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Controlling the Structure and Length of Self-Synthesizing Supramolecular Polymers through Nucleated Growth and Disassembly**

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Abstract: *Directing self-assembly processes out-of-equilibrium to yield kinetically trapped materials with well-defined dimensions remains a considerable challenge. Kinetically controlled assembly of self-synthesizing peptide-functionalized macrocycles through a nucleation–growth mechanism is reported. Spontaneous fiber formation in this system is effectively shut down as most of the material is diverted into metastable non-assembling trimeric and tetrameric macrocycles. However, upon adding seeds to this mixture, well-defined fibers with controllable lengths and narrow polydispersities are obtained. This seeded growth strategy also allows access to supramolecular triblock copolymers. The resulting noncovalent assemblies can be further stabilized through covalent capture. Taken together, these results show that self-synthesizing materials, through their interplay between dynamic covalent bonds and noncovalent interactions, are uniquely suited for out-of-equilibrium self-assembly.*

Supramolecular (bio)polymers[1] have attracted much recent attention and are applied in fields ranging from self-healing materials[1i] to molecular electronics.[1c,m] Most supramolecular polymers are produced by thermodynamically controlled self-assembly, allowing access to only a single state involving assemblies with typically a broad and strongly concentration-dependent length distribution.[1f,2] In contrast, kinetic control of self-assembly pathways[3] can allow access to one of multiple kinetically trapped states, while starting from the same precursors and may also enable control over the dimensions and structure of the resulting assemblies. Through their analogy with initiation–propagation mechanisms in living polymerization, nucleation–growth mechanisms[4] based on noncovalent interactions have the potential of producing assemblies of well-defined lengths. Control over assembly length is important, as length dictates rheological and other physical properties of polymeric products.[1k] Whereas in traditional covalent polymer chemistry many polymerization strategies have been developed aimed at producing polymers with narrow polydispersities, control over dimensions remains rare in colloidal[5] and supramolecular self-assembly[6] and are unprecedented for self-synthesizing materials.[7] Moreover, for supramolecular polymerization control over size and polydispersity of the nuclei, prevention of spontaneous nucleation, and the stability of the resulting materials remain major challenges. In principle, self-synthesizing materials[7] should allow excellent control over nucleation. In self-synthesizing materials, the assembly of the material from its molecular constituents drives the synthesis of more of these constituents from a pool of interconverting molecules. If, prior to assembly, the self-assembling molecule is only a minor component of the interconverting pool, then spontaneous nucleation of the assembly process should be slow and the assembly process may be triggered by seeding.

We now demonstrate that a self-synthesizing material that relies on a self-assembly process based on two levels of hierarchy (covalent and noncovalent) allows nearly perfect control over nucleation. Excellent control over the size and structure of the nuclei can be achieved by mechanical shearing or chemical degradation. Using a nucleation–elongation mechanism, the length of the resulting assemblies can be controlled, resulting in (for supramolecular polymers) unprecedented polydispersity indices. Furthermore, the living nature of the self-synthesizing polymer allows access to B-A-B triblock co-assemblies unprecedented for supramolecular polymers. Finally, the resulting assemblies can be stabilized through covalent capture.

Our approach to controlled self-assembly is based on dynamic combinatorial chemistry,[8] where building blocks combine by forming reversible covalent bonds to give rise to a range of library members that continuously interconvert by exchanging building blocks. The library is initially under thermodynamic control. When molecules in such a library are able to self-assemble, the self-assembly process shifts the library distribution towards the formation of more of the self-assembling library member, giving rise to a self-synthesizing material.[9,10] When the assemblies prevent the molecules within them from further exchange of building blocks, the self-assembling library members become kinetically trapped.

