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Studies of protein folding and structure with model peptides‡

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INTRODUCTION

In his early research work Murray Goodman mainly addressed aspects of protein structure and function by combining the synthesis of poly-α-amino acids and monodispersed oligopeptides with spectroscopic analysis and pioneering the use of CD and NMR for such a purpose. He then extended this research from biopolymers to biomolecules with essential contributions to the basic concept of correlating synthesis with structural analysis and bioassays for investigating molecular crosstalks in (patho)physiological processes. With his scientific contributions he increasingly influenced the research of other laboratories around the world, including the group of E. Scoffone in Padua, where I personally had the occasion to meet him in the 1960s and to know about his pioneering work in the field. It was Goodman and his associates who first discovered the α-helix promoting properties of 2,2,2-trifluoroethanol [1,2], which were immediately applied in our group to analyse the propensities of S-peptide analogues for this ordered structure and to correlate their conformational properties with the binding affinities for S-protein to form active RNase *S* complexes, in those days an excellent model of peptide hormone–receptor interactions [3,4]. Although personally I did not have the privilege of a direct mentorship by Murray Goodman as a student or postdoc in his laboratories, from the first personal contacts in Padua I have continuously profited from his scientific advice and teaching. This led to intensive direct collaborations during his stay in Munich as Humboldt awardee in 1986 and then culminated in the common venture of editing the five-volume Houben-Weyl treatise on *Synthesis of Peptides and Peptidomimetics* in 2002–2003.

Two examples from my present projects, i.e. collagen model peptides and photo-controlled conformational and biophysical properties of peptides, will illustrate exemplarily the strong impact Murray Goodman had on my research activity.

COLLAGEN MODEL PEPTIDES

Upon its discovery by E. Fischer [5], Pro was immediately recognized as unique among the proteinogenic amino acids with its secondary amine function and saturated heterocyclic ring structure. Because of the abundant occurrence of Pro and its (4*R*)-hydroxylated derivative (Hyp) in positions Xaa and Yaa, respectively, of the characteristic (Gly-Xaa-Yaa) collagen repeats, at first mainly random copolymers and sequential polytripeptides with various Pro and Hyp replacements by proteinogenic amino acids were used to characterize the potential key roles of these α-pyrrolidine-carboxylic acids in protein stability and function [6]. Already by this time Goodman had approached this challenging structural aspect by studying the Pro analogues (*S*)- and (*R*)-thiazolidine-4-carboxylic acids in simple derivatives and related polymers in terms of preferences for the *cis*- and *trans*-tertiary peptide bond as well as for the up- and down-puckering of the ring structures [7,8]. Other laboratories investigated the structural properties of Pro analogues of different ring sizes [9] and even of ring-substituted prolines such as (3*R*)-methylproline [10] or (4*R*)-fluoroproline [10,11]. These early studies more recently inspired detailed investigations by various laboratories, including ours, on (4*R*)- and (4*S*)-fluoroprolines with particular attention paid to the conformational effects exerted by the different puckering and *cis-trans N*-alkyl amide bond propensities of the two diastereoisomers in collagen peptides and in proteins [12–15].

With the experience gained from studies of monodispersed amino acid oligomers vs polymers, Goodman turned to model peptides of defined sequence composition also in the collagen field. Taking into account the entropic cost of trimerization of collagen peptides prior to triple-helix formation and inspired by the previous studies of Roth and Heidemann with the trifunctional scaffolds **I** and **II** of Figure 1 [16], he addressed the design of suitable templates for assembly of collagen peptides to *N*-terminally crosslinked homotrimers. With Kemp's triacid (**III**) extended by a Gly residue as a spacer, a template was identified that allowed the $(\text{Gly-Pro-Hyp})_n$ chains to intertwine into stable triple helices already with $n = 5$ [17], although with

