ESRF	**Experiment title:** Structure determination of proton-conducting pathway mutants of cytochrome c oxidase from *Paracoccus denitrificans*	**Experiment number:** LS-946
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Report:

Cytochrome c oxidase (ferrocytochrome c:oxygen oxidoreductase, E.C. 1.9.3.l), a membrane protein complex, is the terminal enzyme of the respiratory chain of mitochondria and many bacteria. It catalyses electron transfer from cytochrome c to oxygen, reducing the latter to water. This redox reaction is coupled to the generation of an electrochemical proton gradient across the membrane that is the outcome of electrons from cytochrome c and protons needed for water formation taken up from opposite sides of the membrane and, additionally, translocation ("pumping") of protons across the membrane.

The crystal structure of cytochrome c oxidase from *Paracoccus dentrificans* (Iwata et al., Nature 376:660-669; Ostermeier et al., Proc. Natl. Acad. Sci. 94: 10547-10553) revealed two putative pathways for protons from the cytoplasm to the binuclear center. Analysis of site-directed mutants (Pfitzner et al., J. Bioenerg. Biomembr. 30, 89-98) showed that specific amino acid residues are crucial for the coupling of electron and proton transfer to occur.

In order to study structural alterations associated with mutations that lead to inhibition of redox-coupled proton transfer, we have crystallised some of these mutants in their two-subunit form. The crystals obtained have the same space group ($P2_12_12_1$) and unit cell dimensions (93.5 x 151.0 x 156.7 A) as crystals of the wild-type enzyme, and diffract to about 2.8 A. However, they were found to be considerably smaller (<50 % in each dimension) and thus prone to substantial radiation damage when mounted in capillaries. Therefore, it was necessary to freeze the crystals to be able to collect a complete data set. Additionally, due to the small size of the crystals, large mosaicity and high background noise derived from detergent micelles, a very fine beam (size, divergence and monochromaticity) and low noise data collection system are required.

However, freezing crystals of cytochrome c oxidase from *Paracoccus denitrificans* is far from trivial, because the crystals are very sensitive to various kinds of cryoprotectants. During the beamtime allocated to LS-946 on ID02B, we collected approximately 400 images from 80 different crystals. All crystals were frozen in liquid propane and data collection was performed at 100K. Most of the crystals, however, were not suitable for extensive data collection as they were either damaged during the freezing process or showed such a high degree of mosaicity that the data could not be processed. From two of the crystals, we have collected complete data sets. Both crystals are “borderline cases” showing freezing damage (e.g. ice rings) as well as high mosaicity (>1.5 degrees). Processing of the data using the HKL processing package (Otwinowski and Minor, Meth. Enzymol. 276:307-326) is under way, but is problematic due to the aforementioned difficulties.