, gene expression. Overexpression of IRF5 is associated with inflammatory and autoimmune diseases. To knockdown IRF5, we identified steric blocking oligonucleotides that modulate splicing, promote out-of-frame shift, and knockdown IRF5 mRNA level. Treatment with an IRF5-targeting EEV-PMO resulted in potent and dose-dependent knockdown of IRF5 *in vitro* and *in vivo*, indicating the applicability of our EEV-PMO approach in modulating gene expression in immune cells.

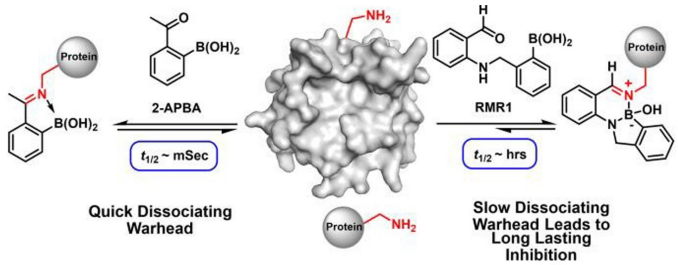
These results demonstrate the ability of our EEV platform to efficiently deliver oligonucleotides to specific target cell and tissue types. In addition, our approach has broad applicability to up- or down-regulate target gene expression through distinct mechanisms of action.

YI-P077 Rahi Massom Reja
Boston College

Lysine-Targeting Reversible Covalent Inhibitors with Long Residence Time

Rahi M. Reja, Wenjian Wang, Yuhan Lyu, Fredrik Haeffner and Jianmin Gao

Reversible covalent inhibitors are one of the promising candidates for modern drug discovery. It holds promise in mitigating toxicity of covalent drugs as the reversibility can minimize off-target reactions and also avoid permanent modification of the target proteins.



In this presentation, I will be discussing a new reversible lysine conjugation that features a novel diazaborine product and significantly slower dissociation kinetics in comparison to the previously known iminoboronate chemistry. Incorporating the diazaborine-forming warhead RMR1, to a peptide ligand gives potent and long-acting reversible covalent inhibitors of the *Staphylococcal* sortase A.

The efficacy of sortase inhibition is demonstrated via biochemical as well as cell-based assays. A comparative study between RMR1 and an iminoboronate-forming warhead towards sortase inhibition will be discussed in this presentation, highlighting the significance and potential of modulating bond dissociation kinetics in achieving long-acting reversible covalent inhibitors.

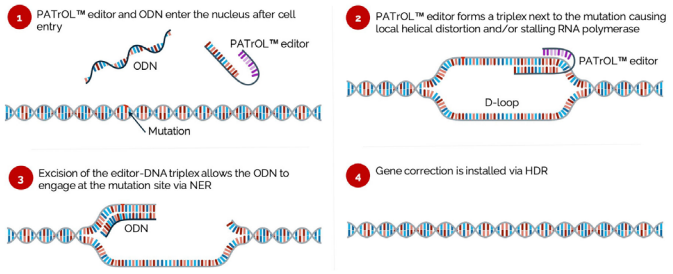
P078 Dani Stoltzfus
Boston College

Nuclease-Free Gene Editing with Peptide Nucleic Acids: A New Class of *In Vivo* Gene Editors

Dani M. Stoltzfus, William Riedl, Lyuqin Chen, Lindsey R Spiker, Steven M Murphy, Nathan Tavenor, Barry Badeau, Jeremy W. Gleaton, and Dietrich A. Stephan

Gene editing promises permanent cures to genetic diseases. Early generations of gene editing technologies, for example, generations of CRISPR/Cas and base editors, have suffered from fidelity, off-target and by-stander edits, and toxicity, including double stranded breaks, issues, which may limit their ability to be successfully administered *in vivo*. Additionally, these first-generation editors have a requirement for a protospacer adjacent motif, PAM, sequence directly adjacent to the causal variant of interest and are constrained to gene disruption or editing transition mutations, meaning the total addressable market of all such editors, combined, is ~20% of known genetic mutations in ClinVar.

The introduction of prime editing, PE, resolves some of the limitations of these earlier technologies, by relaxing the PAM sequence constraint and allowing editing of transversions, insertions, and deletions such that an estimated 90% of all genetic mutations are now technically addressable. Challenges that remain include the ability to be flexible in delivery approaches such that editors can be delivered to a variety of tissues that manifest pathologies, improving fidelity to maximize safety, and overcoming the likely acquired immunogenicity of a bacterial nuclease which could limit the ability to re-dose a patient to address tissue turnover.



We have developed a technology platform, PATrOL™, comprised of modified peptide nucleic acids, PNAs. These editors are PAM-sequence unrestricted, can address all mutational types, have fidelity rates on the order of background mutational rates in human cells, and which have additional advantages over PEs in that we have flexibility in delivery and do not elicit an acquired immune response. PATrOL™ editors engage the double-stranded genome with single base selectivity to recruit endogenous nucleotide excision repair, NER and homology directed repair, HDR, enzymes which replace the disease-causing mutation with the correct sequence. This approach uses multiple layers of sequence selectivity for a locus of interest and relies on the body's own endogenous high-fidelity machinery to reduce off target editing to well below that reported for BEs. The editors themselves are on the order of 100 nucleobases in total length and are charge-tunable to allow use with various delivery technologies.

Finally, due to the synthetic nature of the editors themselves, there has not been an acquired response reported to date. In combination with non- immunogenic lipid nanoparticle delivery, the PATrOL™ editors have the potential to unlock repeat dosing in vivo to address tissue turnover and achieve clinically relevant editing efficiencies. Data will be presented that describes the performance of our nuclease- free editing platform and how the technology in being applied to address several monogenic diseases that remain of high unmet need.

YI-P079 Marcy Robins

University of Utah

D-peptide Inhibitors of Uropathogenic *E. coli* Adhesion Proteins to Treat Urinary Tract Infections

M. M. Robins, S. E. Apple, G. G. Quichocho, S. R. E. Draper, and M. S. Kay

Approximately 80% of urinary tract infections, UTIs, are caused by uropathogenic *E. coli*, UPEC. Adhesion proteins on the pilus-covered surface of UPEC are required for attachment to host cells for colonization and disease progression. The primary adhesion protein associated with UPEC virulence is FimH, which binds D-mannose on uroepithelial cells. Our goal is to develop UTI treatments that prevent the attachment and subsequent internalization of UPEC in uroepithelial cells, eliminating bacteria from the urinary tract. However, it remains challenging to design long-lived inhibitors that reach the urinary tract without disrupting adhesion in commensal gut bacteria.

We are using mirror-image phage display to identify D-peptide inhibitors of UPEC adhesion. Towards this goal, we have synthesized FimH in four peptide segments and used native chemical ligation to assemble them. FimH is densely populated with hydrophobic and negatively charged amino acids, making its synthesis incredibly difficult without the use of removable, solubilizing "Helping Hand," HH, tags developed by our lab that can be added at Lys or Glu positions. Additionally, the use of a newly developed Asp HH proved critical for solubilizing one of the FimH segments. Using recombinant protein, we have validated a refolding protocol and confirmed that refolded FimH retains the same structure and function using circular dichroism and mannose binding assays. We are now synthesizing FimH using D-amino acids to provide the D-target to screen in mirror-image phage display and identify D- peptide inhibitors to prevent and treat UTIs.

P080 Rakesh Tiwari

Chapman University

Silencing of STAT3 Gene in Breast and Ovarian Cancer Cells Using Fatty Acylated Cell-Penetrating Peptides

Mrigank S. Rai, Muhammad Imran Sajid, Keykavous Parang, Rakesh Kumar Tiwari

The delivery of nucleic acid therapeutics across various cell membranes has always been one of the major challenges. Cell-penetrating peptides, CPPs, containing arginine, R, tryptophan, W, and histidine, H, have been promising for siRNA delivery. Therefore, to enhance siRNA delivery and efficient silencing of the STAT3 gene, we added the oleyl chain to our newly designed cell-penetrating peptides $(WRH)_n$ and evaluated their silencing efficiency in breast and ovarian cancer cells.

The oleyl conjugated $(WRH)_n$ (n =1-4) peptides were synthesized using Fmoc/tBu solid- phase peptide chemistry, purified with reverse-phase high-performance liquid chromatography, RP-HPLC, and characterized using matrix-assisted laser desorption/ionization, MALDI, mass spectroscopy. The diameter of synthesized oleyl peptides was <100 nm. The peptides formed nano complexes with siRNA and were relatively stable complexes with a range of 13-18 mV of zeta potential at N/P 40. All peptide:siRNA complexes showed an adequate binding affinity and serum stability at N/P ≥ 40.

The peptide:siRNA complexes were found to be non-cytotoxic up to an N/P ratio of 40, ~20 μM, for 72 h in MDA-MB-231, MCF-7, SK-OV-3, and HEK-293 cells. Along with the evidence of cellular internalization of each siRNA, data for the most potent peptide will be presented in the poster presentation at the meeting to demonstrate gene silencing efficiency using MDA-MB-231 and SK-OV-3 cells.

YI-P081 Rasheda Aktar Samiha

Clark University

Optimization of IEDDA Reaction Parameters

Rasheda Aktar Samiha

Inverse Electron Demands Diels-Alder, IEDDA, reaction is a biorthogonal reaction that potentially can be used for various applications such as bioconjugation, imaging, screening et cetera. The reaction kinetics of the IEDDA reaction varies based on the nature of the substitutions on the tetrazine and diene. So far, IEDDA reaction studies have focused on the use of electron-withdrawing heterocyclic aromatic ring containing tetrazines with various dienophiles, due to the fast kinetics of the reactions. There have been no detailed studies on S, S-disubstituted tetrazines with various dienophiles. While we anticipate these reactions would have slower kinetics, incorporation of S,S-disubstituted tetrazine in biomolecules such as peptides is synthetically much less challenging than the incorporation of an aromatic N,N-tetrazines, and detailed studies of the S,S-tetrazines with various dienophiles would offer valuable knowledge that can lead to new applications that do not need fast kinetics.

We recently developed S, S-tetrazine linked molecules in biocompatible systems in our laboratory that can be easily synthesized. We are studying their reaction kineties with moderately reactive dienophiles like norbornene-containing or cyclopropane-containing molecules under various organic and aqueous conditions using various spectroscopic and analytical techniques and will present our results.

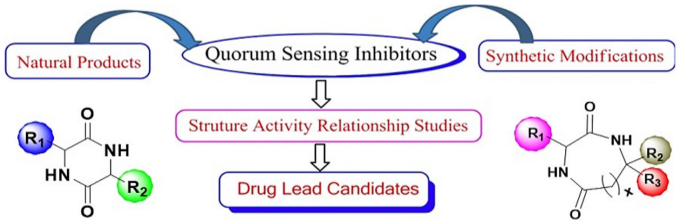
YI-P082 Sudha Shankar

Tel Aviv University

Cyclic Peptide Containing Backbone Modified Amino Acid as Antimicrobial Agents

S. Shankar, and Prof. Ehud Gazit

At this time when we are going through an unprecedented global pandemic, it is an unarguable fact that new and advanced drugs are of paramount importance. A pressing issue is the development of new medicines that treat microbial infections to prevent the growing health threat posed by resistant pathogenic microorganisms. Being able to control drug-resistant strains of gram-negative bacteria, without prolonging the course of treatment is even more challenging.



Amongst the naturally occurring antimicrobial peptides, cyclic peptides have exhibited traits of potential antibiotics due to their diverse biological properties and metabolic stability compared to linear peptides. Their peculiar heterocyclic system, that is, 2,5-diketopiperazine found in several natural products constitutes a rich source of new biologically active compounds. Naturally occurring cyclopeptides isolated from fungi, plants and bacteria have been reported to target and disrupt the functions of microbial cell membranes. Major problems associated with such natural peptides are their low bioavailability, low metabolic, proteolytic stability, complex structures and high cost of goods.

To overcome the inherent limitation, these peptides can be modified using the peptidomimetic approach by introducing synthetic β- and/or γ-amino acid to develop short and potent peptide therapeutic agents against microbial infections. The present work aims to the develop novel cyclic peptides incorporating non-proteinogenic β- and γ-amino acids having non-native diketopiperazine with different side-chain modifications to explore potent antimicrobial agents against microbial infections.

P083 Dieter Willbold

Forschungszentrum Jülich

All-D-Peptide PRI-002 for the Treatment of Alzheimer's Disease is Approaching A Phase II Clinical Trial

Dieter Willbold

Neurodegenerative protein-misfolding diseases, like Alzheimer's, AD, and Parkinson's disease, PD, are driven by prion-like self-replicating and propagating protein assemblies of Aβ, α-synuclein, and many more. The conformation these proteins have in the aggregated state is thermodynamically more stable than their physiological monomer conformation, which is often intrinsically disordered.

The all-D-enantiomeric peptide PRI-002, alias RD2, was developed to bind an stabilize Aβ monomers in their physiological intrinsically disordered structure. By thereby stabilizing intrinsically disordered monomer structure, PRI-002 is not only inhibiting aggregation, but is also eliminating already existing aggregates by disassembling them into monomers. This purely thermodynamic driven mode of action is truly "anti-prionic", because it is eliminating already existing oligomers and fibrils, thus disrupting prion-like replication and propagation of toxic protein aggregates.

PRI-002 demonstrated ex vivo target engagement and disassembled Aβ oligomers obtained from brain tissue of former AD patients. A clinical phase Ib, double-blind, placebo-controlled study with mild cognitively impaired, MCI, due to AD and mild AD patients treated once daily orally with RD2 or placebo for 4 weeks with an additional 4 weeks follow up period has been finished.

I will present the results of this study, especially on good safety and tolerability, which are valuable information for the design of the scheduled phase II study, which will start later this year. I will also acknowledge the many contributors of the development that are too many to be included here in the abstract.

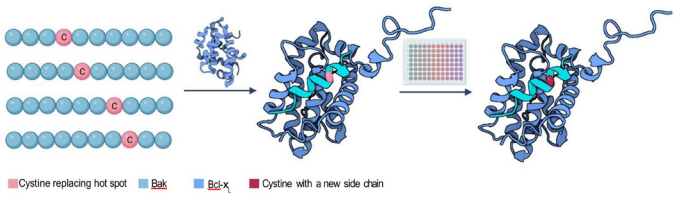
YI-P084 Sachi Sharma

Imperial College London

Target-Directed Synthesis of α-Helix Mimetics

Sachi Sharma, Alan Armstrong, and Anna Barnard

Protein-protein interactions, PPIs, play a significant role in almost all biological processes and, when misregulated, often result in disease. A noteworthy example is the PPI between Bcl-xL and Bak, which has a prominent impact on apoptosis regulation and, thus, is a well validated oncological target.



PPIs are commonly mediated by α-helices, with key hot spot residues contributing the majority of the binding energy. As such, molecules which successfully mimic the hot spot residues found on the native helix, α-helix mimetics, have the potential to behave as a competitive inhibitors. Therefore, α-helix mimicry has long been recognised as a promising strategy for the disruption or recapitulation of relevant PPIs.

This poster presentation will describe a novel approach to the synthesis and identification of α-helix mimetics targeting the Bcl-x_L/Bak PPI via the development of an electrophilic covalent screen. This approach will allow the rapid identification of potent mimetics with high structural diversity. This project employs a target directed strategy via the synthesis and implementation of a thiol functionalised mimetic. The thiol handle is capable of reacting with electrophilic fragments in a 'click' reaction. This will enable screening for highly diverse side chains, in the presence of the target protein, expanding the chemical space beyond fragments found in nature and enabling the rapid identification of high affinity helix mimetic building blocks. By inserting the thiol functionality at different locations within the binding mimetic, the preferred binding motif at several positions can be identified to develop a ready optimised scaffold. The extent to which the electrophilic fragments react with the thiol handle is analysed using the quantitative irreversible tethering assay. This assay is founded on the principle of fluorogenic thiol quantification, whereby the progress of the reaction is monitored through the measurement of residual thiol concentration at various timepoints.

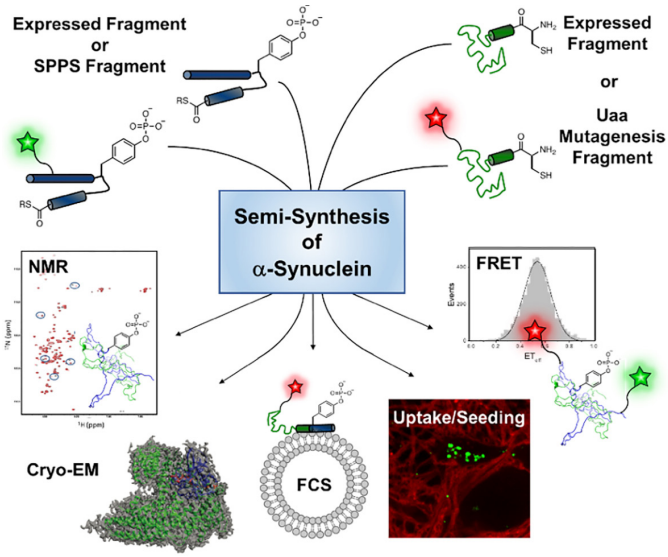
YI-P085 Marie Shimogawa

University of Pennsylvania

Studying the Effects of Post-Translational Modifications On Alpha-Synuclein through Unnatural Amino Acid Mutagenesis and Protein Semi-Synthesis

M. Shimogawa, B. Pan, J. Ramirez, A. Kashina, C. Peng, D. Eliezer, E. Rhoades, and E. J. Petersson

Alpha-synuclein, αS, is a 14kDa protein found at synaptic termini, and it physiologically traffics synaptic vesicles and manages neurotransmitters. Aggresomes of αS are commonly found in neurodegenerative disorders such as Parkinson's Disease, PD, Multiple System Atrophy, MSA, and Dementia with Lewy Bodies, DLB. Recent results show that αS fibrils formed under different disease environments behave differently, but the mechanism of this has not yet been fully understood.



Post-translational modifications, PTMs, have been suggested to contribute to these differences. Recently, we have isolated αS from PD, MSA, and DLB patient tissues and performed mass spectrometry studies to determine which PTMs are differentially present in the soluble αS that might influence its aggregation. Investigating the roles of PTMs helps inform on disease mechanism, identify potential disease biomarkers, and reveal new potential drug targets.

Herein, we synthesized αS with PTMs, glutamate arginylation, lysine acetylation or tyrosine phosphorylation, installed through protein semi-synthesis, chemoenzymatic modification, or unnatural amino acid mutagenesis. We have characterized their aggregation propensity by kinetic or thermodynamic analyses. We further investigated the native or pathological role of the PTM in structural studies, single molecule experiments, cell studies, and in some cases in animal models. This study provides preliminary insight of the role of PTMs at each modification site on αS. We are now using combinations of chemoenzymatic modification, unnatural amino acid mutagenesis and semi-synthesis to install multiple PTMs, enabling first-of- their-kind studies of the crosstalk between PTMs in affecting the function and pathology of αS.

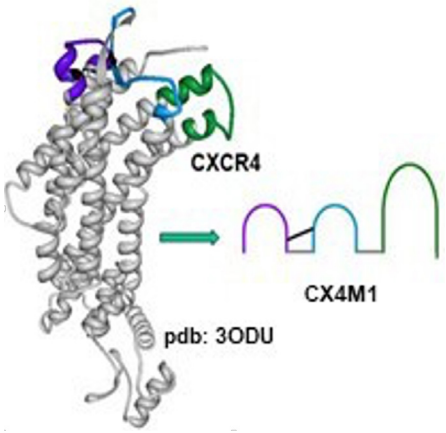
YI-P086 Shoumika Srirangan

Friedrich-Alexander-Universität

Exploring the Potential of CXCR4 Mimetic Peptides to Target Cancer Cells

S. Srirangan, K.M. Popp, M. Franz, S. Mühlich, and J. Eichler

The chemokine receptor CXCR4 is involved in many physiological processes, as well as in diseases, including HIV-1 infection and cancer. CXCR4 interaction with the chemokine CXCL12 has been demonstrated to stimulate different cancer processes. In HIV-1 infection, on the other hand, CXCR4 serves as a coreceptor for virus entry into the host cell.



The extracellular domain of CXCR4, that is, the N- terminus and extracellular loops, ECLs, are important for receptor function and ligand binding. We have previously designed a peptide, see figure below, that presents the three ECLs of

CXCR4, and which was shown to inhibit HIIV-1 infection, as well as CXCR4 signalling.

We are now exploring the potential of CXCR4 mimetic peptides to interfere with the proliferation of CXCR4/CXCL12 depending cancer cells. For this project, the sequence of CX4M1 was optimized for binding to CXCL12 and fused to segments of the CXCR4 N-terminus, which is also involved in the interaction of the receptor with CXCL12.

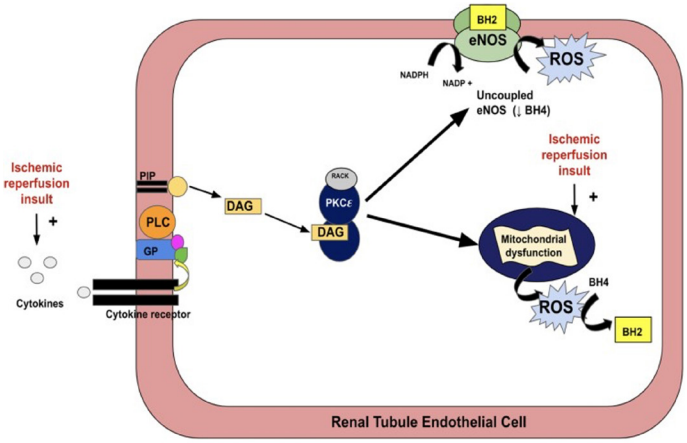
YI-P087 Zinya Talukder

Philadelphia College of Osteopathic Medicine

Myristoylated Protein Kinase C Epsilon Peptide Inhibitor Attenuates Renal Ischemia-Reperfusion Injury and Its Translocation to Epithelial Cell Membranes *In Vivo*

Zinya Talukder, Sunit G. Singh, Alexis B. Verwoert, Tameka C. Dean, Devani Johnson, Lisa Shah, Shino Sleeper, Juliet Melnik, Qian Chen, Robert Barsotti, Yanlin Jiang, James George, Anupam Agarwal, and Lindon Young

Delayed graft function, a post-transplant acute kidney injury, is caused by prolonged ischemia and restoration of blood flow to the ischemic renal tissue, described as renal ischemia-reperfusion injury, IRI. Myristoylated protein C epsilon peptide inhibitor, N-Myr-EAVSLKPT; Myr-PKCε-, is known to have a protective effect in myocardial IRI by inhibiting superoxide production from uncoupled endothelial nitric oxide synthase and mitochondrial ATP-sensitive K+ channels. See figure below. We hypothesized that Myr-PKCε- would attenuate murine renal I, 19min bilateral/R, 96 hours. injury and PKCε expression in renal tubular epithelium when given i.v. at the onset of reperfusion compared to scrambled control peptide, N-Myr-LSETKPAV; Myr-PKCε-scram. Renal pedicles of anesthetized male C57BL/6J mice, 25–30g, were clamped bilaterally for 19 mins. One minute before unclamping, 1.6 mg/kg Myr-PKCε- (n=6) or Myr-PKCε-scram (n=7) was administered by tail vein injection.



Ischemia-reperfusion injury, IRI, from renal tissues increases cytokine release, most prevalent is tumor necrosis factor alpha, TNF-ε, during the first 15 minutes of reperfusion. TNF-α binds to its Gq coupled receptor, which in turn activates of protein kinase C epsilon, PKCε, via diacylglyerol, DAG. PKCε increases reactive oxygen species, ROS, release from uncoupled endothelial nitric oxide synthase, eNOS, and mitochondrial ATP-sensitive K+ channels in renal IRI. Adapted from Perkins et al., 2012.

Glomerular filtration rate, GFR; µl/min, and serum creatinine, Cr; mg/dL, were measured at baseline, 24hrs, 72hrs, and 96hrs post-injury. Myr-PKCε- significantly improved GFR and Cr throughout reperfusion compared to Myr-PKCε-scram control, $p<0.05$. Myr-PKCε- restored final GFR to 52% and Cr to 54% vs. Myr-PKCε-scram, which only recovered to 29%, GFR, and 18%, Cr, compared to initial baseline values; ~222± 15 µl/min and ~0.07± 0.01 mg/dL respectively.

Immunohistochemistry staining of samples using diaminobenzidine chromogen reaction indicated detection of PKCε; defined as positive signal using Aperio ImageScope. Myr-PKCε- ($1.77x10^{8}±3.14x10^{7}$) significantly decreased the number of positive signals compared to Myr-PKCε-scram ($3.58x10^{-1}±5.03x10^{-2}$) ($p<0.05$). Results suggest Myr-PKCε- improved kidney function following renal IRI and attenuated PKCε localization in tubular epithelium compared to Myr-PKCε-scram.

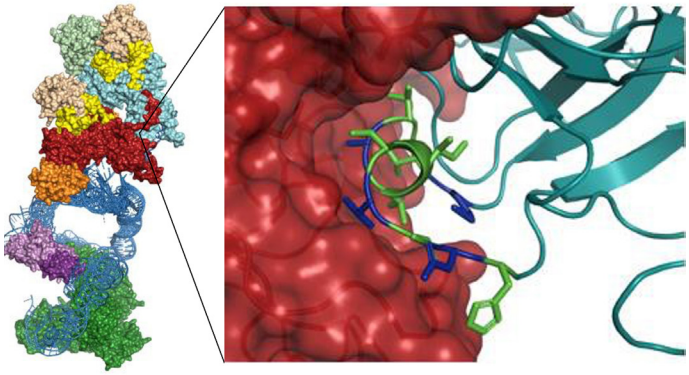
YI-P088 Suzanne van Wier

University of East Anglia

Removing Cancer's Immortality: The Design and Synthesis of Linear and Stapled Peptides Targeting the Dyskerin-Dyskerin PPI in Telomerase

Suzanne van Wier, Mark Searcey & Andrew Beekman

One of the hallmarks of cancers is their ability to replicate limitlessly making them immortal. In 80- 90% of cancer cells this is due to the reactivation of telomerase, a protein complex which elongates telomeres at the end of chromosomes, protecting the chromosomes from degradation and preventing cell senescence.



Left: Full telomerase structure.
Right: dyskerin-dyskerin PPI with derived peptide highlighted in green and the mutated residues commonly found in the disease Dyskeratosis Congenita in this section highlighted in dark blue

The Cryo-EM structure of telomerase published in 2021 provided an opportunity to identify new ways to target telomerase. In patients with Dyskeratosis Congenita, a disease characterised by shortened telomeres, the structure showed that genetic mutations are transcribed to the dyskerin-dyskerin protein-protein interaction, PPI, in telomerase. In this

project we aim to target this PPI, inhibiting the telomerase activity of cancer cells and thus removing cancer's immortality.

We will describe the design and synthesis of a peptide derived from the dyskerin sequence at this PPI. This initial peptide showed low α-helicity and low proteolytic stability as well as not showing any effect on cell viability. To improve these properties hydrocarbon stapling was applied, which increased the α-helicity by 31-48% with the best peptide showing an 83% decrease in cell viability at 200 μM as well as showing an increase in proteolytic stability. Current work is focused on assay development to evaluate the binding of these peptides to dyskerin and their effect on telomerase activity.

When compared to small molecule drugs, peptide therapeutics can have drawbacks such as their limited stability and cell permeability. Therefore, peptide-directed binding will be used to go from peptide to small molecule inhibitor by computationally identifying fragments able to replace parts of the peptide. This method has previously been applied successfully to quickly identify hit compounds for PPIs whilst minimising the organic synthesis and biological screening needed.

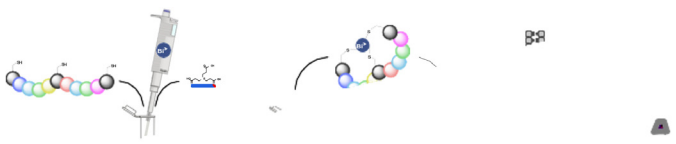
YI-P089 Saan Voss

The Australian National University

Peptide-Bismuth Bicycles

Saan Voss, Jörg Rademann, and Christoph Nitsche

Bicyclic peptides arise as next-generation pharmaceuticals. We introduce peptide-bismuth bicycles as a new class of constrained peptides. Like currently used alkylating agents, bismuth(III) can link three thiols in linear peptide chains. Unlike conventional methods though, *peptide-bismuth bicycles* form instantaneously and selectively at physiological pH, enabling *in-situ* access to highly constrained peptides, directly in biological assays. Two screening campaigns against viral proteases revealed a lead compound which was up to 130 times more active and up to 19 times more proteolytically stable than its linear congener.



Peptide-bismuth bicycles were also stable at physiological pH over several weeks. We next used this technology to discover and explore an array of cell-penetrating peptide, CPP, sequences which allow for the penetration of mammalian cell membranes.

Our new benchtop protocol enables facile generation and purification of cell-penetrating bismuth peptides, which penetrate 20 times better into mammalian cells at identical concentration, then their linear precursors.

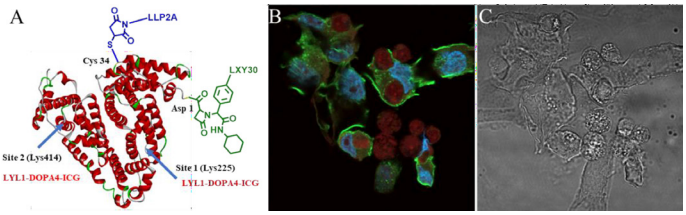
YI-P090 Junwei Zhao

University of California at Davis

Multi-Site-Specific Modification of Human Serum Albumin for Drug Delivery and Cancer Immunotherapy

Junwei Zhao, Asmaa Reda, Xingjian Yu, Ruiwu Liu, and Kit S. Lam

Human Serum Albumin, HSA, is the highest abundant protein in the blood plasma, 40mg/mL. It has good biocompatibility and biodegradability, good oxidation resistance and stability. Its plasma circulating half-life is approximately 21 days and it is a promising drug delivery system, particularly for hydrophobic drugs. LXY30, a α3β1 integrin binding peptide ligand discovered from OBOC libraries, has been shown to target a variety of cancer cells, such as A549, SKOV-3, MDA-MB-231, U87MG, et cetera. We have previously shown that decorating nanodrugs with LXY30 can improve therapeutic efficacy. LLP2A, another peptide discovered from OBOC libraries, is an α4β1 integrin ligand with high binding affinity to malignant lymphoid cells as well as activated normal T and B immune cells.



Scheme 1 A. Schematic representation of LXY30 and LLP2A multi-site-modified, cyanine-loaded HSA; B. and C. multi-site-modified HSA adheres Jurkat cells to MDA-MB-231 cells. Green channel shows MDS-MB-231 cytoskeleton, red channel shows cyanine-loaded HSA, and nuclei stained with DAPI; B. Fluorescence field; C. Bright field.

In this study, HSA was site-specifically grafted with LXY30 and LLP2A through Ugi reaction at Asp1 and maleimide thiol reaction at Cys34, respectively. This bispecific targeting HSA can facilitate the engagement of T-cells with the target cancer cells. Recently, several reactive affinity elements, RAEs, were reported in our lab. Among them, LYL1 has been shown to selectively conjugate to K225 and K414 of HSA via aza-Michael addition. For drug loading, we first conjugated LYL1 to oligo-L-3,4-dihydroxyphenylalanine, DOPA, to form a drug loading element, which was then site-specifically ligated to Lys 414 and Lys225 of HSA. Boronate containing drugs or prodrugs could then be covalently loaded onto HSA via the highly specific boronate-catechol interaction. The loaded drug can then be released at the acidic tumor microenvironment. Cyanine-phenylboronic acid, PBA, conjugate was used as surrogate drug to monitor in vitro cell uptake and in vivo biodistribution of this unique drug delivery system.

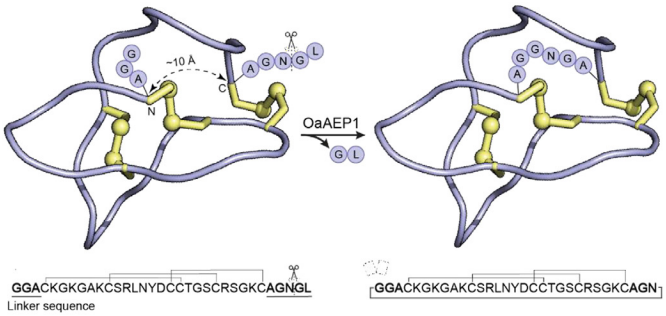
YI-P091 Yan Zhou

The University of Queensland, Australia,
Institute for Molecular Bioscience

A Chemo-Enzymatic Approach to Produce Cyclic MVIIA - Ziconotide

Yan Zhou, Peta J. Harvey, Angeline Chan, Kuok Yap, Åsa Andersson, Alun Jones, Irina Vetter, Thomas Durek, and David J. Craik

Ziconotide, ω-conotoxin MVIIA, was approved by the FDA in 2004 as an analgesic for treating chronic pain, and possesses high therapeutic efficacy in patients who have poor clinical responses to opioids and other analgesics. However, the need for intrathecal administration and adverse effects have limited its widespread application. Backbone cyclization is one way to improve the pharmaceutical properties of conopeptides, but so far chemical synthesis has been unable to produce correctly folded backbone cyclic analogues of MVIIA.



AEP-mediated cyclization of MVIIA. The N and C termini are joined by a linker sequence which is shown in bold.

In this study, an asparaginyl endopeptidase, AEP-mediated cyclization was used to generate backbone cyclic analogues of MVIIA for the first time; see figure. Cyclization using six to nine-residue linkers did not perturb the overall structures of MVIIA, and the cyclic analogues of MVIIA showed inhibition of $Ca_V2.2$ channels and improved serum stability. The results pave the way for further improving the therapeutic value of conotoxins. They also provide insights for manufacturing desired peptide drug leads in a scalable green way and confirm that the use of AEP transpeptidases is a versatile strategy for peptide backbone cyclization.

P092 Luisa Aguiar

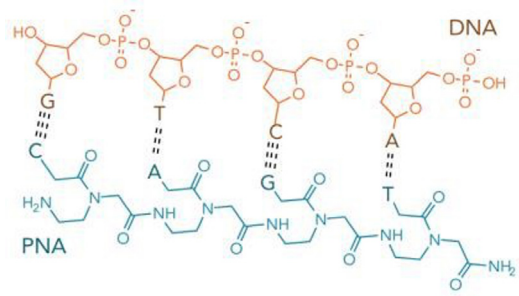
Gyros Protein Technologies

The Flexibility of PurePep® Chorus Enables Optimized Synthesis of Peptide Nucleic Acids

L. Aguiar, S. Lüdtke, M. Muthyala, and R. Zitterbart

Peptide nucleic acids, PNA, are synthetic analogues of DNA and RNA, where the phosphodiester backbone of these natural oligonucleotides, ON, is replaced by a pseudopeptide structure.

PNA hybridize with complementary nucleic acid segments with higher affinity and specificity than natural ON; this allows for a strong, specific hybridization with target genes, with improved sequence mismatch discrimination, rendering them more responsive to single- point mutations than natural oligonucleotides. Moreover, the unnatural backbone of PNA makes them highly resistant to enzymatic degradation.



These characteristics make them valuable tools for numerous applications, from diagnostics to therapeutics. Limitations to their versatility include poor water solubility and low nuclear targeting capability; however, these have been successfully circumvented by the introduction of chemical modifications of PNA sequences, such as cell-penetrating peptides, which have been proven to be highly valuable in drug delivery and gene therapy.

Just as for peptides, PNA can be obtained through standard SPPS procedures. In this work, the optimization of automated PNA synthesis was carried out using PurePep® Chorus. Different coupling conditions were assessed, with the goal of minimizing the number of equivalents used and maximizing the final crude purity.

YI-P093 Skander Abboud

The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology

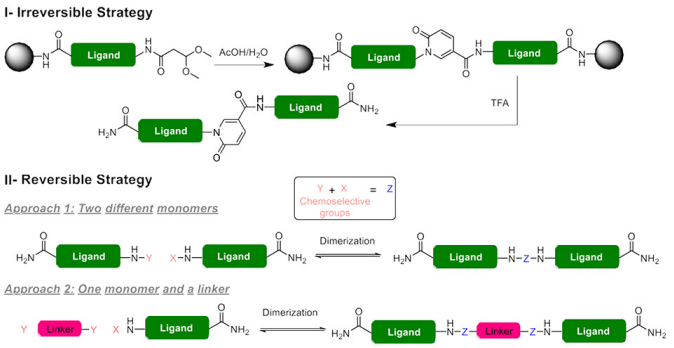
Irreversible and Reversible Strategies for Generating Peptide and Peptoid Dimers

Skander A. Abboud, Weijun Gui and Thomas Kodadek

Small peptides and peptoids have demonstrated potential as drug candidates, but their relatively low affinity for certain targets can impede their practical applications. To overcome this limitation, various strategies have been developed to increase the affinity of these compounds. One effective strategy is the creation of bivalent ligands, which are two monomers linked together to form a compound with a higher affinity. However, this approach requires the synthesis of two separate compounds. A more attractive route from an efficiency perspective is to modify the ligand with a functional group that can homodimerize, thus allowing for the construction of a single modified ligand.

Here, we will present a novel strategy for generating bivalent ligands using acetoacetamide derivatives, which can dimerize

irreversibly into pyridones in the presence of acid as a catalyst. However, these higher molecular weight dimers are generally less bioavailable. To address this limitation, we are exploring a novel strategy to assemble dimers reversibly, allowing the more bioavailable monomeric units to make their way to the target site, then assemble on the dimeric protein to form a stable complex.



To form the dimer, we explored different reversible bioorthogonal reactions and two approaches were considered: one where each monomer is modified with a chemoselective functional group and the second where, and in order to reduce the synthetic burden, a difunctionalized linker was used along with one monomer that was modified with the chemoselective group. In our presentation, we will discuss the advancement of the reversible dimerization strategy and share our discoveries on its effectiveness.

