Supporting information for the article

Methylated Histidines Alter Tautomeric Preferences that Influence the Rates of Cu Nitrite Reductase Catalysis in Designed Peptides

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1. EXAFS Fitting Parameters at pH 5.9 for all constructs reported

Sample	Model	Cu-N R (Å)	Cu-N σ^2 (Å^2) 1 x10^{-3}	Cu-O R (Å)	Cu-O σ^2 (Å^2) 1 x 10^{-3}	F
Cu(I)(TRIW-δ_mH)$_3$	CuN_2	1.93	5.5	N/A	N/A	95
	CuN_3	1.94	7.2	N/A	N/A	58
	CuN_2O	1.93	8.7	1.95	2.8	52
	CuN_3O	1.94	7.6	1.92	17.8	50
Cu(I)(TRIW-δ_mH L19A)$_3$	CuN_2	1.89	8.1	N/A	N/A	53
	CuN_3	1.89	10.4	N/A	N/A	41
	CuN_2O	1.89	10.5	1.90	9.0	38
	CuN_3O	1.89	10.3	1.46	94.8	39
Cu(I)(TRIW-ε_mH)$_3$	CuN_2	1.90	9.4	N/A	N/A	45
	CuN_3	1.90	11.8	N/A	N/A	54
	CuN_2O	1.89	9.1	2.02	31	42
	CuN_3O	1.90	10.9	2.16	8.0	47

Table S1. Cu(I) EXAFS fitting parameters for all constructs reported in main manuscript. Only the parameters that were refined are shown. Histidine outer-shell paths are calculated using a rigid imidazole group as a model (denoted by "N" in the model designation). Fits were performed using all outer-shell paths.

Sample	Model	Cu-N R (Å)	Cu-N σ^2 (Å^2) 1 x 10^{-3}	Cu-O R (Å)	Cu-) σ^2 (Å^2) 1 x 10^{-3}	F
Cu(II)(TRIW-H)$_3$	CuN_2O_2	1.935	9.6	1.990	12.0	160
	CuN_3	1.937	8.1	N/A	N/A	188
	CuN_3O_1	1.910	7.9	2.023	1.04	103
	CuN_3O_2	1.916	11.4	1.987	7.0	121
Cu(II)(TRIW-δ_mH)$_3$	CuN_2O_2	1.912	12.8	1.962	4.2	125
	CuN_3	1.937	7.5	N/A	N/A	177
	CuN_3O_1	1.913	10.5	1.980	1.3	113
	CuN_3O_2	1.942	7.8	1.923	21.2	150
Cu(II)(TRIW-ε_mH)$_3$	CuN_2O_2	1.929	11.3	1.974	4.7	131
	CuN_3	1.951	7.4	N/A	N/A	250
	CuN_3O_1	1.943	10.4	1.972	2.9	157
	CuN_3O_2	1.935	23.1	1.966	3.5	145.2

Table S2. Cu(II) EXAFS fitting parameters for all constructs reported in main manuscript. Only the parameters that were refined are shown. Histidine outer-shell paths are calculated using a rigid imidazole group as a model (denoted by "N" in the model designation). Fits were performed using all outer-shell paths.

