Polymeric Nanostructures

DOI: 10.1002/anie.200805414

A Supramolecularly Assisted Transformation of Block-Copolymer Micelles into Nanotubes**

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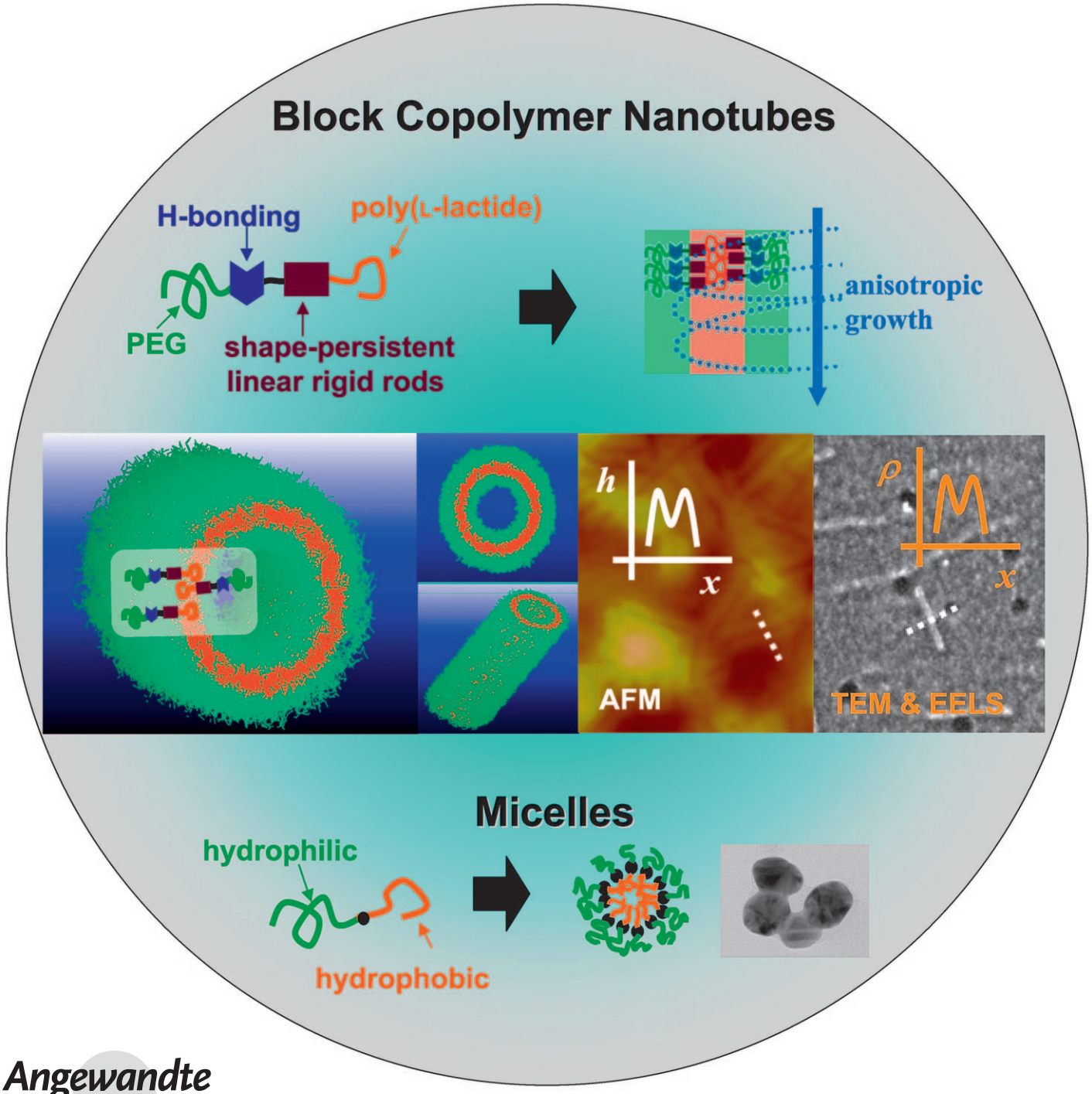

