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Under the influence of phi and psi‡

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When I came to decide on my (MM) postdoctoral work, two names were on my list: Murray Goodman and Paul J. Flory, both outstanding scientists and both in sunny California! PJF welcomed me first, so I made my decision based on getting his response a few days earlier than Murray's warm letter of acceptance. Ever since, whenever I met Murray Goodman, I wondered what path my research would have taken if I had joined his group.

Still, close links to his research were built. And it all started, when I replaced my PhD supervisor Professor Ernst Bayer, at the 4th APS meeting in New York (1975). Immediately after my lecture on the 'Liquid-Phase-Method for Peptide Synthesis', Claudio Toniolo approached me asking for collaboration on PEG-bound oligopeptides. This was the start of an extremely fruitful, joint, research on the onset of helical and notably of β-sheet forming peptides, making use of the solubilizing effect of PEG [1]. We were highly delighted and honored to see Murray using 'our liquid-phase-method' for studying conformational preferences of PEG-peptides by CD and NMR, culminating in a common publication [2].

Most notably, in extending the pioneering work of Murray Goodman and Claudio Toniolo on the critical main-chain length for helix- and β-sheet formation (see C. Toniolo's article in this issue), we could delineate the impact of conformational transitions upon physicochemical properties such as solubility and reaction kinetics during chain elongation (Figure 1). The β-sheet forming sequences were identified later as 'difficult sequences' [4,5].

For the discovery of the close correlation between β-sheet formation, self-aggregation and insolubility, one of the authors (MM) received the 'Max-Bergmann Medaille' on 'Die Bedeutung der Konformation für die Synthese höherer Peptide' in 1982. In 1991, Murray Goodman obtained this same award for his outstanding work on 'Urethan geschützte Aminosäure N-Carboxyanhydride und ihre Bedeutung für die Peptidsynthese'. The finding that conformational transitions of the type random coil/α-helix to β-sheet were at the origin of fundamental problems in solution-phase and SPPS brought us to the concept of pseudo-prolines (ΨPro) used as a structure disrupting methodology in peptide synthesis [6].

Based on the observation that the insertion of a Pro residue results in the disruption of peptide secondary structures [7], we introduced ΨPro building blocks (commercially available) exhibiting a *cis*-bond preceding the ΨPro moiety as a reversible protection technique for Ser, Thr or Cys (Figure 2).

Murray was enthusiastic to see his pioneering work on thiazolidine [8] derivatives being used in new applications! Even more so, as the ΨPro concept could be extended as a tool to elucidate bioactive conformations of Pro-containing peptides, in prodrug design such as explored for *c*yclosporin C derivatives. ΨPro residues also turned out to be inducers of β-turns containing *cis*-amide bonds and useful tools in peptide cyclization [9].

Our systematic studies on amphiphilic peptides [10] triggered the idea of using templates for the *de novo* design of proteins exhibiting tailor-made structural and functional properties (template-assembled synthetic proteins, TASP) [11–14]. In covalently attaching peptide fragments with a high propensity for secondary structure formation onto topological templates, we aimed to bypass the notorious protein-folding problem of linear peptide chains in the design of tertiary structures. As topological templates, cyclic peptides, termed RAFT molecules [15], proved to be especially versatile, due to their potential for orthogonal protection. When chemoselective ligation methods were discovered in peptide synthesis, Dawson and Kent [16] surprised us in preparing a prototype 4α-helix TASP much more efficiently compared with our previous synthetic methodologies. Subsequently, the full potential of the TASP approach became apparent showing that TASP molecules can be used as functional mimetics of native proteins (Figure 3) [17–20]. This was not the whole story on template-assembled proteins. It was our ambition to introduce the TASP approach to San Diego's major peptide laboratories. First, one of the authors (GT) succeeded in becoming a member of the

