Self-Assembling Fibers

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Supramolecular Self-Assembly of *N*-Acetyl-Capped β-Peptides Leads to Nano- to Macroscale Fiber Formation**

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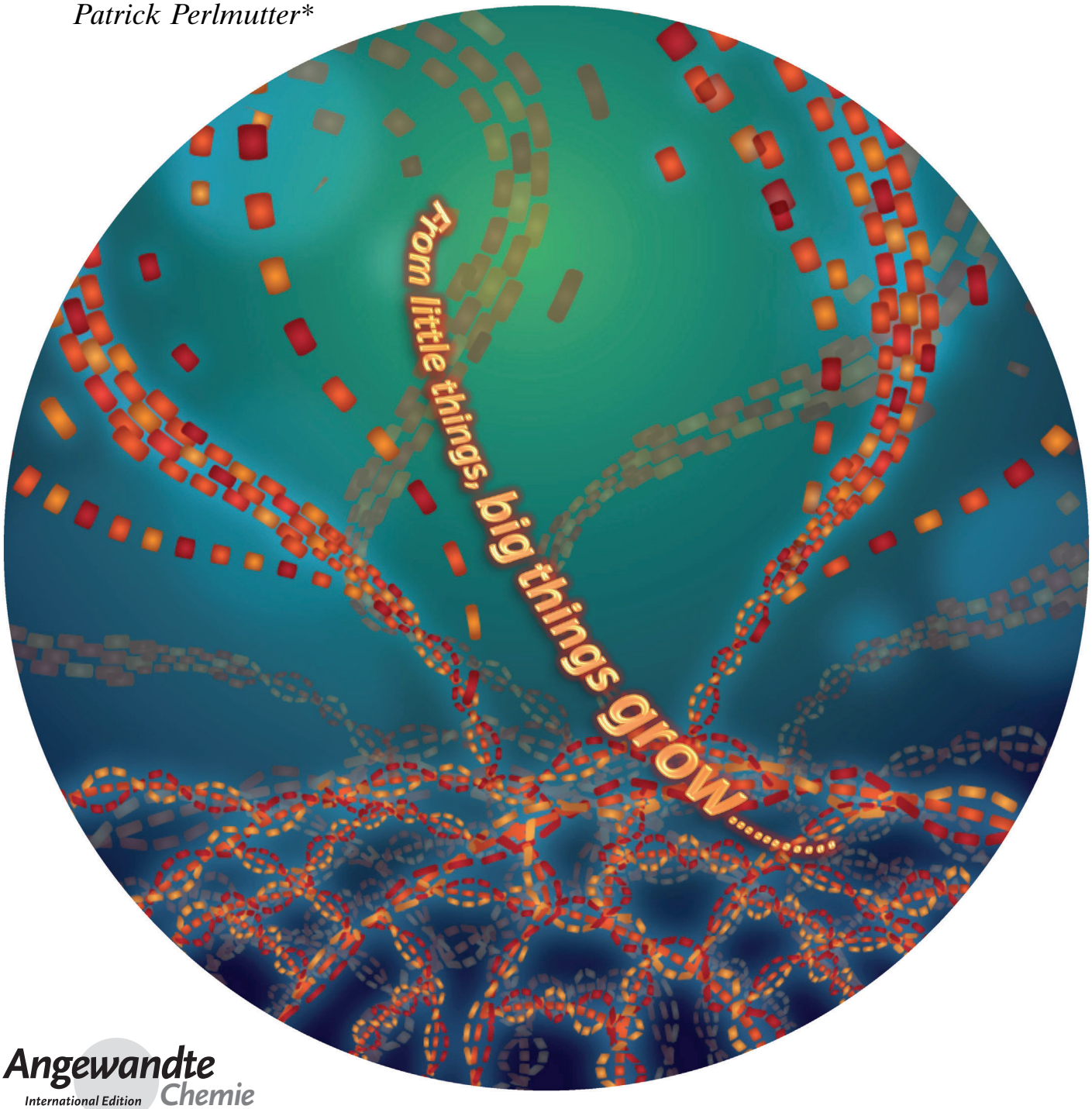

