A Copper-Methionine Interaction Controls the pH-Dependent Activation of Peptidylglycine Monooxygenase†.

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Supporting Information

Table S1. Fits obtained to the EXAFS of the reduced peptidylglycine monooxygenase at pHs between 3 and 8. For the Cu-S component, the Debye-Waller term ($2\sigma^2$) was fixed at 0.010 Å^2, and the shell occupancy was refined in the fits.

	F[a]	No[b]	R (Å)[c]	DW (Å^2)	No[b]	R (Å)[c]	DW (Å^2)	No[b]	R (Å)[c]	DW (Å^2)	E_0
		Cu-N(His)[d]			Cu-O/N[e]			Cu-S			
pH 3	0.325	2.5	1.95	0.019	2	3.30	0.007	1.00	2.27	0.010	0.632
pH 3.5	0.327	2.5	1.95	0.018	2	3.30	0.007	1.05	2.27	0.010	0.665
pH 4	0.344	2.5	1.95	0.018	2	3.30	0.007	0.97	2.27	0.010	0.485
pH 4.5	0.388	2.5	1.94	0.018	2	3.30	0.010	0.79	2.26	0.010	0.436
pH 5	0.357	2.5	1.93	0.018	2	3.31	0.011	0.61	2.25	0.010	0.484
pH 5.5	0.377	2.5	1.93	0.017	1	3.28	0.011	0.51	2.25	0.010	0.626
pH 6	0.395	2.5	1.92	0.017	1	3.28	0.011	0.39	2.24	0.010	0.547
pH 7	0.373	2.5	1.92	0.016	1	3.26	0.011	0.45	2.24	0.010	-0.343
pH 8	0.300	2.5	1.92	0.016	1	3.28	0.011	0.40	2.23	0.010	-0.571

[a] F is a least-squares fitting parameter defined as $F^2 = \frac{1}{N}\sum_{i=1}^{N} k^6 (Data - Model)^2$

[b] Coordination numbers are generally considered accurate to ± 25%

[c] In any one fit, the statistical error in bond-lengths is ±0.005 Å. However, when errors due to imperfect background subtraction, phase-shift calculations, and noise in the data are compounded, the actual error is probably closer to ±0.02 Å.

[d] Fits modeled histidine coordination by an imidazole ring, which included single and multiple scattering contributions from the second shell (C2/C5) and third shell (C3/N4) atoms respectively. The Cu-N-C_x angles were as follows: Cu-N-C2 126°, Cu-N-C3 -126°, Cu-N-N4 163°, Cu-N-C5 -163°.

[e] A shell of single scattering outer shell C atoms, most likely originating from methionine methyl and methylene groups.

Table S2. Apparent kinetic constants of WT and M314H PHMcc for dansyl-YVG. Reactions were performed in a 2mL volume by monitoring the rate of oxygen comsumption in an oxygen-sensitive electrode with the following reagent concentrations. 100 mM NaMES pH 5.5, ascorbate 10 mM, Cu^{2+}(aq) 5 μM, catalase 26,000 units, PHMcc 0.05 μM for WT or 0.5 μM for M314H, dansyl-YVG variable between 5 and 80 μM (WT) or 5 and 400 μM (M314H). Reactions were initiated by addition of dansyl-YVG substrate. Coupling ratios were determined in the oxygen-sensitive electrode by allowing the reaction to run out of oxygen and measuring the amount of substrate consumed by HPLC.

	K'_m (μM)	k'_{cat} (s^{-1})	k'_{cat}/K'_m ($\mu M^{-1}s^{-1}$)	Coupling Ratio
WT	8.2 ± 0.9	13.8 ± 0.4	1.68	
M314H	37 ± 4	2.5 ± 0.1	0.07	1.004

Figure S1. Experimental (black) and simulated (red) EXAFS spectra of WT PHMcc between pHs 3 and 8, using parameters as listed in Table S1.

