

## Supporting Information

### Unprecedented ( $\mu$ -1,1-Peroxo)diferric Structure for the Ambiphilic Orange Peroxo Intermediate of the Nonheme *N*-Oxygenase CmlI

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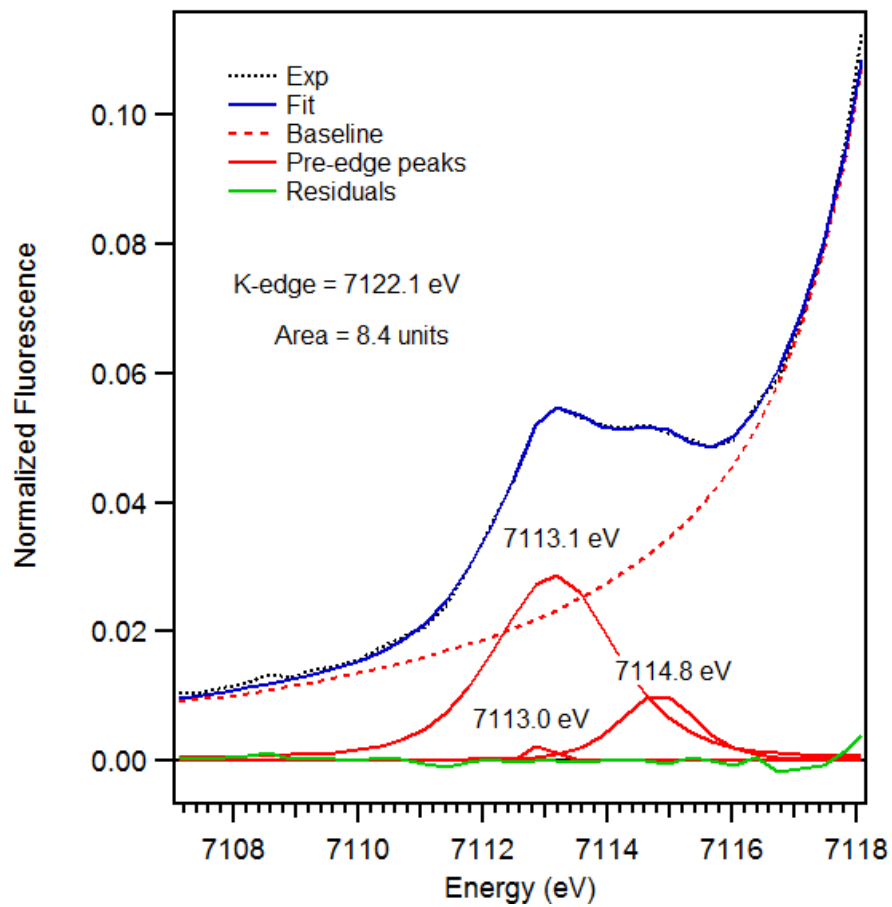
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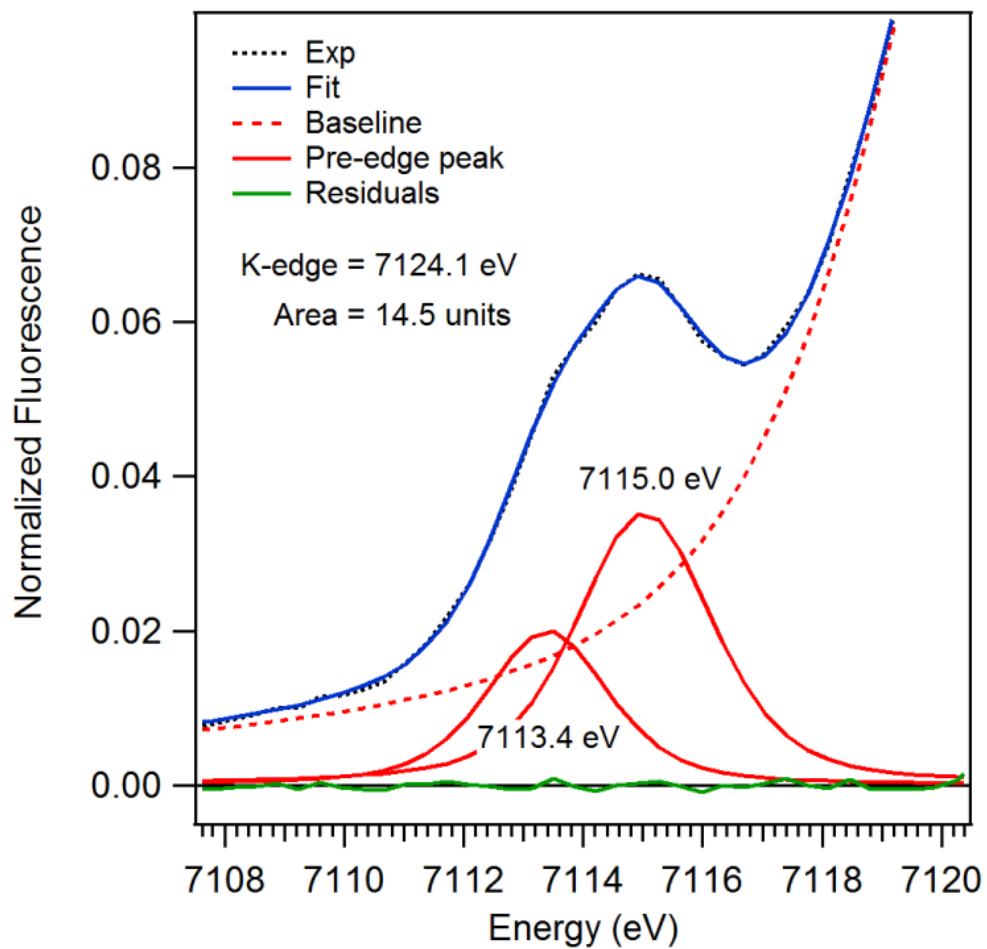
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**General EXAFS considerations.** In the fit tables of EXAFS data, N refers to the number of scatterers used for a particular shell, R is the distance of the scattering shell,  $\sigma^2$  is the mean-squared deviation (or Debye-Waller factor),  $E_0$  is the edge shift parameter, and the goodness of fit (GOF) parameters are calculated as  $F = \sqrt{\sum k^6 (\chi_{\text{exp}} - \chi_{\text{calc}})^2}$ ,

