Polymer Nanostructures

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Mechanically Driven Reorganization of Thermoresponsive Diblock Copolymer Assemblies in Water**

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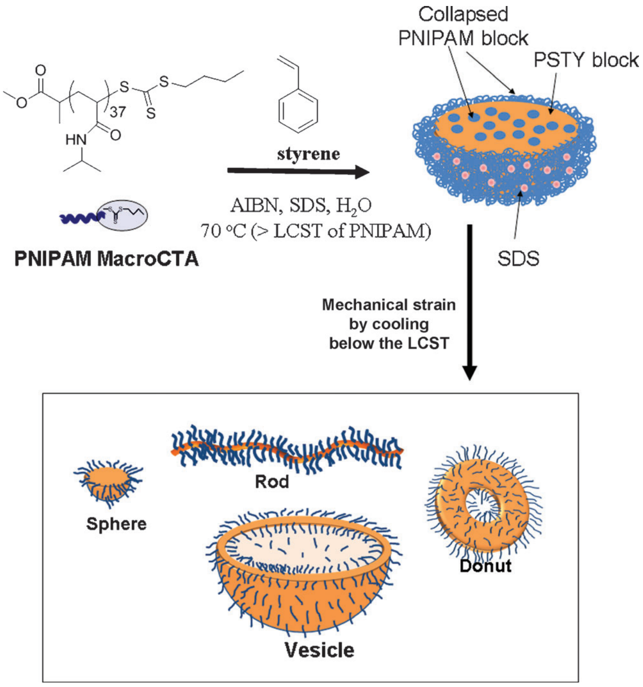
Thermoresponsive amphiphilic diblock copolymers self-assemble into micelles consisting of three-dimensional spheres, cylinders, and bilayer structures capable of responsive change in shape and hydrophobicity.[1] Therefore, these polymers are potentially useful in drug and vaccine delivery,[2] tissue engineering,[3] nanoreactors,[4] and rheology modifiers. Current methods to form these 3D structures require incorporation of thermoresponsive polymer segments into di- or triblock copolymers.[5–7] At temperatures below the lower critical solution temperature (LCST) of the thermoresponsive segment the block polymer is water-soluble. Upon raising the temperature above the LCST, the block polymer assembles into a variety of 3D structures. This process is fully reversible unless the polymer is crosslinked above the LCST.[6,8]

Although this self-assembly approach is not based on the use of harmful organic solvents, the resulting structures strongly depend on the choice of hydrophobic to hydrophilic block lengths[9,10] and are restricted to very high dilutions (<1 wt% of polymers).[5] Some 3D structures prepared by this approach may change in size and structure and require many weeks to reach their equilibrium.[7] To make these 3D species industrially attractive and realize their great potential, these structures must be reproducibly generated at much higher polymer weight fractions, form rapidly, and maintain their structure especially when they are used in vivo. Here, we report a new approach to control the formation of a variety of 3D structures at high weight fractions of the polymer (>8 wt%) in water from a single diblock, consisting of poly(*N*-isopropylacrylamide) and polystyrene, P(NIPAM$_{37}$-b-STY$_{36}$) with approximately equal block lengths. The diblock is produced by emulsion polymerization at 70 °C (Scheme 1). The spherical structures formed after polymerization change their shape through a mechanically driven reorganization of the diblock P(NIPAM$_{37}$-b-STY$_{36}$) upon cooling below the LCST of the PNIPAM block and a combination of cooling and ultrasound. The 3D structures include spheres, rods, vesicles, and donuts. These structures maintain their form in water for over a year, and can be freeze-dried (i.e. sterilized for biological applications) and rehydrated without altering their structures. We can further construct temperature-reversible gel materials, built from the rod structures, above a polymer weight fraction of 5 wt%, which will have great potential in tissue engineering and diagnostic applications.



Scheme 1. In situ polymerization of P(NIPAM$_{37}$-b-STY$_{36}$) using RAFT-mediated emulsion polymerization at 70 °C. Cooling below the LCST produces mechanical strain orginating from the swelling of the PNIPAM segments to form a variety of 3D structures. We speculate that the morphology at 70 °C directly after polymerization is a result of the formation of core–shell structures with incorporated PNIPAM regions.