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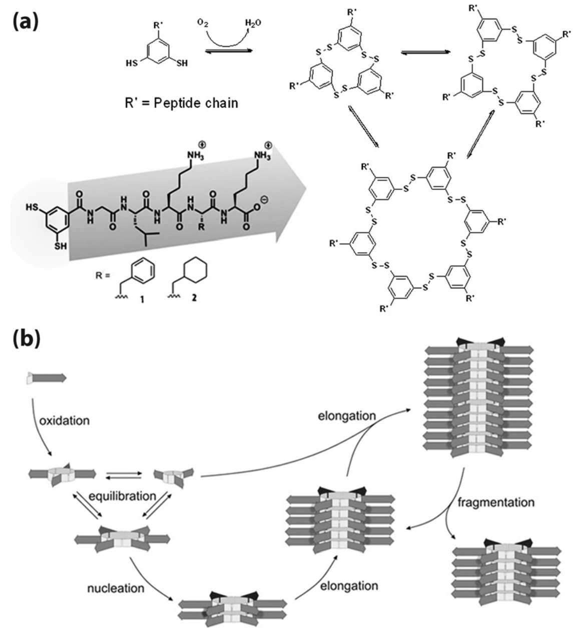
The design of our self-synthesizing material is based on disulfide chemistry, using β-sheet formation between peptides to drive self-assembly. In brief, peptide-functionalized dithiol building block **1** is oxidized by exposure to oxygen from the air to give rise to a small dynamic combinatorial library (DCL) of differently sized macrocyclic disulfides,[11] dominated by trimer and tetramers[12] (Scheme 1). Slow primary nucleation of the stacks of hexamers occurs, but in the absence of agitation the number of such nuclei is too small to allow a significant concentration of hexamer assemblies to build up. However, upon agitating the solution, fibers are formed as the primary nuclei are fragmented into secondary nuclei, increasing the number of ends from which the fibers grow. Thus, agitation enables the exponential growth of the hexamer through a fiber growth–breakage cycle. The outcome is a population of fibers with a broad length distribution (Figure 1a). For example, a system made from **1** that grew while being stirred using a magnetic stirring plate at 1200 rpm, had a polydispersity index (PDI) of 1.25, which is already superior to that of regular thermodynamically controlled supramolecular polymers (PDI ≈ 2).[11f] This mechanism of fiber growth bears some resemblance to amyloid formation, for which an autocatalytic nucleation–growth process has been suggested.[13] However, where amyloid formation involves multiple levels of hierarchy that are difficult to control, our self-synthesizing macrocycles mostly assemble into single fibers.



Scheme 1. a) Oxidation of peptide-functionalized dithiol building blocks **1** or **2** results in the formation of an equilibrium mixture of different sized macrocyclic disulfides. b) Mechanism of replication in a system of bifunctional building blocks **1** or **2**, which initially form an exchanging mixture of macrocycles of different sizes via oxidation of thiols to disulfides and subsequent disulfide exchange. Hexamer macrocycles self-assemble into fibers as the peptide chains (arrows) form β-sheets through a nucleation–elongation mechanism. The fibers grow from their ends and can be broken upon mechanical agitation doubling the number of fiber ends that further promote the formation of the self-replicating hexamer.[7a,14]