The self-assembly of amphiphilic block copolymers in selective solvents provides a defined route to a variety of supramolecular assemblies, including spherical micelles, worm-like micelles, vesicles, multicompartment micelles, toroids, and helices.[1] This versatility has attracted an increasing interest in applications in materials science, microelectronics, bioengineering, and the pharmaceutical industry.[2] However, the dynamic nature of these self-assembled micellar structures limits their use in applications where temperature or solvent fluctuations exist. Efforts to bolster the weak intermolecular interactions that effect micelle formation, include selective cross-linking of the interior (core), exterior (corona), or throughout the micelle, as well as noncovalent interactions.[3,4] The objective of this study is to demonstrate that a hydrogen-bonding motif placed at the interface between block copolymers not only stabilizes self-assembled micellar structures but also modifies the morphology of block copolymer micelles.

There are several approaches that have been reported for creating biomaterial by self-assembly. For example, peptide amphiphiles are a new class of self-assembling biomaterial with applications ranging from regenerative medicine to nanotechnology and engineering.[5] These materials assemble into well-defined nanofibers as a consequence of the formation of β-sheet-like structures. Disruption of the hydrogen bonds precludes fiber formation and favors spherical and other types of morphologies.[6] Similar fiber-like structures have been generated in amphiphilic triblock copolymers having complementary hydrogen-bonding urea groups in the central block.[7] Nanotubes are even more intriguing and formed by specific secondary interactions of amphiphiles, such as diacetylene phospholipid, glycolipids, hexacoronenes, and bolaamphiphiles.[8]

Application of amphiphilic block copolymers as drug carriers has been an active area of research and, among the materials surveyed, polylactide (PLA) based copolymers are the most widely studied. Block copolymers of poly(ethylene glycol) (PEG) and PLA form micelles in aqueous solution and have been investigated in a variety of delivery systems.[9] These micelles are generally spherical in shape with sizes ranging from 20 to 200 nm, depending on the molecular weight, composition, and cargo. Interestingly, recent reports suggest that not only size but also shape plays an important role in circulation lifetime, cellular uptake, and intracellular distribution.[10] Herein, we describe a supramolecularly assisted approach to transform the morphology of block copolymer assemblies by using a rigid hydrogen-bonding motif at the hydrophobic/hydrophilic block junction to affect the curvature of the copolymer assembly.

Our design was inspired by oligo(*p*-benzamides), shape-persistent linear rigid rods with hydrogen bond acceptors and donors, that have been shown to mimic peptide strands in β-sheets.[11,12] The shape persistence allows the prediction of the geometry and simplifies the relationship between shape and supramolecular structure. Copolymers of oligo(*p*-benzamide) with poly(ethylene oxide) form rod-like aggregates, up to 1 μm in length, that mimic a peptide β-sheet architecture. We wanted to design a benzamide motif that could be easily coupled to a functional oligomer and also act as an initiator for lactide polymerization. Towards this goal, we coupled methyl 4-(hydroxymethyl)benzoate with *p*-xylenediamine, by using 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) in THF. The monosubstituted adduct **1** precipitated out of solution in high yields (see the Supporting Information for details). Compound **1** was soluble in aprotic, polar solvents, such as DMF, NMP, and DMSO. Two functional groups were generated; an amine that could be readily coupled to an isocyanate or isothiocyanate functional oligomer and a hydroxy group that could be used to initiate lactide polymerization (see the model coupling reaction of **1** with benzylisothiocyanate to generate the thiourea model compound in the Supporting Information).

