Supporting Information

Direct Observation of Morphological Tranformation from Twisted Ribbons into Helical Ribbons

E. Thomas Pashuck,† and Samuel I. Stupp*,†,‡,§,ǁ

Department of Materials Science and Engineering, Department of Chemistry, Institute for BioNanotechnology in Medicine, and Feinberg School of Medicine, Northwestern University, Evanston, Illinois 60208

Received ; E-mail: s-stupp@northwestern.edu

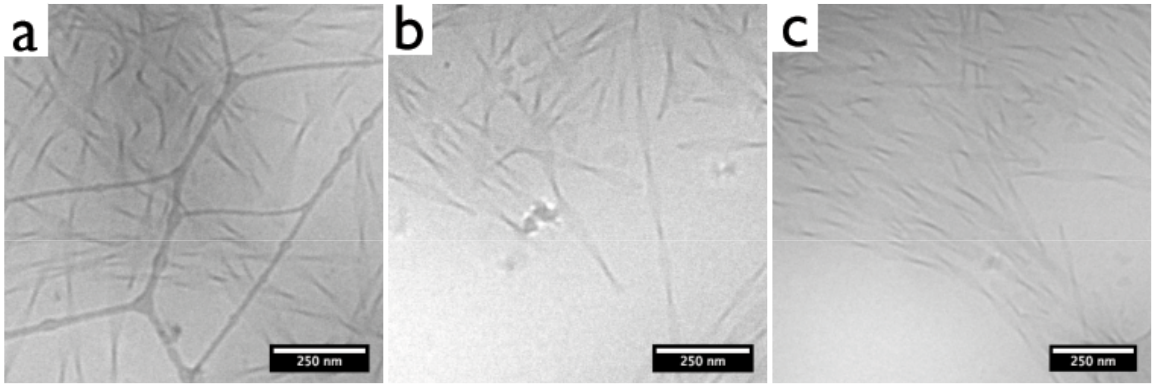


Figure S1. Representative cryoTEM images of PA **1** freshly dissolved in water after three minutes. At early time points the dominant nanostructure of PA **1** is twisted ribbons.

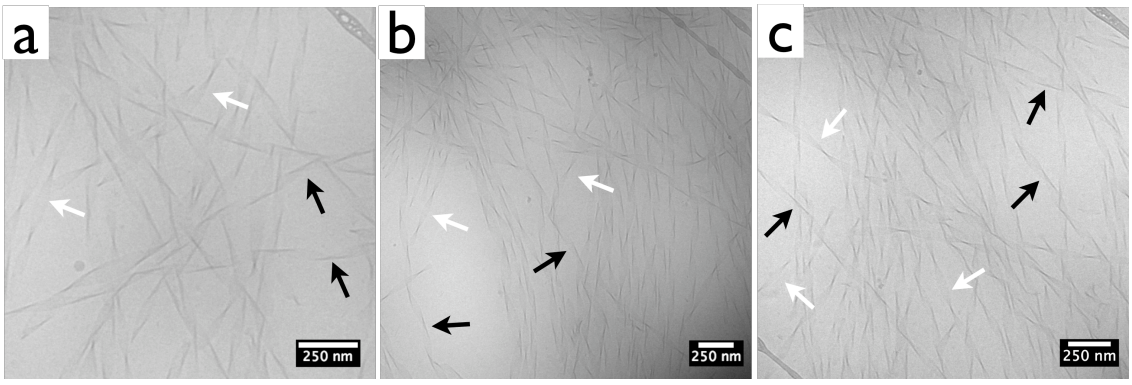


Figure S2. Representative cryoTEM images of PA **1** after being aged for two weeks at 25ºC. A mixed population of twisted ribbons (white arrows) and helical ribbons (black arrows) are present.

