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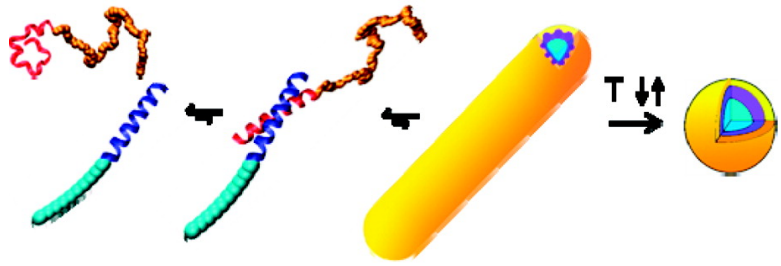

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Noncovalent Triblock Copolymers Based on a Coiled-Coil Peptide Motif

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Abstract: The formation of a noncovalent triblock copolymer based on a coiled-coil peptide motif is demonstrated in solution. A specific peptide pair (**E** and **K**) able to assemble into heterocoiled coils was chosen as the middle block of the polymer and conjugated to poly(ethylene glycol) (PEG) and polystyrene (PS) as the outer blocks. Mixing equimolar amounts of the polymer−peptide block copolymers **PS**−**E** and **K**−**PEG** resulted in the formation of coiled-coil complexes between the peptides and subsequently in the formation of the amphiphilic triblock copolymer **PS**−**E/K**−**PEG**. Aqueous self-assembly of the separate peptides (**E** and **K**), the block copolymers (**PS**−**E** and **K**−**PEG**), and equimolar mixtures thereof was studied by circular dichroism, dynamic light scattering, and cryogenic transmission electron microscopy. It was found that the noncovalent **PS**−**E/K**−**PEG** copolymer assembled into rodlike micelles, while in all other cases, spherical micelles were observed. Temperature-dependent studies revealed the reversible nature of the coiled-coil complex and the influence of this on the morphology of the aggregate. A possible mechanism for these transitions based on the interfacial free energy and the free energy of the hydrophobic blocks is discussed. The self-assembly of the polymer−peptide conjugates is compared to that of polystyrene-*b*-poly(ethylene glycol), emphasizing the importance of the coiled-coil peptide block in determining micellar structure and dynamic behavior.

Introduction

Amphiphilic block copolymers derived from synthetic monomers can self-assemble into well-defined assemblies, such as micelles, vesicles, and networks.[1] Researchers are increasingly focusing on the use of peptide- and protein-based segments as replacements for one of the traditional polymer blocks because polymers based on amino acids have the ability to adopt structures with precisely defined shapes and spatial distributions of functionality, making them attractive building blocks for the bottom-up approach to the production of nanostructures.[2]

It has been demonstrated that the degree of control over the organization of polymer assemblies can be increased not only by using well-defined building blocks but also, more recently, by utilizing noncovalent bonding motifs within the block copolymer.[3] On the basis of this principle, supramolecular polymers have been constructed using metal−ligand coordination, hydrogen bonding, and $\pi-\pi$ interactions.[4] In this manner, it is possible to obtain dynamic systems based on supramolecular interactions in which the association constants are controlled by external parameters such as temperature, solvent, and concentration. Although dynamic reversible assemblies based on noncovalent interactions are ubiquitously present in nature,[5] examples of synthetic assemblies in aqueous systems bearing these properties are rare.[6]

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As an example from nature, several specific peptide sequences have been demonstrated to generate precisely defined noncovalent complexes. A prominent motif in nature is the coiled-coil assembly of helical peptides, which is found in up to 10% of eukaryotic proteins,[7] including transcription factors, motor proteins, chaperone proteins, and viral fusion proteins. Coiled coils are formed through the coiling of two or more α-helical peptides around each other in a very specific manner that produces a stable complex in aqueous solution.[8] The oligomerization state (2−7 peptides[9]), size (∼2 nm[10] to 200 nm[11]), direction of binding (parallel[12] or antiparallel[13]), choice of homo-[14] or heterobinding,[15] and stability[14] can be controlled by careful selection of the amino acids (natural or synthetic[16]) that constitute the peptides. The noncovalent association of these peptides is sensitive to changes in external parameters (e.g., pH, temperature, ionic strength, and solvent), which affect both the electrostatic and hydrophobic interactions, and this responsiveness permits control over the association state of the peptides.[17] As a result, incorporation of coiled-coil-forming peptides into hybrid macromolecules is a vital field of research, as it allows enhanced control over nano-, micro-, and macrostructure.

Coiled coils have previously been applied to connect proteins[18] and hydrophilic polymers,[19] forming hydrogels. In these constructs, coiled-coil motifs flank a water-soluble protein or polymer segment, and the coiled-coil interaction creates a randomly connected network. This peptide motif has also been employed to reassemble complementary protein segments, restoring the original function of the proteins.[20] Aggregation of gold particles decorated with coiled-coil-forming peptides has been accomplished as a result of the interparticle peptide−peptide interaction.[21] Coiled-coil-forming peptides that associate in a staggered way, such that each peptide is involved in two coiled-coil interactions simultaneously, have been used to create fibers[22] having lengths as great as hundreds of micrometers.[23] Fractal structures have also been observed in solutions of coiled coils cross-linked through cysteine residues.[24] In all of these examples, assembly of the nanostructures is driven solely by the coiled-coil peptide interaction. The coiled-coil motif has also been connected to a poly(ethylene glycol) (PEG) block.[25] The PEG wraps around the peptide without inhibiting the formation of coiled-coil complexes. These hydrophilic hybrids do not exhibit any higher-order assembly. In this work, we have extended the complexity in this field by incorporating a hydrophobic block and a hydrophilic block into peptide−polymer hybrids, thereby demonstrating hierarchical self-assembly of "smart" nanostructures in which both coiled-coil formation and, for the first time, hydrophobic-block-induced aggregation into larger assemblies coexist and influence the final structures that form.