We created a thermoresponsive diblock copolymer, P(NIPAM$_{37}$-b-STY$_{36}$), with narrow polydispersity in situ by reversible addition fragmentation chain transfer (RAFT) polymerization at 70 °C, that is, aqueous-phase emulsion polymerization.[4,11] The conversion reached between 50–60 %, and we produced diblock copolymers at polymer weight fractions as high as 15 wt% (see Table S1 in Supporting Information). The thermoresponsive PNIPAM segment was chosen as a component in the diblock copolymer because it contributes to the morphological reorganization of the micelles through its unique transition from being hydrophobic above its LCST to water-soluble below its LCST (around 32 °C[12]).[5,8] The PSTY segment behaves as a nonmobile, elastic solid below its glass transition temperature (T_g of around 82 °C as measure by differential scanning calorimetry;

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see Figure S3 in the Supporting Information), enabling kinetic control over non-equilibrium morphologies and making the resultant structures irreversible.[10] The resulting P(NIPAM$_{37}$-b-STY$_{36}$) diblock copolymer formed (non-equilibrium) spherical particles (hydrodynamic radius, D_h, of around 100 nm) stabilized by sodium dodecylsulfate (SDS) during the polymerization at 70 °C (Scheme 1). Other aqueous-phase emulsion polymerization reactions mediated by "living" radical polymerization (LRP) to form diblock copolymers resulted in equilibrium structures such as fibers, rods,[13] and vesicles[14] during the polymerization process.

Upon cooling our spherical particle mixture (at a polymer weight fraction of 8 wt%, see Reaction 1 in Table S1 in the Supporting Information) rapidly over 5 min from 70 to 25 °C below the LCST of PNIPAM the transmission electron microscopic images display the change from spheres to long rods (Figure 1 B). Cooling the reaction mixture below the LCST of the PNIPAM block results in swelling of the PNIPAM chains, supported by strong binding of SDS to PNIPAM,[15] which induces a mechanical strain to reorganize the spheres into rodlike structures. The core of the rod most probably consists of the hydrophobic PSTY, and the corona as well as the surface of the rod consists of the hydrophilic PNIPAM stratified by strong binding to SDS. In principle, a fast mechanically induced strain should not result in a change of morphology, because the PSTY blocks should behave as a glassy solid and resist any structural change.[16] This behavior is expected because in our case the T_g of the PSTY is far greater than the LCST. However, the unpolymerized styrene monomer trapped within the core of the spherical micelles acts as a plasticizer for the PSTY segments and enables rapid reorganization to their equilibrium structures (i.e. cylinders in this case).[9,10] Taking the rapidly cooled mixture of rods, and then heating and cooling it rapidly over 10 cycles increased the number of individual rods with each consecutive heating and cooling cycle (see Figure S5 in the Supporting Information). This observation shows that a mechanically induced strain drives the micelles towards their equilibrium morphology. The resulting rods consist of a PSTY core, stabilized by hydrophilic PNIPAM block chains, and they have a broad contour-length distribution (CLD) of 2–5 μm and a core width of approximately 10 nm. These rod distributions are similar to those found for other diblock fiber/rod structures.[17] Cryo-TEM, a technique used to observe the nanostructures in solution, shows that indeed rods form in solution (see Figure S4 in the Supporting Information) and not through the interactions with the surface of the TEM grid. Calculations based on a mass (or volume) basis suggest that rods form from a single particle and are not generated through interparticle coalesence as found from previous studies.[10,18] The length of a rod with a 10 nm diameter transformed from a single sphere of an average radius of 45 nm (measured by TEM) was calculated to be 5 μm, in agreement with the length of rods found in the TEM images.

Removal of the unpolymerized styrene monomers by heating the mixture for 4 h at 70 °C (see size exclusion chromatography (SEC) traces indicating the removal of most styrene monomers, Figure S6 in the Supporting Information) followed by cooling did not change the spherical structure (Figure 1 C). These results suggest that by varying the mobility of the core through the amount of plasticizer (in this case styrene), we can trap non-equilibrium structures during the cooling process. The addition of small amounts of toluene as a plasticizer after removal of excess styrene monomers, varying from 3 to 12 μL (corresponding to 6.3 and 25 wt% of toluene relative to the polymer) in 0.5 mL of the latex solution, shows that the morphology changes from spheres to rods with increasing amount of toluene (see Figure 1 D and Figure S7 in the Supporting Information). We could also produce the diblock copolymer, P(NIPAM$_{37}$-b-STY$_{33}$), in situ at a much higher polymer weight fraction of approximately 15 wt% (see Reaction 2 in Table S1 in the

