

Supporting Information for

The transcription regulator RcoM-2 from *Burkholderia xenovorans* is a cysteine-ligated hemoprotein that undergoes a redox-mediated ligand switch

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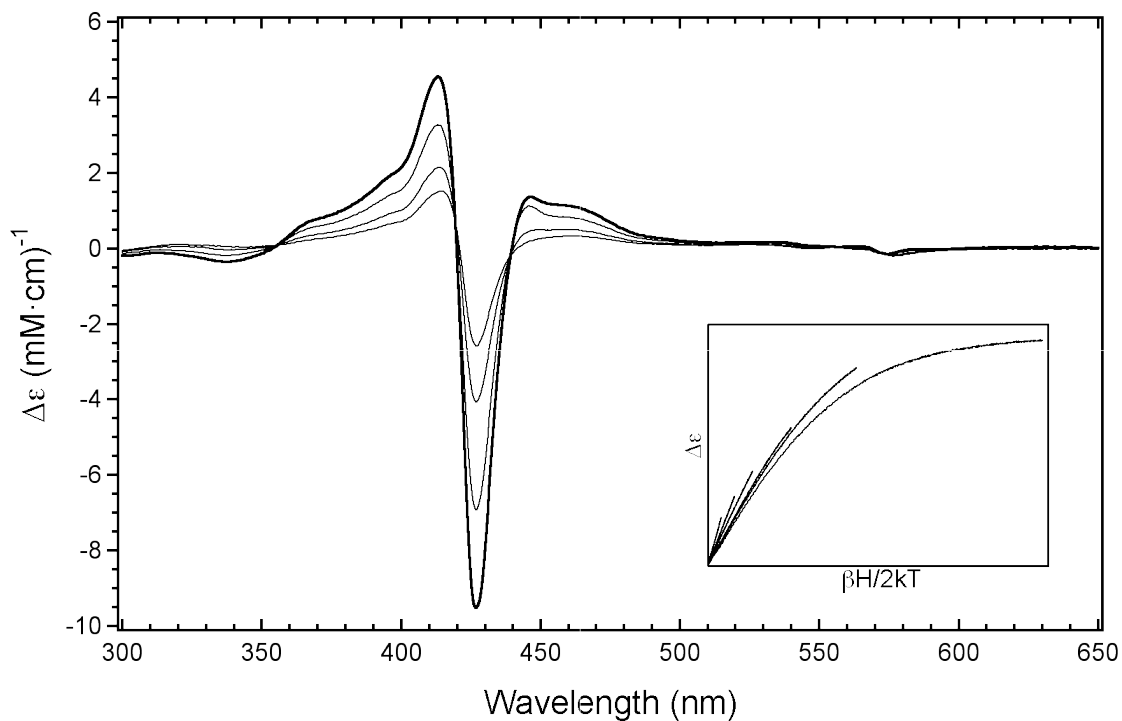
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ABBREVIATIONS

AxPDEA1, heme-containing phosphodiesterase A1 from *Acetobacter xylinum*; hCBS, human cystathionine β -synthase; CCP, cytochrome *c* peroxidase; *ChCooA*, a CooA homologue from the thermophilic bacterium *Carboxydotherrmus hydrogenoformans*; *RrCooA*, a CO-sensing transcription factor in *Rhodospirillum rubrum*; CPO, chloroperoxidase; *EcDos*, a direct oxygen sensor in *Escherichia coli*; EPPS, 3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid; EPR, electron paramagnetic resonance; HRP, horse radish peroxidase; M80C cyt *c*, cytochrome *c* variant in which methionine is replaced by cysteine(thiolate) as an axial ligand to the Fe(III) heme; MCD, magnetic circular dichroism; P450_{CAM}, cytochrome P450 from camphor-hydroxylating *Pseudomonas putida*; P450_{CAM}+ImH, cytochrome P450 from camphor-hydroxylating *Pseudomonas putida* with imidazole as an axial ligand to the Fe(III) heme; RCCP, *Rhodobacter capsulatus* cytochrome *c'*; sGC, soluble guanylyl cyclase, an NO-sensing heme protein that catalyzes the conversion of GTP to cGMP

