	Experiment title: Abeta peptide fibrillation enhancement by the administration of Acetylcholinesterase (AChE) enzyme	**Experiment number**: SC3843
Beamline: ID21 & ID13	**Date of experiment**: from: 23/4/2014 to: 29/4/2014 and from: 15/5/2014 to: 19/5/2014	**Date of report**: 31/07/2014
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Report:

We studied a series of combinations involving: 4 different Aβ fragments, namely Aβ(1-42), Aβ(1-40), Aβ(12-28), Aβ(25-35); 3 side components, namely Acetylcholinestarease (AchE), cholesterol and curcumin; 2 different wetting surfaces (flat and superhydrophilic). All the solutions were prepared in deionized H_2O. We deposited droplets of about 3 µL by a micropipette on superhydrophilic BaF_2 substrate (for FTIR experiments) and Si_3N_4 membranes (for XRD experiments). The droplets formed, after complete evaporation, a coffee-ring residual, which allowed keeping the probed volume for the transmission experiments sufficiently low (Figure 1).

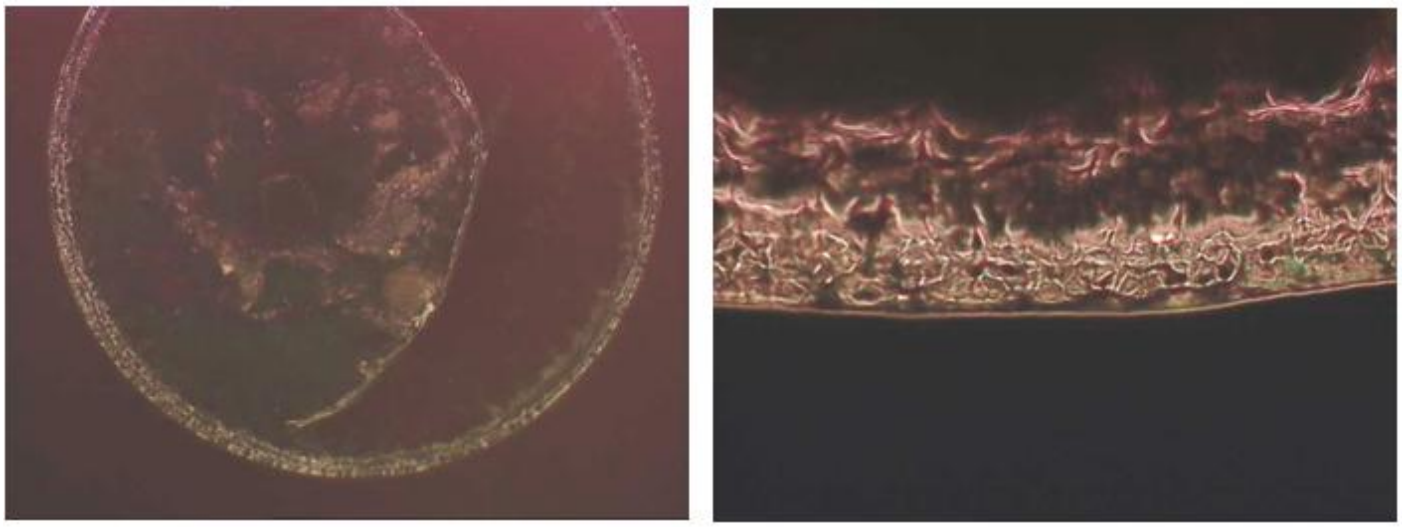
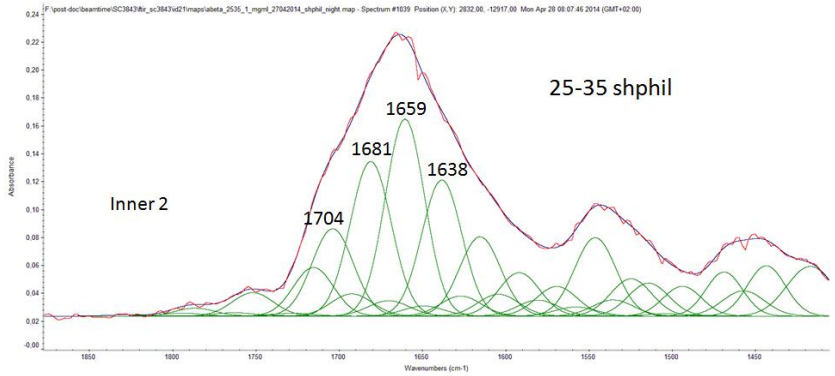
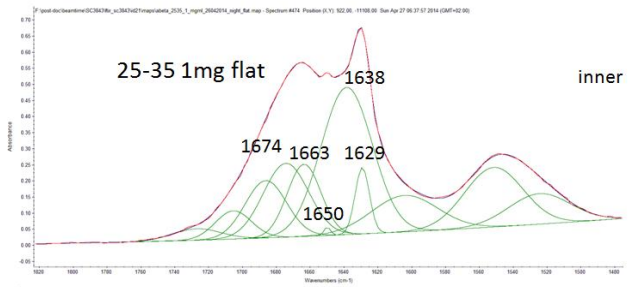
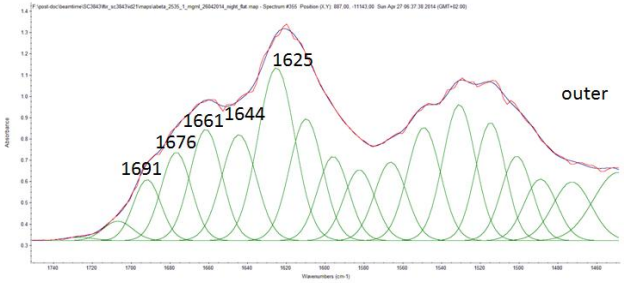


