Additional Narrative for the Narrator, Additional Photos and Visuals and Additional Material from Source Tapes (pages follow the expanded version II E from 9/7/01; Frames follow Jim Pfeiffer's Version 1.0, from May 14). Bold typing indicates additional narration.

(10/02 version). In the nitronuclease S frame, add pictures of Hofmann & Scoffone p. 15

1. Page 17, (or Frame 74-75) to be replaced with new photos of Chinese scientists that directed the insulin synthesis. The collage will consist of 3 to 6 new photos. The photos will be scanned at Rockefeller and sent to Jim as jpg or other files (as instructed by Jim).

- Bajusz and Medzihradsky panel Bruckner during Medzihradsky's video

2. Page 19, after Branderberg's last video and just before Du's interview (immediately after frames 74-75 (or frame 91?), here is the photo of Du that we have:

Yu Cong Du, one of the original young members of the insulin group in Shanghai, summarizes the early work of the Chinese ~~insulin~~ group. photo re Chinese detectives

3. Page 20, (around Frame 100), during Marglin tape insert the scheme of the synthetic strategy for the single-chain insulin synthesis discussed by Marglin (Art has made this graphic)

4. Page 21 after Yanihara's photo and text:" the work showed that ….in a single chain", new Narration (after Frame 104-105):

The insulin story was a landmark chapter in the history of peptide chemistry. Here is the repeated visual of the insulin noodle structure from page 16 (frame 70). While the visual is on:
The research on insulin has continued up to present day, followed by DiMarchi's interview.

5. Page 22: After the title of SPPS (Frame 114-115): FORMAL

In the spring of 1962 the general principle of carrying out peptide synthesis on a solid support was reported by Bruce Merrifield at a Federation Meeting, accompanying visual is a photo of Merrifield ~~in lab coat~~ (296, I am not certain whether this photo is scanned in Jim's computer but apparently is in the folder that Jim has), and is followed by the beginning of RBM interview as is in the San Diego version. This is followed by additional interview from the source tapes. Cecille has the exact order of RBM interview (and times on the VHS) that needs to be edited from the RBM source tapes. Additional narration (see pages 5-6 of this document) is also here. Estimated time: around 3.5-4min.

6. Page 26, after Stewart interview, …"from 3 to 25 in one year" (The frame here doesn't follow Version 1.0, but can be easily identified in the San Diego version)

In recent years Stewart has made effective antagonists of bradykinin that may become important drugs. freeze + fade

7. Page 26, last paragraph after Stewart interview: **In 1964 Garland Marshall joined Merrifield laboratory as his first graduate student.** (Garland's photo as visual, which is already in the San Diego version). **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 the Solid Phase Peptide Method. And he felt strongly about the generality of the solid phase synthesis principle.** Followed by Garland's interview as is in the San Diego version, but needs to be moved in this place. No additional editing from the source tape is required.

8. Page 27, before Manning's interview (Frame 125-126):
~~Shortly after 1964 other peptide chemists began to use the new synthetic method. Maurice Manning came to Rockefeller University from the duVigneu laboratory~~ (this may already be in the San Diego narration).

9. Page 29, frame 132-133: Edit from source tapes part of Merrifield's interview about other home made peptide synthesizers. While RBM talks show photo of Robinson (needs to be scanned, probably at RU and send to Jim), estimated time 0.5-1min

10. Page 29, RBM source tapes continues and talks about Victor Hruby. (Frame 133-134), edit from Hruby source tape about 50-60 sec.

11. Page 30, after the title: Early Commercial Instruments, first frame is a fragment from RBM interview about the many commercial instruments that were built in Europe and Japan (needs to be edited from source tape about 20 sec, followed by the present narration (frame 134) that I inserted in July when I worked with Jim. Then as continuation of the existing narrative:

Bob Hodges while in Merrifield laboratory added a monitoring system for their instrument that automatically did a picrate titration after each coupling, using the assay developed by Baltz Gisin. (need to insert photos of Hodges and 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 the originals** (Visual here from files at Jim's).

Soon, continuous flow machines were constructed. Initially they had the same problems that Bruce Merrifield encountered due to resin compression. But Shepard and Atherton overcome 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 or release of the Fmoc group. Measurement of loss of reagents, 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 (visual here from Art's files).

A segment from RBM interview about more modern instruments and robots follows and concludes this section (needs to be edited from source tape, about 20 sec).

12. Page 33, frame 161: During Veber interview insert (i) a photo of Ralph Hirshman (photo will be scanned at RU and send to Jim) and (ii) a scheme for the Merck synthesis of Rnase (Art made this and is presumably in Jim's computer).

12. A. Towards the end of Veber's segment insert a photo from NY Times front page article about RNase synthesis (Jim has the photo in his folders).

13. Page 35 (after frame 162), after Yajima's RNase synthesis:

Solid Phase approach enabled more detailed studies of the enzymatic properties of Ribonuclease A. Berndt Gutte, Michael Lin, and later Bob Hodges showed that the carboxyl terminal synthetic tetradecapeptide could be used to activate shortened Ribonuclease 1 to 118. They studied the role of different residues at the carboxyl terminal end of the ribonuclease, much as Hoffman and Scoffone had done with the S-peptide at the amino terminus. They also constructed the first non-covalently bound 3-component protein consisting of segments from Ribonuclease A sequence spanning amino acids 1 to 20, 21 to 118 and 111 to 124, which had good enzymatic activity (Visual(s) here from files at Jim's).

