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Time-resolved XFEL crystallography and spectroscopy of cytochrome c oxidase

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Bovine heart cytochrome c oxidase (CcO) is a large membrane enzyme (210 kDa) that catalyzes O2 reduction to water, coupled with proton pump across the mitochondrial inner membrane. The enzyme includes a hydrogen-bond network and a water channel in tandem as a pathway for proton pump (H-pathway). The O2-reduction site is composed of heme a3 and CuB, where O2 binds in the fully-reduced state (Fea3^2+, CuB^1+). After the O=O bond cleavage, CcO sequentially receives four electron equivalents from cytochrome c. Each of the electron transfer processes is coupled with pumping of one proton equivalent, during which the water channel is closed to prevent proton back leak. Recently, high-resolution X-ray structural analysis has revealed that binding of CO (O2 analogue) to Fea3 induces a bulge (unpaired backbone C=O) formation at Ser382 and Met383 in Helix-X, which closes the water channel. To elucidate the coupling mechanism of ligand dynamics with water channel gating, here, we developed novel instruments for time-resolved X-ray crystallography using an X-ray free electron laser (XFEL) at SACLA and time-resolved single-crystal IR spectroscopy, and investigated the structural dynamics following CO-photolysis from Fea3. To induce the CO-photolysis, the visible light pulse was focused onto the loop-mounted crystal from two directions (from the front and back sides of the crystal), allowing us to use the large crystals with a 100% CO-photolysis efficiency. The results of time-resolved X-ray crystallography and IR spectroscopy demonstrate that CO binds to CuB transiently in the crystalline phase as in the solution phase. The communication mechanism between the Fea3-CuB site and the water channel in the H-pathway will be discussed at the presentation.
Keywords: X-ray free electron laser, cytochrome c oxidase, proton pump