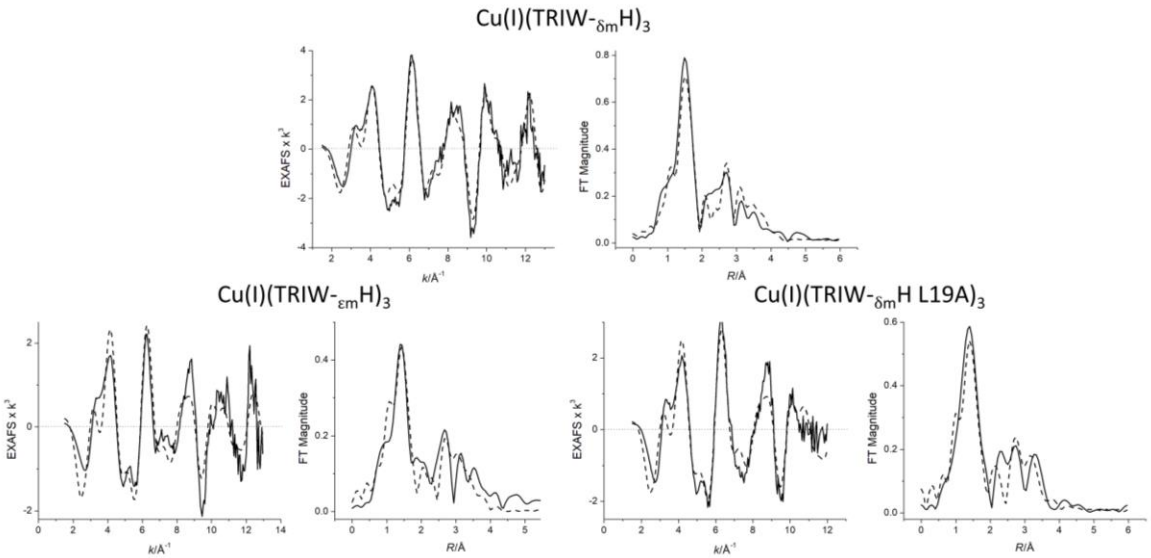

Figure S1. EXAFS and the Fourier Transform of EXAFS of Cu(I) bound forms of all constructs reported at pH 5.9. Fits were created using the best fit models reported in main manuscript ($Cu^{I}(Imid)_3$ for all constructs)

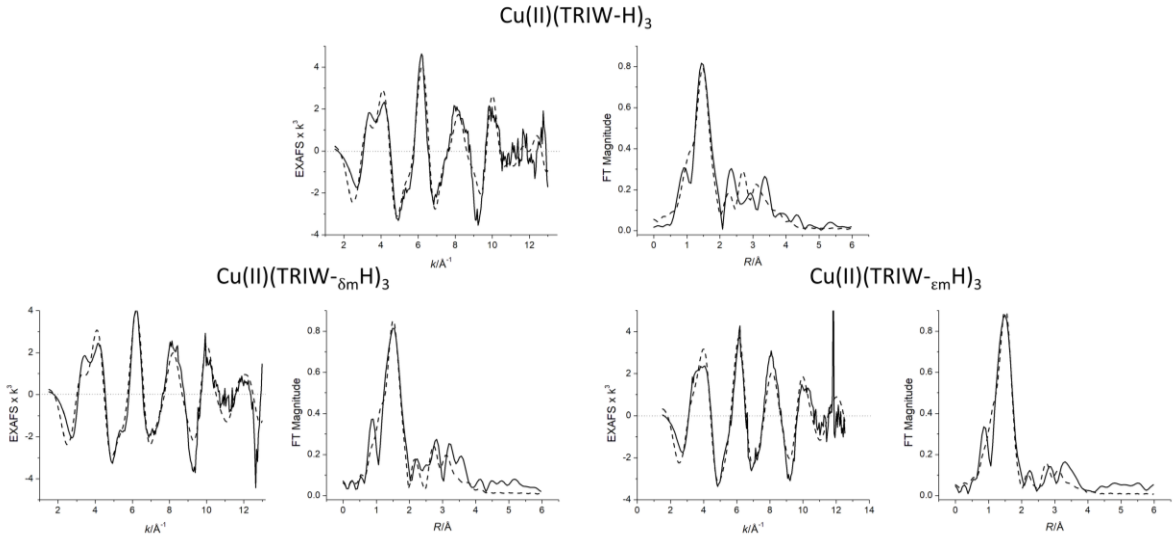

Figure S2. EXAFS and the Fourier Transform of EXAFS of Cu(II) bound forms of TRIW-H, TRIW-δmH, and TRIW-εmH at pH 5.9. Fits were created using the best fit models reported in main manuscript ($Cu^{II}(Imid)_3O_1$ for TRIW-H and TRIW-δmH and $Cu^{II}(Imid)_2O_2$ for TRIW-εmH)