Table 1. Peptide Sequences and Hybrid Compositions

name	structure[a]	M_n (g mol^{-1})[b]	PDI
K	Ac−$(KIAALKE)_3$G−NH_2	2378	−
E	Ac−G$(EIAALEK)_3$−NH_2	2380	−
K−PEG	Ac−$(KIAALKE)_3$G−PEG_{77}	5832	1.05
PS−E	PS_9−G$(EIAALEK)_3$−NH_2	3341	1.01
PS-PEG	PS_{11}−PEG_{74}	4500	1.03

[a] PEG = poly(ethylene glycol), PS = polystyrene, Ac = acetyl. The sequences for the peptides **K** and **E** are written using the one-letter amino acid code. [b] Determined by MALDI−TOF mass spectrometry.

Using as a basis an α-helical coiled-coil pair (**E** and **K**) that exclusively forms parallel heterodimers,[8,26] we designed a pair of polymer−peptide block copolymers **PS−E** and **K−PEG** (Table 1) containing polystyrene (PS) and PEG blocks, respectively, as the synthetic polymers. These molecules undergo two levels of self-assembly upon dispersion in solution: the specific association of the peptide pair leads to the formation of the new amphiphilic hybrid ABC triblock copolymer **PS−E/K−PEG** (Figure 1), which subsequently self-assembles into rodlike micelles. Reversible dissociation of the coiled coil was induced by temperature control, resulting in the transition of the rodlike micelles into spherical micelles. The self-assembly behavior of the peptides and the block copolymer **PS−PEG** was also studied for comparison. These experiments were used to emphasize the influence of the coiled-coil peptide interaction on the formation of the **PS−E/K−PEG** complex and subsequent formation of

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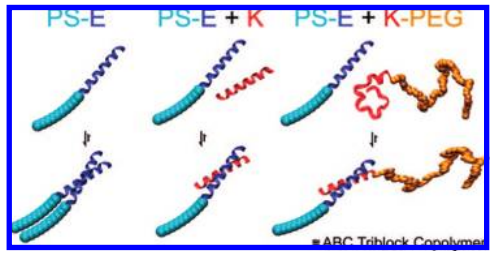

Figure 1. Schematic representation of the hierarchical self-assembly of the hybrids **PS−E** and **K−PEG** containing complementary peptide blocks. PS is polystyrene, PEG is poly(ethylene glycol), and **E** and **K** are peptides.

the rodlike micelles as well as on the ability to change the morphology of the self-assembled nanostructures.

Results and Discussion

Peptide and Polymer-*b*-Peptide Design. The objective was to design a dynamic noncovalent triblock copolymer able to assemble into well-defined aggregates in aqueous solution. In order to create an amphiphile, we chose to design one of the outer blocks to be hydrophobic and the other to be hydrophilic in nature. The coiled-coil motif was chosen to control the self-assembly process and was used as the middle block in order to bring the hydrophobic and hydrophilic blocks together to give the noncovalent triblock copolymer **PS−E/K−PEG**. The pair of 22-mer peptides **E** and **K** was selected on the basis of the well-defined shape, size, and stability of the peptide parallel heterodimer.[8,26] Typically, α-helical peptides able to form a coiled-coil motif consist of a heptad repeat sequence (**abcdefg**).[27] This peptide pair, whose members each contain only three heptad repeats, is the shortest of which we are aware that exclusively forms stable heterodimers. The peptides **E** and **K** chosen for this study are designed to favor heterodimer formation over homodimerization because of the presence of oppositely charged residues at the **e** and **g** positions (Figure S1 in the Supporting Information).[27] In this way, potential homodimer formation is destabilized whereas the **E/K** heterodimer is stabilized through electrostatic interactions. The charged residues at position **f** have charges opposite those at positions **e** and **g** in order to increase the solubilities and reduce the net charges of the peptides. The hydrophobic core (positions **a** and **d**) contains isoleucine and leucine, which pack together well in a "knobs-into-holes" fashion.[28] Alanine is present in the remaining two positions in order to increase the helical propensity of the peptides (Table 1). Since the peptides **E** and **K** are designed to form parallel dimers, the polymers must be conjugated at opposite ends of the peptides if formation of a coiled-coil complex is to result in a linear ABC triblock copolymer. Therefore, peptide **E** was conjugated at the N-terminus with hydrophobic monocarboxy terminated polystyrene to give the block copolymer **PS−E**, which had a polydispersity index (PDI) of 1.01. In order to form a hydrophilic corona upon self-assembly, peptide **K** was conjugated at the C-terminus to poly(ethylene glycol), resulting in **K−PEG** (PDI = 1.05). To show the importance of the coiled-coil motif on the self-assembly process, **PS−PEG** (PDI = 1.03), which has block lengths similar to those in the hybrid **PS−E/K−PEG** complex, was synthesized for comparison. All of the peptides and polymer−peptide hybrids were prepared by solid-phase peptide synthesis protocols using standard Fmoc chemistry. Sieber amide resins were used for the syntheses of **E**, **K**, and **PS−E**. In the synthesis of **PS−E**, monocarboxy-terminated polystyrene (PSCOOH) with a number-average molecular weight (M_n) of 2400 g mol^{-1} and a PDI of 1.20 was coupled to the N-terminus of **E** on the resin for 5 days. For **K−PEG**, **K** was synthesized on a PAP tentagel resin that was preloaded with a PEG block (Fmoc−NH−PEG−OH, M_n = 3400 g mol^{-1}, PDI = 1.02). All of the compounds were purified using precipitation protocols and reversed-phase high-performance liquid chromatography (RP-HPLC) and then characterized using NMR spectroscopy and matrix-assisted laser desorption ionization−time-of-flight (MALDI−TOF) mass spectrometry (Figures S2−S4 in the Supporting Information).