YI-P094 Molly Carter

North Carolina State University

Azapeptoid Synthesis: Determining Amino Acid Compatibility During Late-Stage Azaglycine Alkylations

Molly Carter, Maxwell Bowles, Katelyn Cartrette, and Caroline Proulx

Aza-amino acids are unnatural amino acids that substitute the α-carbon with a nitrogen. This substitution can reinforce secondary structures such as turns and PPII helices, increase stability towards proteases, and alter the hydrogen bonding properties of neighboring NHs. However, there are few examples of azapeptoids and dialkylated azapeptides, despite their interesting conformational properties, atropisomerism. Substitution of azapeptides has been shown to induce atropisomerism due to hindered rotation around the N- N bond. This property of azapeptides could make them interesting candidates for future medicinal applications. Our previous work demonstrated that chemoselective alkylations of internal aza-glycine residues were possible, providing rapid access to azapeptoid and N1, N2-disubstituted azapeptide derivatives of Leu-Enkephalin.

Here, to test the generality of our alkylation procedure, we synthesized a library of aza-tripeptides containing eight different amino acids, Ac-Xaa-azaGly-Phe. For more challenging sequences, we report the impact of **i** amino acid location, N- vs C-terminus, and **ii** side chain protecting groups on the selectivity of the alkylation reaction. Finally, we apply our findings to the alkylation of a more functionally dense Angiotensin derivative.

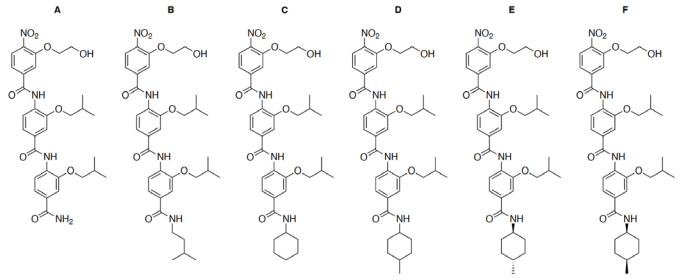
YI-P095 Chia-Yuan Chen

University of Texas at Dallas

A New Therapeutic Strategy for Treating Triple-Negative Breast Cancer via Induction of Endoplasmic Reticulum Stress in LIPA

Chia-Yuan Chen, Kara J. Kassees, Tae-Kyung Lee, Xihui Liu, Suryavathi Viswanadhapalli, Shourya Kumar, Shihong Ma, Liping Chen, Michael Hsieh, Mengxing Li, Gangadhara R. Sareddy, Karla Parra, Eliot B. Blatt, Tanner C. Reese, Yuting Zhao, Annabel Chang, Uday P. Pratap, Zexuan Liu, Carlos M. Roggero, Yan Peng, Rajeshwar R. Tekmal, Carlos L. Arteaga, Ratna K. Vadlamudi, Ganesh V. Raj, and Jung-Mo Ahn

Breast cancer, BC, has become the most common cancer with a rapidly increasing rate throughout the world. Among BC subtypes, triple-negative breast cancer, TNBC, is therapeutically challenging and having the highest mortality rate mainly due to the absence of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 in the tumor cell. Because of the lack of functional receptors, the effective inhibitor drugs have no therapeutic activities in TNBC which result in overexpression of BC cells and subsequent tumor growth and metastases.



However, in this work, we found a therapeutic strategy of treating TNBC. The structure of ERX- 41 was designed from ERX-11, see figure 'A', that was previously shown to effectively block estrogen receptor alpha positive (ERα+ve) BC cell proliferation. However, ERX-11 has poor solubility, and to improve its bioavailability, we progressively substituted the C-terminus from ERX-11 with functional groups which might induce improved flexibility and subsequent solubility in the structure. The expansion of the C-terminus from ERX-11 revealed that ERX-41 induces endoplasmic reticulum, ER, stress resulting in cell death. We defined lysosomal acid lipase A, LIPA, as a viable molecular target and designed and synthesized the molecule ERX-41, see figure 'E', which was further validated by specific LIPA point-mutations to confirm activity via this pathway. Our study implicates a targeted strategy for hormone negative tumors, including breast, brain, pancreatic and ovarian, whereby small, orally bioavailable molecules targeting LIPA block protein folding, induce ER stress and result in tumor cell death.

YI-P096 Canan Durukan

Vrije Universiteit Amsterdam

Peptidomimetic-Fragment Conjugates for Transcription Factor NF-Y Modulations

Canan Durukan, Sadasivam Jeganathan, Mathias Wendt, Sebastian Kiehstaller, Tom Grossmann, Sven Hennig

The Nuclear Factor-Y, NF-Y, transcriptional activator complex consists of three subunits A, B and C. Subunits B and C pre-form a heterodimer. NF-YA is able to bind CCAAT containing DNA promoter regions and to recruit the NF-YB/C heterodimer therefore activating target gene transcription. The development of modulators of such protein-protein interactions is very challenging. Peptides specifically binding to protein complexes such as the NF-YB/C heterodimer are essential for the modulation of biological processes. Binding epitopes derived from PPI interfaces can serve as a starting point for the design of interaction inhibitors.

Mimicking structural motifs can provide an access to novel bioactive compounds, but when excised from the stabilizing context of the tertiary structure, short peptides exhibit flexibility resulting in proteolytic instability and low target affinity. Moreover, peptides show a low tendency to penetrate cells, limiting their therapeutic use. The structure-based design of interaction inhibitors therefore calls for an efficient mimicking of bioactive peptide conformations. The design of so-called 'peptidomimetics' has been a longstanding goal in the chemical sciences.

In my project, we aim to generate fragment-peptidomimetic conjugates: We will covalently attach small- molecular fragment anchors to macrocyclic peptidomimetics. This will lead to a gain in binding affinity, enabling further miniaturization of the peptidomimetic macrocycle. With this we will develop a workflow to improve peptidomimetic based inhibitors. This will enable peptidomimetics with unseen ligand efficiencies suitable for currently "undruggable" biological targets.

YI-P097 Yousra Hamdane

University of Montreal

Turning Peptides with N-Aminoimidazole-2-Ones

Yousra Hamdane, Pradeep S. Chauhan, Suresh Vutla, Darince Truong, Mukandila Mulumba, Huy Ong, William D. Lubell

Lactam dipeptide units have served as conformational constraints in peptide-based drug discovery since the pioneering studies of Freidinger and Veber. The related aza-lactam variants, *N*-aminoimidazolone, Nai, offer similar potential to constrain peptide backbone geometry in β- and γ-turn conformers and are more apt for functionalization with substituents to study side chain orientation.

A general organocatalytic method for the synthesis of 4-, 5- and 4,5-substituted Nai dipeptides will be presented. The synthesis and application of substituted Nai dipeptides will be highlighted in structure-activity relationship studies of cluster of differentiation-36 receptor ligands that modulate innate immunity.

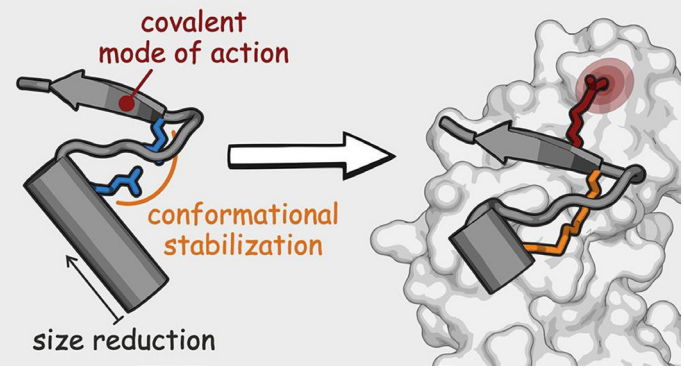
YI-P098 Felix Paulussen

Vrije Universiteit Amsterdam

Covalent Proteomimetic Inhibitor of the Bacterial FtsQB Divisome Complex

Paulussen, F. M., Schouten, G. K., Moertl, C., Verheul, J., Hoekstra, I., Koningstein, G. M., Hutchins G.H., Luirink R.A., Geerke, D.P., van Ulsen, P., den Blaauwen, T., and Grossmann, T. N.

The use of antibiotics is threatened by the emergence and spread of multidrug-resistant strains of bacteria. Thus, there is a need to develop antibiotics that address new targets. In this respect, the bacterial divisome, a multi-protein complex central to cell division, represents a potentially attractive target. Of particular interest is the FtsQB subcomplex that plays a decisive role in divisome assembly and peptidoglycan biogenesis in *E. coli*.



Here, we report the structure-based design of a macrocyclic covalent inhibitor derived from a periplasmic region of FtsB that mediates its binding to FtsQ. The bioactive conformation of this motif was stabilized by a customized crosslink resulting in a tertiary structure mimetic with increased affinity for FtsQ. To increase activity, a covalent handle was incorporated providing an inhibitor that impedes the interaction between FtsQ and FtsB irreversibly. The covalent inhibitor reduced growth of an outer membrane-permeable *E. coli* strain, concurrent with the expected loss of FtsB localization, and also affected the infection of Zebrafish larvae by a clinical *E. coli* strain. This first-in-class inhibitor of a divisome protein-protein interaction highlights the potential of proteomimetic molecules as inhibitors of challenging targets. In particular, the covalent mode-of-action can serve as an inspiration for future antibiotics that target protein-protein interactions.

P099 Michael Ferracane

University of Redlands

Insights into Substrate Recognition and Cleavage by O-Glycoprotease Enzymes

M.J. Ferracane, D.J. Shon, K. Pedram, M.F. Haurat, J. Chongsaritsinsuk, A.D. Steigmeyer, K.E. Mahoney, N.M. Riley, N.E. Scott, M.F. Feldman, S.A. Malaker, and C.R. Bertozzi

O-glycoproteases are a family of enzymes that are secreted by certain commensal, that is, *Akkermansia muciniphila* and pathogenic, like *Escherichia coli* and *Acinetobacter baumannii*, bacteria within the microbiomes of humans and other host organisms. These enzymes facilitate the bacteria's colonization of niche microenvironments, invasion of host tissues, and/or evasion of host immune defense systems. O-glycoproteases selectively cleave the protein backbone of serine/threonine O-linked glycoproteins but not their nonglycosylated counterparts.

Herein, we describe current knowledge of O-glycoproteases and highlight recent X-ray crystallography, molecular modeling, site-directed mutagenesis, and mass spectrometry work investigating the enzymes StcE, CpaA, AM0627, and SmE.

YI-P100 Mónica Aróstica

Pontificia Universidad Católica de Valparaíso y Universidad Técnica Federico Santa María
Valparaíso, Chile

Circular Dichroism Spectroscopy: Peptides Secondary Structure at First Glance

Aróstica Mónica, Rojas Roberto, Cárdenas Constanza, and Guzmán Fanny

Peptides are multifunctional molecules with a broad spectrum of application and different mechanisms of action. Like proteins, their structure or folding is related to their function and mechanism of action. In this context, circular dichroism, CD, spectroscopy becomes a useful and versatile tool for the determination of the secondary structure of peptides, due to the diversity of studies that can be performed.

Circular dichroism spectroscopy is a technique, which has its beginnings in 1960 with the commercialization of instrumentation. The foundation of the technique is in the determination of the excitation of the amide transition n–π* and π–π* in the ultraviolet region, measuring the differential absorbance of light circularly polarized to the left and to the right.

The objective of this work is to give a vision of this technique specifically focused on the study of peptides, based on the data obtained during 8 years of work with the determination of the secondary structure of peptides synthesized in the Peptide Design and Synthesis Laboratory. from NBC-PUCV. The compilation and analysis of the information will be carried out from the treatment of the sample, adequate means, working temperature, structural characteristics, databases and variations with respect to other techniques for the determination of secondary structure.

Finally, with this work it is expected to give the guidelines to be able to do spectroscopy studies more efficiently in the determination of the secondary structure of functional peptides.

Acknowledgment:
Proyecto Fondecyt N°1210056

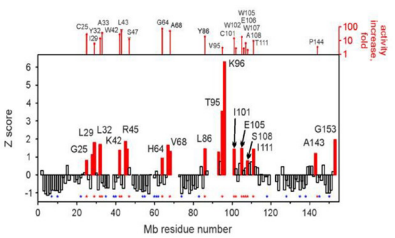
YI-P101 Sagar Bhattacharya

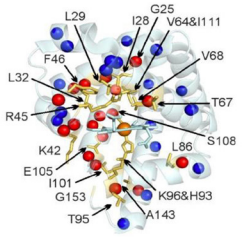
Syracuse University

NMR-Guided Directed Evolution

S. Bhattacharya, E.G. Margheritis, K. Takahashi, A. Kulesha, A. D'Souza, I. Kim, J.H. Yoon, J.R.H. Tame, A.N. Volkov, O.V. Makhlynets, I.V. Korendovych

Directed evolution is a powerful tool for improving existing properties and imparting completely new functionalities onto proteins. Nonetheless, even in small proteins its potential is inherently limited by the hyperastronomical number of possible amino acid sequences. Many computational approaches have been devised to limit the search space in order to improve efficiency of the approach, yet *all* of them rely on extensive previous structural or bioinformatic characterization, which may simply not be available for protein of interest.





We show for the first time that mutagenic hot spots in protein can be experimentally predicted using simple NMR experiments without *any* prior knowledge of structure, bioinformatics, computational modelling, etc. In a proof-of-concept experiment we converted a non- enzymatic protein myoglobin, into FerrElCat, a highly efficient Kemp eliminase using only three mutations. Kemp elimination emerged as an excellent model reaction to test various protein design and evolution approaches, which allows us to directly compare our results to those of others. The catalytic efficiency of FerrElCat is on par with those shown by natural enzymes and only 1-2 orders of magnitude away from the diffusion limit.

This unprecedented 62,000-fold improvement in catalytic efficiency during the course of directed evolution was achieved using instrumentation readily available to the vast majority of scientists and does not require highly specialized facilities.

From the practical point of view, our results drastically expand the possibilities for evolving enzymes to promote new

chemical transformations. From the fundamental standpoint, our results for the first time *prospectively* validate the recent paradigm-shifting work that links protein dynamics and evolution of enzymatic function.

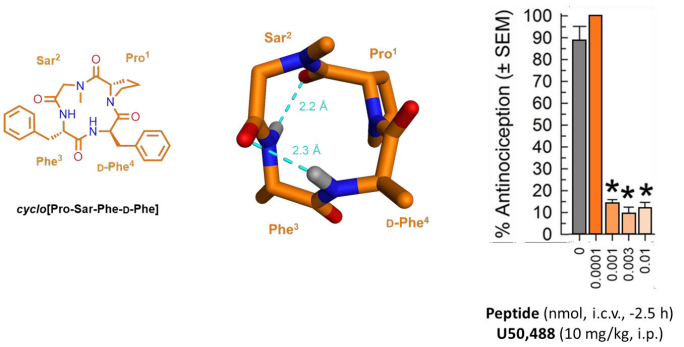
P102 Alejandra Cordova

University of Redlands

Synthesis and Study of Opioid Cyclic Tetrapeptides

A. Cordova, A. Almazo Garcia, D. Plata, Y. Song, C.-T. Zee, B.A. Jenkins, J.J. Cellini, A.C. Brice-Tutt, S.O. Eans, L.L. Wilson, G.G. Simpson, H.W. La Force, B. Okamoto, O. Morán Castro, I. Rodriguez, J.P. McLaughlin, J.V. Aldrich, J.A. Rodriguez, and M.J. Ferracane

The opioid epidemic has been an ongoing issue for decades, with hundreds of thousands of overdose deaths involving an opioid. Opioids act as both agonists and antagonists of the opioid receptors, mu, kappa, delta, which regulate pain and addiction.



The cyclic tetrapeptide *cyclo*[Pro-Sar-Phe-D-Phe] has shown promise in treating pain and addiction in mouse model systems. This peptide also demonstrates blood-brain barrier permeability and oral bioavailability, necessitating further study.

As such, the lead and related analogs have been synthesized and characterized by NMR, molecular modeling, X-ray crystallography, and *in vivo* assays to investigate the structure-activity relationship of opioid cyclic tetrapeptides. Herein, we report preliminary findings.

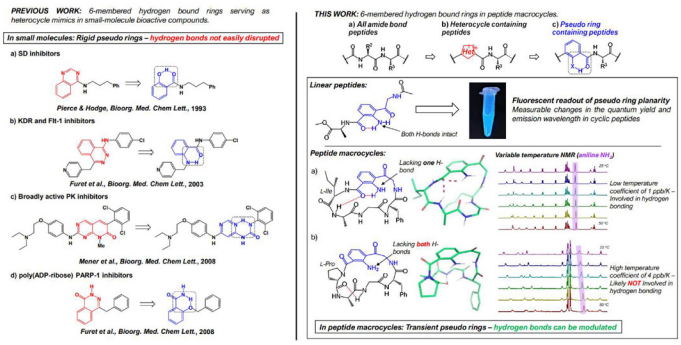
YI-P103 Matthew Diamandas

University of Toronto

Pseudo Rings as a Novel Conformational Control Element in Peptide Macrocycles

M. Diamandas and A. K. Yudin

Heterocycles have played a pivotal role in the discovery of bioactive small-molecules. Emerging modalities such as macrocycles, peptides, unnatural oligonucleotides, and others have adopted some of the findings made in small-molecule chemistry. For instance, the structures of synthetic macrocycles often feature heterocycle grafts that mimic naturally occurring amino acid residues while increasing chemical stability and serve to increase membrane permeability. Despite these advances, one particular small-molecule control element – pseudo rings, comprising of an intramolecular hydrogen bond in place of covalent linkages – has not been applied to control and modulate the structures of complex macrocycles.



This paper examines the unique structural features of pseudo ring containing peptide macrocycles. At the same time, this study offers a methodology to examine conformational equilibria in peptidomimetic macrocycles through computational and spectroscopic techniques. To date, we have shown that we are able to disrupt the intramolecular hydrogen bonding network of a 6-membered pseudo ring in a series of peptide macrocycles. The pseudo rings were disrupted through strain imposed by the peptide backbone itself rather than solvent effects, for example, utilizing polar protic solvents in place of aprotic solvents. This observation is important as these pseudo rings are otherwise quite stable in aprotic solvents. Studies indicate that 6-membered pseudo rings like the ones studied in this article exist in a tightly-bound, rigid conformation in > 90 % entries in the literature. We have shown an ability to control the peptidic system such that specific hydrogen bond donors and acceptors of the pseudo ring system are either embedded within the peptide's core or exposed to the solvent. This is particularly important for the purposes of peptide passive permeability - an ongoing challenge in peptide research - as well as for specific binding of cyclic peptides in enzyme active site binding pockets.

P104 Alexandria Brackbill

CEM

Using Elevated Temperatures to Enable the Purification of Hydrophobic and Difficult Peptides

Alexandria Brackbill

Peptide purification is recognized as a major challenge due to the subtle differences between the desired target and synthetic impurities. This challenge often results in inefficient and time- consuming processes when using chromatography. At the smaller scale range of analytical chromatography,

heating is relatively straightforward to apply due to smaller column IDs and relatively low flow rates. Efficient heating is generally implemented by heating the outside of the column as well as a short section of tubing on the inlet side of the column - in order to pre-heat the mobile phase. However, at preparative scales where larger column IDs and higher flow rates are used, uniform heating of the mobile phase and internal dimensions of the column is more difficult and requires a more sophisticated means of mobile phase pre-heating.

Due to the added difficulty, heating is not often used in preparative chromatography which contributes to decreased performance and efficiency. However, with the introduction of the CEM Prodigy system which includes an integrated heating system, a column oven and mobile phase pre-heater optimized for high flow rates, efficient heating at preparative scales is easy and straightforward.

YI-P105 Md Al-Amin

Arizona State University

Development of DNA-Templated Method for Synthesis of Polypeptides/Pproteins

Md Al-Amin, Nicholas Stephanopoulos

The ability of nucleic acid, DNA, to template protein synthesis in the living system facilitates the advancement of proteins with new structure and functions. We report the development and implementation of a method to couple multiple peptide fragments into proteins using DNA-template directed copper assisted azide-alkyne cycloaddition, CuAAC, reaction. As proof of principle, we synthesized two peptide fragments using a solid phase peptide synthesizer, purified using reverse phase HPLC and characterized by MALDI mass spectrometry.

We introduced cysteine in each peptide to conjugate them with DNA through thiol-maleimide chemistry. We also incorporated two non-natural amino acids such azidolysine in one peptide and propargyl alanine in the other peptide to link them together through CuAAC reaction. The peptides were conjugated with distinct DNA handles through thiol-maleimide bioconjugation reaction and purified them using HPLC and characterized them using running a denaturing polyacrylamide, PAGE, gel. Then we utilized the programmability of DNA to bring the peptides together. The DNA-peptide conjugates were annealed with the template to bring the two peptides into proximity that enhanced the effective molarities of the reactive groups. The peptides were coupled efficiently through use of CuAAC click reaction and the product was characterized by using MALDI mass spectrometry and PAGE gel electrophoresis.

The designed DNA-templated method implemented to synthesize a severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, inhibitor protein called LCB1, which can bind tightly to the human angiotensin-converting enzyme 2, ACE2, receptor and block the interaction to the ACE2. For this purpose, we divided the LCB1 protein into three helices and coupled them into protein using our DNA-templated click reaction. This method allows us to introduce multiple non-natural amino acids in the protein and has the potential to access any large proteins, synthetic polymers and DNA-peptide biomaterials.

P106 Jacob Byerly-Duke

Iowa State University

Thioimidate Solutions to Thioamide Problems: A Generalized Methodology to Incorporating Thioamides in Solid Phase Peptide Synthesis

Byerly-Duke, J., and VanVeller, B.

The prevalence of post-translational modification in natural protein synthesis suggests that expanding beyond the suite of functional groups found in the 20 canonical amino acids can impart specialized utility to peptide-based biomolecules. One modification witnessed in nature is the installment of thioamides over traditional oxoamides along the backbone of peptides. This alteration of the main chain via biosynthetic pathways suggests an evolutionary benefit to exploiting thioamides in peptides.

The perception that current synthetic methods to install thioamides are sufficient is belied by anecdotal reports of failed synthetic campaigns. Such projects to produce thioamidated peptides of interest are often abandoned or require complex methods to sidestep failure to obtain the intended product. We catalogue the major pitfalls associated with current methods to synthesize thioamide-containing peptides and demonstrate the utility of thioimidate protecting groups as a means to side-step many of the problematic synthetic issues surrounding thioamide backbone modifications.

This work designs a more applicable path to access unexplored sequence space for thioamides in peptides: improved methods encourage a 'plug-n-play' synthesis of thioamides, where straightforward inclusion of thioamides during SPPS can require as little troubleshooting as possible and become more routine.

YI-P107 Maxwell Austin

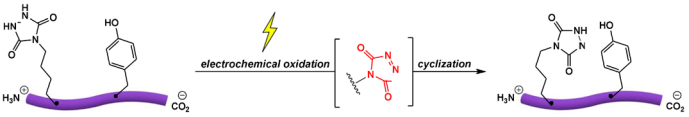
University of Utah

Triazolinedione-Based Cyclization Methods for Antimicrobial Peptide Structural Stabilization

Maxwell J. Austin, Jesus Sandres, E. Dalles Keyes, Zach Nguyen, Georgia J. Brach, Shelley D. Minteer, Ryan E. Looper, and Andrew G. Roberts

Antimicrobial peptides, AMPs, are a promising, yet underdeveloped class of therapeutics with structural characteristics

that go beyond traditional drug discovery guidelines. Though structurally diverse, most AMPs have defined peptide secondary structures that promote their mechanisms of action. A common hypothesis is that the stabilization of these peptide secondary structures may enhance their biological properties. The broad goals of this research are to develop residue-selective cyclization methods that can be applied to mimic and stabilize the secondary structural features of bioactive peptides.



Triazolinediones, TADs, are electrophilic dienophiles with remarkably chemoselective reactivity that has enabled applications in organic synthesis, chemical biology, materials research, and medicine. Substituted TADs are used for tyrosine-selective bioconjugation reactions that satisfy 'click reaction' chemical requirements.

Previously, we showed that the oxidation of 'urazole' peptides using N-chlorosuccinimide can generate TAD- peptides *in situ* that react spontaneously under basic conditions to provide Tyr-linked cyclic peptides. We are working to develop an electrochemical oxidation method to access Tyr-linked cyclic peptides with anticipated expanded scope and native side chain tolerance. Toward antimicrobial peptide therapeutic discovery, we are implementing electrochemical TAD-based cyclization methods to stabilize the structures of bioactive magainin analog peptides.

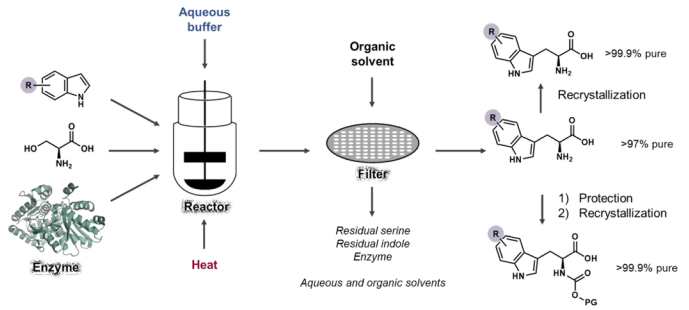
P108 Nat Goldberg

Aralez Bio

Scalable, Green Manufacturing of Noncanonical Amino Acids: Unlocking Hard-to-Make Products through Biocatalysis

Nat Goldberg

The Aralez Bio platform makes novel noncanonical amino acids by leveraging three tried-and-true biotech disciplines: 1 protein engineering, 2 protein production, and 3 biocatalysis. Our process starts with techniques such as directed evolution, which we use to create new, highly active enzyme variants for amino acid synthesis.



Then, these engineered enzyme variants are expressed at large scale using microbial fermentation.

Finally, the enzymes are extracted from their microbial hosts and used as biocatalysts to create a wide variety of noncanonical amino acids. In addition to being a green, environmentally friendly process, our platform is also highly scalable; in particular, we have accomplished kilogram-scale synthesis of ncAAs, and have laid the foundation to perform synthesis at hundreds of kilograms.

YI-P109 Xiaotian Gong

Lafayette College

Developing Solid Phase Synthesis Methods to Synthesize the Cyclic LamD Peptide in *Lactobacillus plantarum*

Xiaotian Gong

Lactobacillus plantarum is a beneficial lactic acid bacterium that is commonly found in the digestive tract and fermented food. It is a commensal gram-positive bacterium that communicates through quorum sensing. LamD is a cyclic peptide with five amino acids, CVGIW, regulating this quorum sensing circuit and is natively cyclic in the form of a thiolactone, LamD-S. LamD-S can proceed to a lactam via an S-to-N acyl shift under mildly basic conditions to form a cyclic amide, LamD-N.

Two kinds of LamD derivatives were synthesized through solid phase peptide synthesis with both on-resin and off-resin methods. The goal of the research is trying to synthesize the LamD peptide in the most efficient way. By synthesizing this peptide derivative, scientists can conduct further research into manipulating density-dependent chemical communication mechanisms in this commensal bacteria. This will allow them to investigate how these mechanisms impact microbial ecology.

YI-P110 Amit Chakraborty

University of Kwazulu-Natal

Expanding Toolbox for Cysteine Chemistry in SPPS

Amit Chakraborty, Sinenhlanhla N. Mthembu, Ralph Schönleber, Fernando Albericio, and Beatriz G. de la Torre

Cys is a unique amino acid, because it is forming part of the two key peptide bonds, the amide and the disulfide. In addition, it is the most susceptible residue for rendering side- reactions, racemization, elimination, alkylation, et cetera, which depend on the protecting groups used for the thiol function and are position and sequence dependent.

Herein, we will review all thiol protecting groups developed by our group and those commercially available in terms of avoiding side-reactions. Those associated to the elongation

of the peptide sequence are minimized by a proper combination of the protecting groups and the base used to remove the Fmoc group. On the other hand, side-reactions produced during the global deprotection are managed by the right combination of scavengers, time and temperature.

Special attention will be paid to the use of protecting group removable under reducing reagents. In this regard, we have engineered the SIT whose use overcome all drawbacks associated to the StBu. Thus, SIT is a secondary thiol, which render a disulfide based protecting group stable in the presence of piperidine and labile towards mild reducing agents such as DTT in both solution and solid-phase.

We have also explored the chemoselective disulfide formation by thiol-disulfide interchange in SIT-protected Cys peptides. SIT facilitates the exchange under mild basic conditions, pH≤8, in absence of any oxidants. Several SIT-protected Cys-peptides comprising one to three disulfide bonds were synthesized - oxytocin, somatostaninamide, conotoxin, linaclotide. In all cases, SIT directed the disulfide based cyclizations in shorter time with impressive HPLC purity profiles.

P111 Samanthreddy Kedika

National Cancer Institute

Chemical Synthesis of Histone Lysine Coenzyme A Adducts to Capture P300 Acetyl Transferase

Samanthreddy Kedika, Tim Strutzenberg, Dmitry Lyumkis, and Terrence Burke Jr.

P300 is a histone acetyl transferase that transfers the acetyl group from coenzyme A, acetyl-CoA, to substrate lysine residues on nucleosomal histones. Currently, there are no crystal structures reporting how full P300 machinery forms complexes with nucleosomes. Since P300 acetylates multiple lysine residues on histones, different structures exist in equilibrium, which is challenging for structural analysis.

Therefore, alternative strategies are needed to capture the P300 complex at a single lysine residue. Previously, lysine acetyl-CoA antagonists have been reported to inhibit P300.2 However, these cannot be employed in generating homogenous P300-nucleosome complexes owing to their small size.

We propose that appending a CoA adduct onto histone lysine residues may be able to specifically "capture" P300 species by: **i** Increasing the histone binding affinity towards P300, since the adduct should be able to interact with both the lysine and CoA substrate-binding pockets and **ii** Inhibiting the P300 from catalyzing reactions at remaining lysine residues.

We have approached synthesizing designer histones having CoA adduct at specific lysine residues using native chemical ligation. Herein, we report both semi-synthetic and total synthetic approaches towards generating CoA-modified histones. This approach should facilitate advancing the current structural understanding P300 nucleosome assembly and mechanisms of acetylation.

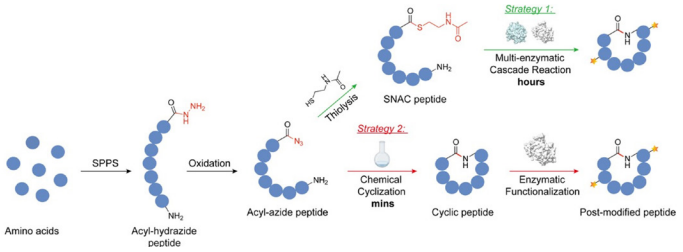
YI-P112 Yaoyu Ding

King's College London

Better than Nature: Facile Chemical Peptide Macrocyclisation

Yaoyu Ding, Gustavo Perez-Ortiz, and Sarah M. Barry

Growing intertest of cyclic peptide has been witnessed in the past two decades, because its rigid structure and moderate molecule size confer it high affinity and selectivity for multiple protein targets. Head-to-tail peptide backbone cyclisation is still challenging, due to undesirable side reaction and low reaction yield. Non-ribosomal peptide Rufomycin, a familty of cyclic heptapeptide show outstanding inhibition, MIC < 0.1 μM, towards *M. tuberculosis* and *M. abscessus* via binding bacteria enzyme ClpC1 proteolysis. In nature, the cyclic precursor is released through macrocyclisation by RufT thioesterase, TE, domain. As antimicrobial resistance is rising up, our goal is to develop a facile cyclisation method that facilitate the production of a rational naturally occuring peptide library.



In this work, we used SPPS to assemble linear peptide precursor and employed the RufT-TE domain to cyclise peptide, as a chemoenzymatic approach to afford rufomycin analogue. Alternatively, we also developed an efficient chemical method as an *in-situ* reaction to give cyclic peptides in ~90% conversion within 15 minutes, then functionalised by two P450s, see figure above. Notably, it can also cyclise peptides that cannot be achieved through enzymatic reaction.

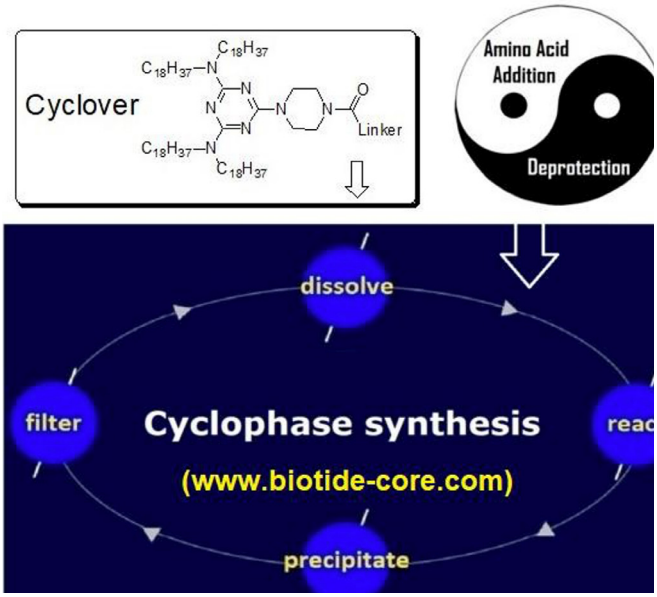
From our work, it indicates that the linear order of modules in non-ribosomal peptide synthetases, and thus the linear peptide sequence, may be vital in dictating the ability of a peptide to cyclise.

P114 **Yongfu Li**
Biotide Core, LLC

Cyclophase Synthesis, a Productive Process for Peptide Manufacturing

Yongfu Li

Success of solid phase peptide synthesis, SPPS, depends largely on the resin, building blocks, reagents and solvents used. Most recently, Amino-Li-resin, the second generation of functionalized polyacrylamide support, has afforded superior results in SPPS, and even in "green" aqueous SPPS. However, SPPS, in general, has been a wasteful process. Greener approaches have attracted pressing attention for protecting the environment and enhancing the productivity.



Cyclover-Amine and its derivatives are soluble in one set of solvent/s. After a desired reaction completes, the resultant product anchored to Cyclover - structure shown in the figure above - can be isolated as a pure solid by adding a miscible solvent, leaving the excess reagents and side-products in solution. With four octadecyl groups attached to a triazine core, Cyclover is more powerful in lipophilicity, more robust in chemical stability than the trialkoxybenzyl tag.

Peptides can thus be manufactured by CYCLOPHASE SYNTHESIS wherein each cycle involves:

- **A -** DISSOLVE the reactants in one volume of solvent/s
- **B -** REACT until the reaction completes
- **C -** PRECIPITATE the product by adding 4 to 5 fold volume of a miscible solvent;
- **D -** FILTER to collect the solid for next cycle.

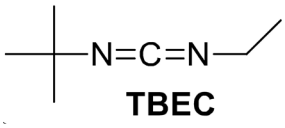
Synthesis of a model peptide has demonstrated that Cyclover can expedite the isolation of solid product of each cycle with ease and certainty, significantly reducing the use of solvents and related chemicals. Therefore, readily available Cyclover- Amine may be developed as a universal lipophilic anchor for efficient synthesis of therapeutic peptides on an industrial scale.

YI-P115 **Srinivasa Rao Manne**
University of KwaZulu-Natal

A New Generation of Efficient and Safer Coupling Reagents for SPPS

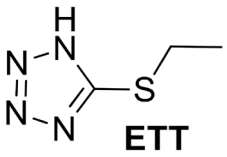
Srinivasa Rao Manne, Beatriz G. de la Torre, Fernando Albericio

For many years, carbodiimide/additive methodology is the most broadly used approach for amide/peptide bond formation. During the last decades, OxymaPure served as a promising alternative to HOBt-based additives in terms of safety, coupling efficiency and optical purity. Recently, Kolis and others. reported that OxymaPure can react with DIC, forming linear adduct and further it can lead to the formation of an oxadiazole and HCN as byproducts.



TBEC

We thoroughly studied the coupling conditions and found that the formation of oxadiazole and HCN can be minimized and even suppressed with an appropriate protocol for the sequence of reagent addition. Further, we studied the reaction of OxymaPure with a series of carbodiimides and reached the conclusion that N-tert-butyl-N'-ethylcarbodiimide, TBEC, has a reactivity similar if not superior DIC and outperforms without any HCN formation.



ETT

In our continuous search of new additives, we have identified 5-(ethylthio)-1*H*-tetrazole, ETT, which has been broadly used in oligonucleotide synthesis, as the most excellent cyano-free alternative to OxymaPure with a comparable efficiency to both OxymaPure and HOAt.

Finally, a new Water-Soluble Carbodiimide special devoted for Green Chemistry will be discussed - patent pending. This new reagent overcomes all drawbacks associated with the use of EDC.Cl, which is translated in a better performance.

YI-P115 **Srinivasa Rao Manne**
University of KwaZulu-Natal

A New Generation of Efficient and Safer Coupling Reagents for SPPS

Srinivasa Rao Manne, Beatriz G. de la Torre, Fernando Albericio

For many years, carbodiimide/additive methodology is the most broadly used approach for amide/peptide bond formation. During the last decades, OxymaPure served as a promising alternative to HOBt-based additives in terms of safety,

coupling efficiency and optical purity. Recently, Kolis and others. reported that OxymaPure can react with DIC, forming linear adduct and further it can lead to the formation of an oxadiazole and HCN as byproducts.

YI-P116 Steven Draper

University of Utah

Biotin Orthogonal Streptavidin System - BOSS: An Application of Mirror-Image Streptavidin and Biotin

S. R. E. Draper, P. Spaltenstein, R. J. Giesler, and M. S. Kay

Streptavidin, SA, and D-biotin have one of the strongest known binding interactions, with a K_D in the fM range. This extraordinary affinity has led to their ubiquitous use in biomedical research and diagnostics, though several challenges limit its ***in vivo*** utility, including interference from endogenous biotin and streptavidin's high immunogenicity as a foreign bacterial protein. Mirror-image proteins — D-proteins, composed of D-amino acids — offer an elegant potential solution to these problems, as they are generally inert to proteases and therefore cannot be digested for MHC presentation to the immune system.

These properties suggest that $_D$-SA will have greatly decreased immunogenicity and increased half-life compared to L-SA. Additionally, by the law of mirror-image symmetry, D-SA and mirror- image biotin, L-biotin, will have identical exceptional affinity as the natural pair, L-SA and D-biotin.

We have discovered through isothermal titration calorimetry that this interaction is highly stereospecific, with nearly a billion-fold preference for the natural ligand over the mirror-image ligand. This result suggests that D-SA and L-biotin can be used in living systems without endogenous biotin interference, thus acting as a biotin orthogonal streptavidin system, BOSS.

