Supplementary Information

Structural insights into functional properties of the oxidized form of cytochrome *c* oxidase

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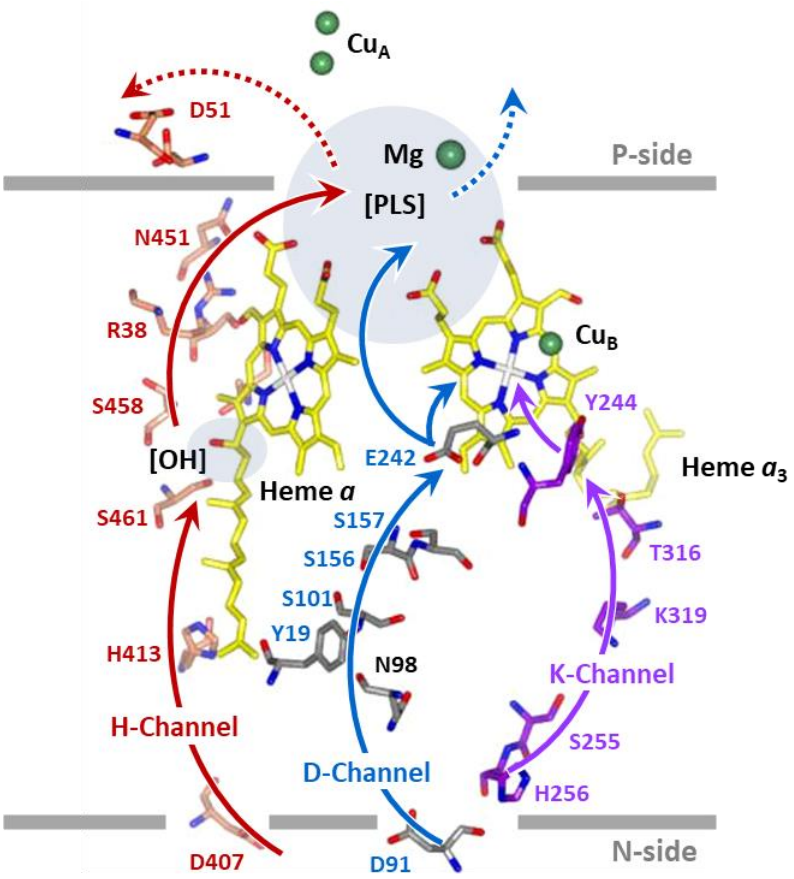
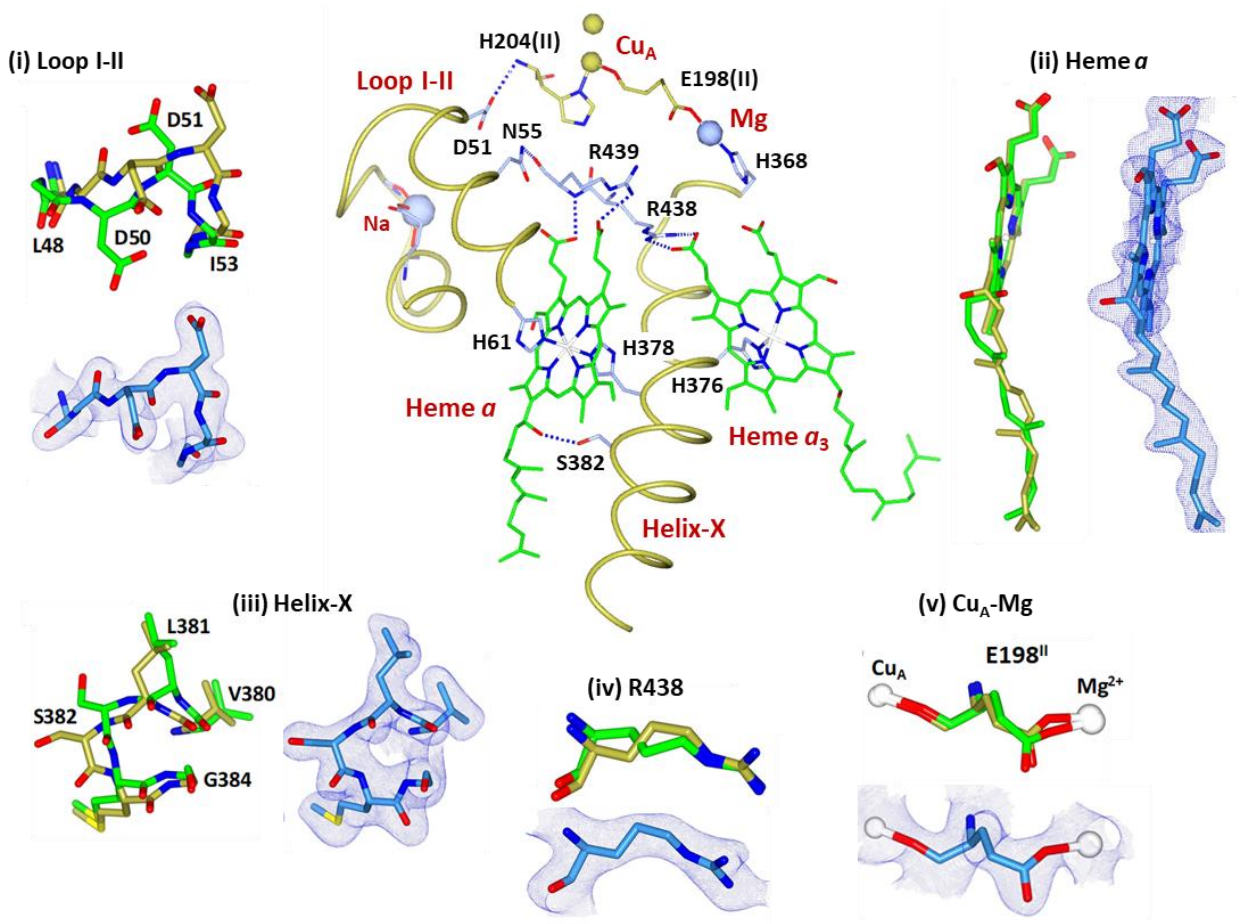
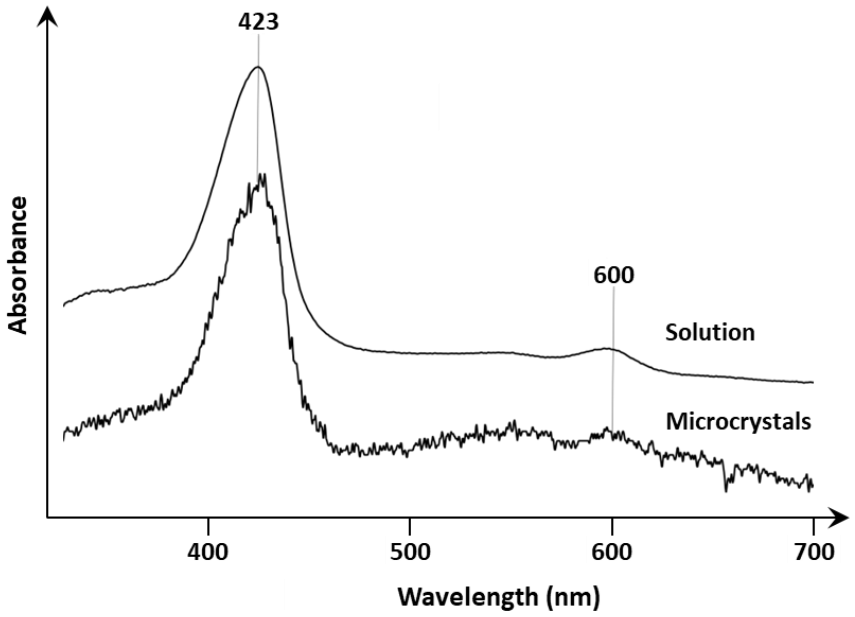


