Structure of Single-Wall Peptide Nanotubes: In situ Flow Aligning X-Ray Diffraction

V. Castelletto, D.R. Nutt, I.W. Hamley* , S. Bucak, Ç. Cenker, U. Olsson

Supporting Info

Experimental

Materials. Peptide A_6K was used as received from CPC Scientific Inc (97% purity) in the form of the TFA salt.

X-ray Diffraction. The capillary shear flow experiments were performed using an in-house built capillary flow device. Details of this machine have been given elsewhere.[1] 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 with R = 0.5 mm internal radius and 0.01 mm wall thickness. 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. X-ray diffraction was also performed on stalks prepared by drying filaments of the peptide. Aqueous solution (17 wt.%) of peptide was 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 XRD data was collected using a Saturn 992 CCD camera.

Fourier Transform Infrared spectroscopy. IR spectra including amide bands were recorded on a Nicolet FTIR Nexus spectrometer equipped with a DTGS detector.

Solutions of A_6K in D_2O were sandwiched in ring spacers between two CaF_2 plate windows (spacer 0.006 mm). Spectra were scanned 128 times over the range of 4000-400 cm^{-1}.

Small-Angle X-ray Scattering (SAXS). Experiments were performed on beamline I711 at the MAX-Lab in Lund, Sweden. Details are provided elsewhere.[2]

Cryogenic Transmission Electron Microscopy (Cryo-TEM). A 15 wt% solution of A_6K was imaged. Experiments were performed using a Philips CM 120 Bio-Twin transmission electron microscope. A small quantity of sample was placed on a copper grid in a thermostatted chamber at 25 ^oC. The sample in the grid was then blotted to create a thin film. Afterward, the copper grid with the sample was quickly vitrified by immersion in liquid ethane and carefully transferred under a liquid-nitrogen environment to the microscope.

