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PFS-\textit{b}-PNIPAM: A first step towards polymeric nanofibrillar hydrogels based on uniform fiber-like micelles

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ABSTRACT

Amphiphilic crystalline-coil di-block copolymers polyferrocenyldimethylsilane-block-poly(N-isopropylacrylamide) of two different block ratios (PFS\textsubscript{56}-b-PNIPAM\textsubscript{190} and PFS\textsubscript{26}-b-PNIPAM\textsubscript{520}) were synthesized by a copper-catalyzed azide-alkyne coupling reaction. They exhibited pronounced differences in self-assembly in alcohol solvents. While PFS\textsubscript{56}-b-PNIPAM\textsubscript{190} formed mixtures of spherical and rod-like micelles in ethanol and 2-propanol, PFS\textsubscript{26}-b-PNIPAM\textsubscript{520} formed long fibers of uniform width in these solvents. We used a seeded growth protocol to grow rod-like PFS\textsubscript{26}-b-PNIPAM\textsubscript{520} micelles of uniform lengths. There were two surprising features of this experiment: First, micelle growth was unusually slow and required a long aging time (40 days) for them to reach their final length. Second, the micelles were characterized by a low number of polymer chains per unit length as determined by multiangle light scattering. This result suggests a loose packing of PFS chains in the micelle core. In an attempt to prepare thermoresponsive nanofibrillar hydrogels from these micelles, we explored approaches to transfer them from 2-propanol to water. These attempts were accompanied by extensive fragmentation of the micelles. We believe the fragility of these micelles is related to the loosely packed nature of the PFS chains in the micelle core. Fragmentation may also be affected by the co-nonsolvency effect of 2-propanol-water mixtures on the PNIPAM corona of the micelles. We could show, however, that the micelle fragments in water retained their anticipated thermoresponsive behavior.
INTRODUCTION

Hydrogels are three-dimensional cross-linked networks swollen with water. They have numerous applications ranging from tissue engineering scaffolds,\textsuperscript{1,2} drug delivery vehicles\textsuperscript{3,4} to cosmetics and food.\textsuperscript{5,6} Compared to chemically cross-linked gels which consist of covalently-bonded networks, physical gels are reversibly cross-linked via non-covalent interactions such as hydrogen bonding, hydrophobic interactions and host-guest interactions.\textsuperscript{7} This property makes physical gel attractive for applications where a non-permanent network is needed.\textsuperscript{8} Both individual molecules and supramolecular assemblies of many molecules can serve as building blocks to induce physical gelation. In the latter case, the assemblies could be spherical micelles\textsuperscript{9,10} or high-aspect-ratio nanofibrils.\textsuperscript{11} For example, many biological polymers like proteins and polysaccharides form hydrogels by reversible association of nanofibrils comprised of multiple polymer chains.\textsuperscript{12,13} The association of nanofibrils into a network can be triggered by changes in temperature or ionic strength, or by the entanglement of nanofibrils at high concentration.\textsuperscript{14,15} The nanofibrillar nature of these hydrogels play a pivotal role in their formation, mechanical properties, and biological activity.\textsuperscript{16} The unique properties and abundant applications of the naturally derived nanofibril hydrogels have attracted chemists to design and prepare synthetic analogues, that is, hydrogels formed by polymeric fiber-like micelles.\textsuperscript{17}

A well-established method to generate polymeric fiber-like micelles is through solution self-assembly of amphiphilic block copolymers in selective solvents.\textsuperscript{18} These fiber-like structures have lengths of a hundred nanometers to tens of micrometers,\textsuperscript{19} and range in stiffness from flexible filamentous worms to rigid rods. Over the past few years, new approaches have been reported for the preparation of fiber-like micelles. Controlled radical polymerization with macroinitiators in aqueous media can lead to dispersions of nanoparticles, also known as polymerization-induced self-assembly (PISA).\textsuperscript{20} Armes and coworkers\textsuperscript{21} have explored the synthesis and applications of fiber-like micelles of poly(hydroxypropyl methacrylate) (PHPMA) generated by RAFT dispersion polymerization in water These fibers, at modest concentrations and slightly elevated temperatures, form thermally reversible hydrogels. The Monteiro group prepared fiber-like micelles with a rigid and glassy polystyrene (PS) core surrounded by a corona of poly(N-isopropyl acrylamide) (PNIPAM).\textsuperscript{22} PNIPAM in water has a lower critical solution temperature (LCST) in the range of 30 – 35 °C, and thus the corona chains undergo a volume-phase (collapse) transition for solutions warmed to higher temperatures. Solutions of these fiber-
like micelles formed hydrogels upon heating. The sol-gel process was reversible over multiple heating and cooling cycles. In a subsequent paper, Kumacheva and coworkers used microfluidics to prepare microgels from these micelles and utilized them to trap living cells at 37 °C.

The PS-b-PNIPAM micelles employed by the Monteiro and Kumacheva groups were characterized by a broad distribution of lengths. We are more interested in hydrogel formation by fiber-like micelles that are uniform in length. These types of uniform micelles can, in principle, be prepared by crystallization driven self-assembly, in which the driving force for micelle formation is the crystallization of the core-forming block. Core crystallization promotes the formation of low curvature nanostructures such as fibers and platelets. Self-assembly has been studied for numerous examples of block copolymers with a crystallizable core-forming block. Examples include poly(ferrocenyldimethylsilane) (PFS), polycaprolactone, poly(L-lactide), polyethylene, polyacrylonitrile, polythiophene, and polyselenophene. In the current state-of-the-art, we have the greatest understanding and the greatest degree of control over rod-like micelle formation of PFS diblock copolymers. Using the seeded growth protocol, one adds a concentrated solution of unimer in a good solvent to a suspension of micelle seed fragments in a selective solvent. In this way, one can obtain uniform micelles in which the length is determined by the ratio of the amount of unimer added to the amount of seeds initially present. Thus we set out to synthesize PFS-b-PNIPAM diblock copolymers, to create dispersions of uniform nanorods in water, and to examine their behavior as the PNIPAM passed through its LCST.