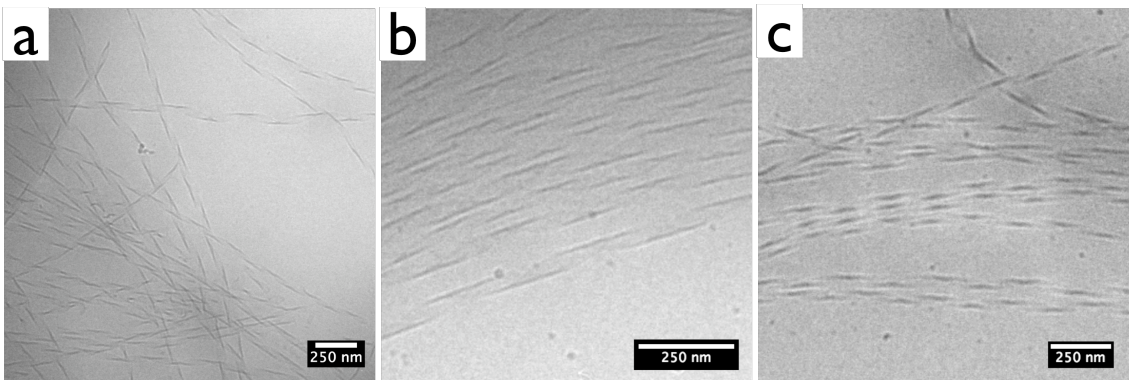
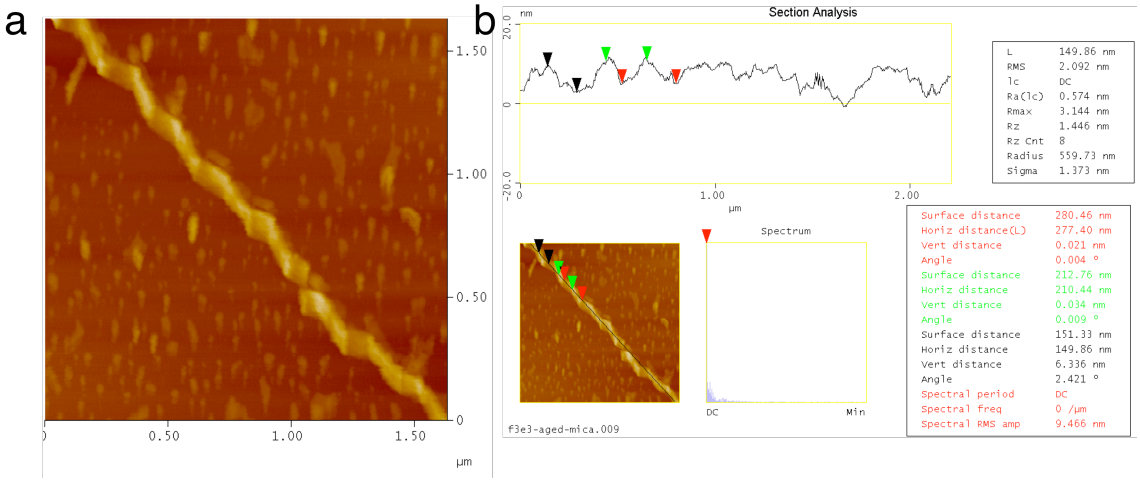


Figure S3. Representative cryoTEM images of PA **1** after being aged for four weeks at 25ºC. At early time points the dominant nanostructure of PA **1** is helical ribbons.



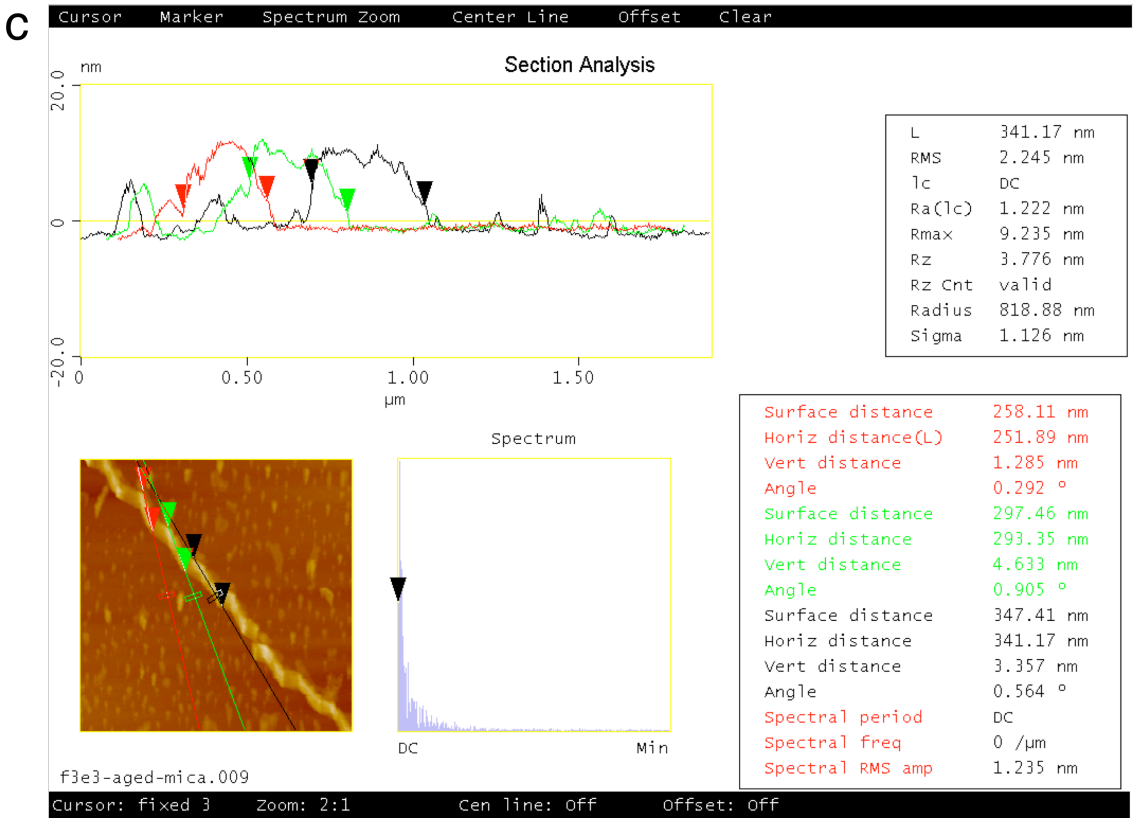


Figure S4. Representative AFM images of PA **1** after being aged for four weeks at 25ºC. a) Image of right-handed helix, b) analysis of helical heights and lengths and c) analysis of ribbon thickness (in green).

