Experimental report

14/08/2017

Proposal:	**8-02-782**	**Council:**	4/2016
Title:	Shear-Induced Orientational Order of the Nematic Phase of Amyloid Fibrils probed by in-situ Rheo –GISANS		
Research area:	Biology		

This proposal is a new proposal

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Samples: Octapeptide RFL4FR
Octapeptide peptide (RF)4

Instrument	Requested days	Allocated days	From	To
FIGARO	4	4	27/06/2016	01/07/2016

Abstract:

The fibrillization of peptides has attracted a lot of interest for many years as a result of its close association with degenerative diseases, for example, Alzheimer’s, Parkinson’s, and diabetes type II. It is widely recognized that such disorders arise from protein misfolding followed by self-assembly into cytotoxic oligomers which form fibrillar structures usually rich in β-strands, which are so-called amyloid fibrils. Understanding the mechanisms involved in amyloid formation is a significant challenge in both fundamental research and in the development of amyloid fibril-based nanomaterials. The proposed experiment aims to perform simultaneous GISANS/rheology on a class of octapeptides including (RF)4 and RFL4FR, which is a bola-amphiphile based also on the Arg-Phe pair but with a tetraleucine “central spacer”. The correlation between the structure and alignment, at the solid liquid surface probed by GISANS, with changes in the rheological response (shear and temperature) will be completed.

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-SANS.[1]

We used a combination of rheo-SANS (D22, ILL), rheo-GISANS (FIGARO, ILL) and rheo-SAXS (ID02, ESRF) 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.

GISANS was performed on beamline FIGARO. The measurements were performed in time-of-flight mode using a wavelength band from 2.2 – 16 Å and a wavelength resolution of 7.4%. The reflection angle was set to 0.62º with a vertical and horizontal angular divergence of 0.022º and 0.1º, respectively. The detector pixel resolution corresponds to an angular spread of 0.018º and 0.16º, respectively. All resolutions are given as Gaussian equivalent full width at half maximum (fwhm). The acquisition time was 5 h per pattern. The specularly reflected beam was masked during the data reduction.
The sample was contained in an Anton-Paar MCR 501 rheometer in cone/plate geometry (1º cone angle, 50 mm cone diameter) to allow *in situ* rheology. The neutrons pass the plate which is a single crystal silicon wafer polished to a roughness of 0.2 nm as determined by specular reflectivity measurements in pure water (NR, not shown). The surface was cleaned by ultrasonication in ethanol, acetone, toluene and Millipore filtered water prior to the measurements. NR was also performed in between the GISANS measurements and no significant adsorption of peptide on the surface was detected during the experiment. Samples were subjected to shear at different shear rates in the range $\dot{\gamma}$ =0.01 s^{-1} to 1000 s^{-1}. The footprint of the neutron beam (45×11 mm^2) was centered to the cone, hence the scattering plane was parallel to the shear gradient and mainly shear flow plane, however with some contributions of the vorticity direction close to the center of the cone. The temperature was kept constant at 20 ºC throughout the GISANS experiment.

Rheo-GISANS measurements were performed in order to correlate rheological behaviour of the sample to structural alignment under flow close to the plate cell wall (70 μm penetration depth into the liquid). Grazing incidence scattering measurements provide a powerful tool to probe interfacial structure.[19] Due to the glancing angle geometry most of the signal comes from a thin liquid layer close to the stationary plate. For the wavelength range used here this layer thickness is on the order of several tens of micrometers. GISANS images obtained from a 1 wt% solution of RFL_4FR under the application of steady shear (and at rest) are shown in Fig.1.

