



	Experiment title: FRANKFURT BAG: ATOMIC MECHANISMS OF MEMBRANE PROTEINS	Experiment number: MX-336
Beamline: ID14-EH1	Date of experiment: from: 02-OCT-2004 8:30 to: 04-OCT-2004 8:00	Date of report: 15-Feb-2005
Shifts: 6	Local contact(s): Dr. Stéphanie MONACO	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Sabine Gemeinhardt * ¹ , Vasundara Srinivasan* ¹ , Hartmut Michel ¹ Volker Zickermann* ² , Carola Hunte ¹ Hanno Juhnke* ¹ Gregor Madej* ¹ , C. Roy D. Lancaster ¹ ¹ Max Planck Institute of Biophysics, Department of Molecular Membrane Biology, Max-von-Laue-Str. 3, D-60438 Frankfurt am Main ² Universität Frankfurt, Fachbereich Medizin, Institut für Biochemie I, D-60590 Frankfurt/M. Germany		

Report:

Cytochrome *cbb*₃ oxidase from *Pseudomonas stutzeri*.

(Sabine Gemeinhardt *¹, Vasundara Srinivasan*¹, Hartmut Michel¹)

Cytochrome *cbb*₃ is a cytochrome c-oxidising enzyme that belongs to the superfamily of respiratory haem/copper oxidases. There is no structural information available for this subfamily of membrane proteins yet. This group of enzymes have a very high affinity for oxygen and forms the basis for bacterial colonization of microaerobic environments. Sequences of *cbb*₃ type oxidases are present in the genomes of human pathogens like *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Helicobacter pylori*.

We have crystallized the 4-subunit transmembrane protein complex from *Pseudomonas stutzeri*. We have tested crystals from various new crystallization conditions in capillaries and under cryo conditions at the beamlines ID14-1 (October 2004) and ID14-3 (November 2004). Most of the crystals showed poor diffraction properties with multiple lattices, anisotropic pattern and could not be used. During the three shifts allocated to this subproject on ID14-1, one dataset has been collected under cryo conditions to around 4.2 Å resolution and could be processed to 4.5 Å resolution with a high overall R_{sym} of 14.7%. The crystal belongs to the space group P2 with cell dimensions of $a=104.42$, $b=167.56$, $c=126.68$, $\alpha=90$, $\beta=94.94$ and $\gamma=90.0$. Improvement of the quality of the crystals is in progress.

Photosynthetic RC from *Rp. viridis*

(Hanno Juhnke* Gregor Madej*, C. Roy D. Lancaster)

The majority of the two shifts devoted to this subproject were used for (ultimately unsuccessful) attempts to improve the previously recorded data set at 2.5 Å resolution of a variant photosynthetic reaction center from *Rhodopseudomonas viridis* (see Aug 2004 EH2 report). The best of three data sets is summarized in Table 1. Higher resolution data are required.

Table 1. Best of three diffraction data sets collected at ESRF ID14-EH1 on a crystal of a variant *Rp. viridis* RC (P4₃2₁2, a = b = 223.5 Å, c = 113.6 Å)

	resol. range [Å]	measured reflections	unique reflections	complete [%]	R _{sym} [%]
RC08_21_2_0	50.0-2.70	314,047	75,597	95.1	7.6
	2.76-2.70	17,177	4,385	84.3	33.5

Complex I from *Yarrowia lipolytica* (Volker Zickermann*, Carola Hunte)

Complex I is the largest and least understood enzyme of the respiratory chain. Structural information is limited to low resolution and is based on electron microscopy of single particles and 2 D crystals. We have obtained crystals of complex I from the strictly aerobic yeast *Yarrowia lipolytica* under several crystallization conditions. Crystals are small and diffraction cannot be screened at the home source. 20 crystals were tested for diffraction. The majority diffracted up to 8 Å resolution. Optimization of the crystallization and freezing conditions is in progress.