

***PEPTIDE AND PROTEIN***

***SYNTHESIS:***

***Origin***

***And***

***Development***

**Final Version**

**Approved for Audio Recording**

**Version 3.0**

May 17, 2001

Jim Pfeiffer

Intersect Incorporated

VIDEO	AUDIO
<p>1</p> <p><b>PEPTIDE AND PROTEIN SYNTHESIS Origin and Development</b></p>	<p>Title:</p>
<p>2</p> <p>Reverse Video opening</p>	<p><b>"Flying Dutchman" music</b></p>
<p>3</p> <p>Reverse video continues</p>	<p><b>NARRATOR (Voice over pictures):</b> It was ten to the tenth B.C. when it all began.</p>
<p>4</p> <p>Reverse video dissolve to evolution footage</p>	<p>Matter and the elements were formed. galaxies, stars, and our Earth were formed. Four million years ago, life appeared.</p>

**VIDEO****AUDIO**

5

Freeze final frame and Dissolve	MUSIC under and out
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6

Photo man on moon photo space station	<b>NARRATOR (Voice over pictures):</b> In our lifetime, there have been remarkable advances in science and technology that have profoundly changed our world.
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7

Hubble telescope image. Earth. Submersible	We have looked to the ends of our universe and mapped the oceans.
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8

Einstein photo 69 Seaborg photo <b><i>Periodic Table</i></b>	We have advanced the laws of relativity and quantum theory and discovered additional elements and subatomic particles.
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**VIDEO****AUDIO**

12

Felix 12 3-D images?	We have synthesized large numbers of naturally occurring peptide hormones and have designed novel peptide structures that are effective therapeutics.
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13

Hold image above	We can synthesize thousands or even millions of peptides in a single experiment, test them for biological activity, and use this information for drug discovery.
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14

Hold image from above	In certain cases, we can produce multikilogram quantities of synthesized peptides. All of this flows from discoveries that are part of our history.
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**VIDEO****AUDIO**

15

<b>Founding Fathers</b>	<b>Title</b>
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16

<b><i>Visual?</i></b>  Fisher Photo	<b>NARRATOR (Voice over pictures):</b> None of this would be possible without the work of many people beginning exactly one hundred years ago, when the great organic chemist, Emil Fischer, synthesized the first peptide.
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17

Fisher photo 2	Fischer coined the term “peptide” and proposed the polyamide structure for proteins.
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German Paragraph and Fisher Photo	<b>Narrator (German Accent):</b> <i>“Whereas professional colleagues fear that a <u>rational</u> study of this class of compounds, because of their complicated structure and their highly inconvenient physical characteristics, would today still uncover insurmountable 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 methods be ascertained.”</i>
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**VIDEO****AUDIO**

19

Fisher Photo 3  Fisher Photo 4	<b>NARRATOR (Voice over pictures):</b> Of course, because no sequence was known at that time, Fischer could not synthesize a natural protein. Some believed that proteins were not single homogeneous compounds. But Fischer laid the groundwork for achievements that came sixty or seventy years later.
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20

Photo Curtius Aberhalden Leuchs  <b>2 Formulas (Page Five 5c.doc)</b>	Fischer and other scientists such as Theodor Curtius, Emil Aberhalden, and Hermann Leuchs, were critically handicapped. There were no reversible protecting groups for the alpha-amine.
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**VIDEO****AUDIO**

21

<b>PROTECTING GROUPS</b>	<b>Title</b>
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22

Bergmann  Zervas  Equation for carbobenzoxy deprotection 186	<b>NARRATOR (Voice over pictures):</b> Fittingly, it was one of Fischer's former students, Max Bergmann, with his associate, Leonidas Zervas, who solved the amino protecting group problem thirty years later. Their carbobenzoxy group can be removed by catalytic hydrogenolysis.  The discovery instantly transformed the field and is still used today.
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23

Fruton and Bergmann Together	In 1937, Joseph Fruton joined Bergmann and used the carbobenzoxy method to synthesize a large number of small free peptides. This allowed him to determine the substrate specificity of proteolytic enzymes.
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**VIDEO****AUDIO**

