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Peptide and Protein Synthesis: Origin and Development

Narrative	Photography
In the year $4X10^9$ BC it all began with a big bang and the formation of the Universe. Expand a little …The elements were formed, The solar system, the earth.	Big bang. Heavy sound effects and very bright light. Then the time scale
In your lifetime and mine we have also seen remarkable advances in science and technology that have profoundly changed our world. The United States has sent spacecraft to circle and explore the planets of the solar system and beyond, and has landed a man on the moon. Progress in the understanding of our universe continues with the contruction of an international manned space station.	Rocket blast off. Solar system, and path of voyager. Spacemen on moon
During the 20th century the laws of relativity and quantum theory were advanced and additional elements and subatomic particles were discovered.	Einstein photo
Electronics from radio to television, the transistor, high-speed computers and the internet have changed the way people live and interact.	Crystal set or telegraph key Philco radio, TV set and historic picture on screen. Laptop with website picture on screen.
During this time period extraordinry developments have been taking place in experimental chemistry and biology. The details of cell division and reproduction have become understood. DNA	Dividing cell drawing DNA helix drawing

was shown to be the fundamental genetic material. The Genetic Code has been deciphered and the transcription of this information to RNA and subsequent translation into protein is being worked out.	DNA → RNA → Protein
In our own specialized field of peptide and protein science advances have also been extensive. The compositions and amino acid sequences of thousands of proteins are known, and many	
three dimensional structures have been deciphered using X-ray and NMR techniques. Peptides and small proteins are now	X-ray picture of hemoglobin etc.
accessible by total chemical synthesis. Large numbers of naturally occurring peptides have been synthesized, antagonists of many peptide hormones and enzymes have been designed and shown to	What should show here????
be pharmacologically effective drugs. Multikilogram quantities of certain peptides have been produced. It is possible to synthesize	Steam shovel (Toy with white solid.)
thousands or even millions of peptides in a single experiment and to test them for their biological activity and to use this information for drug discovery.	Combinatorial experiment
Clearly this progress did not happen overnight, but evolved through the work of many people, over a period of time beginning exactly 100 years ago when the great organic chemist Emil Fischer synthesized the first free peptide. At that time Fischer	
coined the name "peptide" and he soon became known as the	Formula of a dipeptide.
father of peptide chemistry. He was able to prepare several larger peptides and eventually an 18-residue peptide. He explained his	Portrait of Emil Fischer (Pan in and out and across) Leave on.

effort by a famous quotation: "Whereas professional colleagues fear that a rational study of this class of compounds, because of their complicated structure and their highly inconvenient physical difficulties, other optimistically endowed observers, among which I will count myself, are inclined to the view that an attempt should at least be made to besiege this virgin fortress with all the expedients of the present, because only through this hazardous affair can the limitations of the ability of our method be ascertained". Fischer, of course, could not have synthesized a protein because no sequence was known known at the time and some believed that they were not even single compounds. But he laid the groundwork for achievements that eventually came to pass some 60 or 70 years later. Fischer and all the others who entered this exciting field were critically handicapped because they had limited access to amino acids and there were no reversible protecting groups for the α-amine. They had to resort to an indirect route. Fittingly, it was one of Fischer's former students, Max Bergmann and his associate Leonidas Zervas who, 30 years later, solved this problem. Their carbobenzoxy group, which was removable by catalytic hydrogenolysis, instantly transformed the field, and continues to be used today. Within two years Joseph Fruton had begun the synthesis of a large number of small peptides to	Pan on Fischer photo and read the quote. Photo of Bergmann Photo of Zervas Equation for Z deprotection Photo of Fruton. Need video of Fruton discussing early days with

determine the substrate specificity of proteolytic enzymes.	Bergmann and Zervas
For discussion purposes Miklos Bodanszky classified peptides synthesis into two broad division, "Strategy" and "Tactics". Strategy is the general approach to the design of a synthesis e.g. fragment or stepwise coupling using classical synthesis in solution or on a solid support. Tactics deal with the exact details of the chemistry used to implement the synthesis.	Photo of Bodanszky
Further important developments in the tactics of reversible Nα amine protection were very slow and it was another 25 years before another significant advance in amine protection occurred.	
In 1958 Louis Carpino, using modern physical organic principles, proposed the tert butyl function for a urethane protecting group because it would be a better leaving group than benzyl and should be removable in mild acid. Following this, Anderson and Callahan and Albertson and McGregor prepared tert butyloxycarbonyl (Boc) amino acids and showed their value in peptide synthesis.	Photo of Carpino Need a video of Carpino Equation for Boc removal Photo, Anderson, etc.
It took somewhat less time for the next major development in amine protection-again by Carpino in 1972 This time he proposed a base-labile, acid-stable group and selected 9– fluorenylmethyloxycarbonyl (Fmoc) which could be removed by a β -elimination with piperidine or morpholine.	
This was a marked improvement over the previous base-labile phthalyl, trifluoroacetyl, or 2-[9-toluene sulfonylmethyl] -	Equation for Fmoc removal