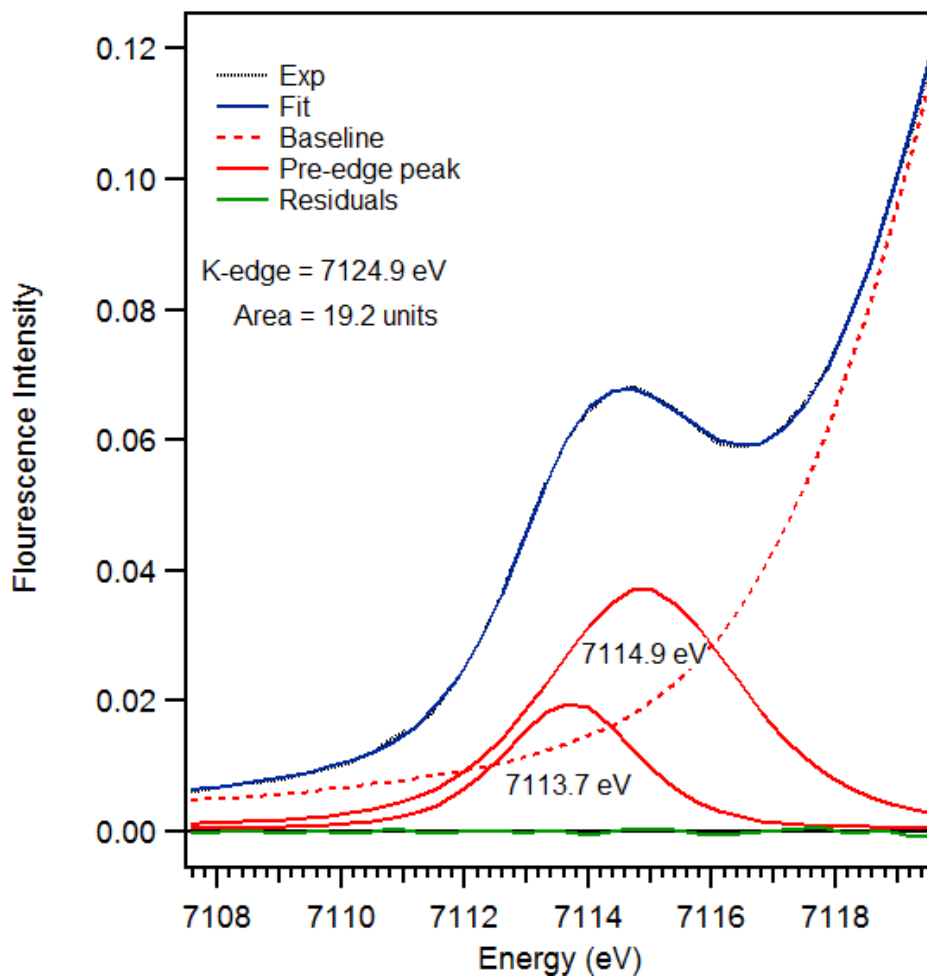
$F' = \sqrt{\sum k^6 (\chi_{\text{exp}} - \chi_{\text{calc}})^2 / \sum k^6 \chi_{\text{exp}}^2}$ . For all fits, the amplitude reduction factor ( $S_0^2$ ) was set to 0.9.



