Spectroscopic studies reveal that the heme regulatory motifs of heme oxygenase-2 are dynamically disordered and exhibit redox-dependent interaction with heme

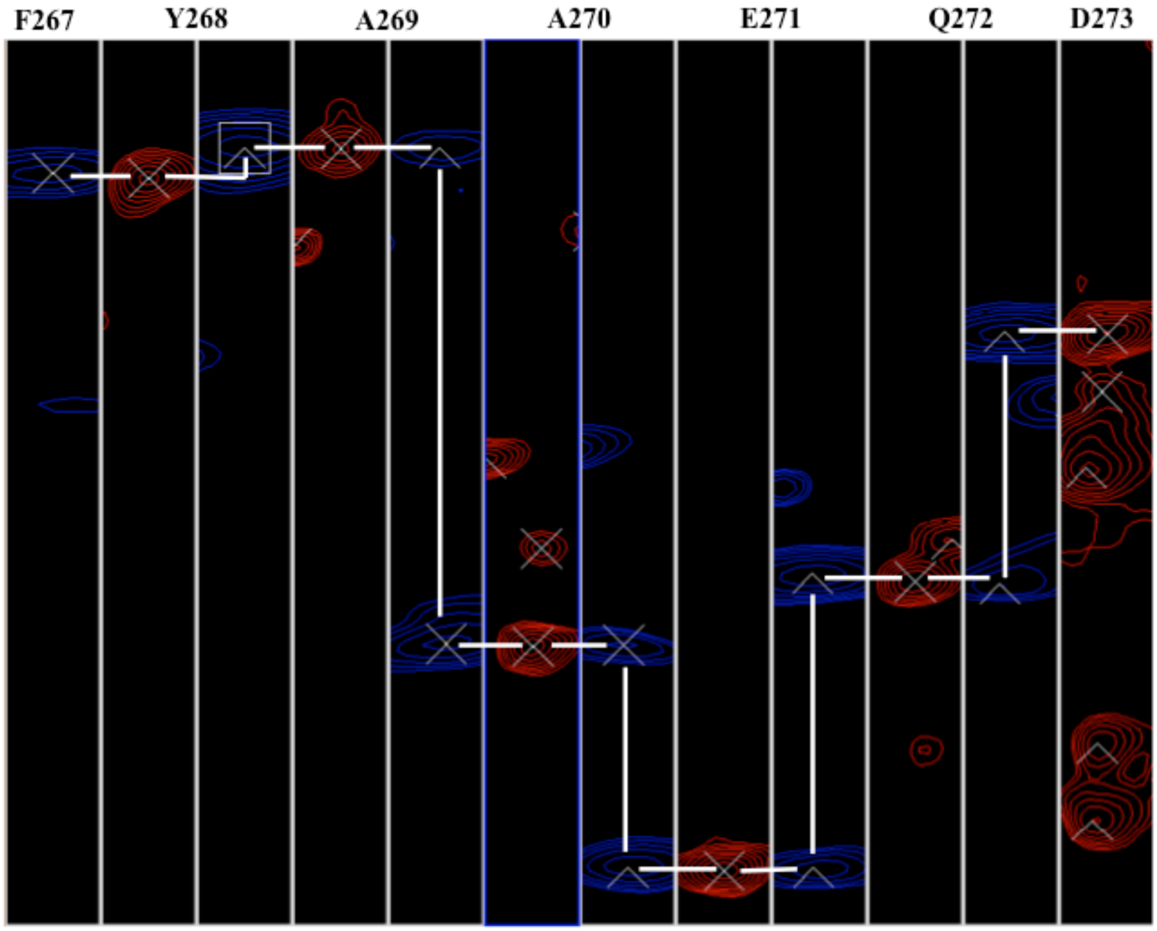
Ireena Bagai[1], Ritimukta Sarangi[2], Angela S. Fleischhacker[1], Ajay Sharma[3], Brian M. Hoffman[3], Erik Zuiderweg[1] and Stephen W. Ragsdale[1]

[1]Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48019

[2]Stanford Synchrotron Radiation Labs, Stanford University, Menlo Park, CA 94025

[3] Department of Chemistry, Northwestern University, Evanston, IL 60208

Supplementary Material



Supp figure 1. Portions of HNCO and HN(CA)CO spectra illustrating the sequential connectivities between F267 and D273

Residue	$^{1}H_N$	$^{15}N_H$	$^{13}C\alpha$	$^{13}C\beta$	^{13}CO
Met1					
Ser2					
Ala3	8.26	126.02	52.03	18.42	177.55
Glu4	8.23	120.18	56.09	29.29	176.43
Val5	7.96	120.73	61.69	32.01	176.04
Glu6	8.40	125.01	56.13	29.52	176.56
Thr7	8.14	115.97	61.16	69.31	174.46
Ser8	8.32	118.56	57.90	63.12	
Glu9	8.37	123.15	56.28	29.47	176.90
Gly10	8.34	110.40	44.89		174.05
Val11	7.86	119.55	61.70	31.96	
Asp12	8.38	124.20	53.94	40.69	176.63
Glu13	8.44	123.20	56.76	29.04	177.18
Ser14	8.31	116.64	59.39	62.88	175.15
Glu15	8.15	122.33	56.66	29.05	176.90
Lys16	7.99	121.84	56.27	31.61	176.98
Lys17					
Asn18	8.11	120.70	53.07		
Ser19	8.31	121.79	57.38		178.16
Gly20	8.23	108.45	45.13		
Ala21	7.96	123.89	52.27	18.26	178.08
Leu22	8.06	120.97	55.16	41.08	177.79
Glu23	8.18	121.48	56.71	29.10	177.01
Lys24	8.09	121.69	56.51	31.80	177.09
Glu25	8.27	121.17	56.81	29.10	176.81
Asn26	8.27	119.27	53.35	38.10	175.59
Gln27	8.17	120.54	56.64		176.36
Met28					
Arg29	8.09	122.26	55.93	29.84	176.18
Met30	8.17	121.46		32.20	175.37
Ala31	7.96	124.47	51.40	19.36	176.45
Asp32	8.29	120.64	53.90	40.33	177.26
Leu33	9.36	126.87	58.15	40.77	177.96
Ser34	10.23	113.65	62.55	60.76	176.74
Glu35	7.22	124.81	57.93	29.03	178.49
Leu36	8.35	120.91	57.21	41.00	181.93
Leu37	8.70	119.65	57.99	40.82	178.31
Lys38					
Glu39					