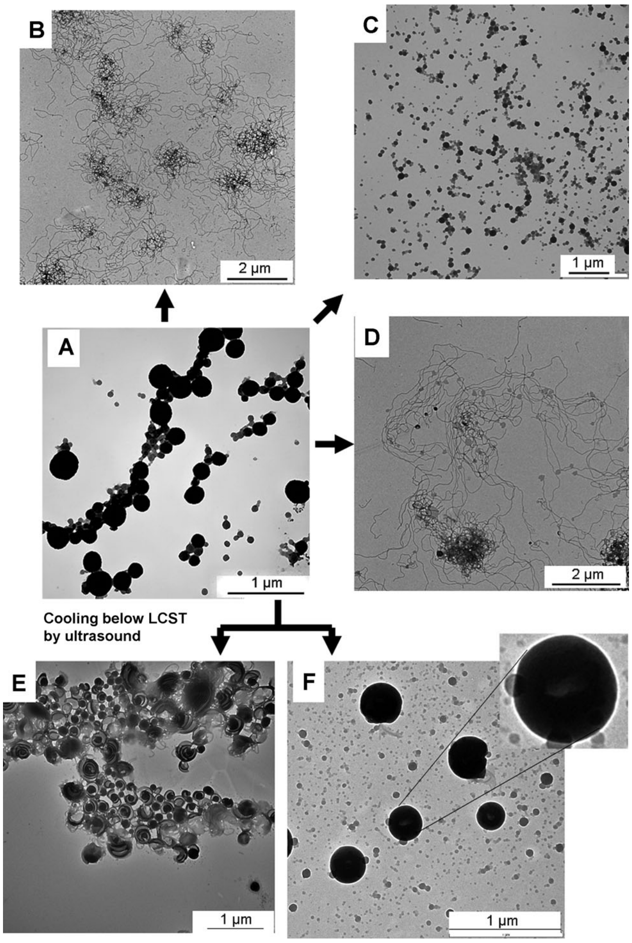

Figure 1. Transmission electron microscopy (TEM) of solutions from Reaction 1d at a conversion of 59% (see Table S1 in the Supporting Information); P(NIPAM$_{37}$-b-STY$_{36}$) at a polymer weight fraction of 8 wt%. Spherical nanoparticles after A) polymerization at 70 °C dried on a hot TEM grid at 50 °C, B) cooling from 70 to 25 °C over 5 min without removal of unpolymerized styrene, C) removal of unpolymerized styrene and rapid cooling from 70 to 25 °C over 5 min, D) removal of styrene monomers, addition of 12 μL toluene (25 wt% toluene relative to the polymer) to the nanoparticle mixture at 70 °C, and rapid cooling to 25 °C over 5 min, and E) sonication and rapid cooling from 70 to 25 °C over 5 min without removal of unpolymerized styrene monomers. F) Removal of styrene followed by addition of 3 μL toluene (6.3 wt% toluene relative to the polymer), followed by sonication, and rapid cooling from 70 to 25 °C over 5 min. Note: The LCST of PNIPAM is approximately 36 °C.

Supporting Information). The resulting transformation from spheres to rods upon cooling was similar to that found for P(NIPAM$_{37}$-b-STY$_{36}$) of 8 wt%. The greater the amount of toluene the more pronounced are the rod structures (see Figure S9 in the Supporting Information).

