ESRF	Experiment title: Frankfurt BAG	Experiment number: LS-1661
Beamline: BM14	Date of experiment: from: 7-Jul-00 8:00 to: 10-Jul-00 7:00	Date of report: 24-Aug-2000
Shifts: 9	Local contact(s): Edward Mitchell	*Received at ESRF:*

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1. Photosynthetic reaction centre (RC) from *Rhodopseudomonas viridis.*

Diffraction data from one tetragonal crystal of a mutant RC (CRD Lancaster, M Bibikova, D Oesterhelt, H Michel) was collected at (see Table 1) using 114 images with a rotation of $\Delta\phi$ = 0.4° per image. Refinement of the structure is currently in progress.

Table 1. Diffraction data collected at ESRF BM14 on a mutant *Rp. viridis* photosynthetic reaction centre crystal ($P4_32_12$; a = b = 223.5 Å, c = 113.6 Å). T= 2°C at the position of the crystal.

resol. range [Å]	measured reflections	unique reflections	complete [%]	I/σ(I)	>2σ [%]	R_{sym}* [%]
50.0-2.45	379,721	105,105	99.1	13.8	79.5	6.2
2.51-2.45	*19,197*	*6343*	*90.9*	*2.4*	*45.1*	*41.4*

2. Data collection at cryogenic temperatures (Christian Lange* and Carola Hunte*)

Short-term changing of the cooling system turned out to be problematic since the nitrogen "warm flow" had previously been disconnected because it was required for the "4°C-cooler" used throughout most of the beam-time. The cryostream was completely blocked with ice and could have only been used after lengthy warming up and recooling. Therefore, we did not, as planned, attempt to intermittently collect data at cryogenic temperatures.

3. Quinol:fumarate reductase from *Wolinella succinogenes*

Quinol:fumarate reductase (QFR), couples the reduction of fumarate to succinate to the oxidation of quinol to quinone, in a reaction opposite to that catalysed by mitochondrial

complex II (succinate dehydrogenase). QFR from the anaerobic bacterium *Wolinella succinogenes* consists of three protein subunits, FrdA, FrdB, and FrdC. Crystals of this bioenergetically important 130 kDa membrane protein complex diffract up to at least 1.8 Å and have previously been shown to 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 β = 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. 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 [1]. During the beam time available for this subproject, four data sets of crystals from a mutant QFR could be collected. Although crystals with the previously determined unit cells were also identified, the only useful data were collected on crystals of a third monoclinic crystal form, form "C", with cell dimensions as listed in Table 2, with four heterotrimeric QFR complexes in the asymmetric unit. The final data set was collected at T= 2°C using 450 images with a rotation of $\Delta\phi = 0.4°$ per image (see Table 2) on a crystal exhibiting the least degree of anisotropy in the diffraction pattern (<2.8 Å resolution along the b* and c* axes, ~3.8 Å along the a* axis).

Table 2. Diffraction data collected at ESRF BM14 on form "C" ($P2_1$; a = 81.1 Å, b= 290.2 Å, c = 153.6 Å, β = 95.7°) crystals of mutant *W. succinogenes* QFR. (Best data set of four)

	resol. range [Å]	measured reflections	unique reflections	complete [%]	I/σ(I)	R_{sym}* [%]
31FR20c012_2	50.0-3.10	277,807	102,882	80.8	11.9	6.0
	3.29-3.10	*25,556*	*12,962*	*61.7*	*2.0*	*26.0*

After solving the structure by molecular replacement, the electron density was of sufficient quality to confirm the general structure of this functionally important mutant QFR [2].

4. Cytochrome *c* oxidase

Diffraction data from one orthorhombic crystal of a modified cytochrome c oxidase (A Kannt, H Michel) was collected at T= 2°C at the position of the crystal (see Table 3) using 106 images with a rotation of $\Delta\phi = 0.75°$ per image. Refinement of the structure is currently in progress.

Table 3. Diffraction data collected at ESRF BM14 on orthorhombic ($P2_12_12_1$; a = 93.5 Å, b= 151.0 Å, c = 156.7 Å) crystals of modified 2-subunit cytochrome *c* oxidase

resol. range [Å]	measured reflections	unique reflections	complete [%]	I/σ(I)	>2σ [%]	R_{sym}* [%]
50.0-3.05	99,419	40,039	91.7	13.1	70.9	5.8
3.12-3.05	*6388*	*14,638*	*94.2*	*2.6*	*42.6*	*35.2*

5. References

[1] CRD. Lancaster, A Kröger, M Auer, H Michel (1999) Nature **402,** 377-385.

[2] CRD Lancaster, R Gross, A Haas, M Ritter, W Mäntele, J Simon, A Kröger, submitted.