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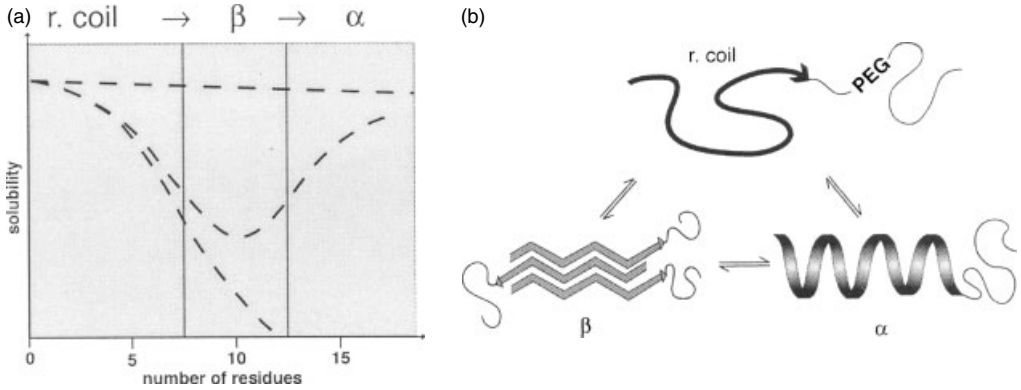

Figure 1 Impact of conformation on solubility in peptide synthesis (a); PEG as solubilizing group for conformational studies (b) (ref. [3]).

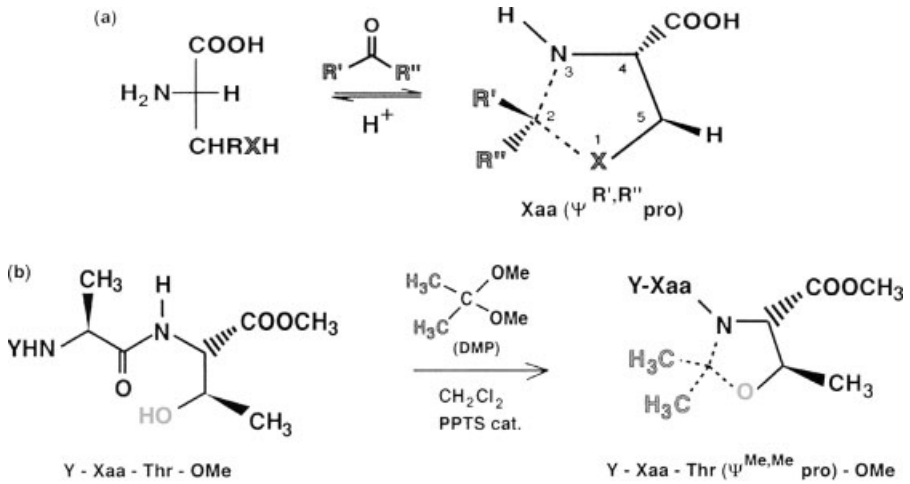

Figure 2 Pseudo-prolines (ΨPro) as a solubilizing, structure-disrupting protection technique in peptide synthesis (ref. [6]). PPTS is 3-(4-phenyl-2-pyridyl)-5-phenyl-1,2,4-triazine disulfonic acid, disodium salt.

Goodman scientific family by spending 6 months in his laboratory synthesizing a two-domain TASP molecule and at the same time learning to appreciate apple juice and potato chips during the late Friday afternoon group seminars. Later, GT joined Jean Rivier's laboratory at the Salk Institute synthesizing a 4-helix bundle TASP containing two different helices and going through the ordeal of extensive HPLC purification [21].

Simply continuing on model TASP molecules was not Murray Goodman's philosophy, rather he explored some fundamental properties of triple helix formation of collagen by making use of solid-phase segment condensation in combination with the covalent template attachment on the resin [22,23]. Unfortunately, he could not finalize this story, but his results on collagen models will undoubtedly stimulate further work.

How can we wrap up the decades of Murray's impact on our research projects better than projecting it to the future? Conformational studies of oligopeptides, critical main-chain length of secondary structure formation, conformational transitions of peptides: Is it just fundamental research, having a touch of 'l'art pour l'art'?