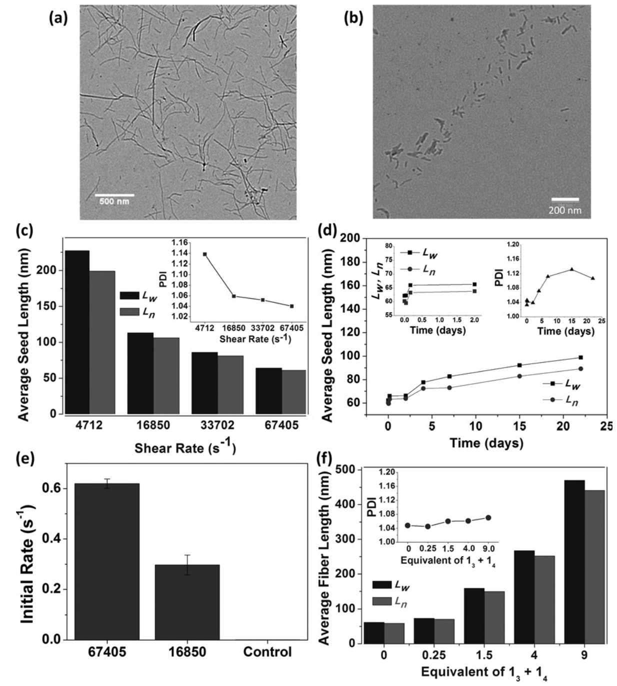


Figure 1. TEM images of fibers of $\mathbf{1}_6$ a) prior to and b) after being sheared in a Couette cell for 30 min at a shear rates of 67 405 s^{-1}. c) Weight-average (L_w) and number-average (L_n) fiber lengths obtained with different shear rates using a Couette cell for generation of fiber seeds. Inset: polydispersity indices (PDIs) of the fiber seeds of $\mathbf{1}_6$ decrease with increasing shear rate. d) Time dependence of the average fiber lengths of non-agitated solutions of seeds obtained after shearing at a shear rate of 67 405 s^{-1} for 30 min. e) Initial rates of fiber growth with seeds sheared with Couette shear rates of 67 405 s^{-1} ($L_n = 106$ nm) or 16 850 s^{-1} ($L_n = 61$ nm) compared with a non-seeded, non-agitated control. For each experiment, 20% v/v seed of hexamer fiber ($\mathbf{1}_6$) was added to a mixture of monomer, trimer, and tetramer (3.8 mM initial monomer concentration, seed : $\mathbf{1}_3/\mathbf{1}_4 = 1:4$). f) Controlled supramolecular polymerization to furnish different fiber lengths by adding 0, 0.25 equiv, 1.5 equiv, 4 equiv, or 9 equiv of a mixture of $\mathbf{1}_3$ and $\mathbf{1}_4$ to the seed. Inset: PDI of the fibers ($\mathbf{1}_6$). The equilibrium mixture of $\mathbf{1}_3$ and $\mathbf{1}_4$ was obtained by oxidizing building block **1**, converting 95% of the thiols into disulfides.

While polydisperse fibers were produced in a stirred solution owing to a poorly controllable combination of primary and secondary nucleation, in the absence of mechanical agitation, and with the use of pre-formed nuclei of uniform size, much narrower fiber length distributions should be obtained. Thus, we set out to add seeds of hexamer fibers to solutions made from building block **1** initially containing mostly cyclic trimers and tetramers. We expected all seeds to grow at equal rates. To achieve maximal control over fiber lengths and obtain small PDIs, it is essential that the seeds are as small and homogeneous as possible. Thus, we used a specially designed Couette cell[14] with which fibers can be subjected to a high shear stress. Samples containing fibers of $\mathbf{1}_6$ were prepared as described previously[9a] and subjected to shear rates of 4712, 16 850, 33 702, and 67 405 s^{-1} respectively, which is well above the Taylor–Couette laminar flow regime.[15]

Fiber length distributions showed that higher shear stress resulted in shorter fibers (Figure 1 a–c; Supporting Information, Figures S1, S2), as evident from the weight-average length (L_w) and number-average length (L_n) values. Applying a shear rate of 67 405 s^{-1} for 30 min produced seeds of $L_n = 60$ nm (Figure 1 b) with a gratifyingly narrow PDI of 1.04. This is a significant improvement over the magnetically stirred seeds (1200 rpm; the maximum stir rate on our magnetic stir plates), which had an L_n of 465 nm with PDI = 1.25 (Figure 1 a). To probe the seed stability on ageing and the kinetics of end-to-end recombination,[16] short seeds of $L_n \approx 60$ nm were incubated without any agitation. The length distributions of the seeds did not change significantly during the first two days (see inset in Figure 1 d), but the average fiber length gradually increased upon prolonged incubation (Figure 1 d; Supporting Information, Figure S3). Thus, the sheared short seeds can be used within two days after shearing, which is a convenient timeframe for seeding-mediated fiber growth experiments. In the absence of spontaneous nucleation, fiber growth kinetics should be solely dictated by the number of fiber ends. Thus, a solution of seeds that, at the same overall monomer concentration, are twice as short have twice the number of fiber ends and therefore should give an overall fiber growth at twice the rate. Indeed, comparing fiber growth (quantified by monitoring the increase in the concentration of hexamer with time) for two samples to which seeds were added that were sheared at 67 405 s^{-1} ($L_n = 61$ nm) or 16 850 s^{-1} ($L_n = 106$ nm) gave initial rates that were twice as fast for the shorter seeds (Figure 1 e; Supporting Information, Figure S4). The non-seeded and non-agitated control did not show any hexamer self-replication nor any fiber formation. Fiber growth was achieved by adding a solution containing mostly cyclic trimers and tetramers (Supporting Information, Figure S5 b), which was prepared by oxidizing the monomer building block to convert 95 % of the thiols into disulfides. The trimer/tetramer solution is stable for at least 7 days under an inert atmosphere and without agitation; that is, spontaneous fiber formation does not occur to a measurable extent in this period, presenting an important advantage over other seeding-mediated supramolecular polymerizations, where avoiding spontaneous aggregation requires diverting the material into another self-assembled state,[6a] which is not readily engineered. To solutions of the short seeds ($L_n = 60$ nm) we added different amounts of the trimer/tetramer mixture (0.25 equiv, 1.5 equiv, 4 equiv, and 9 equiv) and fibers were allowed to grow un-agitated. UPLC analysis[17] showed that at least 95 % of the total material in the mixture was converted into hexamer replicator (Supporting Information, Figure S5). Length distribution analysis based on TEM micrographs of the resulting fibers indicates a linear correlation between fiber length and the amount of trimer/tetramer added (Figure 1 f; Supporting Information, Figures S6, S7), with PDIs in the range of 1.04 to 1.07.