24

<i>H-Gly-Lys-Lcu . . .</i>	This was the first time synthetic peptides were used to solve an existing biochemical problem. It was a landmark achievement.
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25

Bodanszky  Strategy and tactics graphic Highlight each	Miklos Bodanszky contributed to the field by dividing peptide synthesis into “strategy and tactics”. Strategy describes synthesis design while tactics focuses on detailed chemical procedures.
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26

P-NH-C . . .	In spite of this new approach, important developments in reversible N alpha amine protection tactics were slow to emerge.
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27

Carpino photo 169	In 1957 Louis Carpino, using modern physical organic chemistry principles, proposed the tertiary butyl function for a urethane protecting group. It was a better leaving group than benzyl and could be removed in mild acid.
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28

Equation for Boc Removal 185 With name	This led to the development of the Boc-group.
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29

Structure and equation for Fmoc removal (drawing) 184 (labeled: "9-fluorenylmethoxy carbonyl")	The next major development in amine protection occurred in 1972, again by Carpino, who introduced the base-labile, acid-stable Fmoc group. The Fmoc group can be removed by beta-elimination with piperidine.
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**VIDEO****AUDIO**

30

Hans Meienhofer and Bob Sheppard side by side	In 1978 the Meienhofer group and the Sheppard group independently used Fmoc-protection in all steps of a long synthesis.
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31

Fmoc again	The orthogonality of this base labile group and the acid labile side chain tert-butyl group is an important advantage.
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32

<b>Felix Video</b> <b>VT1</b>	<b>Sound on Tape</b>
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33

?? Rich Barrany Patchornick <i>(is this the right visual?)</i>	<b>NARRATOR (Voice over pictures):</b> Other N alpha protecting groups have been introduced including those removable under neutral conditions by photolysis, thiolysis and solvolysis.
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**VIDEO****AUDIO**

34

<b>DEPROTECTION REAGENTS</b>	<b>Title</b>
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35

Photo Young du Vigneaud  I deleted a subordinate clause.	<b>NARRATOR (Voice over pictures):</b> Vincent du Vigneaud used sodium in liquid ammonia to remove benzyl groups instead of catalytic hydrogenolysis.
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36

<b>Felix Video NVTa</b>	<b>SOT</b>
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37

Photo Shumpei Sakakibara	In 1966, Shumpei Sakakibara made the important discovery that anhydrous liquid hydrogen fluoride was a safe and powerful cleavage reagent.
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**VIDEO****AUDIO**

38

Photo Shiro Akabori	Sakakibara was a student of the pioneering protein chemist Shiro Akabori.
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39

<b>Sakakibara Video VT-2</b>	<b>SOT</b>
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40

Tam and Heath photos side by side	<b>NARRATOR (Voice over pictures):</b> In 1982, James Tam and Bill Heath found a way to minimize side reactions with this strong anhydrous acid. They used dimethyl sulfide as a base to reduce acid strength, while retaining the ability to cleave esters, ethers and urethanes.
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**VIDEO****AUDIO**

41

<b>ACTIVATING REAGENTS</b>	<b>Title</b>
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42

Curtius Curtis Equation along with Rudinger equation 189 Rudinger Photo	<b>NARRATOR (Voice over pictures):</b> In 1884 Theodor Curtius was the first to report the activation of a protected amino acid. He used the azide to form the peptide bond. Joseph Rudinger improved this method in 1961
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43

Wieland Equation	During the rapid growth <b>period in</b> peptide synthesis in the 1950's, Theodor Wieland first introduced mixed anhydrides.
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44

Equation  <i>Meaning clear?</i>	The anhydride method was followed by his use of thiophenyl esters as coupling reagents.
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**VIDEO****AUDIO**

45

Schwyzer Bodanszky Together 167	Robert Schwyzer soon proposed cyanomethyl esters, and Miklos Bodanszky then introduced nitrophenyl esters.
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46

<b>Delete these visuals?</b> Pless Boissonas and Kupriszewski together  Weygand Wunsch Konig and Geiger together	Others have introduced a variety of additional activated esters.
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47