Supporting Information Figure S1. MCD spectra of Fe(III)*BxRcoM*-2. The sample was ~30 μ M heme in 25 mM EPPS buffer with 250 mM KCl, pH 8.0, ~55% glycerol. The averages of 3 scans collected at 4.5, 8, 15, and 25 K under constant magnetic field (7 tesla) are shown. **Inset:** Magnetization saturation plot of data acquired at 2, 4.5, 8, 15, 25 and 50 K at the peak maximum, 413 nm.



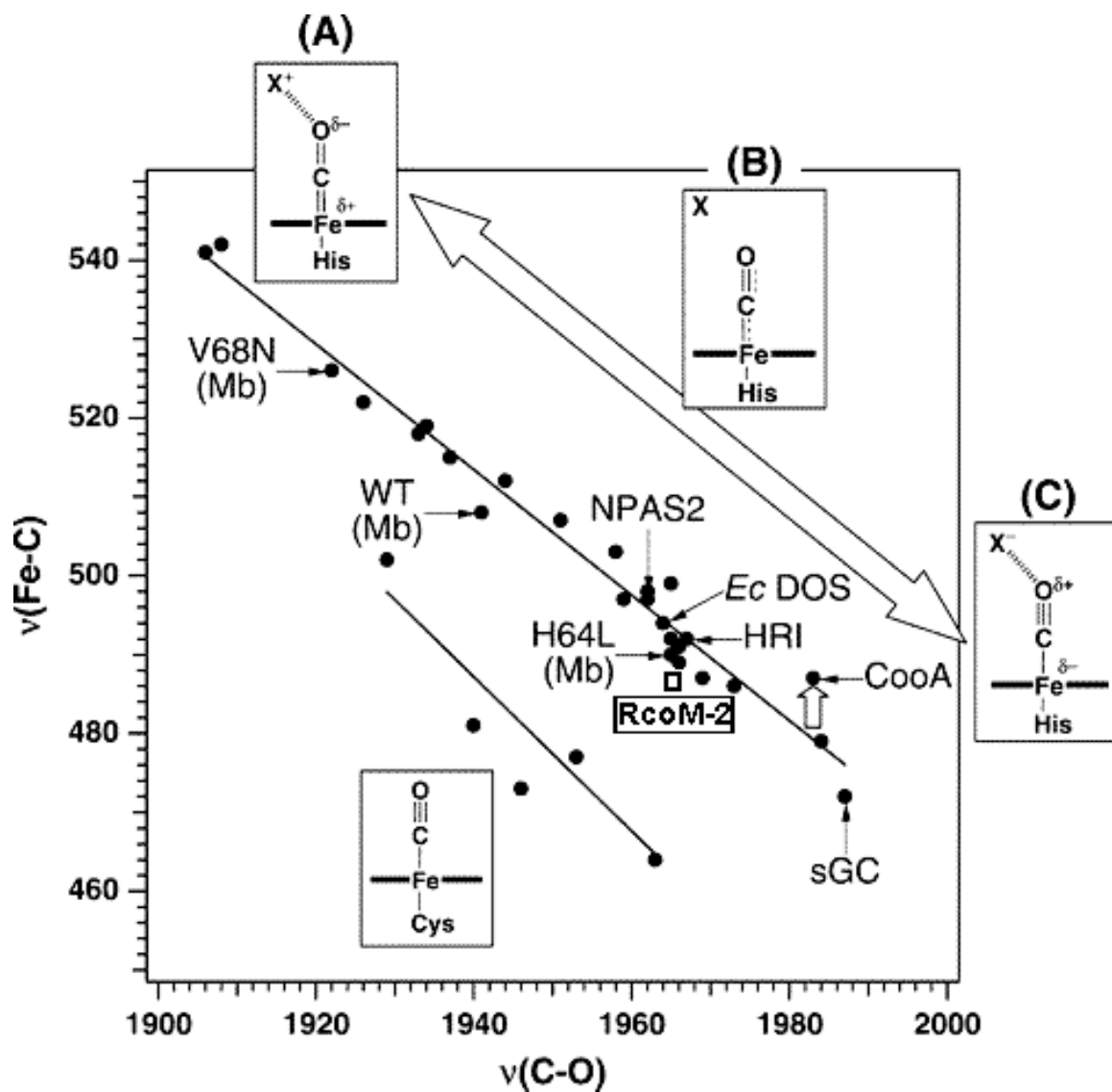
EPR simulation parameters of Fe(III)*BxRcoM-2*