14.A. During Gutte's interview insert a visual showing the structure of the *de novo enzyme*. This was made at RU Media service in power point. Needs to be transferred to Jim's computer.

14.B. Page 34, after Gutte's interview:

This is considered to be the first *de novo synthesis* of designed protein.

15. Section on Multiple Synthesis starts with the existing narration and not with RBM and Marshall interviews. Marshall interview is moved to page 26 (see above under 7) and Merrifield segment to page 31 (see under 21)

16. Page 36, Frame 179: Insert a photo of Geysen together with Houghten. I sent it to Jim by e-mail.

17. Page 36 (around frame 182), after Houghten interview and before Narration about Furka below:

Fodor used photolithography to produce all sequences of a set of amino acids in a given peptide, each with a defined address on a plate (RBM has a slide to be used as visual to accompany this narration; the slide is in Jim's studio. Need to check whether slide can be used or we will need to make a photo).

18. Page 36, existing narrative is completely replaced with the following:
Furka reported in 1990 the design of "divide, couple, and mix" strategy for the combinatorial synthesis on beads of a mixture containing all possible combinations of a given size and fixed number of amino acids. (Furka photo here as in San Diego version). **At about the same time Kit Lam** (photo from the San Diego version) **independently devised a similar strategy to solve the problem of making combinatorial library containing equimolar amounts of peptides.** (Followed by 1st Lam interview, about 2-2.5 min) After this interview go back to the existing narration at "A major insight occurred...." Followed the 2nd part of the interview (about 1-min) and back to the existing narrative on page 35, frames 186-188.

19. Page 38, Frame 192, insert a photo of Blake (I have it needs to be scanned).

20. Page 38; still not clear whether we need additional narration. Perhaps a connecting or introductory sentence. It depends on the sections edited from the Tam interview (estimate: about 1.5-2-min).

21. Page 39. At the end of this section goes RBM interview about the SPPS being a goldmine for organic chemists. This section is moved directly from the beginning of Multiple Synthesis. No editing from source tape is required. (Suggestion: I think that before the start of the RBM video need to have a connecting sentence, before RBM tape. Need to see the San Diego version to determine whether the connecting sentence is needed). At the end of the segment:

It took 25 years before major developments along these lines in organic synthesis were accomplished. An early example is benzodiazopine library (RBM has a slide as a visual to accompany the narration. Art may have to make a graph of the scheme that is shown on the slide). **In recent years K.C. Nicolau has applied the solid phase ~~synthesis~~ approach to the synthesis of more complex organic molecules** (as a visual perhaps the title from Nicolau's article? Or keep the benzodazopine graph still on). **He points out:** need a quote from his paper from the Merrifield symposium. Art found several quotations from the Nicolau paper that can be used). The segment will be on the screen as a visual, while the Narrator is reading the text.

22. Page 39, in the section "Prospects for Peptides in the Pharmaceutical Industry" before Eberle's interview:

Eberle expressed the following opinion.

The current situation has been evaluated by Teresa Kubiak.

23. In Future Section: Page 40

According to Arno Spatola:

Murray Goodman comments on the following:

Page 41:

According to Daniel Veber:

Charles Deber extends these ideas:

The importance of physical chemical methods was extended by Robert Hodges.

Page 42,

But Robin Offord points out

Need to insert Offord's audio

Tom Muir continues along these lines

Page 43,

As the last speaker on the panel, Victor Hruby, offers the final summary of the future of peptide science.

Additional narration by Bruce Merrifield:

1. Around page 24, as a narration to the new SPPS scheme that Art made:

The RBM narration will start after the segment that is already in his video: ...used carbobenzoxy group". While the new RBM narration is on the new SPPS scheme (that Art made) is on the screen. **It was too acid-stable and caused side-reactions. Then I went to the new Boc group, because it could be removed under mild acidic conditions.**

The activating reagent was also very important. Active esters, mixed anhydrides and azides were not satisfactory. The best activating reagent was dicyclohexylcarbodiimide. It gave rapid reactions that went in very high yield.

To synthesize a longer peptide, the sequence of deprotection, neutralization and coupling was repeated over and over for each amino acid. Purification at every step was by rapid and simple thorough washing to remove excess reagents and byproducts.

The last step was cleavage of the peptide from the solid support. Hydrogen bromide (HBr) and, later, hydrogen fluoride (HF) were the best strong-acid reagents.

A final selective purification procedure was then necessary.

2. **At this point I needed a name for the new process and settled on " Solid Phase Peptide Synthesis".** (Here RBM photo with the manual shaker is a visual, it is already in the San Diego version, it just needs to be moved to a different frame).

3. Page 24, bottom. **Initially, I had envisioned a flow system for the synthesis using a pump. I loaded resin into a tube with glass filter at the bottom and a solvent inlet at the top. This was a crude system and did not work well at all** (up to this point visual of the flow system, it has been made in RU Media Service) **I went to a batch process in a closed vessel with a glass filter at the bottom and a rocker to mix reagents.** (Visual is a photo of the manual shaker that is in Jim's files and perhaps in his computer).

Changes in the Credits:

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The Planning Committee is grateful to all who participated in personal video interviews. These tapes have been archived with the American Peptide Society.