When we introduced 'switch-peptides' in the early 1990s for studying conformational transitions in solution [24,25], nobody took much notice of it, apart from the 'folding freaks', of course. However, by the time of the discovery of the impact of structural changes in degenerative diseases, conformational transitions, notably of the type α-helix to β-sheet as a key step in amyloid fibril formation, moved to the center of interest. In exploring new ways to induce such transitions by enzyme-triggered O → N- acyl migrations, we succeeded in elaborating a novel concept for studying structural changes relevant in degenerative diseases [26] (Figure 4), relying on our motto 'Nature's rules and chemists' tools' [11–14], as well as on Murray Goodman's and Claudio Toniolo's pioneering conformational studies on oligopeptides.

When we presented the concept of switch-peptides as a tool for the study of early events in peptide self-assembly and folding at the 28th EPS in Prague we wondered: How would Murray Goodman comment on this? Our reference has been lost!

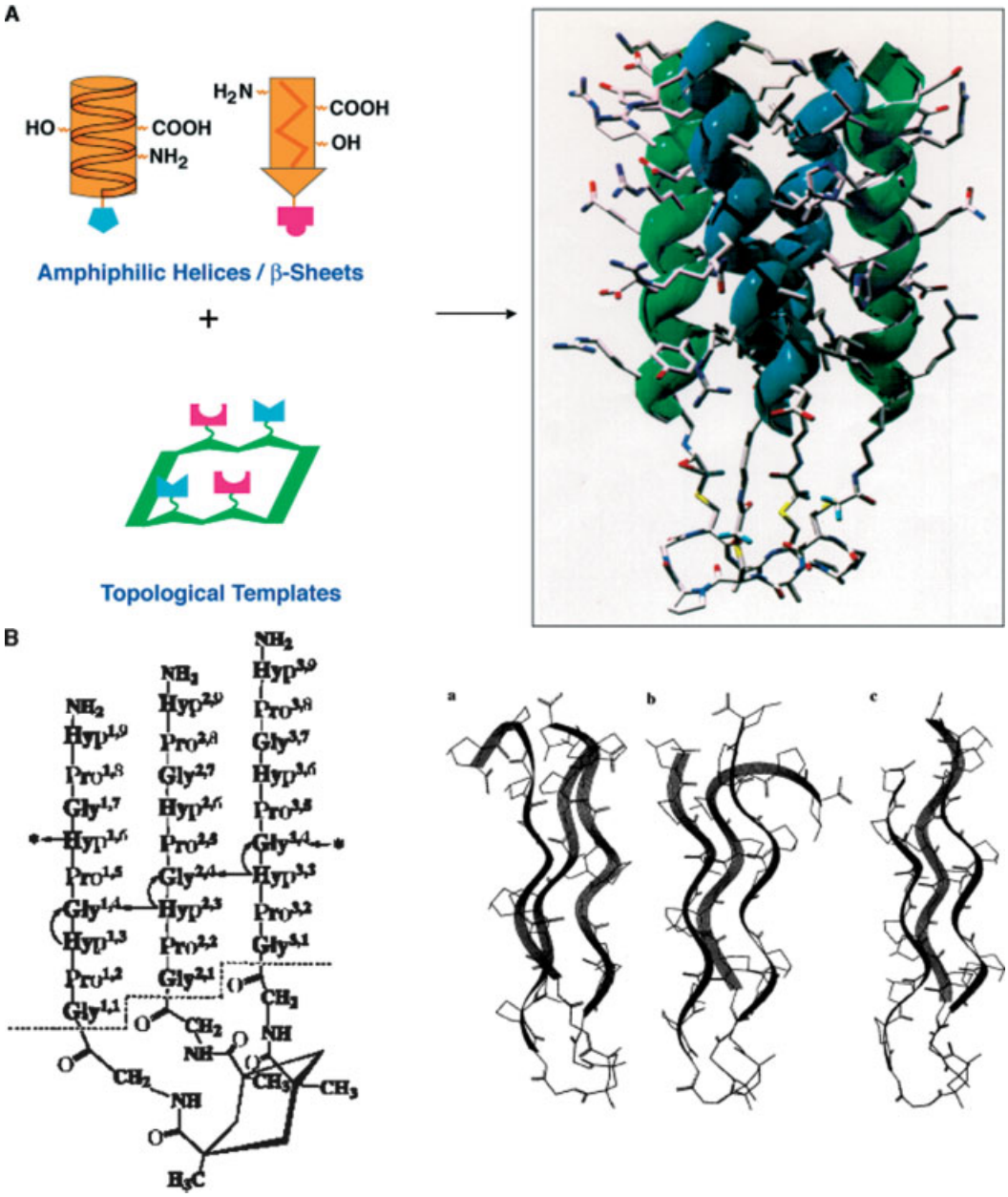

Figure 3 (A) The concept of template-assembled-synthetic proteins (TASP) (ref. [11–14]) (B) A template induced incipient collagen-like triple helical structure (ref. [22,23]).