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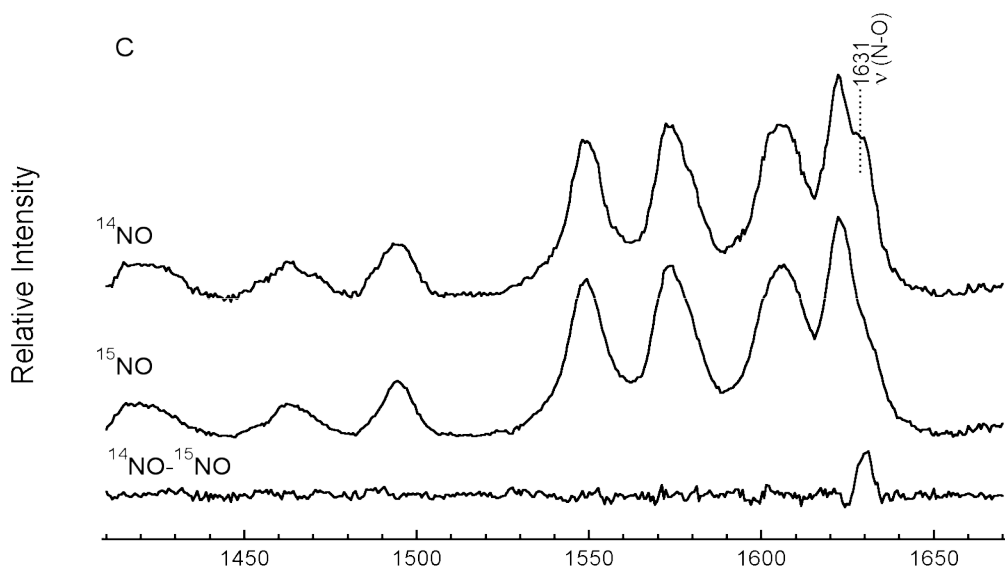
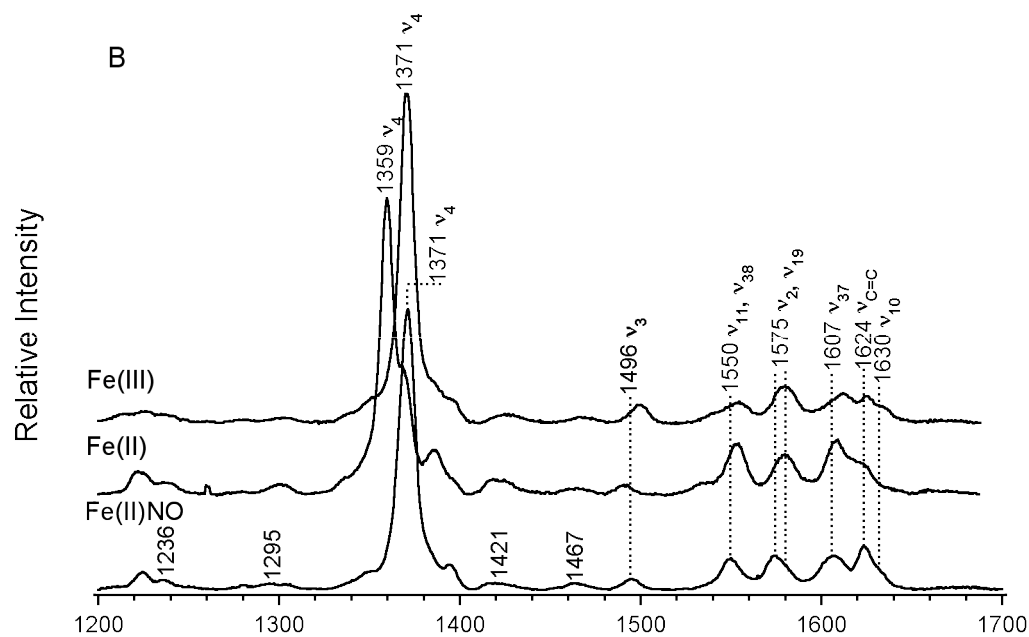
