

# Understanding how the distal environment directs reactivity in chlorite dismutase: spectroscopy and reactivity of Arg183 mutants

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## **Contents:**

**Figure S1.** R183Q mutant UV/visible pH titrations from pH 7 to 3, pH 7 to 11 and pH 8.5 to 12

**Figure S2.** R183A mutant UV/visible pH titrations from pH 6.1 to 4, pH 7 to 10.6 and pH 8.8 to 12.8

**Figure S3.** R183K mutant UV/visible pH titrations from pH 6.1 to 2.8, pH 7 to 10.9 and pH 10 to 12.9

**Figure S4.** Soret-excited, high frequency window rR spectra of Cld mutants as a function of pH.

**Figure S5.** High frequency rR spectra of ferrous Cld(R183Q) at pH 7.8 and 10.0. Spectra were acquired with 406.7 nm excitation and 15 mW at the sample. Inset: The low-frequency window of the rR spectrum of ferrous Cld(R183Q) at pH 7.8 and 10.0 obtained with 441.6 nm excitation.

**Figure S6.** Resonance Raman characterization of ferrous Cld(R183A) in 100 mM sodium phosphate, pH 6.8. (A) The high frequency window rR spectrum acquired with 413.1 nm excitation and 18 mW at the sample. (B) The low-frequency window of the rR spectrum of ferrous Cld(R183A) obtained with 441.6 and 413.1 nm excitation.

**Figure S7.** UV-visible spectra of ferrous Cld(R183K) as a function of pH. Samples were prepared in 100 mM sodium phosphate, pH 6.8 or 100 mM Ches pH 10.0 and reduced with sodium dithionite.

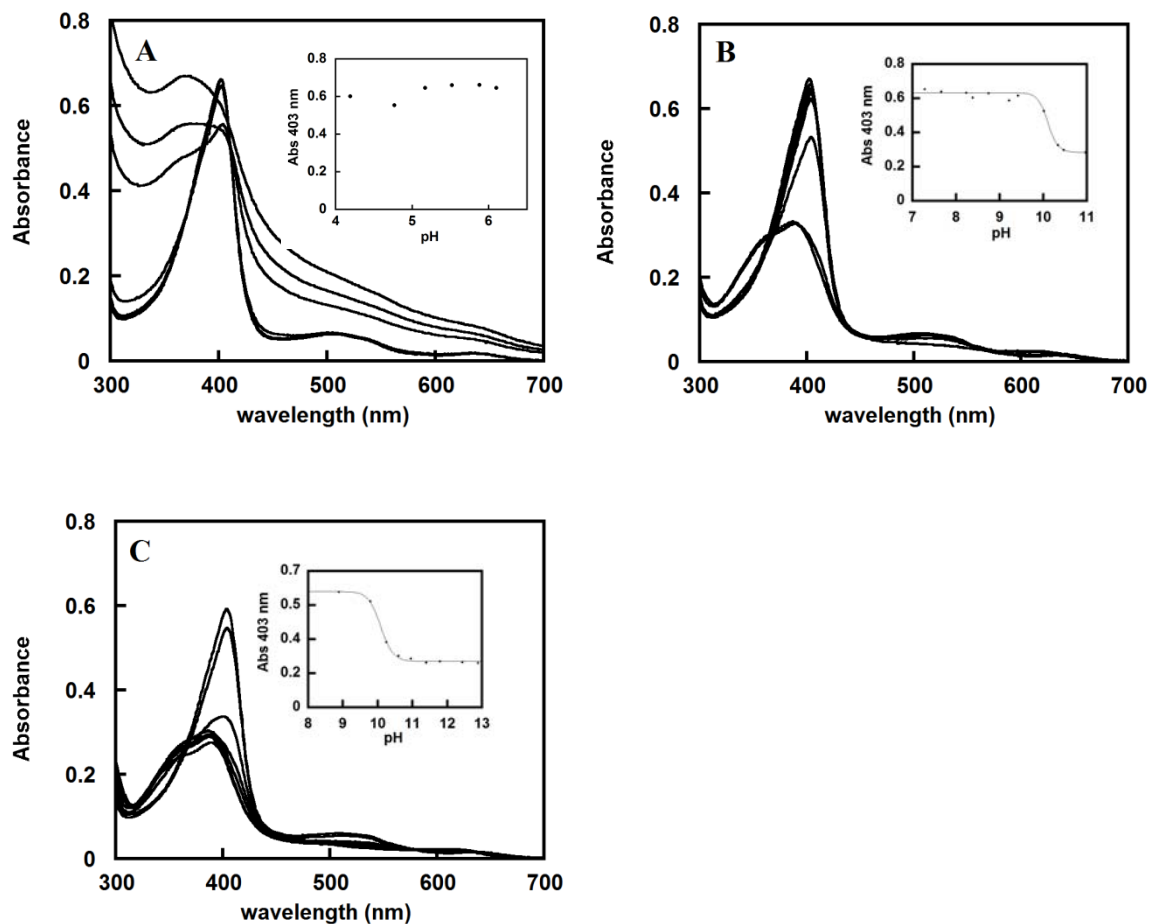
**Figure S8.** Fit of WT *Da*Cld-CO rR data (pH 5.8) for determination of the Fe-C stretching frequency of the two conformers.