ethoxycarbonyl groups, but it still did not become popular for several more years. In 1975, Su Sun Wang made a small peptide using Fmoc – protection for one step. In another application Fmoc chloride was sulfonated by Merrifield and Bach and used to derivatize the growing peptide chains at the end of a solid phase synthesis. After chromatographic separation of the strongly acidic derivative on an ion exchange resin, the peptide was deprotected by base and eluted in highly purified form. The next year, 1979, the Meienhofer group and the Sheppard group each used Fmoc-protection for all steps of a long synthesis. This approach is now widely used. The orthogonality of this base – labile group and the acid – labile side chain tert-butyl group is an important advantage of this system.	Photo of Wang My photo in lab coat. Formula Sulfmoc from p.1 Photo of Meienhofer Photo of Sheppard Interview by Felix or Atherton about Meienhofer and Sheppard
Several other N^{α} protecting groups have been forthcoming including those removable under neutral conditions or by photolysis, or by thiols as with the dithiasuccinoyl group of George Barany.	Photo of Barany
An equally important tactic in peptide synthesis is the choice of activating or coupling reagents. Again, this has been an area of great concern and intensive research. The first report on the activation of a protected amino acid and its reaction with a second amino acid to form an amide bond was by Theodore Curtius using an acid azide. This method was improved in 1961 by Joseph Rudinger.	Photo of Curtius Equation of Curtius Equation of Rudinger Photo Rudinger

Fischer made use of α-amino acid chlorides for coupling, followed by ammonolysis. It was effective, but, erroneously, fell into disfavor. Recently the acid halides, especially acid fluorides have been reported by Carpino to give excellent results. During this very rapid growth period in peptide synthesis, Wieland first introduced mixed anhydrides in 1950, and this was quickly followed by the work from the laboratories of Boissonas, Vaughn and Anderson. This method was immediately adopted in many laboratories. The anhydride method was quickly followed by the introduction of active esters as coupling reagents by Wieland and soon thereafter by several other laboratories. Wieland chose thio phenyl esters, Schwyzer proposed cyanomethyl esters, and Bodanszky introduced nitrophenyl esters. Somewhat later tri-and-penta-chlorophenyl esters were recommended by Pless and Boissonas and by Kupriszewski. Later hydroxysuccinimide and hydroxybenztriazole esters were introduced. One of the greatest advances in coupling reagents came when Sheehan and Hess adapted dicyclohexylcarbodiimide to peptides. Todd, Kenner and Khorana had used carbodiimides successfully for dinucleotide synthesis and had made amide bonds with it but it had not been applied to peptides previously. This soon became very popular and was the method of choice for solid phase peptide synthesis, where an excess of reagent	Video interviews about Rudinger **For all video clips have the narrator ask the question. That helps set the scenes.** Interview with Carpino about acid halides. Photo of Wieland, Boissonas. Schwyzer and Bodanszky, video Schwyzer about early days and about Basel. Use some of Eberle Kupriszewski. Photos Weygand, Wunsch, König, Geiger. Photo of Sheehan Goodman Video. Use part about Sheehan and DCC Photos of Todd, Kenner, Khorana DCC equation