<b>Christian Beir Video VT-3</b>	<b>SOT</b>
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**VIDEO****AUDIO**

48

John Sheehan  DCC equation 182	<b>NARRATOR (Voice over pictures):</b> John Sheehan made one of the greatest advances in coupling reagents. He utilized dicyclohexylcarbodiimide to close the beta lactam in penicillin and extend its application to the formation of the peptide bond.
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49

Alexander Todd, George Kenner and Gobind Khorana together	Previously, Todd, Kenner and Khorana had used carbodiimides successfully for dinucleotide synthesis and had even made amide bonds but they did not apply it to peptides.  Murray Goodman tells the diimide story
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50

<b>Goodman video</b> <b>#6 VT-4</b>	<b>SOT</b>
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**VIDEO****AUDIO**

51

Dissolve out of final freeze frame of interview	<b>NARRATOR (Voice over pictures):</b> In recent years a several more new activating reagents have been developed.
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52

Bertrand Castro 181 BOP formula	Bertrand Castro introduced BOP, a phosphonium salt, as a very reactive coupling reagent.
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53

<b>Felix Video NVTb</b>	<b>SOT</b>
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54

Dourtoglou and Reinhard Knorr Uronium structure.	Dourtoglou, and later Knorr, reported other reagents that replaced phosphonium derivatives with uranium salts.
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55

N-Carboxy structure Leuchs 180	N-Carboxy anhydrides, or NCAs, were introduced by Hermann Leuchs and used to prepare poly alpha-amino acids.
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**VIDEO****AUDIO**

56

Robert Denkewalter and Ralph Hirschmann	Denkewalter and Hirschmann adapted NCAs to a stepwise procedure in aqueous solution under carefully controlled conditions.
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57

UNCA Structure	Goodman and his co-workers obtained urethane-protected N-carboxyanhydrides, or UNCAs, in stable, crystalline condition. They are very reactive, even in hindered couplings.
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58

<b>LANDMARK SYNTHESSES</b>	<b>Title</b>
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59

<i>Show structure?</i> Vincent du Vigneaud Formal (older)	<b>NARRATOR (Voice over pictures):</b> In 1953 the first landmark synthesis of a biologically active peptide occurred when du Vigneaud synthesized the nonapeptide, oxytocin.
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**VIDEO****AUDIO**

60

Charlotte Ressler  Panoyatis Katsoyannis	With associates Charlotte Ressler and Panoyatis Katsoyannis, du Vigneaud drew on the large array of chemical methods available at the time.
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61

<b>Maurice Manning</b>  <b>Video</b>  <b>VT-5</b>	
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62

<b>Victor Hruby</b>  <b>Video</b>  <b>VT-6</b>	
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63

<b><i>Sequence of Oxytocin? Structure</i></b>	<b>NARRATOR (Voice over pictures):</b> The oxytocin synthesis by duVigneaud was the forerunner of a flood of work on peptide hormones by peptide chemists.
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**VIDEO****AUDIO**

64

Rittel Robert Schwyzer 315 <b><i>3-d models of peptides?</i></b>	These included vasopressin, again by du Vigneaud in 1954, angiotensin by Rittel and Schwyzer in 1957 and alpha-MSH by Boissonas in 1958.
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65

<b>Eberle VT-7 (Video #7)</b>	<b>SOT</b>
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66

Klaus Hofmann 207 Ernesto Scoffone 316	<b>NARRATOR (Voice over pictures):</b> In the early 1960s synthetic fragments of the Ribonuclease S-peptide were reported independently by the Hofmann and Scoffone groups.
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67

Kenner Bodanszky Ondetti Wünsch (Panel)	Other notable synthetic achievements include gastrin by Kenner in 1965, secretin by Bodanszky and Ondetti in 1966 and glucagon by Wünsch in 1967.
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**VIDEO****AUDIO**

68

Beyerman Schwyzer. (Together)	By the mid 1960s larger peptides were synthesized such as A C T H one to twenty-four by Beyerman and by Schwyzer.
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69