While mechanical shearing in a Couette cell physically breaks long fibers into short ones with a low PDI, we also developed a second approach to control seed lengths, based on chemical disassembly of fibers by reduction of the disulfide bonds of the macrocycles at the fiber ends mediated by dithiothreitol (DTT; Figure 2 a). DTT was added to hexamer seeds of $\mathbf{1_6}$ ($L_n = 61$ nm) in different amounts to cause a reduction in the concentration of $\mathbf{1_6}$ by 16 %, 31 %, and 42 %, as monitored by UPLC (Supporting Information, Figure S8). TEM analysis of the seed length distribution showed a linear decrease of L_n and L_w with increasing reduction (Figure 2 b). Thus, we produced seeds which are 13 %, 30 %, and 35 % shorter, respectively, than the minimum length that could be obtained by mechanical agitation alone, while retaining excellent PDI values (< 1.05 in all cases). Partial reduction was also performed on the fibers with $L_n = 106$ nm, successfully shortening these without adversely affecting PDI values (Figure 2 c; Supporting Information, Figure S9). Next, we added 1.5 equiv of the $\mathbf{1_3}/\mathbf{1_4}$ solution to the partially reduced seeds and allowed fibers to grow for 24 h without agitation in an inert atmosphere, to prevent thiol re-oxidation. TEM analysis of the resulting hexamer fibers shows the expected fiber growth and excellent PDI values (Figure 2 d), indicating that also chemically reduced seeds can be used to grow fibers with narrow polydispersity.

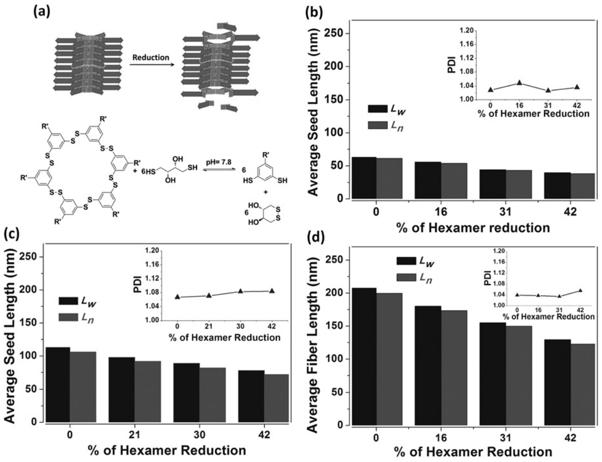


Figure 2. a) DTT-mediated shortening of fibers through reduction of the disulfide bonds. Average seed lengths and PDIs upon DTT-mediated partial reduction for the seeds produced at shear rates of b) 67 405 s^{-1} and c) 16 850 s^{-1}. Percentages of hexamer seed reduction were obtained from UPLC analyses. d) Controlled supramolecular polymerization performed by addition of 1.5 equiv of the $\mathbf{1_3}/\mathbf{1_4}$ mixture to seeds of different lengths as obtained by 16 %, 31 %, and 42 % hexamer reduction by DTT. Fiber elongation was carried out in inert atmosphere to prevent thiol re-oxidation by oxygen from the air. Average lengths of the resulting supramolecular fibers were analyzed by TEM after 24 h (Supporting Information, Figure S9). Insets show that PDIs are retained.

The living nature of the supramolecular polymerization[18] was confirmed by repeated addition of the $\mathbf{1_3}/\mathbf{1_4}$ mixture, which resulted in consecutive elongation of the fibers (Figure 3 a). Short seeds ($L_n = 60$ nm) and the $\mathbf{1_3}/\mathbf{1_4}$ mixture were mixed in a 1:1 ratio and the composition of the mixture was monitored over time by UPLC until the hexamer macrocycles accounted for at least 90 % of the total material in the system. Then half of the resulting fiber solution was removed and analyzed by TEM. To the remaining fiber solution a further equivalent of the $\mathbf{1_3}/\mathbf{1_4}$ mixture was added and the fiber growth and analysis steps were repeated. In this way four consecutive equivalents of the $\mathbf{1_3}/\mathbf{1_4}$ mixture were added. The rate of