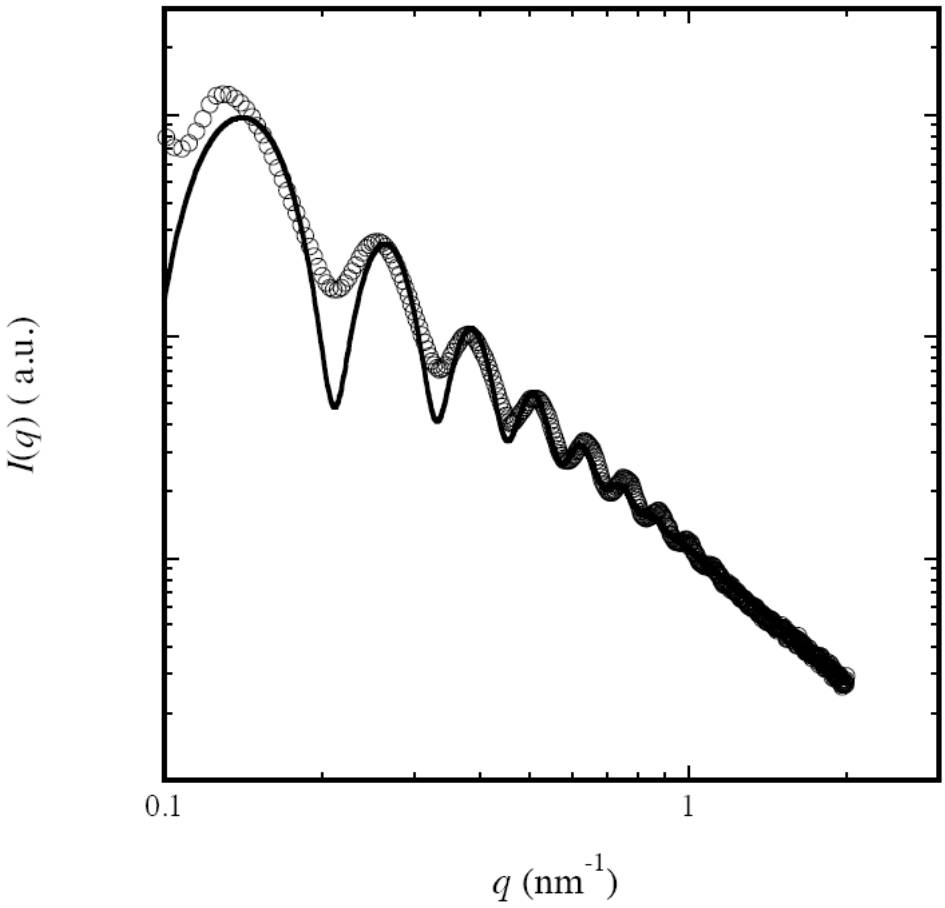

Fig.S1. SAXS profile (circles) with fit to nanotube model form factor (solid line), with a mean radius R = 26 nm, and a relative standard deviation of 8%.[2] The observed q^{-2} decay within the whole q range implies that the wall thickness is smaller than ca. 1 nm.

