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Supplementary Materials for

Time-resolved serial crystallography to track the dynamics of carbon monoxide in the active site of cytochrome *c* oxidase

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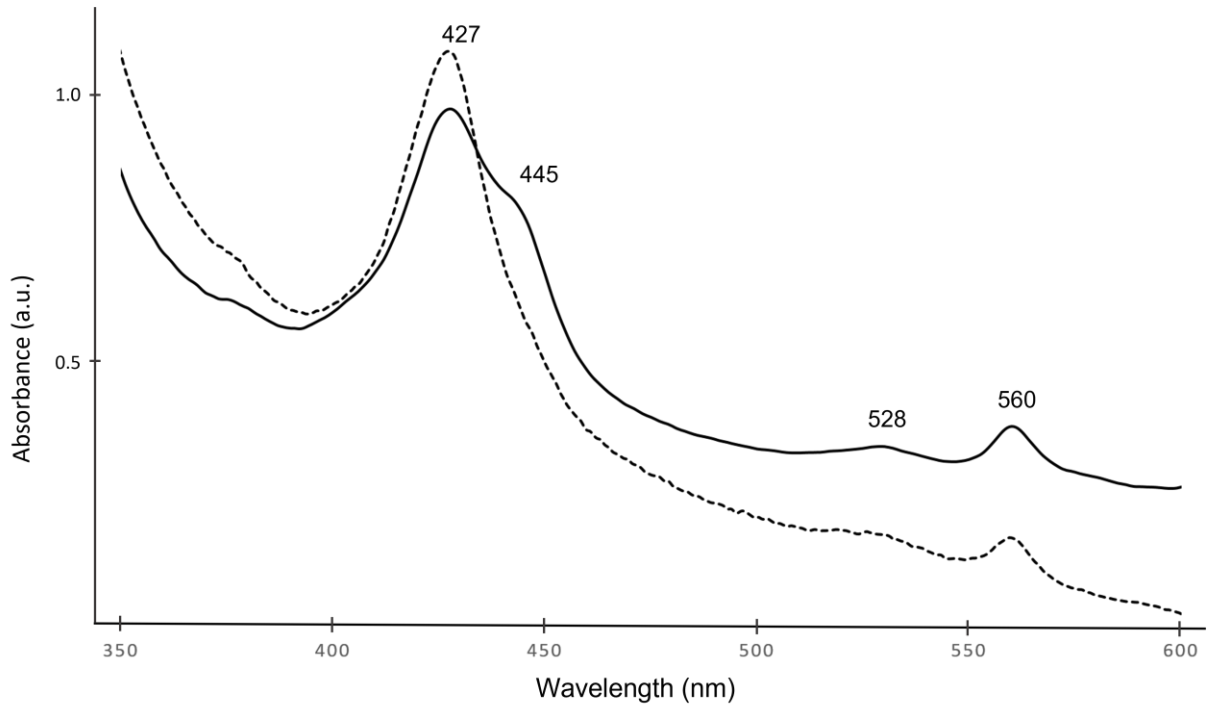

Fig. S1.

Optical absorbance spectra of *ba*$_3$-type C*c*O microcrystals in LCP. The optical absorbance spectra of dithionite-reduced microcrystals (solid line) and reduced CO-bound microcrystals (dashed line).