When we invited him to the 2nd Lausanne Conference on Bioorganic Chemistry in 1999, we made a reservation for him in a hotel situated close to the railway station. After Murray finally arrived exhausted with numerous bags, his comment was: *Manfred, you said the distance to the hotel is only 100 yards, but you forgot to mention the angle!* It was his fine sense of humor, which we will never forget.

REFERENCES

1. Toniolo C, Bonora GM, Mutter M. Conformations of poly(ethylene glycol) bound homooligo-L-alanines and L-valines in aqueous solution. *J. Am. Chem. Soc.* 1979; **101**: 450–454.
2. Ribeiro AA, Saltman RP, Goodman M, Mutter M. 1H-NMR Studies of polyoxyethylene-bound homo-oligo-L-methionines. *Biopolymers* 1982; **21**: 2225–2239.
3. Pillai VNR, Mutter M. Conformational studies of poly(oxyethylene)-bound peptides and protein sequences. *Acc. Chem. Res.* 1981; **14**: 122–130.
4. Kent SBH. Difficult sequences in stepwise peptide synthesis. Common molecular origins in solution and solid phase? In *Peptides, Structure and Function*, Deber CM, Hruby VJ, Kopple KD (eds). Pierce Chemical Company: Rockford, IL, 1985; 407–414.
5. Atherton E, Sheppard RC. Detection of problem sequences in solid phase synthesis. In *Peptides, Structure and Function*, Deber CM, Hruby VJ, Kopple KD (eds). Pierce Chemical Company: Rockford, IL, 1985; 415–418.
6. Wöhr T, Wahl F, Nefzi A, Rohwedder B, Sato T, Sun X, Mutter M. Pseudo-prolines (ΨPro) as a solubilizing, structure disrupting