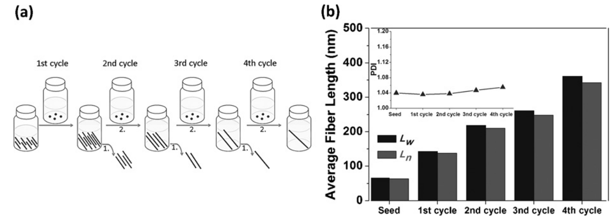


Figure 3. a) Representation of the growth of self-synthesizing fibers of $\mathbf{1}_6$ upon sequential addition of the $\mathbf{1}_3/\mathbf{1}_4$ mixture. In every cycle, half of the volume of fiber solution was removed and replaced by the $\mathbf{1}_3/\mathbf{1}_4$ mixture. (b) Weight-average length (L_w), number-average length (L_n), and PDI values (inset) of the seed and of the fibers after subsequent additions of the $\mathbf{1}_3/\mathbf{1}_4$ mixture.

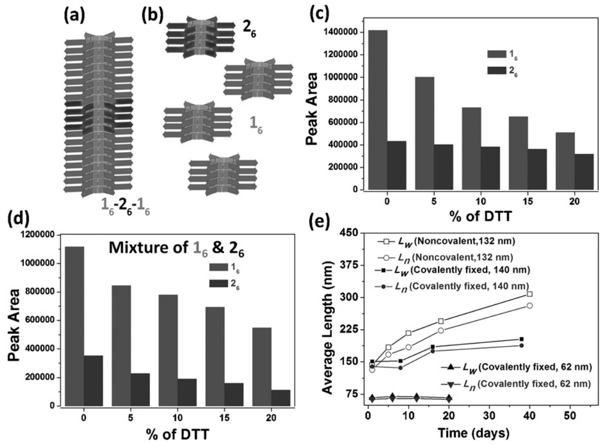


Figure 4. Cartoon representations of a) hexameric triblock co-fibers of $\mathbf{1}_6$-$\mathbf{2}_6$-$\mathbf{1}_6$ and b) composition of a control containing separate hexamer fibers of $\mathbf{1}_6$ and $\mathbf{2}_6$. Hexamer composition upon DTT-mediated partial reduction from the fiber ends for c) the supramolecular triblock co-polymer of composition $\mathbf{1}_6$-$\mathbf{2}_6$-$\mathbf{1}_6$, prepared using a $\mathbf{1}_3/\mathbf{1}_4$-mixture/seed (made from **2**) ratio of 3:1 and d) the mixture of separate hexamer fibers of $\mathbf{1}_6$ and $\mathbf{2}_6$ (molar ratio 3:1). e) Comparison of the time dependence of the average fiber lengths of noncovalent hexamer fibers (L_n = 132 nm) and covalently fixed fibers (L_n = 140 nm and 62 nm). Covalent fixation was done by irradiating the fibers with UV light (312 nm) for 12 h.

hexamer formation decreased with every cycle, consistent with the fact that in each cycle the concentration of the fiber ends was reduced by half (Supporting Information, Figure S10). TEM analysis of the fibers indicates an approximately constant increase in fiber length for each addition of the $\mathbf{1}_3/\mathbf{1}_4$ mixture while retaining excellent PDI values (ca. 1.04–1.06; Figure 3b; Supporting Information, Figures S11, S12).

One of the major advantages of living polymerizations is the precise control over mixed block co-polymer formation by growing the polymer from a first monomer, followed by addition of a second monomer. The living fiber ends of our system enabled us to prepare a supramolecular block co-polymer from building blocks **1** and **2**. We seeded the $\mathbf{1}_3/\mathbf{1}_4$ mixture with hexamer seeds of **2** ($\mathbf{2}_6$) ($\mathbf{1}_3/\mathbf{1}_4$-mixture:seed ratio = 3:1) to generate a mixed supramolecular triblock copolymer in which a central core of hexamers of $\mathbf{2}_6$ is flanked on both sides by hexamers of $\mathbf{1}_6$ (Figure 4a). Direct visualization of the different blocks within the co-polymer by TEM failed as the two blocks have comparable diameters and contrast (Supporting Information, Figure S13). However, formation of the mixed triblock co-polymer could be ascertained by partial reduction of the fiber ends using DTT (Figure 4c; Supporting Information, Figure S14). While monitoring the sample composition using UPLC, upon gradually increasing the extent of reduction from 0–20%, the UPLC peak area for $\mathbf{1}_6$ decreased steadily, while that of $\mathbf{2}_6$ remained almost unchanged. As a control, we mixed separately prepared hexamer fibers of $\mathbf{1}_6$ and $\mathbf{2}_6$ in a 3:1 ratio (Figure 4b). Upon reduction of this sample, the amounts of hexamer of $\mathbf{1}_6$ and $\mathbf{2}_6$ both decreased to comparable relative extents (Figure 4d; Supporting Information, Figure S15), indicating that both hexamers react at comparable rates with DTT. Thus, the data in Figure 4c are consistent with the central block being protected from reduction by the presence of the flanking blocks.