Supramolecular self-assembly represents a powerful approach to the design of functional nanomaterials.[1–13] Peptide-based self-assembled systems offer significant advantages including biological compatibility, ease of synthesis, low toxicity, and functionalizability.[14–16] However, the control over essential features such as chemical, structural, and metabolic stability, the scale, and relatively slow rate of self-assembly remain significant challenges.

Using peptides consisting of only β^3-amino acids offers the means to overcome these limitations. β^3-Peptides exhibit high metabolic stability. Structurally, they adopt stable, helical conformations in solution.[17–20] 14-Helical β^3-peptides in particular contain exactly three residues per complete turn of the helix (i.e. $n=3$) and thus form cylindrical molecules with perfect longitudinal alignment of residues. This provides extraordinary opportunities for designing new materials with functionality located along these faces.[20,21] Perhaps even more significant, is that the perfect pitch offers the opportunity to design a supramolecular self-assembly motif to link the monomers in a highly symmetrical manner reminiscent of one-dimensional crystallization. We hypothesized that a hydrogen-bonding motif similar to that required for 14-helical stabilization (Figure 1 A) would, if propagated intermolecularly (Figure 1 B), mediate axial head-to-tail self-assembly, and ultimately promote fiber formation (Figure 1 C). This mode of fiber self-assembly contrasts with existing approaches which predominantly exploit lateral interactions, rather than axial, assembly motifs.[1–7]

Two critical design elements were identified. The first is peptide length in multiples of three in order to satisfy the requirements of stable 14-helix formation. The second is a total of six axially oriented donor–acceptor interactions, three at each terminus (Figure 1 B). A peptide with a free amino terminus would only provide two of the three required interaction pairs. Thus a small N-terminal acyl group, acetyl, was selected to satisfy these requirements whilst minimizing potential steric perturbation of the hydrogen-bonded interface. Given these considerations, a series of hexa- and tri-β^3-peptides ($n=6$ and $n=3$, respectively) and their *N*-acetylated counterparts was synthesized (see Table 1).

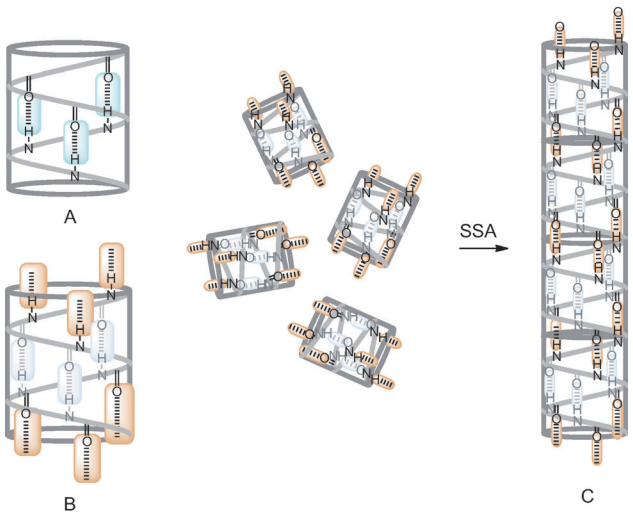

Figure 1. Self-assembly of 14-helical β-peptides. A) Known intramolecular hydrogen-bonding motif for helix stabilization motif, Ref. [17]. B) Intermolecular hydrogen-bonding motif proposed in this work. C) Fiber formation by means of supramolecular self-assembly (SSA) employing the proposed hydrogen-bonding motif.