**Figure S9.** Soret-excited rR spectra of WT Cld-CO as a function of pH.

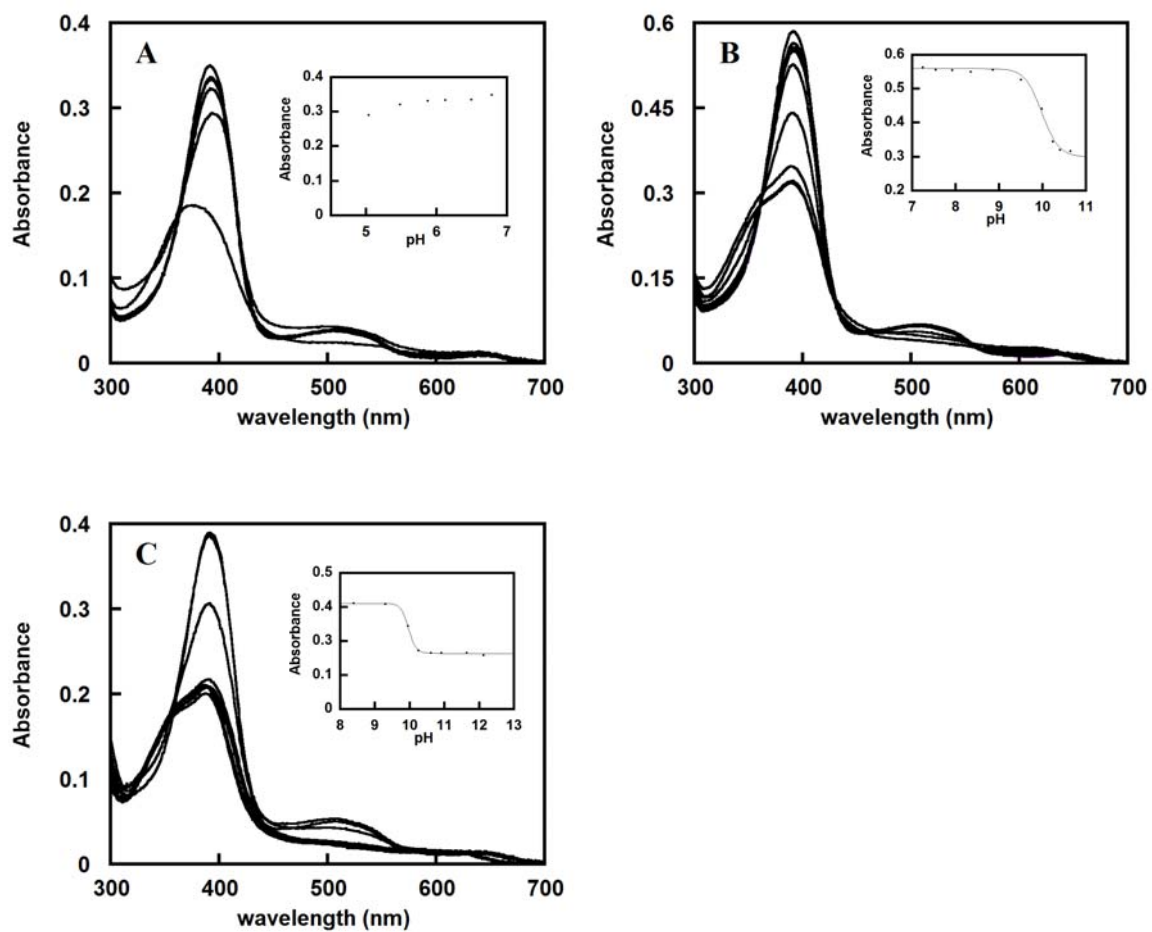
**Figure S10.** Resonance Raman spectra and fits for the low frequency data for the isotopomers of Cld(R183Q)-CO at (A) pH 6.8 and (B) pH 10.0; The original data is in black; blue indicates the component peaks and red shows the calculated fit.