Gly40					
Thr41					
Lys42					
Glu43					
Ala44					
His45					
Asp46					
Arg47					
Ala48					
Glu49					
Asn50					
Thr51					
Gln52					
Phe53					
Val54					
Lys55					
Asp56					
Phe57					
Leu58	8.11	120.73	53.89	40.44	
Lys59	8.20	122.67	56.30	31.68	177.39
Gly60	8.34	109.51	44.96		174.07
Asn61	8.37	120.38	51.62	37.66	
Ile62	7.32	118.32	58.21	39.29	174.20
Lys63	8.37	126.00	54.73	32.36	
Lys64	9.55	116.68	59.34	28.10	
Glu65	7.78	117.67	56.89	27.87	
Leu66	7.61	118.99	52.37	39.63	175.00
Phe67	8.83	119.44	62.96	35.44	
Lys68					
Leu69	8.25	121.81	54.07	40.68	
Ala70	8.12	123.34	55.54		178.04
Thr71					
Thr72					
Ala73					
Leu74					
Tyr75					
Phe76					
Thr77					
Tyr78					
Ser79					
Ala80					
Leu81	8.69	124.24	55.09		
Glu82	8.09	114.18	59.21	31.78	178.52

Glu83	7.45	117.14	58.05	29.46	
Glu84					
Met85	8.71	121.95	59.93	31.52	176.70
Glu86	7.80	117.00	59.21	28.24	180.24
Arg87	7.83	119.80	58.82	29.43	177.78
Asn88	7.08	113.92	53.56	39.58	175.87
Lys89	6.96	120.16	59.38	29.36	174.40
Asp90	8.11	115.70	52.89	41.36	
His91	8.21	127.86	56.27	31.67	175.82
Pro92					
Ala93	7.02	122.46	54.53	18.42	178.18
Phe94	8.14	119.10	57.40	41.63	
Ala95					
Pro96					
Leu97	8.14	113.98	52.46	38.88	175.09
Tyr98	7.00	121.87	56.97	37.56	174.16
Phe99	8.76	129.50	55.66	39.80	171.51
Pro100					
Met101	7.68	116.77	57.26	30.59	178.25
Glu102	9.10	118.43	60.57	28.36	177.17
Leu103	7.47	110.14	54.44	42.24	
His104	6.86	116.26	58.03	27.35	177.78
Arg105					
Lys106					
Glu107	8.58	120.77	59.57	27.68	178.37
Ala108	7.87	125.18	54.57	16.86	179.33
Leu109	8.42	115.69	57.11	41.97	178.73
Thr110					
Lys111					
Asp112					
Met113					
Glu114					
Tyr115					
Phe116	7.98	123.67	55.23		177.33
Phe117	8.05	120.75	54.29	40.41	176.58
Gly118	8.16	109.18	44.81		172.70
Glu119					
Asn120	7.24	123.27	56.48	37.47	
Trp121	8.09	119.05	57.38	27.96	
Glu122	7.93	122.03	56.97		
Glu123	8.14	119.56	56.05		
Gln124	8.36	121.85	56.69		
Val125	8.04	121.71			177.45