2. XANES Comparison of Cu(II) Constructs Reported

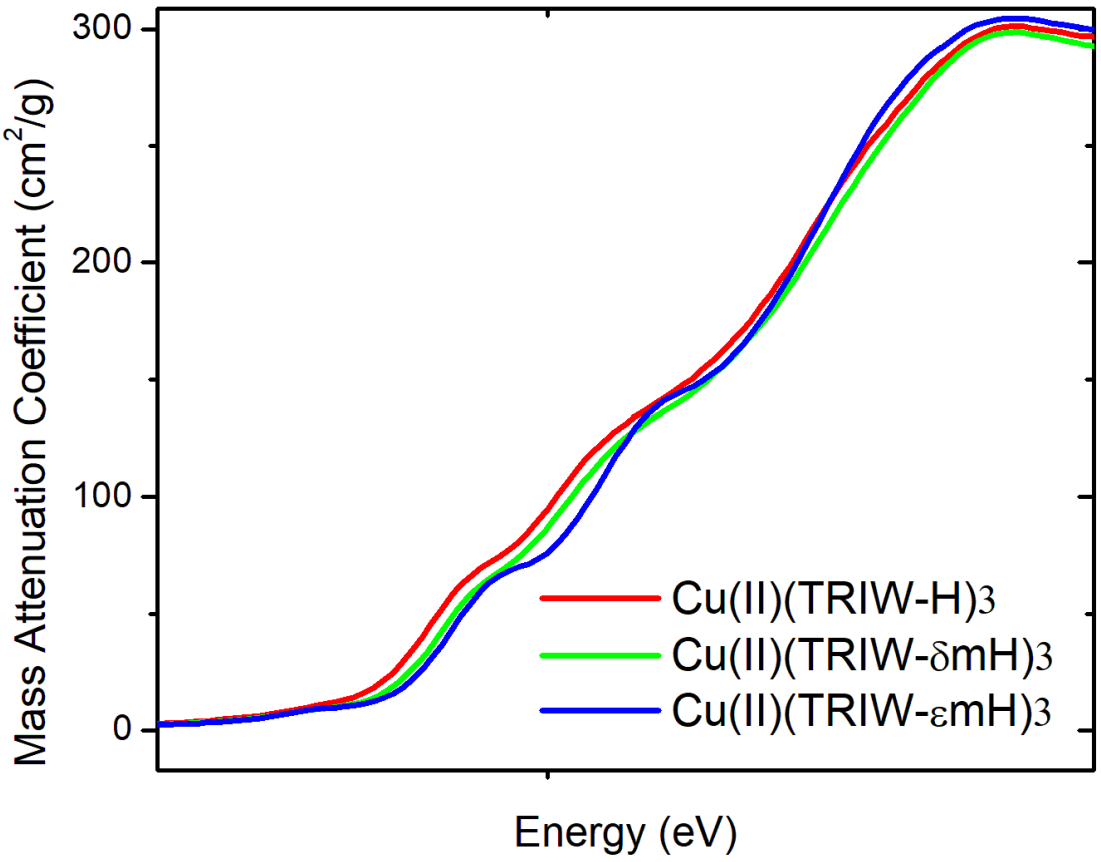

Figure S3. Cu(II) XANES at pH 5.9 of methylated His constructs reported compared to that of TRIW-H

