



	Experiment title: Progress in structural studies of cytochrome <i>c</i> oxidase	Experiment number: MX-27 MX-100
Beamline: ID14-1	Date of experiment: 28/08/2002, 06/12/2002 and 03/07/2003	Date of report: 01-09-2003
Shifts: 4	Local contact(s): Edward Mitchell , Stéphanie Monaco & Joanne McCarthy	<i>Received at ESRF:</i>
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Report:

One of the key problems of molecular bioenergetics is the understanding of the function of redox-driven proton pumps on a molecular level. On such class of proton pumps are the heme-copper oxidases. These enzymes are integral membrane proteins in which proton translocation across the membrane is driven by electron transfer from a low-potential donor, such as cytochrome *c*, to a high-potential acceptor, O₂. We are investigating one member of this group, cytochrome *c* oxidase (CCO) from *Rhodobacter sphaeroides*, using a combination of structural and kinetic studies. We are especially interested in understanding how the proton transfer steps are linked to the oxygen chemistry, and more specifically what the role of the key residues of the two proton pathways (the D- and the K-pathway) in the enzyme is. The structure of wild-type CCO was

solved to a resolution of 2.3/2.8Å (anisotropic resolution) in collaboration with Prof. So Iwata [1]. We also solved the structure of a mutant enzyme where glutamate-286 of the D-pathway was mutated to a glutamine [1]. The data was collected at the ESRF. According to these recently-published structures of the enzyme, the deprotonation of E(I-286) is likely to result in minor structural changes that propagate to protonatable groups on the proton output (positive) side of the protein. We believe that in this way the free energy available from the O₂ reduction is conserved during the proton transfer. On the basis of these observed structural changes, we recently presented a proton-pumping model for the enzyme [2]. During the reviewed period (as part of experiment MX-100) we have also collected 3Å data on another mutant in this position, ED(1-286), which is currently being refined. Fast-kinetics studies on this mutant enzyme suggest that this region of the protein is involved in proton gating, and we hope that the structure will clarify this and support our hypothesis on the proton-pumping mechanism of cytochrome *c* oxidase.

[1] Svensson-Ek, M., Abramson, J., Larsson, G., Törnroth, S., Brzezinski, P. and Iwata, S. (2002) *J Mol Biol* 321, 329-339.

[2] Brzezinski, P. and Larsson, G. (2003) *Biochim Biophys Acta* 1605, 1-13.

