ESRF	**Experiment- title:** FUMARATE REDUCTASE, A MEMBRANE PROTEIN COMPLEX FROM *Wollinella succinogenes*	**Experiment number:** LS-933
Beamline: BM14	**Date of experiment:** from: 12-Apr-98 7:00 to: 14-Apr-97 7:00 17-Apr-98 7:00 to: 19-Apr-98 7:00	**Date of report:** 10-Aug-98
Shifts: 12	**Local contact(s):** G. Leonard	*Received at ESRF:* 31 AOUT 1998

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Report:

Fumarate reductase (menaquinol:fumarate oxidoreductase) from *Wollinella succinogenes* consists of three subunits, FrdA, FrdB, and FrdC, with a total molecular weight of 130 kDa. FrdC (30 kDa) is a dihaem cytochrome *b*, which anchors the enzyme in the membrane and contains the site for menaquinol oxidation. FrdA (73 kDa) contains covalently bound FAD, a trinuclear [3Fe-4S] iron sulfur centre, and carries the site of fumarate reduction. FrdB (27 kDa) contains binuclear [2Fe-2S] and tetranuclear [4Fe-4S] iron-sulfur centres. This enzyme is currently the best investigated system involved in anaerobic respiration.

Crystals of this bioenergetically important 130 kDa membrane protein complex diffract up to at least 2.1 Å resolution and have two different unit cells, both of the monoclinic space group $P2_1$. The unit cell of crystal form "A" is a = 87 A, b = 190 Å, c = 119 Å, with β = 104.6°, the unit cell of form "B" is a = 119 Å, b = 85 Å, c = 190 Å, with β = 96.4°.

Assuming a solvent content of 65%, there are four complexes per unit cell and thus two complexes in the asymmetric units of both unit cells. However, many crystals contain both unit cells, and single crystals of both unit cells are not distinguishable morphologically. This makes data collection from several crystals (beam-)time-consuming, but still feasible.

During the beam time allocated to LS-933, the original two-wavelength experiment on a putative derivative could finally successfully be performed. The reasons for the successful completion of this experiment were:

1. the generous (but necessary) allocation of 12 shifts to the experiment.
2. the availability of a powerful conventional cooling system ("4 °C cooler") to maintain the desired (and necessary) temperature of O-5 °C at the position of the crystal.
3. the improvement of the crystallisation procedure resulting in larger crystals with acceptable mosaicity, thus effectively increasing the wedge of (two-wavelength) data which could be collected from individual crystals.
4. the availability of the CCD detector, thus reducing the time required for read-out to a minimum and also maximising the amount of two-wavelength data which could be collected on individual crystals.
5. the availability of rapid data processing software (DENZO (HKL)) at the beamline to keep up (at least semi-quantitatively) with data acquisition.

The two-wavelength data set was collected at wavelengths of 1.0089 Å and 0.8264 Å, respectively, using the "inverse beam" strategy for the optimisation of the measurement of anomalous pairs. The four passes of data were collected in 6" wedges (eight 0.75" oscillations) from the same crystal. Eventually, data from five crystals of crystal form "A" could be merged to yield two data sets containing 62804 unique reflections (91.4% complete between 10.0 Å and 3.0 Å, with R_{sym} for the intensities of symmetry-related reflections at 8.2% and I/o(I) = 15.2) and 61438 unique reflections (89.3% complete between 10.0 Å and 3.0 A, with R_{sym}= 8.0% and $I/\sigma(I)$ = 16.4%), respectively.

In the remaining beam time, data from another putative derivative could be collected. The crystal could luckily be set perfectly, so no "inverse beam". strategy was required in this case. This meant that a data set, which was 97.0% complete between 10.0 Å and 3.3 Å (R_{sym}= 7.5% and $I/\sigma(I)$ = 17.2%), could be collected from a single crystal. However, the usefulness of this data is questionable since processing indicated a substantial lack of isomorphism.