Supplementary Fig. 1. Proton transfer channels in bCcO. Based on structural and mutagenesis studies of bacterial enzymes, two proton transfer channels, the D and K-channels, have been proposed in C*c*O[1]. The D-channel, starting at D91 (bC*c*O numbering) and ending at E242 (which serves as a diverter valve), delivers either substrate protons to the BNC during the oxidative phase of the catalytic cycle, or pumped protons to the PLS for subsequent translocation to the P-side of the membrane. The K-channel, starting at H256 and ending at Y244, delivers substrate protons to the BNC during the reductive phase of the catalytic cycle. An additional channel, the H-channel, starting at D407 and ending at D51, was proposed in bC*c*O, which translocates pumped protons to the P-side of the membrane *via* the PLS, as gated by either a water pool[2] or the hydroxide group of the farnesyl sidechain (indicated as [OH])[3] of heme *a*. The role of the H-channel, however, remains controversial as it is not conserved in bacterial CcOs[4,5].



Supplementary Fig. 2. SFX structure of the O derivative of bC*c*O in the redox sensitive regions. Inset (i)-(v) show the structural comparison of the oxidized enzyme determined with synchrotron radiation (**O***) (in yellow) and reduced enzyme (**R**) (in green) in the redox sensitive regions. The $2F_O$-F_C electron density map of **O**, contoured at 1.5 σ, is shown in each inset to demonstrate that the structure of **O**, consistent with that of **O***, is in the oxidized state. The PDB IDs of the **O*** and **R** are 7TIE and 7THU, respectively.



Supplementary Fig. 3. UV-Vis absorption spectra of oxidized bC*c*O in the free solution phase and in the microcrystalline state. The Soret maximum at 423 nm indicates that the bC*c*O samples are in the same fast (active) form.[6]

Supplementary Table 1. Crystallographic data collection and refinement statistics (molecular replacement).

	8GCQ
Data collection	
Wavelength(Å)	1.24
Temperature (K)	293
beamline	SLAC LCLS MFX
Space group	P 2_1 2_1 2_1
Cell dimensions	
a, *b*, *c* (Å)	178.60, 189.50, 211.10
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	33.00-2.38(32.80-2.38) *
R_{sprit}	0.2296(0.6388)
I / σI	4.59(1.78)
Completeness (%)	100(100)
Redundancy	2742(2286)
Refinement	
Resolution (Å)	35.00-2.38
No. reflections	270,854
R_{work} / R_{free}	0.232 / 0.266
No. atoms	31,465
Protein	28,514
Ligand/ion	2,350
Water	601
B-factors	34.79
Protein	32.42
Ligand/ion	64.39
Water	31.74
R.m.s. deviations	
Bond lengths (Å)	0.0114
Bond angles (°)	1.742
Ramachandran statistics (%)	
Favored	89.24
Allowed	9.73
Outliers	1.03

*Values in parentheses are for highest-resolution shell.

Supplementary References

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