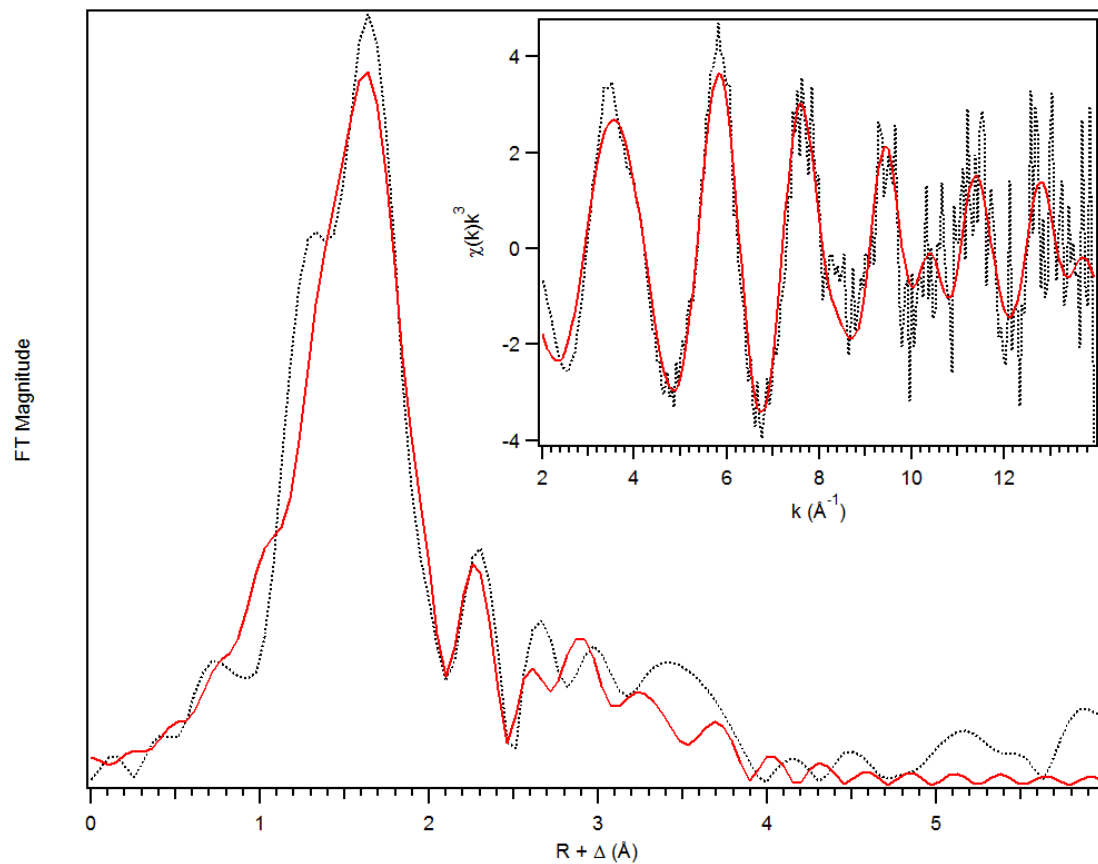
**Figure S1.** Pre-edge region analysis of **CmlI<sup>R</sup>**. The experimental data (black dotted), baseline (red dashed), pre-edge peak components (red solid), residuals (green solid), and total fit (blue solid) are shown.



**Figure S2.** Pre-edge region analysis of  $\text{CmII}^{\text{Ox}}$ . The experimental data (black dotted), baseline (red dashed), pre-edge peak components (red solid), residuals (green solid), and total fit (blue solid) are shown.



**Figure S3.** Pre-edge region analysis of  $\text{CmII}^{\text{P}}$ . The experimental data (black dotted), baseline (red dashed), pre-edge peak components (red solid), residuals (green solid) and total fit (blue solid) are shown.



