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Exploring the local solvation environments of a heme protein using the spectroscopic reporter 4-cyano-L-phenylalanine

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SUPPORTING INFORMATION

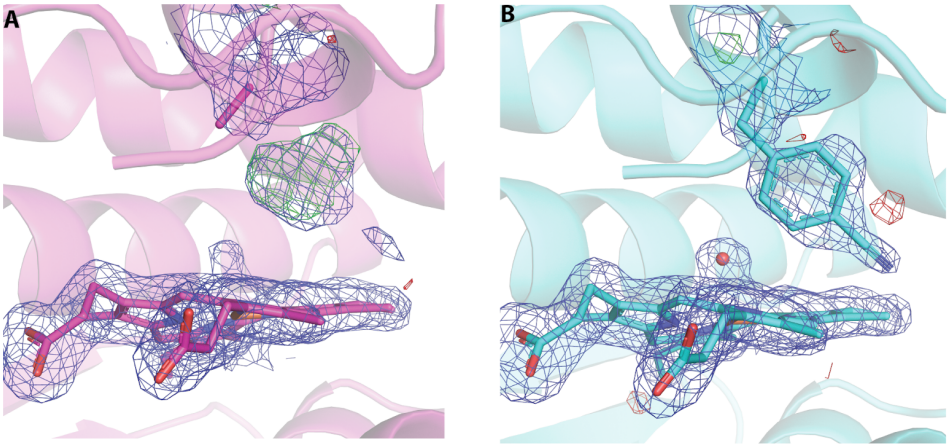

Figure S1. Difference density of pCNF at site 5 in *Cs* H-NOX. Blue mesh represents $2F_O$-F_C electron density map at 1.0σ, green mesh represents F_O-F_C electron density map at +3.0σ, and red mesh represents F_O-F_C electron density map at -3.0σ. (A) Density map after molecular replacement with the heme modeled and alanine modeled at site 5. (B) Density map after final refinement with the heme and water molecule modeled and pCNF modeled at site 5. PDB ID 6CWW.

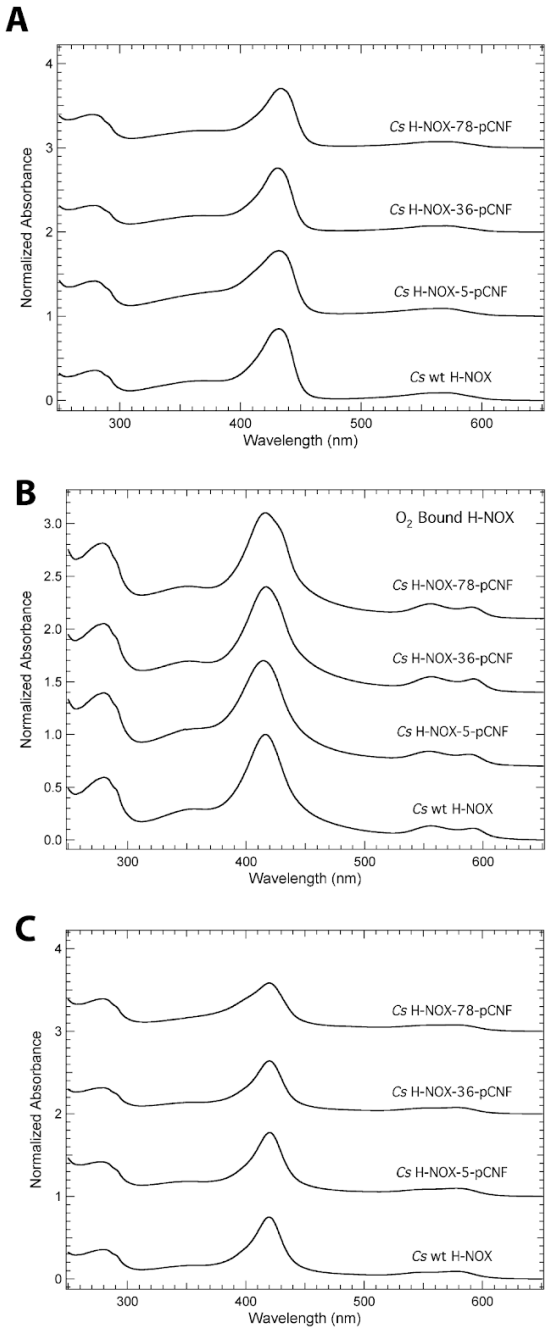

Figure S2. UV-Vis absorbance spectra of WT and pCNF-incorporated H-NOX constructs. UV-Vis spectra (250 – 650 nm) of WT, I5pCNF, V36pCNF, and F78pCNF H-NOX proteins were recorded at various ligation states, including (A) unligated, (B) O_2 bound, and (C) NO bound. All spectra were recorded in a pH 8.0 aqueous buffer solution containing 50 mM TEA and 150 mM NaCl on a Cary 100 UV-vis spectrophotometer. Spectra were intensity normalized to the Soret band.

