MX-2272 (Hunte/Wirth)

Beamtime on ID23-1 of 12th of September 2020

icOS lab on the 11th and 13th of Septembre

Beamtime on ID30A-3 on the 1st of Octobre

Our group aims at understanding the structure as well as the mechanism and regulation of medically relevant membrane proteins. In particular, we aim for an in depth understanding of the mechanism of cytochrome bc_1 (cyt bc_1, complex III, coenzyme Q : cytochrome c oxidoreductase) from the mitochondrial respiratory chain. The redox-cofactor carrying enzyme catalyzes electron transfer from the two-electron two-proton carrier ubiquinol (UQH2) to the single electron carrier cytochrome *c* (cyt *c*). The direct heme-to-heme electron transfer, from subunit cyt c_1 of the cyt bc_1 complex to cyt *c* is accomplished through the formation of a transient complex between the two proteins. The structural basis for transient electron transfer complexes is not well understood. A first structure of cyt bc_1 with the mobile electron carrier cyt *c* was determined at 3-Å resolution in 2002. The resolution was improved later to 1.9-Å resolution (Solmaz and Hunte, 2008). We experimentally address the working hypothesis, that the interaction between bc_1 and cyt *c*, as well as the release of the latter after electron transfer, is controlled via the redox states of the interaction partners.

In order to investigate this, we prepared for this beamtime, crystals frozen in three different redox states (oxidized, reduced and “as purified”). Absorption spectra from the different crystal types were taken at the icOS lab, in collaboration with A. Royant, prior to exposure to X-rays. Clear spectra resolving the typical signatures of *b*- and *c-type* heme cofactors were observed for the different redox states. On the next day, these crystals were exposed to X-rays on ID23-1 and a few data sets were collected from crystals frozen under the same conditions. Unfortunately, full data sets could not be obtained for all crystal types measured at icOS during this beamtime, as there were a few problems with the beamline during these shifts. However, we could expose crystals of each redox state. And absorption spectra were measured of these exposed crystals on the next day. Spectra confirmed that X-rays rapidly reduced the crystals. Finally, datasets from the remaining crystals were collected a few days later, during the beamtime on beamline ID30A-3.

Overall, the experiments were very successful. X-ray diffraction and spectroscopy data are of good quality. The resolution limits of the different datasets collection are better than 3Å. The support from the local contacts was excellent.