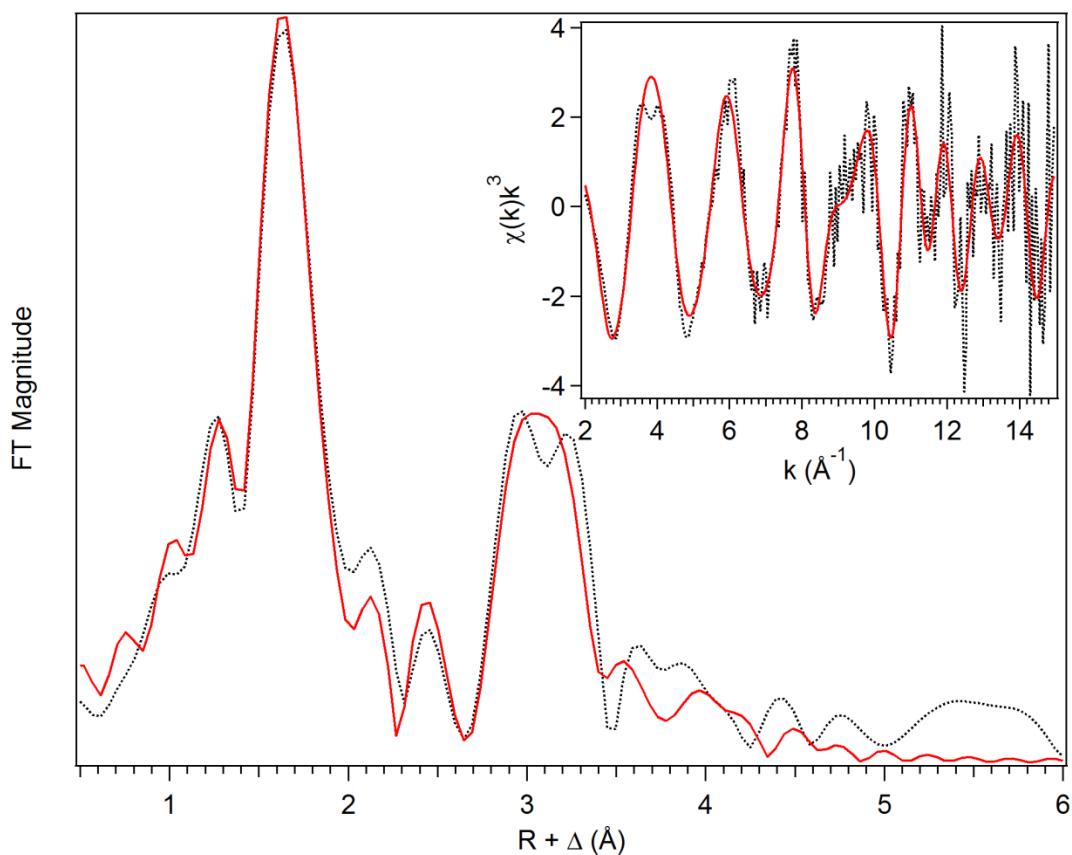
**Figure S4.** Fit (red solid line) of the unfiltered (black dotted) EXAFS data (inset) and corresponding Fourier transform of  $\mathbf{CmII}^{\text{R}}$  (Table S1, Fit 17). Data was fit between  $k = 2\text{--}14 \text{ \AA}^{-1}$ .

**Table S1.** Fit parameters for the unfiltered EXAFS data of **CmII<sup>R</sup>**, between  $k = 2 - 14 \text{ \AA}^{-1}$ .  
Fit 17 gives the most reasonable fit of the experimental data.

Fit	Fe-N		Fe-O		Fe•••Fe		Fe•••C		GOF						
	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	$E_0$	F	F'			
1	6	2.10	7.61							-10.3	300	568			
2	5	2.10	6.23							-9.53	304	572			
3	4	2.11	4.81							-8.82	323	590			
4	3	2.11	3.31							-8.22	365	627			
5	3	2.12	2.60	1	2.01	2.03				-9.71	304	572			
6	4	2.11	4.06	1	1.98	4.40				-11.2	288	557			
7	5	2.10	4.99	1	1.94	5.01				-12.8	281	550			
8	4	2.11	4.03	2	1.97	7.33				-13.6	274	544			
9	4	2.13	4.87	1	2.02	4.61		3	3.11	4.20	-8.72	257	527		
10	4	2.12	4.83	1	2.01	6.35		3	3.14	0.93	-9.05	240	509		
								3	2.99	3.97					
11	4	2.12	4.76	1	2.00	5.61		3	3.15	0.87	-9.66	226	493		
								3	2.99	3.68					
								1	2.59	1.38					
12	4	2.12	4.76	1	2.01	4.90	1	3.34	9.20	3	2.99	2.77	-8.88	221	487
										5	3.15	2.08			
										1	2.60	0.85			
13	4	2.12	5.01	1	2.01	5.90	1	3.35	9.54	5	3.15	2.62	-9.10	210	475
										3	2.99	3.50			
										1	2.60	1.41			
										4	4.01	1.06			
14	4	2.12	4.80	1	2.00	5.54				5	3.13	3.88	-9.61	217	484
										3	2.96	4.31			
										1	2.60	2.07			
										4	4.02	1.30			
15	4	2.12	4.91	1	2.01	6.72	1	3.34	9.73	5	3.15	2.23	-9.04	223	490
										3	2.99	3.16			
										4	4.00	1.49			
16	4	2.13	5.11	1	2.02	3.96	1	3.38	10.36	5	3.13	7.48	-8.09	227	495
										4	4.02	1.32			
										1	2.61	1.36			
<b>17</b>	<b>5</b>	<b>2.10</b>	<b>5.02</b>	<b>1</b>	<b>1.94</b>	<b>5.35</b>	<b>1</b>	<b>3.35</b>	<b>9.90</b>	<b>1</b>	<b>2.58</b>	<b>1.35</b>	<b>-12.0</b>	<b>206</b>	<b>470</b>
										<b>3</b>	<b>2.98</b>	<b>2.76</b>			
										<b>5</b>	<b>3.14</b>	<b>2.48</b>			
										<b>4</b>	<b>3.99</b>	<b>1.15</b>			
18	4	2.11	4.71				1	3.35	8.37	1	2.60	1.08	-7.27	233	501
										3	3.00	2.37			
										5	3.16	1.74			
										4	4.02	0.75			