Table 2. CD Spectrospcopic Data for Synthetic Peptides, Polymer-*b*-Peptides, and Mixtures Thereof

	$[\theta]_{222}/[\theta]_{208}$			
sample[a]	PBS	50% TFE	% α-helicity[b]	coiled coil[c]
K	0.74	0.77	56	−
E	0.59	0.80	22	−
E/K	0.94	0.78	74	+
K−PEG	0.53	0.75	37	−
PS−E	0.95	0.78	48	+
E/K−PEG	0.86	0.79	37	+
PS−E/K	0.91	0.79	33	+
PS−E/K−PEG	0.89	0.77	77	+

[a] **A/B** refers to a mixture having equimolar concentrations of compounds **A** and **B**. [b] The % α-helicity is 100 times the ratio of the $[\theta]_{222}$ value observed in PBS to the $[\theta]_{222}$ value predicted for an α-helical peptide of n residues. The predicted α-helicity is calculated using the formula $[\theta]_{222} = -40000(1 - 4.6/n)$.[30] [c] The + sign signifies a significant decrease in the $[\theta]_{222}/[\theta]_{208}$ ratio in going from PBS to 50% TFE in PBS, indicative of the folded coiled-coil structure in PBS.[8] Conditions: [total peptide] = 150−210 μM, T = 25 °C.

Peptide Self-Assembly. The secondary and quaternary structures of the peptides in buffered solution were evaluated by circular dichroism (CD) spectroscopy. Peptide **E** adopts a predominantly random-coil conformation, while **K** exhibits a predominantly α-helical spectrum. Both peptides exist in the monomeric state, as indicated by the observed ellipticity ratios ($[\theta]_{222}/[\theta]_{208}$) of 0.59 and 0.74, respectively[29] (Table 2 and Figure 2A).

When peptides **E** and **K** were combined in an equimolar ratio, denoted **E/K**, a typical α-helical CD spectrum was exhibited, with minima at 208 and 222 nm (Figure 2A). The ellipticity ratio was determined to be 0.94, consistent with interacting α-helices[29] (Table 2). This clearly shows that **E** and **K** specifically interact to form a heterodimeric α-helical coiled coil. The formation of the dimeric species was confirmed by determining the molecular weights using sedimentation equilibria, revealing that separate solutions of **E** and **K** are purely monomeric while the mixture of **E/K** exists as dimers (Table S1 in the Supporting Information).

Polymer−Peptide Self-Assembly. Circular Dichroism. As discussed in the Introduction, the function of the peptide blocks is to control the behavior of the synthetic polymer blocks, i.e., to assemble them into a supramolecular ABC triblock copolymer. However, the interaction between the peptides **E** and **K** can be influenced by the presence of the synthetic polymers.

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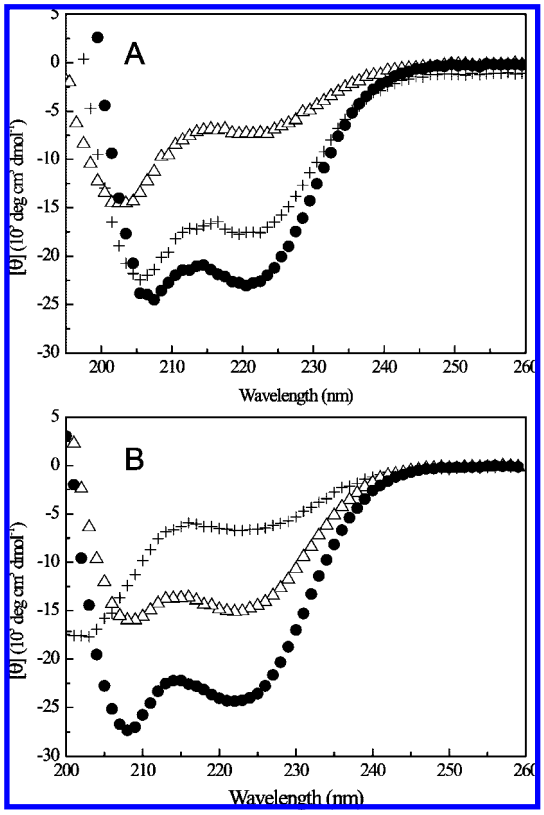

Figure 2. CD spectra of (A) **E** (△), **K** (+), and an equimolar mixture of **E** and **K** (●) and (B) **PS−E** (△), **K−PEG** (+), and an equimolar mixture of **PS−E** and **K−PEG** (●). Conditions: [total peptide] = 150−210 μM, 50 mM phosphate, 100 mM KCl, pH 7.0, 25 °C.

CD spectroscopy (Figure 2b and Table 2) showed that **K−PEG** adopts a random-coil secondary structure, probably due to wrapping of the PEG around the peptide.[25a] Comparison of the hydrodynamic diameters (D_h) of **K** and **K−PEG** (5.1 vs 5.4 nm) supported this interpretation. This tight packing of the PEG chain around **K** results in a different environment for the peptide, disturbing the hydrogen bonding that maintains the helical secondary structure in peptides. An opposite effect was observed when PS was conjugated to peptide **E** to form **PS−E**. While **E** adopts a random-coil conformation in solution, the spectrum of the **PS−E** conjugate was typical of those for interacting helices (ellipticity ratio of 0.95, 48% α-helicity). Stabilization of the α-helical structure in collagen-like peptides by hydrophobic alkyl tails has been demonstrated previously,[31] and in the present study, this observation is attributed to PS-induced aggregation (see below) that results in forced close contact of multiple **E** peptides and subsequent coiled-coil folding between **E** peptides. Mixing **PS−E** with **K−PEG** to form **PS−E/K−PEG** produced increases in the ellipticity ratio and the % α-helicity (to 0.89 and 77%, respectively), indicating the formation of a coiled-coil complex similar to **E/K**.[32] CD spectra were also recorded after the samples were diluted 1:1 (v/v) with trifluoroethanol (TFE), as TFE is known to enhance α-helicity

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(32) It should be noted that the CD spectrum of **PS−E/K−PEG** is not the average of the spectra of **PS−E** and **K−PEG**, as would be observed if the peptide blocks were not interacting. The observed amount of helicity is more than double what would be observed if **PS−E** remained homocoiled in the presence of **K−PEG**.