As a first step towards developing BOSS, we have synthesized L-SA using a three-segment native chemical ligation strategy that employs a "helping hand" semi-permanent solubilizing tag. We will discuss our efforts to optimize this ligation strategy, scale up the synthesis, and optimize yield.

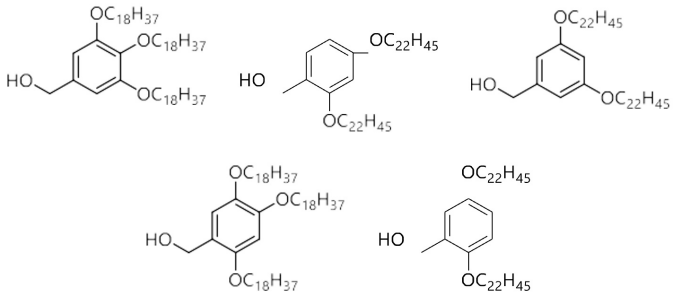
P117 Yohei Okada

Tokyo University of Agriculture and Technology

Tag-Assisted Liquid-Phase Peptide Head-to-Tail Cyclization

Y. Okada, S. Yamagami, Y. Kitano and K. Chiba

In addition to small molecules and antibodies, peptides have proven to be promising therapeutic candidates. In particular, cyclic peptides are effective platforms to design new medicines since they tend to have unique biological activities with improved enzymatic stabilities. However, a chemical synthesis of such cyclic peptides remains challenging for several reasons, including undesired intermolecular side reactions during the target intramolecular cyclization. Although a solid-phase method has been on a first place in the field of peptide synthesis and it has demonstrated efficient and automated production of several bioactive peptides, however, a greener methodology that can also access cyclic peptides is of great importance.



Tag Variations

We have been developing tag-assisted liquid-phase method to prepare several bioactive peptides in larger scale with improved purities. Several hydrophobic benzyl alcohols can be used as soluble supports to facilitate liquid-phase peptide synthesis.

In this work, we apply the tag strategy to synthesize cyclic peptides, which will be presented in detail in this poster.

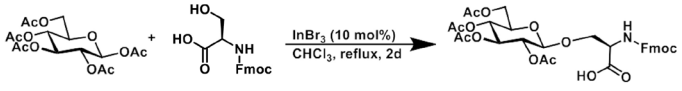
YI-P118 Jacob Dylan Fredman

University of Arizona

Practical Synthesis of Fmoc-Protected Serine Glucosides

J. Fredman, K. Olson, A. Myloserdnyy, T.E. Smith, R. Polt

Membrane impermeability and poor *in vivo* stability of amino acid-based therapeutics has been a prevalent issue for synthetic chemists in the field of peptide drug development. Previous studies addressed this concern through glycosylation of peptides, yet traditional synthetic routes typically require unstable reagents, inert conditions, and inefficient routes for large scale production.



In this poster we present a practical, large-scale method to produce protected serine glucosides for SPPS. The benefits of this methodology include its high yield in a stereoselective manner, without the use of column chromatography. This results in diverse functionality, economic improvement over traditional routes, and more appropriate scalability for synthesis of large quantities. Production of this glucoside is significant for the understanding of glycobiology, drug synthesis, and other demands for glycopeptides.

YI-P119 Garrett Evenson

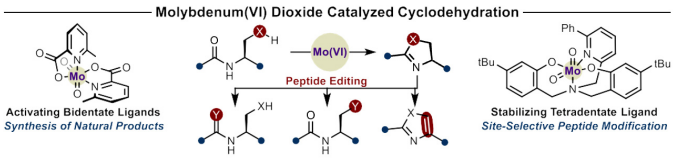
University of Colorado at Boulder

Site-Selective Peptide Editing With Molybdenum(VI) Catalysis

Garrett E. Evenson, Wyatt C. Powell, and Maciej A. Walczak

Post-translational modifications are a common strategy to increase structural and functional diversity of peptides and proteins. While synthetic methods that introduce modifications on side chains have received considerable attention, transformations focused on modifying the peptide backbone remain significantly underdeveloped. Backbone functionalization strategies face challenges due to the stability of the amide bond and the harsh conditions required for activation.

To address these challenges, we focused on azolines as equivalents of reactive amides. Azolines and their oxidized counterparts, azoles, are common in natural products such as metal chelating siderophores and thiopeptide antibiotics. Biosynthetically, these heterocycles are installed by cyclodehydratases, a class of enzymes that activate serine, threonine, and cysteine residues. Inspired by this enzymatic pathway, we developed biomimetic molybdenum(VI) dioxide catalysts that introduce azoline heterocycles into peptides and natural products with high selectivity and broad functional group tolerance.



The first-generation catalyst features a pair of bidentate picolinate ligands and has enabled the synthesis of azole fragments of the thiopeptide antibiotics micrococcin P1 and thiocillin I. The catalyst efficiently prepared oxazoline and thiazoline derivatives containing sensitive functional groups and highly coordinating groups that would deactivate other similar catalysts.

The second-generation catalyst includes a tetradentate ligand that improved thermal stability and facilitated the selective functionalization of natural products, pharmaceuticals, and strained cyclic peptides. In all, we have developed two highly effective catalysts that offer mild conditions for natural product synthesis and for the diversification of natural and unnatural peptides.

P120 Diedra Shorty

CEM

Ultra-Efficient Solid-Phase Peptide Synthesis: Advancements in Microwave-Assisted SPPS

Deidra Shorty

Here, we introduce an ultra-efficient solid-phase peptide synthesis, UE-SPPS, process that, for the first time, eliminates all washing steps for standard Fmoc chemistry while merging the coupling and deprotection steps into a continuous one-pot process under microwave SPPS conditions. A fundamental breakthrough of UE-SPPS is the elimination of the Fmoc removal base without any washing.

This method has been successfully applied to a broad range of sequences, with lengths up to 89 amino acids, including the glucagon-like peptide, (GLP)-1, liraglutide. All peptides were obtained in high purity, fast synthesis times, and without any washing using UE-SPPS.

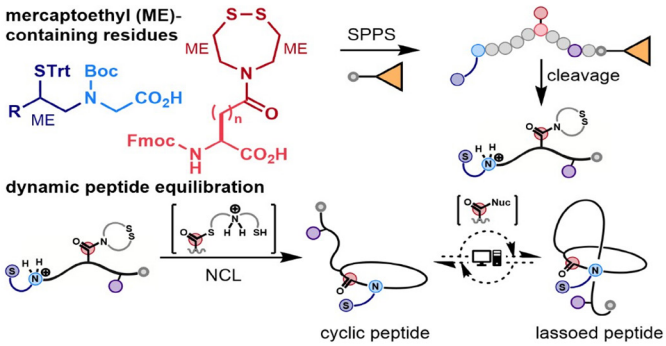
YI-P121 Marcus Mifflin

University of Utah

Scalable Access to Solid-Phase Peptide Synthesis Reagents for Native Chemical Ligation - An Amide Bond Equilibration Approach Toward the Chemical Synthesis of Lasso Peptides

Marcus C. Mifflin, Abagail Darling, and Andrew G. Roberts

Native chemical ligation, NCL, is a versatile chemoselective amide bond formation method used in chemical protein synthesis, peptide modification, and cyclization strategies. The β-mercaptoethylamine, ME, scaffold has emerged as a useful alternative to thiolated sidechain and thioester reactive groups used in NCL. Modification of the *N*-terminus with the ME group , Seitz auxiliaries, permits adaptable access to NCL junctions and can be selectively cleaved to liberate the newly formed amide bond. Moreover, orthogonally disulfide masked bis-ME amides, Melnyk cryptothioesters, provide controlled access to electrophilic thioesters through an acyl transfer equilibration. However, these enabling strategies for NCL are often limited by their synthetic accessibility.



To address this limitation, we developed a scalable method to introduce the ME scaffolds into amino acid reagents that are directly incorporated during solid-phase peptide synthesis. Interestingly, the ME acyl transfer equilibration presents a unique opportunity to study mechanically interlocked molecules, which can be accessed using dynamic bond-forming reactions to provide the thermodynamically favored structure. Accordingly, we are using this approach to understand the folding propensity of peptides toward the threaded structures of lasso peptides through reversible cyclization of the isopeptide bond formed between the N-terminus and a

sidechain acid, which encircles the looped C-terminus in the lassoed conformation.

To guide this approach, we are using Rosetta to design sequences that improve the thermodynamic stability of the native peptide, which will be iteratively evaluated under equilibration conditions to assess their lasso-forming capability and understand the stabilization features that could permit access to this unique class of natural products.

P123 Stephan Kudlacek

Menten AI

Design of Cyclic Peptides with Drug-Like Potency and *In Vivo* Efficacy Using Generative AI

S.T. Kudlacek, N. Hernandez, E. Dolan, D. Kokh, P. Finneran, and H. Melo

Cyclic peptides are a validated therapeutic modality in use for a variety of treatments. 46% of approved peptide drugs are cyclic, and more cyclic peptides are currently in clinical trials. Cyclic peptides can be engineered to overcome the historical challenges peptides have faced in drug development. These engineered cyclic peptides contain the favorable selectivity and high affinity of proteins, and the beneficial features of small molecules, including cell permeability and oral bioavailability.

Menten AI is a drug design company which has developed a proprietary generative AI platform to design drug-like cyclic peptides for internal and external drug discovery programs against disease-relevant targets. Using our platform, we designed cyclic peptides with nanomolar *in vitro* potencies that demonstrate *in vivo* efficacy, without the need for experimental optimization. One peptide demonstrated functional *in vitro* inhibition but no initial *in vivo* efficacy. Following one round of *in silico* optimization, a derivative of this peptide was identified that displayed improved systemic exposure and *in vivo* efficacy. We also present the latest development of the platform to perform *de novo* cell-permeable cyclic peptide design.

YI-P124 Lei Yu

Janssen Research & Development

Cyclic Peptide Linker Design and Optimization by Molecular Dynamics Simulations

Lei Yu, Stephanie A. Barros, Chengzao Sun and Sandeep Somani

Macrocyclic peptide is an emerging therapeutic modality that can target protein-protein interactions with high affinity and selectivity. Designing optimal linkers for macrocyclization of peptide hits is an important problem in rational design of peptide therapeutics We present a method for predicting the impact on the conformation of a peptide by varying linker length and chemistry. The method is based on enhanced sampling molecular dynamics simulations and compares the conformational stability of series of cyclic peptides with different linker lengths, substitution patterns, and linker types.

We applied it to three macrocyclic peptide series that bind to PCSK9, trypsin, and MDM2 with secondary structures spanning helix, β-sheet, and loop. In general, we observe high correlation between the structural rigidity and binding affinity of the peptides. This method may be used to design peptides with increased potency and chemical stability.

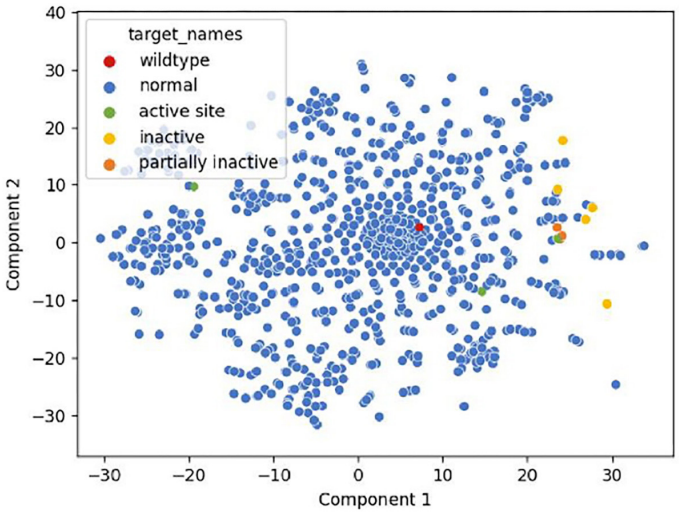
P125 Ewa Lis

Koliber Biosciences

Zero Shot - No Training Data - Machine Learning Approaches for Protein and Peptide Optimization

Lily Lindmeier, and Ewa Lis

The public release of DALL-E in 2021 and ChatGPT in 2023 has ushered in a new era in Artificial Intelligence enabling humans to work along AI to generate images and text. The use of these tools is fundamentally changing the way humans create by providing a variety of starting points, accelerating the process, and eliminating tedious tasks. The awe inspiring success of ChatGPT is based on novel model architectures, transformers, as well as an unsupervised training approach with masked tokens that leverages vast unlabeled datasets.



T7 RNA polymerase was substituted with alanine al all positions and embedded using a pre-trained transformer model. The embeddings were dimension reduced to two components and visualized. Known inactive, partially active and catalytic, active site, mutations as well as wild type sequence are indicated.

This presentation will demonstrate how transformers trained on millions of naturally occurring proteins and peptides can be utilized for protein and peptide optimization going beyond published methods for protein classification and secondary structure prediction. The presentation will focus on zero shot learning that enables *de novo* predictions of substitutions that disrupt or enhance function. Correlations of >65% to measured activity have been observed with this approach.

When visualized in dimension reduced embedding space, see figure above, known inactive substitutions occur on the edges of the evolutionary- learned embedding space and cluster together.

Discovery and optimization of peptides using examples from immuno-modulating and anti-microbial peptides will be shown. Zero shot learning as well as learning on limited datasets will be demonstrated.

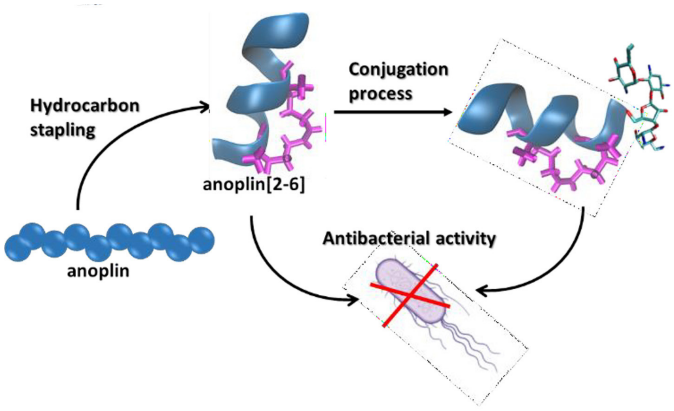
YI-P126 Julia Macyszyn

Centre of New Technologies
University of Warsaw

Antibacterial Activity of Hydrocarbon Stapled Peptides and Peptide Conjugates with Aminoglycosides

J. Macyszyn, M. Wojciechowska, M. Burmistrz, A. Mieczkowski, J. Miszkiewicz, and J. Trylska

The increasing problem of microbial resistance to antibiotics has intensified the search for new antibacterial agents. We joined in this research by modifications of antimicrobial peptides, AMP. We have chosen two approaches: hydrocarbon stapling of the active structure of AMP and conjugation of them with aminoglycosides. We hypothesized that, firstly, the stable helical structure of the peptide could enhance its antimicrobial activity. Secondly, the synthesis of the aminoglycoside-peptide hybrid, will contribute to the eradication of aminoglycoside-resistant bacterial strains.



We selected anoplin - a linear, natural AMP peptide - Gly-Leu-Leu-Lys-Arg-Ile-Lys-Thr-Leu-Leu-NH_2 - that can form an active amphipathic helical structure near the lipid environment. However, anoplin has weak antibacterial activity and poor stability which prompted us to modify its structure. We obtained anoplin analogs by hydrocarbon stapling. Anoplin stands out with an 8-fold enhanced antibacterial activity on *Escherichia coli* K12 cells and higher protease resistance. In a further step, anoplin and anoplin were conjugated to neomycin and amikacin by a non-cleavable triazole ring or cleavable disulfide bond. Aminoglycoside-peptide hybrids showed slightly improved antibacterial activity against strains resistant to amikacin or neomycin.

Although the conjugation of a peptide to an existing antibiotic seems a promising approach, the best antibacterial activity improvement so far was achieved by hydrocarbon stapling. This modification of AMP stabilized its active helical structure and improved permeabilize and destabilize Gram-negative bacterial membranes.

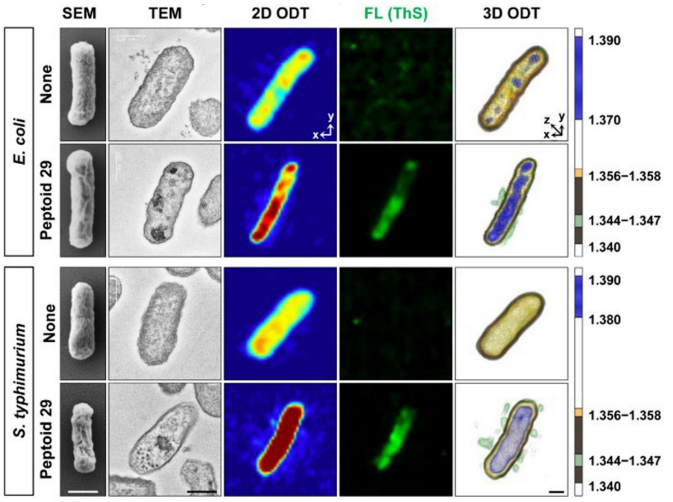
P127 Jiwon Seo

Janssen Research & Development

Multi-Target Engagement of Antimicrobial Peptoids: Synthesis, SAR analysis, and Mechanism Study

Minsang Kim, Yeongmi Cheon, Dongmin Shin, Jieun Choi, Josefine Eilsø Nielsen, Myeong Seon Jeong, Reidar Lund, Håvard Jenssen, Annelise E. Barron, Seongsoo Lee, and Jiwon Seo

Antimicrobials with multiple mechanisms of action have proved successful as natural chemical weapons, and multi-target engagement is considered an effective strategy to overcome the threat of bacterial infection. Antimicrobial peptoids are generally known to kill bacteria by disrupting their membranes. However, non-specific aggregation of intracellular nucleotides and proteins has been proposed as the primary mechanism. This multi-target mechanism was directly associated with fast killing kinetics of antimicrobial peptoids.



To visualize bacteria in the presence of antimicrobial peptoids, we performed a label-free and live-cell imaging of *E. coli* treated with antimicrobial peptoids using optical diffraction tomography, ODT. Structure-activity relationship, SAR, analysis of a library of indole side chain-containing peptoids provided a lead peptoid, compound 29, with potent antimicrobial activity and enhanced selectivity.

With peptoid 29, we demonstrated that membrane disruption and intracellular biomass flocculation in bacteria were the main mechanism of bacterial killing by performing various biochemical assays and imaging experiments, for example, ODT, fluorescence, and TEM.

YI-P128 Jing Wang
Tufts University

Investigating the Cell Penetration of Proteins and Lipid Nanoparticles, LNPs, with the Chloroalkane Penetration Assay, CAPA

Jing Wang, Yamin Li, Qiaobing Xu, Joshua Kritzer

Different chloroalkane tag, CT, and labeling level's affection on protein interacting with cell and LNPs was studied. Protein with more CT has higher cell uptake, but not functional delivery. CT per protein was selected for performing CAPA because both too much and less CT negatively affected protein and LNPs interaction.

72 different unformulated LNP was screened by CAPA. CAPA results varied from one time to three times of positive cells to the cells only treated with CT-protein. 80-O18 had the best cytosolic delivery ability based on CAPA and was approved by GFP biodistribution screen and Cre functional delivery.

P129 Kenji Usui
Faculty of Frontiers of Innovative Research in Science and Technology , FIRST, Konan University

Rapid Sensitization Assessment Using Peptidyl PEGA Resins as Alternatives to Animal Testing

Kenji Usuia, Masae Fukudaa, Aki Nishimuraa, Fumihiro Kayamoria, Hideto Ariumib, and Yoshio Hamada

The direct peptide reactivity assay, DPRA, assesses the binding reaction of chemical substances to Lys or Cys of the peptides as an effective alternative to animal testing for skin sensitization. However, DPRA cannot be utilized in assay for poorly water-soluble chemicals, and HPLC in the protocol is time-consuming and difficult to handle compared to other general analyses. Recently, we had developed a novel sensitization assay, chromophore solid phase peptide reaction assay, C-SPRA, to solve these problems. In this study, we further improved the C-SPRA system and optimized its protocol for assessing numerous types of samples in a short period of time, 10-30 minutes, using PEGA resins.

At first, we selected the resin for immobilization of the peptides for rapid assessment of numerous types of samples. PEGA (Poly[acryloyl-bis(aminopropyl)polyethylene glycol]) resin was chosen as much more suitable for the rapid reaction of chemicals and peptides. Then we optimized the assessing protocol. Especially, the preparation protocol of the peptidyl resin before the assessment was simplified. In the preparation protocol we could skip the swelling step every time before the assay using PEGA resin. Throughout these studies we successfully modified the conventional C-SPRA. Our method allows a next-generation high-throughput measurement for in vitro skin sensitization assessment with easy handling in 10-30 minutes.

P130 Jeahoon Yu
Seoul National University

Cardiolipin-Specific Peptides, Designed to Possess Cell Penetrating Ability, Rescue Mitochondrial Dysfunction

Gwangsu Shin, Soonsil Hyun, Dongwoo Kim, Yoonhwa Choi, Kyu Hong Kim, Dongmin Kim, Soie Kwon, Seung Hee Yang, Kyeong-Ryoon Lee, and Jaehoon Yu

Chemically important events in mitochondria occur in the inner membrane, where cardiolipin, CL, a specific phospholipid, causes curvature needed for functional proteins to function properly However, when abnormal CL remodeling occurs, it compromises this curvature and leads to mitochondrial malfunction. CMP001, an amphipathic dimeric α-helical peptide, penetrates into cells, binds to multiple CLs, and regenerates the mitochondrial inner membrane curvature. CMP001 reduces reactive oxygen species, ROS, and increases ATP production in mitochondria.

The first target indication of CMP001 is acute kidney injury, AKI. AKI is derived from mal-function of the glomerulus and proximal epithelial cells that are lack of glycolysis to generate ATP. Mitochondrial dysfunction is central to the pathogenesis of AKI, regardless of whether I/R injury, sepsis or exposure to toxic reagents is the initiating insult. There is no specific therapeutic available so far. CMP001 attenuates nephrotoxicity induced by colistin in mouse model. CMP001 has the potential to be developed as a promising therapeutic for various diseases related to mitochondrial dysfunction.

YI-P131 Jason Marmorstein
University of Pennsylvania

Peptide Materials for Environmentally Friendly Extraction of Rare Earth Elements

Marmorstein, J. G., Wang, Y. Hummingbird, E. J., Crane, S., Dmochowski, I. D., Stebe, and K., Petersson, E. J.

Industrial uses for rare earth elements, REEs, are increasing exponentially, which has highlighted the importance extracting single REEs from ores. A widely used method for REE separation involves solvent extraction to move the REE of interest from an aqueous phase into an organic phase, but this process is environmentally unfriendly. Calmodulin and lanmodulin are two naturally occurring proteins which have been shown to have affinity for REEs. The binding loops of these proteins has been mimicked as 17 amino acid peptides, retaining high nM to μM affinity for REEs. These peptides, called lanthanide binding tags, LBTs, show little selectivity among REEs, which renders them ineffective for removing one REE ion from a bulk mixture.

Here, we aim to develop peptides with high affinity and selectivity for individual REEs. To do this, we start from the high affinity parent LBT, and mutate residues to examine effects on selectivity. This data, as well as literature data on the selectivity of related peptides, are used as inputs for machine

learning, ML, models that combine experimental data with molecular simulation to create custom score functions for predicting LBT affinity and selectivity. Some mutants tested have shown an increase in selectivity for heavy lanthanides, while others have been able to impart selectivity on light lanthanides. Tb^{3+} is of central importance to this research, as a critical tryptophan residue can participate in energy transfer with Tb^{3+}, resulting in emission at 545nm when Tb^{3+} is bound. In place of tryptophan, an unnatural amino acid, acridon-2-yl alanine, can be inserted and used to instead sensitize Eu^{3+}.

The affinity of the LBTs for the other REEs can be determined through competition assays. In parallel with these ML-guided studies to optimize the LBTs, we are also modifying the peptides to undergo various self-assembly pathways to sequester the REEs to achieve our ultimate goal of a "green" separation strategy for REEs.

YI-P132 Luisa Aguiar

Gyros Protein Technologies

PurePep® Chorus - A Modular All-in-One Platform for Rapid Peptide Synthesis and Purification

R. Zitterbart, S. Lüdtke, D. Sarma

Rapid production of purified peptides is a significant challenge, particularly in neoantigen cancer therapy where time is of the essence to synthesize and purify multiple peptide sequences to help patients awaiting treatment. However, manual transfer from parallel synthesis to sequential chromatography limits throughput, resulting in longer production times. In addition, product loss and low yields for difficult peptides can result in pools with fewer peptides, leading to longer than desired waiting times.

To address these challenges, we introduce a unique integrated workflow that enables peptide synthesis and purification in a single instrument with the flexible PurePep© Chorus synthesizer. With its built-in induction heating technology, followed by automated purification using PurePep® Easy-Clean, PEC, technology, sets of six purified peptides can be produced in less than 24 hours with high reliability.

The PEC technology is based on a catch-and-release, C&R, approach using a universal linker system with a reduction-triggered safety release. The C&R principles are ideally suited for automation in SPPS instruments, as they are based on the same processing principles in fritted reaction tubes.
In this poster presentation, we will introduce this novel and rapid PurePep® workflow and demonstrate the production of selected peptides. This advancement has the potential to significantly reduce the time and effort required for peptide synthesis and purification, making it a valuable tool in the field of neoantigen cancer therapy and beyond.

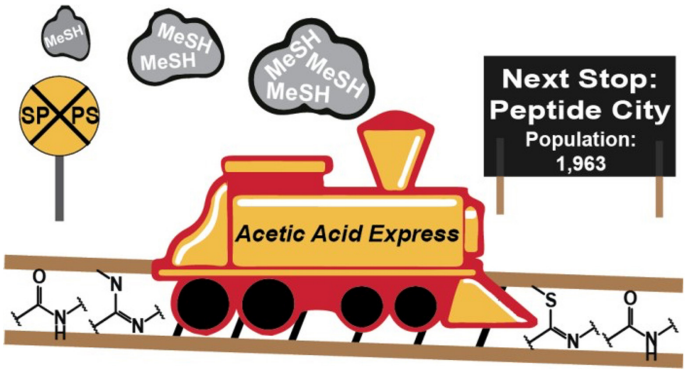
YI-P133 Emily O'Brien

Iowa State University

General Insertion of Amidines along the Peptide Backbone via Solid-Phase and their effects on the Secondary Structure

Emily O'Brien, Krishna K. Sharma, Jacob Byerly-Duke, Luis A. Camacho III, and Brett VanVeller

Post-translational modification in nature has modified proteins via the sidechains and the backbone. These natural processes have changed the tracks of the backbone from poly-amide linkages to incorporate thioamides and amidine connections. Amidines exhibit an analogous structural motif to the amide and are considered an amide isostere. However, what makes amidines unique is the substitution of oxygen for nitrogen, creating an additional site for derivatization.



Unfortunately, amidines have received little attention in linear peptides due to previous inability to access the moiety using standard SPPS techniques. Indeed, this project pushes the boundaries of bio-isosteres and takes amidines to where they have never been before – the backbone of peptide, on resin, during SPPS.

Recently we have developed a general method to insert amidine motifs selectivity along the backbone of peptides. The method has created 'new tracks' for the 'train' of peptide synthesis, and we have leveraged this tool to probe the effects of amidine moieties on peptide helical structure and stability.

P134 Daniel DeOliveira

Mytide Therapeutics

High Throughput Peptide Production: Connecting Synthesis, Analysis, Purification and Delivery with a Digital Thread

Daniel B. DeOliveira, Liam Kelly, William Wiener, Ryan Ballirano, Tori Cordeiro, Vladimir Bellegarde, Matthew Ewing, Abigail Campbell, Mike Valliere, and Dale Thomas III

The fact that more and more peptides and peptido-mimetic products have received FDA approval over the last several years; four in 2020, ten in 2021, and four in 2022, is great news for peptide scientists and the patients awaiting these

peptide-based drugs. Unfortunately, the science, and art, of peptide production has not kept pace like that of other technological advances...that is until now! The recent advances demonstrated by Mytide Therapeutics in being able to provide peptides to organizations that serve a diverse array of applications, immuno-oncology, diagnostics, neoantigen cell therapy, peptide research, et cetera, has demonstrated their commitment to changing the status quo of peptide manufacturing that will enable the peptide drugs of tomorrow.

Using a state-of-the-art fully automated process, Mytide's BioFab2 platform spans all aspects of the peptide manufacturing process steps from synthesis, cleavage, LCMS analysis, purification, lyophilization and even pooling / aliquoting. Mytide has demonstrated the ability to take peptide manufacturing into the 21st century with superior data analytics and produce a product with superior purity and quality.

Our Proprietary peptide solid phase slug flow, SPSF, and disaggregation technology leveraging integrated manufacturing artificial intelligence enables a higher level of purity while operating faster than standard synthesizing practices. The peptide manufacturing process at Mytide is not just simple automation, it enables a complete sequence-to-vial production process that is fully controlled via our NEXUS platform which utilizes machine learning to enable complete peptide production that can be measured in mere hours vs. weeks as is typical for standard peptide production. NEXUS analyzes data from one process step to decide and trigger the next process step and so on until the peptide is finished complete with vialing and lyophilization. Mytide's peptide production process will be presented along with case studies that demonstrate the power and benefit of a fully automated peptide production platform.

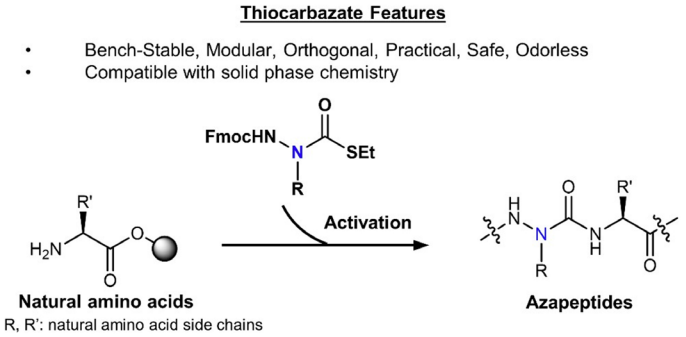
P135 Mingzhu He

Feinstein Institutes for Medical Research

Novel Thiocarbazate Building Blocks: A Universal Synthetic Platform to Construct Azapeptide Libraries

Ahmad Altiti, Mingzhu He, Kai Fan Cheng, Sonya VanPatten, Umair Ahmed, Ibrahim T Mughrabi, Stavros Zanos, and Yousef Al-Abed

Peptides, polymers of amino acids, have a growing role as therapeutics. Their rapid degradation by proteases, however, is an inherent limitation and chemical modifications to native peptides have been used to overcome this weakness. In this work, we describe a functionalized thiocarbazate scaffold as a novel surrogate of natural amino acids, that, once activated, can replace selected amino acid/s in a peptide sequence using standard peptide synthetic methods and machinery.



This methodology facilitates custom editing, replacing targeted amino acid/s with aza-surrogates, of peptides, and has potential to mitigate protease sensitivity, thereby extending half-life/bioavailability while at the same time typically preserving structural features and biological activities. The convenience of this original azapeptide synthesis platform is demonstrated in several bioactive peptides. This novel, bench-stable thiocarbazate-based synthetic platform offers a robust and universal approach to optimize both new and existing peptide-based therapeutics.

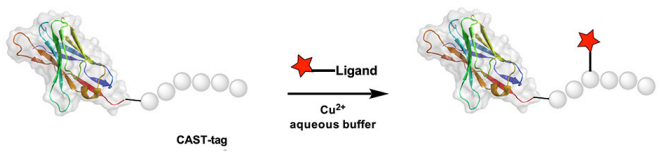
P136 Bobo Dang

L32 Westlake University, China

Copper-Assisted Sequence-Specific Chemical Protein Conjugation

Mengzhun Guo, Kai Zhao, Liang Guo, Qiuju He, Rui Zhou, Kuan Lu, Tian Li, Dandan Liu, Xin Fu, Samuel I. Mann, Jing Huang, Bing Yang, William F. DeGrado, Ting Zhou, Yingjie Lei, and Bobo Dang

Direct site-specific methods of chemical protein functionalization are highly desirable. However, such methods are particularly challenging to develop due to the tremendous difficulty of chemically differentiating the same amino acid type at different protein sites.



Herein, we proposed "metal binding targeting" strategy and developed Copper Assisted Sequence-specific conjugation Tag, CAST to achieve single site-specific protein conjugations. CAST possesses superior reaction kinetics with a rate constant of 8.1 M^{-1} s^{-1} in aqueous buffer.

Importantly, CAST conjugation can be employed as a univer-

sal method for efficient and quantitative payload attachment on different proteins. We also highlight that an antibody drug conjugate prepared using CAST is highly stable in plasma and exhibits potent efficacy *in vitro* and *in vivo*.

YI-P137 Jael Rodriguez

University of Michigan, Ann Arbor

Development and Implementation of a Cell-Permeable Dual-Site Inhibitor of CBP/p300 KIX in AML

Jael A. Rodriguez, Stephen T. Joy, and Anna K. Mapp

AML is characterized by abundant mutations of genes encoding regulators of gene transcription and chromatin structure. Interestingly, a subset of AML with lower survival rates and increased chemotherapy resistance are known as *MLL*-rearranged leukemias. This subset of AML is of interest due to their reported ability to utilize the CBP/p300 protein-protein interaction network to induce leukemogenesis. CBP/p300 is a large 300kDa master coactivator with a primary role as a promiscuous histone acetyltransferase, HAT. A domain of CBP/p300 named KIX recruits the transcription factor Myb to induce stem-cell renewal, cell proliferation and leukemogenesis.

The Mapp lab has developed a dual-site inhibitor of KIX, termed MybLL-tide, used to disrupt its interaction with KIX. This work builds on this inhibitor by increasing its efficacy in cell models through the adaption of a cell-penetrating peptide, CLIP6. Our data suggests that MybLL-tide induces loss viability of leukemia cells and apoptosis through its engagement with KIX. The new MybLL-tide construct synthesized has high cell permeability and sub-nanomolar potency validating CBP/p300 KIX – Myb interaction as a prominent therapeutic target.

P138 Michael Bertucci

Lafayette College

Determining the Active form of the Quorum Sensing Peptide in *Lactobacillus plantarum*

Ryann Carlotz, Rebecca Hartman, Xiaotian Gong, Abigail Skidmore, Jasmine Nguyen, Carter Brand, Fadi Hanna, Jonathan Nadraws, Ashlyn Cantrel, and Michael A. Bertucci

Quorum sensing, QS, is a density-dependent process of chemical communication in bacteria that regulates colony-wide gene expression. In the commensal bacteria *Lactobacillus plantarum*, QS has been demonstrated to control key phenotypes such as adhesin production and biofilm formation. Despite the publication of a QS peptide sequence, the active form of the peptide remains unclear as pH- dependent S-to-N acyl transfer occurs within the cyclic peptide backbone.

Thus, we have synthesized both possible forms of the peptide and developed a reporter strain and bioassay in which to screen these and other QS modulators. Controlling QS in *L. plantarum* to increase the longevity of the species in the gastrointestinal tract has the potential to promote a variety of positive health outcomes.

YI-P139 Baba M. Yussif

University of Nebraska-Lincoln

Endogenous L- to D-Amino Acid Residue Isomerization Modulates Selectivity Between Distinct Neuropeptide Receptor Family Members

Baba M. Yussif, Cole V. Blasing, James W. Checco

Neuropeptides play important biological roles in cell-to-cell communication. Some amino acid residues found within neuropeptides sequences have been shown to undergo post-translational isomerization from the canonical L- stereoisomer to a D- stereoisomer. Historically, difficulties in the identification of post-translational amino acid residue isomerism in neuropeptides has led to very little understanding of the receptor proteins for these D- amino acid containing peptides, DAACPs.

Recently, we discovered that the Aplysia allatotropin-related peptide, ATRP, signaling system employs the use of L- to D- amino acid residue isomerization present in neuropeptide ligand sequence to selectively modulate two distinct G protein-coupled receptors, GPCRs. We observed two distinct GPCRs, ATRPR1 and ATRPR2, signaling through the $G\alpha_q$ and $G\alpha_s$ signaling pathways with different potencies when selectively modulated by the two diastereomers of Aplysia allatotropin-related peptide, L-ATRP and D2-ATRP.

Presently, our discovery represents the first reported case of nature utilizing post-translational isomerization of amino acid residue in endogenous peptide ligands to modulate receptor protein selectivity. The novelty and significance of this discovery is of great importance to understanding the functional consequences of endogenous post-translational isomerization in cell-to-cell communication.

P140 Liv Kaser

Numaferm GmbH

Numaswitch – a Viable Platform to Access Peptides and Proteins

In the previous decade, we observed an increasing number of development projects for peptides and proteins, 30aa – 500aa, as drug substances for pharma and non-pharma applications. This led to challenges in the sourcing of the targets, also showing that there is no viable production approach that ensures the timely delivery of high-quality material.

For almost ten years, Numaferm has been working on a new approach, meeting these needs. We have developed a high-titer expression platform, Numaswitch, which is designed for the release of peptides and proteins within a few weeks, while simultaneously meeting API standards. These developments were peer reviewed and published.

With the production of hundreds of different targets, the reliability of Numaswitch was proven under laboratory conditions, that is, for aggregation-prone Maβ40/42, antimicrobial, LL-37, and pharmaceutical, Teriparatide, PTH1-34 targets. Industry grade, upscaled processes were developed for dozens of targets and the production in the g scale is routine by now.

The first processes were scaled in the multi-kg scale in GMP-conform environments. Our data shows how Numaswitch reduces cost of goods and CO_2-emissions by more than 90% compared to chemical synthesis and how targets with purities >99% become accessible.

At APS 2023, we would like to share selected case studies, demonstrating how Numaswitch is easily adjusted for targets and how mg samples are ready-for-shipment within weeks. With Numaswitch, peptides and proteins are accessible at time, at quality, at cost – meeting the current market demand and enabling the pure innovation potential of peptides and proteins.