**Table S1.** Comparison of Fe-CO vibrational frequencies for several heme proteins that were used to construct Figure 7 in the paper. The frequencies are reported for pH 6.8 unless otherwise indicated. The frequencies for Cld-<sup>13</sup>C CO complexes are in parentheses.

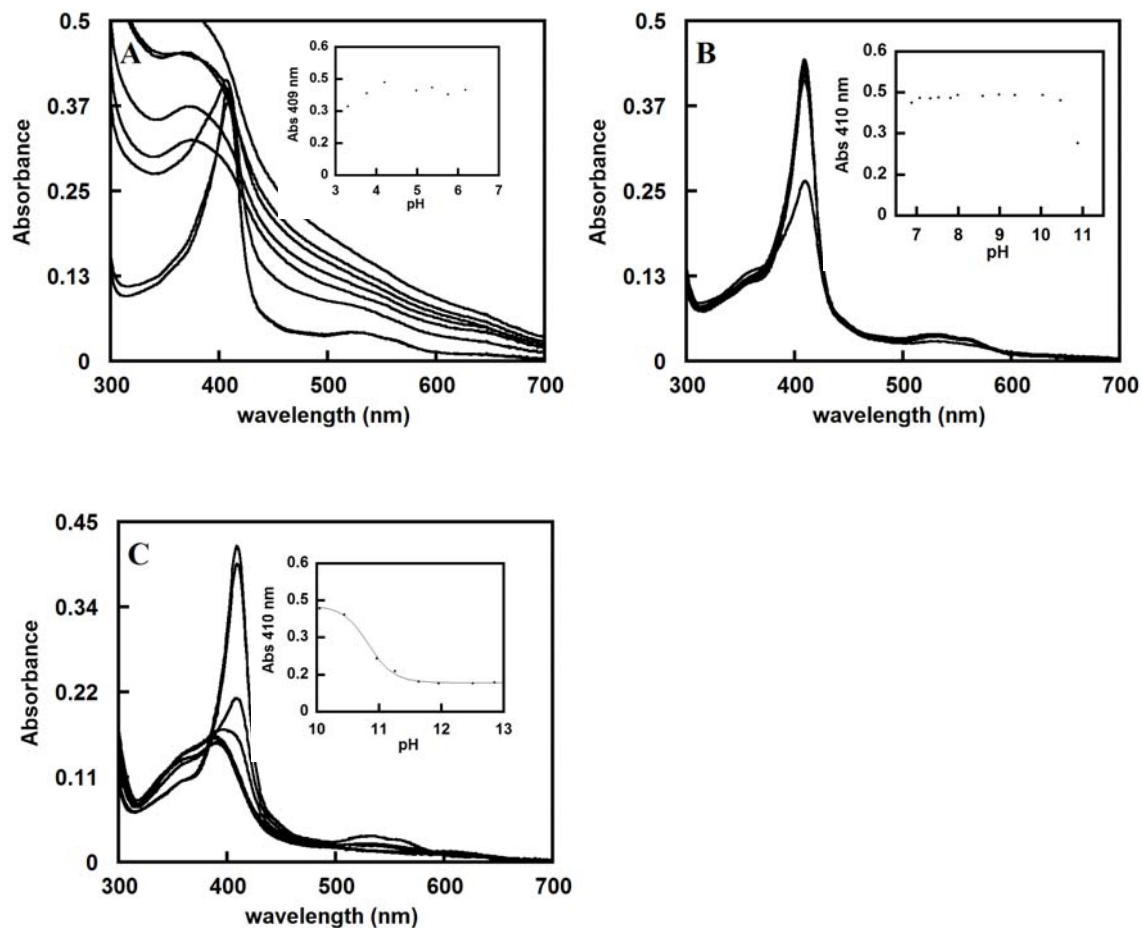
**Figure S1:** UV visible pH titration for the R183Q mutant (A) pH 6.8, 6.5, 6.1, 5.8, 5.5, 5, 3.8 (B) pH 6.8, 7.3, 7.7, 8.2, 8.7, 9.4, 10, 10.5, 10.9 and (C) pH 8.4, 9.3, 9.9, 10.3, 10.6, 10.9, 11.6, 12.1