**Table S1 (continued).** Fit parameters for the unfiltered EXAFS data of  $\text{CmII}^{\text{R}}$ , between  $k = 2 - 14 \text{ \AA}^{-1}$ . Fit 17 gives the most reasonable fit of the experimental data.

Fit	Fe-N			Fe-O			Fe•••Fe			Fe•••C			GOF		
	N	R(Å)	$\sigma^2(10^{-3})$	N	R(Å)	$\sigma^2(10^{-3})$	N	R(Å)	$\sigma^2(10^{-3})$	N	R(Å)	$\sigma^2(10^{-3})$	$E_0$	F	F'
19	5	2.11	4.75	1	1.94	4.36	1	3.34	8.55	0.5	2.58	-2.50	-11.7	198	462
										3	2.99	1.03			
										5	3.14	1.15			
										4	3.99	3.31			



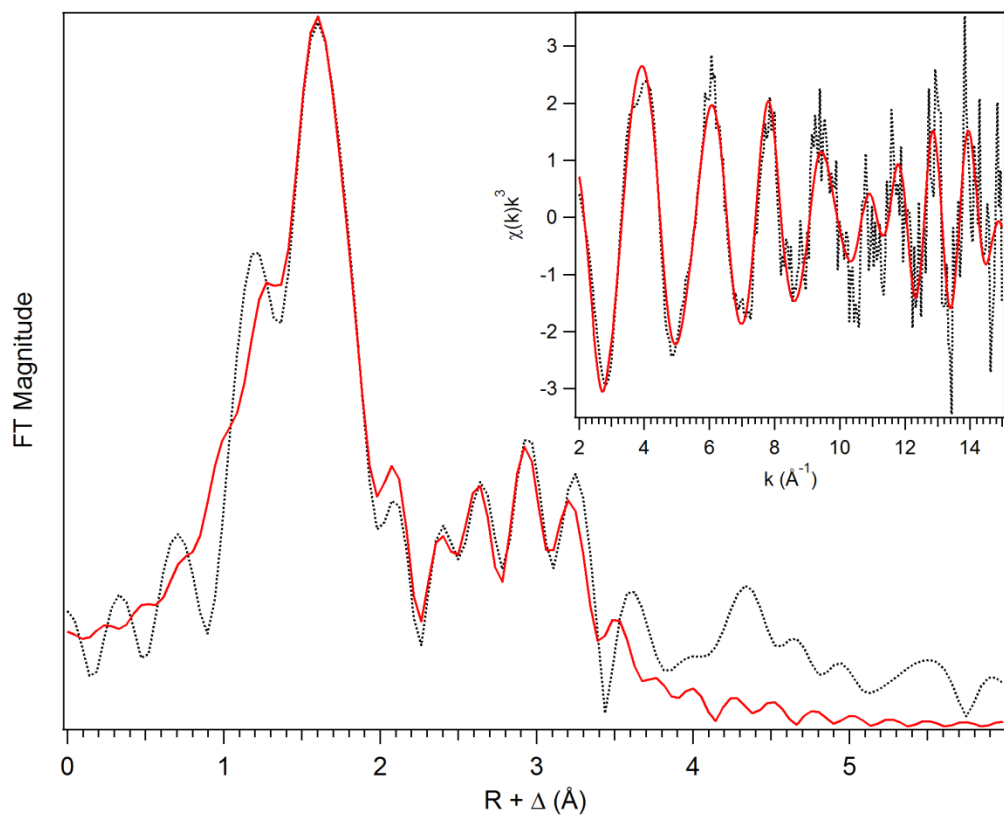
**Figure S5.** Fit (red solid line) of the unfiltered (black dotted) EXAFS data (inset) and corresponding Fourier transform of  $\text{CmII}^{\text{Ox}}$  (Table S2, Fit 15). Data was fit between  $k = 2 - 15 \text{ \AA}^{-1}$ .



**Table S2.** Fit parameters for the unfiltered EXAFS data of **CmII<sup>Ox</sup>**, between  $k = 2 - 15 \text{ \AA}^{-1}$ .