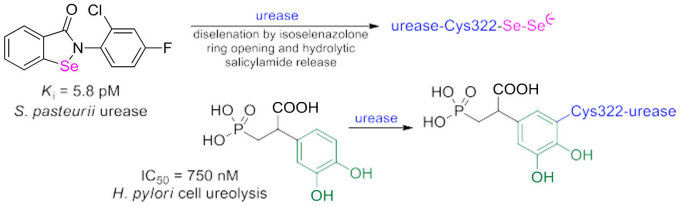
P141 Artur Mucha

Wroclaw University of Science and Technology

Urease Inhibition by Cysteine Modification

A. Mucha, A. Grabowiecka, K. Macegoniuk, M. Maślanka, W. Tabora, M. Giurg, L. Mazzei, S, Ciurli, and Ł. Berlicki

Urease, EC 3.5.1.5, is a nickel-dependent amidohydrolase whose activity is identified as a primary virulence factor in the colonization of persistent microbial infections in humans. The enzymatic mechanism of urea hydrolysis demands a shift of the mobile flap to adopt the closed conformation of the active site. Cys322, *Sporosarcina pasteurii* numbering, the component of this flap, has emerged as an inspiring target for the development of covalent, competitive and uncompetitive enzyme inhibitors.



Here, we present the design, structure, activity, and presumable mode of action of new benzisoselenazolones and catechols, including those functionalized with phosphorus acid moieties, as an approach to structurally optimized cysteine-targeted urease modifiers.

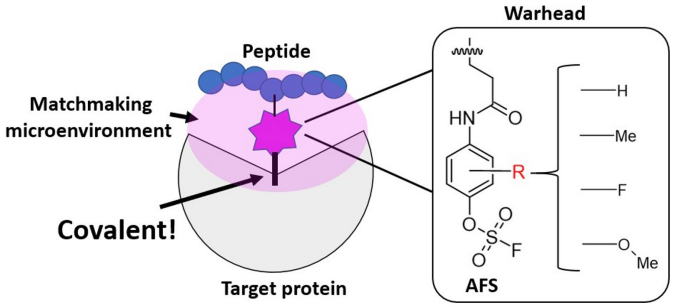
P142 Riku Katsuki

The University of Electro-Communications

Latent Reactivities of Aryl-Fluorosulfate Warheads in a Peptidic-Covalent-Binder / Protein Complex

R. Katsuki and M. Taki

In recent years, middle-molecular covalent drugs are attracting attention because they can form a specific covalent bond to a target protein and show semi-permanent drug effects. Such covalent binders possess a reactive group, that is, warhead, which must react only in a bioactive site of the protein avoiding potential risks of irreversible side effects.



Among the warheads, aryl-fluorosulfate, Ar-OSO_2F; AFS, has a latent reactivity; it is activated only in a matchmaking microenvironment of the bioactive site. In a previous work, we have succeeded in obtaining a peptidic covalent binder possessing AFS via the reactivity/affinity-based co-selection with extended phage display. Still, the activation mechanism of the latent AFS in the special environment is largely unknown.

Here, to reveal how fine structure of the warhead affects the proper activation, we synthesized seven AFS-derivatives, altered the original latent AFS warhead in the peptidic binder to each derivative, and tried to compare the target-protein inhibition by each covalent binder. The activation of AFS was affected by chemical structure, for example, hydrophobicity, hydrogen bonding, or bulkiness, at the meta-position against the -OSO_2F group.

YI-P143 Denise Pinto

Philadelphia College of Osteopathic Medicine

N-terminus Conjugation of Protein Kinase C Beta II Peptide Inhibitor Using Myristic Acid and Trans-Activator of Transcription Improves Intracellular Delivery of Cargo in Isolated Rat Polymorphonuclear Leukocytes

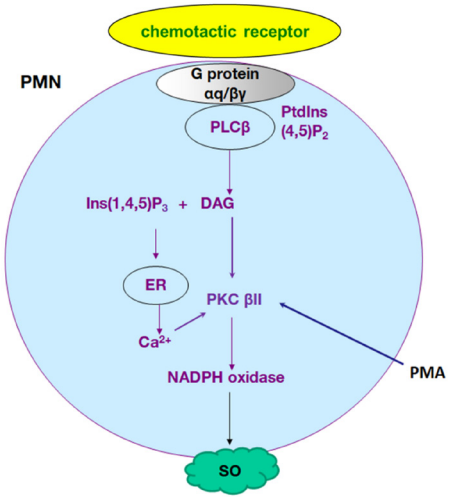
Denise Pinto, Alexandra Barrera, Alexis Verwoert, Sunit Singh, Emily An-

drews, Arjun Nair, Devani Johnson, Taylor DiLisi, Annam Humayun, Zinya Talukder, Tameka Dean, Juliet Melnik, Qian Chen, Robert Barsotti, and Lindon Young

Protein kinase C beta II, PKCβII, activation, see figure, via Ca^{2+} and diacylgylcerol, DAG, promotes polymorphonuclear, PMN, superoxide, SO, production by phosphorylating serine and threonine amino acid residues on NADPH oxidase, NOX-2.

Previously, myristic acid-conjugated, myr PKCβII inhibitor, PKCβII-, SLNPEWNET, 20μM, attenuated phorbol 12-myristate 13-acetate induced, PMA, SO release by 35%. Furthermore, a trans-activator of transcription-conjugated, Tat, NOX-2 inhibitor, Nox2ds-Tat, was also found to attenuate SO release by 37% at 80μM. However, Tat- and myr-Tat-conjugated PKCβII- has not been evaluated by comparison.

We hypothesize myr-Tat- conjugation will significantly attenuate SO release compared to myr- or Tat- conjugation alone.



This study compared the effects of myr-PKCβII-, Tat (YGRKKRRQRRR)-PKCβII-, myr-Tat-PKCβII-, and 0.5% dimethyl sulfoxide vehicle control group. Rat PMNs were incubated for 15min at 37°C with either 5μM myr-PKCβII-, Tat-PKCβII-, or myr-Tat-PKCβII-. SO release was measured by spectrophotometrically at 550nm over 390 sec via ferricytochrome c reduction after PMA stimulation, 100nM. Data were analyzed with ANOVA Fisher's PLSD post-hoc analysis.

Myr-Tat-PKCβII- (n=20, 0.338±0.024) and Myr-PKCβII- (n=8, 0.342±0.041) significantly attenuated SO release by 28% and 27%, respectively, compared to control (n=81, 0.470±0.013). Tat-PKCβII- (n=5, 0.404±0.049) attenuated SO release by 14% at 390s. Cell viability was >85% in all groups.

Results suggest that N-terminus conjugation of PKCβII- using myr-tat improves intracellular delivery of cargo compared to myr- or Tat- alone, whereas myr- is more effective than Tat.

Future studies will utilize immunohistochemistry and western blot analysis to investigate the effects of myr-, Tat-, or myr-Tat-PKCβII- peptides on PKCβII- translocation or activity.

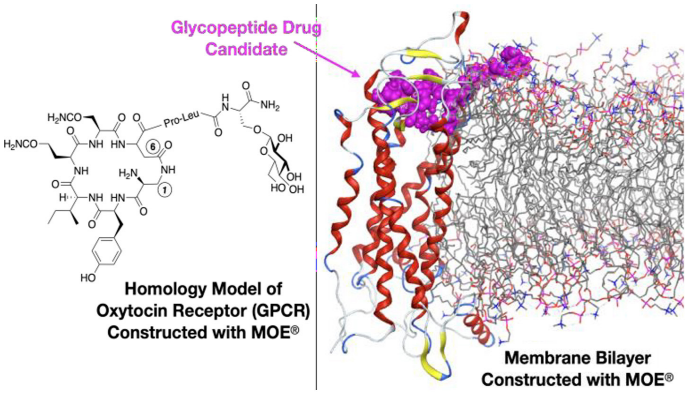
P144 Robin Polt

The University of Arizona

Peptide Hormones as a Source of *Bona Fide* CNS Drugs: Glycosides Promote *In Vivo* Stability and BBB Penetration

L. Szabò, C.R. Apostol, H.J. Goodman, T.E. Smith, F. Al-Obeidi, H.W. Morrison T. Falk, M.L. Heien, J.M. Streicher, and R. Polt

The incorporation of O-linked glycosides into peptide sequences converts peptide neurotransmitters and hormones into CNS-active pharmaceuticals. Hormones leu-enkephalin, deltorphin\dermorphin, endomorphin, β-endorphin\dynorphin, and angiotensin$_{1-7}$ have all provided CNS-active drug candidates, and one, PNA5, is beginning clinical trials for the treatment of vascular dementia.



This paper will focus on the design, synthesis and *in vivo* activity of much larger helical peptides such as the secretin PACAP as well as novel cyclic glycopeptide derivatives related to Oxytocin.

YI-P145 Jessica Tennett

Fordham University

A Rational Crosslinking Approach to Stabilizing Polyproline II Helices

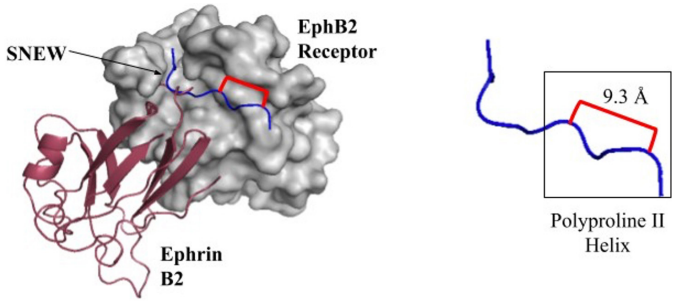
J. C. Tennett, S. R. Epstein, and N. Sawyer

Protein-protein interactions, PPIs, play fundamental roles in all cellular processes, and their misregulation is frequently associated with cellular and/or organismal dysfunction. Molecular modulators of PPIs therefore represent valuable tools to investigate complex biological systems and offer leads for PPI modulation in relevant diseases.

Significant progress has been made in the development of crosslinking approaches to create structured α-helical and β-sheet peptides, which represent ~50% of PPIs. System-

atic targeting of the remaining 50% of PPIs mediated by non-α-helical, non-β-sheet structures remains elusive because comparable rational crosslinking approaches for these structures are lacking.

Here we describe efforts toward a crosslinking approach for polyproline II, polyProII, helices, which are enriched at PPI interfaces, such as in ligands for Src homology 3, SH3, Ena/Vasp Homology 1, EVH1, and WW domains. As a model system, we studied the EphB2 receptor interaction with the peptide SNEW, which specifically binds the receptor in a polyProII helix conformation and competitively inhibits its interaction with ephrin protein ligands. Rational crosslinker design, spanning a single polyProII helical turn within SNEW's C-terminus, resulted in crosslinked SNEW variants with greater inhibitory potency than SNEW. Crosslinked peptides also showed enhanced resistance to proteolysis and retained binding specificity for the EphB2 receptor. NMR experiments support folding of the cyclized region into a polyProII helix conformation.



We anticipate that the cyclization approach may also be transferable to other peptides for specific targeting of other PPIs mediated by polyProII helices.

YI-P146 Mona Mehrani

University of Nevada Reno

Conformational Screening of Competence-Stimulating Peptide, CSP, Analogs in *Streptococcus pneumoniae* to Develop Potent Cyclic Inhibitors

Mona Mehrani, Muralikrishna Lella, and Yftah Tal-Gan

Streptococcus pneumoniae is a Gram-positive bacterium and an opportunistic human pathobiont that has developed resistance to most antibiotics due to genetic competence. To overcome this issue, we seek novel methods to target the non-essential pathways for long-term effectiveness over antibiotics and vaccines. Modulating quorum sensing, QS, is potentially one of these alternative approaches. QS is a cell-cell communication mechanism that governs bacterial competence and virulence in many streptococci bacteria including *S. pneumoniae*.

Competence stimulating peptides, CSPs, are chemical cues that regulate QS in S. pneumoniae. 2 Each of the two significant groups of *S. pneumoniae* group 1, D39 strain, and group 2, TIGR4 strain, has its own CSP, CSP1 and CSP2, which correspond to their respective receptors, ComD1 and ComD2. A single mutation from Glu1 to Ala in CSP1, produces the peptide CSP1-E1A, a potent inhibitor. Despite the ability of this inhibitory peptide to disrupt QS, it is not able to withstand enzymatic degradation, resulting in low stability. Higher stability and helicity could be obtained by cyclizing these peptides.

Throughout this study, we initially screened urea bridge cyclization in different areas of the CSP1-E1A scaffold to determine the most appropriate area to perform side chain-to-side chain peptide cyclization. Furthermore, we examined the effect of cyclization on the bioactivity and helicity of the analogs. We then sought to investigate the effects of bridge position, ring size, and side chain length on the helicity and bioactivity of the peptides.

By leveraging this information, in the future, we can rationally design cyclic CSP QS modulators with therapeutic potential that are highly potent and pharmacologically stable.

P147 Roberto Rojas

Pontificia Universidad Católica de Valparaíso

Effect of Antifreeze Activity and Lipid Interaction of Tryptophane Scanning of 11-Residue Arginine Homo-Peptide

Rojas R., Aróstica M., Cardenas C., Aguilar L. ,Albericio F. ,Carvajal-Rondanelli P., and Guzmán F.

Antifreeze peptides and proteins are present in organisms that survive the cold, including fish that inhabit the polar zones. They are important compounds that perform multiple functions, including cryopreservation. Different types of peptides have been described, with various primary and secondary structures.

In the present work, the study was made of an 11-residue arginine homopeptide, R11, and by means of the scanning technique, each position was replaced by Trp. The peptides were synthesized by the Fmoc/tBu strategy, purified and characterized by RP-HPLC and mass spectrometry.

The antifreeze activity was measured by differential scanning calorimetry, DSC, and quantified through the inhibition of ice recrystallization in a non-colligative way, a phenomenon called thermal hysteresis, THA. The results showed 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. The tryptophan substitution caused the disruption of the secondary structure, which for R11 is a type II polyproline helix, also causing a decrease in antifreeze activity compared to other model peptides.

This work will allow raising the potential effect on the inhibition of ice crystal growth during freezing.

YI-P148 Christopher Travis

University of North Carolina at Chapel Hill

Evaluation of the Differential Binding Preferences of Histone Trimethyllysine Reader Proteins

Christopher R. Travis, Kelsey M. Kean, Katherine I. Albanese, Hanne C. Henriksen, and Marcey L. Waters

Lysine trimethylation, Kme3, is a common post-translational modification, PTM, on histone tails responsible for regulating gene expression and other cellular processes, often facilitated via binding of reader proteins. Dozens of human Kme3 reader proteins have been identified, many of which bind to the same histone sequence but have different biological implications.

Many readers have been identified as therapeutic targets, but selective inhibition of these conserved binding motifs has proven challenging. Methyllysine reader proteins universally bind Kme3 in an aromatic cage. Typically, these binding events are attributed to cation-π interactions but recently exceptions have been suggested.

We investigated the generality of this assumption with the goal of providing insight into novel approaches for selective inhibition. To this end, we utilized high throughput screening, biophysical and mechanistic studies, as well as structural characterization to discover that the ability of readers to bind a peptide containing neutral isostere of Kme3, tBuNle, is more widespread than previously thought with 50% of readers investigated binding with equal or tighter affinity to the neutral mark. Readers capable of binding tBuNle are part of two distinct families, and there is no singular factor that dictates whether a reader binds the neutral isostere, as highly similar proteins differ significantly in their binding preferences.

This discovery establishes a new framework for selective inhibitor design by exploiting differences in charge-dependence among readers in the same family with highly similar aromatic cages. Further, we find that reader proteins that bind both Kme3 and tBuNle do so through different mechanisms, utilizing cation-π interactions to recognize the former and a combination of the hydrophobic effect and dispersion forces to bind the latter.

This finding is significant in the field of molecular recognition, as these readers represent rare examples of proteins recognizing differently charged ligands in the same binding site via distinct mechanisms.

YI-P149 Jonathan Moreno

Chapman University School of Pharmacy

Amphiphilic Cell-Penetrating Peptides Containing Arginine and Hydrophobic Residues as Protein Delivery Agents

Jonathan Moreno, Khalid Zoghebi, David Salehi, Lois Kim, Sorour Khayyatnejad Shoushtari, Rakesh K Tiwari, and Keykavous Parang

The entry of proteins through the cell membrane is challenging, thus limiting their use as potential therapeutics. Seven cell-penetrating peptides, designed in our laboratory, were evaluated for the delivery of proteins. Fmoc solid-phase peptide synthesis was utilized for the synthesis of seven cyclic or hybrid cyclic–linear amphiphilic peptides composed of hydrophobic tryptophan, W, or 3,3-diphenylalanine, Dip, and positively-charged arginine, R, residues, such as $[WR]_4$, [WR]9, [WWRR]4, [WWRR]5, [(RW)5K](RW)5, [R5K]W7, and $[DipR]_5$.

Confocal microscopy was used to screen the peptides at non-cytotoxic concentrations in triple-negative breast cancer cells, MDA-MB-231, as a protein delivery system of model cargo proteins, green and red fluorescein proteins, GFP and RFP. $[WR]_9$, 2–10μM, and $[DipR]_5$, 1–10μM, were the most efficient among all tested peptides. Fluorescence-activated cell sorting, FACS, analysis indicated that the cellular uptake of GFP was concentration-dependent in the presence of $[WR]_9$ in MDA- MB-231 cells after 3 hours of incubation at 37°C. The concentration-dependent uptake of GFP and RFP was also observed in the presence of $[DipR]_5$ in SK-OV-3 and MDA-MB-231 cells after 3 hours of incubation at 37°C. FACS analysis indicated that the cellular uptake of GFP in the presence of $[WR]_9$ was partially decreased by methyl-β-cyclodextrin and nystatin as endocytosis inhibitors after 3 hours of incubation in MDA-MB-231 cells, whereas nystatin and chlorpromazine as endocytosis inhibitors slightly reduced the uptake of GFP in the presence of $[DipR]_5$ after 3 hours of incubation in MDA-MB-231. $[WR]_9$ was able to deliver therapeutically relevant protein, Histone H2A, at different concentrations. These results provide insight into the use of amphiphilic cyclic peptides in the delivery of protein-related therapeutics.

P150 Ewa Rudzinska-Szostak

Wroclaw University of Science and Technology

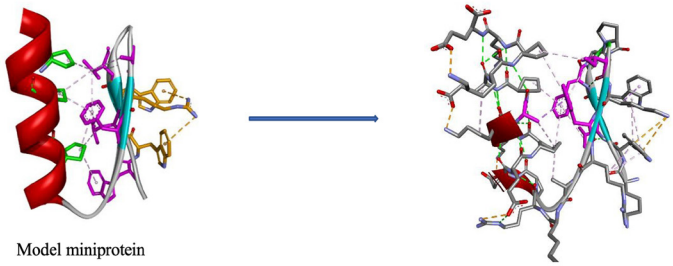
Design, Synthesis and Spectral Analysis of Miniproteins Containing π-Cation Stabilized β-Hairpin and Non-Canonical Helix

E. Rudzińska-Szostak, K. Ożga, M. Kołacki, and Ł. Berlicki

Miniproteins are defined as polypeptides with a molecular weight not exceeding 10 kDa and adopting a tertiary structure in solution. The combination of synthetic availability and expanded structure make them extremely interesting and valuable molecules. The miniprotein fold, similar to that of

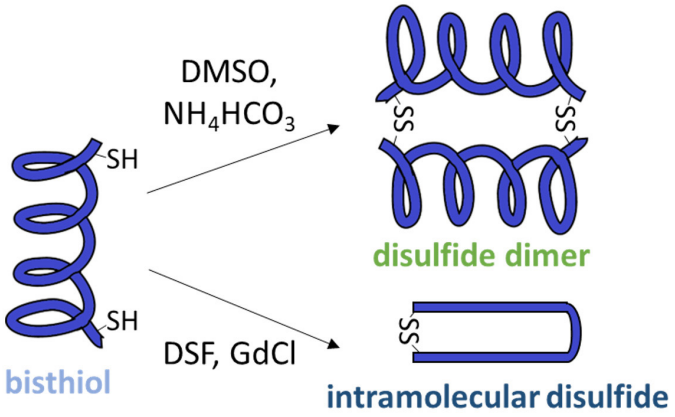
the native protein, is stabilised by any combination of the three main types of interaction, namely, the hydrophobic core, covalent bonds, and metal complexation. As a result of the robustness design and analysis approaches, as well as relatively simple synthesis, that is, up to 50 amino acids and no additional modification steps, the monomeric miniproteins with a hydrophobic core are of special interest.

Many methodologies have been developed to obtain various secondary structures, both native and containing non-canonical building blocks, including helices that contain conformationally restricted β-amino acids. However, the preparation of hybrid tertiary structures containing non- native secondary structures and/or building blocks is mainly limited to native sequence engineering, and *de novo* design still remains challenging.



Here, we report *de novo* designed novel hybrid miniproteins. The compounds presented are a combination of two secondary structures: a β-hairpin stabilised by the π-cation interaction between Trp and Lys/Arg residues and a non-native helix with the sequence motif ααβαααααβ^3, considering hydrophobic interactions within the molecule. The introduction of (1S,2S)- 2-aminocyclopentanecarboxylic acid (*β*, trans-ACPC) residues is reported to stabilise α-helix like fold. Automated solid-phase approach supported by microwaves was used to obtain designed molecules, while the application of circular dichroism, CD, enabled the evaluation of conformational stability. The selected structure was characterised on the atomic level by means of 2D NMR techniques.

tions can lead to monomeric or dimeric species from fully deprotected linear bisthiol peptides. Examination of reaction conditions indicates that the use of disulfiram, DSF, under denaturing conditions leads to the formation of intramolecular disulfide bonds while reactions with dimethyl sulfoxide, DMSO, under aqueous conditions yields disulfide-linked, antiparallel dimeric species. Intramolecular disulfide formation using DSF and denaturing conditions proved to be sequence-independent.



On the other hand, the formation of antiparallel dimers is sequence-dependent and requires partial α-helical folding of the linear peptide and the presence of a hydrophobic triad to encourage peptide association. Treatment with Proteinase K indicates that both disulfide species demonstrate enhanced protease stability compared to the linear precursor. The half-life increases by a factor of 100 for the dimer and even greater for the intramolecular disulfide. Additionally, both disulfide species are fully reduced by tris(2-carboxyethyl)phosphine, TCEP, within 15 minutes to regain the original α-helical structure for targeting proteins.

These results provide an approach for using disulfide bonds to control peptide folding and oligomerization to better understand how these properties influence interactions with protein and membrane targets.

YI-P151 Clara Victorio

Fordham University

Controlling Disulfide Formation and Dimerization to Constrain Alternate Peptide Conformations

Clara G. Victorio and Nicholas Sawyer

Disulfide bonds form covalent bonds between distal regions of peptides and proteins to dramatically impact their folding, stability, and oligomerization. Given the prevalence of disulfide bonds in many natural products, considerable effort has been invested in site-selective disulfide bond formation approaches to control folding of chemically synthesized peptides and proteins.

Here we show that careful choice of thiol oxidation condi-

YI-P152 Mohammad Imtiazur Rahman

Arizona State University

De Novo Design of a Cofactor Binding Peptide that Forms Condensates by Molecular Crowding

Mohammad I. Rahman, Abesh Banerjee, Nicholas R. Halloran, and Giovanna Ghirlanda

Hydrogels derived through the polymerization of structured elements are linked by disordered portions. Several studies showed that reversible hydrogel formation can be achieved out of artificially designed proteins.

Here, we explore whether the hydrogel or its intermediate state, coacervates, could be formed with cofactor binding peptides. Previously, we designed a D2- symmetric diheme protein where a disordered peptide self-assembles into a di-

heme tetrameric four-helix bundle upon coordination of Fe (III) PPIX. Inspired by D_2, we have designed a peptide called M1 which comprises two D_2 motifs connected by a negatively charged disordered linker region $(AGAGAGPEG)_{10}$. The porphyrin binding segments form a helical four-helix bundle by coordinating CoPPIX through histidine residues. The M1-CoPPIX phase separates and forms complex coacervates in the presence of positively charged polyarginine, R10. Additionally, at high concentrations, >7mM, M1 peptide forms hydrogel after cooling from 50°C.

We want to explore whether M1 peptide can perform biomimetics of simple coacervation through molecular crowding. We would also like to investigate whether the M1 protein droplets can catalyze the H_2 evolution reaction by utilizing coordinated CoPPIX.

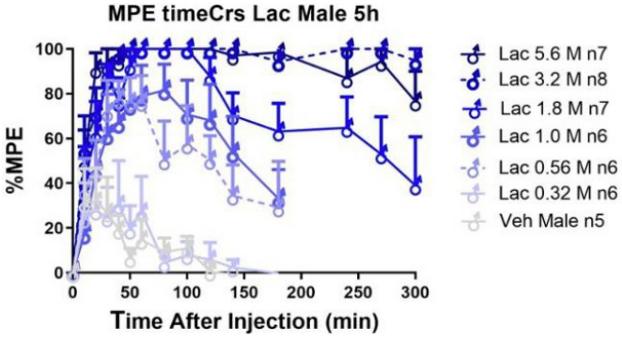
P153 Lajos Szabo

The University of Arizona

Cyclic Glyco-Endomorphins Produce Potent, Long-Lived Antinociception in Mice

Lajos Szabó, Xing Zhang, James E. Zadina, and Robin Polt

Cyclic peptides related to endomorphin are known to be much more stable *in vivo* than linear peptides. This increased stability, combined with the use of glycosylation to promote membrane hopping and the penetration of cellular barriers leads to cyclic glycopeptides with greatly enhanced pharmacological properties.



Application of this drug design approach to the naturally occurring μ-opioid agonists produces drug candidates for analgesia with potent and extremely long-lasting antinociception, as demonstrated by tail flick and hot plate studies in mice. The preparation of these compounds and their testing in mice is described in this poster.

YI-P154 Samilla Rezende

Universidade Católica Dom Bosco, Brazil

Influence of D-arg and D-lys on the Structure and Antibacterial Activities of Computer-Made Peptides

Rezende, S.B., Cândido, E.S., Chan, L.Y.,Oshiro, K.G.N., David J. Craik, Franco, O.L., and Cardoso, M.H.

Incorporating D-amino acid residues into antimicrobial peptides, AMPs, L- sequences, generates diastereoisomers, improving resistance to proteolytic degradation and favoring bioavailability. We studied the influence of D-arg and D-lys in three computationally designed AMPs, PaDBS1R2, R6, and R7.

Two groups were synthesized, Group I, L-aa peptides, and Group II, all-D-arg and D-lys. Both groups were evaluated for their susceptibility to degradation through serum stability assays. Minimal inhibitory concentrations and minimal bactericidal concentration assays were performed against ESKAPE strains. Circular dichroism, CD, nuclear magnetic resonance, NMR, and computational studies were also conducted.

Serum stability assays revealed the group II, after 30 minutes of incubation, was 50% more resistant to degradation than group I. By contrast, group II drastically compromised their antibacterial effects due chirality inversion, whereas group I displayed a broad spectrum of antibacterial activities from 3 to 32 μmol L-1. Cytotoxic/hemolytic properties were not observed up to 100 μmol L-1 for all groups. CD and NMR studies revealed group I adopted α-helix structures in 30% TFE and SDS. Group II displayed random coil at those same conditions. Amide proton temperature coefficient analysis indicated that group I preserved their secondary structure from 285-310 K.

We concluded that group II, although being resistant to proteolysis, does not adopt well-defined secondary structures, abolishing its bioactivity. Further studies are proposed using computational tools to predict punctual chirality modification, and a group containing D-arg and D-lys residues outside the α-helical antibacterial pattern (KK[ILV]x(3)[AILV])) present in all sequences here evaluated.

YI-P155 Naiem Wani

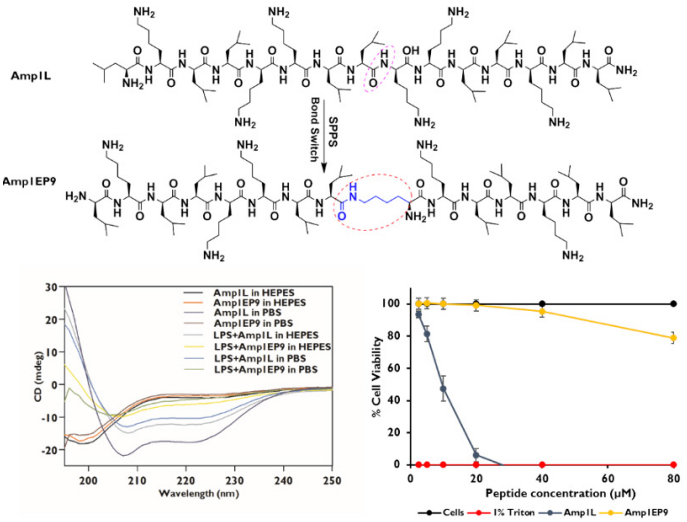
Weizmann Institute of Science

Site-Specific Isopeptide Bond Formation: A Powerful Tool for the Generation of Potent and Nontoxic Antimicrobial Peptides

N. A. Wani, D. Ben Hur, E. Stolovicki, and Y. Shai

Antimicrobial peptides, AMPs, have the potential to treat multi-drug-resistant bacterial infections. However, the clinical application of AMPs is prevented by their toxicity and poor proteolytic stability. A site-specific approach is used to generate new AMPs to improve their efficacy against bacterial pathogens while reducing their toxicity.

We modified and generated a new series of antimicrobial peptides from leucine/lysine rich antimicrobial peptide Amp1L: LKLLKKLLKKLLKLL by the site-specific incorporation of isopeptide bond while retaining size, sequence, charge and molecular weight. This single bond switch provides peptides



with weak helical conformation, strong antimicrobial activity, resistance to proteolytic degradation, accompanied by low toxicity and hemolysis. This new site-specific approach offers a powerful tool for developing potent and nontoxic antimicrobial drugs.

P156 Rumit Maini

PepLib

Peptide Information Compression Technology, PICT, for GPCR Drug Discovery using Cell-Based Functional High-Throughput Screening Assays

Rumit Maini and Henry Liu

Peptides have proven to be excellent drugs against GPCRs, specially to treat diabetes and obesity, due to their high potency and selectivity. However, nearly 60% GPCRs are yet to be explored as drug targets and recently, 87 orphan GPCRs were identified in the human genome. Furthermore, most peptide GPCR drugs have been developed from endogenous peptide ligands and function as agonists. High-throughput screening technologies using a one-well one-peptide library in conjunction with cell-based functional assays have the potential to discover both novel peptide agonists and antagonists and greatly accelerate the drug discovery efforts against the unexplored GPCR target space.

We have developed a functional peptide discovery platform capable of discovering functionally active peptide hits directly from a screen. Based on the proprietary Peptide Information Compression Technology, PICT, a library of cyclic peptides containing half a billion unique sequences has been designed and synthesized. Each member is individually expressed, head-to-tail cyclized, and purified in a genetic tag-free manner to build a one-well one-peptide library. The capabilities of the PepLib discovery platform extend beyond just binding assays to biochemical and cell-based functional assays making it suitable for GPCR drug discovery.

To highlight the success of this platform, results from cell-based functional screens against two GPCR targets will be presented: **i** discovery of cyclic peptide antagonists, 10- to 14-mers, against hMC3R, where 14-mer cyclic peptide, IC50=40nM, demonstrated 1500-fold selectivity over hMC4R and **ii** discovery of a linear 12-mer, EC50=0.45nM, and a cyclic 15-mer, EC50=40nM, peptide agonist against the Apelin receptor, APJ.

YI-P157 Somayeh Mousavi

University of Nebraska-Lincoln

Identification of Cell-Cell Signaling Peptides Within the Central Nervous System of Thirteen-lined Ground Squirrels During Hibernation by Mass Spectrometry-Based Peptidomics

Somayeh Mousavi, Haowen Qiu, Md Shadman Ridwan Abid, Matthew T. Andrews, and James W. Checco

The adaptation of mammalian hibernators to extreme physiological changes during hibernation provides these animals with remarkable capacities to survive dramatic fluctuations during torpor-arousal cycles. Identifying the molecular changes and interactions during this circannual phenotype can lead to development of advanced treatments for brain injuries.

Neuropeptides and peptide hormones are cell-to-cell signaling molecules which facilitate intercellular communication to regulate physiological functions such as appetite, body temperature, reproduction, and more. Despite their known importance in physiology, the roles of individual neuropeptides, including their final processed forms, during hibernation are largely unknown.

In this study, we used tandem mass spectrometry (MS/MS)-based peptidomics methods to measure neuropeptide and peptide hormone changes in the central nervous system of the thirteen-lined ground squirrel, *Ictidomys tridecemlineatus*, throughout its hibernation cycle. Bioinformatics tools were used to interpret the MS/MS data of extracted peptides to provide amino acid sequence information of the neuropeptides and peptide hormones at various times during the year. This approach was used to identify peptides which may play roles in the regulation of hibernation.

Ultimately, this study provides the first peptidomic examination of a mammalian hibernator, directly informing our understanding of hibernation biology.

P158 Christopher Shugrue

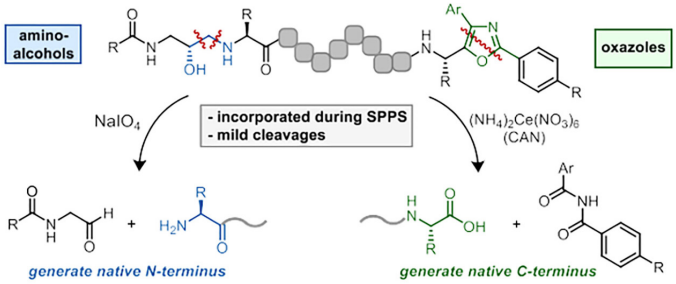
University of Richmond

Oxazoles and Amino-Alcohols: Oxidatively Cleavable Linkers for Peptide Discovery Platforms

Elizabeth Taggart, Evan Wolff, Christopher R. Shugrue

High-throughput screening of large peptides libraries is a powerful technique to develop sequences with novel activity. These assays often necessitate the capture of hit peptides from chemically complex environments. However, effective techniques used for capturing peptides often require harsh conditions to release peptides.

To overcome this challenge, we report the development of two new cleavable linkers, an oxazole and an amino-alcohol, which enable the rapid release of peptides following capture. Both linkers can be added into peptides during solid-phase peptide synthesis through amide coupling and reductive amination respectively. These compounds are stable to most conditions, including harsh acidic cleavages from resin. The linkers can be cleaved in mild, aqueous reactions at physiological conditions using orthogonal oxidizing conditions.



Oxazoles are oxidized using single electron oxidants like cerium ammonium nitrate, CAN. Oxazole cleavage results in the formation of native C-terminal carboxylates. Amino alcohols can be cleaved with sodium periodate, $NaIO_4$. Amino alcohol cleavage produces native *N*-terminal amines. We are currently applying these methodologies as tools for peptide-discovery platform.

P159 L60-YI8 Alexander Lund Nielsen

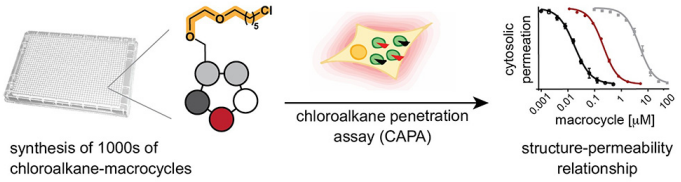
Ecole Polytechnique Fédérale de Lausanne

Assessing the Cellular Permeability of Peptidic Macrocycles in High-Throughput

Alexander L. Nielsen, Christian R.O. Bartling, Kristian Strømgaard, and Christian Heinis

Small cyclic peptides provide an attractive modality for drug development due to their ability to bind challenging targets and their potential to cross membranes for reaching intracellular proteins. In our laboratory, we have recently developed methods to synthesize and screen large combinatorial libraries of small cyclic peptides. For example, *"m"* short linear peptides containing thiol groups at both ends were combinatorially cyclized with *"n"* bis-electrophilic linker reagents to obtain *m* × *n* cyclic peptides that were screened in microwell plates as crude products. While the approaches yielded ligands to several disease targets, not all of them were membrane permeable. A full picture of the membrane permeability of the newly developed format of peptidic macrocycles, and factors that determine their permeability, was lacking.

In this work in progress, we have taken advantage of the chloroalkane permeation assay, CAPA, that has recently emerged as a robust method to determine cytosolic permeability of chloroalkane-tagged biomolecules.



We have established a method to synthesize thousands of diverse chloroalkane-tagged peptidic macrocycles to determine their cytosolic permeability using CAPA. This has given us a new insight into the structure-permeability relationships of an unprecedented number of macrocycles and provides a clearer picture of what features govern permeability of macrocyclic compounds in cellular systems.

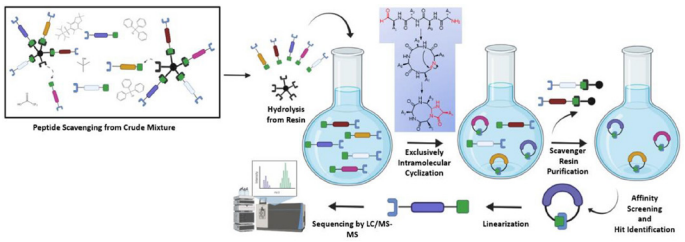
YI-P160 Victor Tolulope Adebomi

University of Washington

Click DeClick Method for Make Pure Libraries of Cyclic Peptides

Victor Adebomi, Angèle Bruce, Patrick Czabala, and Monika Raj

Generation of cyclic peptide libraries — a tool for protein related study — is a powerful tool for drug design, modulating protein interactions and various biological processes. Although there are known methods for the formation of cyclic peptide libraries, they however, have limitations such as peptide oligomerization, limited structural scope and incomplete cyclization reactions which often lead to false positives due to their interactions with target.



We have developed a Click-De-Click method which generates pure cyclic peptide libraries for screening against biological targets. Using a trap and release purification protocol, we can generate large amounts, > 106, of linear peptide-aldehydes with high purity. The linear peptides containing aldehydes at the C-terminal are then cyclized using an exclusively intramolecular CyClick Chemistry strategy.

Uncyclized peptides are removed using free aldehyde scavenging resins. The cyclic peptides would then screened for their binding to biological targets, linearized and characterized by using Tandem Mass Spectrometry.

YI-P161 Leonard Yoon

The Scripps Research Institute

Chemical Synthesis of Fyn SH2 Superbinder Domain Towards Mirror Image Phage Display

L. Yoon, P. A. Cistrone, D. T. Flood, L.T. Nelson, S. Chakraborty, B. P. Balo, J. W. Kelly, and P. E. Dawson

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. However, peptide drugs are limited by their innate immunogenicity and susceptibility to enzymatic hydrolysis.