This paper tells the story of this effort. We were able to synthesize the diblock copolymer by copper-catalyzed coupling of an alkyne-end-capped PFS with samples of azide-terminated PNIPAM. As expected, direct self-assembly of PFS-b-PNIPAM in water did not give useful results, either by heating aqueous suspensions of the polymer or by the “solvent switch” method using mixtures of THF and water. Over the past decade, we have had no success with this approach for PFS diblock copolymers with a variety of water-soluble co-blocks, including polyethylene glycol, poly(N,N’-dimethylaminoethyl methacrylate), and most recently poly(allyl glycidyl ether) decorated with triethylene glycol (TEG), abbreviated as PFS-b-(PEO-g-TEG). In the case of PFS-b-(PEO-g-TEG), micelles of uniform length could be generated in
dimethylformamide and then transferred to water. We found that uniform PFS-\(b\)-PNIPAM micelles could be prepared by seeded growth in ethanol or in 2-propanol, but the self-assembly of this polymer was full of surprises. For example, the micelle growth rate was exceptionally slow, requiring weeks. In addition, static and dynamic light scattering measurements suggest that the PFS in the micelle core is not densely packed. These are features of the self-assembly that we examine in detail. The third surprise is that all attempts to transfer these micelles to water resulted in micelle fragmentation. We explore whether co-nonsolvency of alcohol-water mixtures for PNIPAM played an important role in the fragmentation process.

**EXPERIMENTAL SECTION**

**Materials**

The details of the synthesis and characterization of PFS\(_{56}\)-\(b\)-PNIPAM\(_{190}\) and PFS\(_{26}\)-\(b\)-PNIPAM\(_{520}\) are described in Supporting Information (SI).

**Instrumentation**

Transmission electron microscopy (TEM) measurements were performed on a Hitachi D-7000 microscope operating at an accelerating voltage of 100 kV operating in the bright-field TEM mode. Samples were prepared by placing a drop of solution on a Formvar-coated TEM grid and removing excess liquid with the edge of a filter paper. Images were analyzed with the software Image J (NIH, USA), with ca. 100 micelles traced by hand into the software for each sample. Values of the number (\(L_n\)) and weight average (\(L_w\)) lengths were calculated as described in SI.

Simultaneous static (SLS) and dynamic (DLS) laser light scattering measurements were carried out with an ALV/SP-125 light scattering spectrometer equipped with an ALV-5000 multitau digital time correlator and a He-Ne laser (output power = 35 mW at \(\lambda_0 = 632.8\) nm). Data were collected in increments of 2° for scattering angles of 20° to 60°, 3° increments for scattering angles of 63° to 90°, and 5° increments between 95° and 150°. To determine the molecular weight of PNIPAM\(_{520}\), the Zimm plot (see Figure S3) for the polymer in water was analyzed using the value \(dn/dc = 0.167\) mL/g.\(^{34}\) To analyze SLS experiments of micelles formed by PFS\(_{26}\)-\(b\)-PNIPAM\(_{520}\), \(dn/dc\) values for the block copolymer were calculated from reported \(dn/dc\) values of PFS in ethanol (0.253 mL/g) and in 2-propanol (0.240 mL/g),\(^{35}\) and values of \(dn/dc\) for PNIPAM determined here. These values (0.136 mL/g in ethanol; 0.119 mL/g in 2-propanol) were determined indirectly by measuring the refractive index of a series of PNIPAM
solutions of known concentration in each solvent. Temperature-dependent DLS measurements were carried out with a Malvern Zetasizer Nano ZS instrument at a backscattering angle of 173°.

**Micelle formation**

Micelles were prepared by suspending block copolymer in a solvent at a concentration of 0.2 mg/mL in a 7 mL vial. The vials were placed in an oil bath at 80 °C for 1 h, followed by slow cooling in which the oil bath was allowed to cool to room temperature (RT, 23 °C). Subsequently, the solutions were aged for various times. For seeded growth experiments, samples of micelles of PFS\textsubscript{26}-b-PNIPAM\textsubscript{520} in ethanol or in 2-propanol (0.2 mg/mL) was placed into a 70 watt ultrasonic cleaning bath and sonicated for 30 min at 23 °C to prepare seed solutions. For each seed solution, three replicates of 2 mL solution were transferred to new vials. Aliquots (8 µL, 20 µL and 32 µL) of PFS\textsubscript{26}-b-PNIPAM\textsubscript{520} in THF (10 mg/mL), referred to as “unimer” solutions, were added into each seed solution and swirled for 10s. Then these solutions were aged in the dark for 3 days or 40 days prior to preparing grids for TEM measurements. To test whether the micelles continued to grow after 40 days, selected samples were allowed to age for an additional 50 days, followed by examination by TEM.

**Micelle transfer to water**

Two different methods were adopted to transfer micelle of uniform length to water. i) A solution of micelles approximately 1 µm long was prepared by seeded growth in 2-propanol. An aliquot (1 mL, 0.2 mg/mL) was placed in a pre-activated dialysis cassette (cut-off M\textsubscript{w} = 3,500) and dialyzed against DI water over three days, with water changed every day. The final solution was withdrawn into a new 7 mL vial. ii) Another aliquot (0.1 mL) of the solution of these 1 µm long micelles in 2-propanol was injected with a syringe pump into 1.9 mL water at 0 °C at a rate of 1 mL/h. The solution was allowed to warm to room temperature slowly and characterized by TEM. iii) A control experiment was carried out with another micelle sample in 2-propanol (0.1 mL, 0.18 mg/mL, also 1 µm long). This sample was injected with a syringe pump into 1.9 mL 2-propanol at 0 °C at a rate of 1 mL/h. The solution was allowed to warm to room temperature slowly and characterized by TEM.