Following dissolution of each peptide in either water or methanol, the solutions were visualized under a light microscope. All solutions containing *N*-acetylated β^3-peptides exhibited rapid fiber formation (Figure 2). Fibers from either peptide **1** or **3** grew from several millimeters up to

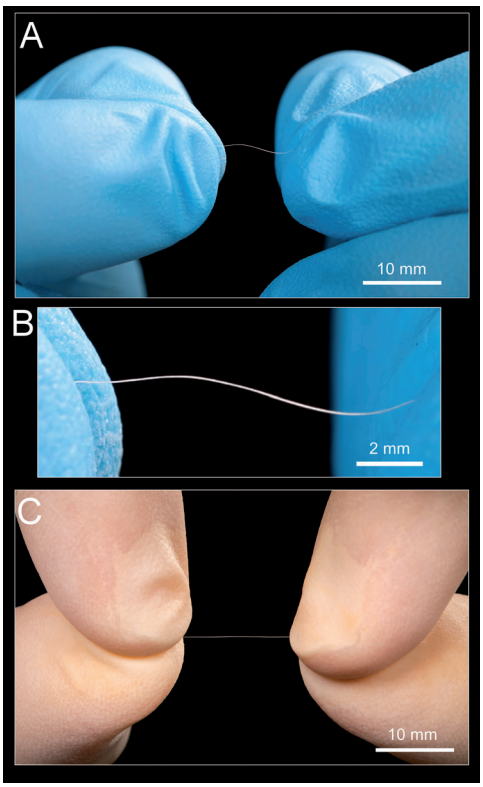

Figure 2. Macroscopic fibers formed by β^3-hexapeptides **1** (A, B) and **3** (C).

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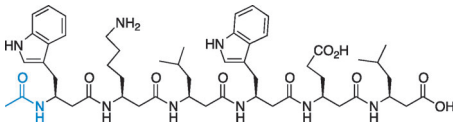
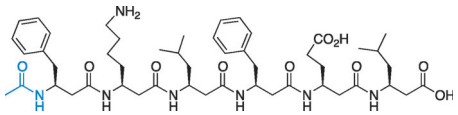
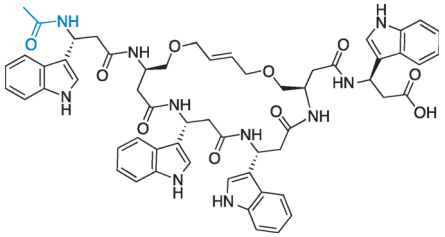
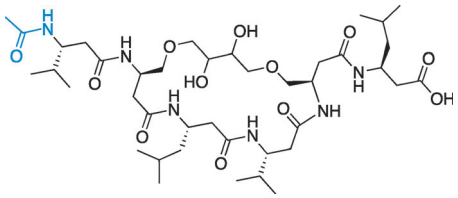
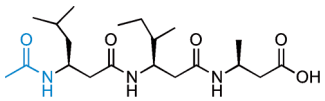
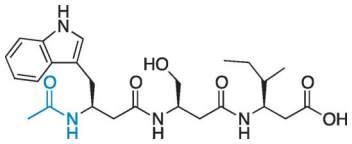
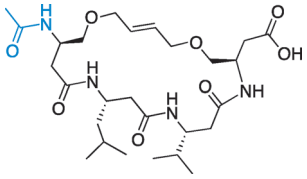
Table 1: β^3-Peptides used in this study (*N*-acetyl caps highlighted in blue).

	β^3-Peptide	Solv.
1		H_2O
2		H_2O
3		MeOH
4		MeOH
5		MeOH
6		MeOH
7		MeOH

three centimeters in length all within one hour and were approximately 0.25 mm in diameter (Figure 2 A–C). Remarkably, these fibers could be easily removed from solution and proved strong and flexible enough to be handled like common thread. For example, these fibers can be bent and/or stretched without damage and retain their original shape. The critical role played by the *N*-acetyl group in promoting axial self-assembly and fiber growth was supported by the complete absence of fiber formation by any of the β^3-peptides with a free *N*-terminus.