Figure 1. Typical coffee-stain residuals of Aβ solution droplets dried on hydrophilic susbtrates

The FTIR analysis of the fragments was performed focusing the attention on the 1600-1700 band responsible for the secondary structure characterization of peptides and proteins in general. The experiments involved: two different kinds of substrates including flat BaF2 and superhydrophilic BaF2 nanostructured (Shphil) windows used already in a previous correlated study (SC3618). The peak fitting of the spectral bands was performed through the use of the OMNIC software which was used as well for the acquisition of the experimental FTIR data. For brevity we are going to show just the results coming from one single fragment, Aβ(25-35).

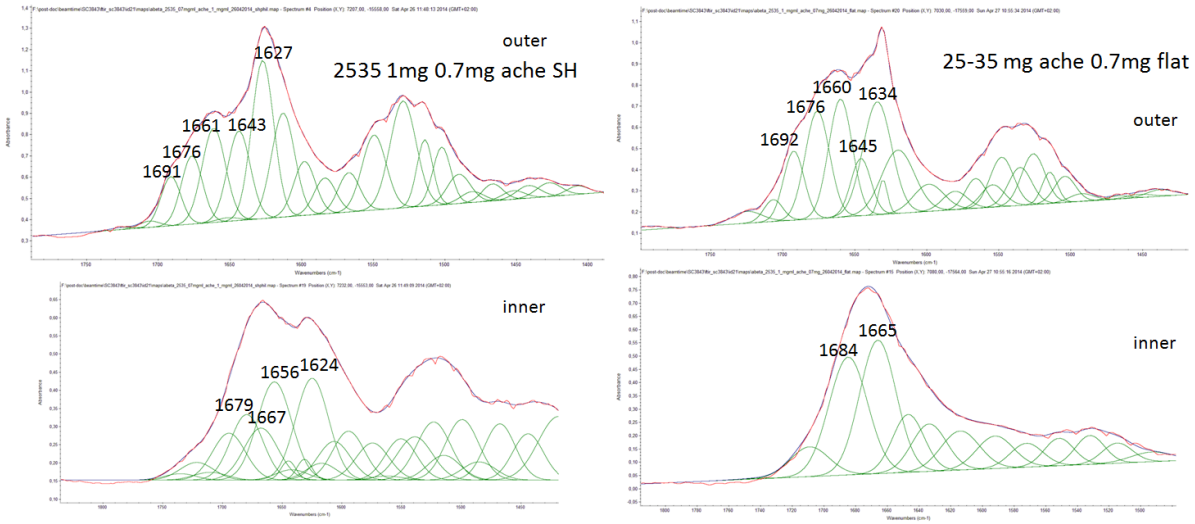




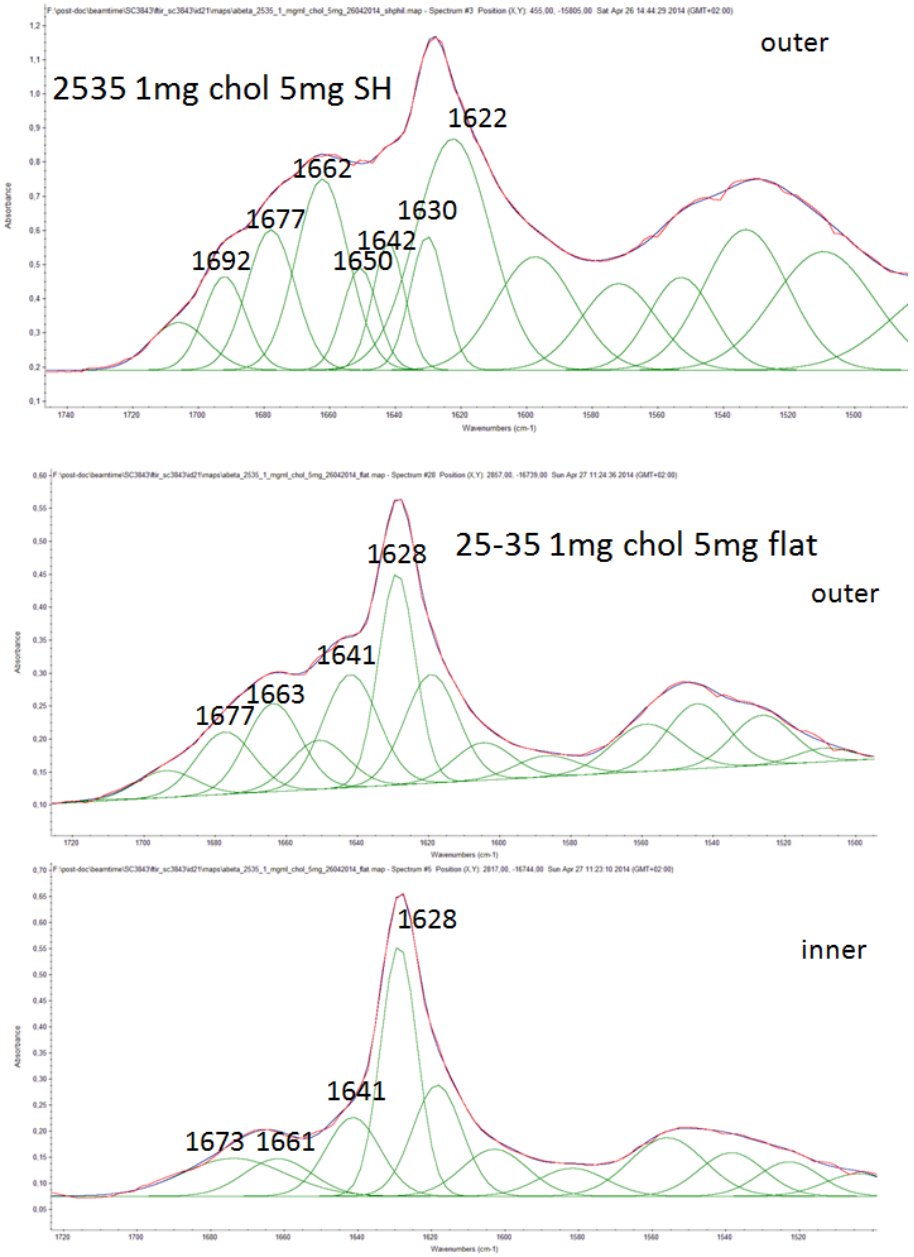


The conformation of the fragment Aβ 25-35 on Sphil supports was already extensively analyzed in a previous work (SC3618). In particular it is possible to see how the parallel β-sheet component is highly pronounced in the outer part (not shown) while the inner part includes a strong attenuation of this component in favour of a α-helical one at 1659 cm-1 and some minor contributions at 1681 cm-1 (β-turn) and 1704 cm-1 (antiparallel β-sheet).

