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Modular Design of Peptide Fibrillar Nano- to Microstructures

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With a growing interest and demand for materials programmed at the nano-to-micrometer scales, biomolecular self-assembly is attracting considerable attention as an efficient tool for building new supramolecular architectures and composites.[1] Self-assembled materials based on polypeptides have been developed extensively over the past decade.[1] Within this area, the design of fibrous systems is particularly notable.[2] Moreover, these are being made with various applications in mind, including templating inorganic nanostructures and as scaffolds for tissue engineering.[1,2] Such materials have been built from three major protein-folding motifs: α-helices,[2a] *β*-pleated structures,[1b,2b] and collagen triple helices.[2c−e] We have focused on α-helical assemblies.[2a]

Previously, we have described two peptides that combine via coiled-coil interactions to form heterodimers that assemble into long (>10 *μ*m), thickened (40−80 nm) fibers.[3] The peptides do not propagate matched or blunt ended but are offset from each other to leave overhanging ends — sticky ends.[3a,b] We have built on this framework to engineer fibers and matrices with altered morphologies and to add functionality.[3c−e] Following this work, others have employed the sticky end concept to assemble both coiled-coil and collagen-based structures.[2d,e,4] Here we show that the approach can be generalized modularly, Figure 1, to render a variety of α-helical and related constructs that assemble into fibrous materials with different morphologies, Figure 2.

The hallmark of coiled-coil assembly is the heptad repeat of hydrophobic (*H*) and polar (*P*) residues, *PHPPHPP*, often designated ***gabcdef***, Figure 1. Our aforementioned designs employed specific combinations of hydrophobic and polar residues at ***a*** and ***d*** sites, and complementary charges at ***g*** and ***e*** sites of successive heptads (***g***−***e′*** interactions) to direct the sticky ended heterodimer. We and others have also described single-peptide fiber-forming designs that avoid the inclusion of polar residues at ***a*** and ***d*** and rely on just the charged ***g***−***e′*** interactions to produce building blocks with sticky ends.[4c,5] Here we explore this concept fully and introduce and compare a variety of constructs based on just two generic heptad repeats. Our aims were to establish the generality of the approach, rather than being limited, as currently, to specific designed sequences and to explore new fiber morphologies and properties that emerge from combining two straightforward heptad building blocks in different ways in a modular strategy.

The two heptads used were EIAALEQ (the anionic module) and KIAALKQ (cationic), Figure 1. The Ile-Leu combination at ***a***−***d*** favors dimers; Ala and Gln were used at the solvent-exposed ***b***, ***c***, and ***f*** sites to minimize side-by-side, uncontrolled association of coiled-coil oligomers; all constructs, except V, were made four heptads long to give stable coiled coils; and a Tyr chromophore

Figure 1. Building blocks for peptide fibers. Two heptad types, or cationic and anionic modules, arranged in an alternating construct (Construct I, Figure 2) (a) and on coiled-coil helical wheels (b). Key: cationic and anionic modules are highlighted in blue and red, respectively; arrows indicate electrostatic interactions between Lys and Glu residues.

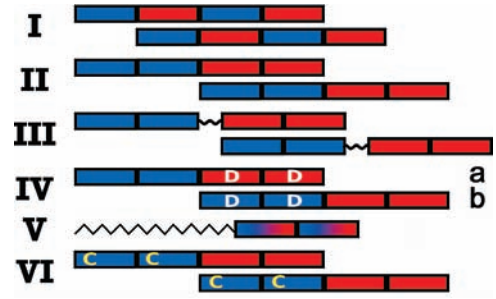

Figure 2. Schematics of modules combined in Constructs I−VI. Key: cationic and anionic modules are in blue and red, respectively; the "D" in Constructs IVa and b signifies that these heptads comprise D-amino acids; and the "C" in Construct VI designates cysteine residues for cross-linking.

was included at a single ***f*** site for determining concentrations. Sequences are given in Table S1, Supporting Information.

In this system, only charge−charge interactions at ***g***−***e′*** can set up the heptad overhang needed to promote fibrillogenesis. Thus, anionic and cationic modules were alternated or paired, Figure 1. For example, in Construct I, Figures 1 and 2, the different modules alternate, and the most-likely sticky ended assembly has a single heptad overhang, whereas, in Construct II, Figure 2, two cationic modules are followed by two anionic ones to direct a two-heptad overhang. In both cases, circular dichroism (CD) spectroscopy showed comparable and high degrees of α-helix expected for helical assemblies[3,4] (Figure S2a). However, and consistent with our design rationale, transmission electron microscopy (TEM) revealed wispy fibers of low density for Construct I and, by contrast, orderly, extended fibers for Construct II, Figures 3 and S1a and b, Supporting Information. Constructs I and II have four contiguous heptads. To test the effect of a break in this pattern, Construct III was made in which a flexible linker of six *β*-alanine residues was inserted between cationic and anionic blocks of Construct II, Figure 2. Though only partially helical, Figure S2a, this construct assembled into bundled networks of thin (∼20 nm) fibrils, Figures 3 and S1c.