The relative thickness of the fibers suggests higher order hierarchical self-assembly. While the substructure of the large fibers could not be resolved by light microscopy, hexa-β^3-peptides **3** and **4**[22] and tri-β^3-peptides **5** and **6** formed thinner fibers which proved more amenable. Images of these fibers clearly show a twisted ribbonlike morphology (Figure 3). Moreover the presence of submicron fibrils, as revealed by

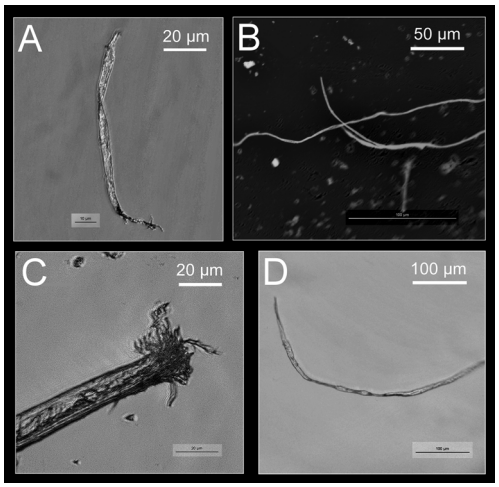


Figure 3. Microscopic fibers formed by β^3-hexapeptides **2** and **4** and β^3-tripeptides **5** and **6**. A) peptide **4**, B) peptide **2**, C) peptide **5,** and D) peptide **6**.

fraying of the ends, provides evidence of hierarchical self-assembly (Figure 3 A, C). Higher resolution analysis of tri-β^3-peptide **6** by atomic force microscopy (AFM) revealed the initial stages of self-assembly. The smallest unit visible in Figure 3 is a helical fibril of approximately 10 nm diameter (labeled 1 and 2 in Figure 4 C) and a surface periodicity of approximately 50 nm (labeled 2 and 3 in Figure 4 D). Assum-

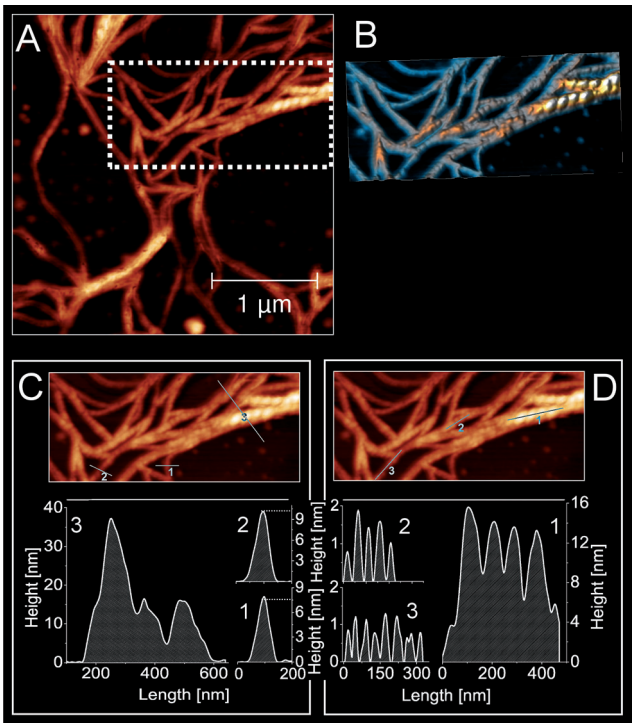


Figure 4. AFM image of sub-microscopic fibrils of **6**. A) large area scan; height scale is approximately 40 nm. B) 3D-rendered representation of the area outlined on (A). Note the periodicity in the smaller fibrils. C, D) The same area; along the lines, height profiles have been measured and depicted below the images. Numbers correspond to the profile below each image.

ing that one nanorod is approximately 0.5 nm in diameter, these fibrils likely arise from assembly of a small number, approximately three to five, individual nanorods.