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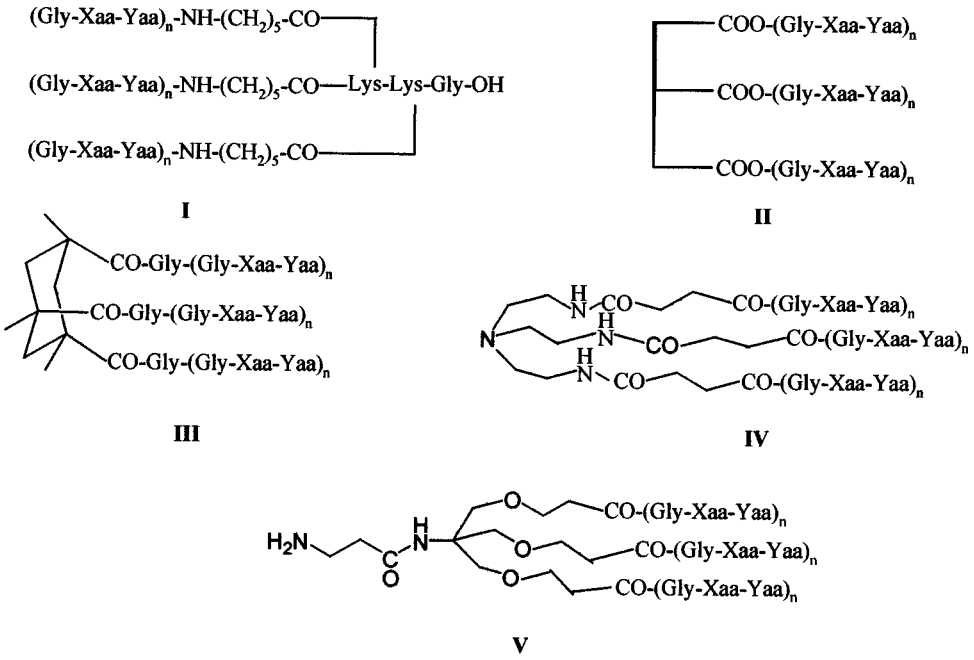

Figure 1 Templates used for the assembly of homotrimeric collagenous molecules: **I** and **II** [16], **III** [17,18], **IV** [19] and **V** [20].

the first triplet only partially included in the triple-helix packing (for a review see reference [18]). Applying the more flexible templates TREN-(suc-OH)$_3$ (**IV**) [19] and TRIS (**V**) [20], respectively, the triple-helix stability was enhanced, with the advantage of template **V** containing an additional functionality that allowed the design of dendrimeric collagens as promising novel biomaterials [20].

To further develop this concept of covalent crosslinking of collagen peptides with optimally designed crosslinking structures, we approached this task with cystine knots in a complementary manner to Goodman's work in order to mimic the more or less complex disulfide crosslinks encountered in native collagens (for reviews see references [21,22]). Exploiting the cystine knot of native collagen type III, which forms in high yields upon prefolding of the chains into a triple helix and air oxidation *C*-terminally crosslinked homotrimeric collagen peptides (Figure 2, **VI**) are readily accessible. Although a well-defined cystine-framework is formed, its disulfide connectivities remain unknown, so far. The interchain disulfide crosslinks lead to triple-helix stabilization similar to the *N*-terminal artificial templates introduced by Goodman, but suffer from thermal instability at higher temperature. Alternatively, an artificial cystine knot (Figure 2, **VII**) was developed as a suitable procedure for assembly of heterotrimeric collagen peptides. Even these artificial cystine knots undergo thermal decomposition, but so far represent the only strategy available for the synthesis of heterotrimeric collagen peptides in the correct native register and containing functional epitopes of the most abundant collagens such as type I and IV [21,22].