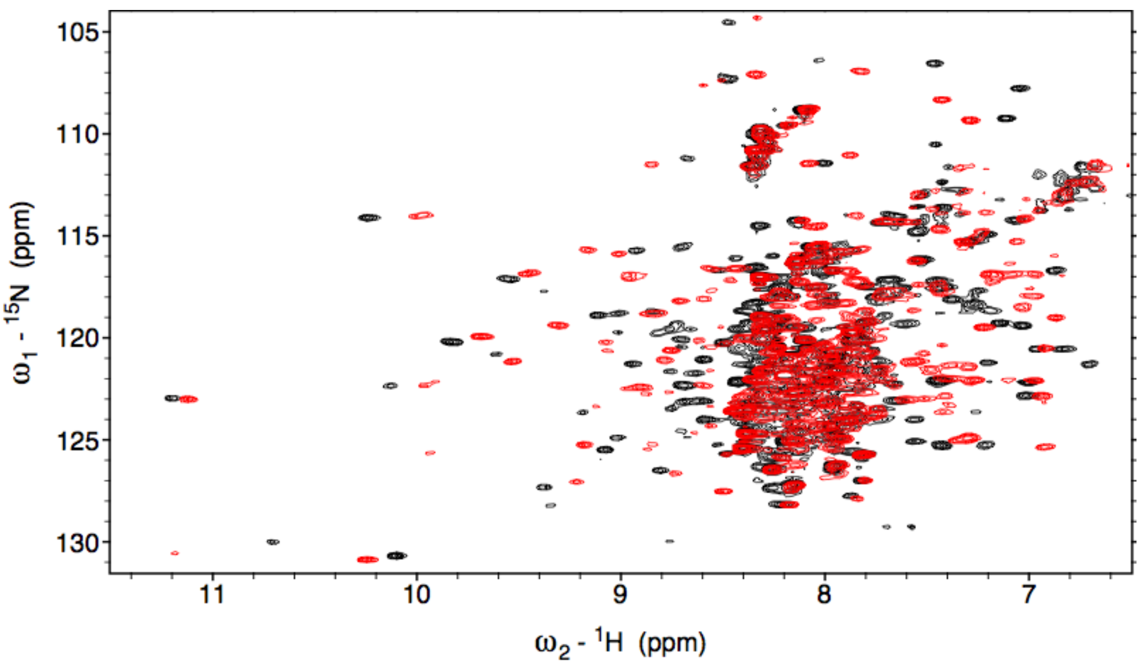
Gln126	8.18	120.88			
Cys127	8.09	120.49	53.80	41.39	172.86
Pro128					
Lys129					
Ala130					
Ala131	8.00	123.33	52.22	18.33	
Gln132	8.25	119.03	56.03	29.27	
Lys133	8.05	122.86	55.77	31.82	176.27
Tyr134	8.21	123.50	55.65	32.42	175.42
Val135					
Glu136	7.70	122.99	59.55	27.61	
Arg137	7.28	114.97	56.47	29.12	176.92
Ile138	8.19	124.55			178.51
His139	8.28	123.22	59.50	31.09	
Tyr140					
Ile141					
Gly142					
Gln143					
Asn144					
Glu145					
Pro146					
Glu147	9.80	119.71	58.62	27.13	175.19
Leu148	8.31	114.04	54.53	41.28	180.18
Leu149	8.26	125.44	58.17	40.74	179.79
Val150	8.98	118.33	64.91	30.33	175.72
Ala151	7.82	121.84	55.47	20.09	
His152	8.06	120.25	55.96	29.87	
Ala153	8.08	122.93	52.23	18.37	177.80
Tyr154					
Thr155					
Arg156					
Tyr157					
Met158					
Gly159					
Asp160					
Leu161					
Ser162					
Gly163					
Gly164					
Gln165					
Val166					
Leu167					
Lys168					

Lys169	8.06	124.46	62.29		176.24
Val170	8.30	124.64	56.53		176.86
Ala171	7.52	115.76	55.72	30.62	176.35
Gln172	7.21	114.86	61.16	32.65	174.69
Arg173	8.10	121.19	53.10	30.59	174.36
Ala174	8.51	124.86	56.86	26.38	
Leu175					
Lys176	7.64	121.69	57.01	28.38	
Leu177	6.71	121.01	52.78	42.31	174.28
Pro178					
Ser179					
Thr180	7.11	108.96	61.41	67.20	176.13
Gly181	7.06	107.37	45.31		174.21
Glu182	9.07	125.13	58.99	26.58	176.52
Gly183	8.67	110.85	45.52		173.12
Thr184	7.45	106.27	59.73	68.92	
Gln185	8.40	120.16	60.20	30.46	
Phe186	8.93	115.37	58.72	39.98	
Tyr187	7.70	123.48	57.98	38.92	
Leu188	7.74	120.83	60.91		177.53
Phe189					
Glu190	8.75	123.08	58.11	28.10	176.81
Asn191	8.69	115.15	52.05	38.19	
Val192	6.84	120.23	60.60	31.15	174.22
Asp193	8.25	126.96	55.75	40.17	176.40
Asn194	7.71	116.78	52.12	38.75	
Ala195	10.14	122.21	54.61	17.78	
Gln196	8.05	117.16	58.90	27.01	179.13
Gln197	7.87	120.00	57.96	27.63	179.03
Phe198	8.34	122.71	61.86	38.52	177.21
Lys199	8.40	118.30	60.33	31.42	178.60
Gln200	7.44	116.76	58.39	27.51	178.82
Leu201	7.56	124.67	57.57	40.10	177.74
Tyr202	8.60	122.65	62.57	38.31	178.86
Arg203	8.51	116.34	59.98	29.93	177.63
Ala204	7.92	120.95	54.84	16.81	181.89
Arg205	8.37	118.97	57.04	29.11	179.18
Met206	8.35	118.24	59.33	33.20	178.65
Asn207	8.37	118.12	53.75	36.87	175.58
Ala208	7.44	121.69	51.41	18.16	178.55
Leu209	7.02	119.06	55.38	40.74	177.75
Asp210	8.81	126.04	53.24	38.91	174.48
Leu211	7.43	124.87	52.15	46.02	176.51