The data show that (1) simple rearrangements of the same modules influences the morphology of resulting fibrillar assemblies and (2) although binding of the overhanging portions of the peptide

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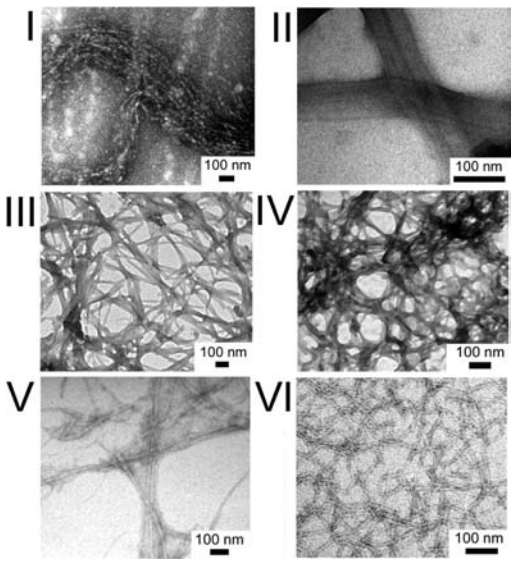

Figure 3. Negatively stained transmission electron micrographs of fibrillar structures assembled from modules I−VI. Assembly conditions: 100 μM in each peptide; 10 mM MOPS, pH 7.4; 20 °C, overnight.

can be independent, cooperativity from contiguous modules is important for the structural integrity of the fibers.

To test the second point in a different way, Constructs IVa and IVb were designed based on Construct II, but with the cationic or anionic modules made from D-amino acids, respectively, Figure 2. These constructs should not be able to assemble alone. This is because the L-amino acids lead to right-handed α-helices and, with canonical heptad repeats, left-handed superhelical packing in the coiled coils, whereas the D-amino acids lead to helices with the opposite sense.[6] Moreover, for D- and L-blocks to assemble into hybrid superhelices, a shift in heptad register would be necessary, compromising the designed overhang.[6a] For such constructs, whether folded or not, CD spectroscopy is uninformative because the signals from the L- and D-components effectively cancel, Figure S2b. Consistent with our hypothesis, TEM did not show any defined supramolecular structures for the individual peptides, Figure S3a and b. In marked contrast, an equimolar mixture of the two peptides gave porous fibrillar meshes, Figures 3 and S1d. These were denser and more closely packed than the networks formed by Construct III suggesting the independent (enantiomeric) binding of the D- and L-fragments.

To confirm that the heptad overhang of modules is necessary for helical fibrillogenesis, we designed Construct V. This takes inspiration from the peptide-amphiphile work of Stupp and colleagues.[7a] The construct has two heptads with mixed charges such that blunt-ended, rather than sticky ended, coiled-coil folding is promoted, Figure 2. The peptide was capped at the N-terminus with stearic acid to give a fixed hydrophobic domain. In contrast to seeding fibrillogenesis with sticky ended helices, the aim was to provide a trigger for nonspecific micelle formation driven by the hydrophobic effect.[7b] Accordingly, the construct adopted an α-helical conformation at pH 7, Figure S2c, but without any signs of higher-order self-assembly, Figure S3c. However, elevated temperatures (melting up to 100 °C) induced an irreversible α→β switch and the formation of amyloid-like fibrils, which did not alter at pH 7−9, Figures 3, S1e, and S2c. Consistent with this and with our earlier observations,[3a−c] the blunt-ended construct lacking the alkyl tail did not assemble under the same conditions, Figure S3d and e.

To this point, the focus has been on sticky end assembly and, therefore, longitudinal fiber growth. As is clear from Figure 3, the resulting fibers are considerably thicker than expected for a linearly extended arrangement of dimeric coiled coils (a fibril), which would have a diameter of ∼2 nm. An ability to tune and control thickening would be of considerable interest.[3−5,8] We attempted to address this with Construct VI, in which alanines in cationic modules are replaced by cysteines, Figure 2 and Table S1. The substitutions generate a thiol-rich outer face on each coiled-coil fibril. When fibrils pack together and these faces contact, thiol cross-linking should limit any further lateral association (Supporting Information). Indeed, by TEM, Construct VI assembled into uniform fibrils of 10−15 nm in diameter, Figures 3 and S1f. However, by CD spectroscopy, these underwent α→β transitions under mild alkaline conditions (pH 7.5−8), Figure S2d. The fibrils remained intact up to pH 8.5, Figure S4a−c, unlike those from Construct II that disassembled at lower pH, Figure S4d. Thus, the conformational transitions to β-structured fibils in Constructs V and VI occur against the hydrophobic effect and the cysteine networks, respectively. However, the latter are seeded by the designed, sticky end helical interactions, whereas for Construct V the initial driving force is nonspecific from the alkyl tails.

In summary, we introduce a concept for the modular design of peptide-based fibers. The approach uses generic self-assembling units and offers a straightforward route to various assembly templates resulting in different fiber morphologies and properties.

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Supporting Information Available: Experimental procedures, synthesis and characterization, CD spectra and additional low resolution electron micrographs. This material is available free of charge via the Internet at http://pubs.acs.org.

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