Understanding Assembly Enables the Better Design of Peptide Conjugate Which May Form Useful and Functional Nanostructures

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Introduction

One of the most facile and versatile approaches for creating artificial dynamic biomaterials lies in the stimulus responsive strategies, in which structurally tunable moieties are conventionally incorporated. Peptides, which consist of the identical structural constituents with proteins, that is, amino acids, have been considered as one of the ideal synthetic building blocks to create stimulus-responsive biomaterials due to their remarkable biocompatibility and the intrinsic sequence−assembly relationship. The structural tunability of natural or non-natural amino acids in hydrophobicity and conformation allows for precisely encoding the sequence of peptides, thus endowing a considerable number of the probability to modulate peptide self-assembly by utilizing biologically external or internal stimuli. Prion are a singular subset of proteins able to switch between a soluble conformation and an amyloid state. The ability to transit between these two conformations is encoded in the so-called “prion domains” which are long and disordered regions of low complexity, enriched in polar and uncharged amino acids. The polar nature of prion domains results in slow amyloid formation, which allow kinetic control of fiber assembly. The cross-β spine is a common motif of amyloid fibrils; it is composed of two sandwiched β-sheets, each resulting from the lateral stacking of peptide segments. At the contact region between the β-sheets, side chains form a tight, solvent-excluded interface called a steric zipper. This regular repeat of stacked strands arranged perpendicular to the fibril growth axis represents the structural basis of amyloid fibrils. Furthermore, this apparently simple organization contains the key to the polymorphism of amyloid fibrils, i.e. the ability of the same polypeptide sequence to yield a variety of different three-dimensional architectures. Indeed, this heterogeneity arises from a multiplicity of possible polypeptide arrangements and conformations, all lying in a narrow energy range. Also, short peptides have the ability to self-assemble in various dispositions with distinct probabilities, giving rise to fibrils with different morphologies; these morphologies, in turn, can translate into different mechanical properties, highlighting the importance of having accurate knowledge of the atomistic structure for revealing the experimental results and designing new systems.

Method.

Conformational behavior of the prion-like heptapeptides HCO-Gly-Phe-Gly-Phe-Gly-Phe-Gly-OMe (GF7). Glycine, which has achiral C alpha, when inserted into a peptide chain can behave as both an L amino acid and a D amino acid. GY7 prefers an antiparallel organization over the parallel one.

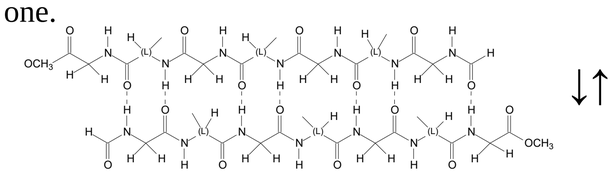

Figure 1. HCO-Gly-Phe-Gly-Phe-Gly-Phe-Gly-OMe (GF7)

Conformational behavior of Boc-L-Phe-(D-Phe-L-Phe)$_3$-OMe Boc-and OMe- protected D,L-alternating oligo-phenylalanine from the hepta-peptide upward, have a very strong preference for an antiparallel organization ↑↓β5.6-helical structure. In chloroform solution the formyl hepta-peptide HCO-L-Phe-(D-Phe-L-Phe)3-OMe forms three major species, namely ↑↓ β5.6- helix, ↑↑β5.6-helix and a tetramer formed by the head-to-head association of the ↑↑β$^{5.6}$-helix having a parallel orientation of the strands (Fig.2). That the oligo-phenylalanine always prefer to form associated species demonstrated that the interaction between side chains is a strong stabilizing factor for self-assembling process.

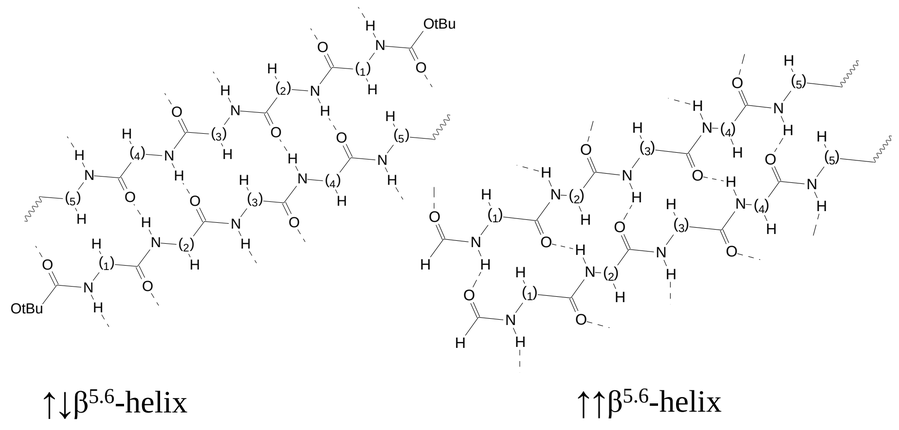

Figure 2. Boc-L-Phe-(D-Phe-L-Phe)$_3$-OMe ↓↑ β$^{5.6}$-helix and HCO-L-Phe-(D-Phe-L-Phe)$_3$-OMe ↑↑ β$^{5.6}$-helix

Synthesis of conjugates **1** and **2**

HCO-L-Phe-(D-Phe-L-Phe)$_3$-OH + H-Gly-Phe-Gly-Phe-Gly-Phe-Gly-OMe ⟶ HCO-L-Phe-(D-Phe-L-Phe)$_3$-Gly-Phe-Gly-Phe-Gly-Phe-Gly-OMe **(1)**