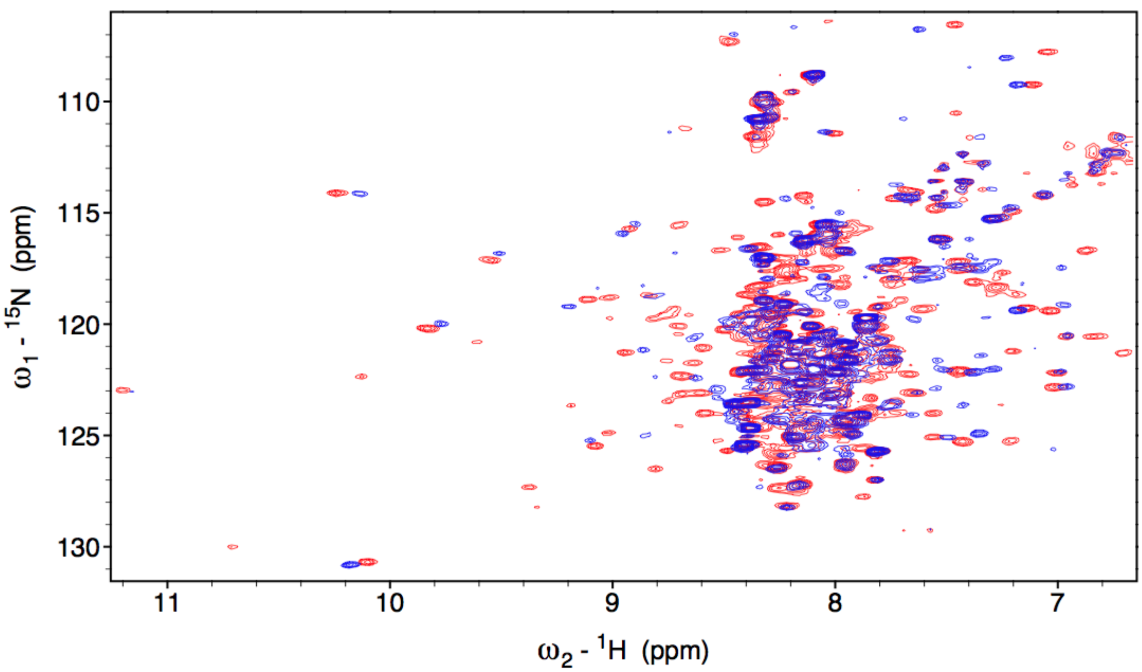
Asn212	8.49	119.80	51.12	37.99	175.66
Met213					
Lys214	8.33	119.13	58.21	30.44	179.61
Thr215	8.20	117.08	66.88		
Lys216	8.31	121.65	61.42	31.18	
Glu217	8.32	117.77	59.58	28.71	180.13
Arg218	7.99	121.36	59.28	29.70	179.80
Ile219	8.58	123.62	65.83	36.72	177.65
Val220	8.07	122.19	66.74		178.92
Glu221	8.10	121.48	59.34		179.79
Glu222	8.69	122.81	57.99	27.22	178.26
Ala223	9.00	124.41	54.80	17.93	180.04
Asn224	8.27	118.53	58.19	38.48	178.63
Lys225	8.28	123.22	59.50	31.09	
Ala226					
Phe227	8.69	115.15	52.05	38.19	
Glu228	8.25	115.73	59.98		
Tyr229	7.55	123.53	58.04	41.36	
Asn230	7.25	123.04	56.45	37.42	
Met231	8.01	123.99			177.60
Gln232	8.04	120.56	59.16	27.10	
Ile233	6.47	112.77	57.09	37.31	
Phe234	7.98	122.73	55.40	30.32	
Asn235	7.99	125.72	54.44	40.38	179.28
Glu236					
Leu237	7.82	121.51	55.40	41.06	177.93
Asp238	7.87	121.17	54.95		177.57
Gln239	8.21	121.67	56.40	29.47	177.03
Ala240	8.33	122.03			176.68
Gly241	8.19	109.05	44.84		172.73
Ser242					
Thr243	8.28	121.80			175.21
Leu244	7.83	123.52	55.33	41.28	177.43
Ala245	8.12	124.19	52.28	18.17	177.82
Arg246					
Glu247	8.42	121.75	56.50	29.47	
Thr248	8.06	115.19	61.53	69.34	174.36
Leu249	8.19	124.65	54.78	41.34	177.45
Glu250	8.31	121.71	56.18	29.38	176.20
Asp251	8.21	121.33	54.24	40.60	176.51
Gly252	8.10	108.38	44.70		173.53
Phe253	7.96	121.32	55.44	38.14	173.84
Pro254					

Val255	8.08	120.47	61.77	31.90	176.06
His256	8.12	121.38	53.15	30.58	
Asp257	8.26	121.84	54.07	40.59	
Gly258	8.30	109.78	45.23		174.52
Lys259	8.11	120.75	56.04	31.75	177.20
Gly260	8.35	109.67	44.96		174.07
Asp261	8.37	120.40	51.62	37.66	
Met262	7.31	118.29	58.21	39.29	174.20
Arg263	8.37	125.95	54.73	32.36	
Lys264	7.80	130.88			182.30
Cys265	8.30	120.79	53.61	40.73	172.84
Pro266					
Phe267	7.83	119.01	57.49	38.65	175.14
Tyr268	7.76	121.28	57.08	38.27	175.04
Ala269	7.95	125.88	51.93	18.53	177.07
Ala270	8.02	122.85	52.30	18.36	177.98
Glu271	8.22	118.77	56.62	28.97	176.78
Gln272	8.09	119.76	55.79	28.50	175.79
Asp273	8.17	120.88		40.54	176.19
Lys274	8.08	121.75	56.34	31.57	177.35
Gly275	8.30	109.42	45.04		174.19
Ala276	7.89	123.73	52.15	18.29	177.91
Leu277	8.03	120.76		41.02	177.70
Glu278	8.21	121.67	56.40	29.47	177.03
Gly279	8.29	110.27	45.08		
Ser280	8.03	115.57	57.59		
Ser281	8.17	117.60	58.38	63.17	
Cys282	8.33	121.56	53.67	40.90	172.73
Pro283					
Phe284	7.86	119.58	57.03	38.66	175.34
Arg285	7.98	122.73	55.40	30.32	
Thr286	8.03	115.78	61.40	69.27	173.82
Ala287	8.18	127.07	51.96	18.42	176.45
Met288	7.82	125.37	56.69	32.97	180.78

