ESRF	**Experiment title:** Structure determination of the bovine heart mitochondrial cytochrome bc_1 complex (cytochrome c reductase)	**Experiment number:** LS-525
Beamline: ID2/BL4	**Date of experiment:** from: 2 Aug, 1996 to: 5 Aug, 1996	**Date of report:** 22 Feb, 1997
Shifts: 9	**Local contact(s):** Dr Jeff Show and Dr Bjarne Rasmussen	*Received at ESRF:* 03 MAR 1997

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Report:

Cytochrome c reductase (also known as Complex III or bc_1 complex) is the middle segment of the mitochondrial respiratory chain which is crucial for aerobic metabolism. Defects in the respiratory chain lead to many mitochondrial myopathies including many neuromuscular disorders such as Parkinson's disease. For the bovine heart mitochondrial bcl complex, this rather large oligomeric enzyme is composed of 11 polypeptide subunits having a total combined weight of 240 kDa. The primary sequences of all eleven subunits are known for the bovine heart system. Three of the subunits contain redox prosthetic groups, specifically, cytochrome *b*, cytochrome c_1, and the Rieske iron-sulfur (2Fe-2S) cluster. Cytochrome c reductase is an integral membrane protein complex and is currently the largest membrane protein complex to be crystallized.

We have isolated, purified, and crystallized the protein complex from bovine heart mitochondria and have collected preliminary data at synchrotron sources in the United States and Japan on two different crystal morphologies. Recently, we have focused our

attention on one particular morphology, long rods up to 4 mm long, which give a primitive hexagonal space group $P6_{1(5)}$ with cell dimensions a = b = 130 A, c = 720 A. These rods seem more promising with lower mosaic spread giving rise to cleaner diffraction patterns as well as increased resolution. All of the previous data collection at other synchrotron sources resulted in unusable data due to a combination of inadequate flux along with inadequate spot separation of our large unit cell.

The high-brilliance at Beamline 4 enabled us to collect high resolution native data that diffract out to 2.8Å (fig.a,b). Our data set was collected after placing the MarResearch 30 cm detector at a 600 mm crystal-to-detector distance with a 6 degree 2-theta offset using a wavelength of 0.99Å. Due to the high flux of this beamline, we were able to crop the beam down to a 100 micron size to help resolve the closely spaced diffraction spots of our 720 Å c-axis. Although the flash-cooled crystals have not been notably sensitive to X-ray damage at other synchrotron sources, our crystals had to be translated throughout the course of the data collection at this intense beamline; fortunately due to the long rod morphology of our crystals, we were able to collect an entire data set from one crystal. We also collected several potential heavy atom derivatives, one of which gives a clear Patterson cross-peak solution (fig. c). This potential derivative was later found to be too weak to give any adequate phase information.