ESRF	**Experiment title:** Microfocus X-Ray Diffraction Studies Of Fibres From Hagfish Slime	**Experiment number**: SC-997
Beamline:	**Date of experiment**: from: Feb 18, 2002 to: Feb 23, 2002	**Date of report**: Aug 31, 2004
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Report:

The purpose of this experiment was to examine the secondary structure of proteins within the intermediate filaments from hagfish slime threads as a function of mechanical stretch. We originally intended to collect WAXS data from single slime threads, but these were too small to get any reasonable x-ray pattern from, even with the remarkable optics available at ID13. To get around this problem, we developed a technique for mounting and straining multiple threads that could then be subsequently mounted on a TEM hole grid for placement in the beam. This worked and yielded excellent data about how the proteins in these important cytoskeletal filaments change their conformation as a function of mechanical strain. These data appeared in the following publication.

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.

One figure and the abstract from this paper appear on the following page.

Abstract: Intermediate filaments (IFs) impart mechanical integrity to cells, yet IF mechanics are poorly understood. It is assumed that IFs in cells are as stiff as hard alpha-keratin, F-actin, and microtubules, but the high bending flexibility of IFs and the low stiffness of soft alpha-keratins suggest that hydrated IFs may be quite soft. To test this hypothesis, we measured the tensile mechanics of the keratin-like threads from hagfish slime, which are an ideal model for exploring the mechanics of IF bundles and IFs because they consist of tightly packed and aligned IFs. Tensile tests suggest that hydrated IF bundles possess low initial stiffness (E_i = 6.4 MPa) and remarkable elasticity (up to strains of 0.34), which we attribute to soft elastomeric IF protein terminal domains in series with stiffer coiled coils. The high tensile strength (180 MPa) and toughness (130 MJ/m^3) of IF bundles support the notion that IFs lend mechanical integrity to cells. Their long-range elasticity suggests that IFs may also allow cells to recover from large deformations. X-ray diffraction and congo-red staining indicate that post-yield deformation leads to an irreversible alpha-->beta conformational transition in IFs, which leads to plastic deformation, and may be used by cells as a mechanosensory cue.

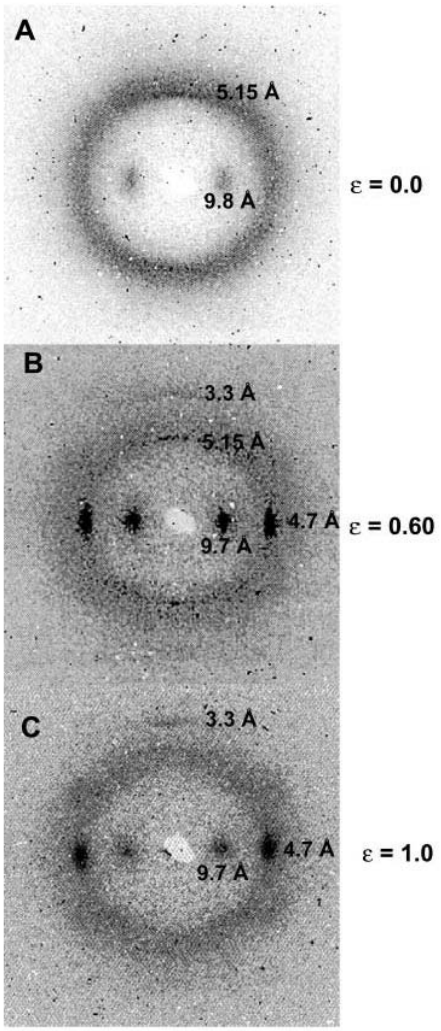

FIGURE 7 X-ray diffraction patterns for hagfish thread bundles strained in seawater. (*A*) Unstrained threads exhibited a typical "α-pattern," whereas threads extended to a strain of 1.0 exhibited a typical "β-pattern" (*C*). Thread extended to a strain of 0.60 exhibited a mixed pattern, suggesting the presence of both α-helix and β-sheet structure (*B*). Diffraction maxima (*dark spots*) are labeled according to the molecular spacings (in Angstroms, Å) to which they correspond.