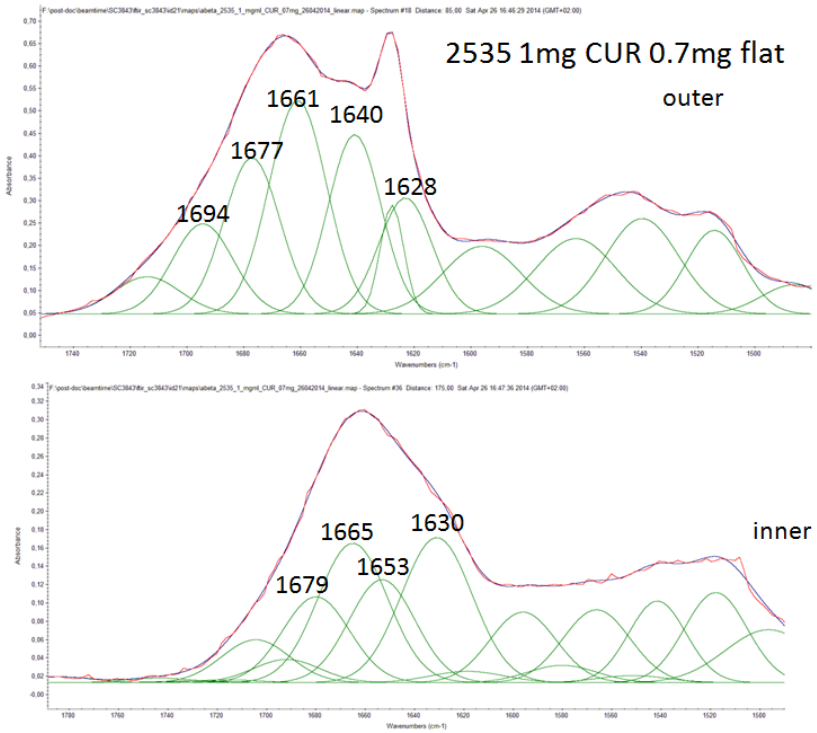
The conformation of the fragment on a flat substrate shows once more how the wettability of the support plays an important role in terms of local variations of concentration. In particular the outer part is always characterized by a main parallel β-sheet component (1625 cm-1) with the contemporary presence of possible 3_{10} helices (1661 cm-1), β-turns (1676 cm-1) and antiparallel β-sheets (1691 cm-1). The inner part in this case, unlike the Shphil case, still shows a prominent β-sheet phase (1638 cm-1) together with α-helices (1650 cm-1), 3_{10} helices (1663 cm-1) and β-turns (1674 cm-1) probably due to the higher local concentration of the peptide due to the lower hydrophilicity of the susbtrate.



The addition of Ache, induced different conformations for the two support environments. In the Shphil case (left panels) the residue is characterized by a strong β-sheet component (1627 cm-1) in the outer rim while the inner one, even if still showing an α-helical one (1656 cm-1) like in the pure peptide case, presents also a minor β-turn (1667-1679 cm-1) contribution and, most of all, a persistent parallel β-sheet phase (1624 cm-1) probably due to the action of the enzyme. In the flat case (right panels) the outer rim is quite similar to the Shphil case while the inner one does not include any α-helical component but it is mainly composed by 3_{10} helix (1665 cm-1) and β-turn (1684 cm-1) components thanks to the combined actions of the enzyme and of the less hydrophilic substrate.



