Supporting Information.
Self-Assembled Arginine-Coated Peptide Nanosheets in Water

Ian W. Hamley,* Ashkan Dehsorkhi and Valeria Castelletto
Department of Chemistry, University of Reading, Reading, RG6 6AD, U.K.

Acknowledgements

This work was supported by EPSRC grants EP/F048114/1 and EP/G026203/1 to IWH. We thank Dr Joe Wall and Dr Beth Lin, Brookhaven National Laboratory for the STEM measurements. We are grateful to Dr Ge Cheng for the synthesis of one batch of A₆R. We thank Narayan Theyencheri (ESRF) for assistance with experiments on beamline ID02, Petra Pernot for help on ID14-3 (beamtime ref. MX-1347). At SOLEIL on beamline SWING we thank Javier Perez (beamtime ref. 20110562). We are grateful to Nick Spencer for assistance with XRD experiments and Claire Moulton for the FTIR. We thank Steve Furzeland and Derek Atkins (Unilever, UK) for the cryo-TEM images.

Experimental

Materials. Four batches of peptide were used. Three batches of NH₂-AAAAAAAR-COOH, referred to as A₆R, were custom synthesized by C.S. Bio Company (USA) and were used as received as a TFA salt. For the first, the purity was 97.01% by HPLC in water/acetonitrile (0.1% TFA). Electrospray-ionisation mass spectroscopy (ESI-MS) indicated a molar mass 600.87 g mol⁻¹ (600.69 g mol⁻¹, expected). For the second, the purity was 98.47% by HPLC in water/acetonitrile (0.1% TFA). ESI-MS indicated a molar mass 600.40 g mol⁻¹. The third batch had a purity of 98.94% by HPLC in water/acetonitrile (0.1% TFA) with a molar mass 600.40 by ESI-MS. The
fourth batch of material was synthesized in house using standard solid phase peptide synthesis methods (ABI 433A automated peptide synthesizer) using Fmoc-amino acids [Fmoc (9-fluorenylmethyloxycarbonyl)] and activation with HBTU/HOBt/DIPEA in NMP [HBTU = O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate, HOBt= 1-hydroxybenzotriazole, DIPEA = N,N-diisopropylethylamine].

To characterize self-assembly in water, solutions were made using amounts of peptide dissolved in Millipore water. The pH for solutions with 13.3-17% A6R was measured (Mettler Toledo FiveEasy pH Meter) to be pH 2.

**FTIR.** Spectra were recorded using a Nexus-FTIR spectrometer equipped with a DTGS detector and a multiple reflection attenuated total reflectance (ATR) system. Solutions of A6R in D2O (2 – 17 wt.%) were sandwiched in ring spacers between two CaF2 plate windows (spacer 0.006 mm). All spectra were scanned 128 times over the range of 4000-950 cm⁻¹.

**Thioflavin T (ThT) Fluorescence Spectroscopy.** Spectra were recorded with a Varian Cary Eclipse Fluorescence Spectrometer with samples in 4 mm inner width quartz cuvettes. The spectra were recorded from 460 to 600 nm using an excitation wavelength λex= 440 nm. ThT assays were performed using a 4.0×10⁻³ wt % ThT solution.

**Scanning Transmission Electron Microscopy (STEM).** STEM imaging and associated quantitative analysis (mass per unit area) were performed at the STEM
facility, in the Biology Department at Brookhaven National Laboratory using a custom-built instrument. Further details are provided elsewhere.¹

**Cryogenic-Transmission Electron Microscopy (Cryo-TEM).** Experiments were performed at Unilever Research, Colworth, Bedford, UK. Sample preparation was carried out using a CryoPlunge 3 unit (Gatan Instruments) employing a double blot technique. 3 µl of sample was pipetted onto a plasma etched (15 s) 400 mesh holey carbon grid (Agar Scientific) held in the plunge chamber at approx. 90% humidity. Samples were blotted from both sides. The samples were then plunged into liquid ethane at a temperature of -170 °C. The grids were blotted to remove excess ethane then transferred, under liquid nitrogen to the cryo TEM specimen holder (Gatan 626 cryo holder) at -170 °C. Samples were examined using a JEOL 2100 TEM operated at 200 kV and imaged using a Gatan Ultrascan 4000 camera and images captured using DigitalMicrograph software (Gatan).