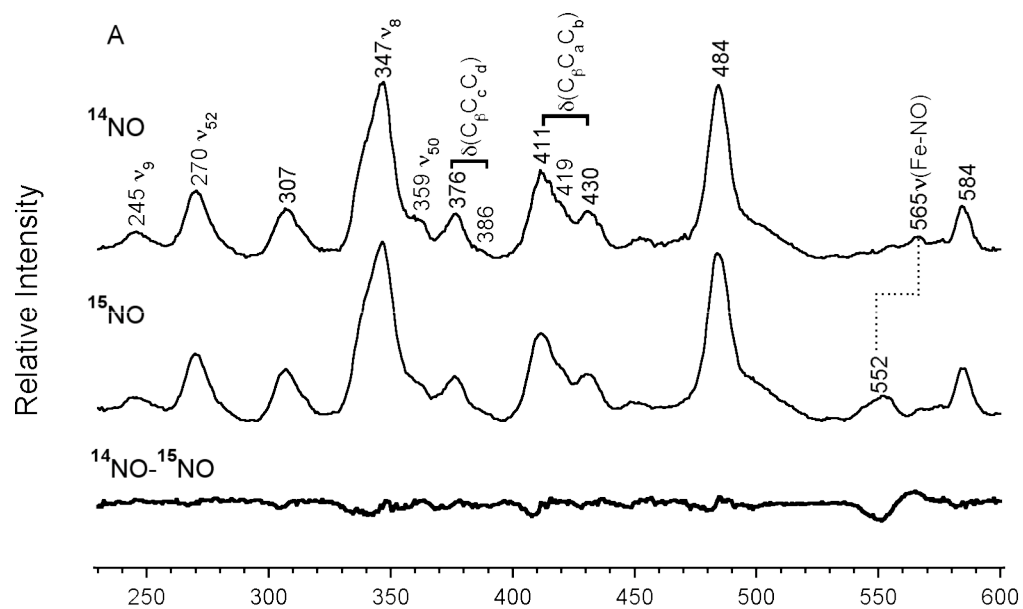
Supporting Information Figure S2. Inverse correlation plot for $\nu(\text{Fe-CO})$ and $\nu(\text{C-O})$