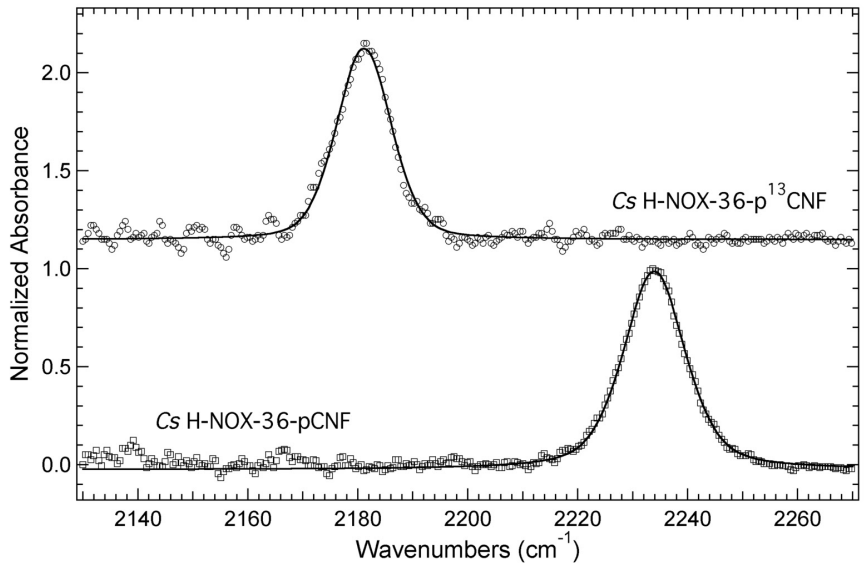

Figure S3. FTIR absorbance spectra of V36pCNF H-NOX containing p^{13}CNF or pCNF. ^{13}C-labeled (top, open circles) and unlabeled (bottom, open squares) FTIR spectra of as-purified H-NOX protein containing pCNF at site 36. The H-NOX proteins were dissolved in a pH 8.0 aqueous buffer solution of 50 mM TEA, 150 mM NaCl, and 5% glycerol. All spectra were recorded at 25 ºC on a Bruker Vertex 70 FTIR spectrometer, baseline corrected, intensity normalized, and fitted (solid lines) to a linear combination of Gaussian and Lorentzian functions.