could be used to drive the reaction and to compensate for the N-acyl urea side reaction. The relatively insoluble diyclohexyl urea by-product was not a problem. Boc-Histidine, cysteine and some other amino acids are quite susceptible to racemization depending on the protecting group and reaction conditions coupling with N^{α}-Boc-His (N^{im} benzyl) OH gives a small amount of enantiomer during DCC activation, but the N^{im}– DNP or N^{im} – Tosyl derivatives do not. Benoiton showed that N^{α} urethane-protected amino acids in the presence of the strong base, N, N dimethyl amino pyridine, go through the oxazoline, which can racemize. Wang found that Boc-Ile was completely resistant, but Boc-Phe was not.	Photo Benoiton, Some of his video.
In recent years a number of new activating reagents have been developed. Castro introduced the phosphonium salt, benzotriazolyloxytris (dimethylamino) phosphonium hexafluorophosphate (BOP), as a very reactive coupling reagent. Coste reported other reagents in which phosphonium derivatives were replaced by uronium salts, which had even better properties. They also replaced the HOBt component of BOP by bromo and obtained BroP which was much more reactive in hindered syntheses because Br is a better leaving group.	Photo of Castro. Need a video about origin of BOP and derivatives of Coste, Knorr etc. BOP formula Photo of Coste, Knorr & Dourtsoylou Uronium Structure
N-Carboxy anhydrides were introduced by Fischer's student, Leuchs, and used to prepare poly amino acids. Adaptation to a stepwise procedure was found to be possible by Veber, but	Photo of Leuchs Photo of Veber

required special, very exact conditions. However, in Goodman's lab, urethane-protected N-carboxyanhydrides have been obtained in stable, crystalline condition and were reported to be very reactive even in hindered couplings. Fmoc-Ala-F was even better for a hindered coupling.

Having available a large array of effective chemical methods, what are the early landmark syntheses that have been achieved with these techniques? The first remarkable example was the synthesis of oxytocin in 1953 by du Vigneaud. This was the forerunner of a flood of work on peptide hormones, including: vasopressin by du Vigneaud, angiotensin by Rittel 1957, and by Schwarz, 1957, α MSH by Boissonas in 1958, gastrin by Kenner, et al, 1965, glucagon by Wunsch, 1967, Secretin by Ondetti, 1968,S- peptide analogs by Hofmann and Scoffone, ACTH (1-24) by Beyerman, and ACTH (1-39) by Medzirhradszky et al, and by Schwyzer and Sieber. The latter peptide was a particularly elegant synthesis using both stepwise assembly of fragments by the nitrophenyl ester technique and coupling of these fragments by azide, mixed anhydride or carbodiimide methods. This was the largest peptide synthesized until 1963.

About this time a variety of amino acid reagents began to become commercially available by Cyclo, Bachem and other companies such as Penninsula, The Protein Research Institute, Vega, Pierce, and others. Virtually every known compound of the

Goodman, Photo or video

Photo of du Vigneaud

Videos of Hruby and Manning about lab.

Li
Boissonas
Hofmann
Scoffone
Kenner
Wunsch
Ondretti
Medzirhradszky
Kisfaludy
Bajus
Schwyzer
Rittel
Sieber

Make a panel of photos

Video of Medzirhradszky on ACTH

Photo of Rao Mackenini

Photo of Barstow

hormone class has now been synthesized and the same can be said of the peptide antibiotics, growth factors, toxins, neuropeptides many antigens, and other biologically active peptides.	Photo of Pierce
The race to the synthesis of insulin was quite remarkable. This 51-residue, two-chain peptide is generally classed as a protein. The group of Katsoyannis in Pittsburgh first reported their synthesis at a meeting in June 1963, but it was not published until 1964. The group of Zahn also completed their synthesis in late 1963 and published their findings later that year. The Chinese group had been publishing progress on their synthesis for several years, but the final synthesis of active insulin was not published by them until 1965. All three groups used standard solution fragment strategies but with somewhat different tactics. The difficult step in all three cases was the combination of the separate by purified A and B chains to give the correct three disulfide bonds. The yields at this step were low but all three groups succeeded in obtaining biologically active insulin.	Photo Katsoyannis Photo Zahn Video of Brandenberg Photo of Niu, Wang, Du, Kung or the Group? Video by Du. Use 2 or 3 minutes about lab and strategy and cooperation and recombination. A+B. Use Tam interview. Show A+B recombination.
About 5 years later Sieber et al, completed a total synthesis of insulin by selective formation of the disulfides. Crystalline human insulin of full activity biological was obtained. Later the oxidation yields were partially improved.	Sieber, show his scheme.
The natural precursor of insulin, proinsulin, was discovered by Steiner. It contains a 31- residue connecting peptide between the C-terminus of the A chain and the N-terminus of the B chain. The	

