	Experiment title:	Experiment number:
	Structural studies on the respiratory membrane proteins	LS1762
Beamline: ID14/EH2 and EH4	**Date of experiment:** from: 15 Sep 2000 to: 17 Sep 2000 25 Nov 2000 27 Nov 2000 15 Feb 2001 16 Feb 2001	**Date of report:** 1
Shifts: 15	**Local contact(s):** Dr. Edward MITCHELL, Dr. Sean MCSWEENEY Dr. Sigrid KOZIELSKI	*Received at ESRF:*

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Report:

(1) Formate dehydrogenase-N from *E.coli*

We have succeeded to crystallize formate dehydrogenase-N from *Escherichia coli*. This molybdenum-containing enzyme, composed of α, β and γ subunits, is the major electron donor to the nitrate respiratory chain of *Escherichia coli*. The obtained crystals belong to a cubic space group $P2_13$ with the unit-cell dimensions of $a = b = c = 203$ Å. An asymmetric unit of the crystals is assumed to contain one formate dehydrogenase-N monomer (Mw. 170kDa). We have collected native and derivative data sets at ID14/EH2. A native data set at 1.6 Å resolution, with 342,711 independent observations (94.4% complete) and 0.08 R_{merge}, has been collected from the crystal (fig. 1). This is the highest resolution data set reported for a membrane protein complex to date. We have recently collected MAD data sets at ID29 in the proposal LS1957 and succeeded to obtain the interpretable electron density map. The model building and refinement

(2) Succinate dehydrogense from *E. coli*

A membrane protein complex, succinate dehydrogenase (SQR) from *Escherichia coli* has been purified and crystallised. This enzyme is composed of four subunits containing FAD, three iron-sulphur clusters and haem *b* as prosthetic groups. The obtained crystals belong to a hexagonal space group $P6_3$ with the unit-cell dimensions of $a = b = 123.8$ Å and $c = 214.6$ Å. An asymmetric unit of the crystals is assumed to contain one SQR monomer (Mw. 129kDa). One data set up to 4.0 Å resolution (88.1% complete) and 0.065 R_{merge}, has been collected from the crystal at ID14/EH4. The structure was solved by molecular replacement method using the soluble domain of fumarate reductase (QFR) from *E. coli* as a search model (fig. 2). The crystal packing strongly suggest the *E .coli* SQR is a trimer unlike a dimer observed in QFR in *E. coli*.

(3) VoV1-ATPase from *Thermus termophilus*

V-ATPases are mainly found in eukaryote and archaebacteria and counterparts of F-ATPases found in eubacteria and mitochondria. V-ATPase from *Thermus thermophilus* is a very stable and suitable sample to study the proton-driven ATP synthesis, particulary in comparison to F-ATPases. To date, we have obtained the crystals of the a_3b_3-subcomplex of V_1-ATPase and V_0-ATPase. The a_3b_3-subcomplex crystals belong to the trigonal space group *P*3 with the unit-cell dimensions of $a = b = 200$ Å c = 180 Å. An asymmetric unit of the crystals is assumed to contain 6 molecules of 500 kDa a_3b_3-subcomplex. A data set at 2.6 Å resolution, with 125,080 independent observations (99.4% complete) and 0.108 R_{merge}, has been collected at ID14/EH2. The V_0-ATPase crystals belong to a cubic space group F432 with the unit-cell dimensions of $a = b = c = 179$ Å. A native data with the size of 30μm diffracted up to 5.0 Å at ID14/EH2. We are currently trying to increase the size of the crystals.

(4) b_2-subunit of K^+-channel.

Kv1 Shaker–related K^+-channels from mammalian brain have been purified using the specific blocker α-dendrotoxin, and shown to be octomeric sialglycoproteins consisting of 4 transmembrane pore-forming α-subunits and 4 cytoplasmic auxiliary β-subunits. Up to date, we have obtained the crystals of full length b_2-subunit (a subtype of b-subunit) diffracting up to 1.8 Å. Data were collected at ID14/EH2 . The crystals belong to a orthorhombic space group $P2_12_12$ with the unit-cell dimensions of $a = b = 222$ Å and c=82 Å. An asymmetric unit of the crystals is assumed to contain 2 molecules of b_2-tertramer. At ID14/EH2, a native data set at 1.8 Å resolution, with 296,287 independent observations (94.4% complete) and 0.076 R_{merge}, has been collected from the crystals.

