ESRF	**Experiment title:** **A Time-Resolved SAXS Study of the Kinetics of Alignment of an Amyloid Peptide Nematic Phase**	**Experiment number**: SC-4267
Beamline:	**Date of experiment**: from: 20/7/16 to: 22/7/16	**Date of report**: 25/1/17
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Report:

The bola-amphiphilic arginine-capped peptide RFL_4RF self-assembles into nanotubes in aqueous solution. The nanostructure and rheology were probed by *in situ* simultaneous rheology/small-angle scattering experiments including rheo-SAXS.[1]

SAXS data were obtained on beamline ID02 at the ESRF, Grenoble, France. Data were collected using a Pilatus 1M detector. Samples were placed into a Haake RS6000 stress-controlled rheometer with modified (polycarbonate) Couette cell of inner diameter 20 mm radius and 2 mm gap. Samples were subjected to shear at different shear rates in the range $\dot{\gamma} = 0.1$ s^{-1} to 1000 s^{-1}. The sample-detector distance was either 1.2 or 10 m. The X-ray wavelength (λ) was 0.995 Å and the wavenumber $q = 4\pi \sin\theta / \lambda$ (where 2θ is the scattering angle) scale was calibrated using silver behenate.

We used a combination of rheo-SANS (D22, ILL), rheo-GISANS (FIGARO, ILL) and rheo-SAXS techniques to examine orientation. Bulk alignment effects in a Couette (concentric cylinder) were examined by rheo-SAXS and rheo-SANS and structure-flow properties were investigated by simultaneous rheology/scattering measurements.

Rheo-SAXS was used to provide information on the alignment of the sample across the entire gap of the Couette call rather than only in the interfacial region. SAXS data obtained from a 1 wt% solution of RFL_4FR under Couette flow in the radial geometry is shown in Fig.1. Significant alignment of the bulk sample was only observed whilst the sample was experiencing shear at higher shear rates at or above $\dot{\gamma} = 1$ s^{-1}, no substantial orientation being observed in the SAXS pattern at lower shear rates studied (an example pattern for a sample under shear at $\dot{\gamma} = 100$ s^{-1} is shown in Fig.1b, data was also measured at $\dot{\gamma} = 0.1$, 1 and 10 s^{-1}). The onset of significant alignment above $\dot{\gamma} = 1$ s^{-1} is in agreement with the GISANS results. Also of note, the anisotropy in the SAXS pattern was immediately lost following cessation of shear as shown in Fig.1d. This is

due to the low viscosity of these samples (discussed quantitatively below) which means that shear-induced alignment at the macroscale is not retained in the absence of shear, although local alignment must be retained in the nematic phase. A previous study[2] using SANS and rheology (performed separately) reported differences in response of fluid and gel nematic phases of peptide fibrils under shear – the fluid nematic phase showing no alignment in the absence of shear as in the present study, but in contrast to the nematic gel structure. More concentrated nematic phases (with higher viscosity due to fibril network formation) as for a PEG-peptide conjugate show retained nematic alignment after shearing.[3-4] No orientation was observed in the tangential direction, even under shear at high shear rates. These patterns are consistent with alignment of the nanotubes in the (**v**, ∇**v**) plane. Similar alignment was observed in the rheo-SANS measurements (also in a Couette geometry).

These results are included in our published paper on shear-alignment and rheo-SAXS/rheo-SANS on these peptide nanotubes.[1]

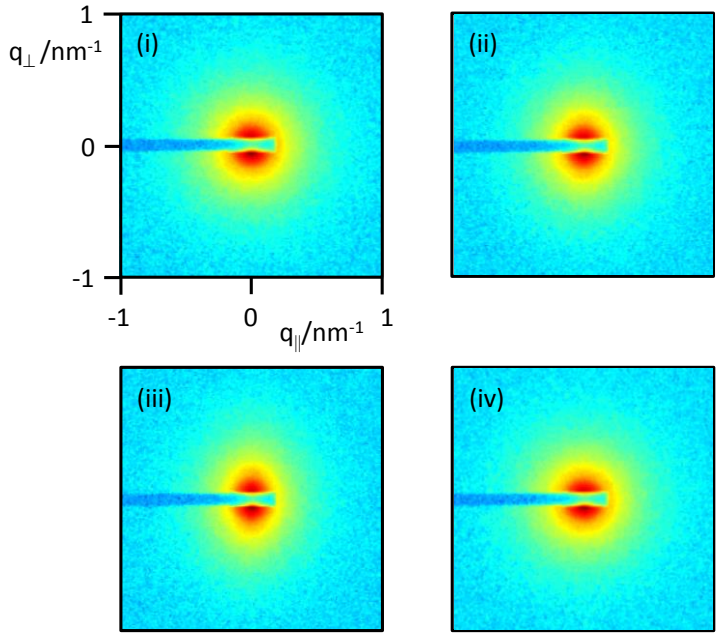

Fig.1. SAXS patterns obtained from a 1 wt% solution of RFL_4FR in D_2O in the radial configuration. (i) Zero shear (ii) under shear at $\dot{\gamma}$ = 100 s^{-1}, (iii) under shear at $\dot{\gamma}$ = 1000 s^{-1}, (iv) Following shear at $\dot{\gamma}$ = 1000 s^{-1}. The shear direction is horizontal and the intensity scale is logarithmic.

References

1. Hamley, I. W.; Burholt, S.; Hutchinson, J.; Castelletto, V.; da Silva, E. R.; Alves, W. A.; Gutfreund, P.; Porcar, L.; Dattani, R.; Hermida-Merino, D.; Newby, G. E.; Reza, M.; Ruokolainen, J.; Stasiak, J., Shear Alignment of Bola-Amphiphilic Arginine-Coated Peptide Nanotubes. *Biomacromolecules* **2017,** *18*, 141-149.
2. Mawer, P. J.; Waigh, T. A.; Harding, R.; McLeish, T. C. B.; King, S. M.; Bell, M.; Boden, N., Small-Angle Neutron Scattering from Peptide Nematic Fluids and Hydrogels under Shear. *Langmuir* **2003,** *19*, 4940-4949.
3. Hamley , I. W.; Krysmann, M. J.; Castelletto, V.; Kelarakis, A.; Noirez, L.; Hule, R. A.; Pochan, D., Nematic and Columnar Ordering of a Peg-Peptide Conjugate in Aqueous Solution *Chem. Eur. J.* **2008,** *14*, 11369-11374.
4. Hamley, I. W.; Krysmann, M. J.; Newby, G. E.; Castelletto, V.; Noirez, L., Orientational Ordering in the Nematic Phase of a Peg-Peptide Conjugate in Aqueous Solution. *Phys. Rev. E* **2008,** *57*, 062901.