**RESULTS AND DISCUSSION**

**Polymer synthesis.** Two alkyne-terminated poly(ferrocenyl(dimethyl)silane) samples were synthesized by anionic ring-opening polymerization described elsewhere.\textsuperscript{36} The degree of
polymerization (DP<sub>n</sub>) for each sample was determined to be 26 and 56 by 1H NMR end-group analysis.

Azide-terminated PNIPAM (1, 2) was synthesized by atom transfer radical polymerization (ATRP) as shown in Scheme 1. The azide-functionalized initiator 2-(2-azidoethoxy) ethyl bromoisobutyrate with Cu(I)/Me₆TREN (tris[2-(dimethylamino)ethyl]amine) as the catalyst was chosen to polymerize NIPAM in a water/DMF mixture at room temperature. The reaction can reach 80% monomer conversion in 10 min. By varying the monomer/initiator ratio, we synthesized two PNIPAM samples of different DP<sub>n</sub> with narrow polydispersity as determined by GPC. For the shorter polymer (PNIPAM<sub>190</sub>), we determined DP<sub>n</sub> = 190 by 1H NMR end-group analysis, comparing the integration of the backbone CH protons next to the amide groups to that of the (−CH₂−O−CH₂−) on the initiator fragment (Figure S1, SI). For the longer polymer (PNIPAM<sub>520</sub>), 1H NMR end-group analysis did not give a meaningful value of DP<sub>n</sub> because the end group signal was too weak (Figure S2). We used static light scattering (SLS) to determine the absolute weight-averaged molecular weight. Four aqueous solutions of the polymer with increasing concentrations were prepared and examined at multiple angles. From the fit of the Zimm plot (Eq. S4 and Figure S3) we obtained <i>M</i><sub>w</sub> = 67,000 g/mol. This value was combined with D = 1.14, determined by GPC, to obtain DP<sub>n</sub> = 520. The PNIPAM<sub>520</sub> sample showed a small peak in the GPC (RI signal) at a retention time consistent with twice the molecular weight. We attribute this peak to a small extent of radical terminated side product during the synthesis. No further purification was carried out as this by-product will not affect the click-coupling reaction.

Two block co-polymers, PFS<sub>56</sub>-<i>b</i>-PNIPAM<sub>190</sub> and PFS<sub>26</sub>-<i>b</i>-PNIPAM<sub>520</sub> were synthesized by copper-catalyzed alkyne-azide “click” coupling at 40 °C in the presence of CuBr/pentamethyldiethylenetriamine (PMDETA) as a catalyst. The coupling reaction was monitored by GPC equipped with RI and UV-Vis detectors, using 450 nm detection to monitor the PFS component in each sample. Figure S5 shows traces of the two mixtures of homopolymers prior to coupling, superimposed on the reaction product after coupling. The coupling reaction led to a nearly complete disappearance of the PFS-alkyne peaks accompanied by a shift to shorter retention time for the block copolymer (Figure S5). To remove traces of PFS homopolymer from the crude product, hexanes were added slowly into a concentrated solution of the reaction product in THF. The diblock copolymer precipitated, leaving traces of the PFS homopolymer dissolved in the solvent mixture. As suggested above, the higher molecular weight
PNIPAM by-product does not appear to interfere with the alkyne-azide coupling reaction, which gives a symmetrical monomodal peak in the GPC monitored at 450 nm (Figure S5D). $^1$H-NMR analysis (Figure S4) of the diblock copolymers, comparing the integration of Cp signals at 4.1 and 4.3 ppm to PNIPAM (-CO-NH-$CH(CH_3)_2$ signals at 4.0 ppm, gave block ratios consistent with the relative DP$_n$ values of the two blocks. We conclude that the amount of homopolymer impurity in the samples is negligible, and no further purification was carried out on the polymers. Details of the characteristics of each polymer are presented in Table S1.

![Scheme 1](image)

**Scheme 1.** Synthesis of PFS$_{56}$-b-PNIPAM$_{190}$ and PFS$_{26}$-b-PNIPAM$_{520}$ by a combination of ATRP and copper-catalyzed azide-alkyne coupling.

**Self-assembly of PFS-b-PNIPAM in solution.** Initial self-assembly experiments with PFS$_{56}$-b-PNIPAM$_{190}$ (3) and PFS$_{26}$-b-PNIPAM$_{520}$ (4) involved attempts to employ water as the selective solvent. These experiments did not lead to well-defined structures. For example, heating small amounts of polymer in water (e.g., to 80 °C) followed by slow cooling and aging for one week at RT, or simply stirring samples in water at RT did not disperse the samples. Adding polymer dissolved in THF to an excess of water or adding water to polymer dissolved in THF led to turbid solutions but no interesting self-assembly products. We attribute these results to the poor solubility of PFS in water. As a consequence, we turned our attention to self-assembly experiments in alcohol solvents, where we have had good success with other PFS block copolymers such as PFS-$b$-P2VP (P2VP = poly(2-vinylpyridine)).$^{37}$
We began with \( \text{PFS}_{56}^-b^-\text{PNIPAM}_{190} \) in methanol, ethanol and 2-propanol. Small amounts of polymer were added to each solvent (2 mL samples, ca. 0.2 mg/mL). The mixtures were heated to 80 °C for 10 min, then slowly cooled to RT. The TEM images in Figure 1, show only spherical micelles for \( \text{PFS}_{56}^-b^-\text{PNIPAM}_{190} \) in methanol and ethanol after aging for 1 day, while a mixture of spherical and cylindrical micelles were formed in 2-propanol. We attribute the dark core of the unstained micelles to the PFS component, and find that the PFS cores of both the spherical and cylindrical structures have diameters \( d \approx 30 \) nm. These micelle solutions were then further aged for two months and re-examined by TEM. For \( \text{PFS}_{56}^-b^-\text{PNIPAM}_{190} \) in methanol, no detectable change was observed, while the sample in ethanol showed a mixture of cylindrical micelles on the grid, along with the spherical micelles. For the sample in 2-propanol, one still sees a mixture of spherical and cylindrical objects, but the cylindrical micelles became longer as a consequence of aging.