**Figure S2:** UV/visible pH titrations for mutant R183A at (A) pH 6.8, 6.5, 6.1, 5.8, 5.5, 5, 3.8 (B) pH 6.8, 7.2, 7.5, 7.9, 8.3, 8.9, 9.5, 9.9, 10.2, 10.4, 10.6 and (C) pH 8.4, 9.3, 9.9, 10.3, 10.6, 10.9, 11.6, 12.1

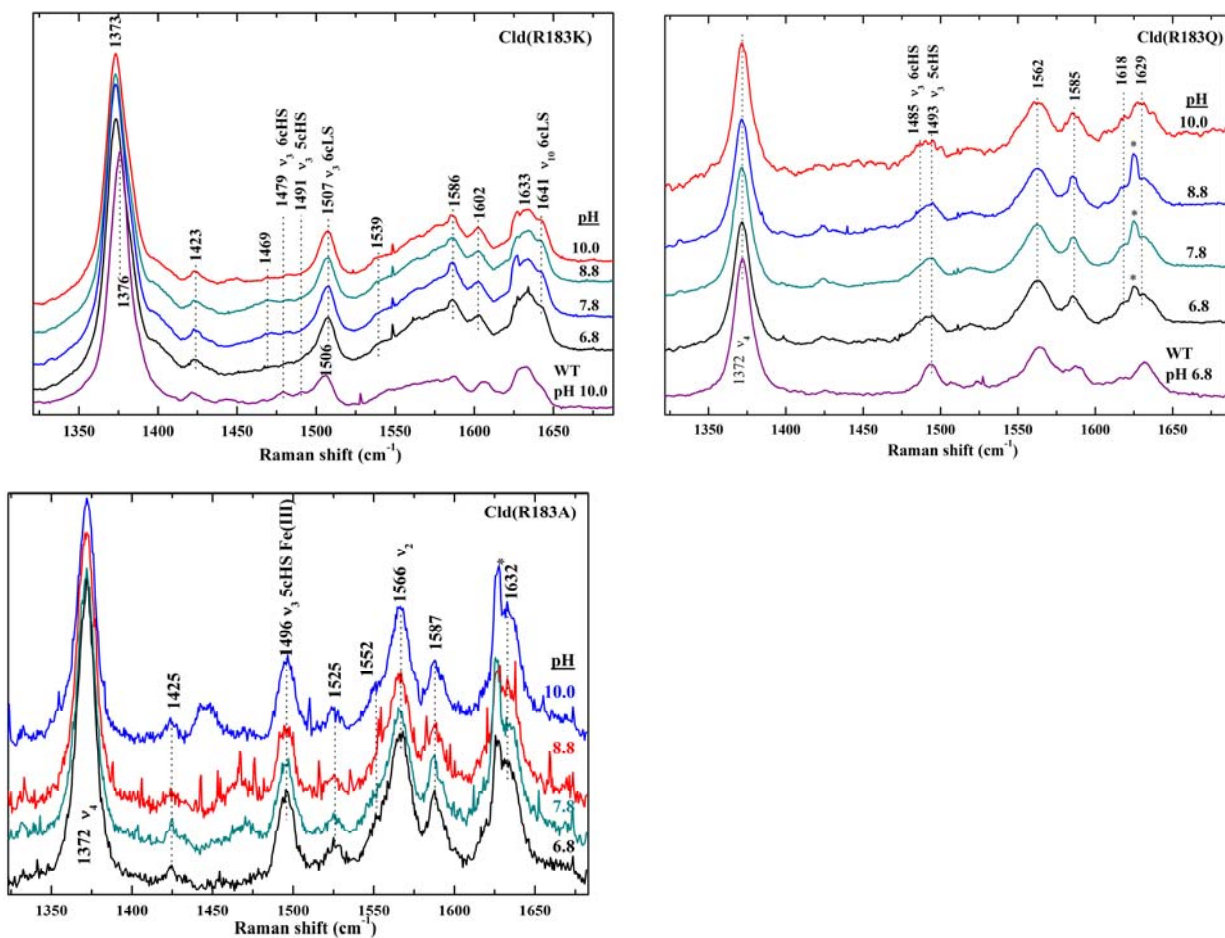


**Figure S3:** UV/visible pH titrations for mutant R183K at pH 6.2, 5.8, 5.4, 4.9, 4.2, 3.8, 3.3, 2.8 (B) at pH 6.9, 7.1, 7.3, 7.5, 7.8, 7.9, 8.9, 9.4, 10. 10.5, 10.9 and (C) at pH 10.04, 10.4, 10.9, 11.2, 11.6, 11.9, 12.5, 12.9

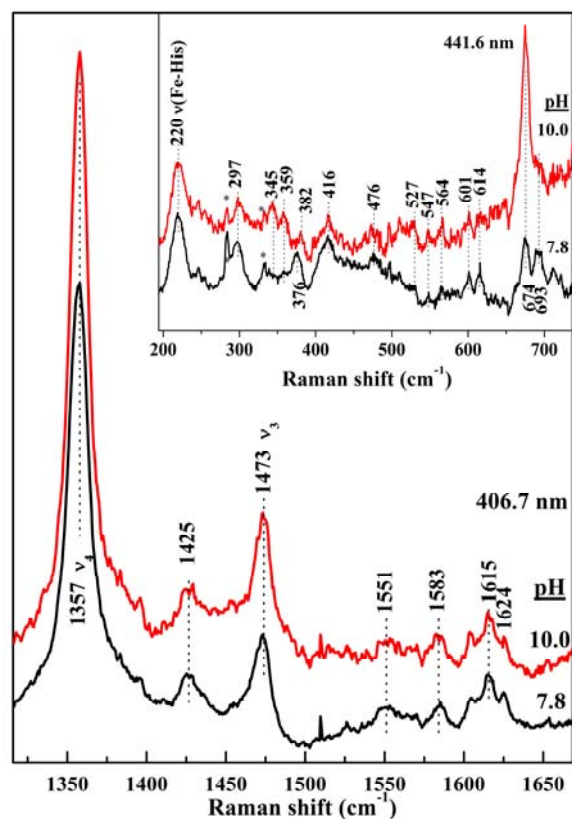


**Figure S4.** Soret-excited, high frequency window rR spectra of Cld mutants as a function of pH. Samples were prepared in 100 mM sodium phosphate, pH 