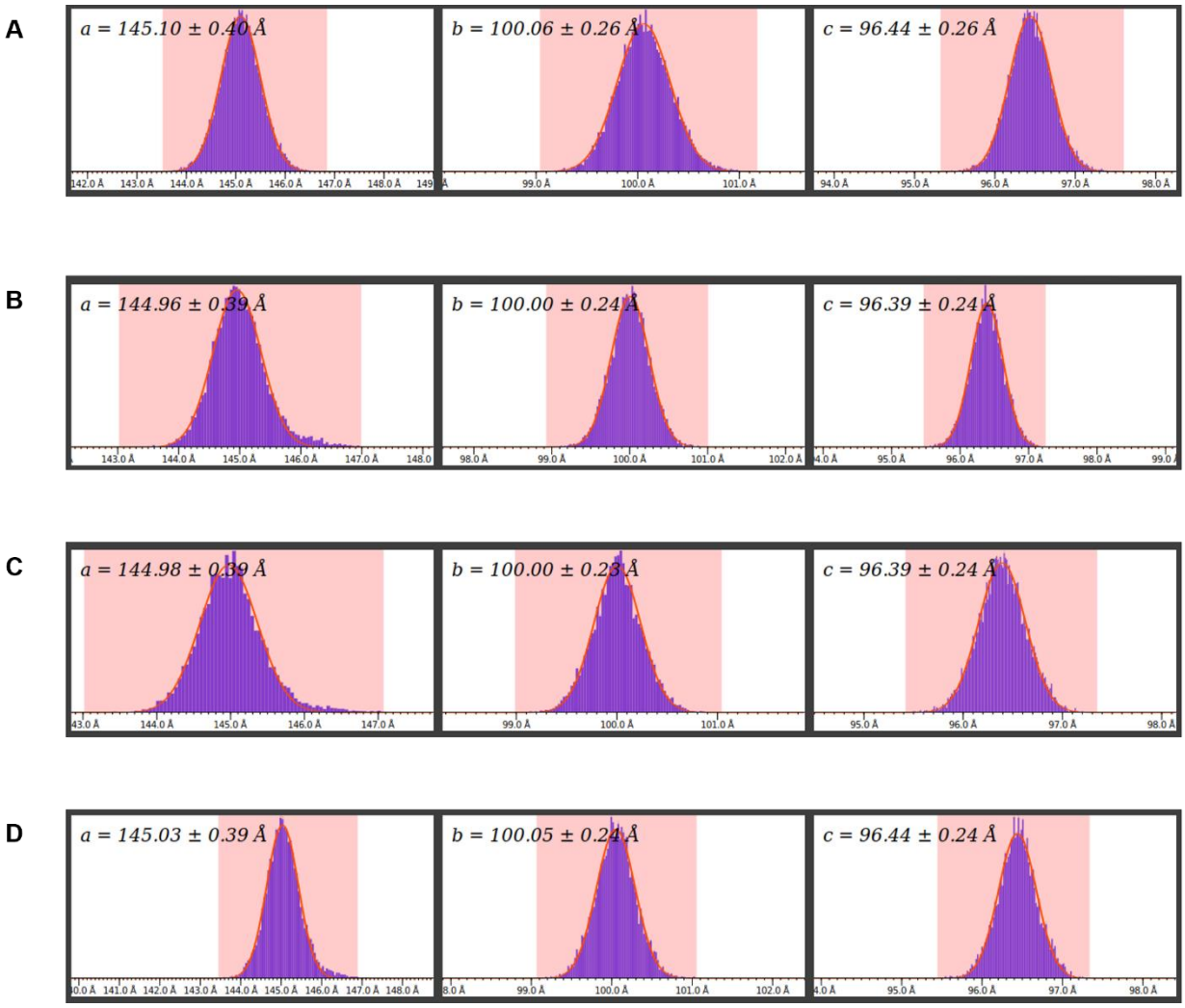

Fig. S2.

Unit cell parameters of the light and dark data sets. Unit cell parameters from indexing using CrystFEL are given for (**A**) the dark-only (collected without interleaved light images) data set, (**B**) the dark 1 (first exposure without laser illumination) data set, (**C**) the dark 2 (second exposure without laser illumination) data set, and (**D**) the light (laser illuminated) data set. The variation of the unit cell axis of the different data sets is much smaller than the estimated uncertainties.

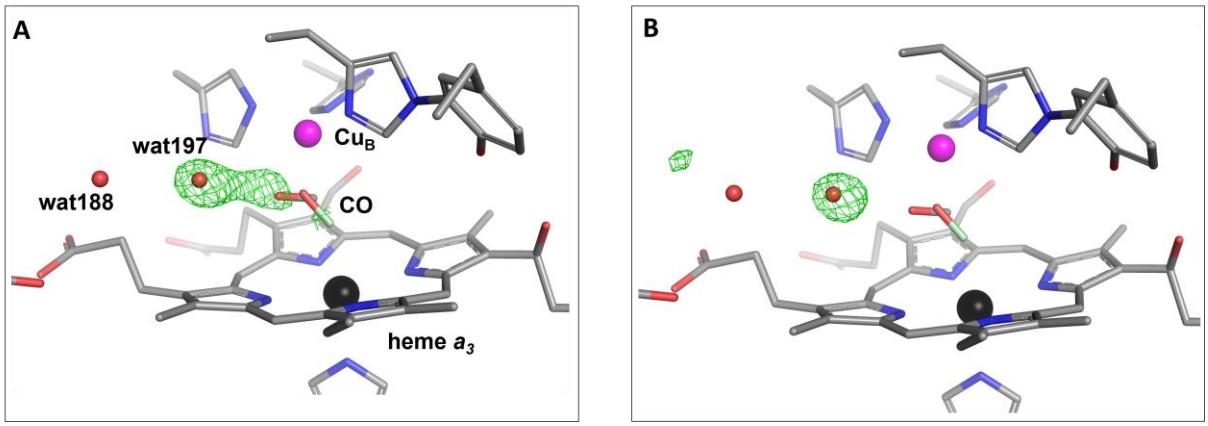

Fig. S3.

Activated-state structure of *ba*$_3$-type C*c*O from partial occupancy refinement. (**A**) The positive F_o-F_c omit map density (green), calculated with CO in the dark state position at an occupancy of 70 % but without the activated state CO molecule or wat197 present in the model, is displayed at 3.0 σ around the CO molecule. The CO molecule from the dark state structure (green) is included for comparison. (**B**) To highlight the position of the new water molecule, the positive F_o-F_c omit map density (green), calculated without wat197 in the model but with the CO molecule present in the two positions, is displayed at 3.5 σ. The CO molecule from the dark state structure (green) is included for comparison.