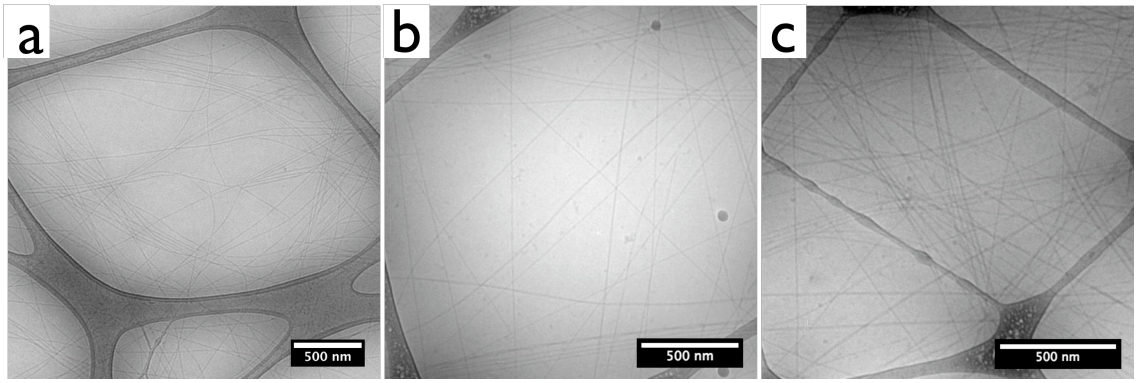


Figure S5. CryoTEM of PA **2** a) freshly dissolved in water b) after two weeks of aging at 25ºC c) after four weeks of aging at 25ºC. PA **2** forms cylindrical nanofibers at all time points.

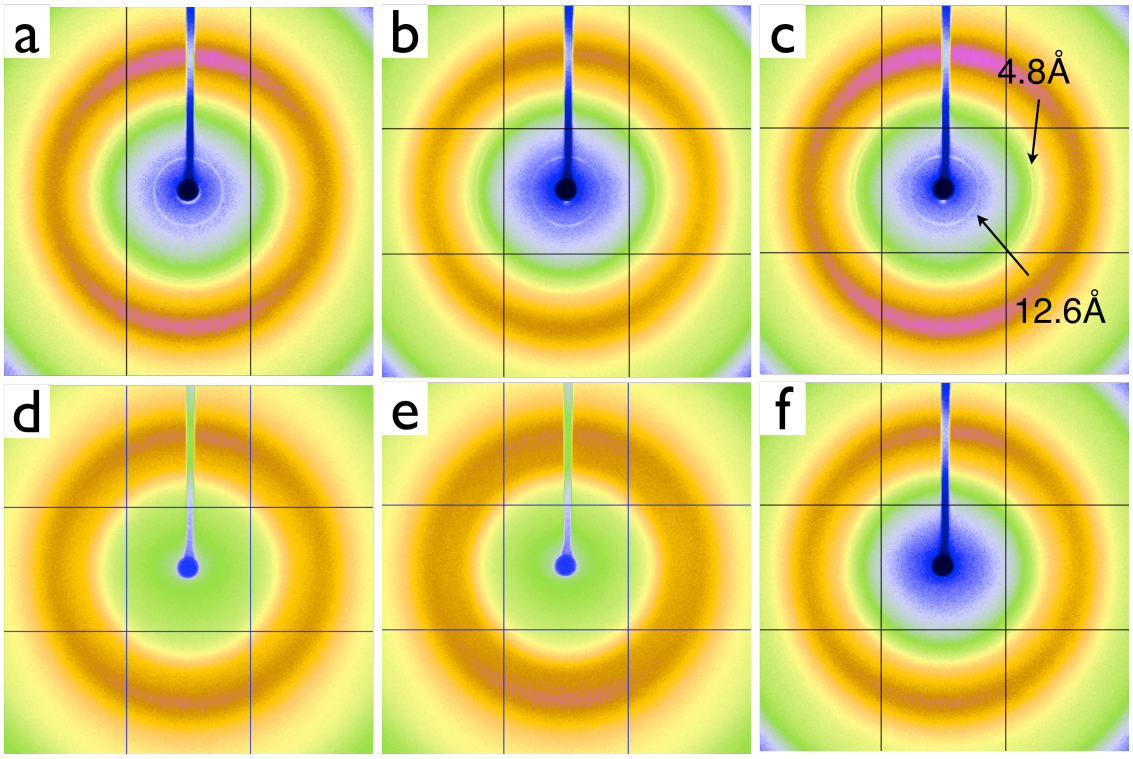


Figure S6. XRD of PA **1** a) freshly dissolved b) after two weeks of aging c) four weeks of aging and PA **2** d) freshly dissolved e) after two weeks of aging f) four weeks of aging.