frequencies for 5- and 6- coordinate heme complexes (*1*). Fe(II)CO BxRcoM-2 is

indicated by an open square ($\square$).



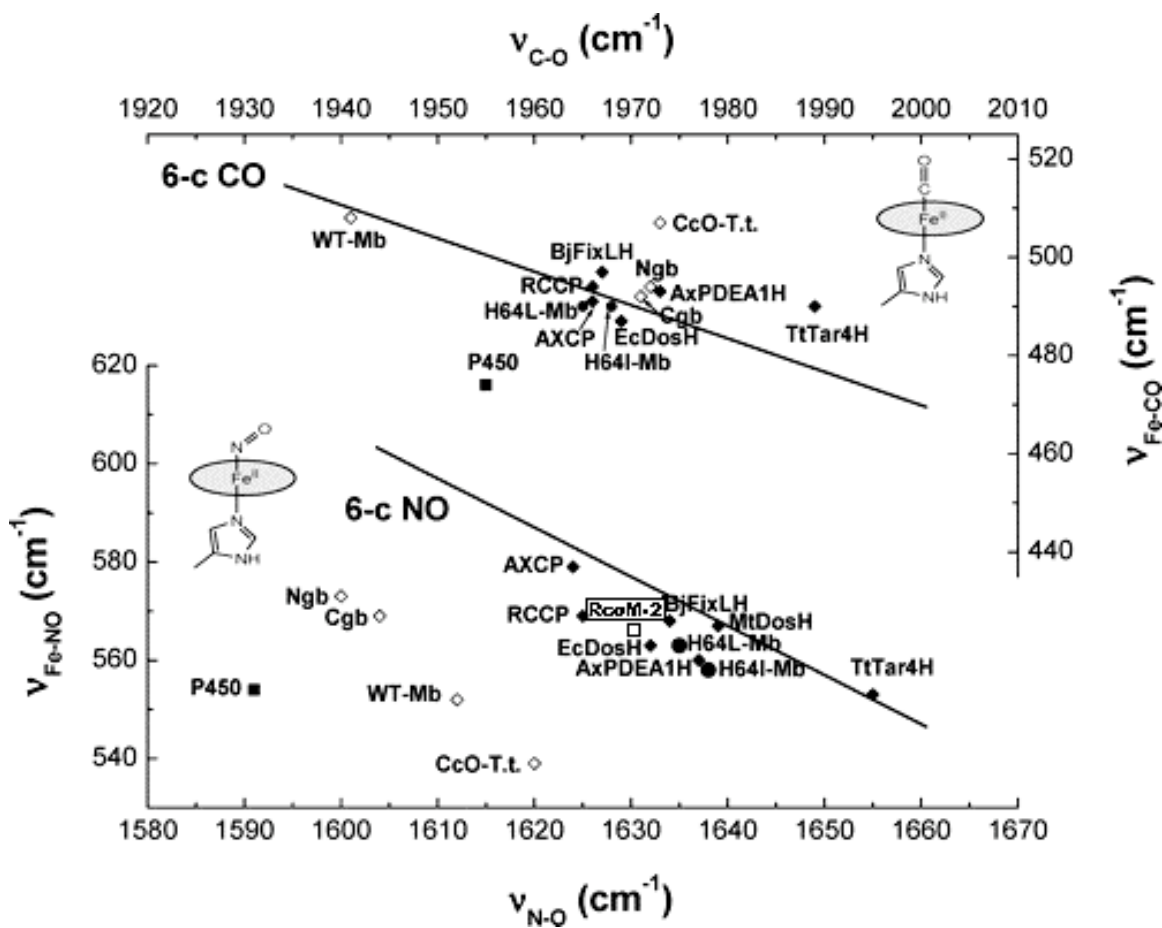
Supporting Information Figure S3. Resonance Raman spectra for Fe(II)(¹⁴NO) and Fe(II)(¹⁵NO) *BxRcoM-2*. The samples were 250 μM heme in 50 mM borate buffer and 500 mM KCl, pH 8.0. The samples were prepared via reduction of Fe(III)*BxRcoM-2* followed by generation of NO_(g). Spectra were acquired on the frozen samples at 77 K using the 413.1 nm Kr⁺ laser line with ~10 mW power at the sample. The difference spectrum (¹⁴NO-¹⁵NO) is shown for the frequency ranges containing ν(Fe-NO) and ν(N-O). **A)** The lowest frequency window, 200-600 cm⁻¹, containing the stretching mode ν(Fe-NO). **B)** The mid-frequency window, 1050-1650 cm⁻¹, containing the major oxidation, spin and coordination state marker bands. Shown is a comparison of the Fe(III), Fe(II) and Fe(II)NO states. **C)** The enlarged spectral window, 1250-1750 cm⁻¹, containing the stretching mode ν(N-O). Key vibrational modes are indicated.