Cryo-TEM images

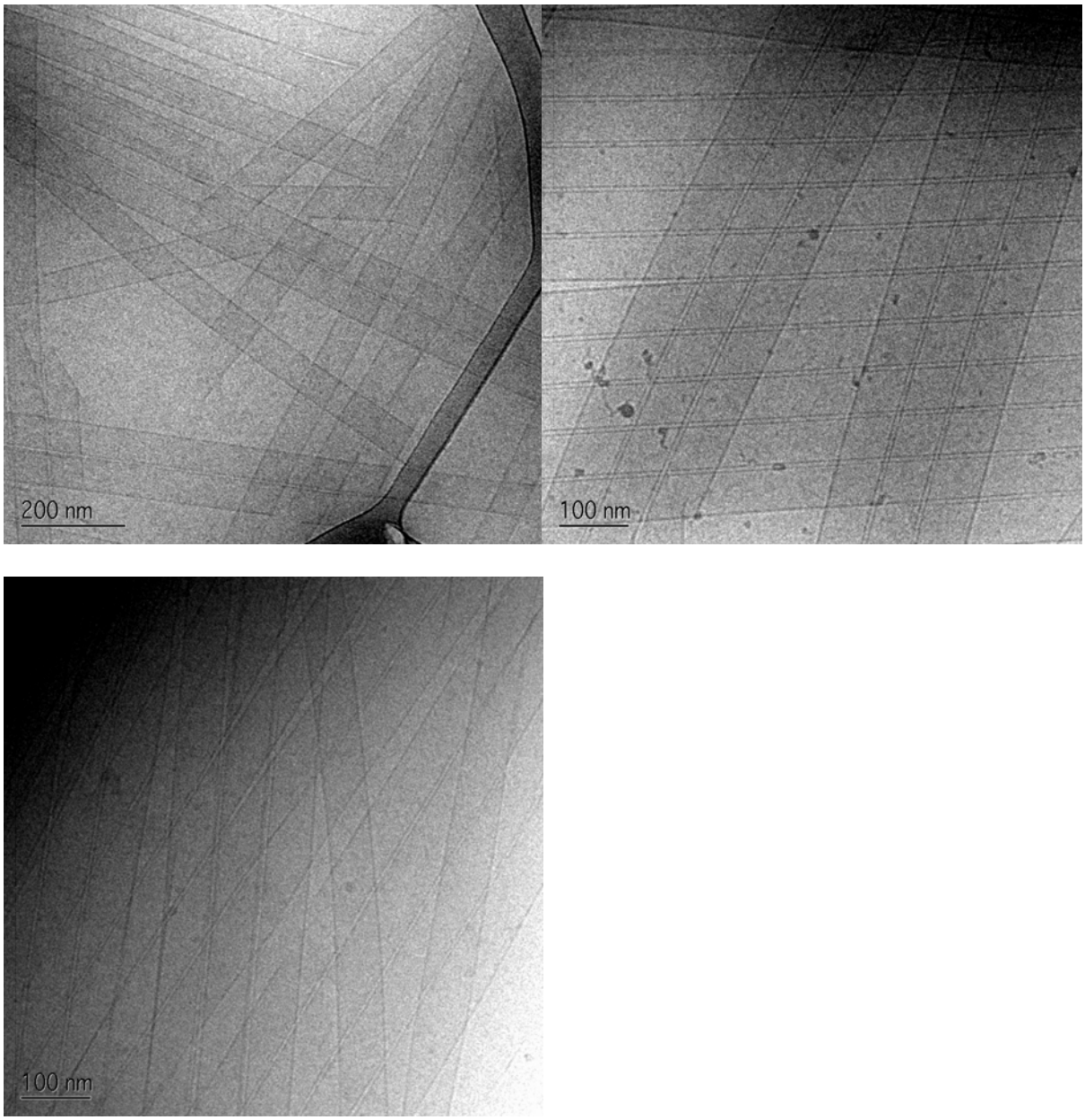

Fig.S2. Representative cryo-TEM images from a 15 wt% aqueous solution of A_6K.