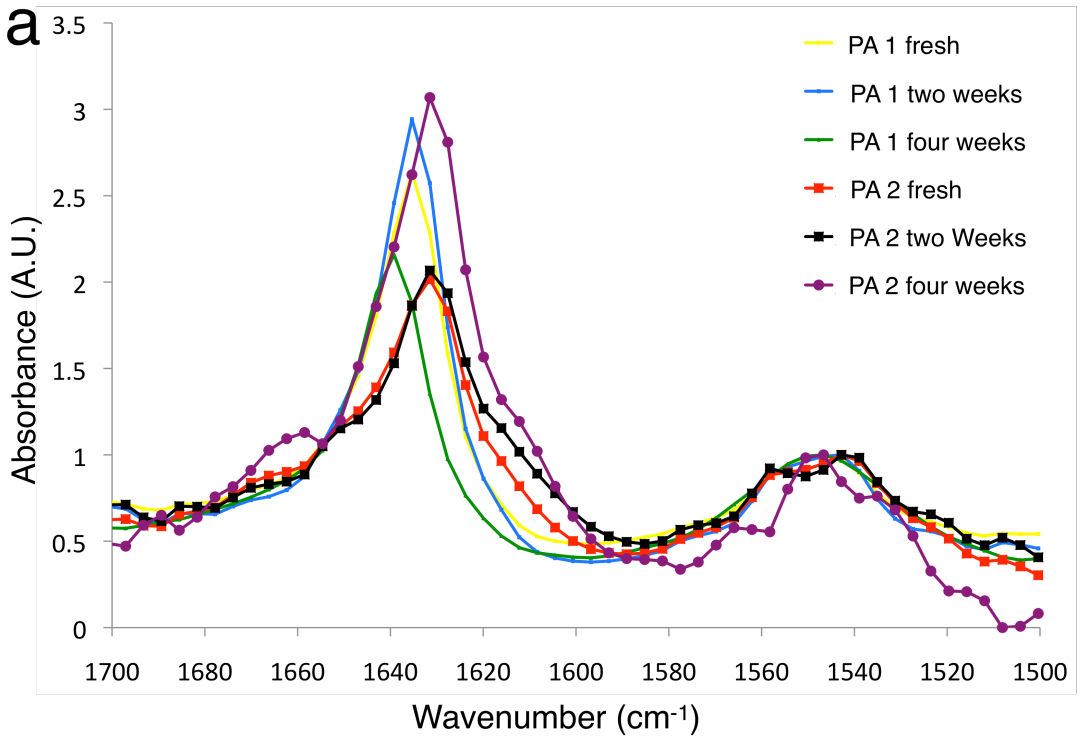


Figure S7. Fourier transform IR spectroscopy of PA **1** and **2** at various time points in the a) amide I and II region and b) CH_2 region.