Looked at another way, the interaction of Stat3 with its receptor could be blocked by a mirror image SH2 superbinder domain with evolved sub-nanomolar affinity by leveraging the achiral pTyr side chain binding motif. This approach harnesses the intrinsic selectivity of a protein for its receptor and limits off-target SH2-peptide interactions.

We report the chemical synthesis of the Fyn SH2 superbinder domain from Sidhu et al. and show that the superbinder binds D-pTyr peptides. This sets the stage for mirror image phage display currently underway, future D-protein synthesis, refolding and delivery into cancer cells.

P162 Hannah Distaffen

University of Rochester

Computational and Experimental Insight into Amyloid-Induced Membrane Damage

Hannah E. Distaffen, Yanxing Yang, Yahui Guo, Loren Cardani, Cristiano L. Dias, and Bradley L. Nilsson

Amyloid disorders are characterized by the aberrant self-assembly of proteins into fibrils with a β-sheet secondary structure. Amyloid aggregates, both cross-β fibrils and prefibril oligomeric aggregates, cause damage to cell membranes, which is hypothesized to account for amyloid-associated cytotoxicity. The exact mechanisms of amyloid-induced membrane damage remain unclear, although direct insertion and pore formation or detergent-like lipid extraction mechanisms have been proposed.

In this work, computational simulations of interactions of model amyloid peptides with a lipid bilayer are described. These simulations suggest various binding modes and membrane interactions that depend on peptide sequence and charge distribution. These simulations are correlated to experimental studies that confirm peptide-induced membrane leakage as a function of peptide charge and charge distribution.

YI-P163 Christopher Jones

University of Rochester

Effect of Peptide Sequence Order on Pleated and Rippled β-Sheet Assembly of Isomeric Peptides

Christopher W. Jones, Loren P. Cardani, and Bradley L. Nilsson

Amphipathic peptides composed of alternating hydrophobic and hydrophilic amino acids display an enhanced propensity to self-assemble into amyloid-like pleated β-sheet fibrils with a cross-β structure. These supramolecular assemblies display interesting emergent properties that facilitate their application as biomaterials for regenerative medicine, tissue engineering, and drug delivery. These self-assembled peptide-based biomaterials are composed of the well-known pleated β-sheet.

In 1953, Pauling and Corey predicted that enantiomeric mixtures of β-sheet peptides would coassemble in an alternating L/D pattern to form so called "rippled β-sheets" with a structure distinct from the naturally occurring pleated β-sheet. In the last decade, experimental evidence consistent with Pauling and Corey's prediction has emerged and it has been observed that putative rippled β-sheets have interesting potential as next-generation biomaterials since they exhibit unique emergent properties from their single enantiomer pleated β-sheet counterparts. The known scope of rippled β-sheet formation is currently very limited.

Herein, we describe studies of the scope of rippled β-sheet formation using sequence isomers of the model Ac-$(FKFE)_2$-NH_2 peptide, including (Ac-(FK)2$(FE)_2$-NH2, Ac-KE(F)4KE-NH_2, Ac-$(KFFE)_2$-NH_2, Ac-FF$(KE)_2$FF-NH_2). We compare the self-assembly of the enantiopure peptides to the coassembly of enantiomeric mixtures to gain further insight into the formation of both pleated and rippled β-sheets. These studies show that, in most cases, mixtures of enantiomers have a significantly higher propensity to assemble than the corresponding single enantiomers. This work provides new insight into the scope of rippled β-sheet formation.

YI-P164 Chan Wook Kim

Yonsei University

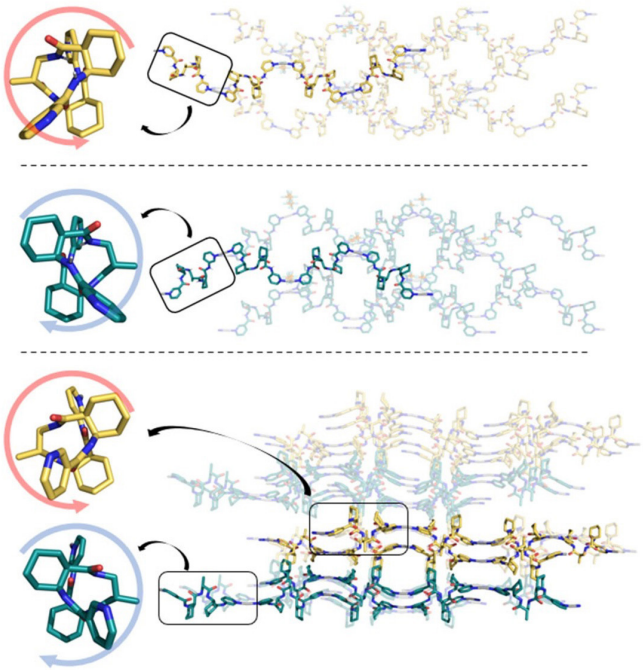
Metal Peptide Networks -MPNs- from a Racemic Mixture of Helical Tripeptides

Chan Wook Kim, and Soo Hyuk Choi

By virtue of modularity and biocompatibility, several studies on Metal-Peptide Networks, MPNs, were reported. Among them, due to versatility of networks originated from flexibility of peptides, designing MPNs consisting of helical peptides is still challenging.

Herein, we synthesized a helical tripeptide containing *cis*-2-aminocyclohexane carboxylic acid, cis-ACHC, a type of ring-constrained unnatural amino acid that promotes mixed

helices in different foldamer backbones. In addition, we synthesized an enantiomeric tripeptide with different chirality, of which the opposite helical handedness was confirmed through circular dichroism. In complexation with Ag(I) ion, each ligand retained its own helical handedness while showing an identical coordination bond between each tripeptide ligand.



We further studied the structure of MPN constructed from a racemic mixture of tripeptides, rac-MPNs. The crystal structure of rac-MPNs revealed an interesting feature that two different helical tripeptides exist in 1:1 ratio in a single network system. Surprisingly, the same helical tripeptide ligands with the same helical handedness are self-sorted as the result of self-assembly process.

This study gives insight for diversifying the structures of MPNs by introducing both helical peptides with opposite helical handedness.

YI-P165 Lucia Lombardi

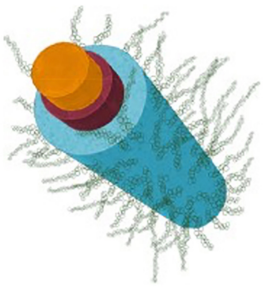
Imperial College London

Self-Assembling Biomaterials with Tunable Antimicrobial Activity

L. Lombardi, Y. Shi, A. Falanga, H.S. Azevedo, and S. Galdiero

With our research, we wanted to combine the advantages of both self-assembling and antimicrobial peptides to create a new biomaterial to be used to treat and eliminate bacterial biofilm formed on medical implants.

We would like to present a new peptide sequence, WMR, identified in our lab and its antimicrobial properties. Above all, we would like to explain how we designed and built a new biomaterial that contains WMR. This biomaterial effectively eradicates resistant biofilms such as *Pseudomonas aeruginosa*, Gram-negative bacteria, and *Candida albicans*, pathogenic fungus. This efficacy is greater than the one of the bare WMR. Furthermore, we designed our self-assembling material to have a tunable activity.



Our biomaterial with antimicrobial action is a multilayer system where each layer plays a specific role that can be structural or functional.

For example, one layer represents the driving force for self-assembly, orange; another one confers stability, burgundy; the third layer is the support and binding coating, light blue of the antimicrobial peptides, green.

Specifically, WMR was used to modify the surface of the self-assembling structures to confer antimicrobial activity. The new design was with WMR linked to a hybrid segment made of aliphatic amino acid residues and a lipid tail to generate a peptide amphiphile, WMR- PA.

The self-assembly of the WMR-PA was used alone or combined with co-assembling shorter amphiphilic peptides, PAs, to tune the activity. All the structures were studied using several spectroscopy and microscopy techniques to investigate the structure, stability and other physicochemical properties in several conditions, for example, different ionic strengths, pH values and concentrations. The designed PAs were shown to self-assemble into stable and robust nanofiber structures, and these nano assemblies significantly inhibit biofilm formation and eradicate the already formed resistant biofilms.

Our results provide insights into the design of peptide-based supramolecular assemblies with antibacterial activity and establish an innovative strategy to develop self-assembled antimicrobial materials for biomedical applications.

YI-P167 Sahabaj Mondal

Indian Institute of Science Education and Research

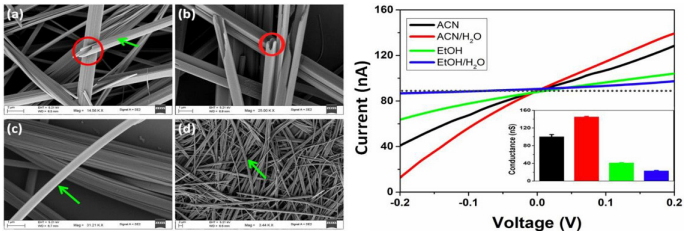
Phenylalanine Microtube: Modulating Semiconductivity and Piezoelectric Properties

Sahabaj Mondal and Debasish Haldar

Phenylalanine derivatives are extremely useful to develop functional materials with special optoelectronic properties for diverse applications in medicine, material science and chemistry. However, the design of synergistically aggregated building blocks to produce tailor-made functional nanomaterial is still challenging.

Herein, we have developed nanotubes by solvent-induced polymorph transformation of a phenylalanine derivative. Using diverse experimental techniques, we demonstrate that in comparison to EtOH and EtOH/water, ACN and ACN/

water induces nanotubes exhibit excellent conductivity and piezoelectric properties. The assembly in the ACN or ACN/water solutions is mostly governed by hydrogen bonding and p-stacking interactions, favoring -sheet formation and leading to long fibrils.



Comparison of semiconducting properties of different microstructures as obtained in various solvents.

Thus, the functionality of a system can be fine-tuned by carefully managing polymorphisms by selecting appropriate environmental assembly circumstances.

YI-P168 Tuan Samdin

National Institutes of Health

X-Ray Crystallographic Studies of Hydrogel Forming Macrocyclic β-Hairpin Peptides Reveal the Formation of Fibril-Like Structures Assembled from Antiparallel and Rippled β-Sheets

T.D. Samdin, Jacek Lubkowski, Caleb F. Anderson, and J.P. Schneider

Rippled β-sheets are a unique and thus far underutilized structural motif in the design of peptide-based materials. First described by Pauling and Corey in 1953, empirical observations of rippled β-sheet interactions have only been made in the last decade by the Raskatov, Nilsson, and Schneider groups.

The Schneider group has discovered that racemic mixtures of the hydrogel- forming β-hairpin peptide MAX1 exhibit a 4-fold greater gel stiffness over enantiopure hydrogels of either L- or D-MAX1. Diffusing wave spectroscopy reveals that these racemic gels are more mechanically rigid because the fibrils that comprise the gel are, themselves, more rigid. Molecular modeling of a rippled β-sheet comprising L- and D-MAX1 shows how the intermolecular nesting of hydrophobic valine side chains facilitates a tighter packing of fibrils and thus their increased stiffness.

The incorporation of rippled β-sheet-interactions into peptide-based hydrogels holds the promise of developing materials with novel biophysical properties and potential therapeutic applications. Developing these materials, however, requires deepening our understanding of the structural determinants of rippled β-sheets using high-resolution techniques such as X-ray crystallography or cryo-EM. This poster describes the efforts of the Schneider group to better understand and characterize rippled β-sheet-interactions observed in racemic mixtures of MAX1 derived peptides using X-ray crystallography.

To facilitate these crystallographic studies and better understand the assembly of rippled β-sheets, we designed and prepared several macrocyclic homologues of MAX1 and D-MAX1. X-ray crystallography reveals the formation of fibril-like structures assembled from antiparallel and rippled β-sheets amongst macrocyclic β-hairpin peptides derived from MAX1 and D-MAX1.

YI-P169 Srijani Sarkar

National Institutes of Health

Enhancement of the Mechanical Rigidity of Beta-Hairpin Peptide Hydrogels by a Library of Dialdehyde Crosslinkers

Srijani Sarkar, Caleb Anderson, Yixin Xie, and Joel Schneider

Peptide hydrogels are viscoelastic materials that have shown potential in drug delivery because of easy synthesis, biocompatibility, and ability to transport a variety of payloads, including protein biologics, microRNA nanoparticles, peptides, and small molecules drugs. However, peptide hydrogels often lack mechanical strength, which limits their applications in 3D printing, and cell/ tissue engineering.

Over the years, there have been multiple reports to increase the mechanical stability of peptide gels by changing amino acid compositions of the peptide sequence, incorporating racemic mixtures of enantiomeric peptides, and attempting different covalent crosslinking. Glutaraldehyde crosslinking to lysine side chains has been one of the popular covalent modifications to fine-tune the rigidity of hydrogels by forming imine bonds.

Here, we have tested a library of dialdehydes to rigidify hydrogels by forming imine covalent crosslinks amongst and between fibers. We studied the crosslinking effect on a previously established β-hairpin peptide in our research group, reported as Max1, which forms very weak gels at physiological pH. We have observed an enormous increase in the storage modulus values, >300-fold, of crosslinked hydrogels depending on the nature and the equivalence of crosslinkers.

Additionally, we determined the effect of a variety of external stimuli on the storage modulus values of crosslinked gels, including pre/post-gelation crosslinking, salt concentrations, pH of the buffer, and sodium borohydride reduction. Finally, we quantitatively determined the linear relationship between the hydrogels' storage modulus values and the number of crosslinked amines within the hydrogel network. Currently, we are testing the biocompatibility of these crosslinked gels employing human neonatal dermal fibroblast, HDFs, cells.

YI-P170 Kevon Stanford

National Institutes of Health

Modulating Peptide hydrogel Rigidity by incorporating Boronic Ester Crosslinks

Kevon Stanford and Joel Schneider

Peptide hydrogels have been attractive tools for biomedical applications owing to their ability to facilitate drug delivery. Amphiphilic peptides can assemble into fibrils, which can undergo higher-order assembly to form physically crosslinked gel networks. Typically, fibril crosslink density and the bending modulus of the fibrils define material stiffness. One way to modulate gel rigidity is to introduce chemical crosslinks into the fibril network.

Herein, we explore the use of boronic esters to crosslink peptide fibers. We have designed and synthesized boronic acid-containing peptide sequences by incorporating different numbers of 3-aminophenylboronic acids, 3-APBA, at varying positions designed to interact with peptides displaying diol moieties, such as L-DOPA to form intermolecular crosslinks. Boronic ester formation was studied using a fluorescence assay by utilizing the boronic acid-diol sensor dye, Alizarin Red S, ARS, which confirms the formation of diol-esters both in solution and gel state. Additionally, diol prodrug was used to demonstrate that the gel network consists of crosslinks that contribute significantly to the rigidity. Further, rheological studies suggest that the mechanical rigidity of corresponding gels increased by boronic ester formation. Lastly, peptide structural changes that occur during self-assembly and the morphology of resulting fibrils was studied by temperature-dependent CD and transmission electron microscopy, TEM, respectively.

YI-P171 Monessha Nambiar

National Institutes of Health

Immune-Responsive Peptide Hydrogels for On-Demand Delivery of Tofacitinib Towards Vascular Composite Allotransplantation Treatment Applications

Monessha Nambiar, Chen Liang, Xiomara Calderon-Colon, Olivia Tiburzi, Alexander Komin, Julia Patrone, Giorgio Raimondi, and Joel Schneider

Vascularized composite allotransplantation, VCA, provides a restorative option for patients requiring the transplantation of multiple tissues as a single functional unit. Further, successful patient outcome after transplantation correlates with effective life-long application of immunosuppressive drugs. However, debilitating side-effects counterbalance the benefits of immunosuppressive therapy, presenting an imminent need for developing therapeutic methods that limit tissue rejection after surgery.

Studies indicate that inhibition of inflammatory cytokines can be particularly effective in controlling the activation of T-cells, which are at the core of transplant rejection. Inflammatory cytokines use multiple signaling pathways to deliver their functions, and studies show that Tofacitinib is very effective at suppressing a key pathway, JAK/STAT, involved in T-cell activation.

We are developing a novel dual component therapeutic platform for the targeted delivery of Tofacitinib, JAK1/3 inhibitor. Our platform utilizes a self-assembling peptide hydrogel injected near the transplant site, that encapsulates crystalline Tofacitinib, cTofa, as well as Tofacitinib-containing nanoparticles, Tofa-LNPs, to facilitate drug release in two phases.

First, the cTofa deposits afford a sustained release via diffusion, to immune cells that infiltrate the grafting site. Second, the Tofa-LNPs are released in response to upregulation of rejection-associated proteases, followed by subsequent accumulation in the draining lymphoid tissues, the physiological sites of alloresponse activation.

The modulated drug release in the second phase is achieved by engineering the peptide hydrogel to be susceptible to degradation by proteases released from the alloresponse. Thus, our immune-responsive peptide gel platform enables the localized and temporal delivery of Tofacitinib, minimizing its associated side-effects, with potential to aid in long-term transplant survival.

YI-P172 Karl Ocius

University of Virginia

Non-Invasive Analysis of Peptidoglycan Biopolymers from Living Animals

Karl Ocius

The gastrointestinal tract houses a diverse population of bacteria that works together to support human health. This diverse population, the microbiome, participates in various physiological processes, all of which allow the body to maintain a steady state equilibrium between its different systems for efficient functioning and maintenance of life.

One of the ways these bacteria interact with its host is through the peptidoglycan, the major component of the bacterial cell wall that confers shape and rigidity. Peptidoglycan from the bacteria in the gut modulate the immune system through activation of the Nucleotide-binding Oligomerization Domain containing protein 2 (NOD2)[3]. The activation of NOD2 results in the downstream activation of a signal transduction pathway, which triggers an innate inflammatory response. Moreover, the whole peptidoglycan, sacculi, and its soluble fragments modulate host physiology including checkpoint anticancer therapy efficacy, body temperature, appetite, and postnatal growth.

The Pires lab has developed a platform that analyzes the peptidoglycan called Saccuflow. We aim to utilize this assay to directly and non- invasively analyze the sacculi of gut bacteria isolated from mice and human fecal samples. This direct

analysis provides a route to probe the gut bacteria community including the level of NOD2 activation, which may be leveraged to assess overall gut health.

YI-P173 Soei Watari

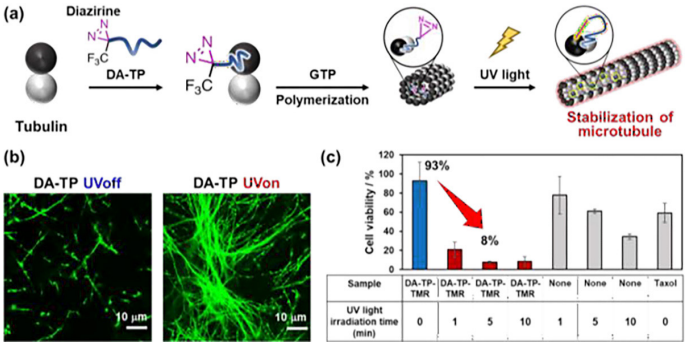
Tottori University, Japan

Non-Invasive Analysis of Peptidoglycan Biopolymers from Living Animals

S. Watari, H. Inaba, A. M. R. Kabir, A. Kakugo, K. Sada, and K. Matsuura

Microtubules are hollow tubule cytoskeletons with a 15 nm inner diameter, which are formed by polymerization of tubulins. Although Taxol, an anti-cancer drug, binds inner pocket and stabilizes the structures of microtubules, it has several limitations including low water solubility and side effects resulting from its adverse outcomes on normal cells.

We have previously designed a Tau-derived peptide (TP)[1] that is soluble in water and binds microtubule inner surface. Its sequence, CGGGKKHVPGGGSVQIVYKPVDL, was based on the repeat domain of the MT-associated protein Tau. Since the binding of TP to tubulin is reversible and not so strong, the stabilization effect of microtubules is moderate.



a - Schematic illustration of stabilization of microtubules by incorporation of DA-TP and subsequent UV light irradiation. **b** - CLSM images of DA-TP-incorporated microtubule with or without UV light irradiation. **c** - Cell viability assay

In this study, we have conjugated a photo-reactive diazirine, DA, which forms a carbene upon UV light irradiation, at the N-terminus of TP (DA-TP)[2]. DA-TP allowed stabilization of MTs via covalent bond formation upon UV light irradiation in vitro and in living cells, se figure above. The DA-conjugated TP (DA-TP) was synthesized by Fmoc solid-phase chemistry followed by coupling of a DA moiety.

We confirmed the binding of DA-TP to microtubules using confocal laser scanning microscopy, CLSM. Competitive binding experiment with Taxol indicated that DA-TP covalently bound inner surface of microtubules upon UV light irradiation. Formation of stable microtubules was observed by irradiation of UV light to DA-TP-incorporated microtubules, se figure above. It was also found that DA-TP bound to intracellular microtubules and the subsequent UV light irradiation induced cytotoxicity.

YI-P174 Mary Villareal

University of Michigan

Defining the Role of Fatty Acid Tails in Lipopeptide Inhibitors of Transcriptional Coactivators

Mary Villarreal, Anna Mapp, and David Sherman

Med25, a subunit of the Mediator complex coactivator, regulates the transcription of stress-response and metastasis genes including HSPA5 and MMP through protein-protein interactions, PPIs, of its Activator Interaction Domain, AcID. Dysregulation of these PPIs is linked to oncogenesis in breast cancer and metastatic phenotypes.

Recent data from the Mapp and Sherman groups suggests that lipopeptide natural products are privileged scaffolds for targeting activator-coactivator PPIs such as those formed between Med25 and its cognate activators. What is not yet known is the role that the lipid architecture plays in affinity and selectivity for a given coactivator. To address this, a library of modified fatty acyl tails using polyhydroxyalkanoates, PHAs, naturally occurring polyesters, as a source of diversification was constructed. PHAs provide an excellent resource for creating enantiomerically pure synthons for the purpose of acyl tail diversification.

Here, I present the successful synthesis of enantiomerically pure (*R*)-3-hydroxyacids from *Cupriavidus necator*, ATCC 17699, a PHA producing microbe, using butanoic and pentanoic acids as the feeding substrates. This workflow yielded (*R*)-3-hydroxybutanoic acid and (*R*)-3-hydroxyvaleric acid, which were coupled to the peptide sequence of the inhibitor. This lipopeptide library was screened for inhibition activity against Med25 AcID PPIs and their selectivity was profiled against other coactivator complexes.

Next steps include expanding the alkanoic acids library (C_6-C_{11}) and investigating acyl tails with and without terminal alkenes, sterically hindered groups and halogenated groups, to assess their potential contribution to the inhibitory activity of the lipopeptide for Med25.

YI-P175 Titia Rixt Oppewal

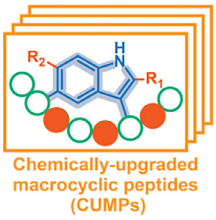
University of Groningen

Selection of Natural-Product-Like Macrocyclic Peptides by Phage Display

Titia Rixt Oppewal, Ivar Daniel Jansen, and Clemens Mayer

Many potent, bioactive natural products are hybrid molecules. For example, α-amanitin or thiostrepton can be regarded as macrocyclic peptides, MPs, that are constructed around small-molecule cores. Produced by organisms to gain a competitive advantage, such hybrid MPs are the result of evolutionary algorithms that fine-tuned both the amino

acid sequence as well as the post-translational processes that enable cyclization with non-peptidic moieties. Leveraging this type of chemogenetic optimization for drug discovery is desirable, yet challenging as synthetic strategies that facilitate peptide diversification and/or macrocyclization are not compatible with the biological selection platforms needed to identify binders from vast libraries.



Chemically-upgraded macrocyclic peptides (CUMPs)

To meet this challenge, I will present strategies to generate billions of chemically-upgraded MPs, CUMPs, via the programmed modification of a unique cysteine residue and a nearby N-terminal amine. We showcased that these cyclization strategies are compatible with the in vitro selection of peptides by phage-display, enabling us to identify nanomolar binders against a model-protein target.

With the prospect of seamlessly combining the favorable traits of small- molecule and peptide-based drugs, our strategies pave the way toward multifaceted opportunities for ligand diversification in drug discovery.

YI-P176 Estefania Martinez Valdivia
L41-YI6

University of Michigan at Ann Arbor

A Lipopeptidomimetic Transcriptional Inhibitor

Estefania Martinez Valdivia, Olivia Pattelli, Matthew Beyersdorf, Clint S. Regan, Mónica Rivas, Tomasz Cierpicki, David H. Sherman, and Anna K. Mapp

Dysregulation in the protein-protein interactions, PPIs, of transcriptional components, such as coactivators and activators, profoundly affects gene expression and is prominent in disease. Coactivators act as regulators of gene expression by bridging activators engaged at enhancer regions of genes to the general transcription machinery. Thus, modulation of gene expression through the inhibition of these PPIs is an avenue to define the role that dysregulated networks play in the development of disease. Due to the intricacy in targeting these complexes, peptidomimetic molecules have the potential to be developed into selective and potent inhibitors.

Here, we report the development of a target-adjustable lipopeptide inhibitor of activator-coactivator complexes based on transcriptional activation domain, TAD, sequences of activators. Structure-activity relationship studies demonstrate adjustability in the selectivity of lipopeptide analogs for various coactivators, including Med25 and CBP/p300. We identified specific moieties on the lipopeptide that dictate the selective disruption of coactivator-activator PPIs. Additionally, the binding mode of potent inhibitors to their main target was determined using 2D NMR.

Our results suggest the occurrence of specific interactions between lipopeptide residues and coactivators, which are crucial for the activity and selectivity of these lipopeptides. Our data positions this molecule as a platform with modifiable selectivity, and it proposes lipopeptides as a novel scaffold for the selective inhibition and characterization of coactivator PPI networks.

P177 Birgit Kosjek

Merck & Co., Inc,. PRD Enabling Technologies

Enzymatic Toolbox for Chemically Defined Functionalization of Peptides and Proteins

Birgit Kosjek

The increasing structural complexity and diversity of novel therapeutic modalities presents a significant synthetic challenge. New methods to enable efficient production of protein or peptide based molecules and conjugates are critical to both discovery and development efforts. Mild, chemo- and regioselective technologies are desired to perform custom, precise peptide modifications, and to differentiate among amino acid residues with similar chemical character while retaining the three-dimensional structure. Biocatalysis has matured into an essential tool in modern chemical synthesis, leveraging the unprecedented chemo, regio-, and stereoselectivity of enzymes. These features enable site-selective bioconjugation as well as modification of biomolecules without the need for protecting groups. Enzyme selectivity, performance and properties, including reaction condition compatibility, can be readily improved through directed evolution, allowing the catalysts to be customized to the synthetic requirements.

Here, we share our work on the development of an enzymatic toolbox for site-selective protein modification. We demonstrate how penicillin G acylases can be engineered to differentiate free amino acid groups in insulin to selectively acylate or deprotect specific sites, enabling defined conjugation with payload molecules. This strategy unlocked access to a full set of insulin bioconjugates for therapeutic evaluation and projects applicability to other proteins and peptides. We discuss how bioconjugation strategies using naturally occurring and engineered enzymes to act on native proteins and peptides can accelerate the discovery and development of diagnostic probes and therapeutics.

YI-P178 Radoslaw Piast

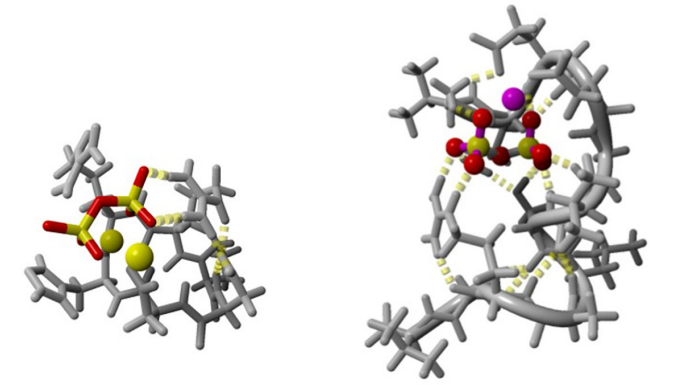
Warsaw University

Obtaining Minimal Phosphate Transferases

Radoslaw Piast, Rafał Wieczorek, Maciej Garstka, and Aleksandra Misicka

Phosphate transferases are key enzymes for life as they incorporate phosphate ions into organic molecules which in turn constitute energy metabolism, build information-storing molecules, activate anabolic substrates, and are a cell signaling pathway. In our study, we were trying to obtain

minimal versions of phosphate-transferring peptides - a few amino acid-long peptides able to mimic the catalytic abilities of a whole enzyme.



C3 - peptide with synthetase-like properties

R25 - peptide with pyrophosphatase-like properties

In my presentation, I will explore our attempt to obtain a minimal peptide synthetase to be used as a catalyst in technological processes and a minimal inorganic pyrophosphatase able to dissolve crystals of calcium pyrophosphate - the etiological factor of chondrocalcinosis.

P179 Leah Witus

Macalester College

Exploration of the Role of Turn Position Residues in β-Hairpin Peptide Catalysts

L.S. Witus

Peptide catalysts are appealing 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. Although short oligomers lack the intricate three-dimensional environment of a folded protein, a commonly used strategy in peptide catalysis is to design sequences that induce a secondary structure conformation, such as a β-hairpin.

We have been investigating the *i+1* and *i+2* turn residues in β-hairpin peptide catalysts, enabling an exploration of the role of the peptide conformation on catalysis. Our findings on the effect of turn-nucleating positions on catalytic activity for aldol and ester hydrolysis reactions will be presented.

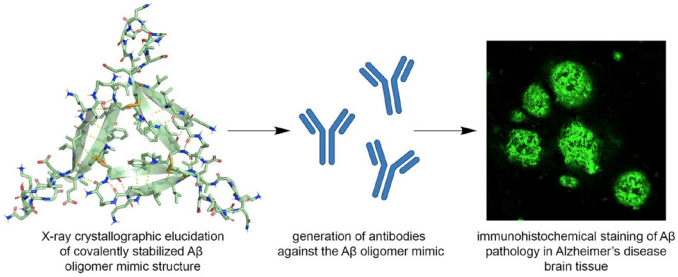
P180 Adam Kreutzer

University of California at Irvine

Antibodies Raised Against an AP Oligomer Mimic Recognize Pathological Features in Alzheimer's Disease and Associated Amyloid-Disease Brain Tissue

Adam G. Kreutzera, Chelsea Marie T. Parrocha, Sepehr Haerianardakani, Gretchen Guaglianone, Jennifer T. Nguyen, Michelle N. Diab, and James S. Nowick

Antibodies that target the B-amyloid peptide, AB, and its associated assemblies are important tools in Alzheimer's disease research and have emerged as promising Alzheimer's disease therapies.



X-ray crystallographic elucidation of covalently stabilized Aβ oligomer mimic structure

generation of antibodies against the Aβ oligomer mimic

immunohistochemical staining of Aβ pathology in Alzheimer's disease brain tissue

This poster reports the creation and characterization of a triangular AB trimer mimic composed of AB_{17-36} B-hairpins, and the generation and study of polyclonal antibodies raised against the AB trimer mimic. The AB trimer mimic is covalently stabilized by three disulfide bonds at the corners of the triangular trimer to create a homogeneous oligomer. Structural, biophysical, and cell-based studies demonstrate that the AB trimer mimic shares characteristics with oligomers of full-length AB: X-ray crystallography elucidates the high-resolution structure of the trimer and reveals that four copies of the trimer assemble to form a dodecamer; SDS-PAGE and size exclusion chromatography demonstrate that the trimer also forms a dodecamer in solution; cell-based toxicity assays show that the trimer elicits LDH release, decreases ATP levels, and activates caspase-3/7 mediated apoptosis.

Immunostaining studies on brain slices from people who lived with Alzheimer's disease as well as people who lived with Down syndrome reveal that the polyclonal antibodies raised against the AB trimer mimic recognize pathological features including different types of AB plaques and cerebral amyloid angiopathy. These findings suggest that the triangular trimer structural motif is important in Alzheimer's disease and may thus constitute a new molecular target for diagnostic and therapy development.

YI-P181 Ajayi Akinwale

Chapman University School of Pharmacy

Mechanism of Action of Cyclic Amphipathic Antimicrobial Peptide [R_4W_4] Against Methicillin-Resistant *Staphylococcus aureus*

Ajayi David Akinwale, Keykavous Parang, Rakesh Kumar Tiwari, and Jason Yamaki

Antimicrobial peptides are an emerging class of potent antibacterial agents that have demonstrated activity against multidrug-resistant pathogens. Cyclic peptide [R_4W_4] containing positively charged arginine, R, and hydrophobic tryptophan, W, residues showed antibacterial activity with a minimum inhibitory concentration, MIC, value of 4 μg/mL against laboratory strains of methicillin resistant *Staphylococcus aureus*, MRSA, and 16 μg/mL against *Escherichia coli*, *E. coli*. How-

ever, the mechanism of action of cyclic peptide [R_4W_4] and its stability has not explored. No change in the antibacterial activity of [R4W4] after heating the peptide at 100°C for 10 min and incubating the peptide under varying physiological salt concentrations, sodium chloride, calcium chloride, and ferric chloride, was observed, suggesting therostability and salt resistance, respectively. [R_4W_4] and vancomycin were tested for antibacterial activity against eight clinical resistant isolates of Gram-positive *Staphylococcus aureus* strains, revealing MIC ranges of 2-8 µg/mL and 1-2 µg/mL, respectively. [R_4W_4] also had activy against vancomycin resistant *Enterococcus,* VRE, with a MIC 4 µg/mL. Gentamicin synergy testing demonstrated fractional inhibitory concentration indices, FICI, for MRSA and *E. coli* of 0.75 and 0.30, respectively, indicating a synergistic effect of [R_4W_4] and gentamicin against *E. coli*, but not MRSA.

We hypothesized that the [R_4W_4] activity may be a selective disruption of bacteria cell wall and membrane due to net positive charge and amphipathic structure centered on tryptophan and arginine constituents. Surface charge neutralization assay revealed statistically significant difference in the zeta potential against E. coli with p-value <0.0001, using vancomycin as a negative control. The result suggests that the positive net charge in the [R_4W_4] may play a significant role in the antibacterial activity.

YI-P182 A'Lester Allen

University of Illinois at Chicago

Mapping the Uptake, On-Target Binding, and Translation Inhibition of Apidaecin 137 Derivatives

A'Lester C. Allen, Weiping Huang, Nora Vàzquez-Laslop, Shura Mankin, and Terry Moore

Multi-drug resistant infections were responsible for 4.95 million deaths in 2019 and are projected to cause 10 million deaths every year by 2050. Currently, none of the approved clinical antibiotics target translation termination. Recently, Apidaecin, an antimicrobial peptide, was found to inhibit translation termination, causing cell death in Gram-negative pathogens. Unlike other antimicrobial peptides, Apidaecin is nonlytic, and it undergoes cellular uptake by the SbmA transporter.

Upon binding to the 70S ribosome, arginine 17 of Apidaecin forms electrostatic interactions that prevent dissociation of the release factor from the ribosome, causing cell death. Approaches based on analyzing the antibacterial activity of chemically synthesized peptides fail to distinguish the effects of cellular uptake from those affecting on-target activity. Raman imaging combines molecular spectroscopy with high-resolution spatial information to create images of molecular distributions.

Herein, we show preliminary antimicrobial activity of Apidaecin analogs with small molecule tags.

P183 Ana Salome Veiga

Instituto de Medicina Molecular

Design and Discovery of pepRF1, a new Potent CXCR4-Targeted Inhibitor of HIV-1 Entry

Iris Cadima-Couto, Alexandra Tauzin, João M. Freire, Tiago N. Figueira, Rúben D. M. Silva, Clara Pérez-Peinado, Catarina Cunha-Santos, Inês Bártolo, Nuno Taveira, Lurdes Gano, Joao D. G. Correia, Joao Goncalves, Fabrizio Mammano, David Andreu, Miguel A.R.B Castanho, Ana Salomé Veiga

There is a need for the development of new anti-HIV drugs that can complement existing medicines. In this work, we show that pepRF1, a human serum-resistant peptide derived from the Dengue virus capsid protein, is able to inhibit HIV-1 infection with a 50% inhibitory concentration, IC_{50} of 1.5nM.

This peptide is specific for CXCR4-tropic HIV-1 strains, preventing viral entry into target cells by binding to the viral co-receptor CXCR4. pepRF1 is more effective than T20, the only peptide-based HIV-1 entry inhibitor approved by FDA for clinical use, and excels in inhibiting an HIV-1 strain resistant to T20 (HIV-$1_{NL4.3}$ DIM) with an IC50 of 2.8nM.

Overall, our study led to the discovery of a peptide highly active against HIV-1, serum-stable, and with low toxicity, that acts as a CXCR4 antagonist. Potentially, pepRF1 can be used alone or in combination with other anti-HIV drugs to fight AIDS. Furthermore, one can also envisage its use as a therapeutic strategy for other CXCR4-related diseases such as various types of cancers, where the receptor plays a pivotal role in tumor development and metastasis.

YI-P184 Othman Almusaimi

Imperial College London

Glial-Specific Blood-Brain Barrier Shuttle Peptide for Brain- Related Diseases

Othman Al Musaimi, Sophie V. Morse, Lucia Lombardi, and Daryl R. Williams

TD2.2 is a blood-brain barrier shuttle peptide that has been reported to target oligodendrocytes and has been shown to not target non-glial cells, such as human neural cells and human dermal fibroblasts. This peptide has therefore high specificity compared to other peptides of the same class. Successful manual synthesis of TD2.2 peptide acting as a blood-brain barrier shuttle has been achieved. TD2.2 was successfully synthesised by sequential condensation of four protected peptide fragments on solid- phase settings after several unsuccessful attempts using the stepwise approach.

These fragments were chosen to minimize the number of demanding amino acids, in terms of coupling, Fmoc-removal, in each fragment that are expected to hamper the overall synthetic process. Thus, the hydrophobic amino acids as well as Arg(Pbf) were strategically spread over multiple fragments rather than having them congested in one fragment.