These images also demonstrate that individual fibrils self-assemble further to form larger helical, ropelike structures with the largest fiber approximately 35 nm in diameter (labeled 3 in Figure 3C) and surface periodicity of 100 nm (labeled 1 in Figure 4D).

The presence of the different-sized fibers with helical periodicity suggests a mechanism of hierarchical self-assembly that follows a multistep "self-twining" process by means of the formation of consecutively higher order ropelike nanofibers from individual fibrils (Figure 4B and see also Figure 6G–I).

At high concentrations two of the peptides which formed fibers, namely **4** and **6**, were also found to crystallize affording the opportunity to analyze the structures by X-ray crystallography. This allowed us to gain further insight into the self-assembly process leading to fiber formation. Hexapeptide **4** exhibits a typical left-handed 14-helical structure of exactly two turns, internally supported by three $i,i+3$ intramolecular N–H–O hydrogen bonds between N(1), N(2), and N(3), and O(4), O(5), and O(6) respectively (Figure 6A, hydrogen bonds highlighted in aqua). The pitch of the helix is approximately 5.0 Å with an internal radius of approximately 1.8 Å. Self-assembly arises when residues from one helical peptide form intermolecular hydrogen bonds with complementary residues from the axially proximal peptide (Figure 6A, hydrogen bonds highlighted in gray).[21,23] Our results demonstrate the crucial role played by the *N*-acetyl cap of a 14-helix in providing both intra- and intermolecular hydrogen bonds.

The importance to fiber morphology of the number of residues per self-assembling peptide is illustrated in the case of **7** where $n=4$ (Figure 5). The result is an apparent curling

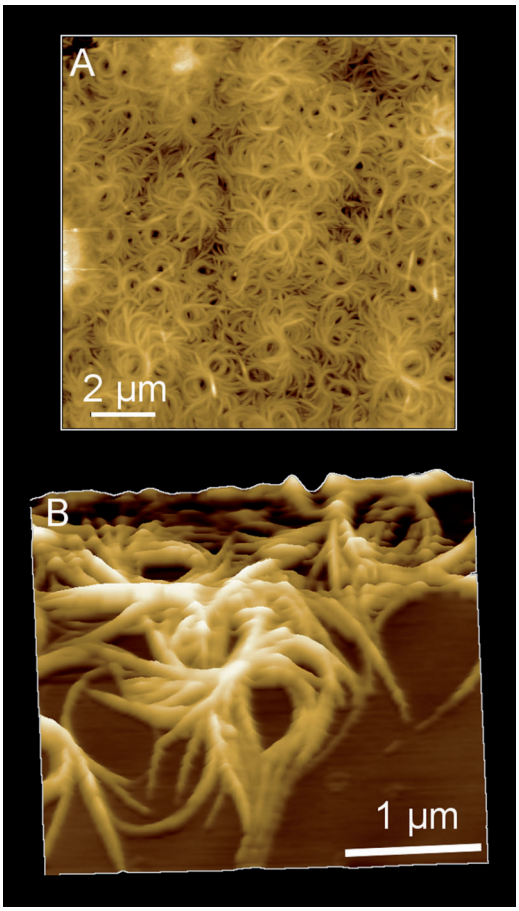


Figure 5. AFM images of stapled tetra β^3-peptide **7** reveal an alternative pattern of self-assembly where small fibrils roll up into coiled superstructures. A) top view; B) 3D-rendered representation.