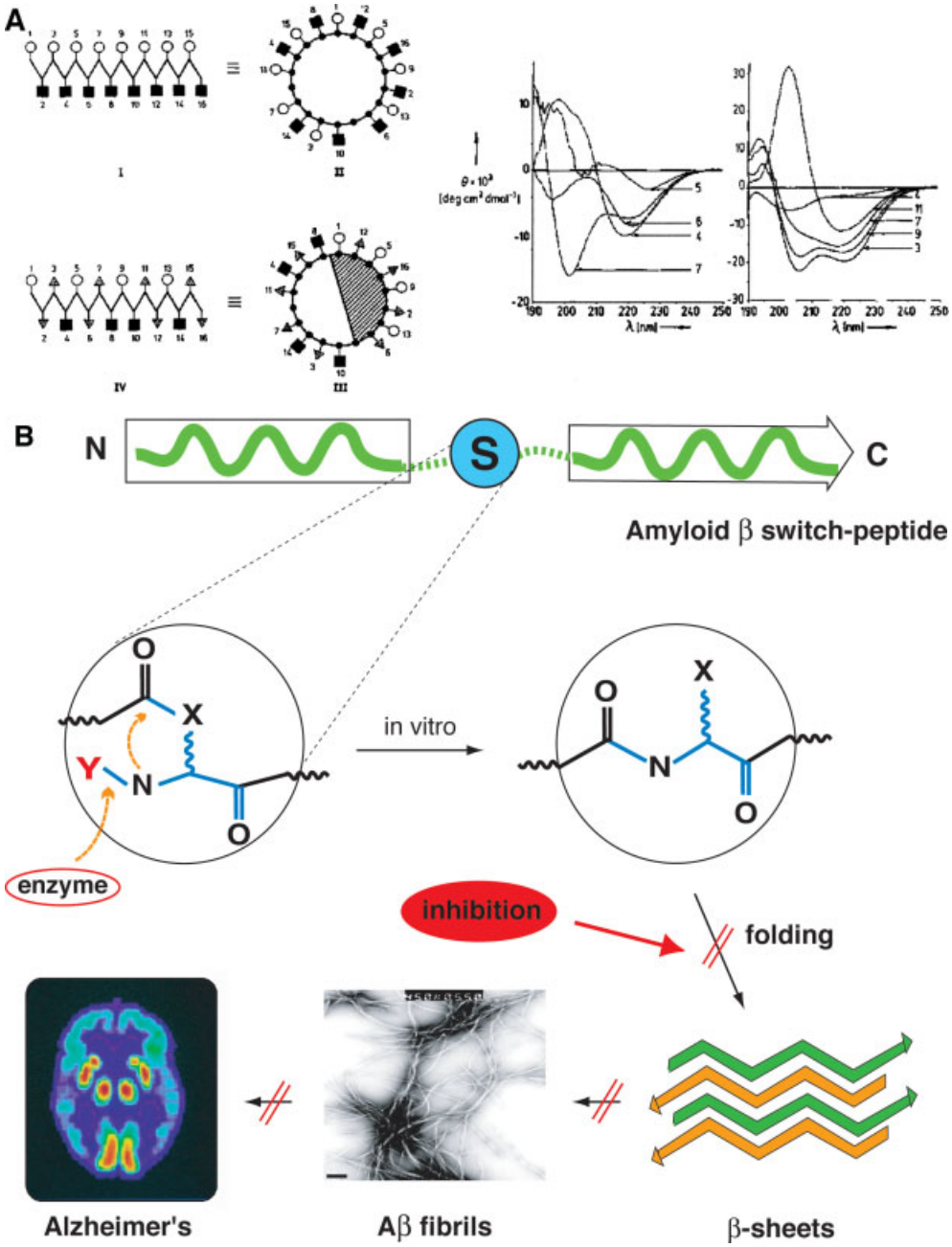

Figure 4 (A) Switch-peptides for studying conformational transitions (ref. [24,25]); (B) Switch-peptides for elucidating early processes in fibril formation (ref. [26]).

protection technique in peptide synthesis. *J. Am. Chem. Soc.* 1996; **118**: 9218–9227.

7. Toniolo C, Bonora GM, Mutter, Pillai VNR. Linear oligopeptides 77. The effect of the insertion of a proline residue on the solution conformation of host peptides. *Makromol. Chem.* 1981; **182**: 2007–2014.
8. Benedetti E, Christensen A, Gilon C, Fuller W, Goodman M. Conformational studies of peptides. Crystal structures of N-acetyl-L-prolinamide and N-acetyl-(S)-thiazolidine-4-carboxamide. *Biopolymers* 1976; **15**: 2523–2534.
9. Tuchscherer G, Mutter M. Peptidomimetics for bridging structure and function: pseudo-prolines in peptide synthesis, molecular recognition and drug design. *Chimia* 2001; **55**: 306–313.
10. Mutter M, Altmann KH, Floersheimer A, Herbert J. Sequence dependence of secondary-structure formation. Part IV. Helix-forming potential of amphiphilic oligopeptides containing Aib residues. *Helv. Chim. Acta* 1986; **69**: 786–792.
11. Mutter M, Tuchscherer G. The construction of new proteins. Template-assembled synthetic 4-helix bundle protein. *Makromol. Chem. Rapid Commun.* 1988; **9**: 437–443.
12. Vuilleumier S, Mutter M. A chemical approach to protein design. Template-Assembled Synthetic Proteins (TASP). *Angew. Chem. Int. Ed.* 1989; **28**: 535–554.
13. Mutter M, Tuchscherer G. Evolution versus design. Template directed self-assembly of peptides to artificial proteins (TASP). *Chimia* 2004; **54**: 552–557.
14. Tuchscherer G, Mutter M. *De novo* peptide structures (protein design). In *Houben-Weyl, Methods of Organic Chemistry. Synthesis*