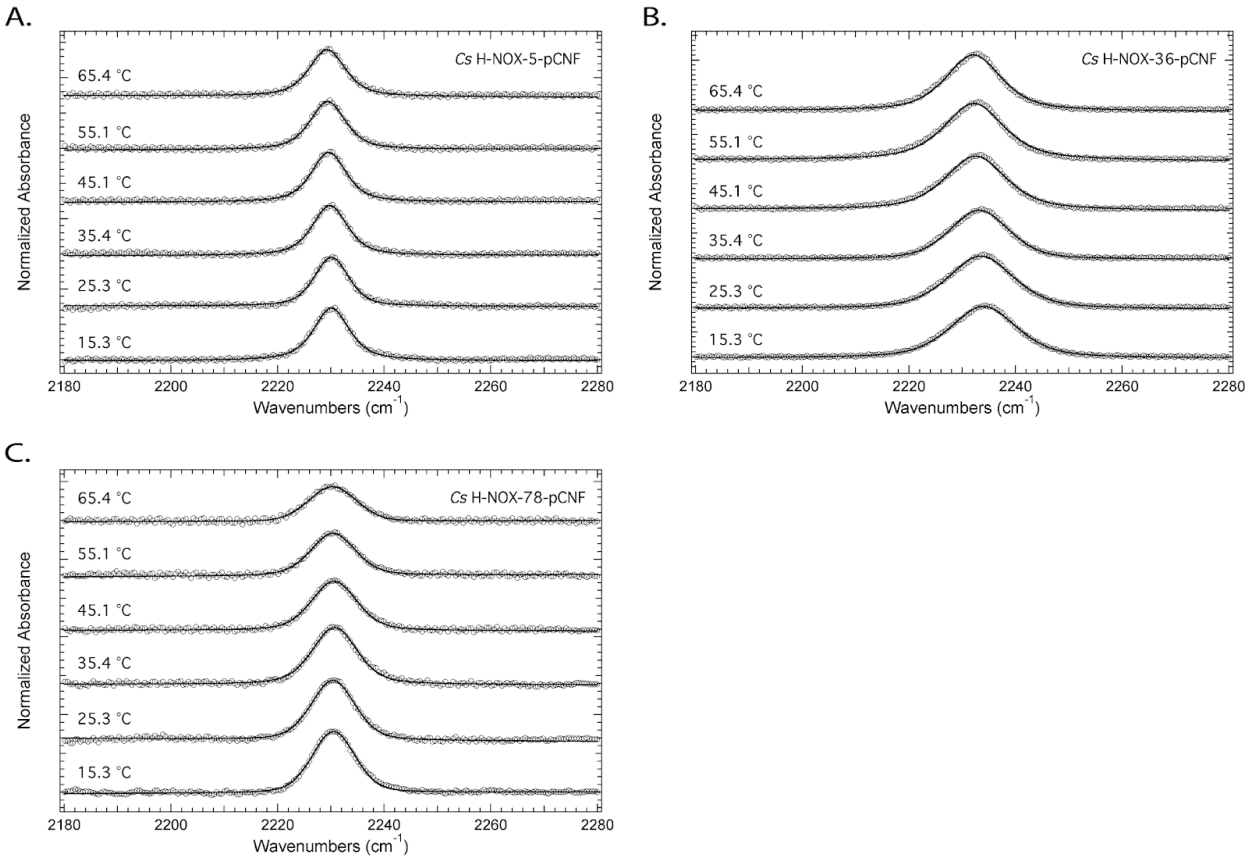

Figure S4. Temperature Dependent FTIR absorbance spectra of ferrous CO bound pCNF mutant H-NOX constructs. FTIR spectra of CO bound H-NOX protein containing pCNF at (A) site 5, (B) site 36, or (C) site 78 at various temperatures ranging from 15 ºC - 65 ºC in the 2180 - 2280 cm^{-1} region. The H-NOX proteins were dissolved in a pH 8.0 aqueous buffer solution containing 50 mM TEA and 150 mM NaCl. All spectra were recorded on a Bruker Vertex 70 FTIR spectrometer, baseline corrected, intensity normalized, and fitted (solid lines) to a linear combination of Gaussian and Lorentzian functions.