Amine-functionalized poly(ethylene glycol) (PEG–NH_2; $M_n = 5000\ g\,mol^{-1}$) was treated with either CS_2 or triphosgene in the presence of base to generate either an isothiocyanate **2a** or an isocyanate **2b**, respectively, as the functional end-group (Scheme 1). This reaction was monitored by infrared spectroscopy, in which the N–H stretching peak at 3400 cm^{-1} disappeared, with the simultaneous appearance of a peak at either 2120 cm^{-1} or 2272 cm^{-1}, indicating the formation of N=C=S or N=C=O groups, respectively. Furthermore, the triplet resonance at 2.88 ppm in the ^1H NMR spectrum, corresponding to CH_2–NH_2, shifted to 3.6 ppm. The polymers could be coupled to the benzamide (BA) **1**, leading to a functionalized PEG–thiourea, **3a**, and PEG–urea, **3b**. The IR signals at 2120 cm^{-1} and 2272 cm^{-1} disappeared and, in the ^1H NMR spectrum, the resonance at 3.68 ppm, corresponding to Ar–CH_2–NH_2 in benzamide **1**, shifted to 4.62 ppm or 4.17 ppm for polymer **3a** or **3b**, respectively. The resulting functionalized polymers were soluble in typical solvents for PEG, and characterized by IR and ^1H NMR spectroscopy.

The alcohol-functionalized PEG oligomers **3a** and **3b** were used as macroinitiators for ring-opening polymerization of L-lactide. Such a polymerization can be catalyzed by strong organic bases. However, a key consideration for this polymerization is the deprotonation of the urea or thiourea functional groups, leading to competing initiation of polymerization by the nitrogen atom. The use of sparteine with a thiourea cocatalyst was found to be strong enough to activate

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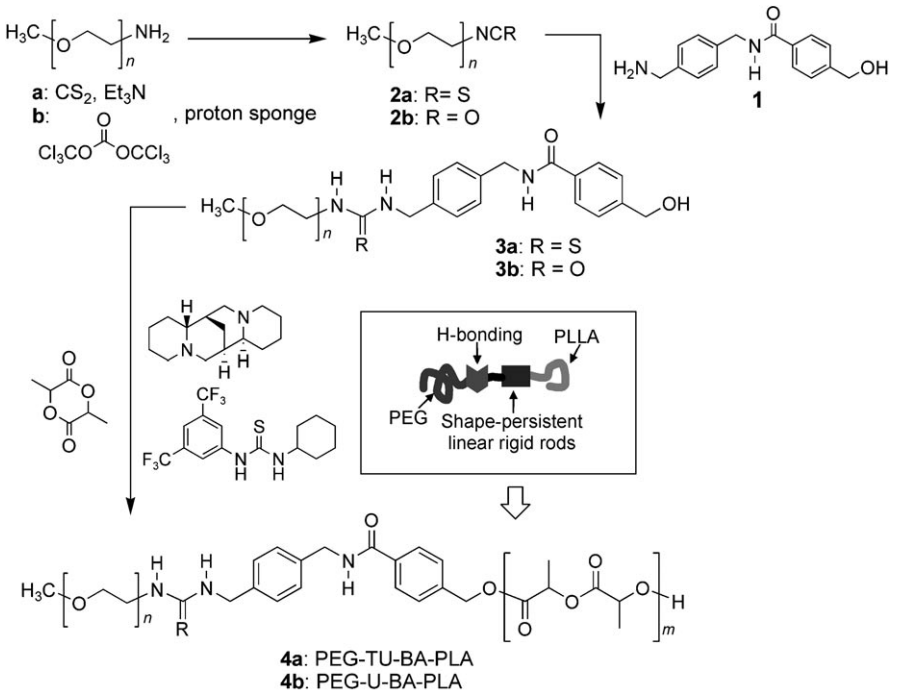
[**] The authors acknowledge support from the NSF Center for Polymer Interfaces and Macromolecular Assemblies (CPIMA: NSF-DMR-0213618). We thank Timothy Yeow and Andy Ong in FEI (Japan) for TEM support.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200805414.