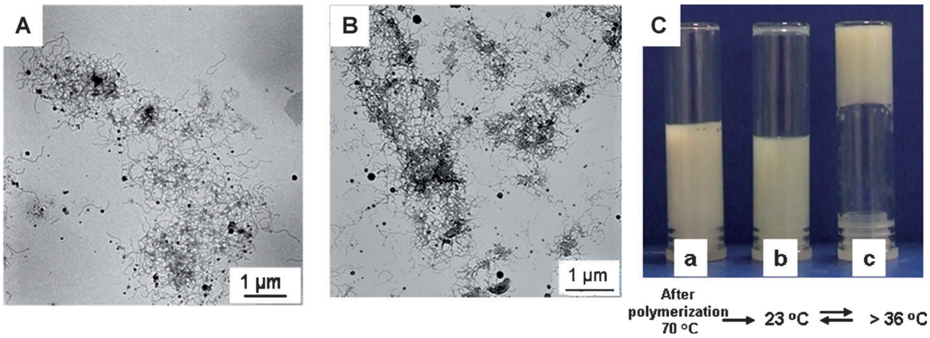

Figure 2. A) A rod/water mixture (Reaction 1d, see the Supporting Information, after cooling without removal of styrene) analyzed after a year of storage in the fridge. B) Rod structures (Reaction 1d after cooling without removal of styrene) after being freeze-dried and redispersed in water. The redispersed rods underwent an identical process of gel formation above the LCST as shown in Figure 2C. C) Temperature-induced gelation of a dispersion made from MacroCTA at a polymer weight fraction of 5 wt% (Reaction 1d after cooling without removal of styrene) containing rodlike nanostructures. This gelation also occurred when MacroCTA at a polymer weight fraction of 10 wt% was used (see Reaction 2d in the Supporting Information).

To be suitable for a wide range of biological applications, the rod structures must possess long-term stability. A rod/water mixture analyzed by TEM after a year of storage in a refrigerator (Figure 2A) showed no observable change in the structure of the rods. Furthermore, for biomedical applications, sterilization of the rods without inducing any changes to their structure and morphology is necessary. The process of freeze-drying represents a simple way to remove residual styrene and toluene and sterilize the polymer structures. A change in the rod structure after freeze-drying and redispersing in water (Figure 2B) was not observed. Heating these rods and rods before being freeze-dried at polymer weight fractions above 6 wt% from room temperature to above 36 °C induced the formation of a solid gel (Figure 2C). This process was reversible over many heating and cooling cycles.

These results show that an induced mechanical strain through swelling of the PNIPAM chains allows us to manipulate the final structure. Ultrasound can lead to quite high mechanical strains for polymers in solutions and in solid phases.[16] In particular, we wanted to study the influence of ultrasound on rods. Winnik and co-workers[18,19] used sonication to fragment cylindrical micelles of poly(isoprene-b-ferrocenylsilane) with an average length of 2 µm and a CLD polydispersity index (PDI$_{\mathrm{CLD}}$) close to 2. After 40 min of sonication, the rods fragmented to a length of 150 nm with a PDI of 1.4, and with no evidence of polymer chain scission.[16] The Gaussian scission model,[19] in which the rate constant for fragmentation shows a strong dependence on the rod length ($k_{\mathrm{Frag}} \approx L^{2.6}$), gave the best fit to the rod length relative to the time of sonication.

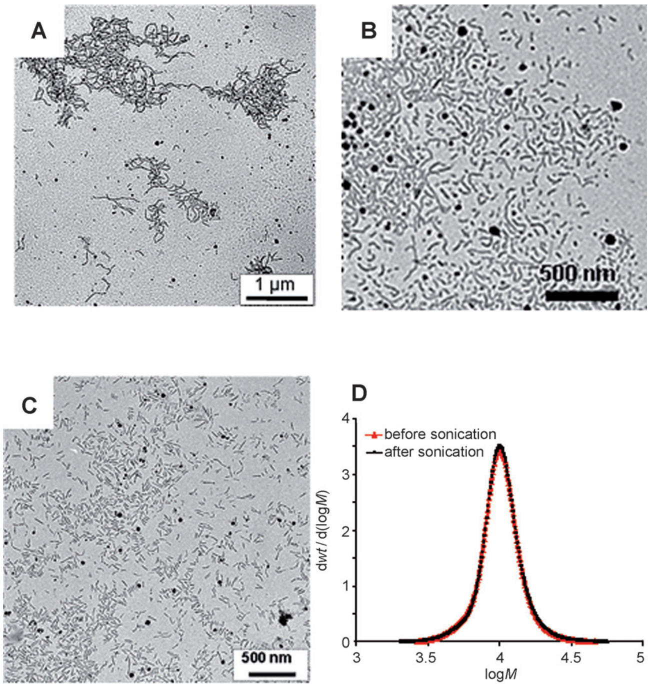

Figure 3. Ultrasound treatment of rod structures from Reaction 1d (see the Supporting Information) formed by cooling without removal of styrene monomers; P(NIPAM$_{37}$-b-STY$_{36}$) at a polymer weight fraction of 8 wt%. A) Sonication in an ultrasound bath at 25 °C (i.e. below the gelation temperature) for 1 h. B) Sonication in an ultrasound bath at 50 °C (i.e. above the gelation temperature) for 1 h. C) Ultrasound probe (Vibra-Cell) at around 50 °C for 30 min. D) Typical size exclusion chromatograms (SEC) before and after sonication (regardless of the type of sonication).