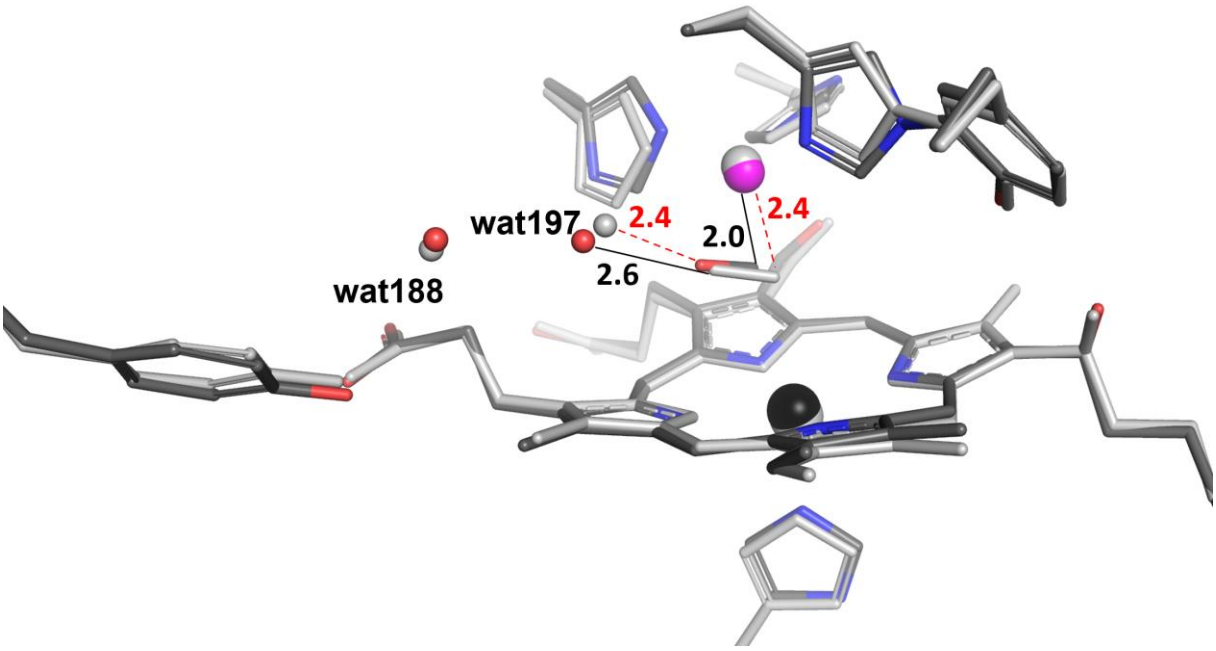

Fig. S4.

Activated-state structure of *ba*$_3$-type C*c*O. Comparison between activated-state structure from partial occupancy refinement (protein dark grey, heme a_3 iron black, Cu_B purple, water molecules red) with that based on extrapolated structure factors (light grey). Distances between the CO molecule and Cu_B as well as the CO molecule and wat197 are indicated in black (structure from partial occupancy refinement) and red (structure refined using extrapolated structure factors), respectively.

Table S1.

X-ray diffraction data collection and refinement statistics.

	Dark	Activated state partial occupancy refinement	Activated state Xtrapol8 refinement
PDB code	8K65	8AJZ	8K6Y
Data Collection			
Collection temperature (K)	293	293	293
Space Group	C121	C121	C121
Cell dimensions			
a, b, c (Å)	145.9, 100.3, 96.6	145.9, 100.3, 96.6	145.9 100.3, 96.6
α, β, γ (°)	90, 126.8, 90	90, 126.8, 90	90 126.8 90
Resolution (Å)‡	43.4 - 2.0	43.4 - 2.0	43.4 - 2.0
R_{split} (%)†‡	18.1 (79.0)	25.4 (73.1)	
I/σ(I)‡	5.4 (1.3)	4.0 (1.4)	3.7 (1.1)
CC($^1/_2$)‡	95.0 (46.6)	90.8 (55.0)	
Completeness (%)	100	100	100
Multiplicity‡	233.9 (81.0)	94.5 (65.7)	
Number of collected images	201274	~97500	~97500
Number of hits	41059	13096	13096
Number of indexed patterns	26999	10844	10844
Indexing rate (%)	13.4	~11.1	~11.1
Number of total reflections	17591236	7109569	7109569
Number of unique reflections	75223	75202	75202
Refinement			
Resolution	37.2 - 2.0	38.7 - 2.0	37.2 - 2.0
R_{work} / R_{free} (%)	17.1 / 19.6	18.7 / 21.3	39.6 / 43.5
Number of atoms	6493	6591	6474
Average B factor (Å^2)	37.8	43.7	37.0
R.m.s deviations			
Bond lengths (Å)	0.016	0.016	0.010
Bond angles (°)	1.82	2.07	1.84

†$R_{split} = 1/\sqrt{2\ \frac{\sum hkl\ |\boldsymbol{I}even-\boldsymbol{I}odd|}{1/2\sum hkl\ |\boldsymbol{I}even+\boldsymbol{I}odd|}}$

‡Values in parenthesis is those of the highest resolution shell