X-Ray Diffraction

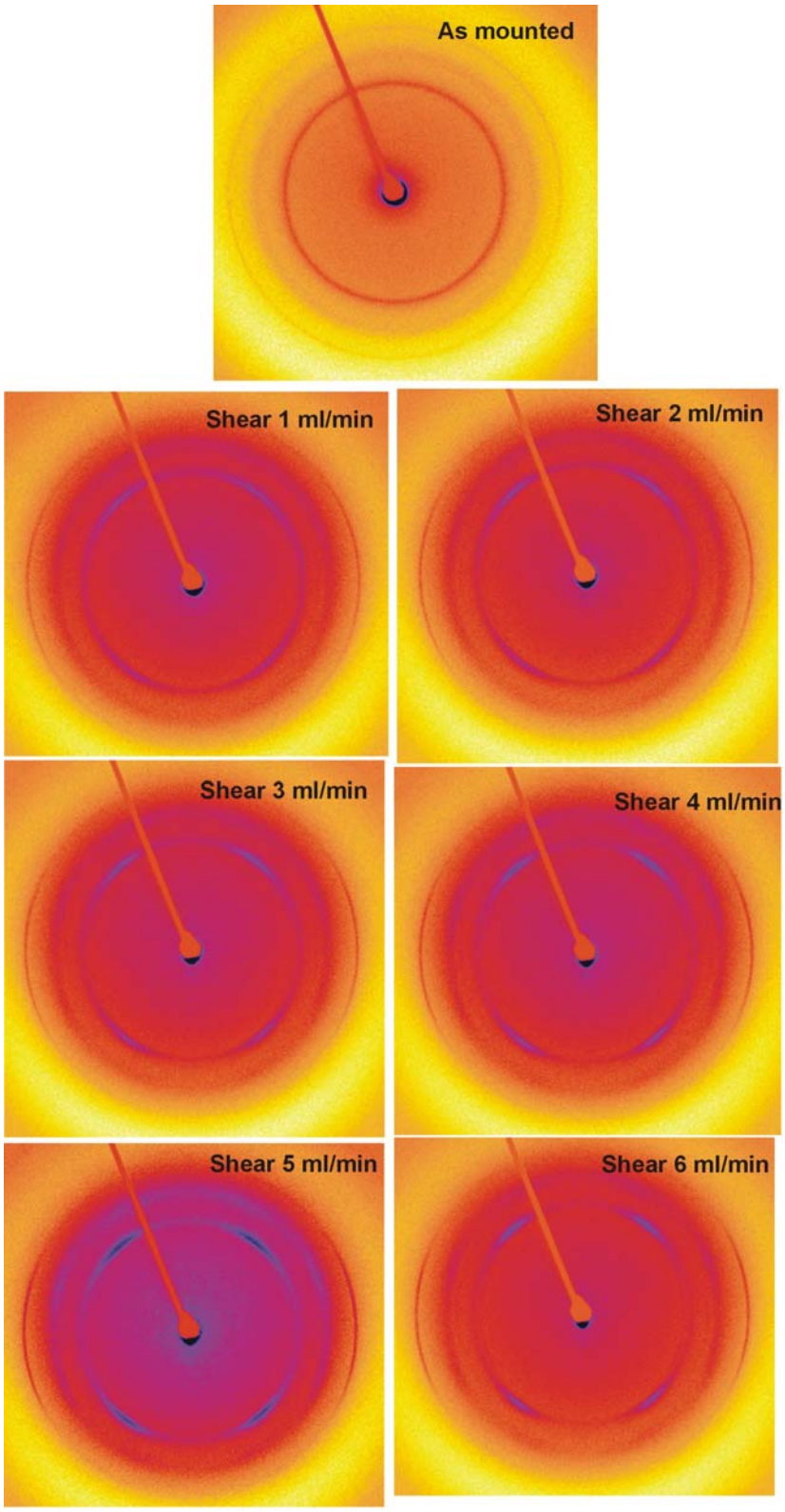

Fig.S3. 2D XRD patterns before application of shear, and as a function of shear rate

Molecular Dynamics Computer Simulations

Molecular dynamics simulations were performed to probe the stability of the proposed structures.

First, an antiparallel dimer of A_6K was constructed using the Charmm program[3] and the Charmm22 all-atom force field[4] (see Figure S4).

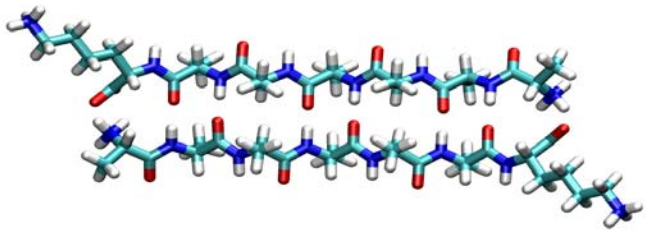

Fig. S4. Initial structure of an antiparallel A_6K dimer.

The dimer was first subject to energy minimisation, followed by 400 ps of equilibration and 200 ps of production at 300 K in implicit solvent.[5] Over this time frame, the structure was found to be stable, forming a structure in which the two peptides wrap around each other like folded arms (Fig. S5). Analysis of specific distances (H-bond lengths, distance between edges of the beta sheets, diagonal distances) were in reasonable agreement with experimental data: 3.9 A between hydrogen-bonded heavy atoms, 4.5 A between the edges of the sheets, and 5.4 A between lines along the direction at 38^o to the hydrogen-bonding axis.

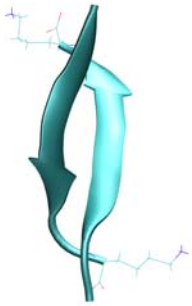

Fig. S5. Final structure of an A_6K dimer from the simulations

Five antiparallel dimers were then built into an extended beta-sheet, as proposed in the model, with two possible offset directions (Fig. S6) and several independent simulations were performed in both implicit and explicit (TIP3P) water[6] at 300 K for up to two nanoseconds. During this period, both structures were found to curve along the hydrogen-bonding direction, as proposed in the initial model. Although some fraying of the structures was observed (mainly at the ends of the sheet, where dimers would occasionally break off), the development of curvature was common to all of the simulations (Fig. S7).

Fig. S6. Possible stacking arrangements of antiparallel A_6K dimers with a two-residue offset.

Fig. S7. Typical structures resulting from simulations of extended A_6K beta sheets with loose (left) and tighter (right) curvatures.

The modeling was then extended to a structure more representative of the proposed peptide nanotube. With a radius of around 26 nm, around 170 dimers would be required to construct a single nanoring and extension of the structure along the tube axis would make the system unfeasibly large for long simulations. Consequently, a unit cell containing a 60° fragment of the nanotube was constructed and replicated in three dimensions using hexagonal, P6 symmetry (see Fig. S8). Lattice parameters were a=b=95.8 nm, c=2.56 nm, α=β=90°, γ=120°. In order to model the physical, constraining effects of solvent (in addition to modeling the electrostatic effects through the implicit solvent model), very weak harmonic constraints were applied to the heavy atom backbone atoms to keep them on the surface of a cylinder. The force constants took the value $0.001*A_r$ kcal mol^{-1} $Å^{-2}$, where A_r is the atomic weight of the constrained atom. This structure was then simulated for 1 ns at 300 K in the same way as described above. The structure obtained at the end of this simulation (Fig. S9) displays reasonable stability and further supports our structural model. Additional

modeling examined the stability of fragments of bilayer structures, which were also found to be stable. A detailed comparison of the stability of model mono- and bi-layer structures will be the subject of future work.