Supporting Information Figure S4. Correlation plot for $\nu(\text{Fe-NO})$ and $\nu(\text{N-O})$

frequencies for 5- and 6- coordinate heme complexes (*I*). Fe(II)NO BxRcoM-2 is

indicated by an open square ($\square$).



Supporting Information Table S1. Comparison of MCD peak positions for low-spin, 6-coordinate Fe(III) heme proteins. The experimental values for *BxRcoM-2* are highlighted.

<i>MCD</i>	Ligands	Peak	Crossover	Peak	Peak	Crossover	Peak	Ref.
<i>BxRcoM-2</i>	?/Cys	413	419	427	565	570	577	a
<i>RrCooA</i>	Pro/Cys	413 ^b	420	427 ^b	562 ^b	571	580 ^b	(2)
P450 _{CAM} +ImH	ImH/Cys	417	426	435	563	569	585	(3)
hCBS ^b	His/Cys	419	425	433	545	555	567	(4)
M80C cyt <i>c</i>	His/Cys	415 ^b	424 ^b	432 ^b	MS ^c	560 ^b	MS ^c	(5)
cytochrome <i>c</i>	His/Met	402	406	413	560	574	590	(6)
<i>EcDos</i>	His/H ₂ O	412	420	426	554	563	569	(7)
cytochrome <i>b</i> ₅	His/His	406	413	419	553	562	570	(8)

^aThis work. ^bPeak and/or crossover positions were not reported in the original paper and are presented from inspection of the data. ^cMultiple peak/crossover/trough signals are present; only that of the central crossover position is indicated.

Supporting Information Table S2: Comparison of electronic absorption and MCD peak positions for selected 6-coordinate Fe(II)CO heme proteins. The experimental values for *BxRcoM-2* are highlighted.

<i>Electronic Absorption</i>								
Protein	Ligands	δ	Soret		β	α	Ref.	
<i>BxRcoM-2</i>	?/CO		423		540	570	a	
<i>RrCooA</i>	His/CO		422		540	569	(2)	
<i>EcDos</i>	His/CO		423		540	570	(9)	
neuroglobin	His/CO		420		537	568	(10)	
myoglobin	His/CO	346	423		540	579	(11)	
P450 _{CAM}	Cys/CO	366	446		551	NR ^b	(12)	
<i>MCD</i>		Peak	Crossover	Trough	Peak	Crossover	Trough	Ref.
<i>BxRcoM-2</i>	?/CO	419	423	427	565	571	577	a
<i>RrCooA</i>	His/CO	417 ^c	420	424 ^c	560 ^c	565	570 ^c	(2)
<i>EcDos</i>	His/CO	420	427	431	564	571	580	(7)
myoglobin	His/CO	417	422	427	565	572	581	(8, 13)
P450 _{CAM}	Cys/CO	442	448	455	530	566	581	(14)

^aThis work. ^bNot reported. ^cPeak positions were not reported in the original paper and are presented from inspection of data.

Supporting Information Table S3. Comparison of resonance Raman stretching frequencies for selected 5- and 6-coordinate Fe(II)CO heme proteins. The experimental values for *BxRcoM-2* are shaded.

<i>Resonance Raman</i>					
Protein	Ligands	$\nu(\text{Fe-CO})$	$\nu(\text{C-O})$	$\delta(\text{Fe-CO})$	Ref.
<i>BxRcoM-2</i>	His/CO	485	1965	565	a
<i>RrCooA</i>	His/CO	487,487	1969,1983	nd ^b ,572	(15, 16)
<i>EcDos</i>	His/CO	487	1969	575	(17)
neuroglobin	His/CO	494	1972	584	(18)
myoglobin	His/CO	512	1944	577	(19)
P450 _{CAM}	Cys/CO	474	1955	558	(20)
sGC+YC1 ^c	CO	521	1965	589	(21)
cytochrome <i>ba</i> ₃	CO	526	1967, 1982	571	(22)

^aThis work. ^bNot detected. ^cComplex of soluble guanylate cyclase and the benzylindazole compound YC-1.