synthesis was achieved by Yanaihara.	
A major step toward bringing peptide chemists together to discuss the advances and problems of the field was the organization of the first European Peptide Symposium. Joseph Rudinger, with the encourgement of Sorm, Director of the Czechoslovakian Academy of Science Institute, in Prague, invited all the leading peptide scientists in Europe to attend a symposium in 1958. Twelve people from Europe and the members of the Institute were in attendance. The discussions were necessarily centered on synthetic chemistry, which was the central concern at that time. The meeting was a great success; in large part because of the personality and experience of Rudinger himself. In June 2000 the 24th European Peptide Symposium was organized in Montpelleir by Martinez. Kalman Medzirhradszky has the distinction of being in attendance at all 24 EPS meetings.	Photo of Yanaihara Photo of Rudinger Maybe group photo's of first symposium and a later group another photo of symposium. Photo of Martinez Maybe part of a video of Martinez Video short clip
These biennial meetings have grown from a handful of participants to over 1000 years. In 1968 the American Peptide Symposium, was organized by Saul Lande and Boris Weinstein. The field is had also been growing rapidly in Japan and China, and both countries have also organized peptide symposia. The emphasis at all the meetings has gradually changed from a focus on chemical synthesis to include physical chemistry and has been extended to include biology, for that is where the ultimate application of peptides lies.	Photos of Lande, Weinstein, Group of one APS. Banner at 4th Chinese Symposium on Hsu Video

In the spring of 1962 the general principle of peptide synthesis on a solid support was reported at a Federation meeting, and the next year Merrifield published the first full paper. It constitutes one of the general strategies of peptide synthesis.	Photo of Merrifield Start video of RBM Show front page of 1963 paper
"I had been synthesizing some pentapeptide growth factors by standard methods and I could get them to work but this required a lot of trial and error and it was labor intensive and gave low yields. So I felt that there must be a better way. After a long time an idea for a new way to approach the problem occurred to me. The idea was to covalently attach the first residue of the peptide to a solid particle. The next step was to add the second amino acid to the first to form a protected dipeptide linked to the solid support. The terminal protecting group would be removed and the third amino acid would be activated and coupled to give a protected tripeptide still linked to the solid support. This process would be continued in a stepwise fashion until the desired peptide sequence was assembled. Finally, protecting groups would be removed and the target peptide cleaved from the solid support and purified. The main advantage seemed to be that at intermediate stages the peptide would be purified simply by thorough washing of the insoluble support containing attached peptide to remove excess of reagents and starting materials, rather than by isolation and chromatographic separation or crystallization at every step. Very high yields and minimal side reactions would be necessary.	Show SPPS scheme (shorten)

If that could be done it would simplify and accelerate the process. There were many variables to consider and they all had to come together at the same time for the procedures to be generally useful. They included finding the right support, which would be physically stable and chemically inert. The support required an appropriate functionality to enable the first attachment of an amino acid. The anchoring bond was critical because it had to be stable during the peptide synthesis, but removable at the conclusion of the synthesis.

Part of the strategy was to decide which terminus of the peptide should be anchored. From my limited experience with peptides, it was clear that it should be the carboxyl group. That would avoid racemization at subsequent coupling steps and it would be the soluble component that would be activated. An ester bond seemed most suitable. Therefore a reversible protecting group would be needed for the α amine which was compatible with the anchor.

Other variables included the coupling reaction and the solvent. I was not aware until later how critical it was to have a solvent that would solvate and swell the solid as much as possible.

My first choice for the support was cellulose because I knew it had been used for chromatographic separations of peptides and proteins. However, early experiments were not very successful. Other available polymers were examined, including

polyacrylamide, polyvinyl alcohol, polymethylmethacrylate, polyethylene chloride, polyethylenglycol, glass, sulfonated crosslinked polystyrene ion exchange resins, and underivatized styrene-divinyl benzene copolymer. The latter as small beads eventually proved to be best.

Many methods of derivatization were examined and chloromethylation proved most effective, so the first C-terminal amino acid could be anchored as a substituted benzyl ester. N^{α}-carbobenzyoxy was first used, but eventually N^{α}-Boc was determined to be preferable..

Active esters were tested for the coupling reaction, but dicyclohexylcarbodiimide proved to be much more satisfactory.

Initially I had envisioned a flow system for the synthesis using a pump, I loaded resin into a tube with a glass filter at the bottom and a solvent inlet at the top. This crude system did not work well at all, so I went to a batch process in a closed vessel with a glass filter at the bottom.