To deliver these peptides to the brain, focused ultrasound and microbubbles have been used to temporarily open the blood-brain barrier. A fluorescent analogues of TD2.2 were delivered to the brain with this technique and detected via fluorescence microscopy. Co-staining for astrocytes and oligodendrocytes shows co-localisation with the peptides.

YI-P185 Keisuke Aoki

Kyoto University

Mirror-Image VHH for Less Immunogenic Protein Therapeutics

K. Aoki, M. Nonaka S. Oda, K. Higashi, A. Manabe, H. Kimura, S. Inuki, H. Ohno and S. Oishi

Immunogenicity is one of the critical issues during development of protein therapeutics. Immune response by administration of protein therapeutics leads to generation of anti-drug antibodies, ADA, and neutralizing antibodies, NAb. Mirror-image peptides and proteins consisting of D-amino acids provide an effective solution because of the resistance to proteolytic degradation in immune cells. There have been a number of examples of mirror-image peptides and proteins in drug discovery.

We focused on mirror-image variable domain of heavy chain of heavy chain antibody, D-VHH, as a protein scaffold for development of novel mirror-image protein therapeutics. Initially, we established a synthetic process of PMP12A2h1, anti-von Willebrand factor VHH, monomeric protein of caplacizumab, as a case study. PMP12A2h1 and its enantiomer were successfully synthesized by sequential native chemical ligation from four peptide fragments.

After folding under optimized conditions, the structures and functions of synthetic VHHs were confirmed by CD spectrometry and SPR analysis, respectively. Immunogenicity testing in mice demonstrated that administration of L-VHH induced the generation of ADAs, while D-VHH-binding antibodies were not observed in D-VHH-immunized mice.

Next, we performed a mirror-image screening using a phage display library to identify a VHH sequence, which bound to mirror-image vascular endothelial growth factor, D-VEGF. The subsequent synthetic study provided the D-VHH, which bound to native VEGF with sub-micromolar affinity.

In the symposium, the details of the mirror-image screening as well as a biodistribution study of mirror-image VHH will also be presented.

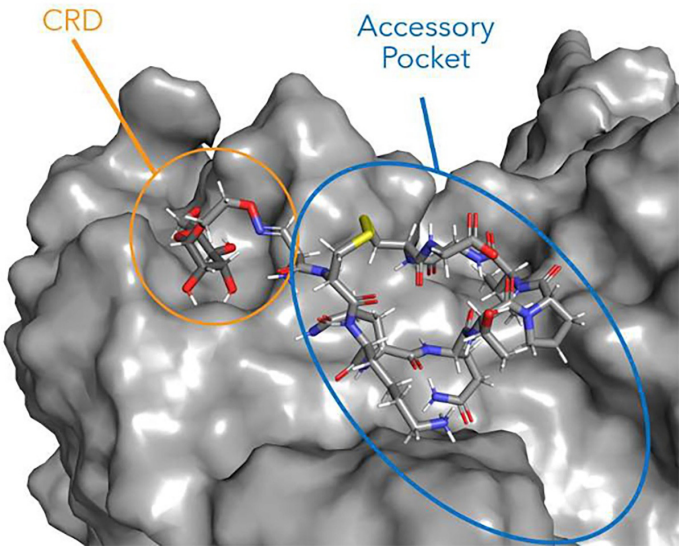
P186 Hassan Boudjelal

University of East Anglia

A Phage Display Method to Identify Novel Glycopeptide Ligands for the *Pseudomonas aeruginosa* lectin, LecB

H. Boudjelal, L. Tucker, P. Hernando, S. Ahmadipour, T. Hicks, D. Baker, A. Beekman, D. Warren, R. A. Field, M. J. Marín and C. J. Morris

Lectins are carbohydrate binding proteins that mediate a wide range of biological processes such as pathogen recognition, cell adhesion and inflammation. The development of inhibitors/agonists for these proteins may therefore be therapeutically valuable in many areas such as treatment of infectious disease and cancer therapy. However, this is challenging due to the typically weak binding, K_D = μM to mM range, between lectins and their cognate carbohydrate ligands. We aim to combine the selectivity of glycan binding to the lectin carbohydrate recognition domain, CRD, with the enhanced affinity of peptide binding to nearby accessory binding sites by using glycopeptide display libraries.



Computational docking of a phage display derived glycopeptide in complex with LecB. The peptide moiety can be observed interacting with a pocket adjacent to the CRD

Phage display is a recombinant screening technique that allows for the selection of high-affinity peptides for a given target molecule. Peptides are specifically selected from libraries containing >106 randomized sequences by iterative screening and enrichment steps. Phage libraries have been engineered to contain an N-terminal serine, which is used to covalently attach a glycan monomer, creating glycopeptide display libraries for screening against specific lectins. In this project, three glycan-conjugated disulfide-constrained peptide libraries, 5-mer, 7-mer and 9-mer, complexity: >106, were cloned and screened against LecB from *P. aeruginosa*, a lectin involved in biofilm formation. Illumina sequencing coupled with MATLAB and Python scripts were used to identify candidate glycopeptides that were significantly enriched, $p < 0.05$, by a ratio of ≥5 compared to controls, which were then sorted by predicted affinity based on molecular docking simulations. Peptides were synthesised by SPPS and glycosylated by oxime ligation to relevant glycan monomer. The affinity

of these glycopeptides to the target lectin is measured by fluorescence polarisation and compared to the native glycan monomer ligand.

YI-P187 Alana Babcock

University of Victoria

Systematic Truncation and Optimization of Antimicrobial Peptides Mined from Amphibian Genomes

Alana Babcock, Darcy Sutherland, Chenkai Li, Hossein Ebrahimikondori, Amalia Richter, Macy Preston, René L. Warren, Linda M.N. Hoang, Fraser Hof, Caren C. Helbing, and Inanc Birol

Antimicrobial resistance is an emerging global health crisis with far-reaching impacts on both animal and human health. Antimicrobial peptides, AMPs, are a promising alternative to conventional small-molecule antibiotics. AMPs can act simultaneously on multiple targets to weaken the bacterial membrane integrity and disrupt macromolecule synthesis. AMPs can also trigger downstream signalling events in immune cells to improve the host response to pathogen infection. Despite their acknowledged potential, in exogeneous applications AMPs often require optimization to become viable therapeutics.

We demonstrate the ability of the deep-learning classifier 'AMPlify' to identify, truncate, and perform site-directed mutagenesis of AMP candidates mined from amphibian genomes. This pipeline produces AMPs with promising therapeutic indices, measured through antibiotic susceptibility testing and hemolysis assays. These combined AI- and chemistry-driven optimizations produce low molecular weight AMP analogues with significant therapeutic potential. This approach can be broadly applied to other genomes, increasing the identification of therapeutically useful antimicrobial alternatives.

YI-P188 Prabhat Bhat

The Ohio State University

Engineering Cell-Permeable Proteins

Prabhat Bhat and Dehua Pei

We engineered a family of membrane translocating domains, MTDs, by replacing the surface loops of fibronectin type III, FN3, scaffold with cell-penetrating peptide motifs.

One of the domains, MTD4, demonstrated unprecedented cytosolic entry efficiencies into eukaryotic cells by several different assays, for example, flow cytometry, confocal microscopy, and protein complementation. Peptide and protein cargoes of diverse sizes, sequences, and physicochemical properties have been efficiently delivered into the cytosol, and nucleus, of mammalian and plant cells by genetically fusing them to the N- or C-terminus of MTD4, resulting in cellular EC_{50}, or IC_{50}, values that are as low as ≤5 nM. MTD4 is proteolytically stable and exhibits broad biodistribution in mice. The MTDs provide a novel, general, highly efficient, and simple approach for delivering peptides and proteins into eukaryotic cells.

P189 Laura Hanold

University of Florida

An Anti-Proliferative Peptide Downregulates CIP2A and Associated Proteins in Ovarian Cancer

L.E. Hanold and J.V. Aldrich

It is estimated that over 19,000 new ovarian cancer cases and over 13,000 ovarian cancer-related deaths will occur in the United States in 2023, American Cancer Society. Although incidence and survival rates have been improving, primary and acquired resistance and severe side effects remain major limitations of current platinum-based and targeted chemotherapies. Thus, there is a need to develop alternative treatment options for patients who do not respond to current therapies and to limit side effects. Cancerous inhibitor of protein phosphatase 2A, CIP2A, which inhibits tumor suppressor protein phosphatase 2A, PP2A, is an oncoprotein that is overexpressed in over 65% of ovarian cancer cases and is associated with poor prognosis in serous ovarian cancer patients. Knockdown of CIP2A has decreased cell proliferation and increased sensitivity to cisplatin in ovarian cancer cells. Thus, downregulating CIP2A may be a promising approach to treat ovarian cancer and overcome chemoresistance.

Our laboratory has identified a lead cyclic peptide that decreases proliferation in ovarian cancer cell lines, with selectivity for these cells over a non-cancer control. This peptide appears to down-regulate CIP2A protein levels and alter levels of post-translationally modified PP2A and Akt, which are frequently dysregulated in cancer. An analog of the lead peptide also demonstrated anti-proliferative activity against multiple cell lines. Preliminary results indicate that the anti-proliferative activity was greatest in the cell lines with high levels of CIP2A. Thus, these anti-proliferative cyclic peptides may have potential for downregulating CIP2A and treating CIP2A-overexpressing ovarian cancer.

Acknowledgement:
This work was supported by the Assistant Secretary of Defense for Health Affairs through the Ovarian Cancer Research Program under Award No. W81XWH-22-1-0268. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

YI-P190 Anamika Biswas

Tata Institute of Fundamental Research

Disrupting AMA1-RON2 Protein-Protein Interaction by Metabolically Stable Mini Protein Inhibitor to Stop the Malaria Parasite Invasion into Red Blood Cells

Anamika Biswas, Mrinal Kanti Bhattacharyya, Maruti Uppalapati, and Kalyaneswar Mandal

Malaria is a mosquito-borne disease caused by *Plasmodium* species, the deadliest of which is *Plasmodium falciparum*. There is no fully effective vaccine reported till date to tackle malaria. Further, the widespread resistance of the frontline medicine 'artemisinin combination therapy' is also responsible for the delayed control over the disease.

Therefore, there is an urgent need to develop an alternative yet effective anti-malarial therapeutic. Unlike small-molecule drugs, peptide or mini-protein based inhibitors are promising due to their larger interacting surface area leading to the high specificity and less toxicity.

Moreover, like antibodies, they do not demand low temperature supply chains. In all apicomplexan parasites a unique invasion mechanism exists that involves moving junction formation between the host cell and the parasite. For *P. falciparum*, two parasitic proteins, named Apical Membrane Antigen 1, (*Pf*AMA1) and Rhoptry Neck Protein 2, *Pf*RON2, strongly interact with each other, and form the moving junction.

Our aim here is to disrupt the *Pf*AMA1-*Pf*RON2 protein-protein interactions by natural or non-natural peptides or proteins, resulting in the inhibition of the *P. falciparum* merozoite invasion into red blood cells. Therefore, we designed potential metabolically stable cyclic peptide inhibitors and D- protein based inhibitors using a unique combination of 'chemical protein synthesis,' and 'mirror image phage display' to target *Pf*AMA1-*Pf*RON2 interactions.

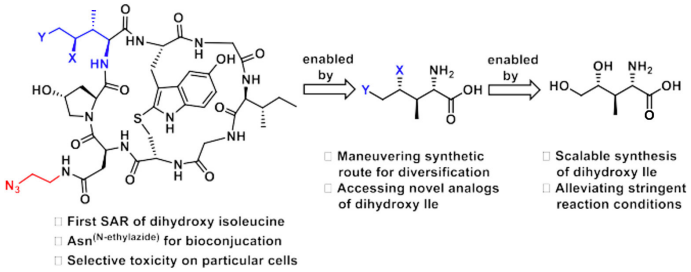
YI-P191 Shambhu Chandra

University of British Columbia

Towards new Amanitin Analogs: Accessing (2*S*,3*R*,4*R*)-Dihydroxy-Isoleucine and its Analogs for Incorporation into α-Amanitin

S.D. Chandra, S. Gunasekera, Antonio A. W. L. Wong, and D. M. Perrin

α-Amanitin is an extremely toxic bicyclic octapeptide extracted from the death-cap mushroom, *Amanita phalloides*. It is an allosteric, selective and potent inhibitor of RNA polymerase II, the enzyme indispensable for cellular function and homeostasis. With the appealing properties of stability, potency, and a unique mechanism of action, α-amanitin has shown formidable promise as a payload in antibody–drug conjugates for cancer treatment. α-Amanitin includes numerous synthetic hurdles with di-hydroxy isoleucine being one of the prominent synthetic challenges. It is a chiral, oxidized non-canonical amino acid indispensable for toxicity. It is a high functional density molecule containing a carboxylic acid, an amine, diols and β-branched methyl on a five-carbon linear main chain, thereby making the synthesis extensive and arduous.



Herein we address the intricacy and challenges of synthesizing di-hydroxy isoleucine in gram-scale quantities, with a rationale of truncating synthetic complexity. We have synthesized di-hydroxy isoleucine, thereby enhancing the application of α-amanitin in cancer therapy. Furthermore assimilating the synthetic route gave us access to 10 novel analogs.

All of them were incorporated in the amanitin core and subsequently, we developed a structure-activity relationship, SAR, study around di-hydroxy isoleucine which is distinctive and the first of its kind. The cytotoxicity of new amanitin analogs was investigated on four different cell lines exhibiting encouraging results for further potential applications.

P192 Dongmin Kim

Seoul National University

A Cardiolipin-Specific Peptide Ameliorates Obesity and NASH through Mitochondrial Function Restoration

Dongmin Kim, Ji Hyeon Kang, Taehyeon Jeong, Yoe-Sik Bae, and Jaehoon Yu

Obesity is a disease characterized by the accumulation of fat in cells and an abnormal increase in weight due to intake of more calories than consumption of them. This fat accumulation in liver cells is the main cause of nonalcoholic fatty liver disease, NAFLD. Even though the disease does not have any pathological symptoms, NAFLD can progress to more severe forms of liver diseases, such as nonalcoholic steatohepatitis, NASH, fibrosis, cirrhosis, and cancer. However, there are currently no FDA-approved drugs to treat NAFLD and NASH.

Mitochondrial dysfunction is strongly associated with the development and progression of NAFLD, and the significant decline in mitochondrial function in fatty liver cells is closely related to the low expression of cardiolipin, CL, a specific phospholipid necessary for maintaining the structure and function of inner membrane of mitochondria, IMM. When CL is oxidized and pathogenically remodeled, protein complexes in IMM cannot carry out their roles properly, gener-

ating dysfunctional mitochondria and progressing NAFLD to NASH.

We have been developing a CL-specific peptide, CMP3013, which can bind to normal or oxidized CLs in IMM, converting leaky to rigid IMM, correcting mitochondrial dysfunction. Treatment of this peptide to high-fat diet induced mouse showed a significant reduction of weight and inflammatory fatty liver damages in liver cells.

Since the CL-specific peptide strongly bind to CLs, it prevents the escape of CL molecules from IMM to cytosol for inflammasome formation, which is known as an essential event from NAFLD to NASH. Taken together, we have shown that the CL- specific peptide can be a plausible therapeutic against obesity related NASH.

YI-P193 Yanan Chang

University of Pennsylvania

Development of NPY Y2-based Thiopeptides for Targeted Imaging of Glioblastomas

Yanan Chang, Hoang Anh T. Phan, Kristen Fiore, and E. James Petersson

The neuropeptide Y Y2 receptor, NPY Y2R, has been found to be highly expressed in human glioblastoma cells and was shown to stimulate cell growth. To develop an effective NPY-based imaging agent probe for glioblastomas, peptide agents were designed as receptor agonists that could be internalized by receptor-mediated endocytosis.

However, the short half-life of most NPY derivatives, <30 minutes, makes them unsuitable as imaging probes. A side-chain lactam bridge was introduced between the 28th and 32nd residues to stabilize the C-terminus of the NPY-based peptides. Elimination of the 24th residue, Arg further enhanced peptide stability.

High-performance liquid chromatography, HPLC was used to identify cleavage sites by analyzing peptides after incubating in mouse serum. Thioamide substitution was then applied to a strategic site to improve proteolytic stability. These thioamide-stabilized cyclized peptide probes showed a significant improvement in half-life from approximately 30 minutes to over 8 hours, albeit with some loss of potency for binding with NPY Y2R.

Overall, these results suggest that stabilized cyclized peptide may have potential for use as Y2R imaging agents in different biological or clinical applications.

YI-P194 Changfeng Cheng

University of Illinois at Chicago

Targeting the PAH2 Domain of Transcriptional Corepressors SIN3A/B for Inhibition of Proliferation in Triple-Negative Breast Cancer

Changfeng Cheng, Dai Horiuchi, Ishwar Radhakrishnan, and Terry Moore

Triple Negative Breast Cancer, TNBC, accounts for 20% of all breast cancer cases and is one of the most clinically challenging subtypes of breast cancer due to its aggressive nature. Recently, Histone Deacetylase-associated transcriptional regulatory proteins SIN3A and SIN3B have emerged as potential molecular targets for TNBC therapeutics. Peptidic inhibitors and small molecules targeting the PAH2 domain, a well-characterized protein-interaction domain of the SIN3 homologs, have shown induction of epigenetic reprogramming along with upregulation of differentiation markers and downregulation of epithelial-to-mesenchymal transition markers in TNBC cell lines.

In this work, we have developed a library of hydrocarbon-stapled, peptidic inhibitors based on a known binding partner, MXD1, with various stapling positions that demonstrate isoform selectivity between SIN3A and SIN3B. Fluorescence Anisotropy assays show varying binding affinity of these stapled peptides towards the PAH2 domain of the paralogs. Initial results show anti-proliferative effects when lead compounds are used as treatment with MDA-MB-231 and BT549 cell lines and acceptable blood plasma concentrations in DMPK studies.

Here we present our methodology for the synthesis and binding affinity characterization for our peptidic inhibitors. These compounds will serve as potential chemical tools for elucidation of the mechanisms of action of SIN3A and SIN3B.

P195 Jiyoun Lee

Sungshin University

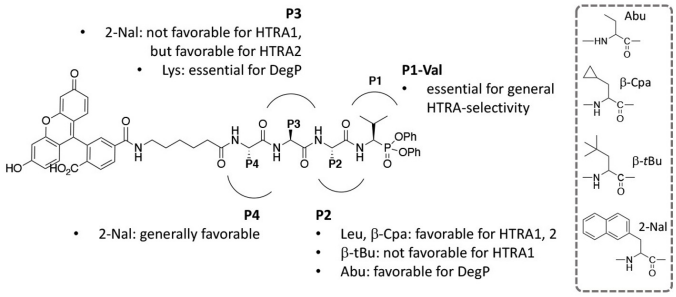
Activity-Based Probes Targeting HTRA Serine Proteases and Their Applications in Biomarker Discovery

J.-Y. Lee, N.-E. Choi, E.-C. Park, S.-J. Han, and Jiyoun Lee

High temperature requirement A serine proteases, HTRA, are ubiquitously expressed and participate in protein quality control and cellular stress responses. They are connected to a number of clinical conditions, such as cancer, age-related macular degeneration, bacterial infections, and neurodegenerative illnesses.

HTRAs have also been identified in a number of recent studies as significant biomarkers and possible therapeutic targets, necessitating the creation of a reliable detection technique to assess their functional status in distinct disease models. With improved subtype selectivity and reactivity, we have

created a new generation of HTRA-targeting activity-based probes and have been able to establish the structure-activity relationship.



We believe that our probes are useful for discovering and validating HTRAs as essential biomarkers since they are cell-permeable and have strong inhibitory effects against HTRA1 and HTRA2. We are improving the probe designs and reporter groups to enable signal amplification with greater selectivity, as well as developing a high-throughput detection method employing these probes. Our focus now is on how we might increase the sensitivity of the probe for practical applications, allowing for the assessment of large quantities of clinical samples with high precision.

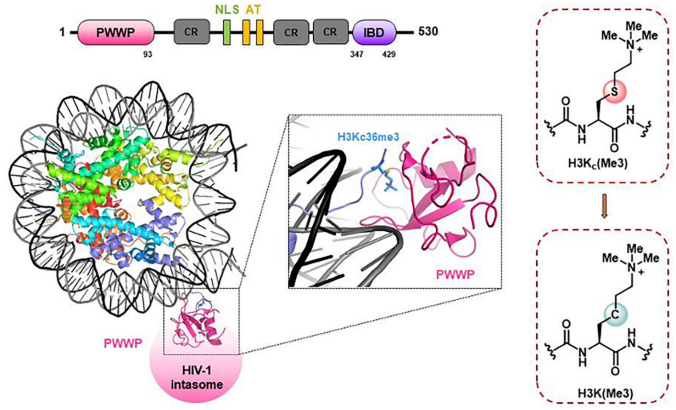
YI-P196 Ramesh Chingle

National Institutes of Health

Total Chemical Synthesis of the Modified Histone H3K36Me3 for Construction of Nucleosomes Bearing a Trimethylated Marker for Enhanced Binding by LEDGF Protein

Ramesh M. Chingle, Samanthreddy Kedika,t and Terrence R. Burke, Jr.

Histone post-translational modifications, PTMs, are critical for epigenetic regulation of chromatin structure and function. In histones methylated histone lysine residues, have important roles in numerous biological processes, including



gene transcription.
Cryo-EM structure of PWWP domain of LEDGF in complex with an H3Kc36me3-modified nucleosomes.

Our work is focused on synthesizing modified histones that have enhanced affinity for the methyl-lysine binding site of the PWWP domain of LEDGF/p75, lens epithelium-derived growth factor, which is an important cellular cofactor of HIV-1 integrase, IN. We are using native chemical ligation, NCL, to introduce non-standard amino acid residues into the histone 3.3 that will be used for further structural and functional studies.

Herein we present optimized protocols for solid-phase peptide synthesis combined with sequential NCL to generate semisynthetic and fully synthetic approaches to prepare the methylated 135-residue protein, H3.3K36me3, in which the K36 residue is present as its trimethyl variant. This histone will be used construct nucleosomes having enhanced affinity for LEDGF for cryo-EM studies.

YI-P197 Yoonhwa Choi

Seoul National University

Mitochondrial Specific Peptides as Novel Therapeutics Against Sarcopenia

Yoonhwa Choi, Dong Heon Lee, Dongmin Kim, Hyeong Woon Choe, Tae Yeon Kim, Hyo Youl Moon, and Jaehoon Yu

Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength by chronic diseases, aging, and lack of exercise for a long period of time. One of the common characters of the disease includes alterations in mitochondrial function. Mitochondrial dysfunction in skeletal muscle cells are mainly derived from an increase of reactive oxygen species, ROS, inside of the organelle, resulting in muscle loss and a decrease in exercise capacity.

We have been developing mitochondria-specific peptides, which can reduce ROS generation, correcting mitochondrial dysfunction derived from ROS.

In this study, we try to reverse muscle dysfunction by our mitochondrial specific peptides in two different pathogenic animal models for sarcopenia. One model is a Drosophila model with phosphatidylserine synthase, Pss, mutation, which generates phenotypes of muscular dystrophy mutation. The other model is an old female mouse, where mitochondria in skeletal muscle are severely damaged in shapes and numbers. Treatment of the mitochondrial specific peptide to the Pss-mutated Drosophila model resulted in enhanced locomotive capacity of larva-stage fly as well as proper development of flight muscle in adult-stage fly. Treatment of the peptide to old mouse also showed enhanced exercise capacity with correcting mitochondrial morphology.

Furthermore, the drug has synergistic effect with common exercise therapy, doubling the exercise capacity relative to the sedimentary control mouse. The improved capacity is owing to increasing mitochondrial number and erasing abnormal mitochondrial morphology in skeletal muscle. Cor-

rectively, mitochondrial-targeted peptides, reversing the mitochondrial dysfunction and improving athletic ability of animals in two different models, may be to clinical trials for therapeutics against sarcopenia.

P198 Yanjie Li
Genentech

A Cell Penetrating Cystine-Knot Peptide for Cargo Delivery

Yanjie Li, Sunhee Hwang, Stéphanie Polderdijk, Kristin Wucherer, Hao Wu, Jakob Fuhrmann, Xinxin Gao, and Rami Hannoush

Cystine-knot peptides, CKPs, have attracted a lot of attention as a promising class of pharmacological ligands. A few CKPs have been shown to penetrate cells. However, the cell penetrating properties and cellular uptake mechanisms of many CKPs remain unclear.

Here we studied the cellular uptake behavior of a model CKP, Imperotoxin A, Imp. We found that a fraction of Imp enters cytosol efficiently via direct translocation and a fraction of Imp enters cells through an active endocytic pathway.

We also demonstrated that Imp efficiently delivers cargoes of various sizes into cells. Overall, our study reports a cell penetrating CKP that could be used to deliver different cargoes into cytosol, and hence enable future exploration of its utility in drug discovery and delivery.

YI-P199 Agnieszka Ciesiolkiewicz
Wroclaw University of Science and Technology

Novel PD-1/PD-L1 Interaction Inhibitors Based on the Engrailed Homeodomain Miniprotein Scaffold

Agnieszka Ciesiołkiewicz, and Łukasz Berlicki

Immunotherapy has been identified as a highly potent cancer treatment. Targeting immune checkpoints, such as PD-1/PD-L1, is considered a particularly beneficial although it is difficult due to the large, flat, hydrophobic interaction surfaces of both proteins. Miniprotein-based therapies have revealed a novel and more effective way to inhibit protein-protein interactions due to their favorable features. Miniprotein drugs are characterized by medium size and relatively stable tertiary structure, allowing the introduction of numerous mutations to interact with the target, with the preservation of an overall structure that can result in high activity and selectivity.

In this work, inhibitors were designed on the basis of the engrailed homeodomain scaffold consisting of three helices. The surface made up of two antiparallel helices was used to create an interaction interface with PD-L1 at the PD-1 binding site. The introduction of mutations that result in interaction with the hydrophobic surface of PD-L1 leads to an increase in the proportion of hydrophobic residues on the surface of the miniprotein and thus to a decrease in polarity and solubility. Therefore, obtaining effective inhibitors of the PD-1/PD-L1 interaction also requires a comprehensive redesign of the structure to introduce more polar residues and increase solubility while preserving the tertiary structure of the original scaffold. This miniprotein design approach has led to the development of inhibitors of the PD-1/PD-L1 interaction with low micromolar activity.

Miniproteins were synthesized using microwave-assisted automated solid-phase synthesis. Their secondary structure and thermal stability were determined by circular dichroism, CD. Binding kinetic analysis and inhibition measurements were performed using biolayer interferometry, BLI, and homogeneous time-resolved fluorescence, HTRF.

YI-P200 Janina Kristin Dahmen
University of Copenhagen

Identifying *De Novo* Cyclic Peptide Correctors of Protein Folding

Janina K. Dahmen, and Joseph M. Rogers

Correct protein folding is essential for many proteins to perform their vital functions. The three-dimensional structure of a protein is encoded in its amino acid sequence, and mutations may lead to loss of fold and function of the protein, followed by degradation in the cell. Such protein folding defects are predicted to be the origin of thousands of. often rare, genetic diseases. Medicines to 'correct' these folding defects are urgently required. In certain cases, traditional small molecule drugs have been used as folding correctors. However, few have reached patients, likely because of limitations of the modality: small molecules can only bind a limited range of protein targets, and even then, they bind in the active sites, inhibit function.

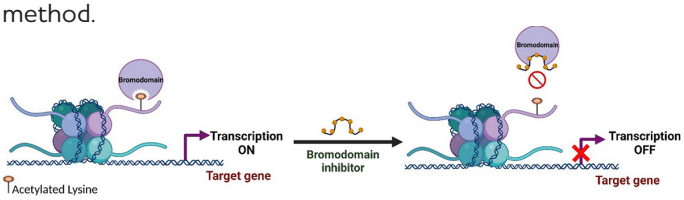
Here, we explore the use of de novo cyclic peptides to correct protein folding problems. We use human dihydrofolate reductase, DHFR, as a model system, as mutants of DHFR have been shown to lead to disease. New correcting cyclic peptides were discovered using the RaPID system, a state-of-the-art chemical biology method which manipulates ribosomes *in vitro* to synthesize and screen trillions of unique cyclic peptides in parallel. We have used and adapted RaPID to isolate *de novo* cyclic peptide compounds with the ability to bind and thermodynamically stabilize DHFR, highlighting the promise of this modality to treat human diseases where protein folding is the root cause.

YI-P202 Gopal Krishan Dubey
Texas A&M University

A Genetically Encoded Phage Display Technique Targeting Bromodomain Protein 9, BRD9, for Discovery of Peptide Inhibitors

Gopal K. Dubey and Wenshe Ray Liu

Acetylation is arguably the most dynamic protein translational modification often associated with increased DNA accessibility and increased transcription. These acetylated histones recruit transcription and remodeling factors, and their deregulation could result in aberrant expression of survival and growth-promoting genes. Recognition of acetylated lysine is principally mediated by bromodomains, BRDs. Recent studies have shown that BRD9 is preferentially used by cancers that harbor SMARCB1 abnormalities such as malignant rhabdoid tumors and several specific types of sarcomas. BRD9 is also an essential component of the SWI/SNF chromatin remodeling complex, and a critical target required in acute myeloid leukemia. As the biological function of BRD9 in tumorigenesis becomes clear, bromodomain of BRD9 has become a new hot target for effective tumor treatment method.



Herein, we utilized Phage-assisted, Active site Directed Ligand Evolution, PADLE, approach to target BRD9. Due to larger hydrophobic cavity of BRD9 than other bromodomains, it can recognize longer propionyl and butyryl marks on lysine. Thus, N^{ε}-butyryl-lysine, BuK, can selectively bind to BRD9 and be genetically encoded for construction of phage library. This experimental design resulted in identification of BRD9 binders with increased specificity and varying affinities.

We aim to study the binding and inhibitory potential of the ligands and further optimize it for in-vivo studies. Conclusion drawn from this work will provide important insights about these epigenetic reader proteins thereby showcasing a new frontier in oncological drug discovery research.

YI-P203 Skylar Harrelson
North Carolina State University

Selective Inhibition of UGDH with a Series of Novel Peptide and Peptoid Anologs

Skylar Harrelson, Kristiana Witte, Emily Allego, Ellen Warner, Caroline Proulx, Joseph J. Barycki, and Melanie A. Simpson

Recurrence of therapy-resistant prostate cancer following androgen deprivation therapy currently has few treatment options and a high mortality rate. To allow cells to regain sensitivity to anti-androgen therapy, the expression of oncogenic proteoglycans needs to be decreased. One way to do this is by reducing the concentration of UDP-glucuronate in the cells. UDP-glucose dehydrogenase, UGDH, converts UDP-glucose to UDP-glucuronate. Knockdown of UGDH decreases the production of UDP-glucuronate, and thus the synthesis of oncogenic proteoglycans. This knockdown still allows UDP-glucuronate to participate in steroid elimination through glucuronidation which further increases the efficacy of anti-androgen therapy. Phage display was used to identify peptides with a high affinity for UGDH and strong inhibitory potential. One peptide co-crystallized with UGDH and was found at the interface between subunits, where there is normally dynamic interaction necessary for activity of the enzyme.

In this work, both alanine and N-substituted glycine scans of this peptide were conducted and the resulting peptides/peptomers were tested for inhibition of UGDH. Peptomers have previously exhibited increased cell permeability and stability to proteolysis when compared to their peptide counterparts. Further studies of this peptide and the peptidomimetic analogs are underway, with future goals to inhibit UGDH in cellular models of prostate cancer.

P204 Keykavous Parang
Chapman University School of Pharmacy

Amphiphilic Membrane-Active Peptides: Broad-Spectrum Antibacterial Activity Alone and in Combination with Antibiotics and Structural Insights

Keykavous Parang

We report the synthesis and antibacterial activities of a library of amphiphilic membrane-active peptides. Lead cyclic peptides showed broad-spectrum activity against drug-resistant Gram-positive, MIC=1.5-6.2μg/mL, and Gram-negative, MIC=12.5-25μg/mL, bacteria. In combination with commercially available antibiotics, tetracycline, tobramycin, clindamycin, kanamycin, levofloxacin, polymyxin B, metronidazole, and vancomycin, lead peptides showed remarkable synergism against a large panel of resistant pathogens.

Cytotoxicity study showed the predominant lethal action of the peptides against bacteria as compared with mammalian cells. A plasma stability study revealed approximately 2-fold higher stability of lead cyclic peptides as compared to their linear counterparts after 24 hours incubation. Calcein dye leakage and scanning electron microscopy studies revealed the membranolytic effect of peptides.

Nuclear magnetic resonance spectroscopy and molecular dynamics simulations studies of the interaction of the peptides with phospholipid bilayer provided a solid structural basis explaining the membranolytic action of the peptides with

atomistic details. *In vivo* animal studies were used to determine the pharmacokinetics and efficacy of the lead peptide utilizing a mouse methicillin-resistant *Staphylococcus aureus*, MRSA, septicemia model. These results highlight the potential of newly designed amphiphilic peptides as the next generation of peptide-based antibiotics.

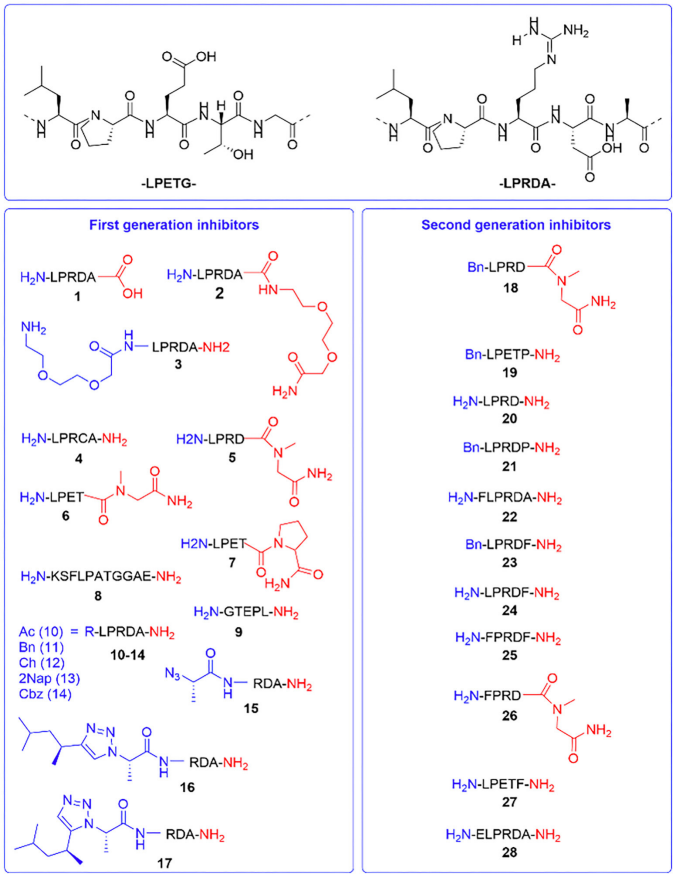
YI-P205 Jordi Hintzen

University of Gothenburg

Substrate-Derived Sortase A Inhibitors: Targeting Antimicrobial Resistance

Jordi C. J. Hintzen, Helal Abujubara, Shadi Rahimi, Ivan Mijakovic, Daniel Tietze, and Alesia A. Tietze

The rapid development of drug-resistant bacteria is a serious healthcare threat that prompted the exploration of new antibacterial targets. Targeting bacterial virulence, instead of developing bactericidal compounds, is a promising strategy that can block pathogenicity by disarming pathogens without affecting bacterial viability. Therefore, this approach exerts less selective pressure to induce the emergence of antibiotic resistance.



In our work, we have targeted the bacterial enzyme Sortase A, known as a promising target for antivirulence treatment. Sortase A is a housekeeping transpeptidase of Gram-positive bacteria that anchors cell wall surface proteins into the peptidoglycan layer of the bacterial coat, a process which is crucial for bacterial adherence and host cell invasion. Sortase A relies on a five amino acid sorting signal, by which it recognizes its natural target.

Here, we represent a series of peptidomimetic inhibitors of Sortase A, supported by computational investigations. *In vitro* inhibition was determined by employing a FRET-compatible substrate. Among our panel, we identified several promising inhibitors. Biofilm inhibition analysis revealed activity against pathogenic *S. aureus* in a low μM range which so far represents the most potent peptidomimetic inhibitors. Our results show great promise for further development as a novel antivirulence treatment.

P206 Jonah Holbrook

University of Utah

D-Peptide Therapeutics for Infectious Diseases

J. Holbrook and M. S. Kay

In the aftermath of a global pandemic, the need for novel therapeutic technologies against infectious diseases has increased. While traditional therapeutics such as small molecules or biologics are currently available for treatment of numerous conditions, they possess key limitations. Notably, biologics are often highly immunogenic and susceptible to degradation, while small molecules often lack specificity. D-peptides, composed of enantiomers of naturally occurring L-amino acids, possess many comparative therapeutic advantages. For example, D-peptides are not prone to proteolysis, can be designed to have desirable binding affinity and specificity, and are less expensive to produce than most antibody therapies.

We have previously reported successful HIV entry inhibition with a novel D-peptide CPT31, a trimeric D-peptide in phase 1 clinical trials. We are now targeting other viral pathogens including SARS-CoV-2 and Henipavirus. While current SARS-CoV-2 therapeutics have mitigated the pandemic, a need remains for potent therapeutics with the potential to address current and future variants. Henipavirus infections are relatively rare but have a 40-70% mortality rate and have high pandemic potential. HIV, Coronaviruses, and Henipaviruses share a similar general viral entry mechanism that exposes a vulnerable prehairpin intermediate. Here we describe our efforts to apply our D-peptide discovery pipeline using mirror-image phage display and affinity maturation against peptide mimics of prehairpin intermediate targets in SARS-CoV-2 and Henipavirus.

P207 Timothy Reichart

Hampden-Sydney College

Determination of the Oligomerization State of the Transmembrane Domains of Membrane Proteins in SARS-CoV-2

T.G. Hobart, A.M. Rehak, and T.M. Reichart

SARS-CoV-2 is the third highly infectious, highly deadly

coronavirus to make the jump to humans in the last two decades. SARS-CoV-2, like other coronaviruses, is an enveloped virus with a small number of integral membrane proteins embedded in a lipid membrane. Each of these proteins has a transmembrane, TM, domain, a portion of the protein approximately 20 amino acids in length. Several proteins, including the Spike protein, are functional proteins only when trimeric – that is, three copies of the protein must self-assemble in order to carry out their function. Six proteins present in SARS-CoV-2 are known or hypothesized to have TM domains. These TM domains are highly conserved, and they are an underexplored region for potential drug discovery.