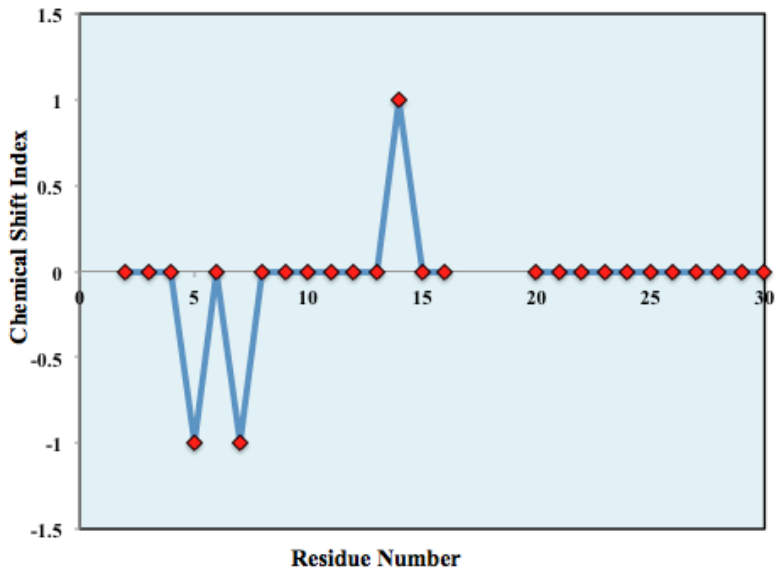
Supp figure 2. Backbone assignments for ^{15}N, ^{13}C, ^{2}H Fe^{3+}-HO2^{O}.



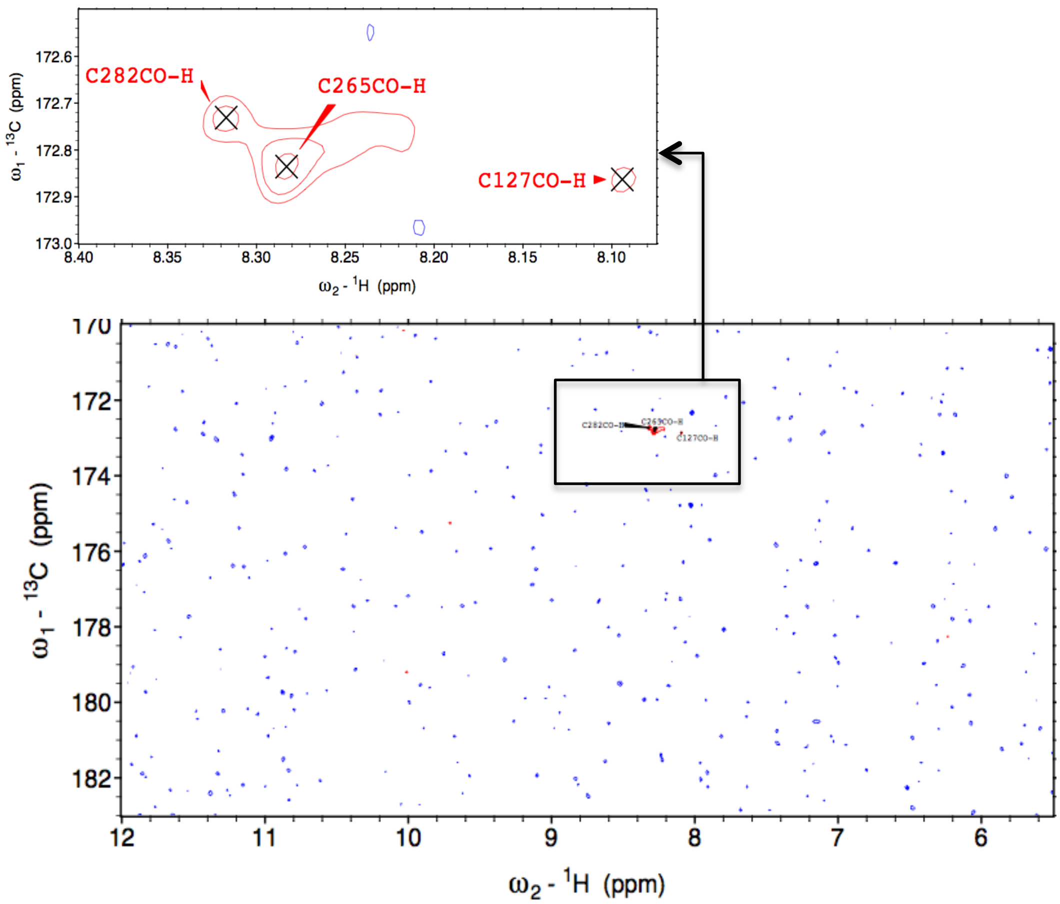
Supp figure 3. Overlay of the ^{1}H- ^{15}N TROSY of aquo-HO2^{O} (black) with N_3-heme-HO2^{O} (red). HO2 was present at a concentration of 250 μM in 50 mM Tris, 50 mM KCl pH 7.0 buffer and NaN_3 was added to a concentration of 17.5 mM (500 times that of HO2).