Fit 15 gives the most reasonable fit of the experimental data. The Fe•••C shells at 3.57 and 4.28 Å are consistent with bound His ligands.

Fit	Fe-N			Fe-O			Fe•••Fe			Fe•••C			GOF		
	N	R(Å)	$\sigma^2(10^{-3})$	N	R(Å)	$\sigma^2(10^{-3})$	N	R(Å)	$\sigma^2(10^{-3})$	N	R(Å)	$\sigma^2(10^{-3})$	E <sub>0</sub>	F	F'
1	6	2.11	10.64										0.50	408	704
2	5	2.11	8.68										1.22	388	687
3	4	2.12	6.92										1.88	383	682
4	3	2.12	5.26										2.46	404	701
5	4	2.11	6.58	1	1.95	18.06							-0.27	378	678
6	3	2.17	2.44	1	2.03	-0.50							2.26	3.57	659
7	3	2.18	4.56	2	2.04	3.92							1.33	388	687
8	3	2.16	1.74	2	2.02	0.74							-2.42	331	635
				1	1.84	3.15									
9	3	2.16	1.96	2	2.02	0.94	1	3.31	2.52				-1.78	248	550
				1	1.85	3.63									
10	3	2.17	2.19	2	2.03	1.16	1	3.31	2.35	2	3.12	3.67	-0.76	239	538
				1	1.85	4.45									
11	3	2.15	1.82	2	2.01	0.98	1	3.31	3.20	2	3.07	10.44	-2.85	209	504
				1	1.84	3.08				3	3.57	1.14			
12	3	2.16	1.77	2	2.01	0.99	1	3.32	3.53	2	3.06	9.28	-2.39	190	481
				1	1.84	3.22				3	3.58	0.55			
										3	4.29	1.07			
13	3	2.15	1.79	2	2.01	0.99	1	3.32	3.22	2	3.07	10.95	-2.65	191	481
				1	1.84	3.16				4	3.57	1.70			
										3	4.29	1.18			
14	3	2.17	2.10	2	2.03	1.11	1	3.31	2.40	2	3.12	3.36	-0.74	230	528
				1	1.85	4.28				3	4.31	3.17			
15	3	2.15	1.73	2	2.00	0.97	1	3.32	3.38	4	3.57	1.39	-3.79	196	488
				1	1.83	2.71				3	4.28	1.04			



**Figure S6.** Fit (red solid line) of the unfiltered (black dotted) EXAFS data (inset) and corresponding Fourier transform of **CmII<sup>P</sup>** (Table S3, Fit 23). Data was fit between  $k = 2 - 15 \text{ \AA}^{-1}$ .

**Table S3.** Fit parameters for the unfiltered EXAFS data of  $\text{CmII}^{\text{P}}$ , between  $k = 2 - 15 \text{ \AA}^{-1}$ .  
Fit 23 gives the most reasonable fit of the experimental data.

