Acta Crystallographica Section D

Volume 70 (2014)

Supporting information for article:

Fingerprinting Redox and Ligand States in Heme Protein Crystal Structures using Resonance Raman Spectroscopy

Demet Kekilli, Florian S. N. Dworkowski, Guillaume Pompidor, Martin R. Fuchs, Colin R. Andrew, Svetlana Antonyuk, Richard W. Strange, Robert R. Eady, S. Samar Hasnain and Michael A. Hough

SUPPORTING INFORMATION

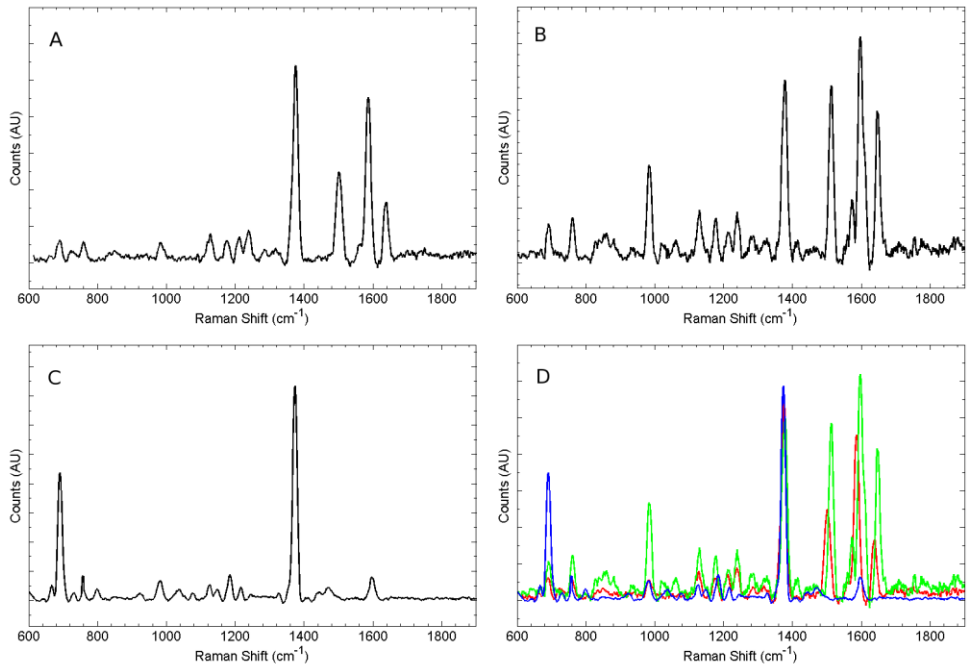

Figure S1 . Examples of the full spectral range measured in all experiments (600-2000 cm^{-1}) for (A) ferric, (B) ferrous-NO, (C) ferrous-CO states and (D) superposition of ferric (red), ferrous-NO (green) and ferrous-CO (blue) states.