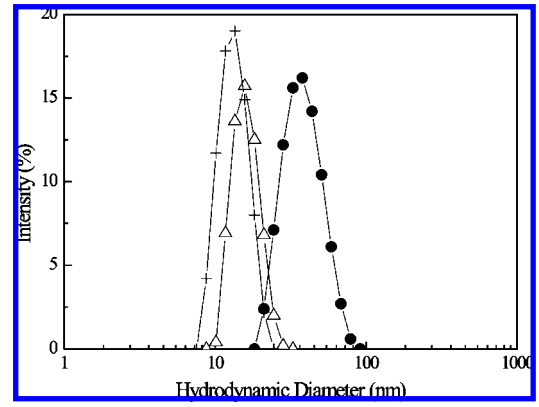

Figure 3. DLS intensity distributions for **PS−E** (△), **PS−E/K** (+), and a mixture of **PS−E** and **K−PEG** (●). Conditions: [total peptide] = 150−210 μM, 50 mM phosphate, 100 mM KCl, pH 7.0, 25 °C.

while disrupting quaternary structures.[33] Therefore, adding TFE to monomeric peptides increases the ellipticity ratio to ~80%, while addition of TFE to coiled coils reduces this ratio to ~80%. As expected for **PS−E/K−PEG**, a significant decrease in the ellipticity ratio (to 0.77) was observed, which is typical of the transition from coiled coils to single helices with nearly maximum α-helicity. Combining these results shows that the ability of the peptides **E** and **K** to form heterocoiled coils is almost completely retained upon conjugation with two vastly different polymer chains. These findings imply the formation of a noncovalent ABC triblock copolymer (**PS−E/K−PEG**) with an amphiphilic nature.

Dynamic Light Scattering. Next, the process of self-assembly of the noncovalent **PS−E/K−PEG** complex into larger structures was studied using dynamic light scattering (DLS) and electron microscopy. The peptides, polymer-*b*-peptide conjugates, and mixtures thereof were dialyzed into phosphate-buffered saline (PBS) starting from dimethylformamide (DMF). As expected, DLS did not show any aggregate formation for water-soluble **E**, **K**, and **K−PEG** or for the complexes **E/K** and **E/K−PEG**. In contrast, all of the samples containing PS assembled into defined aggregates. For **PS−E**, a D_h value of 16.2 ± 3.3 nm was observed (Figure 3 and Table 3). Mixing **PS−E** with **K** resulted in a small decrease in D_h (to 13.7 ± 3.2 nm). This occurred because complexation of **K** with **E** results increases the ratio of the area of the headgroup to the volume of the hydrophobic block and thus effectively decreases the packing parameter, resulting in a reduced radius for the spherical micelles.[34] Combining equimolar amounts of **PS−E** with **K−PEG** increased the area of the hydrophilic headgroup of the resulting **PS−E/K−PEG** complex, and as a result, larger aggregates, having a D_h of 39.7 ± 11.3 nm, were observed. This large increase in micellar size was not expected on the basis of traditional packing-parameter considerations, and another model was adapted in order to explain the observations (see below).

Cryogenic Transmission Electron Microscopy. Further insight into the morphologies of the assemblies was obtained by cryogenic transmission electron microscopy (cryo-TEM). These studies revealed the presence of aggregates for **PS−E** and the complexes **PS−E/K** and **PS−E/K−PEG** (Figure 4A−F). The

(33) Lau, S. Y.; Taneja, A. K.; Hodges, R. S. *J. Biol. Chem.* **1984**, *259*, 13253–13261.

(34) Israelachvili, J. N.; Mitchell, D. J.; Ninham, B. W. *J. Chem. Soc., Faraday Trans. 1* **1976**, *72*, 1525–1568.

Table 3. Number-Average Particle Diameters (with Standard Deviations) and Theoretical Diameters of the Aggregating Systems Investigated in This Study

sample	morphology	D_h (nm)[a]	D_{eff} (nm)[b]	D (nm)[e]
PS−E	spherical	16.2 ± 3.3	15 ± 2	15
PS−E/K	spherical	13.7 ± 3.2	13 ± 3	13
PS−E/K−PEG	rodlike	39.7 ± 11.3	(42 ± 10) × (8 ± 1)[c]	length × 17
PS−E/K−PEG[f]	spherical	21.0 ± 4.4	14 ± 2[d]	18
PS−PEG	spherical	20.6 ± 4.5	12 ± 3	20

[a] Hydrodynamic diameter, as determined by DLS. The DLS data revealed a log-normal distribution of sizes. [b] Mean effective diameter, as determined from measurements of objects in TEM images. TEM size distributions are shown in Figure S9 in the Supporting Information. For PEG-containing samples, the dimensions obtained from TEM are smaller than those from DLS or the model, indicating that the PEG is not observed using TEM. [c] Dimensions (length × width) of the rodlike micelles. [d] PTA-stained sample. [e] Theoretical diameter, as determined using the model explained in the Supporting Information. [f] After annealing.

micrographs showed that **PS−E** and **PS−E/K** assembled into spherical micelles with mean effective diameters (D_{eff}) of 15 ± 2 and 13 ± 3 nm, respectively (the size distributions of these micelles are presented in Figure S9 in the Supporting Information). In contrast, the noncovalent amphiphilic ABC triblock copolymer **PS−E/K−PEG** assembled into rodlike micelles with dimensions (length × width) of (42 ± 10 nm) × (8 ± 1 nm) and an average apparent aspect ratio of 5.[35] Moreover, careful examination of the cryo-TEM images revealed that high-electron-density regions separated by low-density regions were present along the rod. On the basis of the molecular structure of the ABC block copolymer, we propose that the rodlike micelles of **PS−E/K−PEG** are composed of a hydrophobic PS core with a corona of **E/K−PEG**. The high-density regions within the corona are attributed to clustering of multiple coiled-coil segments along the aggregates, while the low-density regions are attributed to hydrated PEG chains that cannot be visualized by cryo-TEM. We therefore also propose that the peptide clusters are separated by PEG-rich domains, as these have been shown to fold around coiled-coil-forming peptides without affecting their ability to associate.[36] Approximately 5% of the observed rods showed a lower intensity in the core of the assembly (Figure 4E and the white box in 4C), likely arising from less-efficient removal of DMF during dialysis. This results in an increased electron-density contrast between the coiled-coil clusters and the PS core, rendering the core visible. In addition, the extra solvation of the PS core most probably allows for better microphase separation of the blocks, in contrast to the majority of the micelles. The mean diameter of the core of these cylinder-like structures, as determined from the density profiles of 16 of these rods, was well-defined, with a well-defined distance between the two walls of 4.7 ± 0.5 nm, which is close to the calculated diameter of a solvated PS_9 core (4.2 nm).[37]