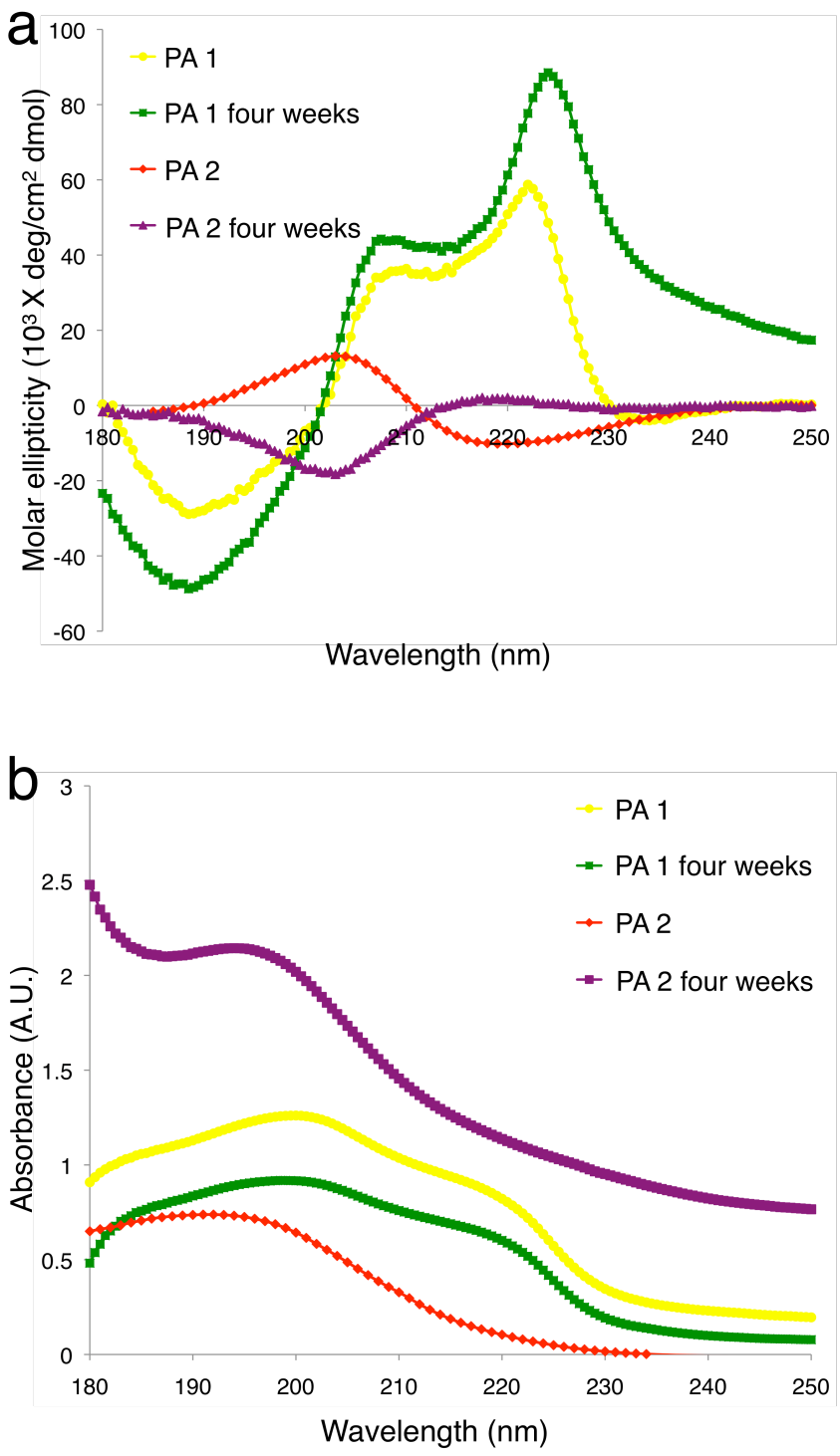


Figure S8. Spectroscopic characterization of the PA **1** and **2** by a) circular dichroism and b) UV-Vis spectroscopy.

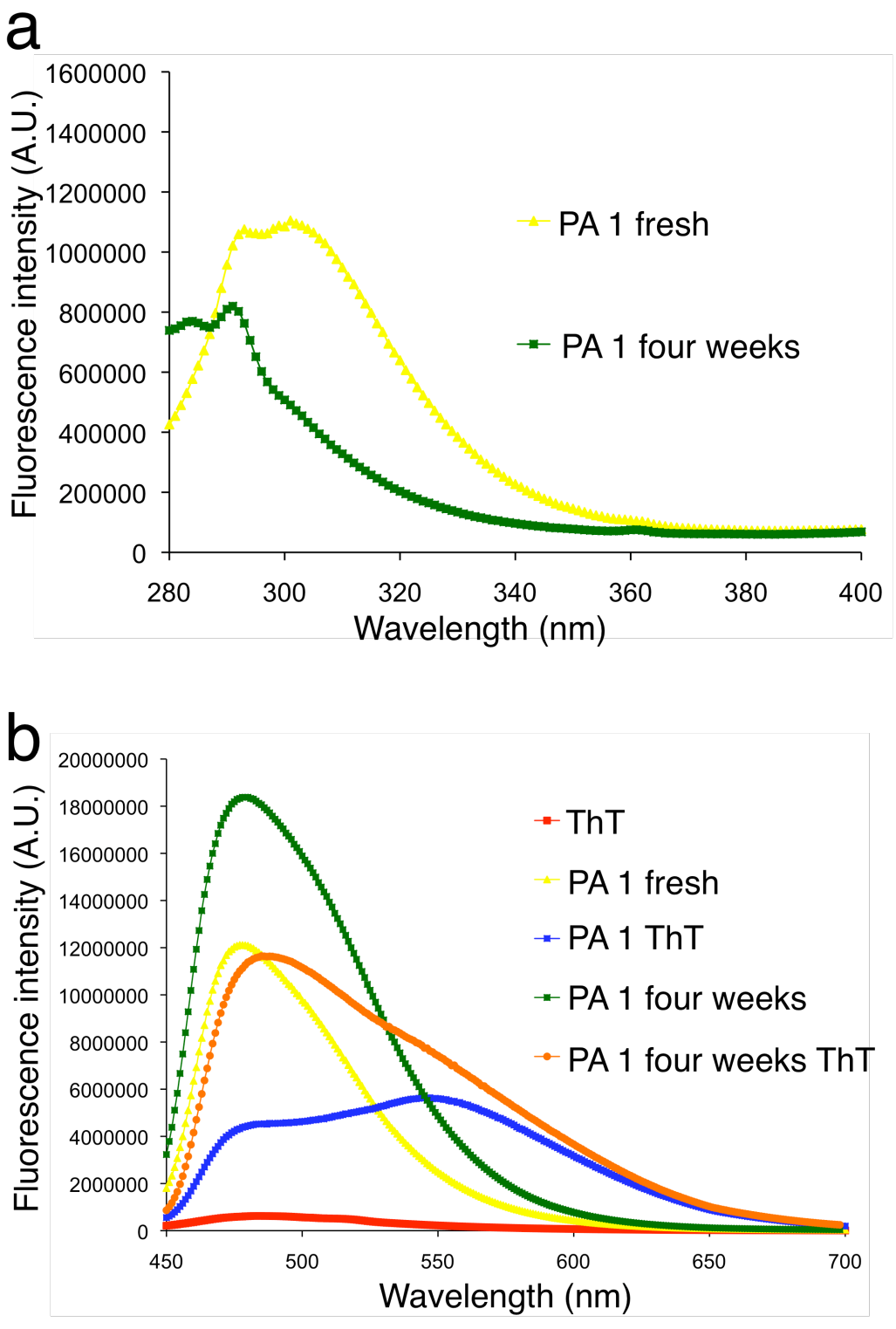


Figure S9. Fluorescence emisssion spectra of PA **1** excited at a) 265 nm and b) 440 nm.