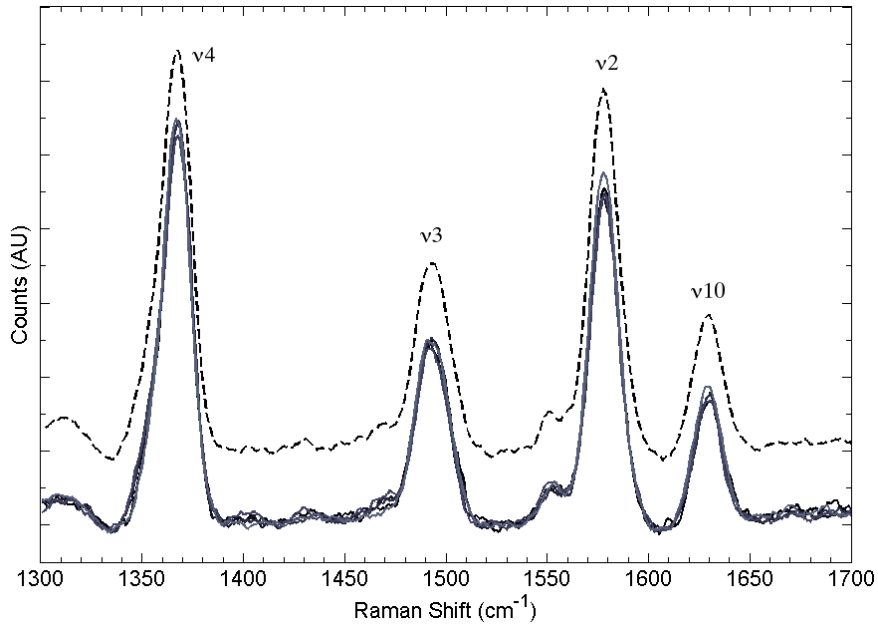

Figure S2 RR spectra from a multi-spot data collection strategy. Multiple positions separated by 150 μm were bookmarked along a ferric crystal. Spectra measured following X-ray data collection at all positions correspond to that of the intact ferric protein. Note that 5 overlaid spectra are shown, together with the spectrum of the crystal prior to any X-ray exposure (dashed line, offset on the y axis for clarity).

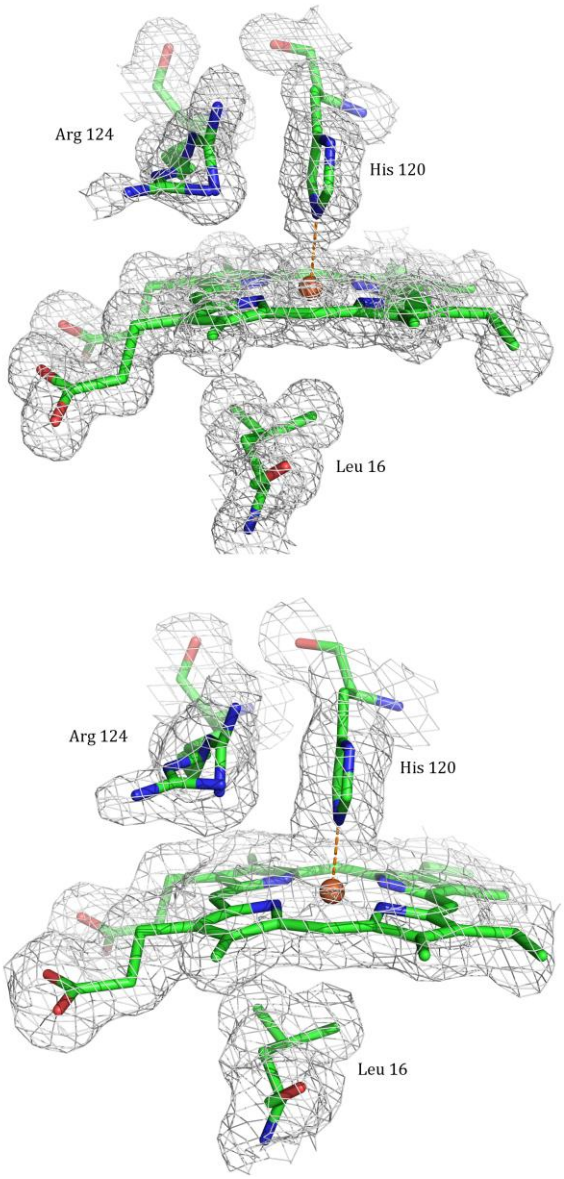

Figure S3 2Fo-Fc electron density maps contoured at 0.5 σ showing (top panel) the chemically-reduced structure and (lower panel), the X-ray reduced 160K structure, in each case showing two alternate conformations for residue Arg 124. The minor conformation in the 180K structure is similar to that of the 160K structure (data not shown).