Robert Schwyzer Peter Sieber (Together)  Sandor Bajusz, Kisfaludy, Kalman Medzirhadszky (Together)	A C T H one to thirty-nine was independently synthesized by both Schwyzer and Sieber and a team composed of the Bajusz, Kisfaludy and Medzirhadszky groups.
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70

<b>Medzirhadszky</b> <b>VT-8</b> (Video #7 )	SOT
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71

<b><i>3D of insulin?</i></b>	<b>NARRATOR (Voice over pictures):</b> The race to synthesize insulin was quite remarkable.
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**VIDEO****AUDIO**

72

Sanger Photo	This 51 residue, two chain peptide was first sequenced by Frederick Sanger in 1952.
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73

Panoyanis Katsoyannis	The Katsoyannis group in Pittsburgh first reported their synthesis in June of 1963, but their publication did not appear until 1964.
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74

Helmut Zahn 330	The Zahn group completed their synthesis in late 1963 and published their findings at the end of that same year.
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75

Yu Wang 227 Yu- Cang Du Kung	The Chinese group had also been reporting progress for several years but didn't publish their final synthesis of active insulin until 1965
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**VIDEO****AUDIO**

76

Primary structure of A+B Chains	All three groups used standard solution fragment strategies, but with somewhat different tactics.
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77

<b><i>PowerPoint slide</i></b>	The difficult step in all three cases was to combine the separate purified A and B chains to give the correct three disulfide bonds. This was the yield limiting step.
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78

<b>Zahn 1 VT-9</b>	<b>SOT</b>
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79

<b>Brandenburg 1 VT-10</b>	<b>SOT</b>
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80

<b>Zahn 2 VT-11</b>	<b>SOT</b>
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81

<b>Brandenburg 2 VT-12</b>	<b>SOT</b>
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82

<b>Zahn 3 VT-13</b>	<b>SOT</b>
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**VIDEO****AUDIO**

83

<b>Brandenburg 3 VT-14</b>	<b>SOT</b>
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84

<b>Zahn 4 VT-15</b>	<b>SOT</b>
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85

<b>Brandenburg 4 VT-16</b>	<b>SOT</b>
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86

<b>Zahn 5 VT-17</b>	<b>SOT</b>
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87

<b>Brandenburg 5 VT-18</b>	<b>SOT</b>
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88

<b>Du 1 VT-19</b>	<b>SOT</b>
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89

<b>Ye 1 VT-20</b>	<b>SOT</b>
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90

<b>Du 2 VT-21</b>	<b>SOT</b>
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**VIDEO****AUDIO**

91

Freeze of Du from interview above. Dissolve to Du photo	<b>NARRATOR (Voice over pictures):</b> Du carried out 600 experiments to find the best conditions to refold native A and B chains. One of the keys to success was operating at four degrees centigrade.
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92

Marglin photo dissolve to freeze first frame of tape.	In 1966 Arnold Marglin showed that the A and B chains could be synthesized by solid phase methods and, when oxidized by Du's method, gave rise to insulin activity similar to that from the other syntheses.
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93

<b>Marglin Video</b> <b>VT-22</b>	<b>SOT</b>
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**VIDEO****AUDIO**

94

Sieber <b><i>Power Point</i></b>	<b>NARRATOR (Voice over pictures):</b> A few years later Sieber's group completed a total synthesis of insulin by selective formation of three disulfides. This produced crystalline human insulin of full biological activity.
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95

<b>Marglin 10</b> <b>Clip 2 VT-23</b>	<b>SOT</b>
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96

<b><i>2-chain-xxx-b-chain</i></b>	<b>NARRATOR (Voice over pictures):</b> Marglin believed that insulin is synthesized in vivo as a single chain and, after folding and disulfide formation, was cleaved into two chains.
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97

Steiner Formula Proinsulin (find in Biochem)	Donald Steiner discovered Proinsulin, a natural substance and the precursor of insulin.
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**VIDEO****AUDIO**

98

<b><i>Hold from above</i></b>	This discovery demonstrated that insulin was, in fact, synthesized as a single chain.
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99