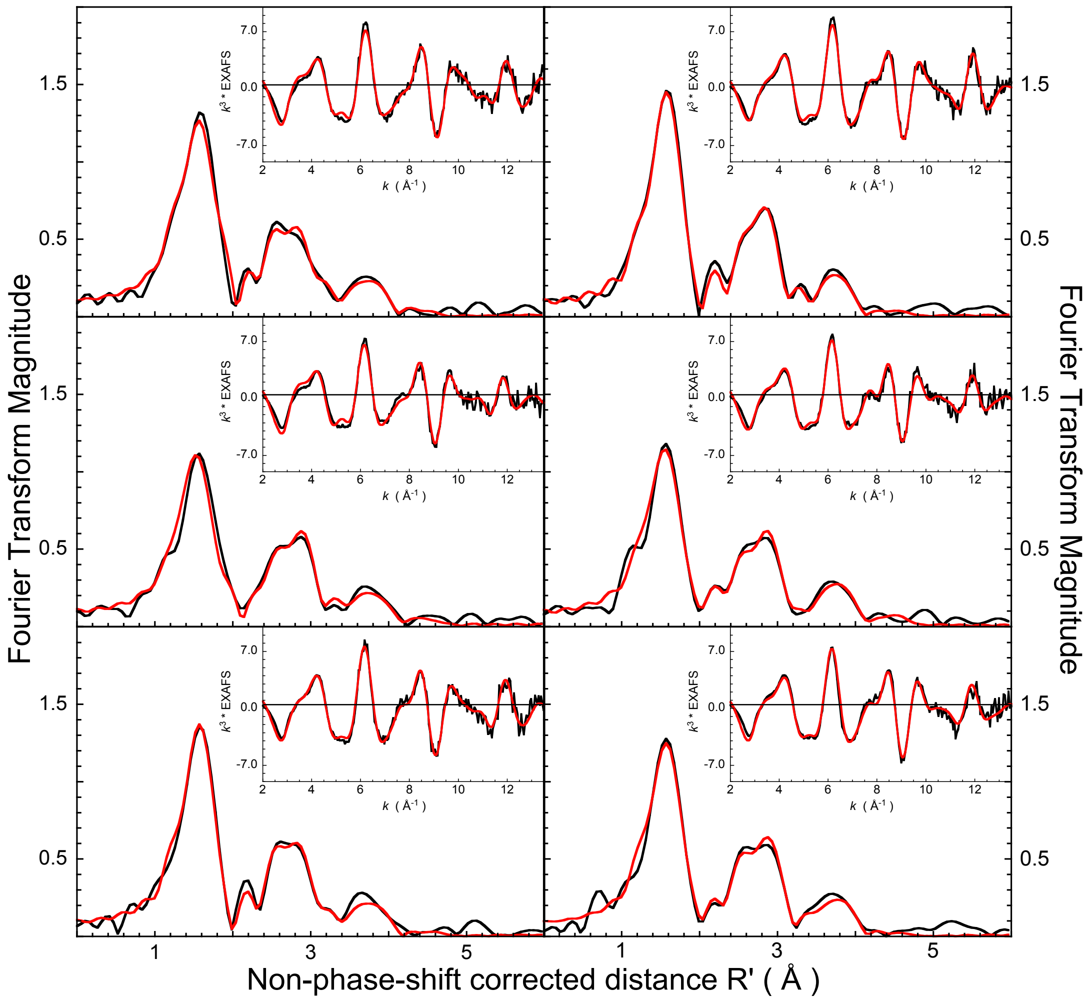
Supp figure 4. Overlay of the ^{1}H- ^{15}N TROSY of Fe^{3+}-HO2^{0} (red) with ZnPP-bound HO2^{0} (blue). Spectrum of ZnPP-bound HO2 ^{0} is not identical with Fe^{3+}-HO2^{0} spectrum. Most peaks in the structured core region revealed chemical shift changes. HO2^{0} was present at a concentration of approximately 250 μM each in 50 mM Tris, 50 mM KCl pH 7.0 buffer.



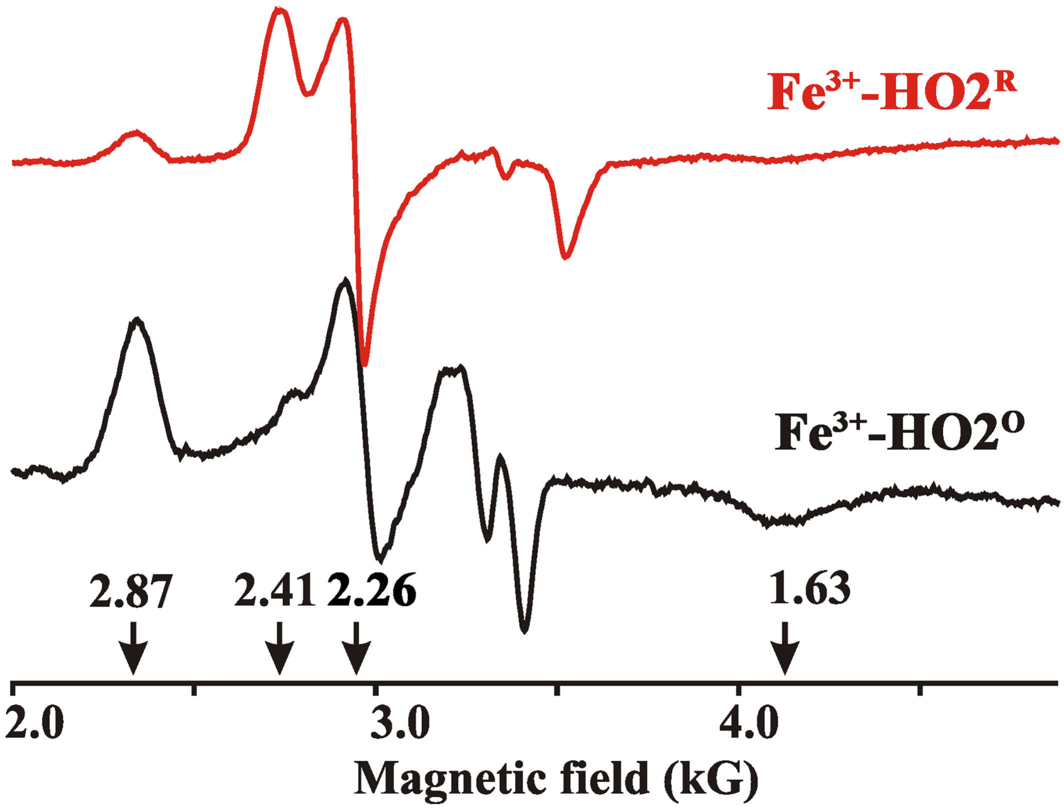
Supp figure 5. Chemical Shift Index (CSI) plot of α-carbons of residues at the N-terminal end of HO2. The values of CSI for β-strand, α-helix and random coil are +1, −1 and 0, respectively, as defined by the program CSI. Missing indices correspond to K17, N18 and S19, which could not be assigned. The assigned secondary structure illustrates N-terminal region to be a random coil in the Fe^{3+}-$HO2^{O}$.