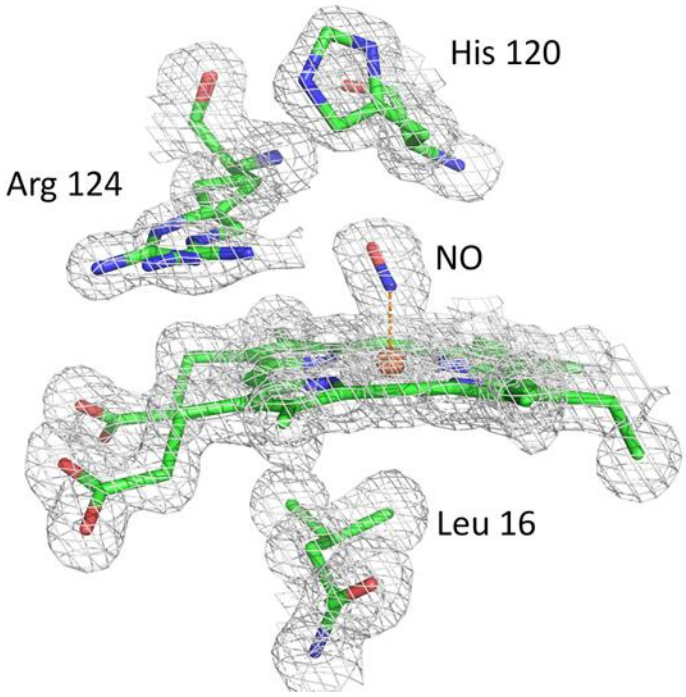

Figure S4 2Fo-Fc electron density map contoured at 1σ for the proximal 5cNO-bound structure. Fe is 5-coordinate with NO bound at the proximal face

Table S1 Data collection and processing statistics for *Ax*CYTcp crystal structures in space group $P6_522$.

For unit cell dimensions, a=b, $\alpha=\beta=90°$, $\gamma=120°$.

Structure	Ferric	Chemically Reduced	X-ray reduced	X-ray reduced	X-ray reduced	NO-complex
Temperature (K)	100	100	100	160	180	100
Wavelength (Å)	0.8	0.8	0.8	1.0	1.0	1.0
Resolution (Å)	46.21-1.30	45.09-1.22	46.10-1.17	45.00-1.77	46.50-1.55	45.00-1.26
Highest resolution shell (Å)	1.37-1.30	1.25-1.20	1.24-1.17	1.87-1.77	1.58-1.55	1.28-1.26
Unit cell dimensions a,c (Å)	53.35, 180.99	53.72, 180.62	53.2, 180.75	53.62, 181.72	53.67, 181.81	53.62, 180.26
Unique reflections	38076	47035	49558	15687	21722	40695
Completeness (%)	99.1 (99.7)	97.8 (88.4)	95.0 (69.7)	98.6 (79.4)	93.9 (96.0)	96.7 (96.7)
Redundancy	4.2 (4.2)	4.5 (3.3)	5.7 (2.6)	4.2 (4.4)	4.7 (4.5)	5.2 (4.8)
R_{merge}	0.110 (0.654)	0.034 (0.510)	0.049 (0.105)	0.053 (0.700)	0.060 (0.662)	0.051 (0.670)
Mn (I/sd)	7.0 (2.1)	20.0 (2.6)	17.1 (5.3)	11.7 (2.0)	12.5 (2.4)	13.2 (2.1)
Wilson B-factor ($Å^2$)	10.2	11.7	7.0	26.6	14.3	11.7
PDB accession code	4cda	4cip	4cdv	4cdy	4cjo	4cjg

Table S2 Refinement statistics for *Ax*CYTcp crystal structures

Structure	Ferric	Chemically	X-ray reduced	X-ray reduced	X-ray reduced	NO-complex
Temperature (K)	100	100	100	160	180	100
R_{cryst}	0.154	0.144	0.140	0.204	0.230	0.162
R_{free}	0.179	0.171	0.160	0.239	0.249	0.195
ESU based on ML (Å)	0.031	0.021	0.018	0.080	0.06	0.034
RMSD bond lengths (Å)	0.012	0.140	0.013	0.012	0.015	0.015
RMSD bond angles (º)	2.1	2.2	2.1	2.1	2.1	2.25
Ramachandran favoured (%)	98.3	98.9	99.0	99.1	98.3	97.8
Number of refined atoms	1308	1311	1328	1067	1073	1303
Average B-factor ($Å^2$)	11.2	14.9	9.9	34.7	19.0	15.9
PDB accession code	4cda	4cip	4cdv	4cdy	4cjo	4cjg