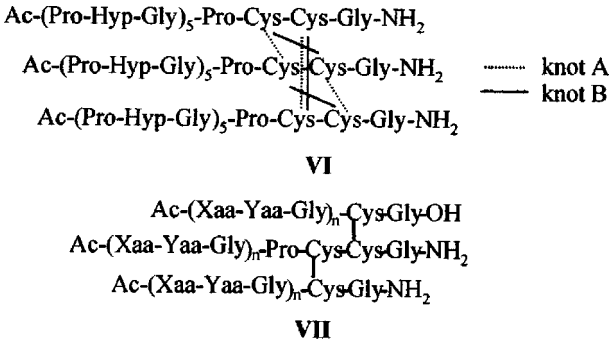

Figure 2 *C*-terminal crosslinking of collagen peptides by collagen type III native cystine knot (**VI**) and artificial cystine knots (**VII**) [21]. With the latter disulfide crosslinks heterotrimers containing natural collagen sequences were obtained in the desired chain register [21].

PHOTOCONTROL OF PEPTIDE CONFORMATIONS

Based on previous conformational studies of photoresponsive azoaromatic vinyl polymers [23], Goodman was the first to recognize the potential of the *cis/trans* photoisomerization of 4-(phenylazo)phenylalanine to photocontrol the conformation of poly-α-amino acids [24,25]. This pioneering work led to extensive follow-up studies reviewed in reference [26]. Since light can be applied with high spatial and temporal resolution with modern laser techniques, the incorporation of optical triggers such as azobenzene, with its geometric changes upon *cis/trans* photoisomerization, into molecular assemblies yields materials with photocontrolled mechanical and optical properties [27], which we exploited for the design of the first light-driven

