



Experiment title:
 Probing peptide fibrillation in the convective flow of an evaporating droplet

Experiment number:
 SC3572

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Report:

We studied 4 materials showing amyloidic fibrillation by FTIR: (a) two designer synthetic peptides which were acetylated at the N-terminus: Ac-ID₃ (IVD), Ac-LD₆ (LIVAGD); (b) human amyloid-β (1-42), KE₇; (c) human IAPP fragment (22-27), NFGAIL). Peptide solutions in D₂O of 1 mg/ml and 0.1 mg/ml were used for the experiments. We deposited droplets of about 4 μL by a micropipette on a superhydrophilic BaF₂ substrate which was produced for the experiments by A.A. at IIT-Genova. Superhydrophilic properties were obtained by plasma deposition of nanostructured poly(methylmethacrylate) (PMMA). The droplets formed during evaporation a coffee-ring which allowed keeping the probed volume for the transmission experiments sufficiently low. Experiments were performed with a 5 μm FTIR beam.

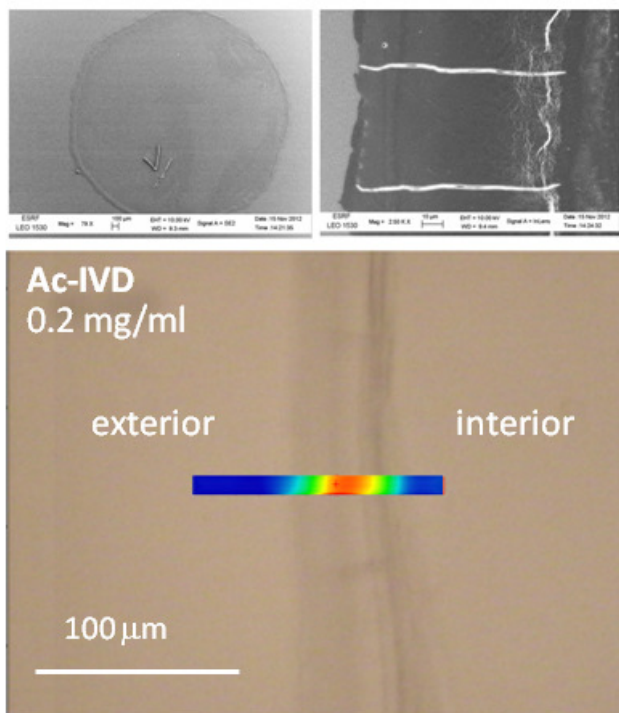


Figure 1. Left: SEM image of Ac-ID₃ coffee-ring residues; Right: Higher-resolution SEM image showing the formation of fibrillar structures at the residue-interior (right side) Bottom: FTIR raster-scan across Ac-IVD coffee-ring with 5 μm(hor)x1 μm(vert) steps. The colour is scaled to the intensity of the Amide I band β-sheet peak (blue: lowest, orange: highest)

The main aim of this experiment was to get information on the mechanistic steps involved in amyloidic fibrillation.^{1,2} The superhydrophilic substrate resulted in a pinning of the triple-phase contact-line during coffee-ring formation which allowed continuously probing of the same area of the droplet during evaporation. We performed a mesh of successive line-scans with 6-8 steps across the triple-phase contact-line until residue formation. Typical drying times were 20-40 minutes for the 0.1 mg/ml concentrations.

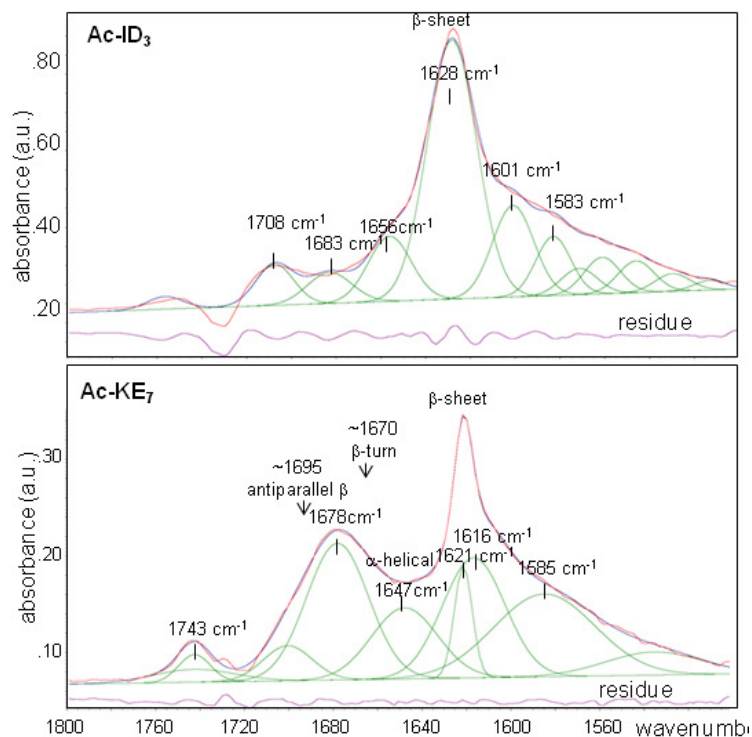


Figure 2. Amide I band of Ac-ID₃ and Ac-KE₇. The Ac-LD₆ and Ac-NFGAIL spectra are practically identical to Ac-ID₃. The spectra have been fitted by Gaussian profiles. The assignment of the main bands to conformations is indicated.

We analyzed the spectra with the ThermoScientific OMNIC 8 software. Parallel β -sheet conformations were identified for Ac-ID₃, Ac-LD₆ and Ac-NFGAIL. (Figure 2,top) The Ac-KE₇ spectrum appears to be more complex as a peak close to an expected antiparallel β -sheet conformation is observed. (Figure 2, bottom) A β -turn conformation can, however, not be completely excluded. We note also the presence of an α -helical band although we do not see a spatial separation of this band from the β -sheet band in the raster-scans. We have not been able observing intermediate conformations (e.g. α -helical) during the raster-scans as the coffee-ring formation was too fast. Whether the α -helical band for Ac-KE₇ is due to a separate phase mixed with the β -sheet phase cannot be determined at present.

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. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 1361-1366, doi:DOI 10.1073/pnas.1014796108 (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, doi:DOI 10.1073/pnas.1217742110 (2013).