All of this took me 3 years before I had a practical method and could demonstrate it with the synthesis of a tetrapeptide. The nonapeptide, bradykinin was the next target and it worked very well. At this point I needed a name for the new process, and settled on "Solid Phase Peptide Synthesis".

A simple manual apparatus was assembled to carry out these syntheses. It consisted of a glass reaction vessel and a shaking

Photo of resin beads

A useful chemical scheme for SPPS

Manual shaker

device. Solvents were added manually and removed by vacuum filtration. A sintered glass funnel was used and John Stewart who was also a member of the Woolley lab sealed an upper tube and side arm to it for me. We used Lyman Craig's torch and oxygen tank. Later vessels were made by the RockefellerUniversity glass blower. My first shaker was an old dialysis rocker provided by Moses Kunitz. It was crude and too big and leaked oil by the quart. So John and I took the parts from a Craig rotary evaporator and adapted them to a much better shaker. Later shakers were made by the University instrument shop. John Stewart had been synthesizing bradykinin by conventional methods and had made three analogs during 1963. When I got the solid phase method working and had documented that it was effective for bradykinin, he immediately took it up and made 25 analogs the next year. In 1964 Garland Marshal came to the lab as my first graduate student. He synthesized the hypertensive octapeptide, angiotensin, which extended the method to include new amino acids. He was the first to realize that a fragment synthesis was possible for SPPS. SPPS caught on with some people, but not with everyone. I am pleased, however, that 39 years later the method is still being used	Photo of Woolley Video of John about Bradykinin Photo of John Stewart Photo or Video of Garland

and its use has actually expanded in recent years.	
Maury Manning joined the Woolley lab about that time from du Vigneauds laboratory and began the solid phase synthesis of vasopressin analogs. Over the next 30 years he made and tested over 2000 derivatives and found some very effective and selective antagonists of vasopressin.	Video of Maury About value of SPPS
I had predicted in my first publication that SPPS should lend itself to automation, especially because all reactions take place in a single vessel without the need to transfer the resin-bound peptide.	Photo of the machine.
In 1965 Dr. Woolley and I obtained a supplement to our NIH grant to build a peptide synthesizer.	More video shots from museum. Keep these on
I invited John Stewart to work with me on the project and he enthusiastically agreed.	
We bought a Tenor stepping drum electromechanical programmer and Nils Jernberg, the head of the University instrument shop, was able to machine two rotary selector valves out of Teflon. John had taken an electronics course in the army and assumed responsibility for the wiring layout of the machine and I focussed more on the plumbing system and the chemistry. In a short time we were able to assemble an automated peptide synthesizer that worked very well. It would automatically add six amino acids per day without the need for personal attention and this enabled us to take on the synthesis of more analogs and larger	John and me and machine Narrator should point out features of the instrument. The reaction vessel, solvent and reagent valve, amino acid valve with alternating rinse ports, a Beckman metering pump, a shaker, and the vacuum line. Then

molecules than before. Very soon many people got interested in the synthesizer and built instruments of their own. Arthur Robinson, a student at UCSD with Martin Kamen, spent about two months in my lab before our instrument was finished and when he returned home constructed what was probably must have been the second synthesizer. Victor Hruby, a post doc across the street with du Vigneaud, built a synthesizer just after he went to the university of Arizona in 1968. The Scoffone lab in Padova, Italy also constructed an instrument. Kay Brunfeld in Copenhagen built an early machine, which became the first commercial synthesizer, built in New York by Schwartz Biochemical. It had the ability to select any amino acid as needed. During this period 1968-72 more than a dozen instruments were built by people in Europe, Japan and the USA. The most successful instrument during this time was built by Beckman Instruments. Bob Hodges added a monitoring system for our instrument that automatically did a picrate titration after each coupling, using the assay developed by Balz Gisin. If significant amounts of uncoupled amino groups were still present, the machine would go on hold until the problem was corrected. Eventually this instrument laid the groundwork for a machine built by Applied Biosystems. All these were discontinuous batch machines like ours. But soon, continuous flow machines were	the programmer with pins that activate micro switches, which in turn operate the mechanical part and timers. One turn of the drum carries out all steps for adding one amino acid. Photo of Robinson Photo of Hruby or short video or his machine. Photo of Brunfeld Video of Rocci Photo of Beckman synthesizer with monitor Photo of Hodges