The presence of cholesterol in the Aβ(25-35) fragment induced, in the case of the Shphil support, a conformation similar to the hybrid Aβ/Ache case while the inner rim (not shown as too noisy to be fitted) did not show a α-helix component but a parallel β-sheet one and a prominent β-turn phase at 1684 cm-1. The situation changes in the flat case where the outer rim includes a general attenuation of the non parallel β-sheet components and the inner one stresses even more this behavior due probably to the formation of some aggregated protofibrils bodies.



The presence of curcumin can be summarized in a strong enhancement of the 3_{10} helix component (1661-1665 cm-1), while the parallel β-sheet component (1628-1630 cm-1) maintains a sharp conformation in the outer rim, then assuming a weaker and broader profile in the inner one.

XRD experiments were performed using a 2.5 x 2.8 μm^2 beam. A preliminary investigation shows how the presence of curcumin can effectively influence the β-sheet material formation as shown in the following picture.

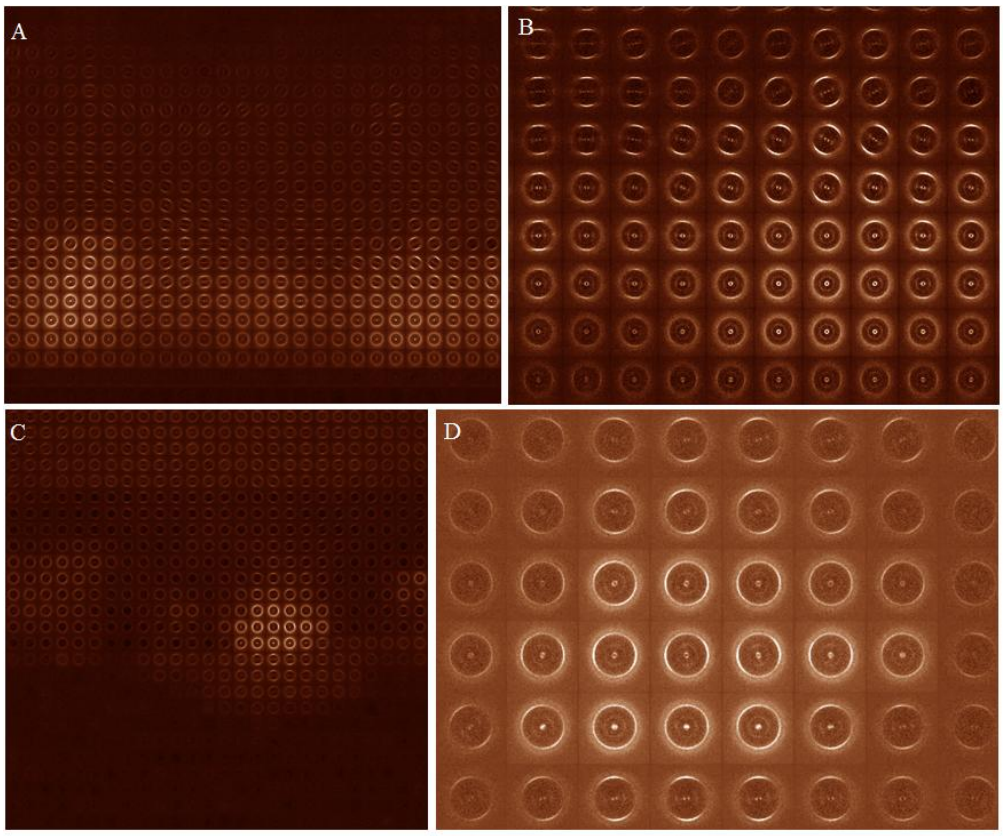


Figure A,B refer to the solid rim of the pure Aβ(25-35) residuals, while C,D to the mixed solution Aβ(25-35)/curcumin. It is possible to see how the typical β-sheet signature, present throughout the whole rim in the pure case (A,B), is less enhanced and limited to single regions for the mixed case (C,D).

Starting from these considerations we are performing a comparative study of the different fragments with different side components which influence in a sensitive way the structural behaviour of the peptides under investigation.