Supporting Information

The Effect of pH on The Self-Assembly of a Collagen Derived Peptide Amphiphile

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Experimental

Materials. Peptide amphiphile C_{16}-KTTKS, Palmitoyl-Lys-Thr-Thr-Lys-Ser, was purchased from CS Bio (Menlo Park, California) as a TFA salt. Purity was 99.15% by analytical HPLC, MS 802.05 (expected) 802.73 (measured).

A sample of 1 wt% C_{16}-KTTKS was made in a sodium citrate buffer of pH 4. Aliquots of pH 4 solutions were adjusted by adding 0.1 M HCl to achieve a pH 2 solution and 0.1 M NaOH to obtain a solution of pH 7.

All characterization experiments were performed at room temperature.

Circular Dichroism Spectroscopy (CD). CD spectra were recorded using a Chirascan spectropolarimeter (Applied Photophysics, UK). Solutions of C_{16}-KTTKS (1 wt%) were loaded in parallel plaque cells (Hellma quartz Suprasil®), with a 0.01 mm

pathlength. The CD data were measured using 1 sec acquisition time per point and 0.5 nm step. The post-acquisition smoothing tool from Chirascan software was used to remove random noise elements from the averaged spectra. A residual plot was generated for each curve in order to verify whether or not the spectrum has been distorted during the smoothing process. The CD signal from the water was subtracted from the CD data of the peptide solutions.

Atomic Force Microscopy (AFM). A 20 µl of solution (1 wt% of C_{16}-KTTKS at pH 2, 4 and 7) were deposited onto freshly cleaved mica, incubated for 1 min and dried by air. Tapping mode AFM was carried out on a Nanoscope 8 Multimode Scanning Force Microscope (Bruker). AFM cantilevers (Bruker) for tapping mode in soft tapping conditions were used at a vibrating frequency of 150 kHz. Images were simply flattened using the Nanoscope 8.1 software, and no further image processing was carried out.

Small-Angle X-ray Scattering (SAXS). SAXS experiments were performed on the EMBL BioSAXS beamline P12 on storage ring PETRA III at the Deutsches Elektronen-Synchtrotron (DESY), Hamburg, Germany. 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 2M detector with a sample-detector distance of 3.1 m. The energy was 10 keV with a wavelength of 1.24 Å. Data were reduced to one-dimensional form and background subtraction was performed using the software PRIMUS.

Fibre X-Ray Diffraction (XRD). X-ray diffraction was performed on stalks prepared

from 1 wt% PA solutions in varying pH. 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 XRD data was collected using a Saturn 992 CCD camera. One-dimensional profiles in the equatorial and meridional reflections (with appropriate re-alignment of images to allow for fibril orientation) were obtained using the software CLEARER[1] which was also used to fit peak positions.

SAXS Data Modelling

The SAXS data for 1 wt% C_{16}-KTTKS at pH 2 formed spherical micelles, which was modelled using the software SASfit.[2] This was fitted to a "spherical shell ii" model with a Gaussian polydispersity in radius and takes into consideration an outer radius R and an inner radius *vR* and relative scattering constrast of the shell $\Delta\eta$ and of the core $\mu\Delta\eta$.