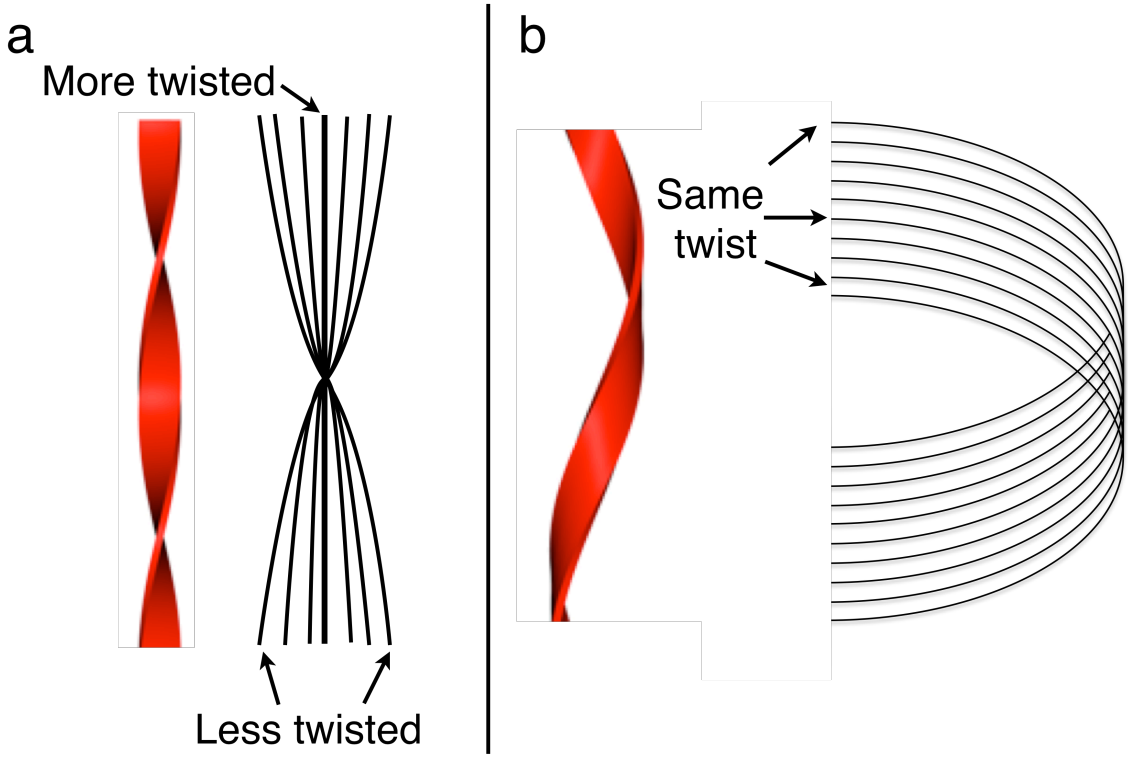
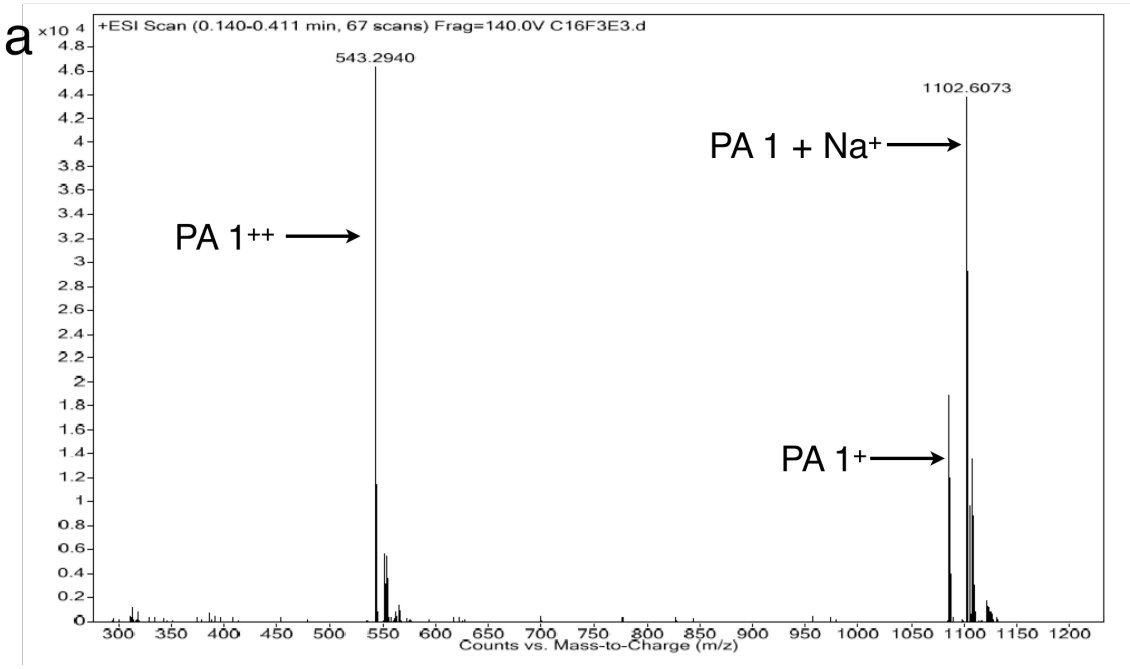


