ESRF	**Experiment title:** Cryocrystallography of cytochrome c oxidase from *Paracoccus denitrificans*	**Experiment number:** LS-545
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Report:

Cytochrome c oxidase (ferrocytochrome c:oxygen oxidoreductase, EC 1.9.3.1) a membrane protein complex is the terminal enzyme of most respiratory chains. It catalyses the final electron transfer steps from cytochrome c to molecular oxygen. In addition to the four protons consumed in water formation per oxygen molecule, up to four protons are electrogenically translocated across the membrane.

We have obtained good crystals of cytochrome c oxidase from *Paracoccus denitrificans. They* belong to space group P4 with a=b=206.7 Å, c=83.5 Å. The protein complex is composed of four subunits (total molecular mass is 125 kDa). Subunit I contains two haem A molecules, called haem a and haem a_3 , and copper B (Cu_B). Haem a_3 and CUB form the binuclear centre where molecular oxygen is reduced to water. Electrons from cytochrome c are first translocated to the copper A centre (Cu_A), which is located in the protein subunit II, and from there to haem a and the binuclear centre.

The structure of cytochrome c oxidase from *Paracoccus denitrificans* has been solved (Iwata, et al. Nature 376: 660-669), but there remain a lot of questions to be studied especially the mechanism of proton pumping.

With the use of cryotechnique (100 K) it is now possible for us to collect better data sets and to stabilize sensitive redox states, what is necessary to detect structural changes between the various states of cytochrome c oxidase.

During the beam time allocated to LS-545, approximately 2000.7° high resolution oscillation images and 100 1.10 low resolution images were collected (including test exposures). The crystals show only weak diffraction, large mosaicity (up to 0.7°) and high background noise derived from detergent micelles. For data collection the finest beam (size, divergence and monochromaticity) and low noise data collection system are necessary. These requests are only fulfilled at ESRF, especially at ID02/BL04.

Processing of the images recorded on the fully oxidized crystals using the HKL processing package (Otwinowski and Minor, to be published) has resulted in a data set of 174,473 measured reflections between 40 Å and 3.0 Å (64,653 unique, 95 % complete), with R_{sym} for intensities of symmetry-related reflections at 10.5%.

Preliminary refinement has resulted in a structural model in which very important details are now defined, for example the so far not detected manganese binding site. The position of His 325 of subunit I, we believe is very important for the reaction mechanism, is now at 100 K visible.