![Figure 1. TEM images of the micelles formed by \( \text{PFS}_{56}^-b^-\text{PNIPAM}_{190} \) in methanol (A), (B), ethanol (C),(D), and 2-propanol (E), (F). Samples were heated to 80 °C for 10 min, cooled slowly to RT, and then aged (A), (C), (E) 1 day and (B),(D),(F) 60 days. Scale bars are 500 nm.](image)

This type of behavior has been observed previously for PFS-b-P2VP self-assembled in alcohol media.\(^{38,35}\) For example, \( \text{PFS}_{23}^-b^-\text{P2VP}_{230} \) formed spherical micelles with an amorphous
PFS core in methanol and elongated fiber-like micelles with a crystalline PFS core in 2-propanol. In ethanol, the spherical micelles that formed initially evolved over a year to very long fiber-like structures. We infer that lower polarity solvents promote the solubility and crystallization of PFS. The very slow transformation of the amorphous spherical micelles to elongated core-crystalline micelles is likely a consequence of the limited solubility of the PFS block copolymer in ethanol compared to 2-propanol.35

\( PFS_{26-b-PNIPAM_{520}} (4) \) has a significantly shorter PFS block and a longer PNIPAM corona-forming block than \( PFS_{56-b-PNIPAM_{190}} (3) \). Under the same self-assembly protocol described above, with aging at RT for 3 days, this polymer formed only long (> 5 µm) fiber-like micelles for all the three solvents, as shown in the TEM images in Figure 2. We attribute the enhanced formation of cylindrical micelles to the higher solubility of this polymer compared to polymer 3 in methanol, ethanol, and 2-propanol. A summary of the self-assembly results of both PFS-b-PNIPAM block copolymers is listed in Table S2.

![Figure 2. TEM images of the fiber-like micelles formed by PFS_{26-b-PNIPAM_{520}} in (A) methanol, (B) ethanol and (C) 2-propanol. Samples were heated to 80 °C for 10 min, cooled slowly to RT, and then aged three days. Scale bars are 500 nm.]

**Micelle formation by seeded growth.** Because samples of \( PFS_{26-b-PNIPAM_{520}} \) exhibited well-behaved self-assembly in alcohol media, this polymer was selected for seeded growth experiments. In this type of experiment, long micelles are subjected to sonication to obtain micelle fragments.39 When additional polymer as a concentrated solution in a common good solvent is added to a suspension of the micelle fragments in a selective solvent, the fragments can serve as seeds for the epitaxial growth of the newly added polymer, leading to longer micelles uniform in length and width. Here we examine seeded growth in ethanol and in 2-propanol.
In the first step, we used mild sonication to obtain seed crystallite fragments (Figure 3A, Figure 4A), characterized (TEM) by $L_{\text{seed},0} = 69.2$ nm in ethanol (Figure 3E) and $L_{\text{seed},0} = 101$ nm, in 2-propanol (Figure 4E). We have carried out many sonication experiments over the past several years to obtain micelle fragments for seeded growth $^{40,41}$ and have never found that the fragment lengths or length distribution evolved over time. Here, however, we looked at the seed samples at longer aging times in order to assure ourselves that the growth was complete. To our surprise, we found that the micelle fragments continued to grow. The solutions were reexamined after aging 40 days. In ethanol the mean length increased from $L_{\text{seed},0} = 69$ to $L_{\text{seed},40} = 84$ nm, and in 2-propanol it increased from $L_{\text{seed},0} = 101$ to $L_{\text{seed},40} = 125$ nm. These results indicate that the seed solutions obtained after sonication, as examined by TEM, had not evolved to their final length. Based on experiments described below, we presume that these solutions contained unimer that grew only slowly onto the micelle fragments formed by sonication. At this time, we do not know whether unimer was present in the samples of the long micelles prepared in ethanol or 2-propanol, or whether it formed by partial dissolution during the sonication-fragmentation step.

In the seeded growth experiments, samples of the freshly prepared micelle fragment seed solution were diluted to $c = 0.020$ mg/mL. Then different amounts of unimer solutions in THF (10 mg/mL) were added to aliquots of the seed solution and allowed to age for various times. It is useful to define a parameter $p$ equal to the ratio of the mass of unimer added in a given experiment to the mass of seed present in that solution. In previous examples from our laboratory with other PFS diblock copolymers, micelles prepared by seeded growth approached their final length after 24 h and were typically allowed to age three days to ensure complete deposition of unimer onto the growing micelles. Under these circumstances, the final length of the micelles could be predicted from the magnitude of $p$ and the mean length of the seed fragments. Because we found that the seed micelles in the seed solution continued to grow after 3 days, this analysis has to be revised as described below. In order to monitor the lengths of the micelles formed by seeded growth, examined the micelles after 3 days and again after 40 days.