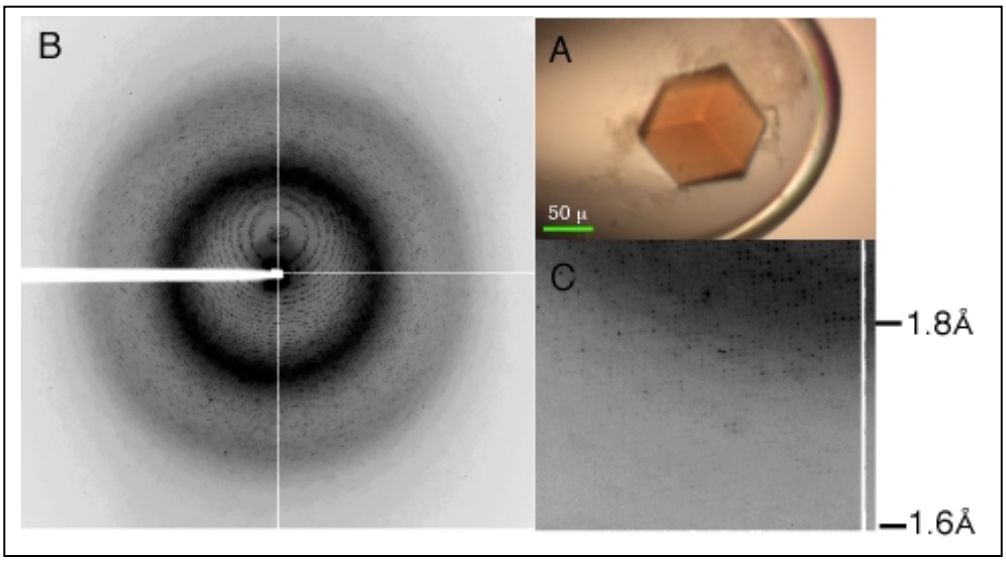

Figure 1. A Crystal of formate dehydrogenase N (Fdh-N) from *E. coli*. **B** X-ray diffraction pattern from the Fdh-N crystal collected at ID14/EH2, ESRF. Beam size = 50 m, exposure time = 20 sec, distance = 140 mm and osscillation angle = 0.3 degrees. A quantum-4 CCD detector was used to record the image. **C** A magnified view of the lower area of panel B. Diffraction spots are extending to 1.6 A.

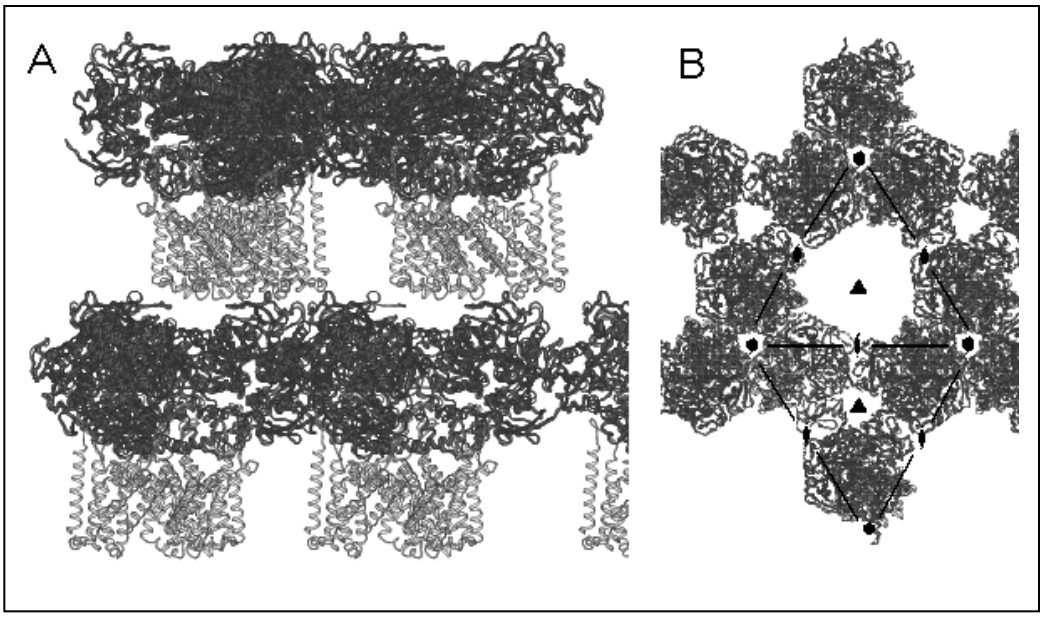

Figure 2. The crystal packing of SQR from *E. coli.* The molecules were drawn using the program Molscript. **(A)** A side-view of the packing showing the stacking of layers of SQR trimers. The soluble part of SQR is shown in the darker shade and the membrane-integral part in the lighter shade.