Our first ultrasound experiment with the rods from P(NIPAM$_{37}$-b-STY$_{36}$) at a polymer weight fraction of 8 wt% in water using a conventional ultrasonic bath at 25 °C for 1 h gave smaller rods with quite a broad CLD as shown in Figure 3A. Interestingly, the diameter of the core did not change and remained close to 10 nm. Sonication of the rods above the temperature of gelation (i.e. at 50 °C) for 1 h led to much smaller and uniform rod structures (Figure 3B). The average size and PDI$_{\mathrm{CLD}}$ was determined by counting 100 rods from different areas of the TEM micrograph. The number-average length was close to 99.8 nm with a narrow CLD (PDI$_{\mathrm{CLD}}$ = 1.04). When the rods are less mobile (in gel state), the rate of fragmentation is faster and more efficient. Sonication of the rod solution at around 50 °C using a Vibra-Cell (Sonics) sonicator at a frequency of 20 kHz and a tip diameter of 13 mm for 30 min fragmented the rods to an average length of 95.7 nm with a PDI$_{\mathrm{CLD}}$ of 1.06 and unchanged core diameters of around 10 nm (Figure 3C). Both sonication methods produced small rods. A thermoreversible gel formed only by heating these small rods above the gelation point of 36 °C when the dispersion was concentrated to above 19 wt %. The temperature-induced gelation in this system is consistent with the formation of a percolated micellar network. The aspect ratio of the rods and the interaction between them influences the weight fraction at which the gels are generated. A greater aspect ratio requires a lower weight fraction of the polymer to obtain a gel. The diblock coppolymer P($NIPAM_{37}$-b-STY_{36}) at a polymer weight fraction of 8 wt % also showed no degradation of the molecular weight (Figure 3D), regardless of the sonication mode and temperature. The rod lengths were all close to 100 nm, which seemed to be in agreement with the Gaussian scission model[19] predicting that the rate of fragmentation below 100 nm becomes extremely slow.

We next study the influence of ultrasound on the resulting 3D structures upon cooling the spheres below the LCST. The Vibra-Cell probe could not be used in this case, because the control of the temperature was problematic. Immersion of the emulsion of P($NIPAM_{37}$-b-STY_{36}) at a polymer weight fraction of 8 wt % in a sealed vial into an ultrasound bath at 65 °C, sonication for 30 min, followed by slow cooling to 25 °C over 1.5 h under sonication gave completely different 3D shapes (Figure 1E). The TEM micrograph reveals that in the neat latex solution (in which styrene monomer is present) unusual vesicle-type structures had formed. The removal of styrene from the latex followed by cooling under sonication gave donut-type structures of approximately 200 nm in size (Figure 1F). Vesicle-type structures formed under slightly different cooling conditions (see Figure S16 in the Supporting Information) showed no change in morphology and shape after freeze-drying and redispersing into water, and after storage for one year in water. Our results clearly demonstrate that a mechanically induced strain by sonication has the potential to produce a much wider range of 3D structures; this is the current focus of work in our laboratory.

In conclusion, the aqueous-phase emulsion polymerization by RAFT to create well-defined diblock copolymers of PNPIAM in situ gives spherical polymer nanoparticles at 70 °C at high weight fractions. Cooling the emulsion with and without sonication below the LCST of the PNIPAM segments reproducibly generates many shapes of the polymer including rods, vesicles, and donuts. At polymer weight fractions higher than 6 wt %, the long rods when heated above the LCST form stable solid gels that are reversible upon cooling. After sonication, the long rods fragment into small uniform rods (around 100 nm in length) that only form a gel at a polymer concentration higher than 19 wt % when reheated. All the shapes are stable in a refrigerator for 1 year, and could be sterilized through a process of freeze-drying followed by rehydration without any change in structure and morphology. Our methodology to influence and control the 3D shape of nanostructures represent a new, highly attractive, and reproducible method to generate shapes, formed at high polymer weight fractions, that are stable and can be sterilized.

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