To examine the oligomerization state, three versions of each peptide of several of the TM proteins have been synthesized. One has a nitrobenzodiazole, NBD, fluorophore active in membranes, one has tetramethylrhodamine, TAMRA, a quencher of NBD, and one is unlabeled. These peptides are dissolved in a detergent above its CMC, which presents the peptides in micelles previously shown to be accurate mimics of membranes for the analysis of oligomerization states of TM domains. The three variants are used in a fluorescence-based assay which monitors the change in fluorescence as a function of added quencher peptide. The shape of the resulting fluorescence curve describes the oligomerization state. We describe the results of this experiment for two TM proteins, ORF 7a and ORF 3a.

YI-P208 Naoya Iwamoto

Graduate School of Pharmaceutical Sciences, Kyoto University

Development of a Mirror-Image Monobody Scaffold for Less Immunogenic Protein Therapeutics

Naoya Iwamoto, Yukino Sato, Asako Manabe, Shinsuke Inuki, Hiroaki Ohno, Motohiro Nonaka, and Shinya Oishi

Mirror-image peptides and proteins, D-peptides and D-proteins, are expected to be promising drug scaffolds due to their favorable pharmacokinetic profiles and less immunogenic properties. For their practical application to D-protein-based therapeutics, facile preparation procedures including chemical protein synthesis and refolding are to be established.

In this study, we developed a simple synthetic process of a monobody scaffold as a proof-of-concept demonstration. To facilitate the synthesis of an anti-GFP monobody, GS2, we designed a novel modified scaffold of monobody, in which Glu38 in the CD loop and Val66 in the EF loop were substituted with cysteines. The full-length GS2 with cysteine substitutions, L-mGS2, was constructed via stepwise NCLs from three peptide segments. The resulting L-mGS2 was subjected to appropriate refolding conditions to provide a functional protein. The biological function of binding to EGFP was verified by SPR analysis.

We also synthesized another GS2 analogue including a disulfide bond between two substituted cysteines, L-mGS2^{SS}. L-mGS2^{SS} showed enhanced binding to EGFP. D-mGS2 was also synthesized by the established protocol. D-mGS2 exhibited the mirror-image CD spectrum to that of L-mGS2. In a preliminary experiment to comparatively assess the immunogenic properties of L-mGS2 and D-mGS2 in BALB/c mice, IgG production by immunization with D-mGS2 was significantly less than that with L-mGS2.

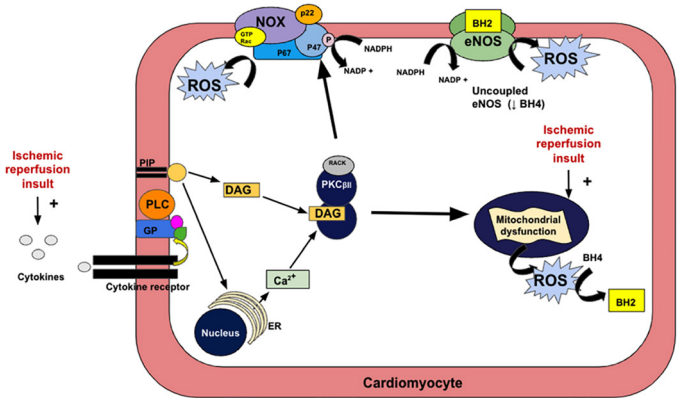
P209 Logan Clair

Philadelphia College of Osteopathic Medicine

Dual Myristic Acid and Transactivator of Transcription Conjugation of Protein Kinase C Beta II Peptide Inhibitor Provides Cardioprotection in Porcine Ischemia-Reperfusion Injury *In Vivo*

Logan Clair, Desmond Boakye Tanoh, James Ramsarran, Juliet Melnik, Kayla P Harrell, Arjun Nair, Sunit Singh, Emily Andrews, Tameka Dean, Qian Chen, Robert Barsotti, and Lindon Young

Prompt reperfusion is vital to resuscitating ischemic myocardium. However, reperfusion of blood induces reactive oxygen species, ROS, mediated ischemia-reperfusion, I/R, injury. Major sources of ROS due to activation by protein kinase C-βII, PKCβII) are shown in the below figure Previously, myristic acid, Myr, trans-activator of transcription, Tat, and Myr-Tat conjugated PKCβII inhibitor, PKCβII-, SLNPEWNET, decreased ROS in neutrophils.



Schematic representation of PKCβII mediated activation of mitochondrial ROS and NOX-2 superoxide, O2-, release along with decreased NO release from endothelial NO synthase, eNOS, in MI/R.

In this study, we hypothesized that Myr-Tat-PKCβII- will mitigate porcine myocardial I/R injury *in-vivo* compared to scrambled peptide controls. Male Yorkshire pigs, 38-50kg, underwent regional I(1hr)/R(3hrs) through occlusion at the second branch of the left anterior descending coronary artery, LAD. During reperfusion, Myr-Tat-PKCβII- or scrambled control was infused into the LAD. Ejection fraction, EF, and cardiac injury markers, creatine phosphokinase, CPK, troponin I and myoglobin, were measured throughout the experiment. Post-reperfused hearts were stained with Evans Blue dye to identify the area at risk, AR, and 1% triphenyltetrazolium chloride to demarcate the area of necrosis, AN. Data were analyzed via Student's t-test. Myr-Tat-PKCβII-

preserved EF with a relative change of 1.4±0.7% compared to 6.4±2.1% in control hearts, p<0.05, from mean baseline EF, 61.4±0.5%. Myr-Tat-PKCβII- significantly decreased serum myoglobin levels at 1hr reperfusion, 135±132ng/mL, n=4, compared to scrambled control, 1022±346ng/mL, n=3 p<0.05. Myr-Tat-PKCβII- reduced infarct size to 10.0±2.8%; n=4; compared to scrambled control hearts, 28.5±8.3%; n=6; p<0.05. CPK and troponin I levels were comparable in both groups.

These results suggest Myr-Tat-PKCβII- mitigates cardiac injury when given at the onset of reperfusion. Future studies will examine a 12 week *in-vivo*, porcine myocardial I/R survival study.

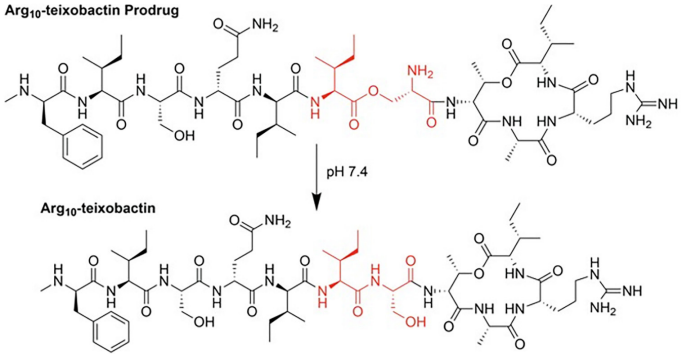
YI-P210 Chelsea Jones

University of California at Irvine

O-Acyl Isopeptide Prodrugs of Teixobactin Derivatives

Chelsea R. Jones, Gretchen Guaglianone, Grant H. Lai, and James S. Nowick

The antibiotic teixobactin is a promising drug candidate against drug-resistant pathogens, such as MRSA and VRE, but forms insoluble gels that may limit intravenous administration. O- Acyl isopeptide prodrug analogues of teixobactin circumvent the problem of gel formation while retaining antibiotic activity. The teixobactin prodrug analogues contain ester linkages between Ile_6 and Ser_7, Ile_2 and Ser_3, or between both Ile_6 and Ser_7 and Ile_2 and Ser_3. Upon exposure to physiological pH, the prodrug analogues undergo clean conversion to the corresponding amides, with half-lives between 13 and 115 min, with a 3 to 5-fold increase at 37°C.



Prodrug analogues containing lysine, arginine, or leucine at position 10 exhibit good antibiotic activity against a variety of Gram-positive bacteria while exhibiting little or no cytotoxicity or hemolytic activity. A mouse thigh infection model against MRSA demonstrates *in vivo* efficacy. Because O-acyl isopeptide prodrug analogues of teixobactin exhibit clean conversion to the corresponding teixobactin analogues with reduced propensity to form gels, it is anticipated that teixobactin prodrugs will be superior to teixobactin as drug candidates.

P211 Tanya Roman

Pontificia Universidad Catolica de Valparaiso

Identification of Chitin-Binding Peptides in Teleost Fish Mucosa as Potential Antiparasitic Agents

Camila Arancibia, Tanya Roman, Laura Tamayo, Claudio Álvarez, Fanny Guzmán, and Paula Santana

Chile is the second world producer of salmon, whose industry has grown steadily producing a significant burden in the ecosystem because of the high amount of biomass produced and the opportunistic pathogens causing high mortality rates of fish due to the conditions of cultivation. Mortality rates have increased being infectious diseases the predominant cause. The challenge in salmon farming in Chile is caligidosis, an infection produced by the ectoparasite *Caligus rogercresseyi*. The infection occurs in the copepodite stage producing tissue damage by fixation through a chitin filament.

The first immunity barrier that the pathogen faces is the epithelial mucosa, which contains components of the innate immunity including antiparasitic peptides, APPs. Caligidosis control in Chile is exerted with antiparasitic chemicals, producing severe environmental damage and health risk for being neurotoxic and generate resistance. Despite APPs being a sound alternative to antiparasitic chemicals, few studies exist on the effect of APPS in epithelial mucosa and its antiparasitic action. Broad spectrum antimicrobial peptides have been described, but chitin binding APPS have not been reported in salmonids.

This work reports the search of chitin binding peptides from teleost fish with antiparasitic activity to be used in salmon farming. Five peptides identified in teleost fish were synthesized, characterized and *in-silico* analysis to further evaluate their chitin binding capacity. Piscidin and hepcidin were effective chitin binders and therefore potentially effective against *C. rogercresseyi*. Therefore, their hemolytic and cytotoxic effects on salmonid cells was evaluated. No cytotoxic effect on RTS- 11, RT-gut, RTgill-W1 and SHK-1 cells was detected at concentrations lower than 75μM. However, they produce hemolysis in salmon erythrocytes at concentrations higher than 5 and 50μM, respectively. It is concluded that hepcidin is a candidate peptide to be used against *C. rogercresseyi*.

Funding Grant ANID FONDECYT 1210056

YI-P212 Yi Zhang

Stanford University

Omniligase-1-mediated Ligation for Insulin Analog Synthesis in Solution and On Phage Surface

Yi Wolf Zhang, Nai-Pin Lin, and Danny Hung-Chieh Chou

The B-chain C-terminal region of insulin has been mutated or modified to achieve improved therapeutic efficacies.

For example, all FDA-approved insulin analogs have altered C-terminal segments, leading to improved pharmacokinetic properties and significant clinical benefits on blood sugar regulation. Nonetheless, there is still no efficient method to synthesize insulin analogs with the altered C-terminal region.

Herein, we report a facile synthesis using omniligase-1 to ligate an insulin core with a peptide segment in high conversion. We further apply this ligation to M13 phage surface modifications and demonstrate that the phage-displayed insulin molecules can bind to insulin receptor ectodomain in an insulin-dependent manner. More interestingly, omniligase-1 could only selectively mediate the ligation to phage PIII protein, and we showed that insulin phage libraries could be generated with commercially available phage libraries. These results pave the way for engineering new insulin analogs from phage display with therapeutic properties and demonstrate the feasibility of using omniligase-1 to display and screen disulfide-rich peptides and proteins on phage, which traditional methods cannot achieve.

P213 Zoe O'Gara

48Hour Discovery Inc.

Applications of 48Hour Discovery's Platform in Drug Discovery

Z. O'Gara

The discovery of macrocyclic peptides against specific targets offers opportunities for new drug development that benefit from a lower entropic cost of binding compared to acyclic congeners, while offering more selectivity against challenging intracellular targets which are often inaccessible to small molecules.

Alpha 4 beta 7, a4B7, integrin has been shown to play a role in Inflammatory Bowel Disease, and in this study our goal is to identify antagonists for a4B7 to inhibit its binding to Mucosal addressing Cell Adhesion Molecule, MadCAM, which has been shown to help resolve IBD symptoms. To this end, the DNA encoded library SxCxxxxxx(x)C, where X represents a random natural amino acid excluding cysteine, modified with two-fold symmetric chemical linkers, TSL-6, was used to discover peptide binders to a4B7 integrin in a three rounds of selection strategy. In this case, the library contained peptides with six or seven randomized amino acids between the two cysteine residues, and the diversity of the library is ~108 unique peptide sequences.

In another study, the goal was to identify binders to O-linked N-acetylglucosamine, O-GlcNAc, transferase, which has been found to play an important role in cell physiology as it installs O- GlcNAc to thousands of nuclear and cytoplasmic proteins. In this case, the linear library of 15 randomized amino acids in length, X15 library, excluding any cysteine residues was used. in a three rounds of selection strategy, similar to the a4B7 project.
After three rounds of selection, samples before and after selection from each round were submitted for next generation sequencing, NGS, and bioinformatic analysis was applied on the NGS data. A consensus motif, [Y/F]-x-P-x-Y-x-[I/M/F], that drives peptide binding to OGT was identified in the OGT project. Two consensus sequences, PMD and PLD, were identified for a4B7.

These consensus motifs and sequences highlight the potential for phage display in peptide binder discovery, and both projects have since been pushed to the next stage of drug development, lead optimization.

YI-P214 Luis Prent

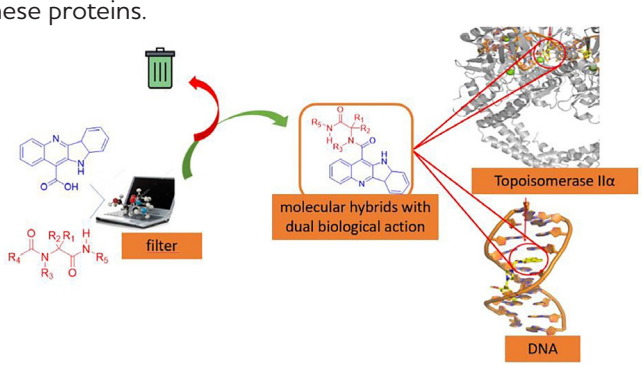
Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andrés Bello

Rational Design, Synthesis of Peptidomimetic-Cryptolepine Hybrids and their Biological Evaluation as Potential Anticancer Agents

Luis Prent-Peñaloza, Jorge Soto-delgado, Laura Aros-Sánchez, and Fanny Guzmán

Cancer is a disease that constitutes the first or second leading cause of premature death in approximately 183 countries. In 2018 more than 18 million people suffered from some type of cancer and about 50% of these died as a result of this disease. Among the most common cancers are lung cancer, breast cancer, gastric cancer, colorectal cancer, prostate cancer and liver cancer. In Chile, breast cancer is the leading cause of death in women, while gastric and prostate cancer are the most common causes of death in men.

Among the most common strategies for dealing with cancer are: treatment by surgery, treatment by chemotherapy, treatment by radiotherapy and treatment by immunotherapy. A disadvantage of pharmacological therapy is its poor selectivity and toxic effects on patients. Therefore, the search for compounds that act specifically on tumor cells and not on normal cells is a relevant problem, with no solution at present. One of the strategies to solve this problem is to identify proteins whose levels or function are increased in tumor cells and thus develop specific pharmacological inhibitors for these proteins.



In this project, it was hypothesized that the biological activity of hybrid compounds will be enhanced compared to the individual fragments that compose them. Therefore, the main objective of this interdisciplinary proposal is the **rational design and synthesis of peptidomimetic-cryptolepine hybrids, as well as to evaluate their antitumor activity and inhibitory capacity against topoisomerase IIα and the DNA molecule**. In the initial stage, a library of molecules will be generated using in silico analysis as a filter, then the synthesis, through the proposed synthetic routes, of this library of compounds will be carried out and, finally, their inhibitory capacity, as well as their mechanism of action, will be evaluated.

P215 Younghee Shin

Technical University of Korea

Exploration of Sequence-Based Design for Helical α/β/γ-Peptidomimetics

Y.H. Shin

Fine-tuned modulation of protein-protein interactions, PPIs, involving helical contacts with helical peptidomimetics is highly desirable for pharmacological applications. Natural α-amino acid-based helical peptidomimetics have critical limitations with respect to biomedical applications, mainly for their rapid proteolytic degradation under physiological conditions. Helical peptidomimetics incorporated with non-natural amino acids such as β- and γ-residues are less prone to proteolytic degradation; however, the additional backbone carbons in β- and γ-residues yield flexible helical backbone, increase the entropic penalty upon binding, resulting in decreased affinity to the targets. This could be addressed by the systematic incorporation of ring-constrained β- and γ-amino acid residues to α-helix mimetics, engendering stable helical secondary structures.

We applied the α/β/γ- peptide foldamer design strategy to mimicry of BH3 helical domain, Bim, as a pioneering study for functional α/β/γ-peptides development. The Bim-based α/β/γ-peptides in an αγααβα-hexad repeat with five helical turns inhibited the interaction between Bak and Bcl-xL with excellent resistance against proteolytic digestion. Our study suggests that α/β/γ-foldamer can be developed as an alternative general platform for the sequence-based peptide design researches especially with prominent resistance to enzymatic digestion.

YI-P216 Ellen Warner

North Carolina State University

Head-to-Tail Peptide Cyclizations using *N*-Aryl Peptide Hydrazides

E.J.T. Warner, Q.A.E. Guthrie, B. Humphrey, and C. Proulx

Bioorthogonal chemistry has become a powerful tool in the manipulation of biomolecules under mild conditions. In our lab, we previously demonstrated the use of *N*-aryl peptides as novel precursors for oxime ligations at neutral pH, taking advantage of their site selective oxidation into a reactive, protonated, Schiff base intermediate. Among the various advantages of this method, tuning the electronics of the phenyl ring in electron rich *N*-aryl peptides has allowed for the orthogonal reactivity at varying pHs, efficient ketoxime peptide ligations, and an increase in diversity at the site of ligation.

Here, we have explored the use of *N*-aryl peptide hydrazides in head-to-tail peptide macrocyclization reactions, possessing substituents at the alpha-carbon. A scope will be presented, showcasing >50 macrocyclic peptides with sequence diversity and variable ring sizes. In addition, conditions for isomerization, reduction, ring opening, and the stability of the resulting kethydrazone-linked cyclic peptides will be presented.

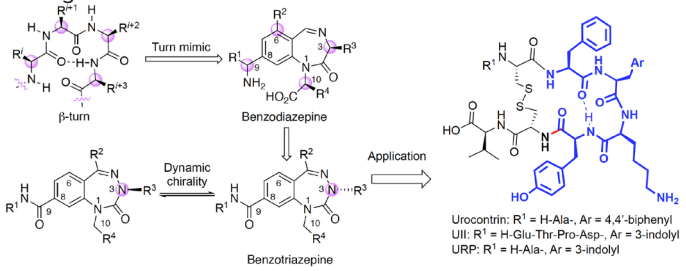
YI-P217 Xiaozheng Wei

University of Montreal

β-Turn Mimicry Without Chiral Carbons Using Tetrasubstituted Benzotriazepinones

Xiaozheng Wei and William D. Lubell

The importance of β-turns in peptide recognition drives interest to mimic these secondary structures for drug discovery. Efforts to employ nonpeptide small molecules to replicate the form and function of β-turns in so-called topographic mimics has led to designs base on benzodiazepines, see figure.



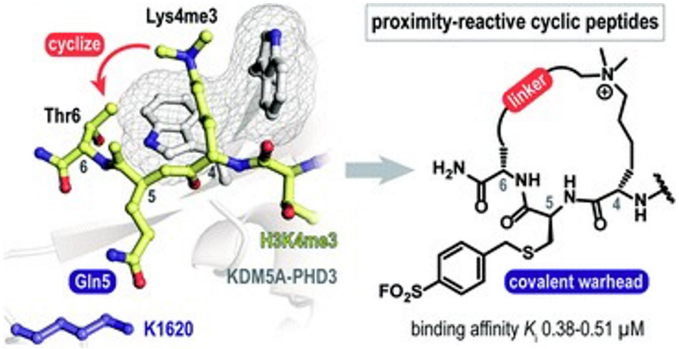
Considering that the low barrier for nitrogen isomerization offers potential for dynamic chirality, tetrasubstituted benzotriazepines were synthesized and analyzed for potential to serve as topographic mimics without chiral carbons. Benzotriazepine analysis by X-ray crystallography illustrated potential to replicate type I and I' β-turn geometry. Synthesis, conformational analysis, and application of tetrasubstituted benzotriazepinones as mimics of peptide modulators, for example, Urocontrin, of the urontensin-II receptor will be described.

P218 Hyunjun Yang
University of California at San Francisco

Covalent Labeling of a Chromatin Reader Domain Using Proximity-Reactive Cyclic Peptides

Meng Yao Zhang, Hyunjun Yang, Gloria Ortiz, Michael J. Trnka, Nektaria Petronikolou, Alma L. Burlingame, William F. DeGrado, and Danica Galonic Fujimori

Chemical probes for chromatin reader proteins are valuable tools for investigating epigenetic regulatory mechanisms and evaluating whether the target of interest holds therapeutic potential. Developing potent inhibitors for the plant homeodomain, PHD, family of methylation readers remains a difficult task due to the charged, shallow, and extended nature of the histone binding site that precludes effective engagement of conventional small molecules.



Herein, we describe the development of novel proximity reactive cyclopeptide inhibitors for PHD3 — a trimethyl lysine reader domain of histone demethylase KDM5A. Guided by the PHD3-histone co-crystal structure, we designed a side-chain-to-sidechain linking strategy to improve peptide proteolytic stability whilst maintaining binding affinity. We have developed an operationally simple solid-phase macrocyclization pathway, capitalizing on the inherent reactivity of the dimethyl lysine 3-amino group to generate scaffolds bearing charged tetraalkylammonium functionalities that effectively engage the shallow aromatic 'groove' of PHD3.

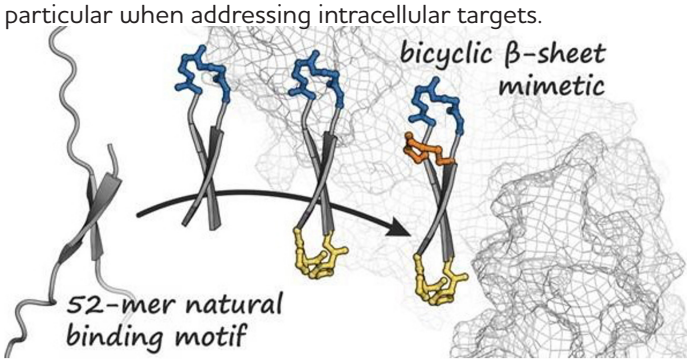
Leveraging a surface-exposed lysine residue on PHD3 adjacent to the ligand binding site, cyclic peptides were rendered covalent through installation of an aryl sulfonyl fluoride warhead. The resulting lysine-reactive cyclic peptides demonstrated rapid and efficient labeling of the PHD3 domain in HEK293T lysates, showcasing the feasibility of employing proximity-induced reactivity for covalent labeling of this challenging family of reader domains.

YI-P219 Alejandro Yeste Vazquez
Vrije Universiteit Amsterdam

β-Sheet mimetics that Target the Transcriptional Coactivator β-Catenin

M. Wendt, A. Yeste-Vazquez, T. van Ramshorst, R. J. Hana, N. M. Pearce, S. Hennig, and T. N. Grossmann

Protein complexes are defined by the three-dimensional structure of participating binding partners. Knowledge about these structures can facilitate the design of peptidomimetics which have been applied for example, as inhibitors of protein–protein interactions, PPIs. Even though β-sheets participate widely in PPIs, they have only rarely served as the basis for peptidomimetic PPI inhibitors, in particular when addressing intracellular targets.



Here, we present the structure-based design of β-sheet mimetics targeting the intracellular protein β-catenin, a central component of the Wnt signalling pathway. Based on a protein binding partner of β-catenin, a macrocyclic peptide was designed, and its crystal structure in complex with β catenin obtained. The presented design strategy can support the development of inhibitors for other β-sheet-mediate.

YI-P220 Charity D. Yongo-Luwawa
Université de Montréal

Peptidomimetic Strategy for Interleukin 1 Receptor Modulation Towards Tocolytic Innovation for Delaying Birth and Improving Neonatal Outcomes

Charity D. Yongo-Luwawa, Christiane Quiniou, Sylvain Chemtob, and William D. Lubell

Premature birth occurs in about 10% of all pregnancies worldwide with short- and long-term consequence on newborn health. For example, neonatal incubator care in elevated oxygen environment can lead to retinopathy of prematurity the leading cause of infant vision loss. Current birth delaying "tocolytic" agents are used for limited time due to potential detrimental effects on mother and fetus. Moreover, such tocolytic drugs do not address the inflammatory component of premature birth.

Interleukin-1β, IL-1β, is a central pro-inflammatory cytokine, which acts in key roles in labor, inflammation, and the immune response against pathogen invasion. Binding the IL- 1 receptor, IL-1R, IL-1β activates transcription of the nuclear factor kappa light chain enhancer of B activated B cells, NF-ϰB. NF-ϰB is critical for immune vigilance.

The peptide **101.10,** H-D-Arg-D-Tyr-D-Thr-D-Val-D-

Glu-D-Leu-D-Ala-NH_2, binds to the IL-1R and biases downstream signaling to curb inflammation and retain immune vigilance.

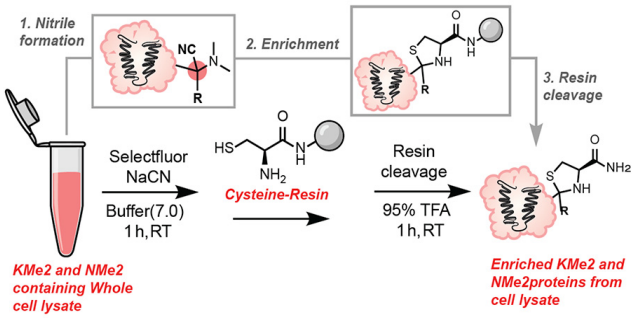
Our presentation describes recent progress on employing lactam dipeptides to restrict the conformation of **101.10** in structure-activity relationship studies that have defined active conformers for controlling biased IL-1R signalling. Lactam IL-1R modulators can delay labour and mitigate oxygen-induced retinopathy in animal models.

P221 **Benjamin Emenike**
L16-YI2 Emory University

Chemical Tools for Profiling Lysine Dimethylation and N-Terminal Dimethylation of Proteins

Benjamin Emenike

One of the ways through which nature increases the functional diversity of the proteome is through a process known as posttranslational modifications, PTMs. Dimethylation of lysine, Kme2, and N-termini, NMe2, of proteins is a post-translational modification which involves the catalytic attachment of two methyl groups onto lysine side chain or the N-termini amines.



Despite the surging interest towards this modification and its emergence as a key component of diverse set of biological events, its global identification has remained an unachieved goal. This is due to the lack of pan specific chemical tools for tagging of Kme2 and NMe2.

Our approach towards the global profiling of Kme2 and NMe2 involves the use of two independent tertiary amine trapping reactions: tertiary amine coupling via oxidation, TACO, and tertiary amine nucleophilic substitution, TANS.

Preliminary data highlights the efficiency of both techniques in tagging KMe2 and NMe2 in a pan-specific manner, with the added advantage of faster reaction kinetics and near-quantitative conversions. Our long-term goal is to utilize these technologies for the global profiling of KMe2 and NMe2 sites, and identification of disease related KMe2 and NMe2 biomarkers in the human proteome. To the best of our knowledge, this represents the first attempt towards labeling of KMe2 and NMe2 using a chemical strategy, thus

YI-P222 **Majedul Islam**
Florida Atlantic University

Development of a Novel Tau Aggregation Model for the Study of Fibrillation and Drug Screening Against Tauopathies

Majedul Islam, and Deguo Du

Tau, a microtubule-associated protein, aids in the maintenance of the axonal transport tract's integrity by promoting tubulin self-assembly into microtubule polymer. Tauopathies are characterized by abnormal tau buildup in the brain. This study uses a small tau fragment and its mutants to better understand tau aggregation and then uses that insight to develop aggregation inhibitors.

First, a 20-mer peptide sequence, $tau_{298-317}$ was selected from the longest tau isomer, 2N4R. Then, using Fmoc chemistry, the wild-type peptide fragment was synthesized and purified with HPLC. In order to conduct the aggregation studies, a variety of biophysical characterizations were used, including thioflavin-T, ThT, binding using fluorescence spectroscopy, secondary structural changes using circular dichroism, CD, and fibril morphology using atomic force microscopy, AFM. The kinetic analysis of the fragment showed that the fibrillation of the fragments required an external force such as palmitic acid, PA, to induce fibrillation. AFM studies also confirmed the presence of fibrils in PA-facilitated $tau_{298-317}$. A circular dichroism study showed that $tau_{298-317}$ adopted beta-sheet structures upon incubation along with PA. Then, a monovalent salt, NaCl, 8- Anilinonaphthalene-1-sulfonic acid, ANS, dye, and urea studies confirmed the presence of electrostatic interaction, hydrophobic interaction, hydrogen bond, and Van der Waals force forces.

Then we studied inhibitory activities of polyphenolic compounds against PA mediated tau298-317 aggregation. We tested resveratrol, rosmarinic acid, naringenin, EGCG, baicalein, myricetin, and luteolin as probable candidates. While multiple candidates were able to suppress the PA-mediated tau aggregation process, myricetin and luteolin were highly effective in disintegrating preformed fibrils. Thus, our new model serves as a standard method to screen drug compound against tauopathic disorder.

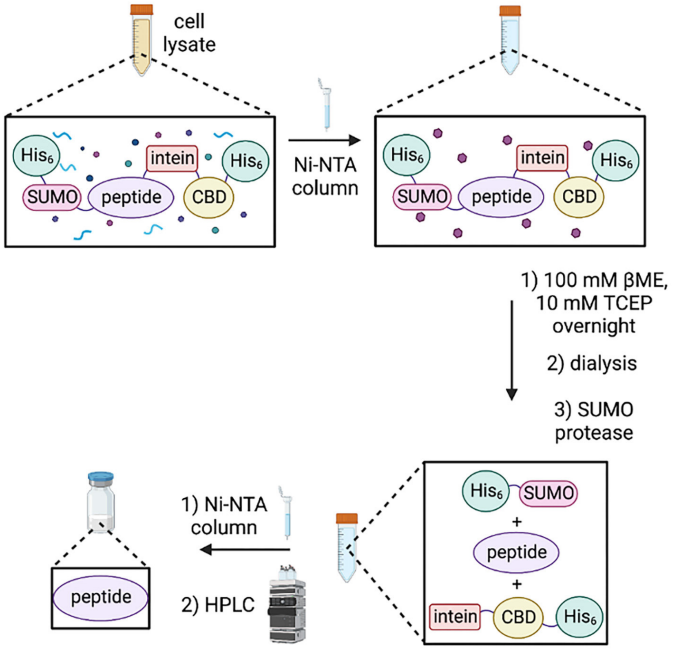
YI-P223 **Tess Lamer**
University of Alberta

"Sandwiched" *E. coli* Expression System for Isotopic Labeling and NMR Structural Elucidation of Bacteriocin Peptides

T. Lamer, M. J. van Belkum, R.T. McKay, and J. C. Vederas

Bacteriocins are antimicrobial ribosomally synthesized peptides produced by bacteria to help the host strain carve

out an ecological niche. Studies of ribosomally synthesized peptides, such as bacteriocins, often require recombinant production in *Escherichia coli* as fusion proteins. However, heterologously expressed peptides can suffer from degradation in *E. coli*, which can pose a major issue for applications requiring a large amount of purified peptide, such as NMR structural elucidation or biochemical assays. In this work, we describe an updated, easy to use, "sandwiched" expression system that makes use of dual N-terminal and C-terminal fusion proteins to heterologously overexpress and purify peptides from E. coli. This "sandwiched" expression system requires only a single cloning vector, available through Addgene, and the cloning and isolation methodology is rapid, versatile, and can be implemented by any biochemistry lab.



We demonstrate this updated "sandwiched" fusion protein strategy's use in production of several bacteriocin peptides with good yield. We then describe the use of this expression system to produce a bacteriocin peptide with unknown structure in 13C/15N isotopically labeled media, and discuss efforts towards NMR structural elucidation of this peptide natural product, along with other mechanism of action studies.

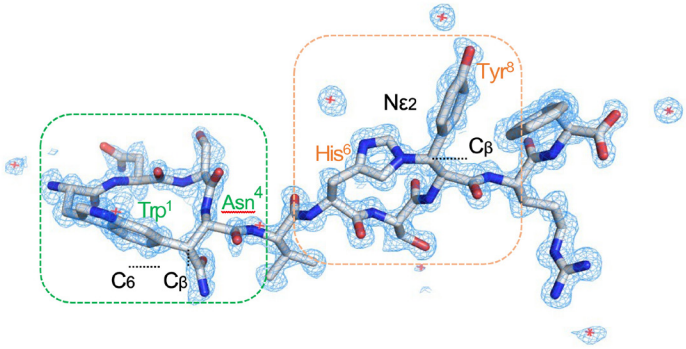
P224 Byung-Kuk Yoo

Thermo Fisher Scientific

MicroED Structure of a Novel Macrocyclic Antibiotic Targeting BamA Against Gram-Negative Pathogens

Ryan D. Miller, Akira Iinishi, Seyed Majed Modaresi, Byung-Kuk Yoo, Thomas D. Curtis, Patrick J. Lariviere, Libang Liang, Sangkeun Son, Samantha Nicolau, Rachel Bargabos, Madeleine Morrissette, Michael F. Gates, Norman Pitt, Roman P. Jakob, Parthasarathi Rath, Timm Maier, Andrey G. Mayutin, Jens T. Kaiser, Samantha Niles, Blake Karavas, Meghan Ghiglieri, Sarah Bowman, Douglas C. Rees, Sebastian Hiller, and Kim Lewis

Discovery of antibiotics against Gram-negative species is uniquely challenging due to their restrictive penetration barrier. BamA, which inserts proteins into the outer membrane, is an attractive target with its surface location. In this study, we identified dynobactin A, a novel peptide antibiotic from *Photorhabdus australis* and unveiled two unique unlinked rings by MicroED. The novel compound is the first natural product antibiotic of unknown structure solved *de novo* with this approach, PDB 7T3H. It is a decapeptide of sequence $W^1N^2S^3N^4V^5H^6S^7Y^8R^9F^{10}$, having two closed rings: **i** a carbon-carbon bond formed between the Trp1 C6 and the β-carbon of Asn^4, green box; **ii** an unusual nitrogen-carbon linkage between the His^6 imidazole $N\varepsilon_2$ and the β-carbon of Tyr^8, orange box.



These connections create unfused 4- and 3-constituent rings respectively, resulting in a flexible peptide, contrasting the fused rings of darobactins. Dynobactin is one of examples of natural-product antibiotics acting against the undruggable surface proteins of Gram-negative bacteria. This study demonstrates how MicroED accelerates antibiotic discovery by providing unambiguous structures from submicron-sized crystals.

YI-P225 Diane Rafizadeh

University of Pennsylvania

Covalent Capture of Collagen Triple Helices via Photocrosslinking

Diane N. Rafizadeh, Jia Deng, and David M. Chenoweth

Collagen lays the foundation of bodily tissues, serving to strengthen, connect, and signal from the micro to the macro scale. As a permutated, triple helical polymer involved in biochemical signaling, collagen has potential for manipulation as a tool for modulating protein-protein interactions. However, these applications have been limited by its tripartite nature, which restrict its thermal and entropic stability. Synthetic linkage and/or cyclization of the three collagen strands may overcome these limitations. Reducing entropy of termolecular collagen to a unimolecular system by inter-strand linkage can offer improved stability through preorganized structure. Literature reports have previously shown covalent capture via a protecting group strategy.

Here we demonstrate a novel approach taking advantage of

photoactivatable crosslinking agents incorporated into our collagen mimetic peptides, CMPs, during solid phase peptide synthesis. CMPs are crosslinked with UV light following self-assembly of the individually synthesized collagen strands into triple helices. These CMPs are expected to exhibit improved thermal and proteolytic stability. Capacity of the covalently captured CMPs to bind CNA, a staphylococcal collagen-binding protein implicated in the pathogenesis of bacterial infection, will be examined, lending potential for wound healing applications to our molecules.

P227 **Hui Lao**
WuXi

Overcoming Synthetic Challenges for one KRas Inhibitor, a Highly N-Alkylated Cyclic Peptide

Hui Lao

Macrocyclic peptides, among the structurally diverse peptide drug candidates, have demonstrated remarkable efficacy towards high-value therapeutic targets such as the KRas-SOS1 through intracellular protein-protein interactions. Among the different types of macrocyclic peptides, the synthesis of highly N-alkylated ones is particularly challenging. Here we present a case study of the synthesis of one KRas inhibitor, a particularly challenging cyclic peptide of this category due to the many bulky unnatural amino acids in the structure.

We successfully developed a robust route for the preparation of this inhibitor within six weeks through parallel cyclization position screening, hybrid SPPS/LPPS approach and comprehensive purification method screening. This strategy has also been demonstrated to be effective in the preparation of other N-alkylation-rich cyclic peptides with good yields and high purity.

YI-P228 **Nassim Maarouf**
University of Montreal

Copper Catalyzed S_N2' Alkylation Entry to Peptide Mimics

Nassim Maarouf, Ramakotaiah Mulamreddy, N. D. Prasad Atmuri, and William D. Lubel

Copper catalyzed reactions of organozincates and allylic halides have broad utility for making carbon-carbon bonds. Studying the application of such copper chemistry, our laboratory has developed catalytic and scalable methods for synthesizing a variety of enantiomerically pure amino acids and dipeptides. For example, vinyl β-homoproline, vinyl proline, and indolidizinone analogs have been respectively synthesized through copper-catalyzed reactions of allylic halides on aspartate- and serine-derived zincates. The vinyl prolines have served as building blocks in the synthesis of constrained analogs for the study of peptide chemical biology. Moreover, β-homoproline/isoproline chimeras derived from vinyl β-homoproline offer potential to nucleate anti-parallel β-sheets contingent of configuration. Indolidizinone dipeptides were shown to adopt dihedral angle geometry analogous to the central residues of type II' β-turn secondary structures.