6.8, 100 mM Tris/HCl pH 7.8, 100 mM Tris/HCl pH 8.8, 100 mM Ches pH 10.0 and 100 mM MES pH 5.5. Spectra were collected with 406.7 nm excitation and 14 mW at the sample. A) Cld(R183K) spectra compared to the spectrum of WT Cld at pH 10, B) Cld(R183A) spectra, and C) Cld(R183Q) spectra compared to WT Cld at pH 6.8.

\* indicates plasma emission lines.

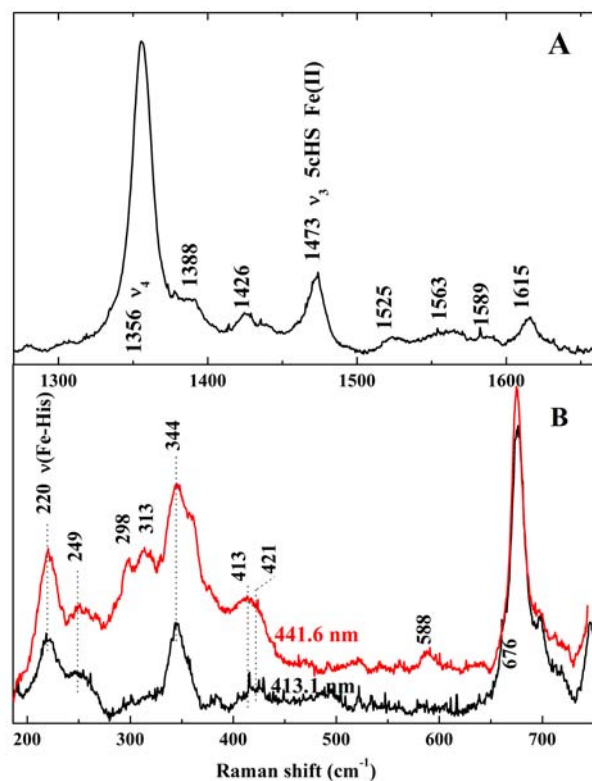


**Figure S5.** High frequency rR spectra of ferrous Cld(R183Q) at pH 7.8 and 10.0. Spectra were acquired with 406.7 nm excitation and 15 mW at the sample. Inset: The low-frequency window of the rR spectrum of ferrous Cld(R183Q) at pH 7.8 and 10.0 obtained with 441.6 nm excitation.