**X-ray Diffraction (XRD).** Measurements were performed on stalks prepared by drying filaments of the peptide from 13.3 or 16.8 wt% solutions. Solutions of the peptide were suspended between the ends of wax-coated capillaries and dried. The stalks were mounted (vertically) onto the four axis goniometer of a RAXIS IV++ X-ray diffractometer (Rigaku) equipped with a rotating anode generator. The sample-detector distance was 60 mm. The X-ray wavelength was \( \lambda = 1.54 \ \text{Å} \). The wavenumber scale \( (q = 4\pi\sin \theta/\lambda, \text{where } 2\theta \text{ is the scattering angle}) \) was geometrically calculated using the size of each pixel in the detector screen (0.0898 mm) and the sample-detector distance. The XRD data was collected using a Saturn 992 CCD camera. XRD was also performed using the same system on hydrated gels containing
17 wt% A₈R, mounted in borosilicate capillaries with $D = 1$ mm internal diameter and 0.01 mm wall thickness. The capillary flow cell was positioned horizontally and the sample-detector distance was 70 mm.

**Flow-Aligning X-ray Diffraction.** A capillary flow device was used, details of which have been given elsewhere.² Briefly, the central part of the capillary flow device is a computer controlled peristaltic pump that allows controlled volume and time dispensing. The flow rate is recorded and the unit is interfaced to a PC for acquisition of flow rate data. We used borosilicate capillaries. The measured flow rates were in the range $Q = 1$ to 6 ml min⁻¹. These correspond to Newtonian shear rates at the wall of $\dot{\gamma} = 32Q/\pi R^3 = 170$ s⁻¹ to 1020 s⁻¹. The actual flow rate will differ for non-Newtonian fluids, and for this reason we quote flow rates $Q$. The XRD experiments were carried out using a four axis goniometer of a RAXIS IV++ x-ray diffractometer (Rigaku) equipped with a rotating anode generator. The XRD data was collected using a Saturn 992 CCD camera. The capillary flow cell was positioned with the flow direction $v$ parallel to the horizontal (x-axis) in the 2D detector plane.

**Small-Angle X-Ray Scattering.** SAXS data for dilute solutions were measured using beamline ID14-3 at the European Synchrotron Radiation Facility, Grenoble, France. Samples were loaded in PCR tubes in a multi-well plate in a robotic sample changer and delivered automatically into a flow-through capillary tube. SAXS patterns were recorded using a Pilatus 1M detector with a sample-detector distance of 2.43 m. The X-ray wavelength was 0.931 Å. Data were reduced to one-dimensional form and background subtraction was performed using the software SAXSUtilities (www.sztucki.de/SAXSUtilities). Repeat experiments were performed on beamline SWING at SOLEIL. (L’Orme des Merisiers, France). A few microlitres of sample
(0.1, 1 and 2 wt% solutions) were injected at a slow and very reproducible flux into a quartz capillary, placed in front of the X-ray beam. After the sample was injected in the capillary and delivered in front of the X-ray beam, the flow was stopped during the SAXS data acquisition. The wavenumber \( q \) range was set to 0.004-0.5 Å\(^{-1} \), with \( \lambda = 1.03 \) Å (12 keV). The images captured by the AVIEX170170 CCD detector were radially averaged and corrected for transmitted intensity and water background using the software \textit{Foxtrot}.