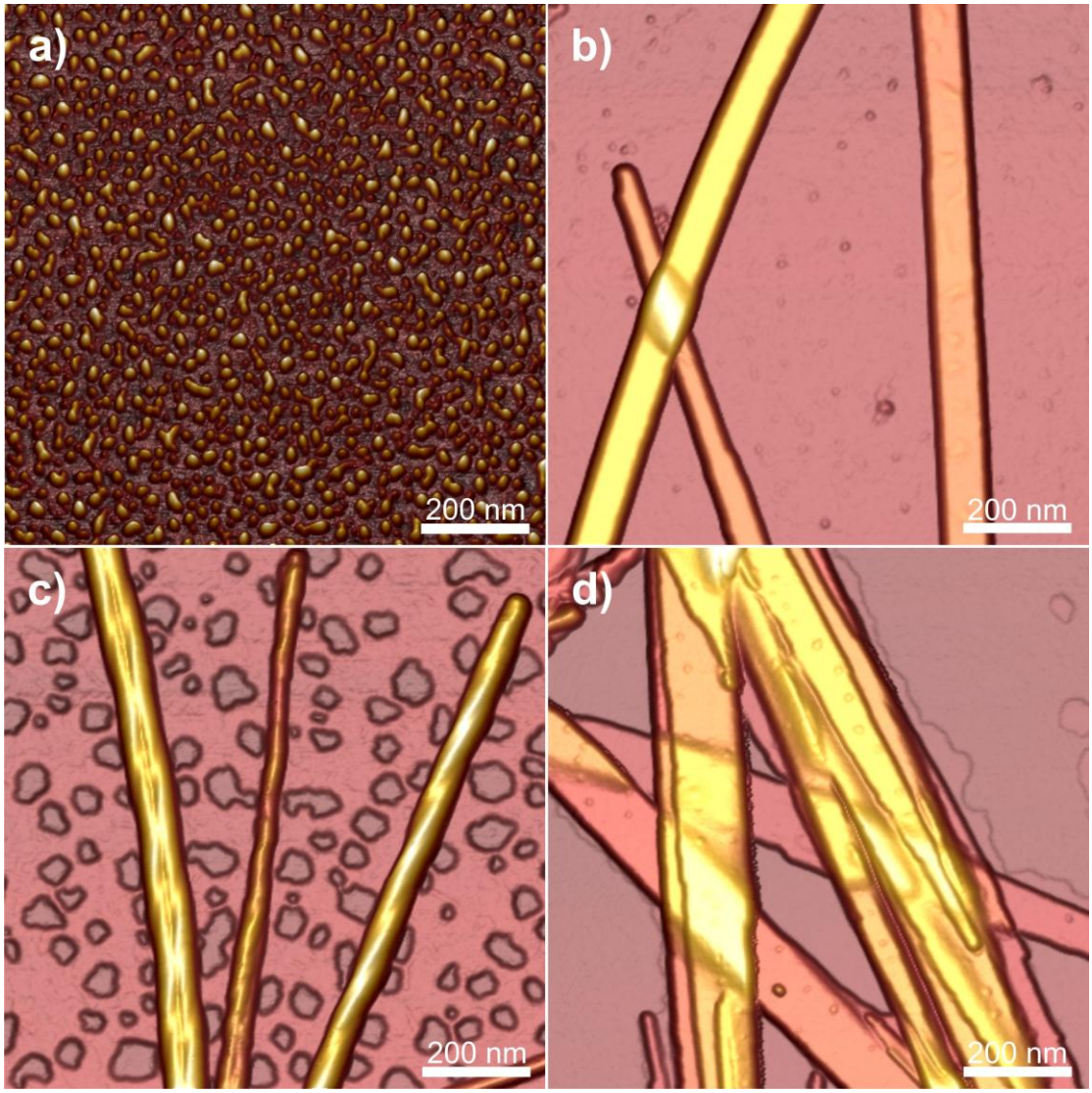
$$I(q) = \left(K(q,R,\Delta\eta) - K(q,vR,\Delta\eta(1-\mu))\right)^2,$$

