Small-angle X-ray scattering of magnetically aligned amyloid fibrils at extreme pressures

Experiment 26-01-827 carried out at DUBBLE in March 2009 aimed to obtain further structural information of amyloid fibrils at atmospheric pressure and at high pressures by the use of synchrotron small-angle X-ray scattering (SAXS) in combination with a diamond anvil cell (DAC). Reflections observed in the course of preliminary tests in a DAC had been interpreted in terms of the helical arrangement of the protofilaments that form the fibrils. Thus, we proposed to use SAXS to investigate protofilament-protofilament interactions and structural features on longer length scales.

Preliminary tests in July 2008 had focused on the possibility of doing SAXS measurements using a DAC, making use of the convenient selection of energies and extremely long sample-to-detector distances available at DUBBLE to avoid the otherwise interfering Kossel lines. In particular, it was found that using a beam energy of 15keV ($\lambda = 0.83$Å), a Bragg angle of 7.37 and a sample-to-detector distance of 8 meters strongly reduced the presence of the Kossel lines. Moreover, under these conditions we could observe weak reflections that we took to correspond to a helical arrangement of the protofilaments. During our measurements in March 2009, however, we realised that under the given setup we could only observe a faint reflection corresponding to a fibril-fibril spacing of 230Å. This is in agreement with previous work by Yagi *et al.* (2006). In order to observe structural features such as the protofilament-protofilament spacing we had to change sample-to-detector distance from 8m to 1.8m, which allowed us to obtain patterns from the fibrils at ambient conditions. These showed a peak around 30Å which corresponds to the protofilament-protofilament distance within a single fibre (Yagi *et al.*, 2006). Unfortunately, the diffraction peak was very weak and we found that highly-concentrated solutions of non-aligned fibrils were required in order to obtain patterns with a good resolution. This was observed for all the various protein/peptide samples tested and it was a constraint for the DAC experiments (which only use very small volumes of material). Furthermore, increasing the exposure time of the runs to 20-30 minutes resulted in a a yellow-brownish colouring of the sample, suggesting that exposure for such long time period led to radiation damage of the samples.

The experience of the beamline scientists at DUBBLE for fibril samples of a similar nature indicates that the intensity of the patterns significantly increases with the alignment of the fibrils within the same exposure time and sample concentration as used in our experiments. The necessity for aligned material was insufficiently clear to us at the outset. As we have previously successfully aligned amyloid fibrils in a magnetic field at University of Nijmegen's *High Field Magnetic Laboratory* (Meersman *et al.*), we will use these samples to proceed with the present SAXS studies.

Meersman *et al.*, unpublished observations
Yagi *et al.*, *J. Mol. Biol.* 2006, **362**, 327-333