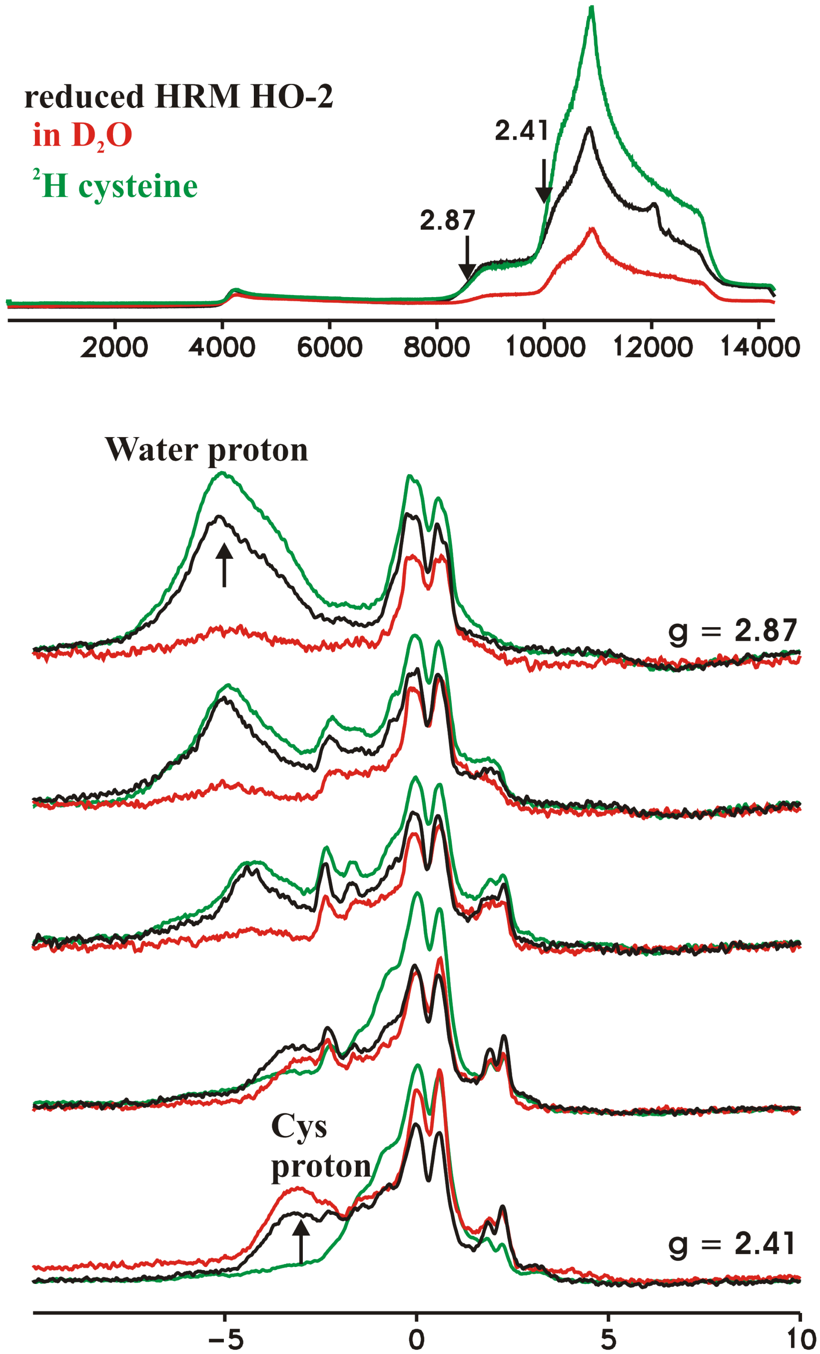
Supp figure 6. 2D HNCACO overlay of Fe^{3+}-HO2^{O} (red) and Fe^{3+}-HO2^{R} (blue) with selective cysteine labeling. The experiments were performed in 50mM Tris, 50mM KCl pH 7.0 buffer at 30 °C. No cysteines were observed in the Fe^{3+}-HO2^{R} spectrum.



Supp figure 7: Comparison of the non phase-shift corrected Fourier transforms and the corresponding k^3-weighted EXAFS data (inset) for (top panel; left) WT Fe^{3+}-HO2^{O}, (top panel; right) WT Fe^{3+}-HO2^{R}, (middle panel; left) C127A/C265A Fe^{3+}-HO2^{O}, (middle panel; right) C127A/C265A Fe^{3+}-HO2^{R}, (bottom panel; left) C127A/C282A Fe^{3+}-HO2^{O} and (bottom panel; right) C127A/C282A Fe^{3+}-HO2^{R}. Data (—), FEFF best-fit (—).



Supp Figure 8. X-band CW EPR spectra of Fe^{3+}-HO2. Fe^{3+}-HO2^{O} (black), Fe^{3+}-HO2^{R} (red). The g-values are shown above the y-axis. Experimental conditions: temperature, 20K; microwave frequency, 9.374 GHz; microwave power, 2 mW; modulation amplitude, 10 G; scan time, 120 s.