Yanaihara	The Yanaihara group in Japan achieved a synthesis of Proinsulin..
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100

<b><i>Visual??</i></b>	This showed that insulin was folded and oxidized in high yield while in a single chain
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101

DiMarchi Video	
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**VIDEO****AUDIO**

102

<b>PEPTIDE SYMPOSIA</b>	<b>Title</b>
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103

Joseph Rudinger Frantisek Sorm	<b>NARRATOR (Voice over pictures):</b> Joseph Rudinger in 1958, with the encouragement of Sorm, Director of the Institute of Chemistry in Prague, organized the first European Peptide Symposium. Rudinger extended invitations to all leading peptide scientists in Europe.
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104

<b>Photo???</b> <b>Wives?</b>	<b>Twelve peptide chemists</b> attended to discuss the advancements and challenges within the field.
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105

Hold photos	The symposium naturally centered on synthetic chemistry, the central concern at the time.
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**VIDEO****AUDIO**

106

Literature from the 26 <sup>th</sup> symposium Martinez photo	In September, 2000, the twenty-sixth Symposium, organized by Jean Martinez, was held in Montpellier.
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107

Large group photo Holland France Spain	These biennial meetings now host over 1000 scientists.
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108

Saul Lande and Boris Weinstein photos Symbol of amer symposium?	In 1968 the American Peptide Symposium, was founded by Saul Lande and Boris Weinstein.
<b>2<sup>nd</sup> International Logo</b>	In the Early 1990s Japan, China and Australia each organized peptide societies These societies also organized periodic international peptide symposia.

**VIDEO****AUDIO**

109

<b>Solid Phase Peptide Synthesis</b>	<b>Title</b>
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110

<b>Merrifield video VT-24</b>	<b>SOT</b>
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111

Other covering video: SPPS Scheme	
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112

Photo resin beads Manual shaker and vessel	
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113

Photo Woolley Photo Stewart	
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VIDEO	AUDIO
<p>114</p> <p><b>Stewart Video</b> <b>VT-25</b></p>	<p><b>SOT</b></p>
<p>115</p> <p><b>Merrifield Video 2</b> <b>VT-26</b> <b>Best vessels and shakers</b> <b>Photo Marshall</b></p>	<p><b>SOT</b></p>
<p>116</p> <p><b>Marshall Video</b> <b>VT-27</b></p>	<p><b>SOT</b></p>
<p>117</p> <p>Photo Marshall</p>	<p><b>NARRATOR (Voice over pictures):</b> Garland Marshall was the Merrifield Laboratory's first graduate student.</p>
<p>118</p> <p>Manning photo</p>	<p>Maurice Manning was among the many peptide chemists who soon used the new synthetic method.</p>

**VIDEO****AUDIO**

119

**Manning 2 video  
VT-28****SOT**

120

**Hruby 2 VT-29****SOT**

121

**Automation****Title**

122

**Merrifield 3  
VT-30****SOT****Photo John  
Stewart and RBM**

123

**Stewart 2 VT-31****SOT**

124

**Merrifield 4  
VT-32****SOT****Machine photo**

**VIDEO****AUDIO**

125

<b>Merrifield 5</b> <b>VT-33</b> <b>Photo Robinson</b>	<b>SOT</b>
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126

<b>Hruby 3</b> <b>VT-34</b>	<b>SOT</b>
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127

Brunfeld  <i><b>Photo of machine?</b></i>	<b>NARRATOR (Voice over pictures):</b> In Copenhagen, Kai Brunfeld built an early machine that became the first commercial synthesizer. Schwarz Biochemical Company in New York built the prototype.
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128

<i><b>Photo??</b></i>	The Beckman Synthesizer was the most successful commercial machine among the many built between 1968 and 1972.
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**VIDEO****AUDIO**

129

<p>Photo Hodges (Informal) Balz Gisin.</p>	<p>Bob Hodges, while in Merrifield's lab, added a monitoring system to their Beckman Synthesizer. The monitor automatically performed a picrate titration after each coupling using the assay developed by Balz Gisin.</p>
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130