constructed. At first they encountered the same flow problems I had found in my first effort along these lines. But Sheppard and Atherton overcame this difficulty by putting their polyamide resin inside a rigid porous zeolite cage. They built a photometric monitoring system to estimate the extent of coupling and deprotection. It was based on the uptake of the Fmoc group. Measurement of loss of reagent is, of course, is inherently less sensitive than the measurement of unreacted amino groups. This instrument was marketed by Pharmacia and by Millipore and became quite popular. Bernd Gutte quickly put our new synthesizer to use. In 1968 he undertook the total synthesis of ribonuclease A. The synthesis of this 124-residue protein was successful, although the yield was low. The specific activity was increased to 80% by chromatographic separations, salting out and enzyme treatment. It had the correct amino acid composition, substrate specificity and antibody specificity. We also isolated key intermediates consisting of residues 26-124 and 21-124 Ribonuclease (S-Protein). They were biologically inactive but became active when combined non-covalently with synthetic or natural Ribonuclease S-Peptide. The work demonstrated that a real protein with true enzymatic activity could be assembled from simple amino acid derivatives, and confirmed Anfinsin's hypothesis that the primary	Photo of Sheppard or video Photo of Atherton Photo or diagram of machine Photo of Gutte. Maybe a video clip. 3X 2X RNase Structure Photo of Anfinsin

structure of a protein can determine its tertiary structure.

Analogs of the C-terminal end of RNase were synthesized by Gutte and by Hodges and combined with various shortened RNase, e.g. RNase (1-118), showed the role of different residues at this end of the molecule, much as Hofmann and Scoffone had done with the S-peptide at the N-terminus.

At this same time the Merck group, under, the direction of Denkewalter and Hirschman, using solution methods, synthesized RNase S-protein (21-124) and obtained biologicalactivity after itscombination with S-peptide. About 10 years later Yajima and Fujii also made RNase A by solution fragment synthesis. After purification of the crude product by affinity chromatography they obtained fully active enzyme.

Photo of Denkewalter, Hirshman, Gutte, Merrifield and model and machine.

Photo of Yajima

In general, early attempts to synthesize proteins by solution methods were labor intensive and not always very successful, mainly because of solubility problems, but in recent years some notable syntheses have been achieved. This is best exemplified by the developments in the Sakakibara laboratories at the Protein Institute in Osaka, Japan. They realized that the main obstacle was solubility of large protected intermediates and set out to devise better solvent mixtures. Although mixtures of DMF and dimethylsulfoxide were good solvents, the best were mixtures of trifluoroethanol and chloroform; hexafluoroisopropanol and chloroform; and phenol and chloroform. Bayer's magic solvent, is

Photo of Sakakibara & Kimura

Check on Bayer solvents in Wunschl Symposium.

also effective. With these solvents, virtually all of the several hundred medium and large peptides examined were soluble and could be satisfactorily handled in fragment syntheses. Examples of their successful protein syntheses include human midkine (121 residues) and the 238-residue precursor of the *Aequorea* green fluorescent protein. The final coupling of the latter involved fully protected fragments (1-116) and (117-238). The deprotected protein had the correct molecular weight, amino acid composition and chromatographic properties. The possibilities using the technology seem almost unlimited. There have been a number of lengthy stepwise syntheses using solid phase methods. Here the solubility of intermediates does not seem to be a problem, but peptide aggregation and the formation of deletion peptides remains a problem that has not been fully solved. Examples of proteins synthesized in this way include: HIV protease (99 residues) by Schneider and Kent; chaparonin 10 (100 res.) by Ball and Mascagni; migration inhibitor factor (115 res.) by Kaiser and Voelter; leukemia protease (126 res.) by Blake et al; intergin (126 res.) by Muir et al; and colony stimulating factor (127 res.) by Clark-Lewis, et al. During the past several years two major new developments have occurred. The simultaneous multiple synthesis of peptides	Bayer Photo Synth. scheme (see my fig. 12 for midkine) Fig. 12 for midkine Kent Mascagni Blake Muir Clark-Lewis Group Photo