Figure S10. Model of β-sheets in (a) twisted ribbon and (b) helical ribbon geometries. In a twisted ribbon the β-sheet going down the center of the ribbon is best visualized as a twisted line, while those on the outside of the ribbon are helical and less twisted, since it takes a longer β-sheet distance (and thus more PA molecules) to complete a single rotation in a helix than in a straight line. In the helical ribbon every β-sheet is helical, with an identical amount of twist.



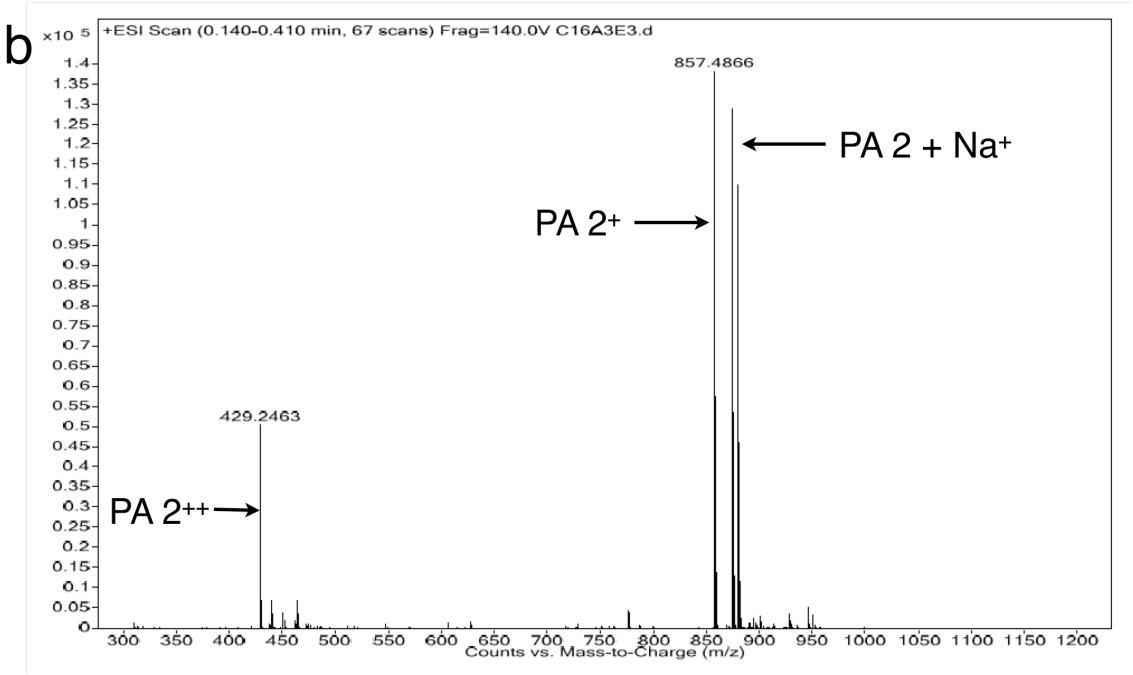


Figure S11. Electrospray ionication mass spectroscopy of a) PA **1** and b) PA **2.**

Methods and Materials

Peptide Amphiphile Synthesis and Purification PAs were synthesized using resins and Fmoc-protected amino acids purchased from Novabiochem Corporation. All other solvents were ACS reagent grade and purchased from Mallinckrodt and reagents were purchased from Aldrich and used as received. Manual solid-phase peptide synthesis was performed on a 0.5 mM scale using 50mL peptide synthesis vessels (Chemglass) and a wrist-action shaker. In each molecule the first glutamic acid was purchased preloaded as a Fmoc - Glu (OtBu) Wang resin. For each coupling the Fmoc protecting group was removed by shaking the resin in 30% piperidine in *N*,*N*-dimethylformamide (DMF) for ten minutes, rinsed and repeated a second time. The resin was washed with

dichloromethane (DCM) and DMF, and allowed to swell in DCM for 15 minutes before coupling. Amino acids were activated by adding 4 molar equivalents of the Fmoc-protected amino acids to 4 molar equivalents of *O*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyluronium-hexafluorophosphate (HBTU) and dissolving in 30 ml of DMF. Six molar equivalents of *N*,*N*-diisopropylethylamine (DIEA) were added to the amino acid solution, which was allowed to sit for a minute before being added to the resin. The coupling reaction was allowed to proceed for three hours, at the end of which the resin was washed in DCM and DMF and ninhydrin tests were done to check for the presence of free amines. If the ninhydrin test yielded a positive result the coupling was repeated. The palmitoyl tail was added using same molar ratio of palmitic acid/HBTU/DIEA of 4:4:6. PAs were cleaved by shaking the resin in a peptide cleavage solution of 95% trifluoroacetic acid (TFA), 2.5% triisopropyl silane (TIS) and 2.5% H_2O for three hours. The cleavage solution was drained into a round bottom flask and the resin was rinsed several times with DCM. All liquid was removed using rotory evaporation, and the PA residue was washed with cold diethyl ether and poured into a fritted filter. After the diethyl ether passed through the filter, the PA flakes were rinsed again with diethyl ether, allowed to dry and then placed in a vacuum dessicator until HPLC purification.