Finally, we used photochemistry to stabilize the fibers by covalent capture utilizing previously established procedures.[19] UV irradiation of fiber samples results in homolytic cleavage of the disulfide bonds. The resulting sulfur-based radicals can react with adjacent disulfide bonds, causing disulfide macrocycles to convert into larger disulfide oligomers, enhancing the stability of the fibers. Figure 4e and supplementary Figure S16 show how the L_w and L_n of the 140 and 62 nm long irradiated fibers change with time. Comparison with the corresponding data for non-irradiated controls (see Figure 1d for the 62 nm fibers and Figure 4e for the 132 nm long fibers) demonstrates that photo-irradiation indeed imparts additional stability on the fibers.

In conclusion, we have demonstrated that self-synthesizing systems allow access to supramolecular polymers of well-defined and controllable lengths, including block copolymers. We have also demonstrated that the resulting assemblies may be covalently fixed by photoirradiation. The fact that spontaneous self-assembly (that is, unassisted by seeds) is extremely slow is essential for the success of this approach. Nucleation of the assemblies constitutes a kinetic bottleneck when the self-assembling molecules are only minor species in the dynamic mixture prior to nucleation, while most of the materials remains kinetically trapped in the non-assembling trimer and tetramer macrocycles. Control over fiber length could be exerted by mechanical and chemical means to produce highly uniform and sufficiently stable seeds, from which well-defined materials can then be grown, with reversible covalent chemistry producing more of the assembling molecules as the growth proceeds. This work establishes a new strategy for the production of supramolecular polymers with well-controlled dimensions, combining the advantages of dynamic covalent chemistry and self-assembly.

Experimental Section

Library preparation and seeding: Dynamic combinatorial libraries were prepared by dissolving building block **1** in 50 mM potassium borate buffer to a final concentration of 3.8 mM. The pH of the resulting solution was adjusted to 7.8 by addition of small amounts of 2.0 M KOH solution. The library was then oxidized up to 70% using a freshly prepared solution of sodium perborate (40 mM, pH 7.8) to give a mixture of monomer, trimer and tetramer. This mixture was seeded with 20 mol% (in terms of equivalents of **1**) of a pre-formed library rich in the hexamer of $\mathbf{1_6}$ which had been continuously stirred at 1200 rpm. All libraries were contained in HPLC vials tightly closed with Teflon-lined snap caps. The libraries were stirred at 1200 rpm using a Teflon coated magnetic stirrer bar (5 × 2 mm, obtained from VWR), on an IKA RCT basic magnetic stir plate. Library compositions were monitored by UPLC.

Mixed-block supramolecular co-polymer formation: A solution containing pre-existing hexamer seeds of $\mathbf{2_6}$ (72 µL, 3.4 mM) was added to a solution of **1** containing mostly trimer and tetramer (192 µL, 3.8 mM) obtained by oxidizing a solution of **1** with sodium perborate. The mixture was incubated without agitation to give mixed block co-fibers $\mathbf{1_6}$-$\mathbf{2_6}$-$\mathbf{1_6}$. As a control sample, to a solution containing hexamer fibers of $\mathbf{1_6}$ (120 µL, 3.8 mM), a solution of pre-existing hexamer seeds of $\mathbf{2_6}$ (45 µL, 3.4 mM) was added and the mixture was incubated without agitation. Both samples were monitored by UPLC over a period of two days following the addition of the seeds.

Partial reduction of the mixed block co-fiber by DTT: An aliquot of 6 µL of mixed block co-fiber sample ($\mathbf{1_6}$-$\mathbf{2_6}$-$\mathbf{1_6}$) solution (3.69 mM) was added to 180 µL H_2O, and then different aliquots of 0.58, 1.15, 1.73, 2.31 µL of DDT solution (1.9 mM) were added to reduce 5, 10, 15, or 20% of the fibers, respectively. The solution was incubated for 25–30 min and then analyzed by UPLC.

Keywords: block co-fibers · dynamic covalent chemistry · nucleation and growth · self-replication · systems chemistry

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