These results complement the DLS measurements, as demonstrated by the calculation of effective diameter distributions for the aggregates in cryo-TEM images (Table 3). Significantly, as the ellipticity ratios of **PS−E/K** and **PS−E/K−PEG** are

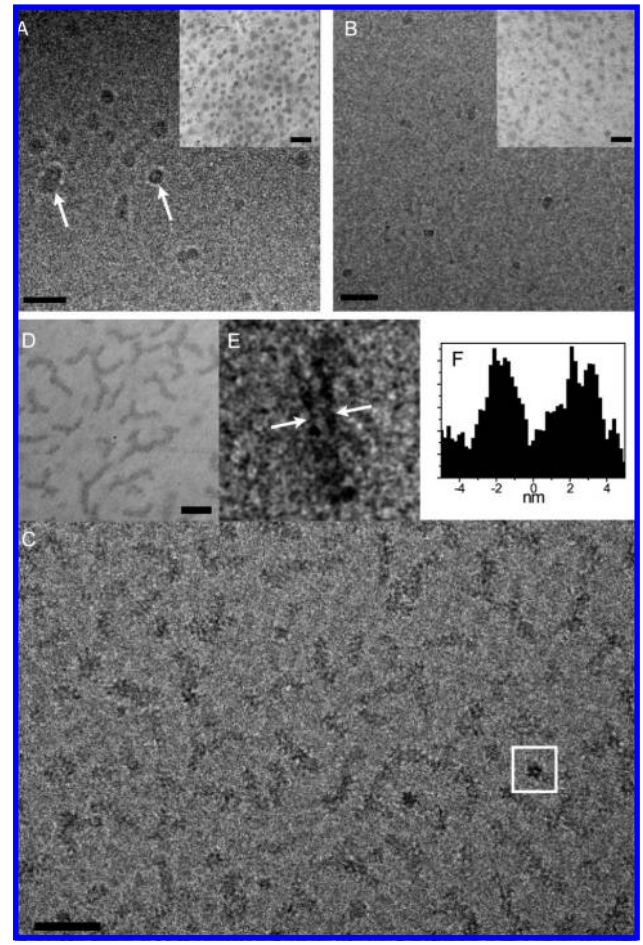

Figure 4. Cryo-TEM images of (A) **PS−E**, (B) **PS−E/K**, and (C) **PS−E/K−PEG**, with 50 nm scale bars. Conditions: [total peptide] = ~1500 μM, 50 mM phosphate, 100 mM KCl, pH 7.0. Arrows in (A) show ice particles arising from vacuum contamination. The insets in (A) and (B) and the image in (D) are phosphotungstic acid (PTA)-stained samples ([total peptide] = 150−210 μM, 50 mM phosphate, 100 mM KCl, pH 7.0, 25 °C). The micrographs in (C) and (E) show that the rods of **PS−E/K−PEG** are formed by small dots organized along the rod. Approximately 5% of the rods show a lower electron density in the core [viewed lengthwise in (E) and perpendicularly down the cylinder axis in the white box in (C)]. (F) The intensity profile of these rods shows a mean core diameter of 4.7 nm (representing an average calculated from 16 different profiles with a standard deviation of 0.5 nm).

nearly identical (Table 2), the observed morphological differences can be attributed completely to the presence of the PEG segment in the latter complex. Finally, the structural integrities of all of the micellar forms were maintained for at least 9 months when the micelles were stored at 4 °C.

In order to study the influence of the coiled-coil complex on the self-assembly behavior, $PS_{11}-PEG_{74}$ was studied. Upon dispersion in PBS, the exclusive formation of spherical micelles was observed. Cryo-TEM (Figure S6 in the Supporting Information) revealed a polystyrene core with a mean effective diameter of 12 ± 3 nm, and DLS gave an overall hydrodynamic diameter of 20.6 ± 4.5 nm.

Temperature-Dependent Self-Assembly. Circular Dichroism. The temperature dependence of the assemblies was tested in order to gain insight into the dynamics and reversibility of the systems. Coiled-coil peptide complexes typically are temperature sensitive,[17] and the mixture **E/K** showed a melting temperature of 50 °C, as determined from the inflection point of the observed decrease in ellipticity at 222 nm (Figure 5A). The rodlike

(35) On the basis of a comparison of the DLS and TEM data (Table 3), we believe that the PEG segments do not contribute to the contrast observed in TEM; therefore, the true aspect ratio would be smaller.
(36) The measured D_h of **K−PEG** (5.4 nm) is only slightly larger than that of **K** (5.1 nm). Also see ref 25a.
(37) See the theoretical model section in the Supporting Information.