Scheme 1. PEG–TU–BA–PLA and PEG–U–BA–PLA block copolymers.

the alcohol functionality, without deprotonation of the functional group, and the polymers were thus initiated solely from the hydroxy terminus.[13] Polymers of predictable molecular weight and end-group fidelity were prepared (Table 1). Most

Table 1: General characteristics of block copolymers.

Polymer	DP_{PLLA}[a]	M_n[a] [g mol^{-1}]	M_n[b] [g mol^{-1}]	PDI[b]
PEG–NH_2	–	5000	4284	1.10[c]
PEG–U–BA–PLLA (5k–5k)	36	10500	18879	1.11[c]
PEG–TU–BA–PLLA (5k–5k)	32	9900	13420	1.12
PEG–TU–BA–PLLA (5k–2k)	8	6500	8380	1.06
PEG–PLLA (5k–5k)	27	8900	11223	1.12
PEG–PLLA (5k–2k)	11	6600	8962	1.05

[a] Degree of polymerization and molecular weight, as determined by ^{1}H NMR spectroscopy. [b] Molecular weight and polydispersity index, as determined by gel-permeation chromatography in THF. [c] Shouldered peaks were observed.

of the gel-permeation chromatographs (GPCs) were obtained in THF, giving narrowly dispersed products with the expected molecular weights. However, chromatographs of the urea-based copolymers in THF showed multimodal peaks, which suggested aggregation. Carrying out the measurements in DMF mitigated this problem, leading to a single-peak product with a polydispersity of 1.16 (see Figure S1 in the Supporting Information). The block copolymer with the thiourea functional group, **4a** (PEG–TU–BA–PLLA; PLLA = poly(L-lactide)), was prepared having both symmetric ($M_{n,PEG} = 5000$ and $M_{n,PLLA} = 5000$; 5 K–5 K) and asymmetric ($M_{n,PEG} = 5000$ and $M_{n,PLLA} = 2000$; 5 K–2 K) block lengths. In the case of the urea-functionalized block copolymer, **4b** (PEG–U–BA–PLLA, 5 K–5 K), only the symmetric polymer was made. As a reference, block copolymers without the hydrogen-bonding block junction (PEG–PLLA) were also synthesized. In all cases, the polymerization was detected through a shift of a peak in the ^{1}H NMR spectrum from 5.0 ppm, corresponding to O–C*H*–CH_3 of the L-lactide monomer, to 5.2 ppm for poly(L-lactide) (see the Supporting Information for details).

Block copolymer assemblies were prepared by membrane dialysis with subsequent sonication and equilibration of micelle solution. Once the dialysis was over, cotton-like aggregates of cylindrical micelles were formed (see Figure S3 in the Supporting Information). The solution was then sonicated to facilitate an aqueous dispersion of the block copolymer micelles and maintained at ambient condition to equilibrate the micelle formation.

The PEG–PLLA diblock copolymer, without a benzamide group at the block junction, formed spherical micelles about 20–30 nm in diameter, consistent with the reported values (Figure 1 a).[9] However, the incorporation of a rigid hydrogen-bonding motif at the block junction affected the curvature of the block copolymer assembly (Figure 1 b and Figure S3 in the Supporting Information), such that the morphology of micelles was transformed.