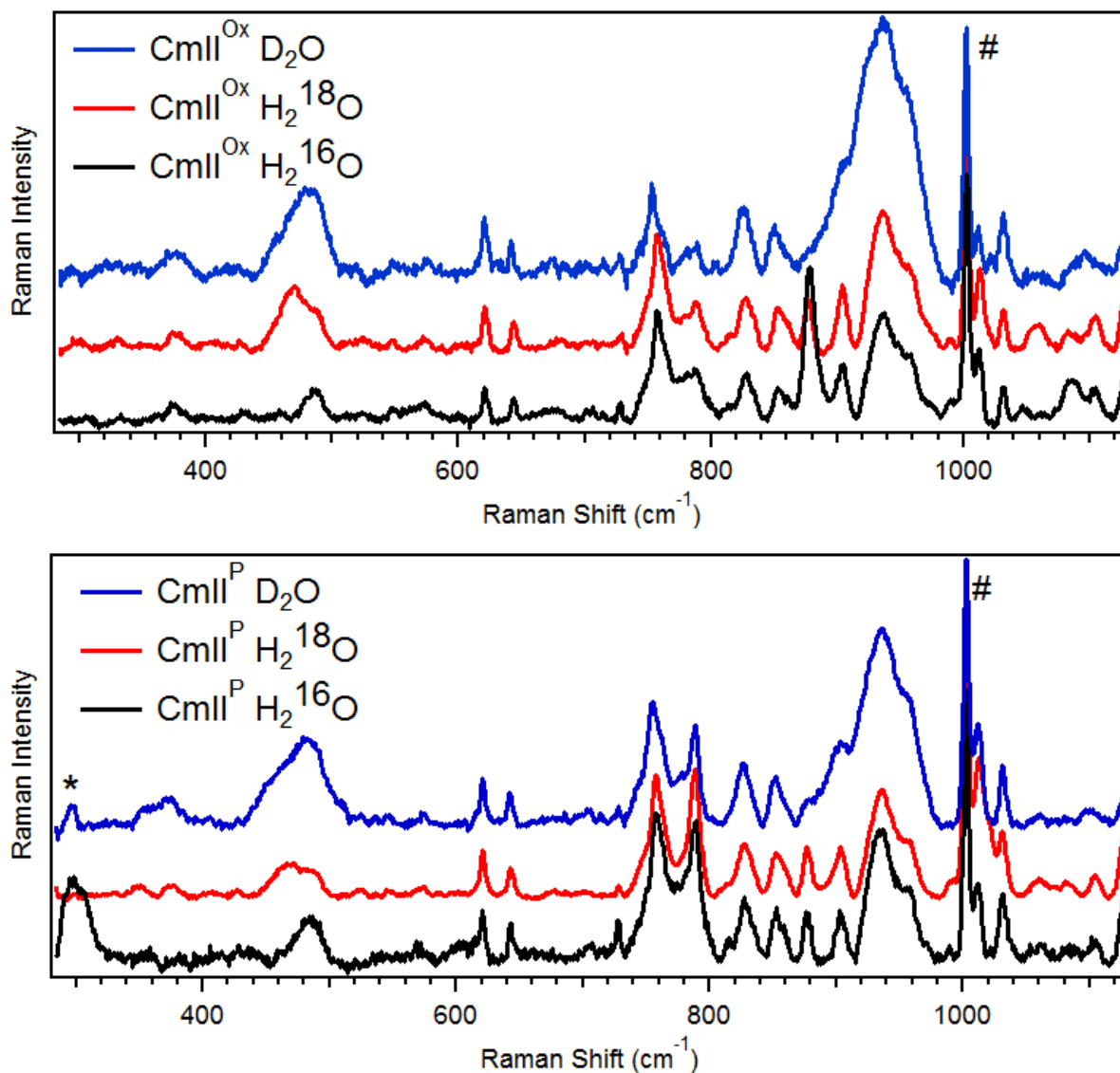
Fit	Fe-N		Fe-O		Fe•••Fe		Fe•••C		GOF						
	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	$E_0$	F	F'			
1	5	2.08	12.13							1.63	202	615			
2	4	2.09	9.45							2.62	199	610			
3	3	2.10	6.99							3.64	211	629			
4	5	2.08	9.28	1	1.86	9.72				-0.65	188	594			
5	5	2.08	8.96	2	1.86	14.71				-2.53	190	599			
6	4	2.10	7.67	1	1.91	9.84				0.24	187	593			
7	4	2.10	7.77	2	1.91	15.33				-1.50	187	592			
8	3	2.12	5.25	1	1.97	4.90				1.71	191	599			
9	3	2.11	6.41	2	1.96	13.93				-0.59	187	593			
10	3	2.13	2.58	2	1.98	2.05				-3.21	175	573			
				1	1.82	3.58									
11	3	2.12	2.10	1	1.99	-0.20				-3.64	176	574			
				2	1.88	9.04									
12	3	2.12	2.54	2	1.97	1.97	1	2.83	17.88	-3.29	172	568			
				1	1.82	3.39									
13	3	2.13	2.54	2	1.98	1.98	1	3.37	35.99	-3.14	175	572			
				1	1.82	3.54									
14	3	2.13	3.32	2	1.99	2.76	1	3.11	21.89	-2.23	154	536			
				1	1.83	4.53									
				1	2.83	0.21									
15	3	2.13	2.72	2	1.98	2.53	1	3.29	10.99	1	3.11	-2.30	-2.29	124	483
				1	1.82	4.54									
				1	2.82	3.75									
16	3	2.14	3.22	2	1.99	3.02	1	3.30	6.34	3	3.12	1.69	-0.99	133	499
				1	1.83	5.44									
				1	2.84	1.50									
17	3	2.13	2.84	2	1.98	2.50	1	3.11	7.61	3	3.34	0.91	-2.36	122	478
				1	1.83	4.47									
				1	2.83	1.23									
18	3	2.13	2.41	2	1.98	1.98	1	3.34	4.23	3	3.14	4.38	-2.40	118	469
				1	1.82	3.80				3	3.55	1.85			
				1	2.82	1.81									
19	3	2.12	6.25	2	1.96	12.77	1	3.30	5.03	3	3.12	1.55	0.43	130	494
				1	2.85	1.14				3	3.49	9.49			
20	3	2.13	1.98	2	1.97	1.52	1	3.33	2.88	3	3.15	2.58			
				1	1.8	3.09									
21	3	2.13	2.81	2	1.98	2.45	1	3.1	7.66	3	3.34	0.90	-2.42	118	470
				1	1.82	4.36				3	3.78	23.62			
				1	2.83	1.22									

**Table S3 (continued).** Fit parameters for the unfiltered EXAFS data of  $\text{CmII}^{\text{P}}$ , between  $k = 2 - 15 \text{ \AA}^{-1}$ . Fit 23 gives the most reasonable fit of the experimental data. The  $\text{Fe}\cdots\text{C}$  shells at 3.14, 3.56 and 4.28  $\text{\AA}$  are consistent with bound His ligands.