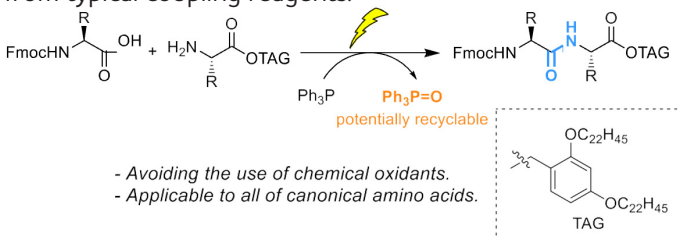
Our presentation will feature recent developments in the copper catalyzed synthesis of unsaturated amino acids and their subsequent incorporation into rigid mimics of different peptide secondary structures.

YI-P229 **Shingo Nagahara**
Tokyo University of Agriculture and Technology

Biphasic Electrochemical Peptide Synthesis Utilizing Triphenylphosphine as a Recyclable Coupling Reagent

S. Nagahara, Y. Okada, Y. Kitano, and K. Chiba

While peptide is one of the promising candidates for medium molecular medicines, there is room for improving the synthetic process. In conventional peptide synthesis, the large amount of waste is produced, which have been recognized as urgent issue from green chemistry viewpoint. To address the challenge, we have developed an electrochemical peptide synthesis utilizing triphenylphosphine, Ph_3P, in a biphasic system, MeCN-c-Hex, aiming for reducing the amount of waste derived from coupling reagents. Anodic oxidation of Ph_3P generates a phosphine radical cation, which serves as the coupling reagent to activate carboxylic acids followed by peptide bond formation. In this reaction, triphenylphosphine oxide, Ph_3PO, is produced as a stoichiometric byproduct. Given that methods to reduce Ph_3PO to Ph_3P have been reported, Ph_3P can be a recyclable byproduct unlike those from typical coupling reagents.



We found that the electrochemical peptide synthesis is applicable to all of the canonical amino acids. In addition, the selective recovery of desired peptides and Ph_3PO was achieved in combination with a soluble tag-assisted liquid-phase peptide synthesis. Moreover, pharmaceutical nonapeptide, leuprorelin, was successfully synthesized by the electrochemical way.

P230 Gosuke Hayashi
Nagoya University

N-Terminal Deprotection Strategies for One-Pot Multiple Peptide Ligation

Gosuke Hayashi

Peptide ligation is an essential step in chemical protein synthesis that can produce proteins of interest from chemically synthesized peptide building blocks. The most popular peptide ligation is native chemical ligation, NCL, in which unprotected C-terminal peptide thioesters and N-terminal cysteinyl peptides are coupled to form an amide bond in mild aqueous conditions. NCL and its expanded methods have been used to create not only naturally occurring proteins with/without site-specific posttranslational modifications, PTMs, but also artificial proteins such as mirror-image proteins and fluorophore-labeled proteins. However, chemical protein synthesis still has issues to be solved such as the low-yield in large and/or hydrophobic protein synthesis and the time-consuming procedures. To upgrade and streamline the procedure of chemical protein synthesis, many sophisticated strategies about thioester preparation, peptide solubilization, and one-pot ligation have been developed.

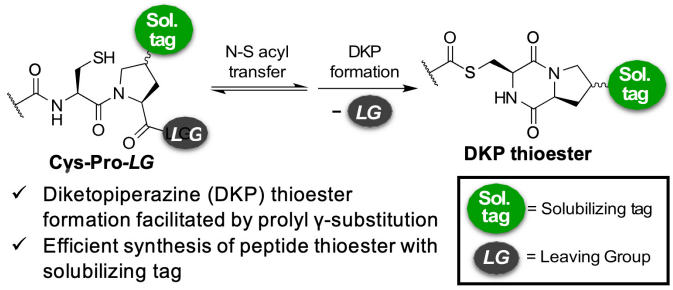
We have been engaged in developing novel strategies of one-pot peptide ligation via efficient deprotection of peptide N-terminal cysteine. One-pot ligation of 5 peptide segments was demonstrated for the first time by removing N-terminal allyloxycarbonyl, Alloc, group with palladium or ruthenium complex and multiple roles of a thiophenol derivative. More recently, we have developed C-to-N one-pot peptide ligation through repetitive removal of N-terminal thiazolidine by newly designed aldehyde scavengers. In this presentation, we share our recent progress about new chemical tools enhancing chemical protein synthesis.

YI-P231 Koki Nakatsu
Nagoya University

β-Substituted-Proline-Prompted Diketopiperazine Formation for Efficient Synthesis of Tag-Attached Peptide Thioester

Koki Nakatsu, Gosuke Hayashi, and Hiroshi Murakami

Native chemical ligation, NCL, is a powerful strategy to ligate side-chain unprotected peptide segments between an N-terminal cysteine peptide and a C-terminal peptide thioester in neutral aqueous conditions. To introduce a base-liable thioester moiety through Fmoc solid-phase peptide synthesis, SPPS, peptide chemists have designed and reported many thioester surrogates. Among them, the Cysteinyl- Prolyl-(Leaving Group), Cys-Pro-*LG*, is easily introduced to synthetic peptides in Fmoc-SPPS, and it converts into thioester through the sequential reactions consisting of N-S acyl transfer at cysteine and 2,5- diketopiperazine, DKP, formation. The effects of leaving group on the reaction rate in DKP formation have been reported intensively by previous studies. However, few studies have reported the effect of substitutions at prolyl moiety on DKP formation. Among many types of modified prolines, γ-substituted prolines are relatively readily available and have been intensively investigated mainly in the context of collagen mimetic peptides.5 These substitutions significantly affect *cis*-trans isomerization of prolyl amide and puckering of the pyrrolidine ring of proline. Therefore, we hypothesized that γ-modified proline is an effective tool for tuning the DKP formation.



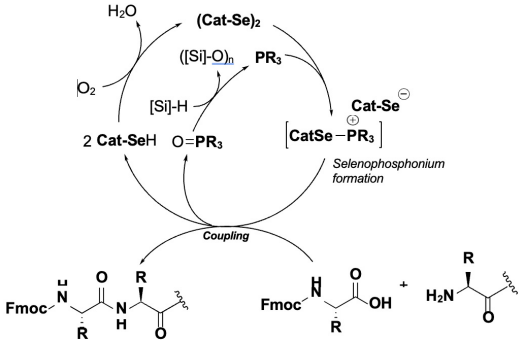
Herein, we will discuss the effect of prolyl γ-substitutions of Cys-Pro-*LG* on DKP-thioester formation using Cys-Pro-pyrazole[7] model peptides. By comparing various substitutions, we revealed that stereochemistry, electronegativity, steric bulkiness, and intramolecular hydrogen bond were crucial factors for DKP-thioester formation. Notably, some modifications showed a great improvement, up to ~6-fold, in the rate of DKP-thioester formation. Moreover, we will report chemical protein synthesis using peptide DKP-thioester with a solubilizing tag connected via an optimized linker to facilitate handling of peptides during ligation reaction and purification process.

YI-P232 Nihar Panigrahi
New York University

Rational Design of an Organocatalyst for Solid-Phase Peptide Synthesis

Nihar R. Panigrahi and Paramjit S. Arora

Contemporary solid-phase peptide synthesis is a highly wasteful process with typical conditions utilizing five equivalents or more of the coupling agents for every amide bond synthesized. A catalyst that could replace requirement for an



excess of activating reagents would be highly desirable. We have developed a two-component organoreductant/organooxidant-recycling strategy to catalyze amide bond formation. This strategy utilizes a diselenide organocatalyst and catalytic phosphine for solution and solid-phase peptide synthesis. The optimized catalyst is active on a diverse range of amino acid substrates with undetectable amount of epimerization. NMR analysis of the catalytic cycle reveals key role for a selenophosphonium intermediate in carboxylic acid activation. Based on our mechanistic studies, we are optimizing the organocatalyst for potential application in solid phase peptide synthesis. These studies will be described.

P233 **Ruiwu Liu**
University of California Davis

Comparison of Microwave and Conduction Heating for Solid Phase Peptide Synthesis

R. Liu, Y. Ajena, L.N. Solano and K.S. Lam

Solid phase peptide synthesis, SPPS, has become a standard approach for synthesis of peptides, especially in a laboratory setting. Heating the reactions in SPPS could significantly reduce the coupling and deprotection times. One of the heating methods is to use microwave which is becoming increasingly popular because it not only dramatically reduces the synthesis times, but also increases the crude peptide purity. However, microwave peptide synthesizers are relatively expensive. In this study, we investigated whether SPPS using conduction heating can achieve similar result as microwave irradiation.

CSBIO II and CEM Liberty Blue were used as heating resource of conduction and microwave heating, respectively. Four peptides with length of 18mer, TMEDKIYDQQVTKQCLCF, 19mer, YSYPETPLYMQTASTSYYE, 20mer, Bivalirudin, and 39mer, Exenatide, were selected as examples. The peptides were synthesized using the same synthesis protocol at 90°C including identical coupling, deprotection and washing cycles. The differences between the two approaches are the temperature of washing DMF, 90°C vs. 23°C for conduction and microwave heating, respectively, and overall synthesis cycle time, 17 min vs. 10 min in conduction and microwave heating, respectively.

Both conduction and microwave heating generated comparable results with crude purity of 52.7% vs. 53.6%, 56.3% vs. 58.3%, 62.8% vs. 61.5%, 38.2% vs. 39.9% for 18mer, 19mer, 20mer, and 39mer, respectively. One of the advantages of conduction heating is the uniformly and consistently delivered temperature during the synthesis which could minimize racemization and side reactions caused by spikes and hotspots typically associated with microwave heating. In addition, conduction heating is also a more cost-efficient heating method when compared to expensive microwave heating technology.

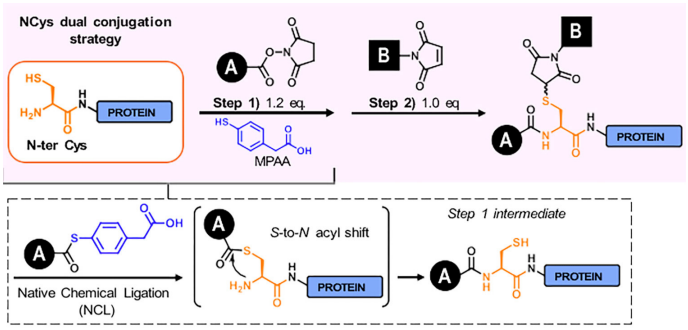
YI-P234 **Carlo Pifferi**
Center for Molecular Biophysics

N-Terminal Cysteine as Minimalistic Handle for Dual, Site-Selective Bioconjugation: Case Study on an Anti-HER2 Affibody

C. Pifferi, A. Novak, F. Kersaudy, S. Berger, S. Morisset-Lopez, F. Lefoulonc, and V. Aucagne

Site-selective, dual-conjugation approaches for the incorporation of distinct payloads are key for the development of molecularly targeted biomolecules endowed with optimized properties. The unique reactivity of the 1,2-aminothiol group of N-terminal cysteines, NCys, have been largely exploited for chemoselective reactions using a variety of tailored reagents, leading either to a *N,S*-cosubstituted product, or a to a *N*-acylated one. In the latter case the free cysteine side chain can be further engaged in a second, thiol-selective conjugation yielding a dual conjugate. Amongst NCys-selective conjugations, a recent strategy caught our attention, inspired by Native Chemical Ligation, NCL, and based on the *in situ* generation of a thioester from a *N*-hydroxysuccinimidyl, NHS, ester.

The scope of such approach indeed resonates with the large portfolio of commercially-available NHS-functionalized reagents, commonly used for non-selective protein acylation. However a very large excess, 50- 500 equivalent, of the NHS ester is required, and the thioester must be pre- formed.



We report here a methodology for dual-bioconjugation at NCys, involving direct addition of a slight excess of the NHS-moiety to the protein solution containing a thiol additive; thioester formation takes place with extremely fast kinetics, enabling for subsequent selective NCL-mediated functionalization at the NCys. The second step is carried out without intermediate chromatographic purification, *via* thiol-Michael addition to a stoichiometric amount of *N*-maleimido- functionalized cargos, another class of readily available reagents. Protein dual conjugates are obtained in excellent yields, under very mild conditions and using extremely reduced amounts of NHS and maleimide reagents, as exemplified with the generation of anti-HER2 affibody derivatives equipped with a variety of cytotoxic drugs and probes, and which functionality was demonstrated on several cell lines.

YI-P235 Wyatt Powell
University of Colorado at Boulder

The Role of *N*-linked Glycans in Phase Transitions of Tau

Wyatt C. Powell, Ruiheng Jing, McKinley Nahum, and Maciej A. Walczak

Filaments composed of microtubule associate protein tau, MAPT, are a hallmark of Alzheimer's disease, AD, and other dementias collectively known as tauopathies. Many post-translational modifications regulate the structure and function of tau, among them *N*-glycosylation found in tau from AD brains but not in healthy controls. However, it is poorly understood how these disease-specific modifications impact the pathological aggregation of tau and its neurotoxicity. To address these questions, we developed a robust chemical synthesis of tau and the major N-linked glycans from AD patients.

Synthetic peptides were coupled with the (oligo)saccharide amines at two confirmed glycosylation sites, and the building blocks were assembled into several glycosylated variants of full-length tau and the repeat domain K18. Biophysical studies established that glycosylation promotes polyanion induced liquid-liquid phase separation and in vitro aggregation. Furthermore, glycosylation likely facilitates hydration of the backbone, increases the overall flexibility due to hinderance and limited conformational freedom.

Glycosylation of tau also stabilizes the filaments because it provides a protective hydration shield of the hydrophobic region. Taken together, the combined synthetic and biophysical studies provide a molecular insight into the role of *N*-linked glycans in tau biology and can serve as a platform to develop new diagnostic tools and therapeutic strategies.

P236 Dominik Sarma
Gyros Protein Technologies

Green Solvents and Orthogonal Catch-and-Release Purification Boost Eco-Friendly Peptide Production

L. Pacini, A. M. Papini, S. Lüdtke, M. Muthyala, and R. Zitterbart

Over the past few decades, environmental concerns have become increasingly urgent, particularly in the context of manufacturing processes involving chemicals. Traditionally, solvents such as DMF, NMP, and TFA have been used in solid-phase peptide synthesis, SPPS, despite their negative impact on the environment. However, the use of such solvents conflicts with the current trend toward green solvents.

In this study, we investigated the use of PurePep® Chorus to produce model peptides, ACP, and poly-Ala while avoiding the use of DMF. We tested binary solvent mixtures that varied in polarity, viscosity, boiling points, safety, and sustainability. We focused on incorporating green chemistry principles into our process. Despite the absence of DMF, our yields and crude purity remained comparable to those obtained using traditional methods.

We further improved the sustainability of the process by implementing the orthogonal PurePep® EasyClean purification technique. This catch-and-release method resulted in high quality peptides while minimizing the impact of solvents on the purification step. Overall, our approach demonstrates the feasibility of using green solvents and implementing green chemistry principles in peptide synthesis and purification using the PurePep® solutions.

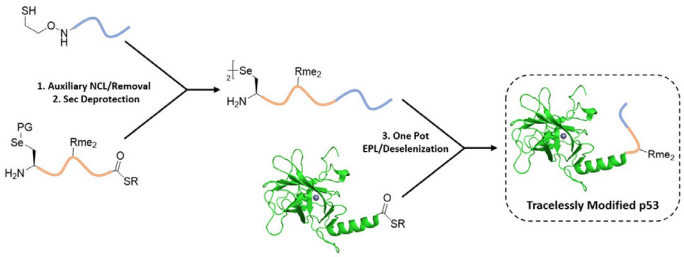
YI-P237 Alexander Russell
University of Washington

Mechanistic Interrogation of p53 Arginine Methylation via Protein Semisynthesis

Alexander Russell

The tumor suppressor protein p53 is a critical regulator of cell fate and is the premier oncogene, bearing mutations in approximately 50% of cancers. In the remaining 50%, however, it is unclear why p53 is unable to effectively fulfill its role in arresting aberrant growth.

One hypothesis is that dysregulation of post-translational modifications of p53, which are critical for its function and regulation, may enable cancer cell proliferation. In particular, arginine methylation within the tetramerization domain, TD, of p53 has been shown to have significant effects on phenotypic outcomes in cellular models and is installed by the putative oncogene PRMT5. Despite this potential relevance to p53's role in cancer, no direct molecular-level characterization of this modification state or its direct effects on p53 activity has been reported. Through a combination of protein semisynthesis and biochemical/biophysical assays we are investigating the role of arginine methylation of the TD in regulating p53 function.



We have found that methylation at these sites decreases the stability of the tetrameric form of the TD in peptide models, which may significantly impact the ability of p53 to activate transcription of target genes. In addition, we have developed a semisynthetic approach to access milligram-scale quantities of tracelessly and site-specifically modified p53 via a novel 3-component strategy.

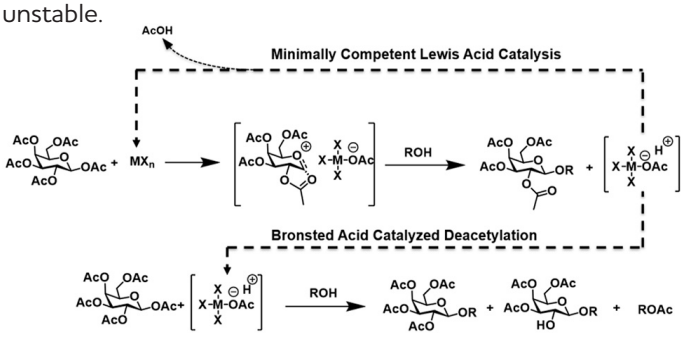
With this material in hand, we intend to assay its ability to bind DNA, activate transcription, and associate with other proteins as compared to unmodified p53. The results of these experiments will give vital insight into the mechanisms by which TD arginine methylation affects p53 activity as well as downstream cellular outcomes.

P238 **Troy Smith**
L51-YI7 University of Arizona

Minimally Competent Lewis Acids: Effective and Efficient Catalysts for β-Glycosylation of Peptide Hormones

T.E. Smith, A, Myloserdnyy, and R. Polt

The synthesis of amino acid glycosides has been an ongoing synthetic challenge. Glycosylation of peptides has been shown to be an important way to enhance stability and transport of peptide-based drugs. Unfortunately, there are no universal conditions for the variety of glycosyl donors and acceptors commonly used, and the donors typically used are unstable.



Previous methods often require lengthy synthetic routes, unstable reagents, and low temperatures to provide the required glycosides. This study examines the

use of minimally competent Lewis acids, MCLAs, as robust catalysts for β-glycosylation of serine using stable sugar peracetates with the goal of developing more finely tuned catalysts for glycopeptides and other types of glycosides.

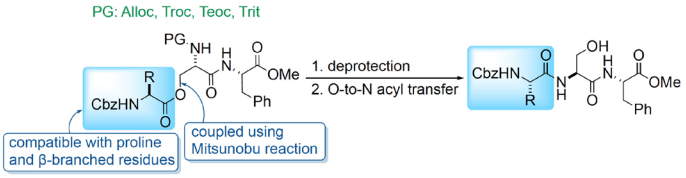
YI-P239 **Regina So**
Brigham Young University

Understanding the Utility and Scope of a novel Serine Ligation Method

Regina C. So, Ankur Jalan, Michael Kopach, and Steven L. Castle

Native chemical ligation, NCL, presents a powerful method to prepare unprotected peptides or proteins. However, the requirement of a N-terminal cysteine, C, residue poses a limitation for this method since there is a low abundance of cysteine present in protein segments. Alternatively, a desulfurization step is necessary to couple at an alanine, A, site.

The Li group has reported a serine/threonine ligation, STL, as a complement to NCL. However, this strategy is not suitable for aspartic acid, glutamic acid, lysine and bulky β-branched amino acids such as, threonine, valine, and isoleucine - slow reactions.



Our strategy is based on a Mitsunobu reaction to form the tripeptide ester, followed by a deprotection and O-to-N acyl transfer. We used a model tripeptide, **G**-O-SF, in order to investigate the scope and the limitations of the O-to-N acyl transfer. Initial studies showed facile transfer of G from the serine oxygen to the nitrogen moiety. The scope of the serine ligation with model tripeptides will be discussed along with extensions to more complex coupling partners. Notably, the ligation is viable with proline and β-branched amino acids.

P240 **Jack Silver**
Teledyne ISCO

Can Large Peptides be Purified Using Only Flash Chromatography?

J. Silver

Peptides with a mass range from ~1200 through ~8000 Daltons, Da, were successfully purified using only flash chromatography. Flash purification traditionally provides an initial purification to peptides that then require final polishing via prep HPLC or other techniques. Scouting gradients were run on HPLC or on the flash chromatography system to calculate the focused gradients used for purification. The scouting gradients were also used for column and mobile phase screening. The scouting runs are very quick, allowing calculations of fast, efficient, focused gradients. A mass spectrometer was used to verify the elution of the peptides for both the scout and purification runs. Flash chromatography has the advantages of being inexpensive compared to preparative HPLC, allowing large sample loads, and quick runs. The peptides were clean after flash chromatography, surprisingly so for the 7.8 kDa peptide.

YI-P241 **Paul Spaltenstein**
University of Utah

Multi-Segment Click-Assisted Native Chemical Ligations

P. Spaltenstein and M. S. Kay

Chemical protein synthesis, CPS, enables access to proteins and peptides that would otherwise be difficult or impossible

to access via recombinant expression, unlocking biophysical and biochemical studies such as mirror-image phage display. Peptides containing ~50 AAs are routinely obtained by solid-phase peptide synthesis, SPPS, and can be joined together by chemical ligations to produce larger proteins. Native chemical ligation, NCL, is widely used, but can suffer from slow kinetics and typically requires mM concentrations unattainable for hydrophobic peptides. We developed Click-Assisted NCL, CAN, to overcome slow kinetics by "clicking" two peptides together to increase their effective concentrations.

We expand on the CAN methodology to perform templated NCL at multiple junctions. To do so, we established a second conjugation reaction that is compatible with NCL conditions and can be used in tandem with CAN. In addition, we demonstrate selective traceless modifications of peptides at two distinct sites to enable multi-segment templated ligations. For a three-segment templated ligation system, we explore selective thioester activation via orthogonal thioester surrogates to minimize side reactions. We further discuss the additional steps to enable TEMplated Peptide-Oligo, TEMPO, NCL for simultaneous ligations extending beyond three segments. Peptides are functionalized with traceless linkers and conjugated to oligos that are designed to hybridize to a complementary DNA template, positioning the peptide segments in close proximity for one-pot templated NCL. The templated NCL approaches discussed here are anticipated to greatly facilitate syntheses of proteins that are currently unattainable by CPS.

YI-P242 Sheryl Sharma

L33-YI4 University of Nebraska at Lincoln

Identifying Peptide-Receptor Interactions using Aryl Diazonium-Labeled Peptide Ligands

Sheryl Sharma, Makayla Gill, and James W. Checco

Endogenous bioactive peptides, for example, neuropeptides and peptide hormones, are a class of signaling molecules that play pivotal roles in maintaining cardiovascular, reproductive, gastrointestinal, and metabolic health. Dysregulation of peptide signaling is associated with diseases such as cancer, multiple sclerosis, Alzheimer's, diabetic nephropathy, and many more. However, despite the growing interest, the receptor proteins for many disease-relevant bioactive peptides are currently unknown, severely limiting their potential as therapeutic targets.

In a quest to discover novel peptide-protein interactions, and unveil molecular mechanisms underlying diseases, we report a proximity induced labeling strategy using aryl diazonium-modified bioactive peptides to covalently label corresponding membrane-bound receptors.

We describe the design principles needed for the synthesis of aryl-diazonium modified bioactive peptides to achieve efficient receptor labeling and demonstrate that a peptide's affinity to a given receptor can be harnessed to specifically label interacting proteins on living cells under physiological conditions.

We hypothesize that the developed protein labeling strategy can be used for the enrichment and isolation of membrane proteins in an unbiased fashion. Long-term, this research may aid in identifying new therapeutic targets for a number of diseases caused by dysregulated cell-cell signaling peptides.

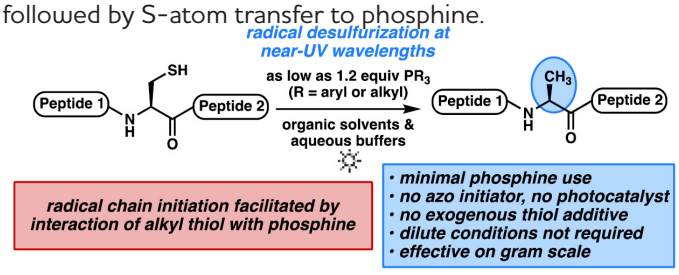
YI-P243 Naresh Murty Venneti

Wayne State University

Photo and Catalytic Desulfurization: Application to Organic Molecules, Peptides, and Proteins

Naresh M. Venneti, Ganesh Samala, Rana M. I. Morsy, Lawrence G. Mendoza, Albert Isidro- Llobet, Janine K. Tom, Subha Mukherjee, Michael E. Kopach, and Jennifer L. Stockdill

A high yielding photodesulfurization of cysteine containing peptides reported with minimum equivalents use of the phosphine reagents. This facile desulfurization method doesn't demand the use of radical initiator or photocatalyst or exogenous thiols or other H-atom donors. It has wide applicability in organic and aqueous solvent conditions. This method was successfully examined with unprotected and protected peptides with different combinations of amino acids. The method is also compatible with NCL buffer and applied in one-pot disulfide reduction/multidesulfurization of linaclotide, aprotinin, and wheat protein. An electron donor-acceptor, EDA, complex between phosphine and alkyl thiol is predicted that could initiate the thiyl radical formation followed by S-atom transfer to phosphine.



Continuation to this investigation, we developed a catalytic thiyl-radical mediated desulfurization method, and this method utilizes a catalytic phosphite reagent and thermal initiator with stoichiometric silane as a hydrogen donor. This method was applicable to organic molecules and small protected peptides with good functional group compatibility, including free carboxylic acid, ester, carbamate, amide, steroid, and carbohydrate moieties.

P244 Aimin Song

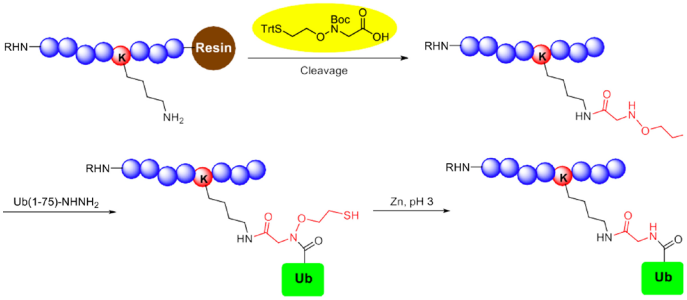
Genentech, Inc.

Site-Specific Chemical Ubiquitination of Synthetic Peptides Using an *N*-(2-Mercaptoethoxy)Glycine Building Block

Lingling Peng, Elizabeth Helgason, Rafael Miranda, Jeffrey Tom, Erin C. Dueber, and Aimin Song

Ubiquitination, a versatile post-translational modification, PTM, of proteins in eukaryotes, has profound impacts on a variety of physiological and pathological processes. The preparation of ubiquitin, Ub, conjugates with high homogeneity and sufficient quantities is critical for studying ubiquitination. Chemical synthesis approaches have been developed that offer attractive solutions to overcome limitations of the enzymatic approach to generate precise and homogeneous site-specifically ubiquitinated peptides. Most of the published methodologies for chemical ubiquitination involve native chemical ligation, NCL, via a glycyl-auxiliary or a mercaptolysine building block.

Weller and others reported a semisynthetic strategy using a 2-(aminooxy)ethanethiol auxiliary for chemical ubiquitination. This strategy offers an advantage of being compatible with the presence of protein thiol groups, but suffers from low yields with long peptides and incompatibility with other modifications such as phosphorylation.



In order to address the limitations, we have developed a novel *N*-(2-mercaptoethoxy)glycine building block that enables convenient site-specific chemical ubiquitination of synthetic peptides. The orthogonally protected building block is fully compatible with solid phase peptide synthesis, SPPS and a variety of peptide modifications including phosphorylation, methylation, acetylation, biotinylation and fluorescent labeling.

The efficiency and advantages of this methodology have been demonstrated by the synthesis of ubiquitinated histone and Tau peptides bearing PTMs and fluorescent labeling. Free cysteine residues in the peptide sequence were well tolerated. The correct structure and folding of Ub in the synthetic ubiquitinated peptides were confirmed by their reactivity towards deubiquitinases, DUBs. This methodology is ideally suited for the preparation of Ub conjugates with diverse and complex structures.

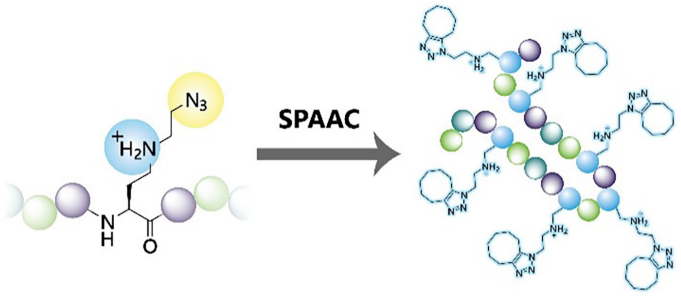
YI-P245 Yixin Xie

National Cancer Institute

Hydrophilic Azide-Containing Amino Acid to Enhance the Solubility of Peptides for Strain-Promoted Azide Alkyne Cycloaddition

Yixin Xie, Tania L. Lopez-Silva, and Joel P. Schneider

Strain-Promoted Azide Alkyne Cycloaddition, SPAAC, one of the bioorthogonal reactions, has become a widespread tool for modifications of biomolecules in living system, due to its chemoselectivity and compatibility with physiological environments. The modification of peptides and other macromolecules with azido groups for SPAAC reactions can affect the physical, structural, and functional properties, particularly when residues in the native primary sequence are altered or when multiple modifications are required.



Amino acid: *Efficient synthesis • Compatible with SPPS*
Side-chain: *Hydrophilic • Efficient cycloaddition • Affords highly decorated systems*

In this work, we developed a new unnatural amino acid that contains both a secondary amine and azide in its side chain, which provides the positive charge at physiological pH and improves solubility while allowing efficient SPAAC ligation. The two-step synthesis of the new unnatural amino acid is simple, high yielding, scalable, and compatible with Fmoc SPPS employing a variety of coupling conditions. This new side-chain offers efficient second-order rate constants in SPAAC employing DBCO and BCN. Importantly, numerous azide residue can be incorporated into a single peptide while minimally affecting solubility. This new amino represents a new tool in the SPAAC toolbox to facilitate macromolecular ligation where solubility and maintenance of primary charge are of concern, especially in highly decorated systems.

P246 Michael Taylor

University of Arizona

Radical Photocages for Rapid Biomolecular Perfluoroalkylation

Michael T. Taylor

Owing to its electronegativity, small size, and orthogonality to biology, the incorporation of fluorine-containing functional groups into biomolecular structure has become essential in chemical- and structural biology, imaging, and drug discovery.

Here, we report the development of a photocage for trifluoromethyl radicals that enables ultra-rapid trifluoromethylation of peptides and proteins. Our photocage design features a cationic, aromatic, sulfonate ester that features both a weak S–OAr bond and an aromatic chromophore that absorbs visible light. Absorption of a photon of light results in homolytic cleavage of the labile S–OAr bond that ultimately leads to the generation of a free trifluoromethyl radical that then is trapped by π-nucleophiles in biomolecules. This photo-decaging process is unimolecular which, when combined with an efficient quantum yield of decaging and the ultra-rapid kinetics of trifluoromethyl radical trapping with π-nucleophiles, allows for very short reaction times, <1s, and very mild labelling conditions, for example, low μM of photocage, no oxidants et cetera.

We explored this capability through chemical mapping experiments with individual proteins, protein-ligand complexes, and protein-protein complexes. These experiments enabled the labelling of Tryptophan, Trp, Histidine, and Tyrosine residues in a context-dependent fashion, where changes in amino acid microenvironments can be detected by changes in labelling outcomes.

We also show that our trifluoromethyl radical photocages can be adapted to preparative scale trifluoromethylation of peptides; enabling a 6-minute synthesis of milligram quantities of peptide conjugates that harbor trifluoromethyl groups on Trp residues. We anticipate that this approach will enable rapid access to bespoke perfluoroalkylated peptides for therapeutic and imaging purposes as well as enable new approaches to probing the interactions of proteins.

YI-P247 Evan Yanagawa
University of Pennsylvania

Investigating the Effects of Thioamide Incorporation on the Aggregation of Alpha-Synuclei

Evan Yanagawa

Alpha-synuclein is an intrinsically disordered protein that is implicated in various neurological diseases such as Parkinson's disease, PD, dementia with Lewy bodies, DLB, and multiple system atrophy, MSA. A hallmark of PD pathology is the increased formation of amyloid fibrils, an aggregated form of alpha-synuclein. The formation of these fibrillar Lewy bodies arise from a complex hydrogen bonding network between beta-sheet alpha-synuclein protein.

Because thioamides have different hydrogen bonding properties than their oxoamide counterparts, we hypothesize that their incorporation in specific amino acid sites of alpha-synuclein will have profound destabilizing effect on beta-sheet formation and amyloid aggregation. The thioamide moiety is a molecular isotere of the peptide backbone that is intriguing to chemical biologists because of their various effects on protein secondary structure. The extent to which thioamides impact the secondary structure of proteins is especially attractive to biochemists because of the single atom substitution modification of such studies.

The most common methods of incorporating unnatural amino acids into protein sequences involves the semi synthesis of peptide fragments following by a ligation-desulfurization procedure to append the fragments into a full-length protein. Because this ligation strategy typically ends with a desulfurization step to restore an alanine residue on the C-terminal fragment, it is limited to either terminal alanine or cysteine residues. Other methods have been generated to restore other amino acids, for example, glutamine and phenylalanine, upon desulfurization, with B-thiol amino acid derivatives.

We report a synthetic method of generating a B-thiol tyrosine derivative, to expand the scope of peptide semi-synthesis and chemical ligation so that incorporation of thioamides at more positions is possible.

P248 Dirk Winkler
Kinexus Bioinformatics Information

SPOT Synthesis – Advantages, Challenges, Limitations

D.F.H. Winkler and S. Pelech

Three years ago, SPOT synthesis celebrated its 30th anniversary – undeservedly quietly. Invented by Ronald Frank and presented first in 1990, the SPOT technique is an easy and inexpensive method for the synthesis of small and medium-sized peptide arrays with up to 1200 peptides on a membrane with a size of approximately 9 cm x 14 cm.

Example of a peptide array produced by SPOT synthesis with overlapping peptides derived from the spike, nucleocapsid and membrane proteins of SARS-CoV-2 that was probed with serum from a COVID-19 positive individual.

The produced arrays can be used for a wide diversity of applications, including the investigation of protein-peptide interactions or, in vivo, to study effect on cancer cells or bacteria. The peptides produced by SPOT synthesis can be used in different ways: either as solutions or, attached to cellulose membranes, either as macroarrays or as individual cellulose discs.

This presentation will provide an overview about the observations of 30 years of our research using the SPOT technique for the synthesis of arrays and their application for the investigation of protein-protein interaction. It will show the advantages of this technique and its limitations. Additionally, some examples of the use of the SPOT technique presented by other research groups will be mentioned to show other applications.

P249 Ankur Jalan

Lilly

Methods for Peptide Synthesis

Ankur Jalan

Peptide APIs can be manufactured by biological and chemical methods. Synthetic peptides are made by either traditional linear solid phase peptide synthesis, SPPS, or hybrid solid-phase and solution-phase. Synthesis and manufacture of peptides by linear SPPS can be challenging because of lower yields and purities with increasing peptide length. Linear SPPS is also associated with an inherent risk of total loss of the peptide asset with a single mistake.

The convergent hybrid synthesis is a very appealing substitute to the linear SPPS. It allows for parallel synthesis of likely high purity fragments for condensation and can allow for faster production of the API. Native chemical ligation and liquid phase peptide synthesis methods are also gaining popularity.

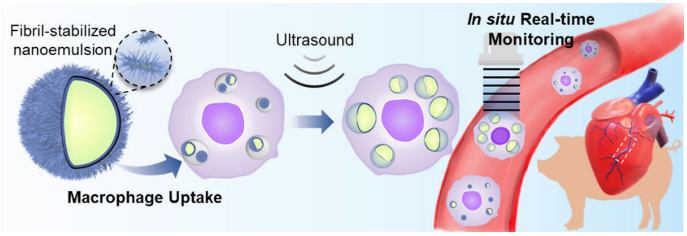
L24 Scott Medina

Penn State College of Engineering

Real-Time, In Situ Imaging of Macrophages via Phase-Change Peptide Nanoemulsions

Scott Medina

Macrophages are specialized phagocytes that play central roles in immunity and tissue repair. Their diverse functionalities have led to an evolution of new allogenic and autologous macrophage products. However, realizing the full therapeutic potential of these cell-based therapies requires development of imaging technologies that can track immune cell migration within tissues in real-time. Such innovations would not only inform treatment regimens and empower interpretation of therapeutic outcomes, but also enable prediction and early intervention during adverse events.



In this talk, I will summarize our recent efforts to develop phase-changing nanoemulsion contrast agents that permit real-time, continuous, and high-fidelity ultrasound imaging of macrophages in situ. Using a de novo designed peptide emulsifier, we prepare liquid perfluorocarbon nanoemulsions and show that rational control over interfacial peptide assembly affords formulations with tunable acoustic sensitivity, macrophage internalization, and in cellulo stability.

Imaging experiments demonstrate that emulsion-loaded macrophages can be readily visualized using standard diagnostic B-mode and Doppler ultrasound modalities. This allows on-demand and long-term tracking of macrophages within porcine coronary arteries, as an exemplary model. Our results demonstrate that this platform is poised to open new opportunities for non-invasive, contrast-enhanced imaging of cell-based immunotherapies in tissues, while leveraging the low-cost, portable, and safe nature of diagnostic ultrasound.

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