Figure 3 shows results from experiments in ethanol. Panels 3B-D show representative TEM images of grids prepared from micelle solutions after 3 days aging accompanied by length distribution histograms in Panels 3F-H. Results after aging 40 days are presented in panels 3J-L and 3N-P. Figure 4 shows the results for similar seeded growth experiments in 2-propanol. In both sets of experiments, one can see that after 3 days, longer micelles of relatively uniform
length formed as more unimer was added to a fixed amount of seed crystallites. There was, however, a large further increase in micelle length after aging the micelle suspensions for 40 days. We would like to emphasize, as mentioned in the previous paragraph, how unexpected it was to find such a slow increase in length in a seeded growth experiment. This situation is very different from the observation of slow micelle evolution for PFS-b-P2VP in ethanol, where the solution contains a mixture core-crystalline rod-like micelles and amorphous spherical micelles. For this example, we believe that the slow dissolution of polymer from the insoluble amorphous material acts as the limiting step in the growth of the rod-like micelles.

To ensure that 40 days was sufficient for complete unimer deposition on seeds present in solution, we re-examined the micelle solutions in ethanol and 2-propanol after aging for 90 days. Analysis of these TEM images (Figure S7) showed that no further growth in length had occurred.
Figure 3. TEM images of PFS_{26-b}-PNIPAM_{520} micelles formed by seeded-growth in ethanol after aging for 3 days (B-D) and 40 days (I-L). (A) Seed micelles after sonication of fiber-like long micelles in ethanol ($c = 0.21$ mg/mL). (B-D) Uniform micelles of different lengths aged for three days in ethanol. (E-H) Length distribution histograms of the micelles shown in (A-D). (E) $L_n = 69.2\text{ nm}$, $L_w = 79.4\text{ nm}$, $L_w/L_n = 1.14$, and $\sigma/L_n = 0.39$. (F) $L_n = 204\text{ nm}$, $L_w = 213\text{ nm}$, $L_w/L_n = 1.04$, and $\sigma/L_n = 0.21$. (G) $L_n = 375\text{ nm}$, $L_w = 385\text{ nm}$, $L_w/L_n = 1.03$, and $\sigma/L_n = 0.16$. (H) $L_n = 552\text{ nm}$, $L_w = 563\text{ nm}$, $L_w/L_n = 1.02$, and $\sigma/L_n = 0.15$. (I) Seed micelles after aging for 40 days in ethanol ($c = 0.21$ mg/mL). (J-L) Uniform micelles of different lengths aged for 40 days in ethanol. (M-P) Length distribution histograms of the micelles shown in (E-H). (M) $L_n = 125\text{ nm}$, $L_w = 131\text{ nm}$, $L_w/L_n = 1.05$, and $\sigma/L_n = 0.23$. (N) $L_n = 364\text{ nm}$, $L_w = 377\text{ nm}$, $L_w/L_n = 1.04$, and $\sigma/L_n = 0.19$. (O) $L_n = 708\text{ nm}$, $L_w = 724\text{ nm}$, $L_w/L_n = 1.02$, and $\sigma/L_n = 0.15$. (L) $L_n = 1106\text{ nm}$, $L_w = 1122\text{ nm}$, $L_w/L_n = 1.01$, and $\sigma/L_n = 0.12$. Scale bars are all 500 nm.
Figure 4. TEM images of PFS_{26}-b-PNIPAM_{520} micelles formed by seeded-growth in 2-propanol after aging for 3 days (B-D) and 40 days (I-L). (A) Seed micelles after sonication of fiber-like long micelles in 2-propanol (c = 0.18 mg/mL). (B-D) Uniform micelles of different lengths aged for 3 days in 2-propanol. (E-H) Length distribution histograms of the micelles shown in (A-D). (E) $L_n = 83.8$ nm, $L_w = 90.4$ nm, $L_w/L_n = 1.08$, and $\sigma/L_n = 0.28$. (F) $L_n = 252$ nm, $L_w = 265$ nm, $L_w/L_n = 1.05$, and $\sigma/L_n = 0.23$. (G) $L_n = 474$ nm, $L_w = 488$ nm, $L_w/L_n = 1.03$, and $\sigma/L_n = 0.17$. (H) $L_n = 688$ nm, $L_w = 703$ nm, $L_w/L_n = 1.02$, and $\sigma/L_n = 0.15$. (I) Seed micelles aged for 40 days in 2-propanol (c = 0.18 mg/mL). (J-L) Uniform micelles of different lengths aged for 40 days in 2-propanol. (M-P) Length distribution histograms of the micelles shown in (I-L). (M) $L_n = 101$ nm, $L_w = 108$ nm, $L_w/L_n = 1.06$, and $\sigma/L_n = 0.25$. (N) $L_n = 273$ nm, $L_w = 283$ nm, $L_w/L_n = 1.03$, and $\sigma/L_n = 0.19$. (O) $L_n = 503$ nm, $L_w = 512$ nm, $L_w/L_n = 1.02$, and $\sigma/L_n = 0.14$. (P) $L_n = 733$ nm, $L_w = 747$ nm, $L_w/L_n = 1.02$, and $\sigma/L_n = 0.14$. Scale bars are all 500 nm.
The implication of these results is that the initial seed solution contained more unimer and a smaller mass of seeds than what we expected. For example, if the seeds formed immediately upon sonication maintained their length, we could calculate a “theoretically expected” length ($L_{ex}$) from the ratio $p = p_{ex} = \frac{\text{amount of added unimer}}{\text{mass of seed crystallites}}$ presumed to be present in the solution.

$$L_{ex} = \left(\frac{m_{uni,added}}{m_{seed,ex}} + 1\right) \times L_{seed,0} = (p_{ex} + 1) \times L_{seed,0} \quad (1)$$

This analysis assumes that the mass per unit length of the micelles does not change. When we carried out this analysis using $p_{ex}$ and the values of $L_{seed,0}$ obtained immediately after sonication, we found values of the mean micelle length after 40 days ($L_{40}$) larger than the $L_{ex}$ values predicted by eq. 1 (Figure S6).

