

Unraveling structural intermediates in ba3-type cytochrome c oxidase using time-resolved X-ray methods

Content

Cytochrome c oxidase (CcO) is an integral membrane protein that participates as terminal acceptor of electrons during cellular respiration. It receives electrons from upstream biological transporters that reduces molecular oxygen to water. The free energy stored in this process is then used for transmembrane transport and ATP synthesis. A large amount of biochemical data and equilibrium-states structures of CcO are available to guide the proton translocation pathway of this enzyme, however, the mechanism of redox-linked proton translocation still remains elusive. Using time-resolved serial crystallography (TrSX) and solution scattering (TrXSS) and, we have determined structural changes in ba3- type CcO from *Thermus thermophilus* at ultrafast time-scales. TrSX studies on CO- bound CcO show significant structural changes at the catalytic center at 50ns and 2ms after illuminating CO with a green laser. We also explored the oxygen- reduction reaction with a photolabile caged-oxygen compound using TrXSS. We observed characteristic changes in the difference scattering plots at different time-points during the enzymatic reaction. Further, we want to study structural intermediates during oxygen-reduction in micro-crystals of CcO. With that goal in mind, we have developed simple devices for mix-and-inject serial synchrotron crystallography (SSX) for mixing micro-crystals (in lipidic cubic phase) with oxygen and delivering to the X-ray beam. We have performed preliminary experiments and validated our set-up at MAXIV laboratories (Sweden) and collected data-sets of resting state, reduced- and CO-bound (on and off) states of ba3- type CcO from *Thermus thermophilus*. SSX structure of resting state is solved at 2.3Å, similar to earlier reported structure obtained at X-ray free electron laser (Andersson et. al,2017). Overall, we aim is to create a molecular movie, elucidating the structural mechanisms of redox-linked proton- pumping and gating of proton channel in ba3- type CcO.

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