with

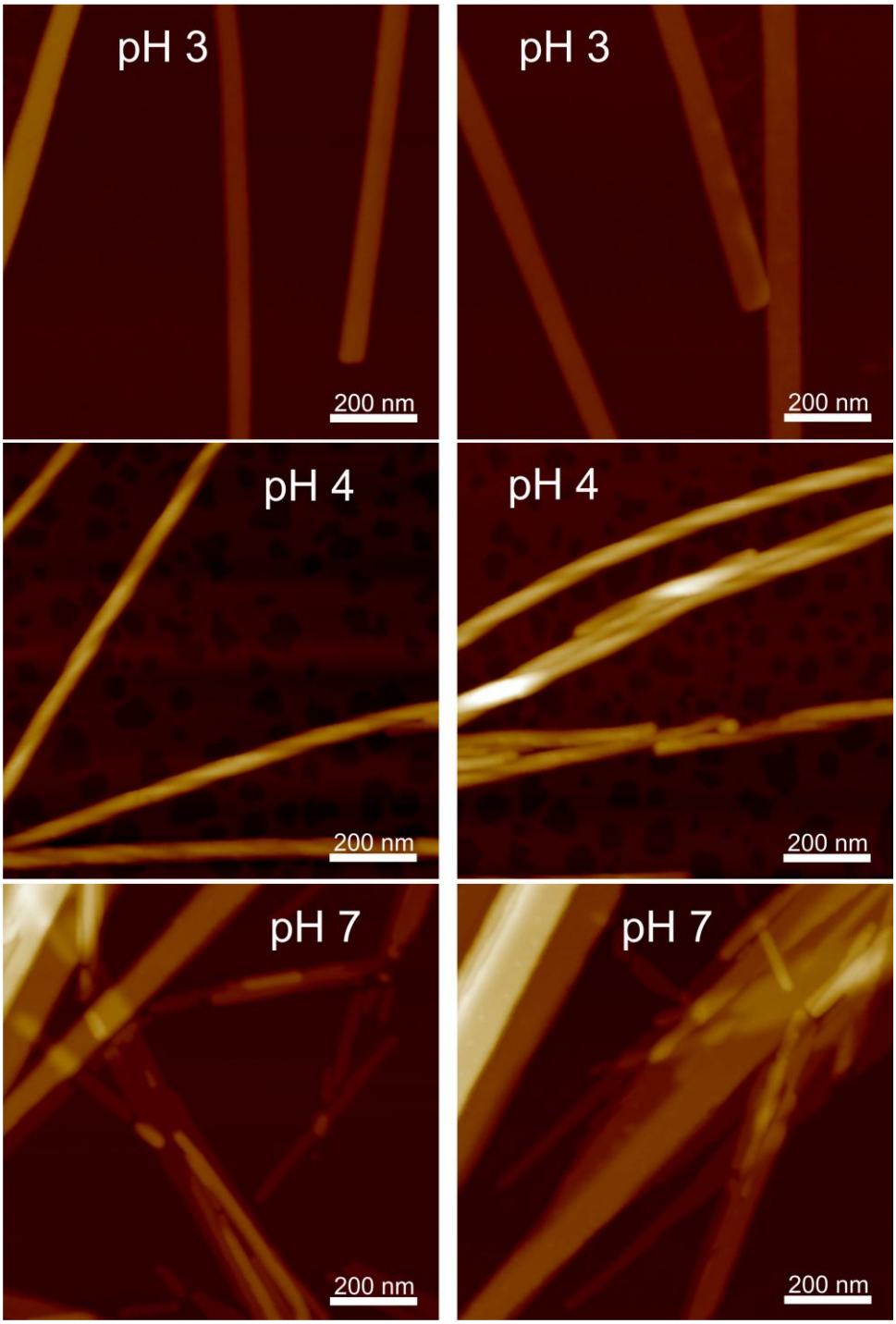
$$K(q,R,\Delta\eta) = \frac{4}{3}\pi R^3 \Delta\eta . 3\frac{\sin qR - qR\cos qR}{(qR)^3}$$

The parameters to fit the data were; N = 1, σ = 0.24, R = 2.79, v = 0.61, μ = -0.81, $\Delta\eta$ = 0.053. A constant background was required, BG = 0.05.