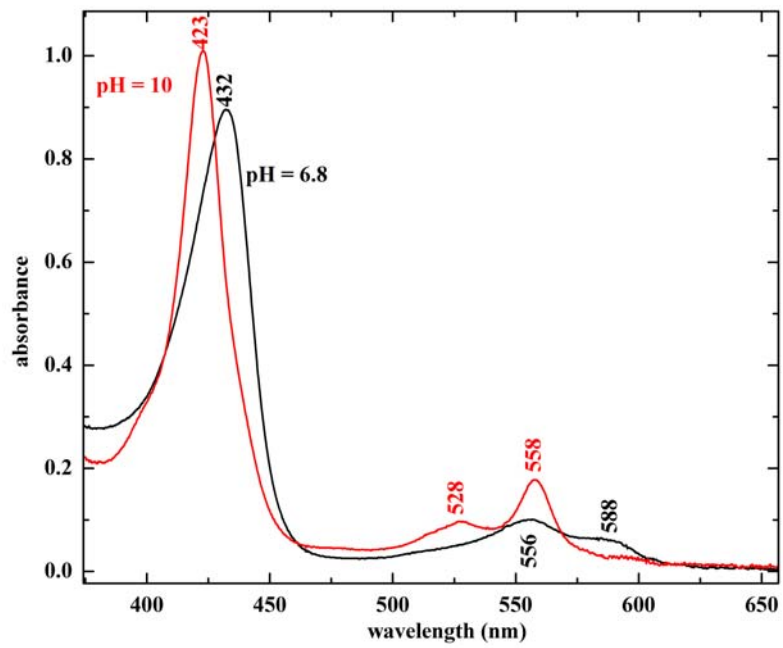
**Figure S6.** Resonance Raman characterization of ferrous Cld(R183A) in 100 mM sodium phosphate, pH 6.8. (A) The high frequency window rR spectrum acquired with 413.1 nm excitation and 18 mW at

the sample. (B) The low-frequency window of the rR spectrum of ferrous Cld(R183A) obtained with 441.6 and 413.1 nm excitation.

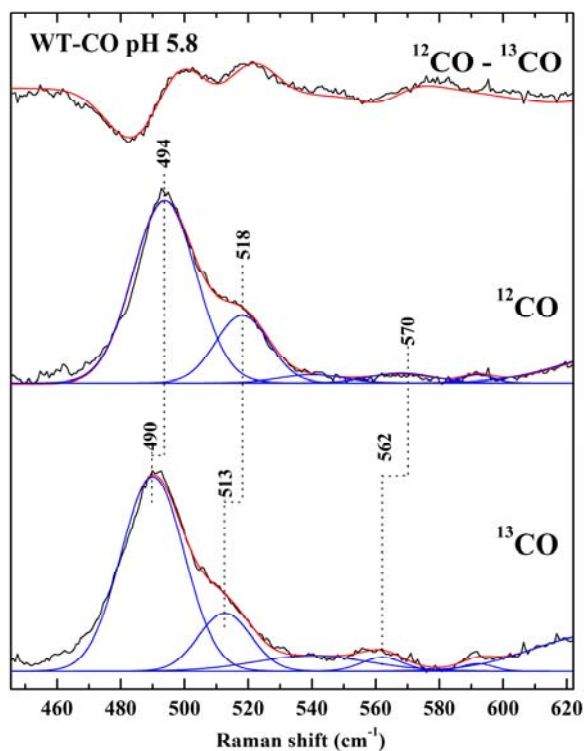


**Figure S7.** UV-visible spectra of ferrous Cld(R183K) as a function of pH. Samples were prepared in 100 mM sodium phosphate, pH 6.8 or 100 mM Ches pH 10.0 and reduced with sodium dithionite.