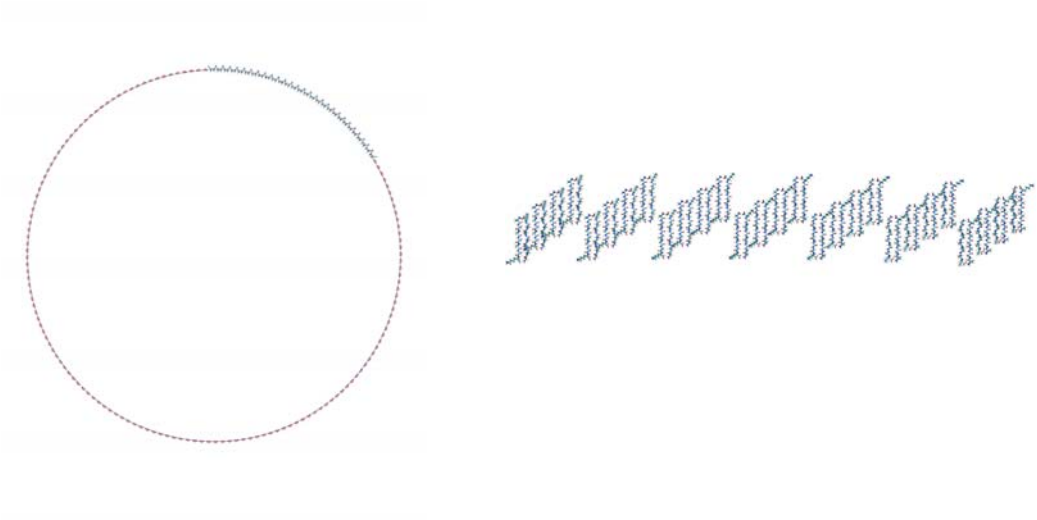

Fig. S8. Left – top view of the nanotube, with the 60° fragment (asymmetric unit) at the top-right of the figure. The rest of the structure is generated by applying six-fold rotational symmetry. Right – side view of the unit cell looking from the inside of the tube (the left and right sides curve outwards towards the viewer).

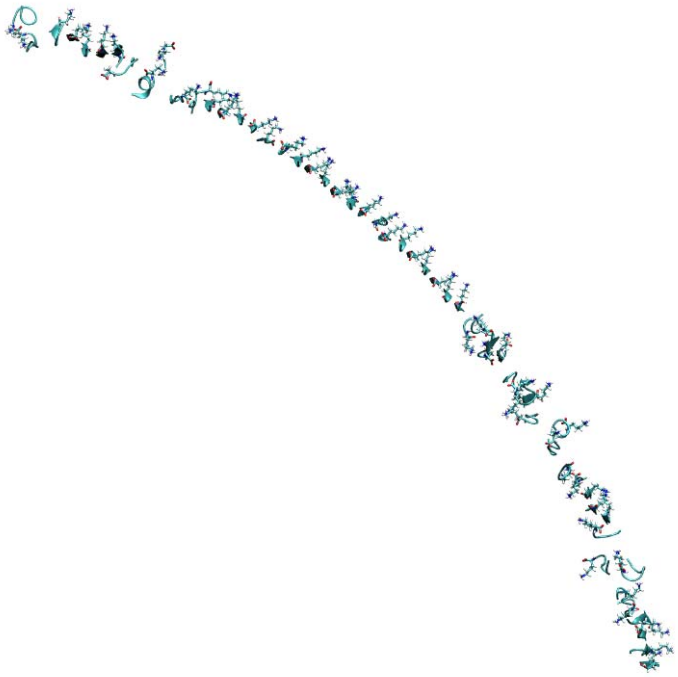

Fig. S9. Top view of the structure of the asymmetric unit after 1 ns simulation at 300 K. The lysine side chains can be seen projecting outwards on the outer surface of the tube.

Further modelling will be required in order to probe the details of side-chain packing, registry and the presence of salt bridges between strands, however the basic model has been demonstrated to be structurally sound.

References

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