HCO-Gly-Phe-Gly-Phe-Gly-Phe-Gly-OH + H-L-Phe-(D-Phe-L-Phe)$_3$-OMe ⟶ HCO-Gly-Phe-Gly-Phe-Gly-Phe-Gly-L-Phe-(D-Phe-L-Phe)$_3$-OMe **(2)**

Hydrogen bonding vs aromatic(π-π) interactions: there are many occasions in the literature where conformational propensity is varied through aromatic interactions, even at the expense of hydrogen-bonds. The balance of these interactions leads to a propensity to self-assembly, even for conjugates of different peptide chains and these hybrid molecules often form gels from a network of β-sheet fibrils. The relative importance of hydrophobic and polar interactions is especially important when attempting to predict the orientation of conformational behavior before synthesis. In order to study the self-assembling mechanism of a D,L-hexapeptide that forms an organogel we have studied a “model peptide” that have only the possibility to form a dimer with parallel orientation of the strands [1]. This study emphasized that π-π interactions facilitated the gelation. Noncovalent interactions between the orbitals of aromatic rings lead to π-π stacking with several configurations of rings including face-to-face or edge-to-face interactions between rings.

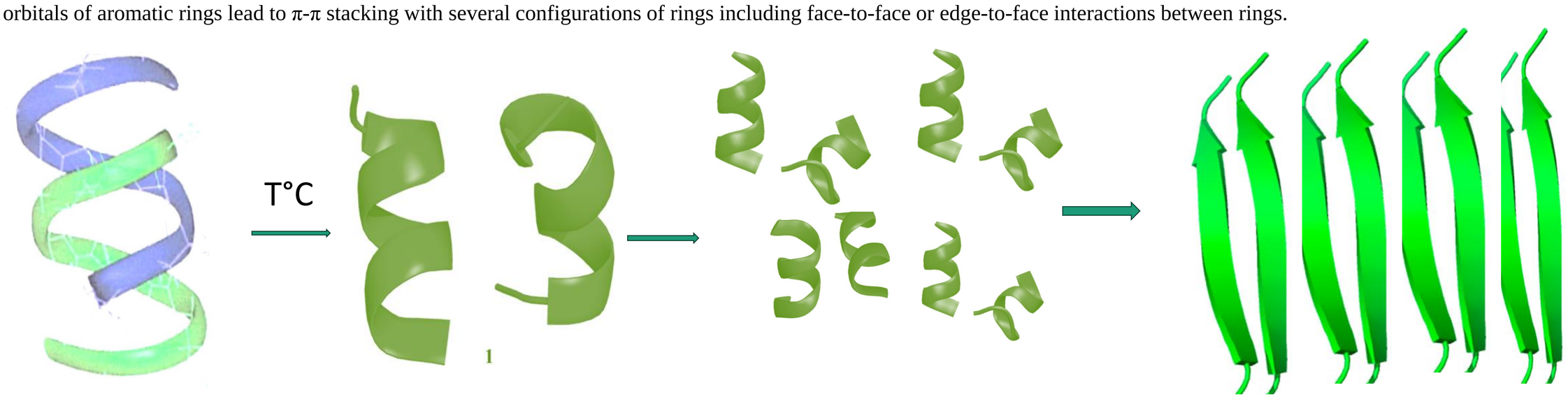

Figure 3. Conformational behavior of HCO-L-Phe-(D-Phe-L-Phe)$_3$-Gly-(L-Phe-Gly)$_3$-OMe in CDCl3 solution at high concentration.

Hybrid peptides **1** and **2** have a conformational behavior coherent with D,L-oligopeptides because Gly behaves as if it were D residue; therefore, the preferred conformation of those peptides is a double stranded antiparallel ↑↓β$^{5.6}$-helix. As the side chains of glycine are hydrogen atoms the β-helix conformation formed by peptides **1** and **2** is less stable compared to D,L-oligophenylalanine due to the decreased number of π-π interactions between the side chains. Therefore, applying an external stimulus the helical structure dissociates (Fig.3). Peptide HCO-Gly-Phe-Gly-Phe-Gly-Phe-Gly-L-Phe-(D-Phe-L-Phe)$_3$-OMe **(2)** is slightly soluble in chloroform at room temperature but when a suspension of this peptide is heated close to 100°C and then cooled slowly a transparent gel is obtained. The maximum number of π-π interactions is reached when the backbone are parallel oriented. The results indicate that on average, parallel fibrils also have more favourable inter-sheet contacts than antiparallel ones. Overall, both intra- and inter-sheet side chain contacts are favoured in parallel organizations over antiparallel ones. As far as backbone hydrogen bond are concerned, the trend is opposite with stronger backbone intra-sheets contacts in antiparallel fibrils over parallel ones, as expected.

Conclusions

Supramolecular biomaterials leverage motif based on supramolecular chemistry in order to produce functional materials that have applications in therapy, diagnostics or devices to advance healthcare. The specific benefits of supramolecular biomaterials arise from the nature of these supramolecular interactions, which confer control over properties in a reversible, highly tunable dynamic and modular fashion. Supramolecular biomaterials may also enable biomimetic recapitulation of native biological signalling or structural cues. As such supramolecular biomaterials provide a diverse toolbox that could help to address important unmet medical needs.

References

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