Experiment Report

(We had 18 shifts on ID10C at the ESRF in October 2008. During that run, we ran into some problems with the beamline optics and could not use the cryo CDI instrument. The main problem is that the pinhole, guard slit and beamstop were not optimized for getting a very clean beam. When we inserted small biological specimens such as cells and cellular organelles into the beam, we could not obtain clean diffraction patterns. We hence studied relatively large specimens (silk fibrils, ~ 20 μm in size). Since then, the cryo CDI instrument on ID10C has been significantly improved and a paper has recently been published on CDI of frozen-hydrated D. radiodurans bacteria [E. Lima et al., PRL 103, 198102 (2009)]. In addition, we have purchased optimized pinholes, guard slits and beamstops, and will bring them to the ESRF in our next run.)

Silks are natural protein polymers assembled into fiber forms, produced by lepidoptera larvae such as silkworms, spiders and flies. Due to the excellent mechanical properties and biocompatibility, silks have been used as biomedical suture material for centuries and have recently gained extensive applications as industry materials and biomaterials, such as scaffolds for tissue engineering. Spider silk fibers are of practical interest because of the high-performance mechanical property. They are strong and tough, designed by nature in an optimal way. The hierarchical structure, especially, the molecular structure that leads to the functional properties of the material, however, is far from being understood.

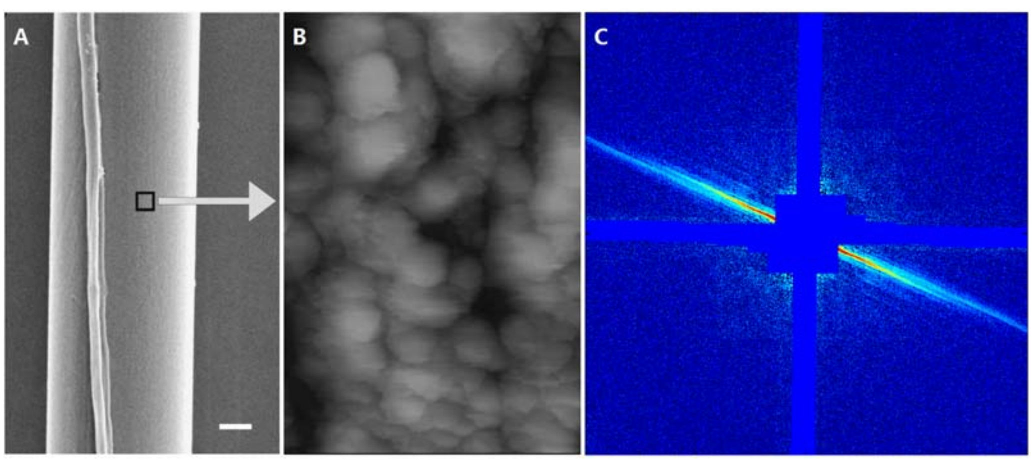

Fig. 1 Hierarchical structure of spider dragline silk *N. pilipes*. (A) SEM image of spider dragline silk. Scale bar, 1 μm. (B) AFM image showing each silk fibril is composed of interconnected "silk fibril segments" with a size of 40–80 nm. (C) Coherent X-ray diffraction pattern of a silk fibril of 20 μm measured from ID10C at the ESRF, showing a high order internal structure of the fiber.

Based on the previous observations by SEM and other microscopy, the spider

dragline silk has a hierarchical structure. It is composed of a large number of silk fibrils along the silk thread axis. According to AFM images, a silk fibril is not a cylindrical fibril, but a lot of silk fibril segments, and some of its segments are interlinked with each other. To obtain the comprehensive understanding of the microstructure of silk fibers, we performed coherent diffractive imaging experiment on a spider dragline silk on ID10C at the ESRF. Fig. 1C shows a diffraction pattern with strong anisotropy distribution, which implies a high order internal structure of the fiber. Fibrils inside the fiber are well aligned with one another, which is consistent with the other observations. This high-order structure of protein macromecules in a nanometer scale may lead to extraordinary mechanical properties. However, the diffraction of a silk section (Fig. 2) shows a random pattern. This indicates the degree of the order structure inside fiber along the equatorial direction is less than that along the meridional direction (fiber axis). The result is also consistent with the AFM observation.

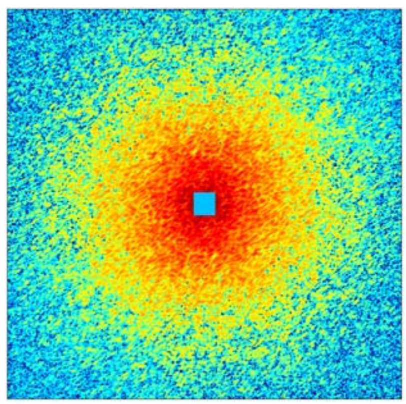

Fig. 2 Coherent X-ray diffraction pattern measured from a silk fibril section.