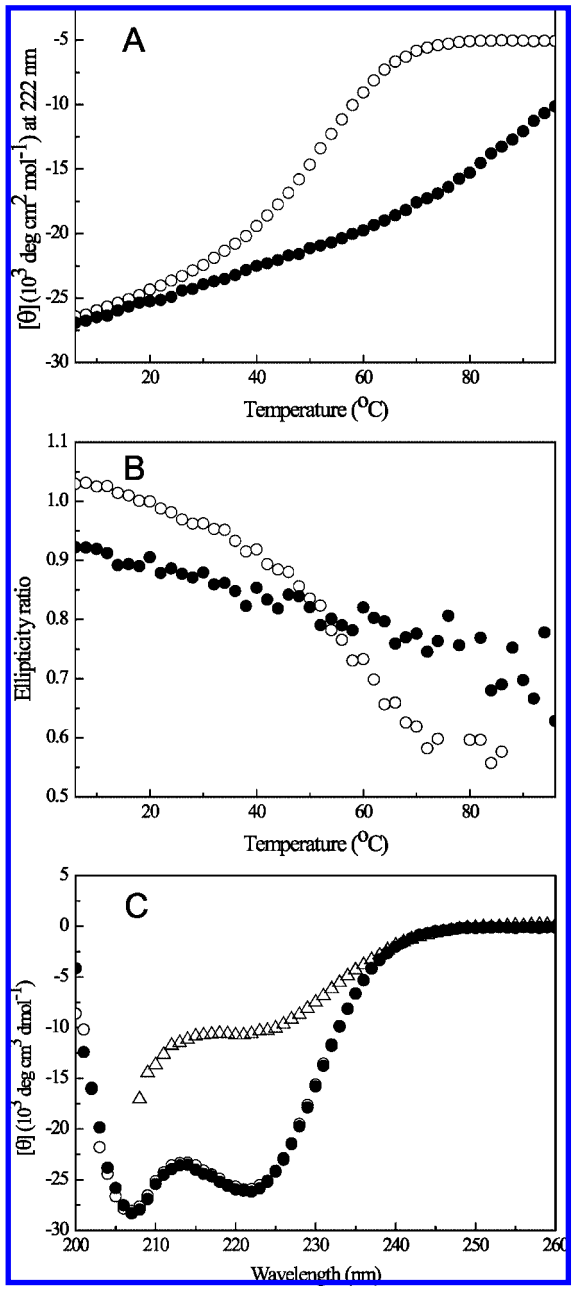

Figure 5. (A) Temperature dependence of the ellipticities ([θ]) at 222 nm for **E/K** (○) and **PS−E/K−PEG** (●). (B) Temperature dependence of the ellipticity ratios for **E/K** (○) and **PS−E/K−PEG** (●). (C) CD spectra of **PS−E/K−PEG** at 6 °C (●), 96 °C (△), and after cooling back to 6 °C (○). Conditions: heating and cooling rates of 2 °C min^{-1}, [total peptide] = 150−210 μM, 50 mM phosphate, 100 mM KCl, pH 7.0.

micelles composed of **PS−E/K−PEG** did not have a clear melting temperature, underlining the stabilizing effect of the micelles (Figure 5A). However, when the temperature reached 96 °C, the ellipticity ratio of **PS−E/K−PEG** mixture had decreased to less than 0.75 (Figure 5B), indicating that the peptides were no longer forming a coiled-coil complex. At this high temperature, the mixture exhibited a random-coil spectrum (Figure 5C), confirming that the specific interaction between **E** and **K** was lost, resulting in a full separation of **PS−E** from **K−PEG** (see below). When the mixture was cooled, the coiled-coil structure of **PS−E/K−PEG** was fully regained, as demonstrated by both the reproduction of the melting profile without hysteresis and the identical CD spectra before and after annealing (Figure 5C). These results show that the process of coiled-coil unfolding/dissociation is reversible and fast, and that the peptide structures are in equilibrium.

The different shapes of the melting curves for the hybrids compared to that for **E/K** (Figure 5A) indicates that the hybrids unfold and dissociate in a way that is atypical for coiled-coil motifs. The initial linear part of the CD melting curves corresponds to changes in helicity in the peptide complex (for example, end-fraying or unimolecular rearrangement) that occur before the onset of the cooperative unfolding and dissociation corresponding to the sigmoidal decrease in helicity seen in the **E/K** profile.[38] The fact that the initial linear parts of the CD melting curves for the hybrids extended from 30 °C for **E/K** to 70 °C for all three forms of micelle means that the PS core imparts stability with respect to cooperative folding of the peptide components by retaining them in close proximity. Although there was no clear cooperative dissociation phase for the peptides in the micellar form, when the temperature reached 70 °C, the ellipticity ratio had decreased to a value less than that for which one can expect the coiled-coil motif to exist, indicating that the peptide complexes do not dissociate in the usual cooperative way but instead fray from one end to another or that the increasing magnitude of structural vibrations gradually dissociates the peptide complexes.[38]

Dynamic Light Scattering. DLS of the annealed solutions of **PS−E/K−PEG** revealed that D_h for the rodlike micelles decreased to 21.0 ± 4.4 nm after cooling to 4 °C (Figure 6A, Table 3). Indeed, TEM revealed a conversion to spherical micelles (Figure 6B) having an average size of 14 ± 2 nm, and rodlike micelles were no longer observed. These results indicate that although the temperature-dependent assembly of the coiled-coil peptide motif is fully reversible, morphological transitions of the entire assembly are subject to more-complex kinetics.

A more detailed inspection of the temperature-dependent DLS data (Figure S11 in the Supporting Information) revealed that the rodlike micelles are stable up to ∼45 °C. Between 45 and 75 °C, a transition of the aggregate morphology occurs, resulting in a 50% decrease in D_h. As 97% of the PS blocks have a glass-transition temperature (T_g) lower than 45 °C, melting of the polystyrene core is unlikely to be the cause for this transition.[39] Temperature-dependent DLS data for the PEG homopolymer (PEG_{77}) did not reveal a significant decrease in size with increasing temperature (Figure S11), so a temperature-induced collapse of PEG chains is also unlikely to trigger the change in aggregate size. Hence, the observed size decrease must be a consequence of the temperature-dependent dissociation of the peptides (Figure 5A).

In contrast, thermal cycling of **PS−PEG** micelles revealed the stability of these assemblies, with only a slight, reversible decrease of 2 nm in D_h (from 23 to 21 nm) upon cycling of the temperature between 4 and 90 °C (Figure S8 in the Supporting Information). The formation of thermally stable spherical **PS−PEG** micelles is in accord with the results of previous studies of the aqueous self-assembly of **PS−PEG** with block lengths or polystyrene weight fractions similar to those used in this study.[40]

Summary and Discussion

Experimental. Our experimental results have shown that the hybrids **PS−E** and **K−PEG** undergo two levels of self-

(38) Dragan, A. I.; Privalov, P. L. *J. Mol. Biol.* **2002**, *321*, 891–908.
(39) Claudy, P.; Létoffé, J. M.; Camberlain, Y.; Pascault, J. P. *Polym. Bull.* **1983**, *9*, 208–215.