Supp Figure 9. ^{1}H 35 GHz CW ENDOR spectra of Fe3+-HO2^{R} recorded at several fields between g values 2.87-2.41. Spectra were recorded in H_2O (black), D_2O (red), or in H_2O with ^{2}H-Cys labeled HO2 (green). The braces show the range of the proton coupling. Experimental conditions: Temperature, 2K; Microwave frequency, 34.9 GHz; modulation amplitude, 2 G; RF sweep rate,1 MHz/s; rf excitation was broadened to 100 KHz.

Supp table 1. Number of generic spin systems (GS) identified for each of the backbone atom types for a previous (i-1)th and current ith residues.

Atom Type	Redox State	Number of GS	GS with 'x' number of rungs					
			6	5	4	3	2	1
COi-1	S-S	279	106	72	54	29	14	4
	SH	279	62	57	68	51	20	21
COi	S-S	187	106	62	10	5	4	0
	SH	129	62	40	17	6	4	0
CAi-1	S-S	250	106	72	53	16	3	0
	SH	189	62	56	55	11	4	1
CAi	S-S	256	106	68	51	24	7	0
	SH	242	62	53	66	49	12	0
CBi-1	S-S	148	106	30	11	1	0	0
	SH	105	62	28	14	1	0	0
CBi	S-S	210	106	54	37	13	0	0
	SH	201	62	50	52	35	2	0

'S-S' and 'SH' denote oxidized and reduced HO-2 respectively. Last 6 columns specify the number of peaks or GS with 6, 5, 4, 3, 2 and 1 rungs. Total number of peaks for S-S and SH forms are same for COi-1 atom and close for CAi and CBi atoms. However, most spin systems in SH HO-2 have fewer matching rungs compared to the S-S form.

Supp Table 2. EXAFS Least Squares Fitting Results for the Oxidized Proteins

Complex	Coordination/Path	R(Å)[a]	σ^2(Å^2)[b]	E_0 (eV)	F[c]
WT	4 Fe-N	1.98	407	-4.22	0.32
	2 Fe-N	2.05	343		
	2 Fe-C	2.99	421		
	8 Fe-C	3.04	/421		
	16 Fe-C-N	/3.27	/421		
	4 Fe-C	3.74	850		
	8 Fe-C	4.28	426		
	16 Fe-C-N	/4.33	/426		
	16 Fe-C-N	4.83	794		
C127A/C265A	4 Fe-N	1.99	180	-2.90	0.32
	2 Fe-N	2.08	346		
	2 Fe-C	3.00	470		
	8 Fe-C	3.07	/470		
	16 Fe-C-N	/3.29	/470		
	4 Fe-C	3.77	1190		
	8 Fe-C	4.30	325		
	16 Fe-C-N	/4.37	/325		
	16 Fe-C-N	4.88	770		
C127A/C282A	5 Fe-N/O	2.00	429	-4.39	0.33
	1 Fe-S	2.27	854		
	2 Fe-C	2.98	340		
	8 Fe-C	3.06	/340		
	16 Fe-C-N	/3.28	/340		
	4 Fe-C	3.74	984		
	8 Fe-C	4.29	413		
	16 Fe-C-N	/4.35	/413		
	16 Fe-C-N	4.84	869		

[a]The estimated standard deviations for the distances are in the order of ± 0.02 Å.

[b]The σ^2 values are multiplied by 10^5.

[c]Error is given by $\Sigma[(\chi_{obsd} - \chi_{calcd})^2 k^6]/\Sigma[(\chi_{obsd})^2 k^6]$.

/ indicates the σ^2 value for the path is linked to preceding path. The S_0^2 factor was set at 1.0.

Supp Table 3**.** EXAFS Least Squares Fitting Results for the Reduced Proteins

Complex	Coordination/Path	R(Å)[a]	σ^2(Å^2)[b]	E_0 (eV)	*F*[c]
WT	5 Fe-N/O	2.01	375	-4.50	0.31
	1 Fe-S	2.26	807		
	2 Fe-C	2.93	191		
	8 Fe-C	3.06	/191		
	16 Fe-C-N	/3.26	/191		
	4 Fe-C	3.73	906		
	8 Fe-C	4.29	441		
	16 Fe-C-N	/4.34	/441		
	16 Fe-C-N	4.82	695		
C127A/C265A	5 Fe-N/O	2.01	569	-4.83	0.33
	1 Fe-S	2.27	883		
	2 Fe-C	2.95	275		
	8 Fe-C	3.07	/275		
	16 Fe-C-N	/3.28	/275		
	4 Fe-C	3.71	724		
	8 Fe-C	4.28	320		
	16 Fe-C-N	/4.34	/320		
	16 Fe-C-N	4.81	404		
C127A/C282A	5 Fe-N/O	2.01	512	-4.78	0.33
	1 Fe-S	2.26	820		
	2 Fe-C	3.02	451		
	8 Fe-C	3.06	/451		
	16 Fe-C-N	/3.29	/451		
	4 Fe-C	3.70	906		
	8 Fe-C	4.28	397		
	16 Fe-C-N	/4.34	/397		
	16 Fe-C-N	4.84	927		

[a]The estimated standard deviations for the distances are in the order of ± 0.02 Å.

[b]The σ^2 values are multiplied by 10^5.

[c]Error is given by $\Sigma[(\chi_{obsd} - \chi_{calcd})^2 k^6]/\Sigma[(\chi_{obsd})^2 k^6]$.

/ indicates the σ^2 value for the path is linked to the preceding path. The S_0^2 factor was set at 1.0.