Co-assembly of Cyclic Peptide Nanotube and Block Copolymer in Thin Films: Controlling the Kinetic Pathway

Chen Zhang[§] and Ting $Xu^{\S,\dagger,\ddagger*}$

[§] Department of Materials Science and Engineering, University of California, Berkeley, CA 94720-1760

[†] Department of Chemistry, University of California, Berkeley, CA 94720-1460 [‡] Material Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA

94720

*To whom correspondence should be addressed

Methods

Reversed-Phase High-Pressure Liquid Chromatography (RP-HPLC) The reaction mixture from conjugation was purified by RP-HPLC (Beckman Coulter) to obtain AK₄-4P2K with reasonable purity using a C4 column (Vydac 22 mm x 250 mm) at a flow rate of 8 mL min⁻¹. Conjugates were eluted with a linear AB gradient, where solvent A consisted of water containing 0.1% (v/v) TFA and solvent B consisted of acetonitrile (ACN) containing 0.1% (v/v) TFA. A linear gradient of 20 to 65% solvent B over 45 min was used and the elution was monitored using a diode array detector at wavelengths of 200 nm and 220 nm. AK₄-4P2K conjugates were eluted at ~55% ACN.

MALDI-TOF Mass Spectrometry. The identity and purity of the CPs and CP-PEG conjugates were verified by MALDI-TOF MS using α -cyano-4-hydroxycinnamic acid matrix. Mass spectra were recorded on an Applied BioSystems Voyager-DE Pro.

Nuclear Magnetic Resonance Spectroscopy (NMR) NMR experiments were performed using Bruker AVQ-400 with 5mm QNP probe. Chemical shifts were taken relative to $CFCl_3$ at 0 ppm and D_2O was used as the solvent. 2,2,2-trifluoroethanol was added as the reference to estimate the amount of trifluoroacetic acid in solution.

Thin Film Si wafers were modified using a random copolymer of styrene and methyl methacrylate as described previously.¹ A \sim 12-14 nm layer of BCB was deposited onto the wafer and subsequently heated to 250°C for 20 minutes to anchor the polymer chains to the substrate and also to crosslink the chains randomly. This brush layer was then rinsed with toluene three times at 3000 rpm to remove any uncrosslinked chains. AK₄-4P2k conjugates and PS-b-PMMA BCPs were dissolved in toluene at 1 % w/v and the solutions was sonicated for 30 minutes. Thin films were prepared by spin casting the sonicated solution at 2500 rpm at room temperature. For the benzene thin films, the preparation was similar except the solution was spin casted at 8000 rpm instead of 2500 rpm. All films were annealed in vacuum for 4 hours and slowly cooled down to room temperature by placing the films in a Styrofoam box. Freestanding films were also prepared for TEM in which the thin films were prepared on special Si wafers with 200nm thermal oxide layer. After annealing, the films were immersed in a 5 wt % HF in H₂O solution to etch away the thermal oxide layer and then transferred to a DI water bath to float the films off the substrates. Finally, freestanding films were transferred onto copper grids for TEM measurements.

^{1.} Ryu, D. Y., Shin, K., Drockenmuller, E., Hawker, C. J., and Russell, T. P. *Science* **2005**, *308*, 236.

Figure S1. Purification and characterization of AK_4 -PEG conjugates. Figure S1 shows the RP-HPLC trace of PEG conjugation reaction mixtures, where 4 peaks are observed corresponding to AK_4 -PEG conjugates with 1, 2, 3, and 4 PEG chains attached respectively. The fraction collected between 32-34 min corresponds to AK_4 -4P2k (4arms), whose purify is confirmed via MALDI-TOF (Fig. 1a) and RP-HPLC (Fig. 1b).



Figure S2. Characterization of as-casted thin films from benzene and toluene solution. Left panel shows the height-contrast and the right panel shows the corresponding phasecontrast AFM image of the same sample. For good solvent benzene, no large aggregates are observed for f = 1-3 wt% as shown in Figure S2 a-c). In contrast, aggregates shown as darker regions in the phase images are observed for films casted from toluene (Figure S2 d-f). Moreover, the sizes of the aggregates are correlated to the value of *f*, demonstrating a concentration dependence of self-aggregation of AK₄-4P2k conjugates in toluene solution.



Figure S3. 19F NMR spectra of AK₄-4P2k conjugates (a) post TFA vapor treatment and (b) first treated with TFA vapor and then thermally heated at 180 °C for 4 hours in vacuum. The triplet peak ~ -76 ppm corresponds to TFE and the single -74 ppm peak corresponds to TFA. The lack of the peak around ~ -74 ppm in (b) indicates that TFA molecules are mostly removed during thermal treatment to the level below the detection limit of the NMR. From the integrated areas of TFA and TFE in (a), the amount of TFA in the sample can be estimated.



Figure S4. Calculation of the critical AK_4 -4P2k loading of the system. As shown in Figure 3, a hexagonal prism is considered as a unit cell. The mass of BCP in the unit cell

can be estimated using $M_{BCP} = \rho \cdot \frac{3\sqrt{3}}{2} L_o^2 \cdot h$, where ρ is the density, L_o is the lateral periodicity of the BCP respectively; and h is the thin film thickness. Under the constraint of the hydrogen bond distance and known film thickness, the number of AK₄-4P2k conjugates in each PMMA cylinder can be estimated by taking the ratio of the two. The

$$M_{AK4-4P2K} = 3 \cdot \frac{h}{D_{H \text{ bond}}} \cdot m_{AK4-4P2K}$$

where $D_{H \text{ bond}}$ is the hydrogen bond distance, $m_{AK4-4P2K}$ is the mass of one AK₄-4P2K conjugate molecule. The ratio of the conjugate to the BCP gives an estimate of the loading limit ~ 2.6 wt%.

amount of AK₄-4P2k in the unit cell becomes:

Figure S5. a) GISAXS intensity profiles of AK₄-4P2k/PS-*b*-PMMA blends (solid lines) and homopolymer PEG/PS-*b*-PMMA blends (dotted lines) at the same loading values. b) Changes in the periodicities of the two different blends at the same loading values with respect to the periodicity of PS-*b*-PMMA (L_o). The incorporation of AK₄-4P2k conjugates results in much bigger change, indicating larger volume occupied by the conjugates. c) AFM phase contrast image of 3 *wt*% AK₄-4P2k in PS-*b*-PMMA processed using good solvent benzene. Surface defects of PEG covered CPNs are seen at several places, likely due to overloading as the critical loading is ~ 2.6 *wt*%.



Figure S6. TFA treatment setup. Two separate glass vials containing 1mL TFA solution and the freshly lyophilized conjugate powder were placed in a glass jar. After the cover is tightly closed, highly volatile TFA molecules escape from the solution and form TFA vapor inside the jar. The treatment lasts for 2 minutes and the vial containing the conjugate powder will be removed from the jar and settled for 1 minute before mixing with BCP in toluene.