In order to correct the predicted micelle lengths in each solvent, we need to consider not only the increase in length of the seeds but also how the unimer present in the seed solution affects the value of $p$. Using results for the increase in the mean length of seeds ($L_{seed,40}$) after 40 days, we can calculate a corrected mass of seeds ($m_{seed,co}$).

$$m_{seed,co} = \frac{L_{seed,0}}{L_{seed,40}} \times m_{seed,ex} \quad (2)$$

Similarly, we can calculate a corrected value for the total amount of unimer ($m_{uni,co}$) present at the beginning of each seeded growth experiment.

$$m_{uni,co} = \left(1 - \frac{L_{seed,0}}{L_{seed,40}}\right) \times m_{seed,ex} + m_{uni,added} \quad (3)$$

In this way, a corrected predicted length ($L_{co}$) can be calculated.

$$L_{co} = \left(\frac{m_{uni,co}}{m_{seed,co}} + 1\right) \times L_{seed,0} = (p_{co} + 1) \times L_{seed,0} \quad (4)$$

where the ratio $p_{co} = \frac{m_{uni,co}}{m_{seed,co}}$ is the total amount of unimer to the corrected mass of seeds ($m_{seed,co}$) in the solution. From eq 1-4, we can also derive a relationship between $p_{co}$ and $p_{ex}$.

$$p_{co} = \frac{L_{seed,40}}{L_{seed,0}} \times (p_{ex} + 1) - 1 \quad (5)$$

In Figure 5 we plot the measured $L_n$ values for micelle samples aged 40 days against the corrected $p$ values ($p_{co}$) and find excellent agreement with the theoretical values predicted by eq 4.
Figure 5. Number average length $L_n$ of the PFS$_{26}$-b-PNIPAM$_{520}$ micelles obtained in seeded growth experiments plotted against the corrected unimer-added-to-seed ratio ($p_{co}$) for experiments in ethanol (A) and 2-propanol (B) after aging for 40 days. The dashed line represents the predicted lengths (eq 4) based on the assumptions that (i) all additional polymer grew onto the seed micelles without forming new micelles and (ii) the linear aggregation number did not change during the micelle growth.

**Laser light scattering measurements.** Simultaneous multi-angle static (SLS) and dynamic (DLS) light scattering measurements were carried out on micelle samples aged 40 days in ethanol and 2-propanol (diluted to $c\sim 0.05$ mg/mL) to obtain a deeper understanding of the micelle structure. For elongated structures like rigid rods, SLS data are well represented by Holtzer-Casassa (HC) plots of $qR_θ/πM_0Kc$ as a function of $q$ (eq S6), where the scattering vector $q = (4πn/λ_0)/\sin(θ/2)$. $R_θ$ is the Rayleigh ratio; $λ_0$ is the wavelength in vacuum of the laser light; $K = 4π^2n^2(dn/dc)^2/(N_Λλ_0^4)$ is an optical constant, and $M_0$ is the molecular weight of the block copolymer. These plots reach a plateau at high $q$ whose magnitude is a measure of the mass per unit length of the object. The HC plots are presented in Figure 6.
Three important parameters can be determined from these plots: (i) the weight average length of the micelle $L_{w,40}$, (ii) the radius of the cylinder cross section, $R_{SLS}$, and (iii) the number of block copolymers per unit length of the micelles, a value we refer to as the linear aggregation number, $N_{agg/L}$. These values are summarized in Table 1. From the table we can see that values of $L_w$ obtained from the HC plots are in good agreement with corresponding values obtained from analysis of TEM images of the same micelle solutions. This provides confidence in the quality of the fits. Values of $R_{SLS} = 17$ nm are essentially identical for micelles in both solvents and of different lengths. $R_{SLS}$ is sensitive both to the core radius and to the distribution of corona segments around the core. The most surprising results are the values of $N_{agg/L}$, which are approximately 0.95 chains/nm for PFS$_{26}$-b-PNIPAM$_{520}$ micelles grown in ethanol and 0.75 chains/nm for micelles grown in 2-propanol. Previous examples examined in our laboratory were characterized by much larger values of $N_{agg/L}$, on the order of 2 to 3 PFS block copolymer molecules per nm. For example, we have reported values of 1.9 chains/nm for PI$_{1000}$-b-PFS$_{50}$, 2.0 chains/nm for PFS$_{35}$-b-P2VP$_{400}$ and 3.2 chains/nm for PFS$_{76}$-b-PDMS$_{456}$. While we do not yet have a clear understanding of what factors affect the magnitude of $N_{agg/L}$, the low value found for this PFS$_{26}$-b-PNIPAM$_{520}$ sample may point to important differences in how the PFS chains pack in the semicrystalline micelle core.

For comparison, in Figure S8, we show Holtzer-Casassa plots of measurements made on the micelle solutions aged 3 days and collect values of the weight average lengths ($L_{w,3}$) determined by SLS and TEM in Table S4. While these values agree with each other, they are shorter than those obtained on samples aged 40 days.
Figure 6. Holtzer-Casassa plots of $q R / \pi M_0 K_c$ as a function of $q$ for the micelles in ethanol (A) and 2-propanol (B) after aging for 40 days. The different color data points refer to PFS$_{26}$-b-PNIPAM$_{520}$ micelles formed at different corrected ratios of unimer-added-to-seed ($p_{co}$). Each line represents the best fit of the corresponding data set to eq. S6 for thick rigid rods.