Data for a more concentrated sample were collected on beamline ID02 at the ESRF, Grenoble, France. A 17 wt% sample of A\(_6\)R in water was mounted in a flow-through capillary cell (diameter ~2 mm). This cell provides lower background, such that the sample and solvent scattering can be measured in the same position in the cell, allowing very reliable subtraction. SAXS data was collected using a FReLoN Kodak CCD detector. Sample-detector distances of 5m and 1.5 m were used. The X-ray wavelength was 0.995 Å.

\textbf{SAXS Data Modelling.} The SAXS intensity from a finite stack of unoriented bilayers can formally be written, within the monodisperse approximation, as:

\[ I(q) \propto \langle F^2(q)S(q) \rangle \]  

(1)

where \( F^2(q) \) is the form factor, and \( S(q) \) is the interference or structure factor, which tends to unity for weakly interacting systems.

The form factor was modelled as for a lipid bilayer (the A\(_6\) unit being the “lipid” chain), based on a sum of Gaussian functions to represent the electron density profile. The details of the model are given elsewhere. The model assumes an electron density
profile comprising Gaussians for the headgroups on either side of the bilayer and another Gaussian for the hydrocarbon chain. Scheme 2 shows a scheme of the electron density distribution along the lamellar normal and illustrates the parameters in the model. The total form factor, is taken as the contributions from the headgroup \(F_H(q)\) and the hydrocarbon chain \(F_C(q)\):

\[
F(q) = 2F_H(q) + F_C(q)
\]

where

\[
F_H(q) = \sqrt{2\pi}\sigma_H \rho_H \exp\left(-\frac{\sigma_H^2q^2}{2}\right) \cos(qz_H)
\]

and

\[
F_C(q) = \sqrt{2\pi}\sigma_C \rho_C \exp\left(-\frac{\sigma_C^2q^2}{2}\right)
\]

The fitting parameters of the model in Equations 3 and 4 are the electron densities of the headgroup \(\rho_H\), the thickness \(z_H\), the electron density of the hydrocarbon chains \(\rho_C\), the standard deviation of the position of the Gaussian peak \(z_H\) \(\sigma_H\) and the standard deviation of the position of the Gaussian peak at \(z_C\) \(\sigma_C\) (SI Scheme 1). The midpoint of the bilayer is defined as \(z = 0\). In our model we assumed a Gaussian distribution of inter-headgroup thicknesses \(z_H\), with an associated degree of polydispersity \(\Delta_H\). For the dilute solution of AβR analysed (data shown in Fig.3b) to a good approximation \(S(q) = 1\). Fitting was performed using the software SASfit. The parameters from the fit were as follows: \(\sigma_H = 0.3\) nm, \(\rho_H = 0.0011\), \(\sigma_C = 0.4\) nm, \(\rho_C = -8.3\times10^{-5}\), thickness \(t = 2Z_H = 2.89\) nm, with Gaussian polydispersity \(\text{HWHM} = 2\) nm, \(D = 500\) nm (diameter of bilayer disk, since \(D \gg t\), this only provides a scaling factor), constant background \(B = 0.5\).
For the more concentrated A₆R solution (Fig.3c), the form factor was modelled as a weighted sum of a bilayer Gaussian form factor (Eqs. 2-4) and a nanotube form factor:

\[ P_{\text{tot}}(q) = w_{\text{layer}}P_{\text{layer}}(q) + w_{\text{tube}}P_{\text{tube}}(q) . \]  

(5)

Again, we took \( S(q) = 1 \) although this is a significant approximation at the high concentration studied.