of Peptides and Peptidomimetics, Goodman M, Felix A, Moroder L, Toniolo C (eds). Thieme: Stuttgart, 2003; **E22d**: 7–67.
15. Peluso S, Rückle T, Lehmann C, Mutter M, Peggion C, Crisma M. Crystal structure of a synthetic cyclopeptide template for TASP design. *Chem. Bio. Chem.* 2001; **2**: 432–437.
16. Dawson PE, Kent SBH. Convenient total synthesis of a 4-helix bundle TASP molecule by chemoselective ligation. *J. Am. Chem. Soc.* 1993; **115**: 7263–7266.
17. Rau HK, Haehnel W. Design, synthesis and properties of a novel cytochrome b model. *J. Am. Chem. Soc.* 1998; **120**: 468–476.
18. Schnepf R, Haehnel W, Wieghardt K, Hildebrandt P. Spectroscopic identification of different types of copper centers generated in synthetic four-helix bundle proteins. *J. Am. Chem. Soc.* 2004; **126**: 14 389–14 399.
19. Hauert J, Fernandez-Carneado J, Michielin O, Mathieu S, Grell D, Schapira M, Spertini O, Mutter M, Tuchscherer G, Kovacsovics T. A template-assembled synthetic protein surface mimetic of the von Willebrand factor A1 domain inhibits botrocetin-induced platelet aggregation. *Chem. Bio. Chem.* 2004; **5**: 856–864.
20. Scheibler L, Dumy P, Boncheva M, Leufgen K, Mathieu H-J, Mutter M, Vogel H. Functional molecular thin films: Topological templates for the chemoselective ligation of antigenic peptides to self-assembled monolayers. *Angew. Chem. Int. Ed.* 1999; **38**: 696–699.
21. Mutter M, Tuchscherer G, Miller C, Altmann K-H, Carey RI, Wyss DF, Labhardt AM, Rivier JE. Template-assembled synthetic proteins with four-helix-bundle topology. Total chemical synthesis and conformational studies. *J. Am. Chem. Soc.* 1992; **114**: 1463–1470.
22. Goodman M, Feng Y, Melacini G, Taulane JP. A template-induced incipient collagen-like triple helical structure. *J. Am. Chem. Soc.* 1996; **118**: 5156–5157.
23. Feng Y, Melacini G, Taulane JP, Goodman M. Acetyl-terminated and template-assembled collagen-based polypeptides composed of Gly-Pro-Hyp sequences. 2. Synthesis and conformational analysis by circular dichroism, ultraviolet absorbance, and optical rotation. *J. Am. Chem. Soc.* 1996; **118**: 10 351–10 358.
24. Mutter M, Hersperger R. Peptides as conformational switch. Medium-induced conformational transitions of designed peptides. *Angew. Chem. Int. Ed.* 1990; **29**: 185–187.
25. Mutter M, Gassmann R, Buttkus U, Altmann KH. Switch-peptides. pH-induced α-helix to β-sheet transitions of bis-amphiphilic oligopeptides. *Angew. Chem. Int. Ed.* 1991; **30**: 1514–1516.
26. Mutter M, Chandravarkar A, Boyat C, Lopez J, Dos Santos S, Mandal B, Mimna R, Murat K, Patiny L, Saucède L, Tuchscherer G. Switch-peptides in statu nascendi. Induction of conformational transitions relevant in degenerative diseases. *Angew. Chem. Int. Ed.* 2004; **43**: 4172–4178.