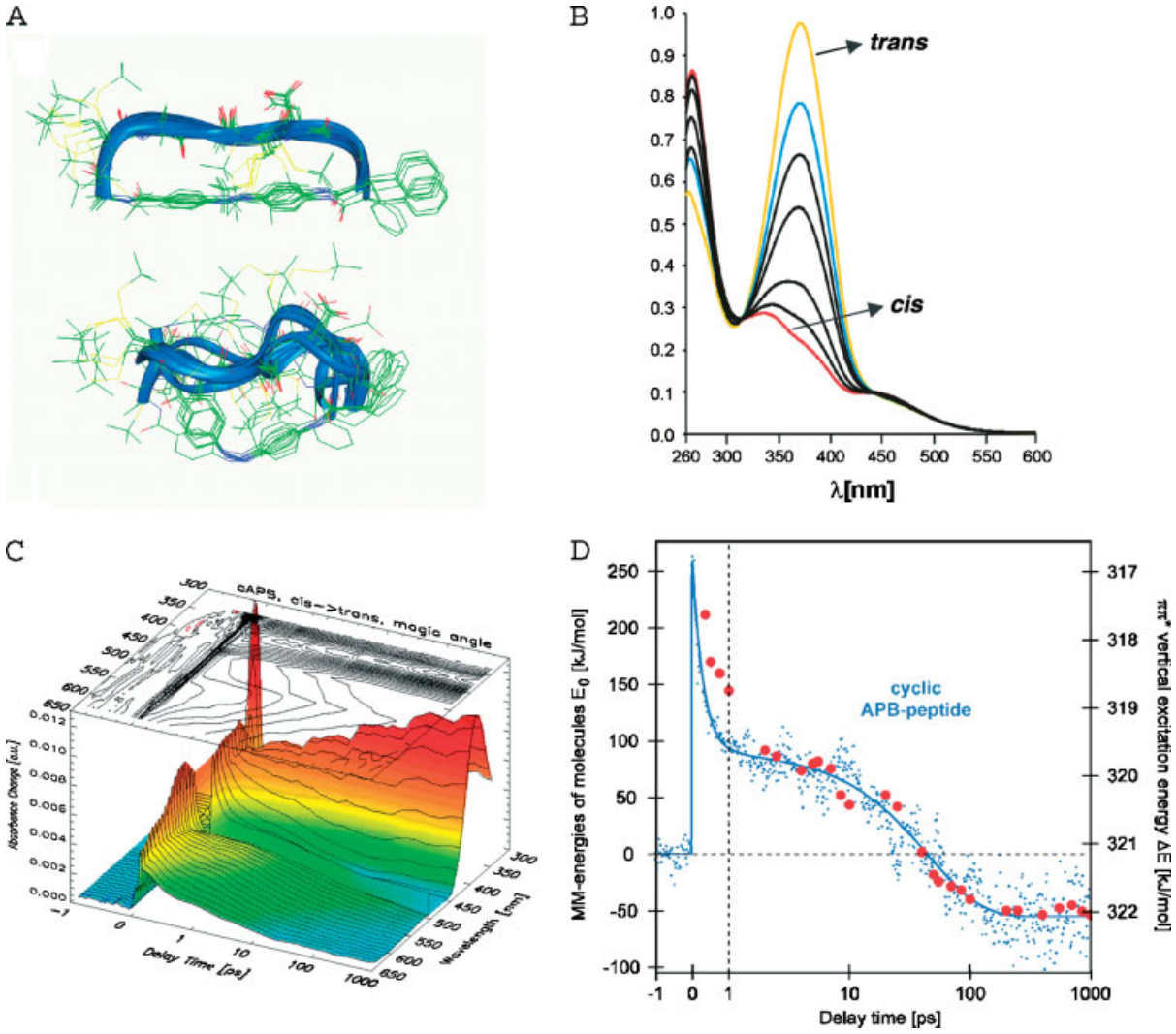

Figure 3 (A) NMR-derived solution structures of the *trans*- (upper panel) and *cis*-azoisomer (lower panel) of the monocyclic *c*[Phe-APB-Ala-Cys(StBu)-Ala-Thr-Cys(StBu)-Asp-Gly-] pseudopeptide in DMSO [30]; (B) UV spectra of the *trans*-to-*cis* photoisomerization upon irradiation of the cyclic azobenzene-peptide at 360 nm [30]; (C) transient UV spectra of the *cis*-to-*trans* photoisomerization of the cyclic azobenzene peptide by irradiation at 450 nm [31]; (D) MD simulations of the *cis*-to-*trans* photoisomerization process [31].

nano-machines [28]. In addition, our group and others have recently focused on combining the azobenzene chromophores with peptide sequences for investigating protein folding and function in a time resolved manner after excitation with light of an appropriate wavelength (for review see reference [29]).

Backbone cyclization of octapeptides via (4-amino) phenylazobenzoic acid (APB) [29,30] or (4-aminomethyl) phenylazobenzoic acid [29] yields cyclic photoresponsive peptides with pronounced conformational transitions upon isomerization, which provoke significant changes in biophysical properties such as redox potentials or receptor binding affinities [32,34]. In addition, the extremely fast isomerization process of the chromophore itself makes these peptides ideal model systems for studying the fast events in protein folding by UV and IR ultrafast absorption spectroscopies [31,34],

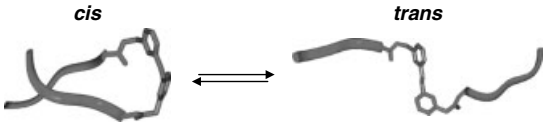

Figure 4 Unfolding of a β-hairpin azobenzenepeptide by *cis*-to-*trans* photoisomerization [37].

as illustrated in Figure 3 with a cyclic azobenzene-peptide.

While the *trans*-to-*cis* photoisomerization of cyclic azobenzene peptides generally relaxes the molecules from rigid and well-defined structures into a large conformational space, Woolley and associates exploited side chain-to-side chain crossbridged α-helical peptides to photocontrol efficiently the onset of this secondary

structure element [35,36]. To further develop light-switchable ordered structures our laboratory succeeded recently, after many failures, in the design of a β-hairpin peptide in which the *cis*-isomer of a suitable azobenzene derivative efficiently simulates the dipeptide unit of the chain reversal (Figure 4) [37].

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