ESRF	**Experiment title:** X-ray data collection from single crystals of the FimCH chaperone/adhesin preassembly complex from E. coli	**Experiment number:** LS-299
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Report:

Bacterial adhesins are often assembled into hair-like fibers called pili. Often the adhesins are components of specialized structures called tip fibrillae that are joined to the distal ends of thicker pilus rods. Some proteins serve as both the structural component of the pilus fiber and the adhesin.

Type 1 pili which mediate binding to mannose-containing glycoproteins on eukaryotic cell surfaces are produced by nearly all *Enterobacteriaceae. The* major component of type 1 pili is repeating FimA subunits arranged in a right-handed helix to form a 7-nw-wide fiber with an axial hole. Apart from FimA and the adhesin FimH, type 1 pili also contain small amounts of FimF and FimG. As for all other known pilus systems from Gram negative bacteria, type 1 pilus biogenesis requires the presence of two non-structural assembly proteins: a periplasmic chaperone (FimC) and an usher (FimD).

In collaboration with Scott Hultgrens group in St Louis, USA, the pre-assembly

complex between the type 1 pilus chaperone FimC and the type 1 pilus adhesin FimH from *E. coli* has been purified to homogeneity and crystallized from ammonium sulphate solution using the hanging drop vapour diffusion method.

Initial data collection and characterization of the crystals was carried out at the Xl 1 beam line at DESY (Hamburg, Germany). The diffraction limit of a fresh crystal was about 2.7 Å Data to 3.2 Å resolution were collected from one crystal at T=288K. Auto indexing showed that the crystals belong to one of the space groups $P4_12_12$ or $P4_32_12$ with unit cell dimensions a = b = 97.7 Å c = 215.9 Å as had previously been determined from low resolution data. Due to limited time, and radiation sensitivity of the crystals, only a 75% complete data set was obtained. A complete native data set was later collected under cryogenic conditions at the BL- 19 beam line at ESRF (Grenoble, France). Using 30% glycerol as cryoprotectant, 3.0 Å data were collected from a single crystal at T = 100K. Due to the high temperature factor of the crystals with rapid decrease in diffracted intensities in the 3.2-2.8 Å resolution shell it was not considered feasible to collect higher resolution data given the relatively low intensity of the beam on the BL19 beam line.As is often found, freezing resulted in a shrinking of the unit cell with a reduction of the c-axis to c = 213.2 Å. The final data set comprises 107577 observations of 20836 independent reflections corresponding to 97.1% of the possible data with RS_Y~ = 8.4 and <I/sigma(I)> = 9.2.

Due to the small size and the limited diffraction of the FimC-FimH crystals it has not been possible to screen for heavy atom derivatives using a laboratory source. Thus, hoping for serendipity, a second crystal was soaked in 2 mM ethyl mercuri thiosalicylate (EMTS) overnight. Setting the wavelength at λ = 1.0 Å, 76887 measurements of 20148 unique reflections (including 14597 Bijvoet pairs) to 3.0 Å Bragg spacing were collected from this crystal. However, this crystal turned out not to provide a useful derivative with an anomalous signal of only 3.7% and isomorphous difference R_{iso} = 14.5%, although both the normal probability plot and χ^2-analysis indicate some degree of binding. It is thus not impossible that soaking in a higher concentration of EMTS will give a useful derivative.