<p>Sheppard Photo Diagram of Flow machine</p>	<p>Sheppard and Atherton developed a continuous flow instrument by putting their resin inside a rigid porous zeolite cage.</p>
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131

<p><b>Synthesis of proteins</b></p>	<p><b>Title</b></p>
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132

<p>3x RNase (slide)</p>	<p><b>NARRATOR (Voice over pictures):</b> With the availability of the new solid phase method and the automated synthesizer, it was possible to undertake the synthesis of much larger and more complicated molecules</p>
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## VIDEO

## AUDIO

133

Gutte	When Bernd Gutte joined the Merrifield group, he quickly put the new technique to use. In 1968, he undertook the total synthesis of ribonuclease A.
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134

2x Ribonuclease	Although the yield was low, he successfully synthesized this one hundred and twenty four residue protein.
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135

3x Ribonuclease	His Ribonuclease A had a specific activity of eighty percent. It had the correct amino acid composition, substrate specificity and antibody specificity.
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136

<p><b>Visual??</b></p> <p>Christian Anfinsen</p>	This work showed that a real protein with true enzymatic activity could be assembled from simple amino acid derivatives. It confirmed Anfinsen's hypothesis that the primary structure of a protein can determine its tertiary structure.
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	VIDEO	AUDIO
137	<i>Fischer</i>	With this achievement, Emil Fischer's dream of protein synthesis was finally realized.
138	<b>Gutte 1 Video VT-35</b>	<b>SOT</b>
139	Group Photo Both Teams	At this same time, under Robert Denkwalter, the exploratory research group of Merck, Sharp and Dohme, undertook the synthesis of the ribonucleaase S-protein by using fragment synthesis in solution.  After combination with S-peptide they obtained some enzymatic activity.
140	<b>Veber 1 video VT-36</b>	<b>SOT</b>

VIDEO	AUDIO
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141

<p>Hiruaki, Yajima and Fujii</p>	<p><b>NARRATOR (Voice over pictures):</b> About ten years later Yajima and Fujii also prepared Ribonuclease A by solution fragment synthesis.</p> <p>They obtained a fully active enzyme after purifying the crude product by affinity chromatography.</p>
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142

<p>Gutte</p>	<p>In 1974 Gutte undertook a de novo synthesis of a shortened protein with ribonuclease specificity.</p>
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143

<p><b>Gutte 2 Video</b> <b>VT-37</b></p>	<p><b>SOT</b></p>
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144

145

<p><i>Graphic:</i> Inhibin 96 residues Yamashiro, Blake, Li</p>	<p>Examples of proteins synthesized by the solid phase method include:  Inhibin</p>
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146

<p>HIV Protease 99 Residues Nutt Schneider and Kent</p>	<p>HIV protease;</p>
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**VIDEO****AUDIO**

147

Chaparonin 100 Residues Ball and Mascagni	Chaparonin 10
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148

Migration inhibitor factor 115 residues E. Kaiser and Voelter	Migration inhibitor factor
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149

Lukemia protease 126 residues Blake	Lukemia protease
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150

Integrin 126 residues Muir	Integrin;
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151

Colony stimulating factor 127 residues Clark-Lewis	And Colony stimulating factor
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**VIDEO**

**AUDIO**

152

Sakakibara	Sakakibara realized that the main obstacle to the synthesis of proteins by solution methods was the insolubility of large protected intermediates. He set out to devise better solvent mixtures.
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153

CHCl <sub>3</sub> . . .	The best solvent mixture used chloroform with trifluoroethanol hexafluoroisopropanol or phenol
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154

<b>Visual??</b>	Virtually all of the several hundred medium and large peptides examined were now soluble.
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155