The corresponding DLS data on micelle samples aged 40 days provide information on fluctuations in the scattered intensity due to Brownian motion. Values of the autocorrelation decay rates ($\Gamma_1$) at different scattering angles were analyzed as plots of $(\Gamma_1 / q^2)$ as a function of $qL$ (Figure S9) based on a model developed by Wilcoxon and Schurr (eq S10). For $L$ we used values of $L_w$ obtained from the SLS data. In this way we calculated characteristic hydrodynamic radii $R_{DLS}$ for the micelles. For micelles in ethanol, we found $R_{DLS} = 31$ nm and $R_{DLS} = 33$ nm for micelles in 2-propanol, independent of the micelle length. The data (collected in Table 1) are internally consistent, which provides confidence in both the scattering data and the data analysis. The fact that $R_{DLS}$ is much larger than $R_{SLS}$ is expected and easily explained. $R_{SLS}$ depends on the mass distribution perpendicular to the micelle. As the corona chains spread out from the core surface, they become more diffuse and swollen by the solvent, thus decreasing their contribution.
to the mass distribution. In contrast, chain segments far from the corona surface contribute hydrodynamic resistance to micelle motion and contribute to the magnitude of $R_{DLS}$.

**Table 1. Summary of structural parameters characterized by TEM and light scattering of micelles aged for 40 days in ethanol and in 2-propanol**

| Solvent    | $p_{co}$ | $L_{w,40 \text{TEM}}$ (nm) | $L_{w,40 \text{SLS}}$ (nm) | $N_{\text{agg,L}}$ (chains/nm) | $R_{\text{SLS}}$ (nm) | $R_{DLS}$ (nm) |
|------------|----------|----------------------------|----------------------------|-------------------------------|------------------------|-----------------|
| Ethanol    | 2.6      | 265                        | 270                        | 0.99                          | 17                     | 29              |
|            | 5.8      | 488                        | 460                        | 0.97                          | 17                     | 31              |
|            | 9.1      | 703                        | 680                        | 0.94                          | 17                     | 31              |
| 2-Propanol | 2.7      | 377                        | 360                        | 0.75                          | 17                     | 33              |
|            | 6.1      | 724                        | 720                        | 0.73                          | 17                     | 33              |
|            | 9.7      | 1122                       | 1120                       | 0.76                          | 17                     | 33              |

**Complications in micelle transfer to water.** As we stated in the introduction, the original goal of this project was to prepare nanofibrillar hydrogels with rod-like micelles of controlled and uniform length. Since direct assembly of PFS$_{26}$-b-PNIPAM$_{520}$ in water to prepare uniform rod-like micelles was not possible, we examined ways to transfer the uniform micelles from 2-propanol solvent into water. For these experiments, we used a sample of uniform rod-like micelles prepared by seeded growth ($c = 0.19$ mg/mL) as shown in Figure 4H. These micelles were characterized as $L_n = 1106$ nm, $L_w/L_n = 1.01$. The results turned out to be unexpectedly complicated.

In our initial experiments, a dilute micelle solution in 2-propanol was placed in a dialysis cassette and immersed in water. The liquid in the cassette rapidly became cloudy. The turbidity decreased over time but even after 3 days, the cloudiness persisted. We were aware that PNIPAM in many alcohol-water mixtures is subject to co-nonsolvency effects, but has not been reported for 2-propanol. This term refers to solvent mixtures that are a precipitant for a polymer, whereas the individual solvents are each good solvents for the polymer. As shown in the phase diagram in Figure S10, PNIPAM also exhibits co-nonsolvency in mixtures of 2-propanol and water. One of the characteristics of co-nonsolvency for PNIPAM is that the solvent mixture decreases the lower critical solution temperature (LCST). There are examples in the literature where solvent mixtures that are precipitants for PNIPAM at RT give clear solutions at lower temperatures.$^{44,45}$ The next
experiments were designed to carry out the transfer into water at 0 °C instead of room temperature. In these experiments, we used a syringe pump to inject the micelle solution at a slow rate (1 mL/h) into a large excess of water, cooled in an ice-water mixture (Scheme 2). The idea was to maintain the mixture in the one-phase region of the phase diagram. The final solvent composition after the addition of the micelle solution was 5 vol % 2-propanol. As a testament to our design strategy, we noted that the aqueous solution remained clear, with no indication of turbidity.

Scheme 2. The set-up to transfer PFS$_{26}$-b-PNIPAM$_{520}$ micelle solution from alcohol solvent to water. The pump ensures a constant injection rate at 1 mL/h.

Unfortunately, we encountered another complication. Although micelle transfer to water was “successful”, it was accompanied by fragmentation. As shown by the TEM images and the histogram of the length distributions (Figure S11), most of the micelles fragmented into much shorter species, ca. 100–200 nm long, while some longer micelles exceeding 700 nm in length persisted in the solution. To test the possibility that shear-forces associated with passage through the syringe induced fragmentation, a control dilution experiment was carried out. Here the micelle solution in 2-propanol was injected into 2-propanol while features of the set-up (Scheme 2) were kept the same. TEM images (Figure S12) of the sample before and after passage through the syringe show identical lengths and length distributions and confirmed that the injection step does not induce fragmentation.

**Temperature-dependent behavior of fragmented micelles in water.** Although transfer to water resulted in fragmented PFS$_{26}$-b-PNIPAM$_{520}$ micelles, we still wished to examine their thermoresponsive behavior. To proceed, the solution of micelle fragments was concentrated to 0.18 mg/mL. Upon heating this solution from 23 °C to 45 °C, we were unable to observe any indication of turbidity or gel formation. This result is not surprising, considering the low concentration and short length of the micelle fragment solution. A more sensitive experiment was
to examine the solution by DLS, using the temperature control feature of a Malvern Zetasizer (173 ° scattering angle). These results are plotted in Figure 7.

