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Report:

We are analyzing structure-function relationships of the cytochrome bc_1-complex (QCR) from *S. cerevisiae*, of which we determined the structure with a bound antibody fragment at 2.3 Å resolution [1,2]. This mitochondrial multisubunit membrane protein is one of the fundamental components of the respiratory chain. It catalyzes electron transfer from ubiquinol to cytochrome *c*, in a process that is coupled to electrogenic translocation of protons across the inner mitochondrial membrane. Still, the molecular basis of fundamental processes of the enzyme mechanism is not known, e.g. the oxidation of quinol, the identity of primary proton donors during quinone reduction or the importance of buried water molecules for proton transfer pathways.

We analyzed substrate and inhibitor binding at the site of ubiquinone reduction. We collected four data sets of crystals soaked with a substrate analogue for different incubation times: I. 3.0 Å, 84.9 % completeness, 8.1 % R_{merge}; II. 3.0 Å, 93.6 % completeness, 5.9 % R_{merge}; III. 3.1 Å, 83.4 % completeness, 7.1 % R_{merge}; IV. 2.7 Å, 96.9 % completeness, 7.5 % R_{merge}. We attempted to collect three data sets of crystals soaked with a Qi-site specific inhibitor (qi3) for

different incubation times: I. 2.5 Å, 98.3 % completeness, 6.9 % R_{merge}. Data sets of crystals with long soaking periods could not be scaled properly. Two data sets were collected at pH 7.5 as a control for the QCR structure with a bound Qo-inhibitor obtained with a previously collected data set (qo3, manus. in prep.): I. 2.8 Å, 93.0 % completeness, 6.5 % R_{merge}; II. 2.5 Å, 94.4 % completeness, 6.4 % R_{merge}. Data collection was performed at 4 °C. For crystals of best quality and size, 16-bunch mode in combination with moderate attenuation allowed the collection of 2.5 Å resolution data sets with good statistics from a single crystal. Refinement is in progress.

We recently determined the structure at 2.97 Å resolution of yeast QCR with its substrate cytochrome *c* bound [3,4; see highlight report]. Crystals belong to the space group P21 with unit cell parameters a=147Å, b=166Å, c=196Å, β=104°. Structure determination was achieved using a single crystal with one data set collected at 4 °C. High resolution data and cryo-conditions are required, to address open questions of substrate binding and catalysis. We tested several cryo-conditions and for the first time, we could establish cryo-conditions for yeast QCR crystals. A data set of a crystal (0.3 x 0.3 x 0.4 mm^3) of the ternary complex consisting of QCR, cytochrome c and Fv fragment was collected: 2.8 Å, 7.1% R_{merge}, 85.2 % completeness. Refinement is in progress to analyze ordered binding of cytochrome *c* under these conditions.

More than 70 crystals of the TOM complex from *N. crassa* and of the sodium/proton antiporter NhaA from *E.coli* have been tested for diffraction quality. The crystals are generally smaller than 0.2 x 0.2 x 0.2 mm^3 and diffraction cannot be tested at home radiation sources. For both projects, crystal quality has to be improved to allow structure determination. Suitable cryo-conditions were found for the sodium/proton antiporter.

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[3] Lange, C. and Hunte, C. (2002). Crystal structure of the yeast cytochrome bc_1 complex with its bound substrate cytochrome *c*. *Proc. Natl. Acad. Sci. USA* **99**, 2800-2805.

[4] Hunte, C., Solmaz, S., Lange, C. (2002). Electron transfer between yeast cytochrome bc_1 complex and cytochrome c: a structural analysis. *Biochim. Biophys. Acta*, in press