The form factor of a tube was computed using SASfit⁵ using the long cylindrical shell model:

\[ P_{\text{tube}}(q) = P'(q)P_{cs}(q) . \]  

(6)

Where the term arising from the length of the tube is

\[ P'(q) = 2 \frac{\text{Si}(qL)}{qL} - \left( \frac{\sin(qL/2)}{qL/2} \right) . \]  

(7)

\[ \text{Si}(x) = \int_{0}^{x} \sin t dt . \]  

(8)

And the form factor of the cross-section is

\[ P_{cs}(q) = \left( 2 \frac{J_1(qR)}{qR} (\sigma_{\text{core}} - \sigma_{\text{shell}}) R^2 L \pi + 2 \frac{J_1(q(R+\Delta R))}{q(R+\Delta R)} (\sigma_{\text{shell}} - \sigma_{\text{solv}})(R+\Delta R)^2 L \pi \right)^2 \]  

(9)
In Eq. 6 – Eq. 9, $R$ is the core radius, $\Delta R$ is the shell thickness, $L$ is the nanotube length (since $L >> R$ this parameter provides a scaling factor only), $\sigma_{\text{core}}$, $\sigma_{\text{shell}}$, $\sigma_{\text{solv}}$ are (relative) electron densities, for a tube $\sigma_{\text{core}} = \sigma_{\text{solv}}$ (these parameters were fixed).

The model parameters were as follows. For the layer structure, $w_{\text{layer}} = 5.52$, $\sigma_{\text{H}} = 0.33$ nm, $\rho_{\text{H}} = 1.36 \times 10^{-5}$, $\sigma_{\text{C}} = 0.33$ nm, $\rho_{\text{C}} = 5.4 \times 10^{-6}$, thickness $t = 2Z_{\text{H}} = 0.87$ nm, with Gaussian polydispersity (HWHM = 0.53 nm), $D = 500$ nm (diameter of bilayer disk, since $D >> t$, this only provides a scaling factor), constant background $B = 0.004$. For the nanotube, $w_{\text{tube}} = 1.63$, $R = 29.8$ with Gaussian polydispersity (HWHM = 2.97 nm), $\Delta R = 3.63$ nm, $\sigma_{\text{core}} = 1 \times 10^{-6}$, $\sigma_{\text{shell}} = 1.48 \times 10^{-5}$, $\sigma_{\text{solv}} = 1 \times 10^{-6}$. 
Tables

**SI Table 1** Calculated and observed X-ray diffraction peaks
(orthorhombic space group P2₁2₁2₁, \( a = 5.54 \text{ Å} \), \( b = 17.59 \text{ Å} \), \( c = 32.72 \text{ Å} \))

<table>
<thead>
<tr>
<th>Reflection</th>
<th>Calculated</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>32.72</td>
<td>32.3</td>
</tr>
<tr>
<td>002</td>
<td>16.36</td>
<td>16.5</td>
</tr>
<tr>
<td>003</td>
<td>10.91</td>
<td>10.9</td>
</tr>
<tr>
<td>100</td>
<td>5.54</td>
<td>5.54</td>
</tr>
<tr>
<td>114</td>
<td>4.44</td>
<td>4.44</td>
</tr>
<tr>
<td>125</td>
<td>3.81</td>
<td>3.81</td>
</tr>
<tr>
<td>150</td>
<td>2.97</td>
<td>2.97</td>
</tr>
<tr>
<td>201</td>
<td>2.76</td>
<td>2.76</td>
</tr>
</tbody>
</table>
SI Scheme 1. Gaussian model of the electron density profile used to describe \( F(q) \) in Eq. 2.
SI Fig.1. (a) Fibre X-ray diffraction pattern obtained from a stalk dried from a 17 wt% solution of A₆R, (b) Equatorial intensity profile with d spacings (Å) indicated, the 5.5 Å peak is truncated, to emphasize the other observed reflections, (c) XRD pattern from a 17 wt% sample of A₆R under flow at 6 ml/min.
SI Fig. 2. FTIR spectra for A₆R in aqueous solution at the concentrations shown.
**SI Fig.3.** Critical aggregation concentration determined via thioflavin T fluorescence experiments. Dependence of $I/I_0$ on A₆R concentration ($I$ = intensity of the ThT fluorescence peak for samples containing peptide, $I_0$ = the intensity of the fluorescence peak for a pure ThT solution). The inset shows two representative fluorescence emission curves for A₆R solutions containing $4 \times 10^{-3}$ wt% ThT.

**References**