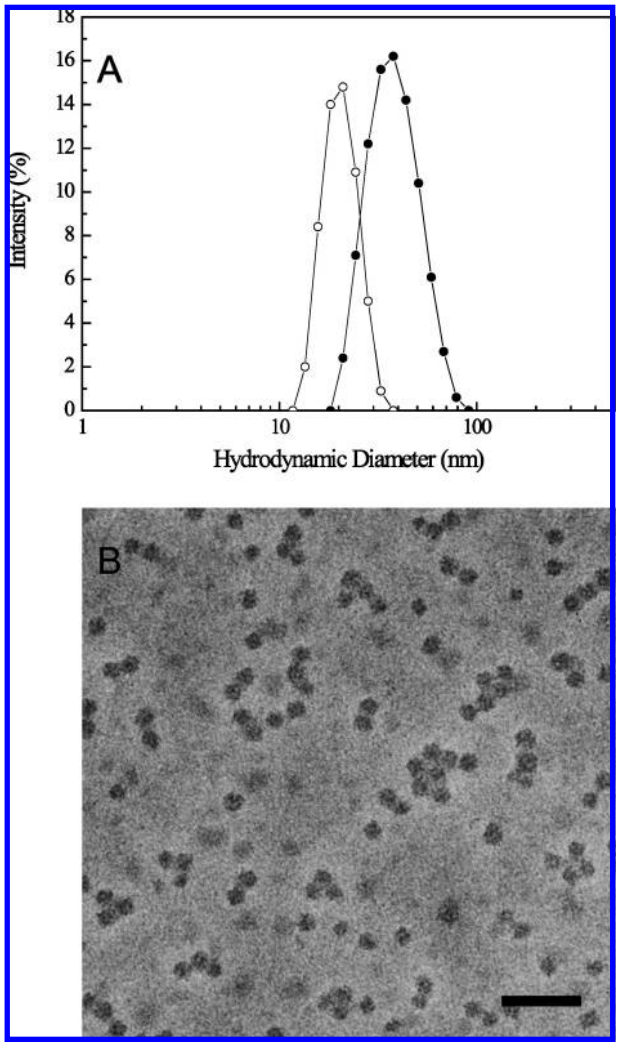

Figure 6. (A) Size distributions obtained from DLS of **PS−E/K−PEG** before (●) and after (○) a heating−cooling cycle. (B) PTA-stained TEM image of **PS−E/K−PEG** after annealing, with a scale bar of 50 nm. Conditions: [total peptide] = 270 μM, 50 mM phosphate, 100 mM KCl, pH 7.0, 25 °C.

assembly: the peptides interact to produce a noncovalent triblock copolymer, which then arranges into rodlike micelles. The hydrophobic PS block forms the core, which is shielded from the aqueous buffer by clusters of coiled-coil peptides around which PEG is closely folded. The dissociation of the **PS−E/K−PEG** complex at high temperature leads to a change in the micellar morphology, yielding spherical micelles composed of **PS−E** with **K−PEG** free in solution, i.e., the system now

(40) (a) Mortensen, K.; Brown, W.; Almdal, K.; Alami, E.; Jada, A. *Langmuir* **1997**, *13*, 3635–3645. **PS−PEG** with block sizes similar to those used in this study (1000 and 3000 g mol^{-1} for PS and PEG, respectively, compared to 1000 and 3500 g mol^{-1} in **PS−E** and **K−PEG**) forms spherical micelles in D_2O with no intermicellar interactions in our concentration range that were detectable by small-angle neutron scattering or static or dynamic light scattering. The micelles were very stable with respect to temperature, showing minimal structural changes (a 5% reduction in core size and a somewhat swollen corona) upon heating from ambient temperature to 95 °C. (b) Brown, G. J.; Richards, R. W.; Heenan, R. K. *Polymer* **2001**, *42*, 7663–7673. Small-angle neutron scattering experiments studying aqueous self-assembly of **PS−PEG** (M = 8500 g mol^{-1}) with a PS weight fraction of 0.15 were found to assemble exclusively into spherical micelles. In contrast, our **PS−E/K−PEG** has a similar PS weight fraction (0.11) and forms rodlike micelles.

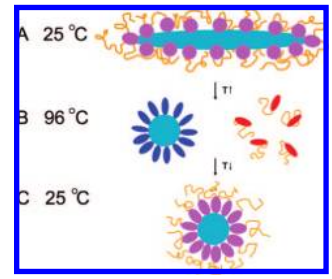

Figure 7. Idealized schematic cross-section representation of the temperature-dependent self-assembly of **PS−E/K−PEG**. (A) Rodlike micelles are composed of a PS core and an **E/K−PEG** corona. (B) Heating leads to the formation of spherical micelles with a PS core and an **E** corona. **K−PEG** is in solution. (C) When the spherical micelles are cooled, they have a PS core and an **E/K−PEG** corona. Color key: PS core, light-blue; PEG, yellow; peptide **E**, blue; peptide **K**, red; **E/K**, purple.

behaves like the **PS−E** sample. When the peptides are cooled, they re-coil (as seen with CD), resulting in spherical micelles coated with PEG. The high-temperature rearrangement of the rodlike micelles studded with clusters of coiled coils into spherical **PS−E** micelles results in homogenous dispersion of **E** over the surface. Hence, when **K−PEG** starts to re-coil with the **PS−E** micelles, the PEG block forms a layer outside the peptide shell. These conclusions are supported by DLS and cryo-TEM data. DLS shows that the hydrodynamic diameter of the annealed **PS−E/K−PEG** spherical micelles is larger than that of **PS−E/K**, and the difference fits with the dimensions of the PEG block used. In contrast, similar diameters were found with TEM since PEG is not visible, further confirming that **PS−E/K−PEG** micelles are composed of a PS core, a peptide layer, and a PEG outer layer. The micelles remain spherical upon subsequent heating and cooling cycles, indicating that these micelles are at thermodynamic equilibrium. A schematic representation of the behavior of the triblock copolymer is shown in Figure 7.

The distinct difference in the self-assembly behaviors of noncovalent **PS−E/K−PEG** and covalent **PS−PEG** highlights the influence of the coiled-coil block on these processes, as shown by the observed morphologies and temperature-dependent dynamic behaviors. In summary, **PS−E/K−PEG** self-assembles into dynamic rodlike micelles that are able to undergo a transition in micellar morphology, while **PS−PEG** organizes into static spherical micelles (see below). Thus, inclusion of the reversible noncovalent connecting block provides access to an unusual micellar morphology and encodes "smartness" into the nanostructures, allowing them to respond to environmental changes.