![Graph of DLS light intensity and apparent hydrodynamic diameter vs temperature](image)

**Figure 7.** DLS light intensity (blue triangles) and apparent hydrodynamic diameter ($D_{h,\text{app}}$, black squares) of fragmented micelles (0.18 mg/mL) in water as a function of temperature. The signals were monitored at a scattering angle of 173°.

On the left-hand axis of Figure 7 we plot the total scattering intensity vs temperature, and on the right-hand axis we show the corresponding apparent hydrodynamic diameters ($D_{h,\text{app}}$) of the scattering objects in solution. The autocorrelation decay plots and CONTIN distribution at each temperature are given in Figure S13. At low temperatures, for example, below 30 °C, the intensity of scattered light was low with $D_{h,\text{app}}$ values of ca. 200 nm. This value is close to that expected at this scattering angle for rigid rod micelles with lengths on the order of 700 nm, consistent with a scattering signal dominated by the longer micelles in the solution. An onset of light intensity increase can be seen at around 35 °C, close to the LCST of PNIAPM. The increase in light intensity at the higher temperatures reflects micelle aggregation and is accompanied by an increase of $D_{h,\text{app}}$ ca. 400 nm at higher temperatures. TEM images of the micelles remeasured after cooling the sample showed the same length distribution histogram to the one before heating-cooling cycle (Figure S11D-F). The TEM results indicate that no further fragmentation of the micelles occurred during the heating-cooling cycle.

**SUMMARY AND CONCLUSIONS**

Two samples of the hydrophobic-hydrophilic block copolymer PFS-$b$-PNIPAM were synthesized by a combination of ATRP synthesis of azide-terminated PNIPAM and copper-
catalyzed “click” coupling to alkyne-terminated PFS. These polymers, PFS$_{56}$-$b$-PNIPAM$_{190}$ and PFS$_{26}$-$b$-PNIPAM$_{520}$, had different block ratios and exhibited pronounced differences in self-assembly in alcohol solvents. PFS$_{56}$-$b$-PNIPAM$_{190}$ formed spherical micelles in methanol and in ethanol and mixture of spherical and elongated micelles in 2-propanol. Over time (60 days), some fiber-like micelles could be seen in the ethanol solution and in 2-propanol, the fiber-like micelles grew longer, although the spherical micelles, which we presume to have an amorphous core,$^{35}$ remained the prominent objects in the TEM images. For this situation, the slow rate of evolution of the micelle morphology is likely determined by the rate at which polymer molecules exit from the spherical micelles and deposit epitaxially on the ends of the core-crystalline cylindrical micelles.

In contrast, PFS$_{26}$-$b$-PNIPAM$_{520}$, with a shorter PFS block and a much longer polar block, formed long fiber-like micelles in all three solvents, and we see no evidence of polymer trapped as amorphous aggregates. Seeded growth experiment were carried out in ethanol and in 2-propanol, in which suspensions of short micelle fragments obtained by mild sonication were treated with different amounts of unimer as a concentrated solution in THF. As expected, uniform micelles were obtained with lengths that increased with increasing amounts of unimer added. The unexpected feature of this experiment was that 3 days of aging at RT was not sufficient for the micelles to complete their growth. The micelles only reached their final length after prolonged aging (40 days). We also showed that the micelle fragment seeds obtained after sonication also increased in length after prolonged aging in solution. We believe that these micelle solutions contain free unimer, which is slow to deposit epitaxially during the crystallization-driven self-assembly process. The final length of the micelles after prolonged aging is consistent with the predictions of eq 4, based on the length of the seed fragments and the amount of unimer added.

We used multiangle light scattering to characterize the rigid-rod micelle samples. A Holtzer-Casassa analysis of the SLS data showed relatively low values of the mass per unit length of these micelles, with $N_{agg,L} = 0.95$ molecules per nm for micelles in ethanol and 0.75 molecules per nm for the micelles in 2-propanol. These values are substantially smaller than values of $N_{agg,L} = 2$ to 3 molecules per nm for other rod-like PFS block copolymer micelle samples. We believe that these low values of $N_{agg}$ indicate important differences in how the PFS chains pack in the micelle core.
Attempts to transfer samples of uniform length from 2-propanol to water were not successful. Dialysis led to turbid solutions and ill-formed aggregates. Careful injection of a micelle solution into cold (~ 0 °C) water at concentrations low enough to avoid the co-nonsolvency effect of water-2-propanol mixtures on PNIPAM led to transparent solutions. Transfer was accompanied by extensive fragmentation of the micelles. Recent result with PFS-b-(PEO-g-TEG) show that micelles formed in DMF can be transferred to water by simple dialysis. We suspect that the fragility of the PFS_{26}-b-PNIPAM_{520} micelles is related to way in which the PFS chains for this polymer pack in the micelle core. We did show by DLS measurements that the fragmented sample of PFS_{26}-b-PNIPAM_{520} micelles in water exhibit the thermoresponsive behavior expected for colloidal objects with a PNIPAM corona, exhibiting aggregation as the solution temperature was increased above 35 °C.

Current experiments in our laboratory are examining other PFS diblock copolymers with polar blocks that are also thermoresponsive in water. These samples appear to be more robust and maintain their length upon transfer to water. This topic will be the subject of a future publication.

ASSOCIATED CONTENT

Supporting Information. Experimental details and characterization data (NMR, GPC, DLS), preliminary photo-irradiation experiments, quantitative analysis of GPC traces from irradiated micelle solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

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