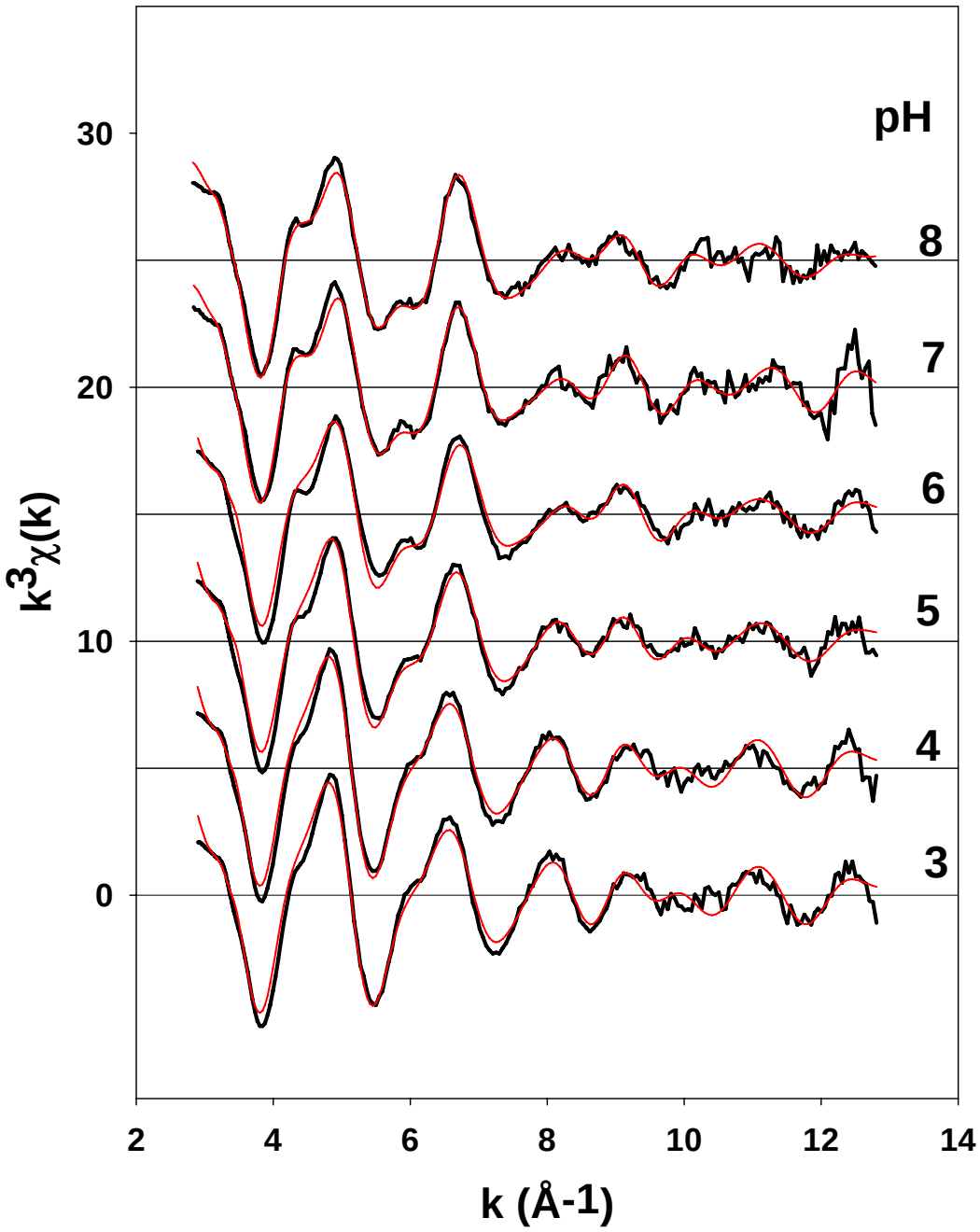


Figure S2. Experimental (black) and simulated (red) Fourier transform spectra of WT PHMcc between pHs 3 and 8, using parameters as listed in Table S1.