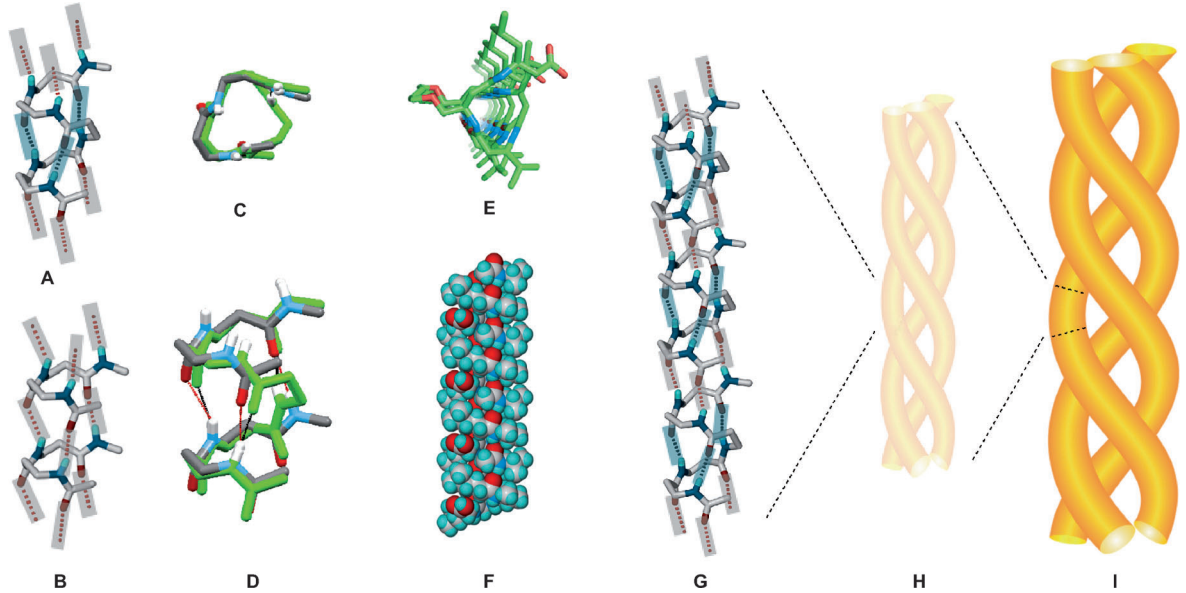


Figure 6. A, B) Crystal structures of, respectively, hexa-β^3-peptide **4** showing intermolecular- (gray) and intramolecular (aqua) hydrogen bonds and tri-β^3-peptide **6** showing intermolecular hydrogen bonds (side chains omitted for clarity); C, D) axial and lateral views, respectively, of the backbones of **4** and **6** superimposed; E–G) different views of the crystal structure of a nanorod section of crystalline β-peptides; H, I) entwining of individual fibrils (H) generates larger fibers (I).

of the self-assembled structures leading to a unique morphology remarkably different to those generated from peptides **1**–**6**.

The X-ray crystal structure of the β^3-tripeptide **6** also shows a three-point intermolecular hydrogen-bonding motif equivalent to that found in β^3-hexapeptide **4** (Figure 6B). Each residue forms a hydrogen bond from the backbone NH to the carbonyl of the same amino acid residue of the axially proximal peptide. Thus, overall, each peptide has six intermolecular hydrogen bonds driving the self-assembly. The acetyl cap again plays a crucial role in the formation of the three-point hydrogen-bonding motif.

Superimposition of their structures clearly shows that the dimensions of **4** and **6** are remarkably similar in the crystal (Figure 6C,D < xfigr6). This similarity in dimensions also explains why the fibers formed from either peptide are so similar in appearance. Figure 6E,F highlight the perfect alignment of side chains along the edges of each nanorod. Figure 6G–I show how the nanorods are spontaneously woven into fibrils and fibers.

In summary, we have demonstrated that *N*-acetylated β^3-peptides of suitable length spontaneously self-assemble into fibers from solution in a unique head-to-tail fashion as proposed in Figure 1. We also show that these fibers are able to grow into large structures that can be seen with the naked eye and handled easily. The inherent flexibility in this unique design as well as ease of synthesis provide powerful new avenues for the development of novel bio- and nanomaterials via supramolecular self-assembly.

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