Model. Because direct dissolution of **PS−E** in buffer is not possible, micelles are produced by the gradual removal of the organic cosolvent from the amphiphile solutions. However, the presence of solvent in the core[41] and the low degree of polymerization of the PS block (which has a low average T_g of 11 °C)[39] may lead to equilibrated micelles with mobile hydrophobic blocks. As a result, the micellar morphology becomes sensitive to external factors such as temperature, buffer concentration, interaction between monomers, and so on.

The dimensions of the **PS-PEG**, **PS−E**, and **PS−E/K** micelles, as measured by DLS and TEM at temperatures above the T_g of the PS part, are in accordance with those of micelles having a disordered polystyrene core surrounded by a PEG or

(41) Zhang, L. F.; Eisenberg, A. *Polym. Adv. Technol.* **1998**, *9*, 677–699.

coiled-coil peptide shell. The packing parameters of **PS-PEG**, **PS−E**, and **PS−E/K** micelles predict the formation of micelles with spherical morphology. **PS−E/K** self-assembles into slightly smaller micelles than **PS−E** does, as the larger size of the hydrophilic block causes greater curvature in the self-assembled nanostructures and, in addition, **E/K** charge neutralization reduces stretching of the PS core. For the **PS−E/K−PEG** complex, a spherical micellar morphology for the thermodynamically favored structures is *expected* on the basis of the simple packing parameter model, as the ratio of the hydrophilic and hydrophobic block lengths increases.[34] However, for this complex, the observed morphology formed during dialysis is the rodlike structure.

The packing parameter was originally designed to predict the morphology and size of nanostructures formed from lipids.[34] However, this approach has proven to be ill-suited for block copolymers like the ones considered here, as it cannot accommodate headgroup complexity such as flexibility and dynamic behavior. To qualitatively explain all of the observed morphologies and the transition from rodlike to spherical micelles, we adapted a theoretical model (see the Supporting Information) initially designed for charged, flexible diblock copolymers.[42] Currently, no simple model for determining the phase diagram of triblock copolymers with a rigid middle block exists. In view of the limitations of available models, we adapted the most practical model that can predict the different morphologies and transitions between them as well as the micellar dimensions that arise from the complex, noncovalent hybrids studied in this work. In order to treat the **PS−E/K−PEG** triblock copolymer as a diblock one, we considered **E/K−PEG** to be a single block. We also assumed that the excess of salt present in the buffer neutralizes the charges of the **E**, **K**, and **E/K** blocks and that polymers follow the statistics of flexible neutral chains. The size of the PS core and the overall size of the micelle are established from the interplay between the free energy of the hydrophilic block (**E**, **E/K**, or **E/K−PEG**) and the interfacial energy between the core and corona. For **PS-PEG**, **PS−E**, and **PS−E/K** complexes, the free energy of the hydrophilic group comprises the free energies of the PEG or peptide conformations and of short-range interactions between molecules.

All of the spherical morphologies observed in this study are predicted by this model. **PS−E** and **PS−E/K** complexes are either in the region of spherical micelles or in the region of degenerate structures where rodlike micelles, spherical micelles, and lamella have the same free energy. We observe only spheres because the translational entropy favors the formation of smaller objects. For comparison, **PS−PEG** is in a region where micelles can only be spherical.

The model predicts that increasing the length of the water-soluble block (through complexation of **PS−E** with **K−PEG**) leads exclusively to rodlike morphology. This is due to the the low free energy of the short-range interactions between monomers per hydrophilic **E/K−PEG** block. This means that the PEG chains are more compact than those in **PS−PEG**.

(42) Netz, R. R. *Europhys. Lett.* **1999**, *47*, 391–397.

Temperature annealing of the rodlike complex transforms the structure into degenerate spheres. This is possible because at high temperature, the **E/K** complex dissociates and **K−PEG** leaves the micelle. The reduced amount of organic solvent in the PS core decreases the conformational entropy of PS and increases the interfacial energy. Therefore, the **PS−E** rearranges into degenerate spheres with a tightly packed polystyrene core. When the system is cooled, **K−PEG** re-coils with the **PS−E** micelles, but because the polystyrene core is now locked in a frozen equilibrium (tightly packed), the PS molecules are unable to rearrange into the rodlike structure upon condensation of **K−PEG** onto the spherical **PS−E** micelles or with further temperature annealing.

As summarized in Table 3, the model correctly predicts the form of **PS−PEG**, **PS−E**, **PS−E/K**, and **PS−E/K−PEG** micelles and includes the transition of **PS−E/K−PEG** from rodlike to spherical micelles upon annealing. The micellar diameters estimated by this model are in surprisingly close agreement with the experimental results. However, the model overestimates the diameter of the rodlike micelles, indicating that it requires further tuning in order to account for the complexity of the triblock copolymer and the end-capping energy.

Conclusions

To our knowledge, this work represents the first account of a noncovalent triblock copolymer composed of hydrophobic and hydrophilic polymers united via a peptide complex. The hierarchical self-assembly in solution of two complementary polymer-*b*-peptides (**PS−E** and **K−PEG**) utilizes the coiled-coil-forming propensities of the peptides, resulting in the formation of an amphiphilic triblock copolymer (**PS−E/K−PEG**) able to assemble into rodlike micelles. The dynamic nature of these micelles was shown by annealing of the amphiphile above the coiled-coil transition temperature. The release of the hydrophilic hybrid led to a transformation to spherical micelles that persisted upon re-coiling of the peptides, demonstrating the reversibility of the noncovalent block linker. As the macromolecular entities that are connected to the peptides are open to choice and additional methods for influencing the peptidic interaction exist, this peptide motif is a promising building block for the bottom-up approach to the formation of "smart" block copolymer structures in aqueous solutions.

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Supporting Information Available: Synthesis and characterization (HPLC, ^{1}H NMR, MALDI−TOF MS, CD) of the compounds, sedimentation equilibrium data and additional temperature-dependent CD and DLS data for self-assembled nanostructures, negative-staining TEM data for **PS−E/K−PEG**, and cryo-TEM data for **PS−PEG**. This material is available free of charge via the Internet at http://pubs.acs.org.

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