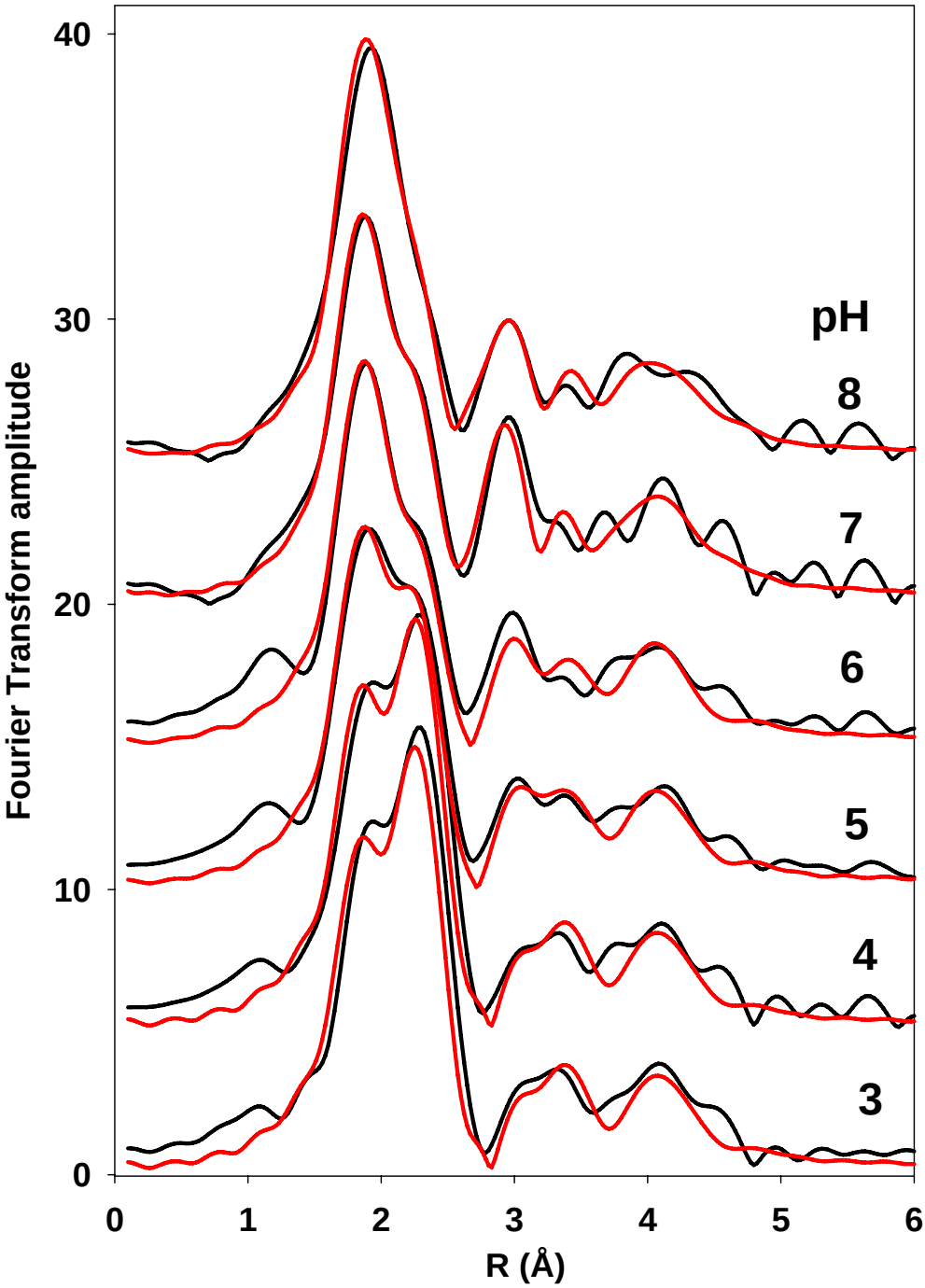
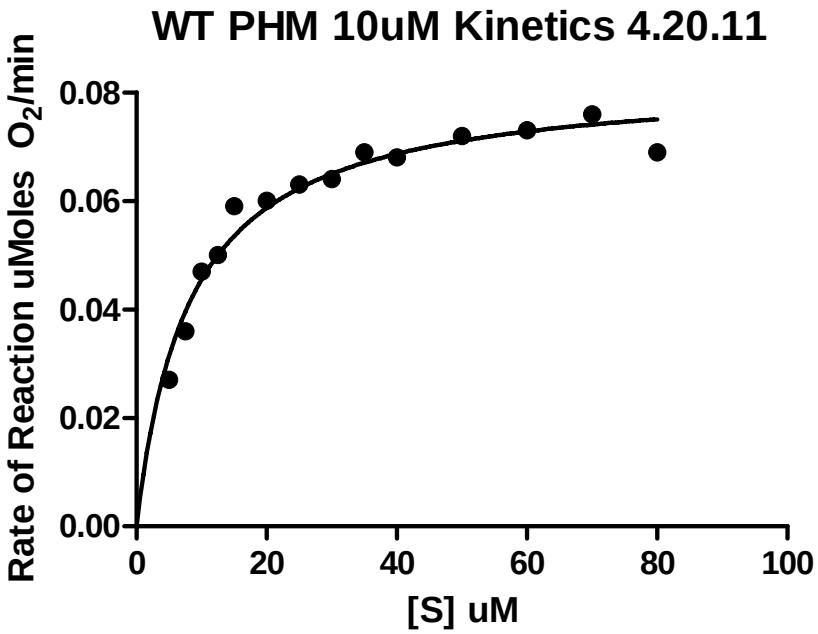


Figure S3. Dependence of the rate of oxygen consumption on dansyl-YVG concentration for WT and the M314H variant using the assay conditions listed in Table S2.