Fit	Fe-N			Fe-O			Fe $\cdots$ Fe			Fe $\cdots$ C			GOF			
	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	N	R( $\text{\AA}$ )	$\sigma^2(10^{-3})$	$E_0$	F	F'	
22	3	2.13	2.47	2	1.98	2.03	1	3.34	4.44	3	3.14	4.84	-2.11	109	451	
				1	1.83	3.93					3	3.56				1.65
				1	2.82	1.54					3	4.39				4.64
<b>23</b>	<b>3</b>	<b>2.13</b>	<b>2.50</b>	<b>2</b>	<b>1.98</b>	<b>2.09</b>	<b>1</b>	<b>3.35</b>	<b>4.47</b>	<b>3</b>	<b>3.14</b>	<b>4.87</b>	<b>-1.86</b>	<b>111</b>	<b>456</b>	
				<b>1</b>	<b>1.83</b>	<b>4.05</b>					<b>3</b>	<b>3.56</b>				<b>1.46</b>
				<b>1</b>	<b>2.82</b>	<b>1.50</b>					<b>3</b>	<b>4.28</b>				<b>5.12</b>
24	3	2.13	2.43	2	1.97	1.95	1	3.12	8.77	3	3.34	0.43	-3.59	116	465	
				1	1.82	3.50					3	3.54				8.78
				1	2.82	1.47					3	4.08				14.75
25	3	2.14	2.89	2	1.99	2.52	1	3.57	7.75	3	3.12	4.61	-177	113	459	
				1	1.83	4.66					3	3.34				1.43
				1	2.82	0.98					3	3.97				5.76
26	3	2.13	2.05	2	1.98	1.66	1	3.33	3.03	3	3.15	2.90	-2.79	130	493	
				1	1.82	3.51					3	3.54				1.85
											3	4.25				4.99
27	3	2.14	3.25	2	1.99	3.03	1	3.30	6.45	3	3.12	1.71	-0.87	131	496	
				1	1.84	5.59					3	4.29				8.00
				1	2.84	1.43										
28	3	2.14	3.38	2	1.99	2.97				3	3.09	3.59	-1.24	142	516	
				1	1.84	5.24	3	3.60	2.10							
				1	2.83	0.17	3	4.30	5.64							
29	3	2.14	2.81	2	1.99	2.37	1	3.36	4.55	3	3.15	7.15	-1.70	111	456	
				1	1.83	4.56					3	3.57				0.86
				0.5	2.82	-1.50					3	4.28				4.91

**Table S4.** Component Analysis of Pre-edge Features of CmlI Intermediates.

<b>Species</b>	<b>K-edge (eV)</b>	<b>Peak Position (eV)</b>	<b>Peak Area (units)</b>	<b>Ratio</b>
<b>CmlI<sup>R</sup></b>	7122.1	7113.0	0.14	0.1
		7113.1	6.7	4.4
		7114.8	1.5	1.0
			<b>Total = 8.3</b>	
<b>CmlI<sup>Ox</sup></b>	7124.1	7113.4	4.9	1.0
		7115.0	9.6	2.0
			<b>Total = 14.5</b>	
<b>CmlI<sup>P</sup></b>	7124.9	7113.7	5.1	1.0
		7114.9	14.1	2.8
			<b>Total = 19.2</b>	



**Figure S7.** Full resonance Raman spectra for **CmlI<sup>Ox</sup>** (top) and **CmlI<sup>P</sup>** (bottom) in H<sub>2</sub><sup>16</sup>O (black), H<sub>2</sub><sup>18</sup>O (red) and D<sub>2</sub><sup>16</sup>O (blue).  $\lambda_{\text{ex}} = 561 \text{ nm}$ , Power =  $\sim 100 \text{ mW}$ . All spectra were collected in solution at  $\sim 4 \text{ }^\circ\text{C}$ . Protein concentration  $\sim 1 \text{ mM}$  for each sample, 50 mM Bicine pH/pD = 9. H<sub>2</sub><sup>18</sup>O enrichment of the samples was  $\sim 60\%$ . All spectra were normalized to the sharp protein feature at  $1002 \text{ cm}^{-1}$  marked with #. Removal of the fluorescence background affected the normalization such that the intensities of the resonance Raman peaks are not necessarily comparable. The peak marked with \* is an artifact of the fluorescence background subtraction.