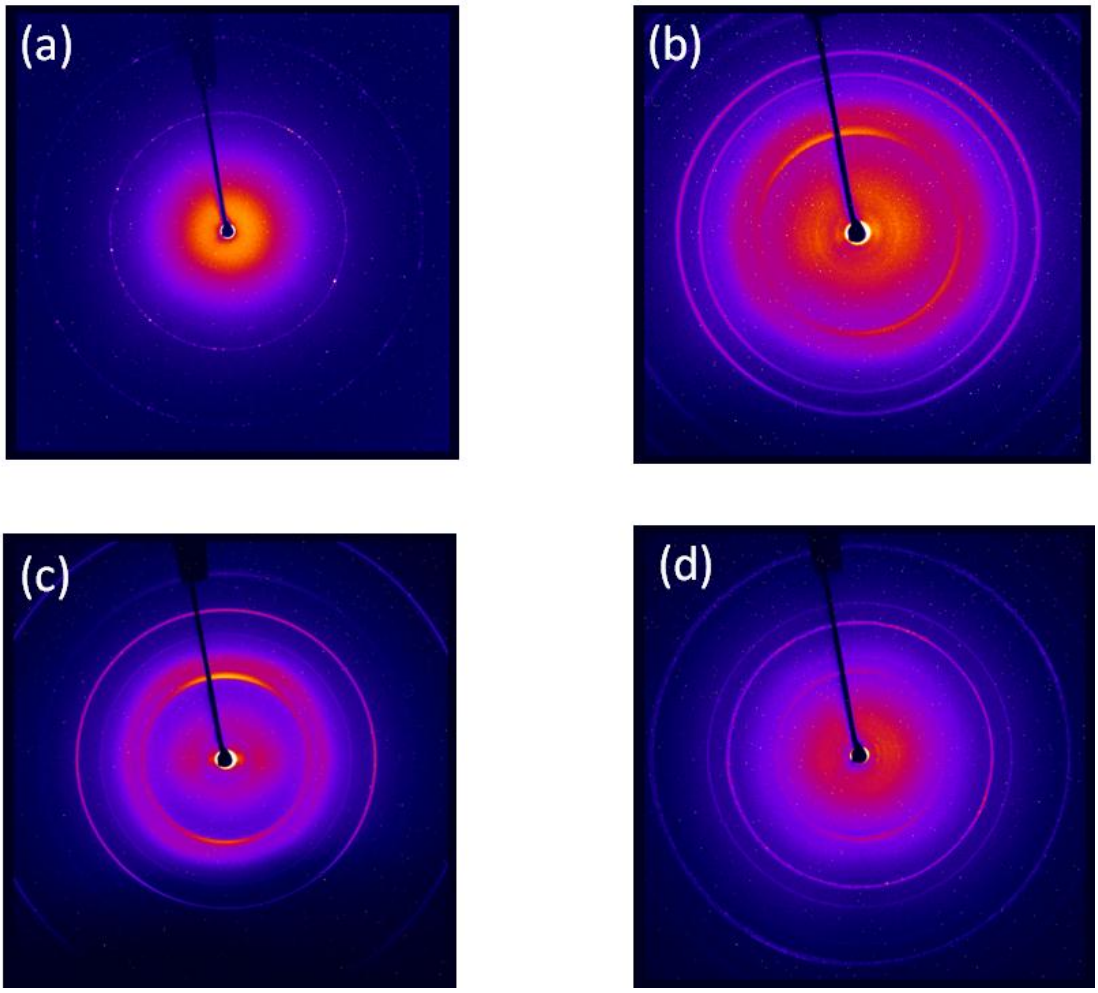
Results



SI Fig.1. 3D AFM images of of 1 wt% C_{16}-KTTKS at a) pH 2, b) native pH 3, c) pH 4 and d) pH 7. Z scale is 6 nm for a) and 60 nm for b), c) and d).



SI Fig. 2. AFM images of of 1 wt% C_{16}-KTTKS at pH 3, pH 4 and pH 7. Z scale is 60 nm for all images.



SI Fig.2. Fibre XRD patterns for 1 wt% C_{16}-KTTKS. (a) pH 2; (b) pH 3; (c) pH 4; (d) pH 7.

References

1. Makin, O. S.; Sikorski, P.; Serpell, L. C., *J. Appl. Crystallogr* **2007,** *40*, 966 -972.

2. http://kur.web.psi.ch/sans1/SANSSoft/sasfit.html', in 2013