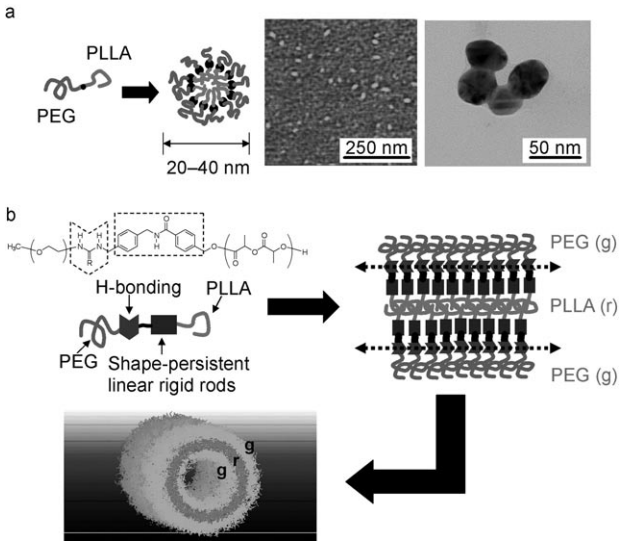


Figure 1. Schematic representations of possible supramolecular structures of a) PEG–PLLA and b) PEG–TU (or U)–PLLA assemblies.

Transmission electron microscopy (TEM) and cryo-TEM images (Figure 2 a and b) for PEG–TU–BA–PLLA micelles

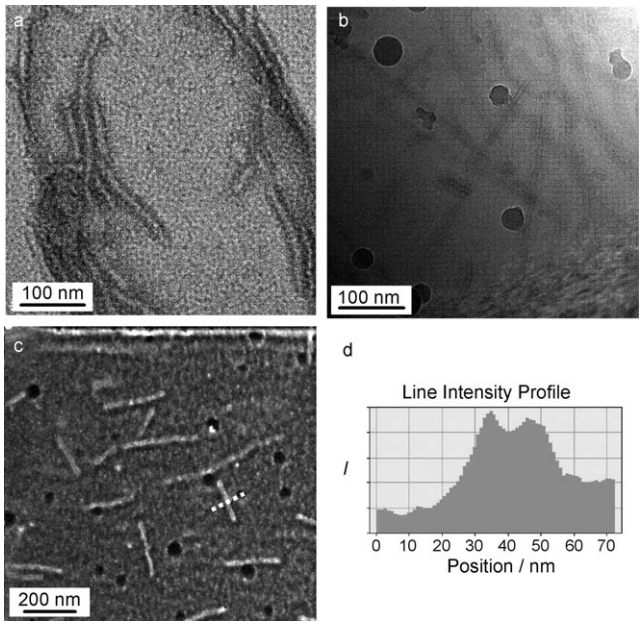


Figure 2. PEG–TU–BA–PLLA (5k–5k) nanostructure observed with a) TEM, b) cryo-TEM, c) STEM, and d) electron energy loss spectrum profile for the selected line in (c).

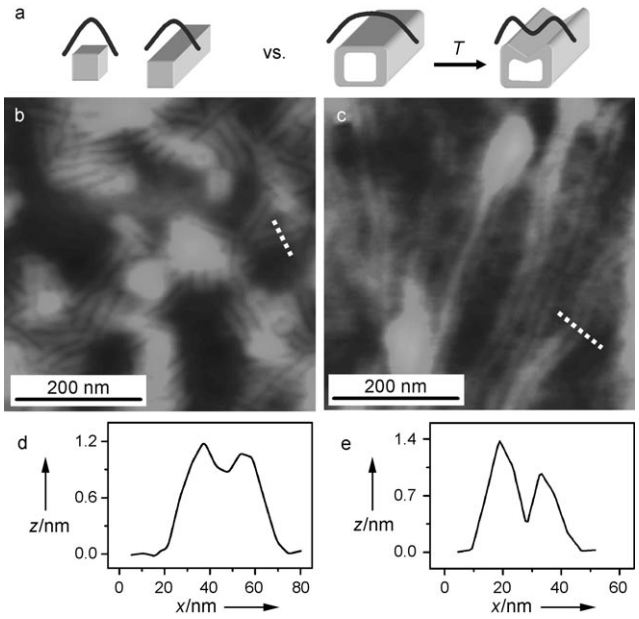


Figure 3. a) Schematic representation of possible structural change in nanotube morphology. b–e) Height-contrast AFM images and cross sections of nanostructures prepared by micellization of PEG–TU–BA–PLLA (b, d), and PEG–U–BA–PLLA (5k–5k) (c, e) followed by thermal annealing.