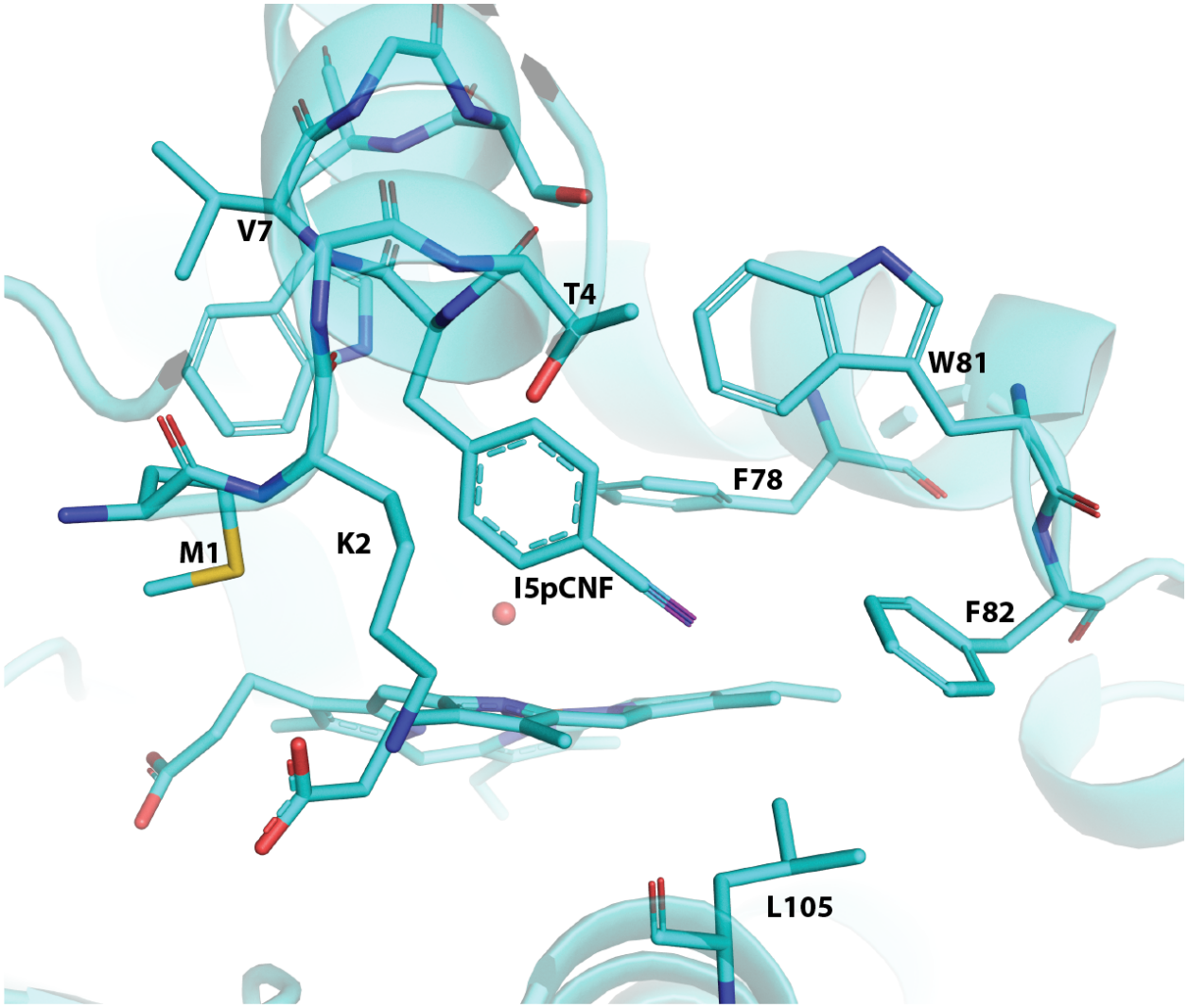

Figure S5. Local I5pCNF environment. All side chain and backbone atoms within 5 Å are represented in sticks. Note that the N in the CN of pCNF is 7.0 Å away from the water molecule above the heme and 6.8 Å away from the iron in the heme. PDB ID 6CWW.