Supporting Information Table S4. Comparison of electronic absorption peak positions for selected 5- and 6-coordinate Fe(II)NO heme proteins. The experimental values for *BxRcoM-2* are highlighted.

<i>Electronic Absorption</i>					
Protein	Ligands	Soret	β	α	Ref.
<i>BxRcoM-2</i>	?/NO	422	544	577	a
<i>EcDos</i>	His/NO	419	537	563	(23)
neuroglobin	His/NO	421	542	572	(24)
<i>ChCooA</i>	His/NO	417	541	571	(25)
CCP	His/NO	423	544	576	(26)
myoglobin	His/NO	420	548	580	(27)
HRP	His/NO	420	540	570	(27)
CPO	Cys/NO	441	560	585	(28)
P450 _{CAM}	Cys/NO	438	557		(28)
sGC	NO	398	537	572	(29)
<i>RrCooA</i>	NO	399	544	572	(30)

^aThis work.

Supporting Information Table S5. Comparison of EPR g values for selected 5- and 6-coordinate Fe(II)NO heme proteins. The experimental values for *BxRcoM-2* are shaded.

<i>EPR</i>						
Protein	Ligands	g_z	g_y	g_x	A_1/A_2^b (Gauss)	Ref.
<i>BxRcoM-2</i>	His/¹⁴NO	2.076	2.005	1.976	22.5/7.5	a
<i>BxRcoM-2</i>	His/¹⁵NO	2.076	2.005	1.976	32.5/6.5	a
<i>ChCooA</i>	His/NO	2.08	2.004	1.98	22/7	(30)
neuroglobin	His/NO	2.077	2.0037	1.970	22.4/8.7	(24)
HRP	His/NO	2.08	2.004	1.955	20.5/6.5	(31)
CCP	His/NO	2.080	2.004	1.960	21/6.4	(31)
P450 _{CAM}	Cys/NO	2.076	2.004	1.975	20/nd ^d	(32)
CPO	Cys/NO	2.082	2.004	1.966	20/nd ^d	(32)
A_3^c (Gauss)						
sGC	NO	2.076	2.029	2.005	16.0	(29)
<i>RrCooA</i>	¹⁴ NO	2.106	2.050	2.015	16.0	(25)
<i>RrCooA</i>	¹⁵ NO	2.100	2.045	2.015	21.8	(25)

^aThis work. ^b A_1/A_2 , the hyperfine and superhyperfine coupling constants arising from coupling of an electron with NO and an additional nitrogen atom. ^c A_3 , the hyperfine coupling constant arising from NO (¹⁴N, $I = 1$, ¹⁵N, $I = 1/2$) coupling to an electron. ^dNot detected.

Supporting Information Table S6. Comparison of resonance Raman stretching frequencies for selected 5- and 6-coordinate Fe(II)NO heme proteins. The experimental values for *BxRcoM-2* are highlighted.

<i>Resonance Raman</i>				
Protein	Ligands	$\nu(\text{Fe-NO})$	$\nu(\text{N-O})$	Ref.
<i>BxRcoM-2</i>	His/NO	565	1631	a
<i>EcDos</i>	His/NO	563	1632	(17)
<i>AxPDEA1</i>	His/NO	560	1637	(17)
RCCP	His/NO	569	1625	(33)
neuroglobin	His/NO	573	1600	(18)
P450 _{CAM}	Cys/NO	554	1591	(34)
CPO	Cys/NO	552	NR ^b	(35)
sGC	NO	521	1681	(36)
<i>RrCooA</i>	NO	523	1672	(25)

^aThis work. ^bNot reported.

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