ESRF	**Experiment title:** Probing the assembly mechanisms of short peptides forming functional amyloids fibers	**Experiment number**: SC-4163
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Report:

The aim of this run was first to explore the self-assembling properties of two families of short peptides in different physico-chemical conditions. We have thus been able to scan a hundred capillaries of six different peptides solubilized in different pH and ionic strength. This resulted in 3000 spectra resulting from the stitching of SAXS and WAXS detectors (*Figure 1A*). The WAXS detector was used to collect the characteristic amyloid reflection at 4.7 Å (interstrand reflection). The second characteristic amyloid reflection at 9.7 Å (intersheet reflection) was at the overlap between the two detectors. The lower angles on the SAXS detector may give information on the form factor of the scattering objects. We had planned to look for aligned domains. Except for a few samples where the fibers spontaneously aligned themselves because of the crowding, which results in a pattern were the typical interstrand and intersheet reflections of the amyloid β-sheets were perpendicular (*Figure 1A*), our attemps to align the fibers with a magnetic field have failed. Most of the scattering signals observed during the run were isotropic. It seems to have only accelerated the sedimentation of the fibrils at the bottom of the capillaries as indicated by an enhanced signal-to-noise ration. We did not spend too much time on shearing the samples in the capillaries as we planned to do because we only had small volumes and it is a painstaking process. In some specific conditions we have obtained powder diffraction spectra that are still under analysis for cell indexation (*Figure 1B*). this is particularly interesting because it suggests that there is a structural continuity between the amyloid fibers and the microcrystals observed in TEM.

The second objective was to study in situ the kinetics of assembly of at least one representative peptide and assess the reversibility of the assembly. The best results were obtained by placing a drop of ammonia solution on top of an acidic solution of the peptide monomer, with air in between. The capillary was sealed with wax so that the ammonia vapours remained in the capillary and slowly increase the pH of the peptide solution. We could thus scan the peptide solution as a function of time (over 24 hours) and follow the spatial progression of the ammonia in the solution. This series of spectra was rich in mechanistic information and are still being analyzed and fitted (*Figure 1C-D*). Apart from the progressive apparition of the typical amyloid reflections, we have followed the quick peptide aggregation at low *q* and identified at least three steps occuring during the assembly process. Conversely, we were also able to probe the reversibility of these functional amyloid fibres by submitting the gels to acidic solutions and/or vapours and observe their

disassembly.

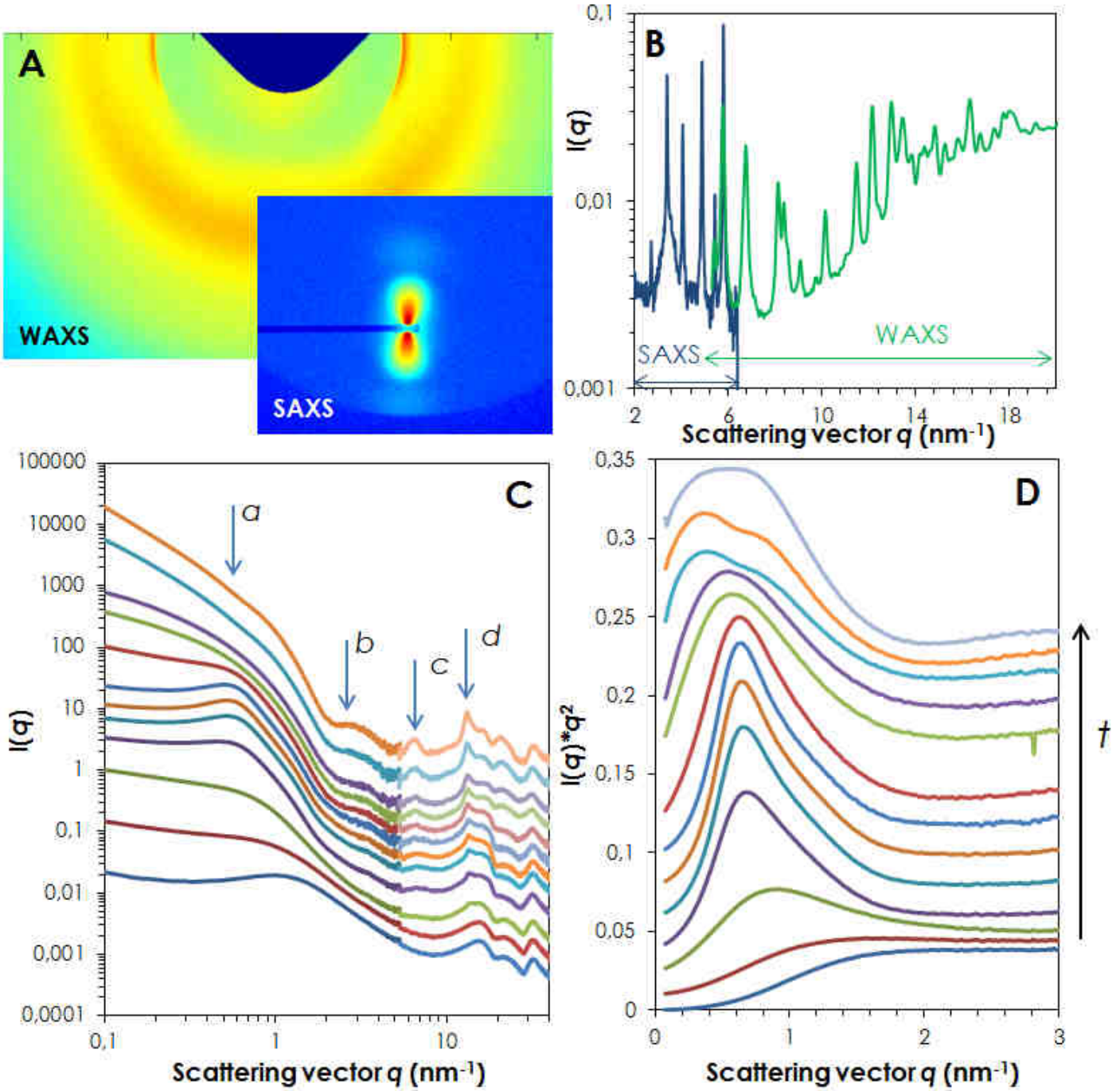

Figure 1: A) 2D-patterns from aligned amyloid fibrillar sample collected on SAXS-WAXS detectors proving the cross-beta nature our fibrillar gels. B) Diffraction pattern of crystallizing samples.C-D) In-situ monitoring of the kinetics of assembly upon pH increase plotted in log-log (C) and for a Kratky analysis (D).

We have also characterized cellulose-silica hybrid nanocomposites. Their synthesis route is based on the colloidal association of elongated polysaccharide nanoparticles and partially pre-condensed silica oligomers. WAXS intensity profiles were recorded on ID02 for cellulose-silica hybrid materials prepared over a wide range of composition. As the volume fraction of cellulose decreases, the main Bragg reflections of type I cellulose are shifting to higher *q*, indicating the contraction of the crystalline lattice upon condensation of the silica network. Since the same behavior was also recorded with chitosan crystalline nanoparticles, this, along with other experimental evidences (NMR, TEM), suggests an intimate interaction of silica oligomers with the polysaccharide surface.