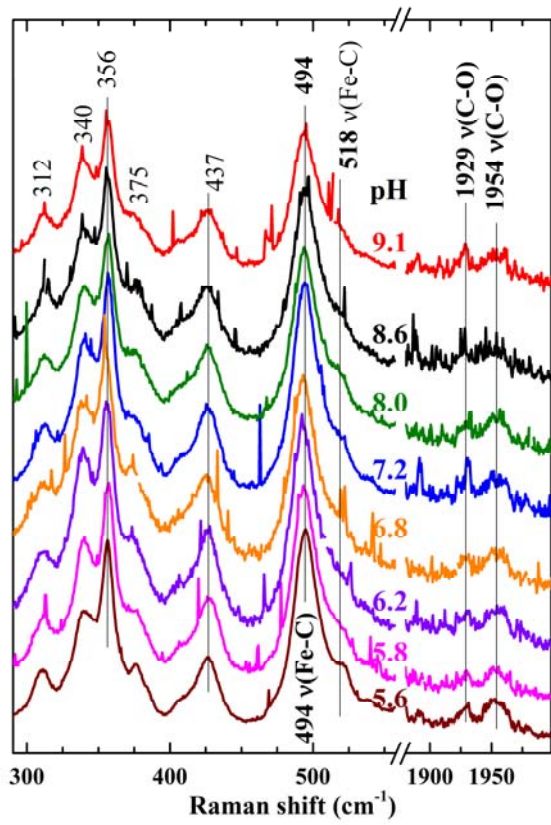




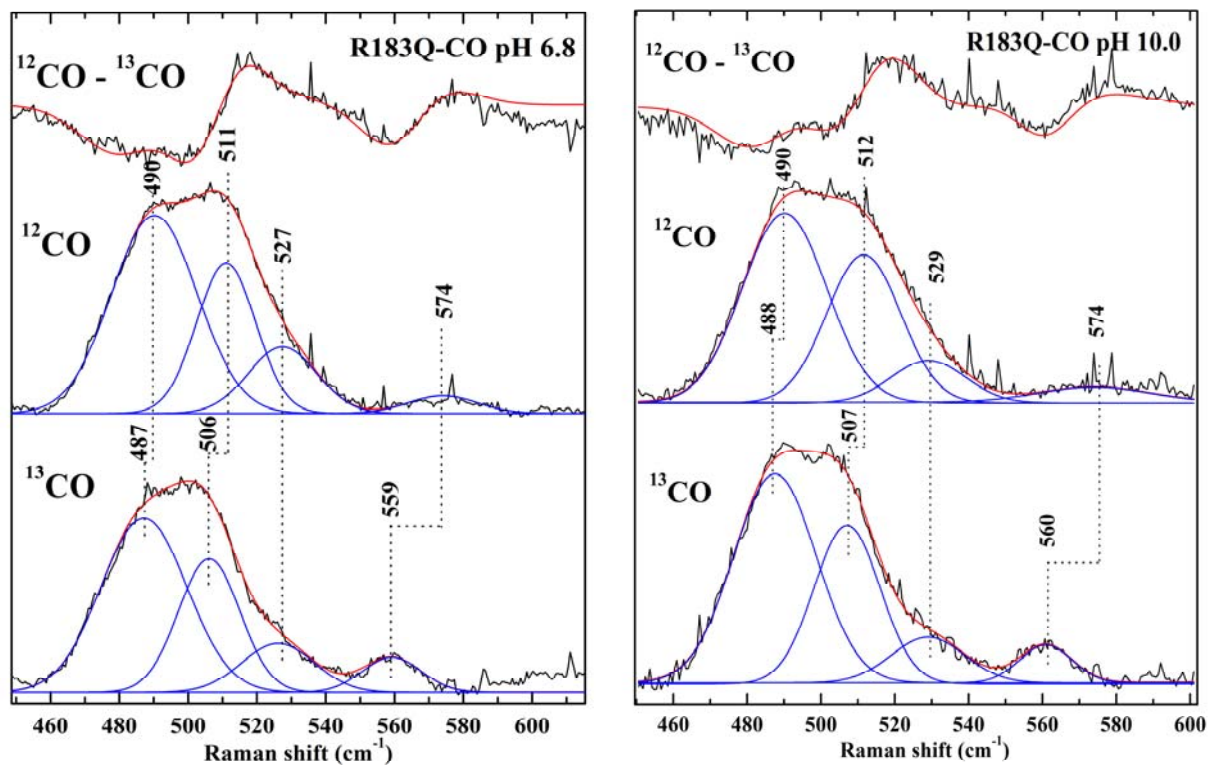
**Figure S8.** Fit of WT DaCld-CO rR data (pH 5.8) determine the Fe-C stretching frequency of the two conformers. Spectra were acquired with 413.1 nm excitation. Original spectrum is in black, fit peaks are in blue, and the simulated spectrum is in red. The simulated difference spectrum was generated by subtraction of the simulated spectra.