To dissolve the PA after cleavage, ultrapure water was added to make the PA 20 millimolar and ammonia hydroxide was added until the pH was raised to 8. The solution was passed through a 0.22 micrometer filter and injected into a preparative-scale reverse-phase HPLC running a mobile phase gradient of 98% H_2O and 2% acetonitrile (spectroscopic grade, Mallinckrodt) to 100% acetonitrile. 0.1% NH_4OH was added to all mobile phases to aid PA solubility. The Phenomenex C_{18} Gemini NX column had a 5

micron pore size, a 110Å particle size and was 150 x 30 mm HPLC fractions were checked for the correct compound using electrospray ionization mass spectroscopy (ESI-MS), rotary evaporated to remove acetonitrile and lyophilized (Labconco, FreezeZone6) at a pressure 0.015 torr. To remove any excess salts, PAs were dissolved in water and dialyzed in 500 molecular weight cutoff dialysis tubing (Spectrum Laboratories). After dialysis the PAs were lyophilized. PAs were then solubilized by adding the appropriate amount of H_2O and adjusting the pH to 7.4 using ammonium hydroxide.

Electron Microscopy Samples for vitreous ice cryo-transmission electron microscopy (cryo-TEM) were prepared by pipetting 10 μL of 10 mM PA solution onto a plasma cleaned holey carbon TEM grid (Electron Microscopy Sciences), blotted and plunged into liquid ethane using a FEI Vitrobot Mark IV. Samples were kept at -180°C and imaged using a JEOL 1230 TEM. For the time dependent studies the PAs were dissolved in hexaflouroisopropanol (HFIP) and then heated to 65 °C to remove the HFIP. Due to the carboxylic groups on the PA molecules, H_2O has to be pH adjusted to 10 to get final pH of 7-8 when mixed with PA powder to get a 10mM final concentration. This pre-pH adjusted H_2O was added to the HFIP treated PA and immediately pippetted onto a TEM grid to get samples at various time points after solubilization.

Circular Dichroism and UV-Vis: CD and UV-Vis were simultaneously done using a model J-815 Jasco Circular Dichroism Spectrometer. The PAs were studied at a concentration of 10 mM using a 0.1 mm cuvette. Each trace represents the average of three scans.

FTIR: Undoped silicon wafers (University Wafer, 3,000-3,500 ohm-cm resistivity) were cut into 10×20 mm sections and sonicated for ten minutes in water (Millipore filtered,

resistivity 18.2 MΩ cm) and ten minutes in isoproanol. 20 μL of a one mM PA solution was spread onto the substrate and allowed to dry in air. Transmission FTIR absorption spectra were collected using a Thermo Nicolet model Nexus 870 FT-IR spectrometer. Spectra are the average of 128 scans taken using a mercury-cadmium-telluride detector. FTIR spectra were baseline corrected using the Omnic FTIR software and normalized to the Amide II peak at 1550 cm^{-1}.

X-ray Diffraction: 10 mmolar PA solutions were pipetted into 2.0 mm capillary tubes (Charles Supper Company). All diffraction was performed at the Advanced Photon Source at Argonne National Laboratory, using the BioCARS beamline. The X-ray wavelength was 0.9787 Å with a detector distance of 400 mm. An AdSC Quantum-315 CCD detector was used to capture the diffraction patterns, and Fit2D software was used to analyze the data. The black lines are from the edges of the nine smaller detectors in the Quantum detector array. A background subtration using a water filled capillary tube was done for all samples, but due to variations in the thickness of the capillary glass some broad peaks are still present.

Fluorescence Spectroscopy: PAs were studied at 0.1 mmolar concentrations using a Nanolog HJ fluorometer. For studies with thioflavin T (ThT), ThT was dissolved at 1 mg/ml and filtered through a 0.2 μm syringe filter to make a 0.1 wt% stock solution. It was then diluted 1:50 in either milliQ water or 0.1 mmolar PA solutions. Spectroscopic studies were done by exciting at 265 nm and recording emission spectra from 280 nm to 400 nm, and by exciting at 440 nm and recording the emission spectra from 450 nm to 700 nm.

Atomic Force Microscopy: Samples were imaged with a Veeco Bioscope in tapping mode and Asylum Research AC 240TS cantilevers. PA was initially dissolved at 10 mmolar and then diluted 1:50 in ultrapure water before 20 μL was pipetted, spread on a mica surface and allowed to dry before imaging.