ESRF	**Experiment title:** Frankfurt BAG subproject: Fumarate reductase, a membrane protein complex from *Wolinella succinogenes*	**Experiment number:** LS-1514
Beamline: BM14	**Date of experiment:** from: 1-Dec-99 8:00 to: 3-Dec-99 7:00	**Date of report:** 29-Feb-2000
Shifts: 6	**Local contact(s):** Gordon Leonard	*Received at ESRF:*

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Report:

Fumarate reductase, a bacterial version of complex II from the respiratory chain, catalyses the reduction of fumarate to succinate, in a reaction opposite to that catalysed by complex II (succinate dehydrogenase). Fumarate reductase (menaquinol:fumarate oxidoreductase) from the anaerobic bacterium *Wolinella succinogenes* consists of three protein 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 andd contains the site for menaquinol oxidation. FrdA (73kDa) contains covalently bound FAD and carries the site of fumarate reduction. FrdB (27 kDa) contains a binuclear [2Fe-2S], a trinuclear [3Fe-4S], and a tetranuclear [4Fe-4S] iron-sulphur centre. 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 and have two different unit cells, both of the monoclinic space group $P2_1$. The unit cell of crystal form "A" is a = 85.2 Å, b = 189.0 Å, c = 117.9 Å, and $\beta = 104.5°$. Crystal form "B" has the unit cell dimensions a = 118.4 Å, b = 85.1 Å, c = 188.9 Å, β = 96.5°. 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. Using data collected earlier at ESRF BM14 (cf. experimental reports for LS-1369), the structure of crystal form A has been solved by multiple isomorphous replacement and anomalous scattering (MIRAS) and refined to 2.2 Å resolution, and that of crystal form B has been solved by molecular replacement (MR) and refined to 2.33 Å resolution.

During the beam time allocated to LS-1514 in December, four higher quality data sets (all for crystal form A, none for crystal form B) could be collected:

Table: Diffraction data collected at ESRF BM14 (1-3 December 1999)

	resol. range [Å]	measured reflections	unique reflections	complete [%]	I/σ(I)	>2σ [%]	R_{sym}* [%]
inhibitor complexes							
proj24/346a505_1	40.0-2.30	342,397	145,484	92.2	8.5	72.0	9.3
	2.35-2.30	*21,499*	*9641*	*91.9*	*2.3*	*45.1*	*35.5*
proj25/340a502_1	40.0-2.35	425,487	140,281	94.0	8.1	74.0	8.9
	2.40-2.35	*29,989*	*9429*	*95.4*	*2.5*	*50.2*	*39.5*
proj26/351b555_1	40.0-2.25	646,589	169,368	99.8	12.9	84.6	8.1
	2.30-2.25	*38,983*	*11,253*	*99.3*	*4.1*	*64.7*	*35.5*
proj27/352a505_1	40.0-2.35	439,847	135,421	91.9	8.9	69.8	8.6
	2.40-2.35	*29,233*	*9287*	*95.0*	*2.5*	*46.4*	*37.4*

Due to the optimum conditions for data collection at BM14 first established in November 1998 (cf. our previous reports for LS-1137 and LS-1369), only one crystal was required for each dataset listed above.

The remaining beamtime was used for testing crystals of the membrane protein complex quinol oxidase (Th. Ostermann* and H. Michel), both at 4°C and at cryogenic temperatures. The results, however, were disappointing and no data set was collected.