GÖTEBORGS UNIVERSITET

INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Microcrystallization in lipidic cubic phase and serial crystallography studies of cytochrome *c* oxidase

Cecilia Safari
Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredag den 06-12-19 kl. 13.00 i Åke Göransson, Institutionen för kemi och molekylärbiologi , Medicinaregatan 11, Göteborg.

ISBN: 978-91-7833-678-4
ISBN: 978-91-7833-679-1
Tillgänglig via http://handle.net/2077/62148

Life in all living organisms depend on chemical processes performed by proteins, which are molecular machineries encoded in the DNA. Cytochrome *c* oxidase (C*c*O) is a membrane protein essential for cellular respiration, a process in which chemical energy enters the electron transport chain in the form of electrons and is transformed to ATP via a proton gradient. In the active site of C*c*O a redox-reaction takes place, where oxygen that we breathe is reduced to two water molecules at the same time as protons are pumped over the membrane.

This thesis focuses on structural investigations performed by serial crystallography (SX) of C*c*O in lipidic cubic phase (LCP). The advances of bright X-ray sources in the form of X-ray Free Electron Lasers with short pulse durations and 4:th generation synchrotrons have evolved the structural biology field in the sense that high-quality data can be collected at room temperature with minimal X-ray induced radiation damage. These advancements enable the capturing of structural intermediates of proteins in a time-resolved manner.

Well-diffracting microcrystals are of essence in serial crystallography experiments, and the study of membrane proteins in lipidic cubic phase has proven to be favorable compared to detergent-based crystallization. This thesis focuses on developing procedures to produce microcrystals in LCP, and its use in studies of ba_3-type C*c*O from *Thermus thermophilus* as well as reaction centre from *Blastrochloris viridis*. The room-temperature structure of ba_3-type C*c*O is determined in both the resting oxidized state and the reduced CO-bound state. The resting state structure reveals the active site ligand as a single oxygen species, and in comparisons to previously published structures of C*c*O we show structural differences between ba_3-type C*c*O and bovine aa_3-type C*c*O upon CO-binding. The work presented in this thesis provides the groundwork for future time-resolved CO-photolysis experiments of ba_3-type C*c*O by the pump-probe approach, which may reveal more about the structural mechanisms that explain proton pumping in C*c*O.