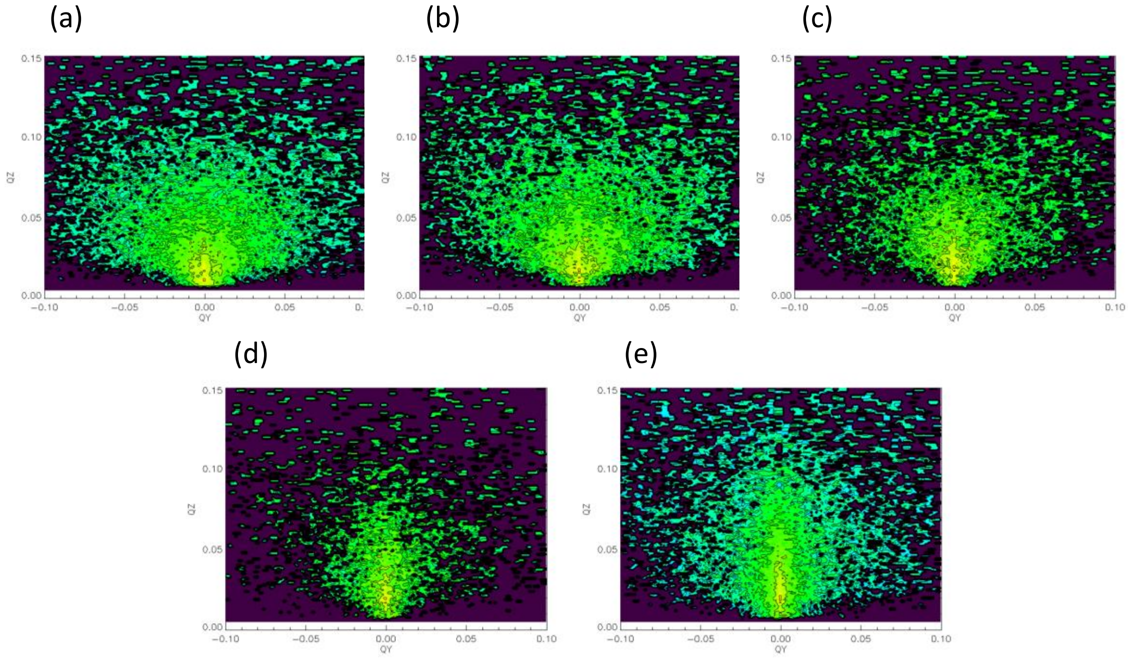

Fig.1. GISANS data from a 1 wt% solution of RFL_4FR at rest or under steady shear (a) at rest (following shear at 1000 s^{-1}), (b) 0.01 s^{-1}, (c) 1 s^{-1}, (d) 100 s^{-1}, (e) 1000 s^{-1}. The QZ axis is along the shear gradient and the QY direction is mainly oriented along the shear flow. The axis units are in $Å^{-1}$. The intensity is plotted on a logarithmic color scale.

A slight degree of anisotropy becomes apparent at a shear rate of 1 s^{-1} but only at 100 s^{-1} and 1000 s^{-1} was pronounced anisotropy due to flow alignment noted. The anisotropy is in the form of prolate elliptical scattering contours, consistent with alignment of the peptide nanotubes along the flow direction (horizontal, QY). Due to the reflection geometry only about half of the scattering plane is accessible in GISANS as compared to SANS patterns. The rest is blocked by the sample horizon. The alignment is immediately lost following the cessation of shear as exemplified by the GISANS pattern in Fig.1a (obtained after shearing at 1000 s^{-1}) which is isotropic. The corresponding bulk flow curves (viscosity *vs*. shear rate) are shown in Fig.2 for two concentrations of the sample. The viscosity decreases dramatically with increasing shear rate above $\dot{\gamma} = 10^{-3}$ s^{-1} indicating strong shear thinning behaviour. However, as noted above, strong anisotropy in the GISANS patterns is not observed until much higher shear rates, i.e. in the strongly shear non-linear flow regime. The flow curves also show an enhancement of the zero shear viscosity with increasing peptide concentration.

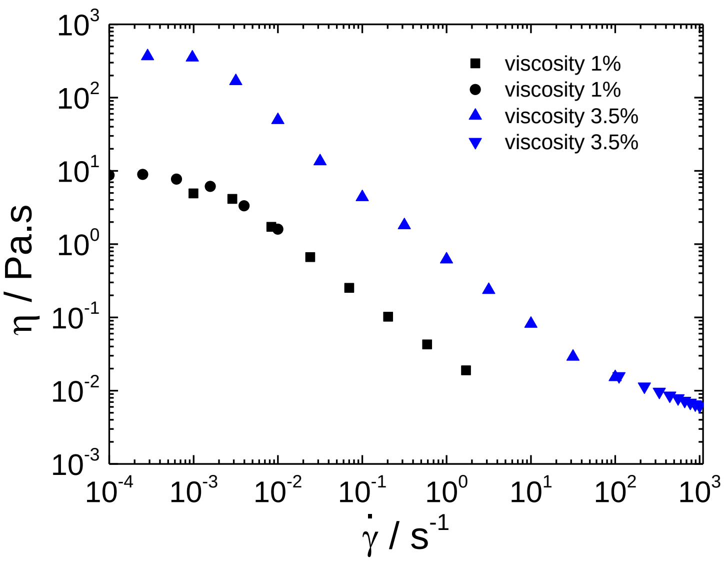

Fig.2. Steady state flow curves (1 wt% and 3.5 wt% RFL_4FR) in cone/plate geometry as used for the GISANS experiments. Flow curves (1 wt% and 3.5 wt% RFL_4FR) were measured for each sample over two ranges of shear rate (shown by different shaped symbols).

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

Reference

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.