Midkine (Scheme) 121 Residues  Aequorea green 238 Residues	Examples of their successful protein synthesis include human midkine and the 238 residue precursor of the Green Fluorescent Protein
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	<b>VIDEO</b>	<b>AUDIO</b>
156	<b>Multiple syntheses</b>	<b>Title</b>
157	Gaysen and Houghten	<b>NARRATOR (Voice over pictures):</b> The simultaneous multiple synthesis of peptides was anticipated by a number of laboratories, but it remained for Gaysen and Houghten to independently achieve this goal.
158	<b>Tea Bag Photo (video?)</b>	Geysen used multiple plastic pins to build a large set of peptides, while Houghten used small porous plastic bags
159	<b>Houghten 1 Video VT-38</b>	<b>SOT</b>
160	Furka  Diagram of Divide couple and mix	Furka designed a “divide, couple, and mix” strategy for the combinational synthesis on beads of a peptide mixture containing all possible combinations of a given size and a fixed number of amino acids.

	VIDEO	AUDIO
161	$7^6 = 117,649$	A hexapeptide containing all combinations of just seven amino acids gives rise to over one hundred thousand peptides.
162	Kit Lam Photo	Lam contributed a major insight when he realized that in such a mixture each individual bead contained multiple copies of only a single peptide sequence.
163	<b><i>Lam Photo</i></b>	An array of peptide beads could be screened by a suitable binding assay
164	<b><i>Visual?</i></b>	The fluorescent beads are separated, micro sequenced and synthesized.
165	<b>Peptide Ligation</b>	<b>Title</b>
166	Kemp Photo	<b>NARRATOR (Voice over pictures):</b> Dan Kemp devised a “thiol capture” followed by an intra-molecular first order reaction to produce a link two peptides at high effective concentration.

## VIDEO

## AUDIO

167

<b>Wieland</b> <b>Wieland's</b> <b>reaction</b>	In the early fifties Wieland's work showing that thio acids and thiophenyl esters are highly activated was the forerunner of the field now called peptide ligation
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168

James Blake	Some thirty years later, Blake reacted a minimally protected peptide thio acid with silver ion and a second peptide to give a peptide amide bond
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169

Kent and Tam	In 1994 both the Kent and Tam groups independently used thio ester chemistry to effect a fragment coupling between two fully <b>unprotected</b> peptides.
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170

Tam Equation	The method departs from the concept introduced by Bergmann sixty years earlier because it is conducted in water, without an added coupling reagent, and does not use protecting groups even for cysteinyl or lysyl residues
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	VIDEO	AUDIO
171	<b>Visual?</b>	With the purpose of producing much larger proteins, a semisynthetic procedure has been devised independently at Rockefeller University by Muir and Cole and at New England Biolabs by Evans, Benner and Xu.
172	<b>Visual??</b>	The process uses synthetic peptides and large proteins produced by molecular biological procedures. These peptides are then ligated to the proteins using a method called intein-mediated protein ligation
173	<b>Visual??</b>	Proteins as large as 600 residues have been produced in this way. Currently, there is no upper limit in sight.
174	<b>Visual??</b>	The value of peptides as drugs is still an open question that generates vigorous debate.
175	<b>Eberle 2Video #3 VT-39</b>	<b>SOT</b>

	<b>VIDEO</b>	<b>AUDIO</b>
176	<b>Kubiak1 Video VT-40</b>	<b>SOT</b>
177	<b>The Future</b>	<b>Title</b>
178	Photo of roundtable group	<b>NARRATOR (Voice over pictures):</b> Recently, during a roundtable discussion, several leaders in the field predicted some of the likely directions of research and potential findings in peptide science.
179	Spatola photo	Spatola Audio
180	Goodman photo	Murray Goodman Audio
181	Veber Photo	Dan Veber Audio
182	Hodges Photo	Robert Hodges Audio
183	Muir Photo	Tom Muir Audio
184	Deber Photo	Deber Audio
185	Hurby Photo	Hruby Audio

VIDEO	AUDIO
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<p>Merrifield Photo Reverse Video “THE END”</p>	<p><b>MERRIFIELD (Voice over pictures):</b>  It is clear that the peptide field is alive  and well. We can't predict the next new  discoveries in this field, but we can all  be sure that many exciting  developments lie ahead.</p> <p><b>Music Up and Under</b></p>
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<p>Credits</p>	<p>Music Under: Peptide dreams</p>
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