**Figure S9.** Soret-excited rR spectra of WT Cld-CO as a function of pH.



**Figure S10.** Resonance Raman spectra and fits for the low frequency data for the isotopomers of Cld(R183Q)-CO at (A) pH 6.8 and (B) pH 10.0. The original data is in black; blue indicates the component peaks and red shows the calculated fit.



**Table S1:** Comparison of Fe-CO vibrational frequencies for several heme proteins that were used to construct Figure 7 in the paper. The frequencies are reported for pH 6.8 unless otherwise indicated. The frequencies for Cld-<sup>13</sup>CO complexes are in parentheses.

<b>Fe(II)-CO</b>		<b><math>\nu_{\text{Fe-C}}</math></b>	<b><math>\nu_{\text{C-O}}</math></b>	$\frac{\Delta\nu_{\text{Fe-C Mut}} - \nu_{\text{Fe-C WT}}}{\Delta\nu_{\text{C-O Mut}} - \nu_{\text{C-O WT}}}$	<b>Ref</b>
WT <i>Da</i> Cld	closed	518(513)	1929(1984)		This work
	open	494(491)	1954(1908)	-24/+25 <sup>a</sup>	
<i>Da</i> Cld(R183K)	open	491(488)	1956(1914)	-3/+2 <sup>b</sup> -27/+27 <sup>c</sup>	
<i>Da</i> Cld(R183Q)	closed	511(506)	1935(1891)	-7/+6	
	open	490(487)	1958(1915)	-3/+4 <sup>b</sup> -28/+29 <sup>c</sup>	
<i>Da</i> Cld(R183A)	open	488(486)	1964(1918)	-6/+10 <sup>b</sup> -30/+35 <sup>c</sup>	
HRPC acidic pH		492	1967		(1,2)
HRPC pH 6	form I	539	1906		
	form II	516	1934		
HRPC alkaline pH		530	1934		
HRPC(R38L) pH 6.0		515	1941.5	-24/+35.5 -1/+7.5	
HRPC(R38L) alkaline pH		496	1944	-34/+10	
HRPC(H42L) pH 6-9.5		525	1924	-14/+18 (form I) +9/-10 (form II)	
CcP pH 7.0		530	1922		(3)

CcP alkaline pH		503	1948		
CcP(R48L) acidic pH		500	1941	-30/+19	(4)
CcP(R48L) alkaline pH		500	1951	-3/+3	
CcP(H52L) pH 6		508	1944	-22/+22	(5)
SW Mb pH 8.4		512	1944		(6)
SW Mb pH 7.0		507	1947		
SW Mb pH 2.6		489	1966		
SW Mb(H64A)		490	1966	-17/+19	(7,8)
SW Mb(H64L)		489	1966	-18/+19	(7-9)
Mtb KatG pH 7.2		525	1923		(10)
Mtb KatG pH 8.0		522	1926		(11)
		500	1956		
Tea peroxidase		544	1892		(12)
Coprinus cinereus peroxidase		519	1930.5		(12)

$$^a \Delta(v_{\text{Fe-C WTopen}} - v_{\text{Fe-C WTclosed}}) / \Delta(v_{\text{C-O WTopen}} - v_{\text{C-O WTclosed}}).$$

$$^b \Delta(v_{\text{Fe-C mut}} - v_{\text{Fe-C WTopen}}) / \Delta(v_{\text{C-O mut}} - v_{\text{C-O WTopen}}).$$

$$^c \Delta(v_{\text{Fe-C mut}} - v_{\text{Fe-C WTclosed}}) / \Delta(v_{\text{C-O mut}} - v_{\text{C-O WTclosed}}).$$

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