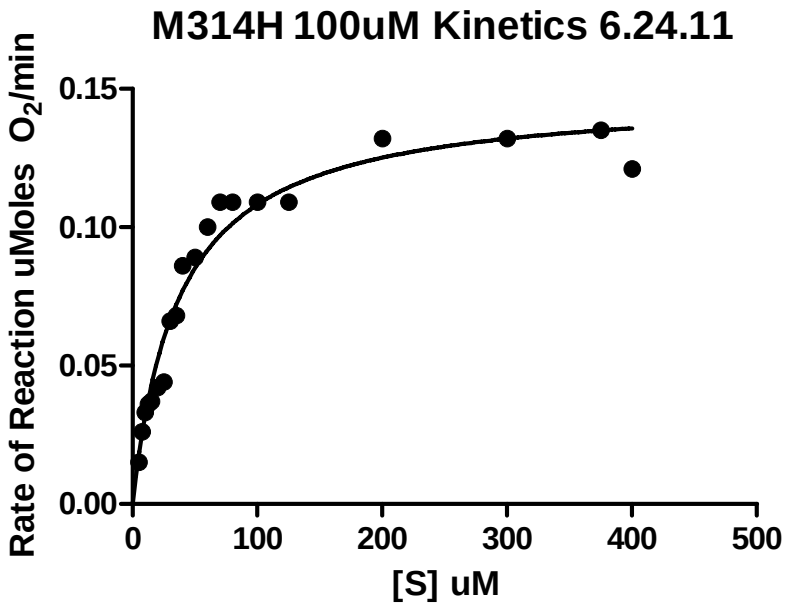


Figure S4. Experimental (black) and simulated (red) Fourier transform and EXAFS spectra (inset) of the oxidized M314H variant of PHMcc in formate-MES-HEPES-CHES mixed buffer system at pH 7.0

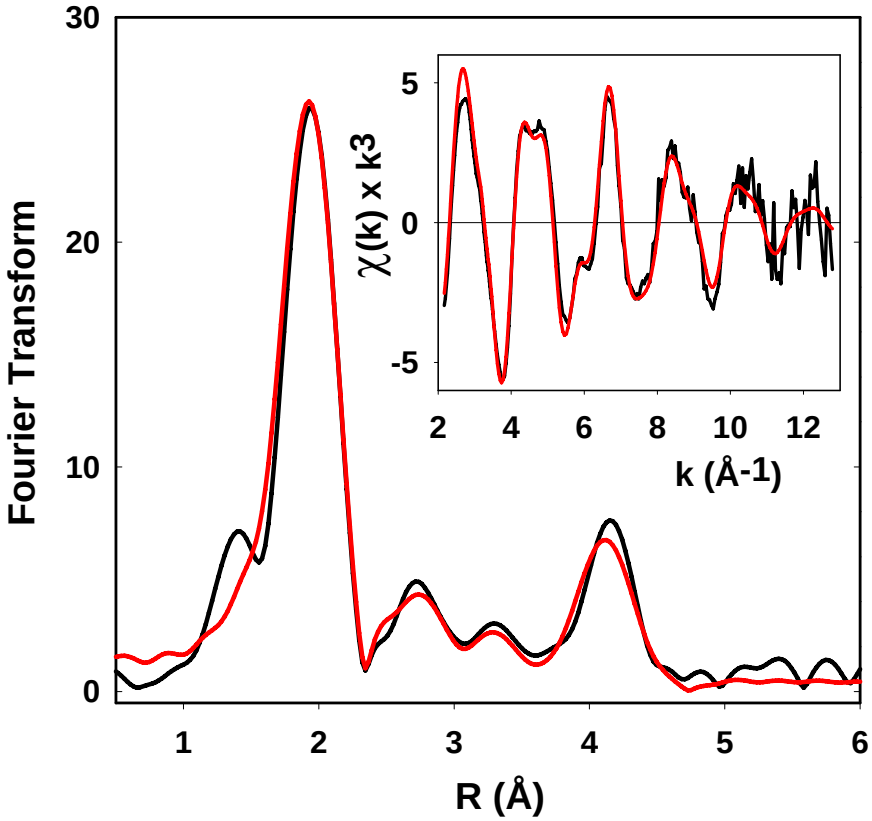


Figure S5. Comparison of the Fourier transforms and EXAFS data for WT PHMcc in formate-MES-HEPES-CHES mixed buffer system at pH 4.0, reduced with either 1 equivalent (top) or 5 equivalents (bottom) ascorbate per copper. The top spectrum was simulated by 2.5 Cu-N(His) at 1.95 Å and 0.92 Cu-S at 2.27 Å while the bottom spectrum was simulated by 2.5 Cu-N(His) at 1.95 Å and 0.99 Cu-S at 2.26 Å. The Debye-Waller term for the Cu-S interaction was fixed at 0.010 $Å^2$.