ESRF	**Experiment title:** Structural Mechanics of Neurofilament Intermediate Filaments from Myxicola Axons	**Experiment number**: SC 1317
Beamline:	**Date of experiment**: from: Mar 8, 2004 to: Mar 12, 2004	**Date of report**: Aug 31, 2004
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Report:

In a previous study carried out in part at the ESRF, we demonstrated that intermediate filament (IF) proteins from the slime of the hagfish undergo an alpha-to-beta structural transition when they are stretched (Fudge et al. 2003). The purpose of this experiment was to test the hypothesis that other IFs, such as the neurofilaments that occur within axons, behave in a similar manner. To do this, we prepared samples of giant axon from the marine worm *Myxicola infundibulum* that were either unstretched (controls) or stretched to 80% strain. We conducted these experiments at ESRF using a 5 μm beam in scanning mode, which yielded high quality WAXS images. We found that control axons yielded a typical 'alpha' diffraction pattern similar to that seen in unstretched hair samples as well as hagfish slime threads. Axons stretched to 80% in a humidity chamber exhibited a predominantly 'alpha' WAXS pattern as well, with little evidence of beta sheet secondary structure. This result was contrary to our expectations, which were that stretching the axons to a strain greater than 35% would yield proteins with significant beta character. To test whether the IFs in axons are capable of undergoing an alpha-to-beta transition at all, we stretched the strained axons even further, and allowed them to 'anneal' overnight in a hydration chamber. Treating the axons in this way yielded sharp beta WAXS patterns. Two images from these experiments are shown at the end of this report.

In the months following these experiments, we have significantly improved our techniques for isolating and handling *Myxicola* giant axons. Our latest data suggest that our initial tensile tests were most likely performed on partly dehydrated samples. Hydration is known to have dramatic effects on IF mechanics (Fudge & Gosline, 2004), so the mechanical properties we obtained from these experiments likely have little relevance to the behavior of IFs in vivo. For this reason, we would like to repeat these experiments using axons that were strained under more physiological conditions.

References:

Fudge, D.S. and Gosline, J.M., 2004. Molecular design of the alpha-keratin composite: Insights from a matrix-free model, hagfish slime threads. Proceedings of the Royal Society of London B 271: 291-299.

Fudge, D.S., Gardner, K.H., Forsyth, V.T., Riekel, C. and Gosline, J.M., 2003. The mechanical properties of hydrated intermediate filaments: Insights from hagfish slime threads. Biophysical Journal 85: 2015-27.

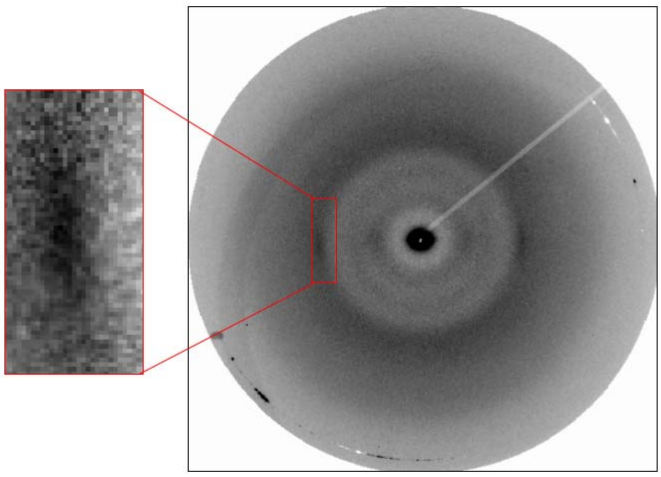

Figure 1. WAXS pattern obtained at ID13 in March 2004 for a 50 μm giant axon from *Myxicola* showing significant beta sheet character. The axon was stretched to a strain of 80% and held overnight in a hydration chamber. Inset shows detail from the 4.7 Å equatorial peak, which is the spacing between chains in beta pleated sheets.