was anticipated by a number of laboratories, but it remained for Geysen and Houghten to independently achieve this goal. Geysen used multiple plastic pins to build a large set of peptides that could be assayed in situ by fluorescent binding methods while Houghten used small porous plastic containing separate batches of the usual styrene-divinylbenzene beads. The individual peptides could be cleaved and assayed by the usual methods. These were important advances for those interested in defining antigenic epitopes or in screening structure activity relations.	Photo of Geysen And picture of pins Photo of Houghten Picture of tea bags Maybe a video
It was soon found that a very much larger array of peptides could be produced by combinatorial methods. Fodor et al used photolithography to produce all sequences of a set of amino acids in a given peptide, each with a defined address on a plate.	Picture of photolithography
Houghten expanded his method to make very large mixtures or libraries of peptides and used iterative methods to define the most active component.	
Furka designed a "divide, couple, and mix" strategy for the combinatorial synthesis on beads of a peptide mixture containing all possible combinations of a given size and a fixed number of amino acids. A hexapeptide containing all combinations of six amino acids gives rise to 4.66×10^4 peptides. A major insight occurred when Kit Lam realized that in such a mixture each individual bead contained only a single peptide sequence. An array of the peptide-beads could be screened by a suitable binding	Photo of Furka Diagram of the divide and mix idea Photo of Lam

assay. The most active beads were separated and sequenced by available very sensitive methods. The most active peptide could then by synthesized in larger amount by standard methods. At the present time, new methods for assay and identification of the active components of these combinatorial libraries are under study.	
The second major development during the past few years is now known as peptide ligation. It began with Wieland in 1951 when he showed that thiophenyl esters are highly activated. Some 30 years later Blake reacted, in aqueous solution, minimally protected peptide thio acids with silver ion and the amine of a second peptide through a second order bimolecular reaction to give the peptide amide bond. In 1992 Schnolzer and Kent reacted a fully unprotected peptide thio ester with an α- bromoacetyl peptide to give a larger peptide containing a thio ester at the ligation site. Using a complex template Kemp devised a "thio capture" followed by an intramolecular first order reaction to produce a peptide bond between two peptides at high effective concentration. Liu and Tam devised a similar "aldehyde capture" method.	Photo of Blake Photo of Kent Photo of Kemp
Wieland in 1953 showed that a cysteine thio ester would react with cysteine to give an internal amino acyl thiol ester which could undergo a cyclic intramolecular displacement by the α-amine of Cys and form a peptide bond.	Photo of Wieland. Show his reaction.

This seems to have gone unnoticed until 1994 when the Kent and Tam groups used this chemistry to effect a fragment coupling (ligation) between two fully unprotected peptides. They prepared by solid phase methods a peptide thio ester on a resin support. After HF cleavage, Kent converted the resulting peptide thioacid in a second step with 5,5[1] dithio-bis (2-nitro) benzoic acid to give the peptide α-CO thio nitrobenzoic acid. This intermediate reacts spontaneously with the thiol of an incoming cysteinyl peptide and quickly undergoes intramolecular rearrangement with the amine to form the amide bond.	Photo of Kent
Tam treated a resin-bound peptide thioester in one step with an alkyl phosphine and an alkyl thiol. The resulting alkyl thio ester of the first peptide then underwent the ligation reaction to give the final larger unprotected peptide product. This ligation method goes quickly with high yields and a minimum of side reactions. It proceeds selectively in aqueous solution even in the presence of other Cys or Lys residues. This technique has been successfully applied to the synthesis of a number of proteins.	Photo of Tam
With the purpose of producing much larger proteins, a semisynthetic procedure has been devised by Muir. It makes use of synthetic peptides, which are ligated, to large proteins produced by methods of molecular biology. The gene for the protein is cloned into a plasmid and followed in-phase with the coding sequences for an inteine-chitin-binding site. [An inteine is	Photo of Muir

a naturally occurring splicing element.] The coded proteins are expressed and undergo conversion to a thio ester of the N-terminal cysteine of the inteine. The product is then bound to a chitin column and the column is treated with thiophenol to give the free protein thiophenyl ester. It will react spontaneously with a small synthetic peptide to give the large ligated protein. Proteins as large as 600 residues have been produced in this way and no upper limit appears to be in sight. In a recent round table discussion by several leaders in the field some of the likely directions of research and potential findings were predicted. The next new discovery in this field cannot be predicted, but it would not be unreasonable to think that there will be another exciting development ahead. EXPAND	Show solar system. Pan over to show entry into black region (50:50) then pan to total black. The End.

Go through a list of people and include the most important ones.

Look through my slides and photographs.

Write for missing photographs.