from the dilute solution depict typical nanotube morphologies with a significant contrast of electron density between bright central channel and dark wall areas. The diameter of the tube is about 30–40 nm and the inner diameter of the tubules is about 10 nm, which remains constant along the entire length (see Figure S4 in the Supporting Information). Additional evidence for the nanostructure was obtained from scanning TEM (STEM) and the resulting electron energy loss spectrum of the selected line (Figure 2c and d). The characteristic energy losses of electron beams penetrating through samples allows quick and reliable measurement of relative local thickness (or density) although the exact evaluation requires more details on the mean free path of electron inelastic scattering. The intensity profile of the electron energy loss spectrum (Figure 2d) indicates that the nanostructure is hollow through the center.

Through tapping mode atomic force microscopy (AFM) analysis, we expected to detect the difference in structural change between solid (such as spheres or rods) and hollow (such as vesicles or tubes) nanostructures (Figure 3a). To detect the morphology by AFM, the block copolymer micelle solution was spin-coated onto a silicon wafer and annealed at 70 °C for 2 hr under nitrogen. PEG–PLLA diblock copolymer formed single dots of 20–30 nm in diameter (see Figure S5 in the Supporting Information). However, AFM images and cross sections of PEG–TU–BA–PLLA and PEG–U–PLLA nanostructures (Figure 3b–e) show the morphology of parallel lines with a central sunken channel, which might be due to a slight collapse of hollow tubular nanostructures during thermal annealing, similarly to the profiles obtained from TEM analysis.

The anisotropic assembly of these polymers into nanotubes is reminiscent of molecular assemblies of glycolipids and other amphiphiles.[8] These molecular systems are seemingly complex as they are dependent upon factors such as chirality, size of the head group, and molecular packing, in determining the resultant morphological structure. In comparison, morphological control in block copolymer systems is largely controlled by the Flory–Huggins interaction parameter χ, molecular weight, and block fraction. However, most block copolymers have higher chain flexibility and fewer anisotropic interactions than low-molecular-weight lipids which places limitations upon the formation of anisotropic polymeric assemblies.[14] Our approach, wherein a rigid hydrogen-bonding component is incorporated at the block junction, offers a strategy for further control over polymeric assemblies by instituting changes at the molecular level.

In summary, we have identified hydrogen-bonding motifs that, when placed at the interface of an amphiphilic block copolymer, have a pronounced effect upon the assembly morphology. Both the urea–benzamide and thiourea–benzamide motifs offer simple building blocks capable of strong anisotropic hydrogen-bonding interactions. These hydrogen-bonding motifs transformed the morphology of PEG–PLA diblock copolymers from spherical to nanotubular morphologies. Block copolymer nanotubes can easily enhance the solubility and mechanical/thermal stabilities of the supramolecular architectures, compared to well-studied examples of low-molecular-weight amphiphiles.[8] This supramolecularly assisted polymer assembly opens a new arena for the construction of well-defined nanoarchitectures with anticipated applications in nanomedicine.

Experimental Section

The synthetic procedure for the block copolymers is shown in Scheme 1. Micellization of the block copolymer was carried out by membrane dialysis with subsequent sonication and equilibration of the micelle solution. Typically, the block copolymer (20 mg) was dissolved in DMF (2 mL) and dialyzed against water for 2 days to form an aqueous block copolymer solution. The solution was then sonicated for 1 h and maintained at ambient conditions for 3 days to equilibrate micelle formation before further analyses. Nanostructure morphologies were observed with TEM, cryo-TEM, STEM, electron energy loss spectroscopy (EELS), and AFM. Details on the preparation and characterization are described in the Supporting Information.

Received: November 5, 2008
Revised: February 26, 2009
Published online: May 4, 2009

Keywords: block copolymers · hydrogen bonds · micelles · nanotubes · self-assembly

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