ESRF	**Experiment title:** Probing peptide fibrillation in the convective flow of an evaporating droplet	**Experiment number**: SC3572
Beamline: ID13	**Date of experiment**: from: 28.2.2013 to: 2.3.2013	**Date of report**: 30.8.2013
Shifts: 9	**Local contact(s)**: Emanuela Di Cola	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Christian Riekel: ESRF

Enzo Di Fabrizio,*Angelo Accardo and *Giovanni Marinaro: Istituto Italiano di Tecnologia (IIT), Nanostructures Department, via Morego 30, 16163 Genova, Italy

Report:

We studied three amyloid forming peptides with acylated N-termini using a 200 nm beam at ID13: Ac-ID_3 (IVD), Ac-LD_6 (LIVAGD) and Ac-KE_7 (Ac-KLVFFAE).[1,2] Peptide solutions of up to 5 mg/ml were deposited by ~4-µL droplets on a 200 nm thick Si_3N_4 substrate with a thin superhydrophilic PMMA layer (see ID21 SC3572 report for details on substrate) or a hydrophilic Si_3N_4 substrate.

The residues revealed cross-β structures as already reported elsewhere.[1,2] The cross-β structures formed immediately after depositing the droplets by a syringe. The time required for localizing the sample in the beam and starting the raster-scan did not allow capturing structural events preceding β-sheet formation. We could also not detect inhomogeneities across the coffee-rings indicating a sequence of precipitated phases (e.g. α-helical preceding β-sheet suggested by MD-modeling and CD-spectroscopy[1]).

A 5 mg/ml Ac-ID_3 solution droplet forms a well-defined coffee-ring (Figure 1A) The raster-scan image is completely homogeneous and composed of highly oriented cross-β patterns. (Figures 1B,C) The orientational order of the cross-β peak in all single patterns (~56^0) is similar to that observed for residues on a superhydrophobic substrate.[1] Ac-LD_6 on hydrophilic Si_3N_4 does not form a regular coffee-ring but rather island-morphologies. Cross-β patterns were identified within the islands (not shown). A 5 mg/ml Ac-KE_7 solution shows a complex morphology on a superhydrophilic substrate with a very thin coffee-ring and a fibrous phase in the bulk of the residue. (Figure 1D) Diffraction from the coffee-ring was too weak but FTIR data show β-sheets with an antiparallel conformation although β-turns cannot be completely excluded. (see report ID21-SC3572) There is no evidence for an α-helical phase although a corresponding band is present in the FTIR spectra. The fibrous residue can be identified as cross-β material while a distorted cross-β phase is formed at the interface of the droplet to a superhydrophobic substrate[2]. The diference may be due to shear-induced orientation effects on the superhydrophobic substrate.

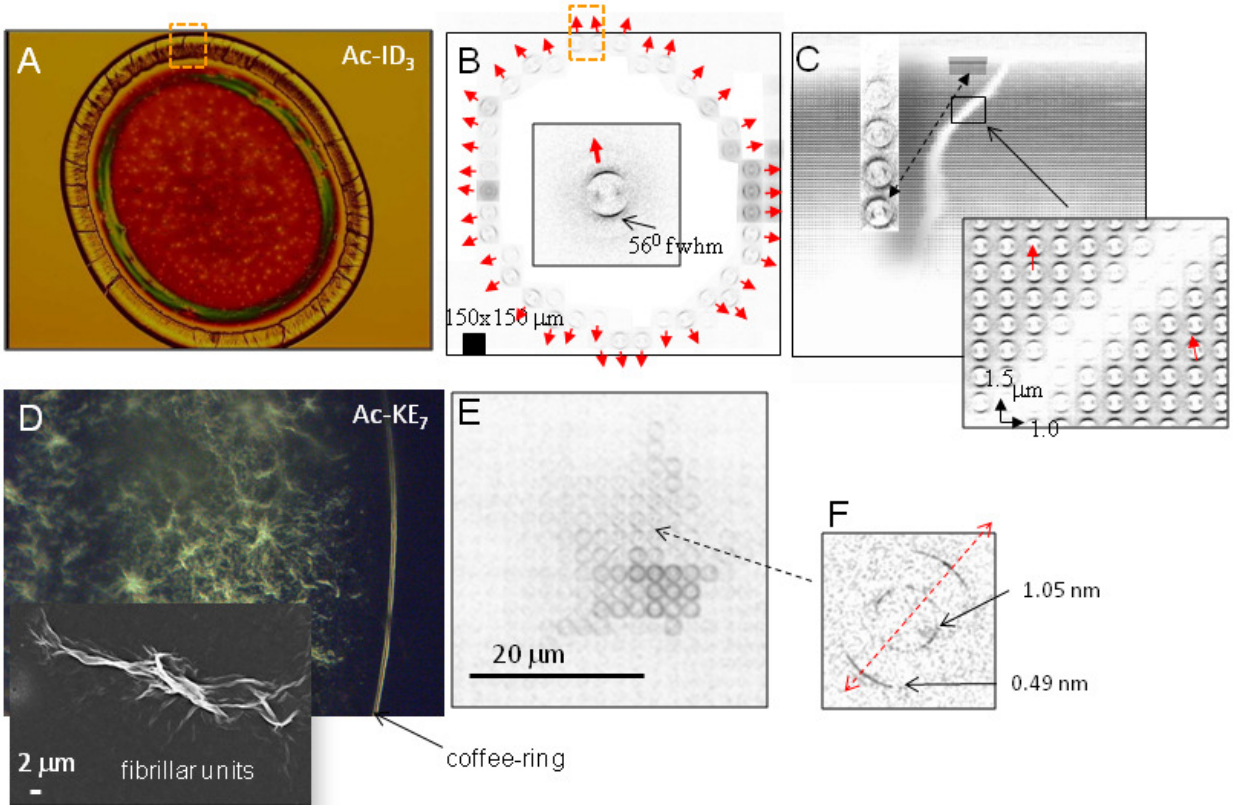

Figure 1 A: Optical image of Ac-ID_3 coffee-ring on hydrophilic Si_3N_4 substrate. B: Coarse raster-scan image of residue revealing orientation of cross-β fiber axis in the coffee ring (red arrows). A typical cross-β pattern is shown in the center. C: High resolution raster-scan image within the rectangle shown in (A). Selected patterns normal to the interface are shown as inset. A subset of diffraction patterns from the rectangle in (C) is shown to the right. D: Optical image of Ac-KE_7 residue on superhydrophilic Si_3N_4 substrate with a thin coffee-ring and fibrillar bulk-deposits. The inset shows an SEM image of a fibrillar unit. E: Highly binned raster-scan of a fibrillar unit with "arms" radiating from the center. Right: The cross-β pattern was derived by averaging patterns from an arm. The fiber axis is indicated by an arrow.

References

1 Hauser, C. A. E. *et al.* Natural tri- to hexapeptides self-assemble in water to amyloid beta-type fiber aggregates by unexpected alpha-helical intermediate structures. *PNAS* **108**, 1361-1366, (2011).

2 Lakshmanan, A. *et al.* Aliphatic peptides show similar self-assembly to amyloid core sequences, challenging the importance of aromatic interactions in amyloidosis. *PNAS* **110**, 519-524, (2013).