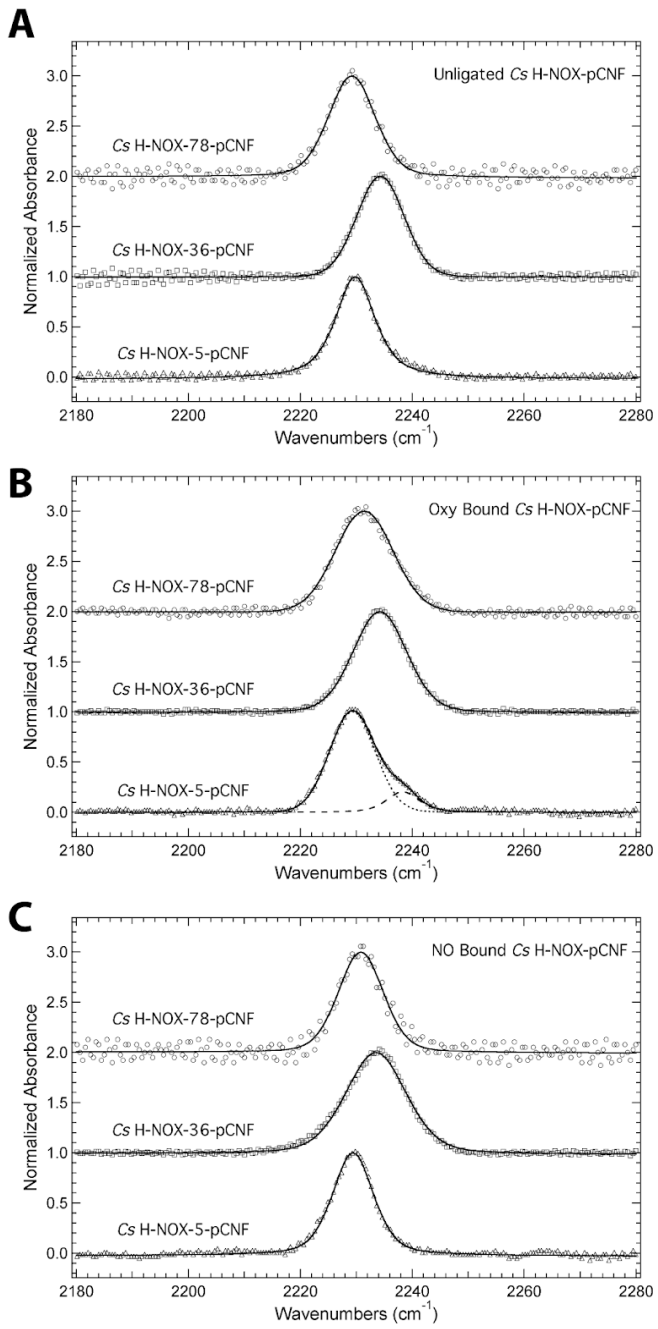

Figure S6. FTIR absorbance spectra of pCNF mutant H-NOX constructs at various ligation states. FTIR spectra of H-NOX protein containing pCNF at site 5 (open triangles), site 36 (open squares), or site 78 (open circles) at various ligation states, including (A) unligated, (B) O_2 bound, and (C) NO bound states. The H-NOX proteins were dissolved in a pH 8.0 aqueous buffer solution containing 50 mM TEA and 150 mM NaCl. All spectra were recorded at 25 ºC on a Bruker Vertex 70 FTIR spectrometer, baseline corrected, intensity normalized, and fitted (solid lines) to a linear combination of Gaussian and Lorentzian functions.

Table S1. X-ray data collection and refinement statistics. Data were processed in HKL2000 and the structure refined in Phenix with physical refinement in COOT.

Crystal	**I5pCNF *Cs* HNOX**
Space Group	$P2_1$
Cell Dimensions	
a (Å)	51.06
b (Å)	67.13
c (Å)	57.67
ɣ (º)	105.33
Wavelength (Å)	0.979180
Temperature (K)	100
Unique Reflections[1]	31078 (1527)
Resolution Range (Å)[1]	50 – 1.85 (1.88 – 1.85)
Average Redundancy[1]	3.2 (2.9)
Completeness (%)[1]	96.8 (95.7)
I / σ(I)[1]	25 (1.9)
# *Cs* HNOX molecules per asu	2
R_{crvst} (R_{free}) (%)[2]	19.1 (25.87)
rms deviations	
bond lengths (Å)	0.007
bond angles (°)	0.978
Ramachandran plot (%)[3]	
preferred	97.72 (343)
allowed	2.28 (8)
outliers	0.00 (0)

[1]The number in parentheses is for the highest resolution shell.

[2]$R_{\text{cryst}} = \Sigma_{hkl} \, ||F_o(hkl)| - |F_c(hkl)|| \, / \, \Sigma_{hkl} \, |F_o(hkl)|$. $R_{\text{free}} = R_{\text{cryst}}$ for a test set of reflections (5%) not included in refinement.

[3]Numbers in parentheses are the number of residues in each category.

Table S2. Fit parameters from the line shape analysis of the nitrile IR absorbance band of the pCNF incorporated H-NOX proteins. Nitrile stretching frequencies, full-width half-maximum (FWHM) and fraction Lorentzian of the Lorentzian/Gaussian line shape for the I5pCNF, V36pCNF, and F78pCNF H-NOX constructs at various ligation states, including CO bound, NO bound, O_2 bound, and unligated. Note: The nitrile IR absorbance band of O_2 bound I5pCNF contained two components. The relative percentage of each component is given in parentheses.

		I5pCNF			
Ligand	**CO**	**NO**	**O_2**		**Unligated**
Frequency (cm^{-1})	2230.0	2229.4	2229.3 (85%)	2238.3 (15%)	2229.8
FWHM	7.70	8.68	9.59	6.95	8.41
Fraction	0.52	0.53	0.07	0.53	0.76

		V36pCNF		
Ligand	**CO**	**NO**	**O_2**	**Unligated**
Frequency (cm^{-1})	2233.6	2233.5	2234.2	2234.3
FWHM	13.8	12.7	11.3	9.98
Fraction	0.41	0.38	0.21	0.08

		F78pCNF		
Ligand	**CO**	**NO**	**O_2**	**Unligated**
Frequency (cm^{-1})	2230.7	2230.8	2231.4	2229.2
FWHM	9.56	9.48	12.2	9.83
Fraction	0.024	0.32	0.048	0.34