3. EXAFS Signal Comparison at pH 5.9

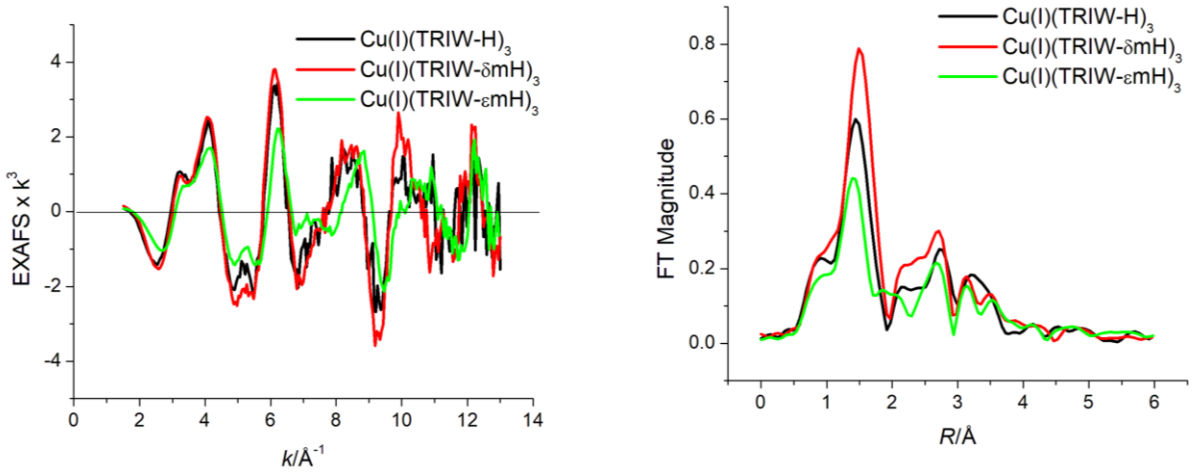

Figure S4. Cu(I) EXAFS at pH 5.9 of methylated His constructs reported compared to that of TRIW-H

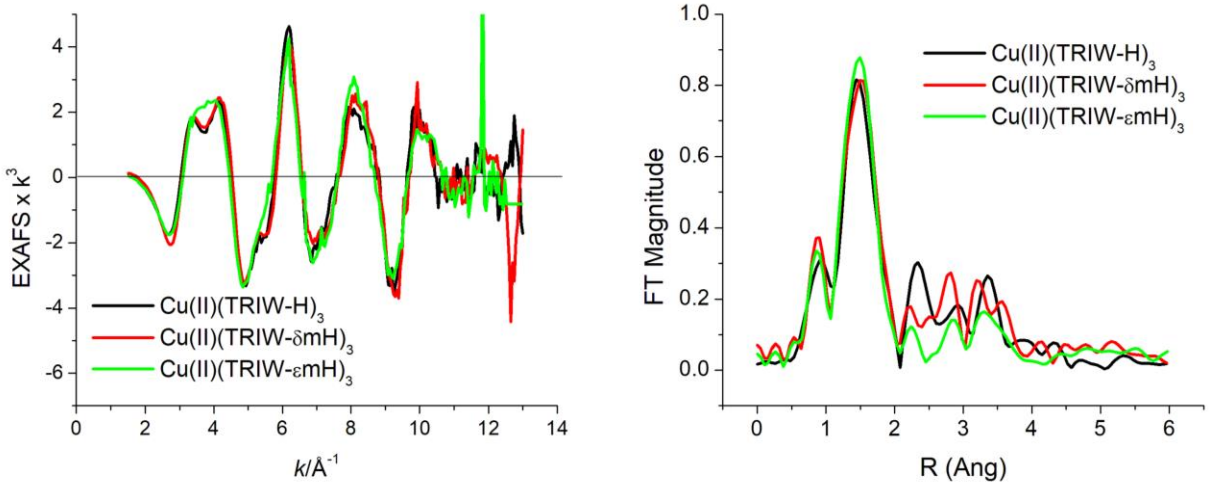

Figure S5. Cu(II) EXAFS at pH 5.9 of methylated His constructs reported compared to that of TRIW-H

4. Example Cu(II) Titration for K_D determination

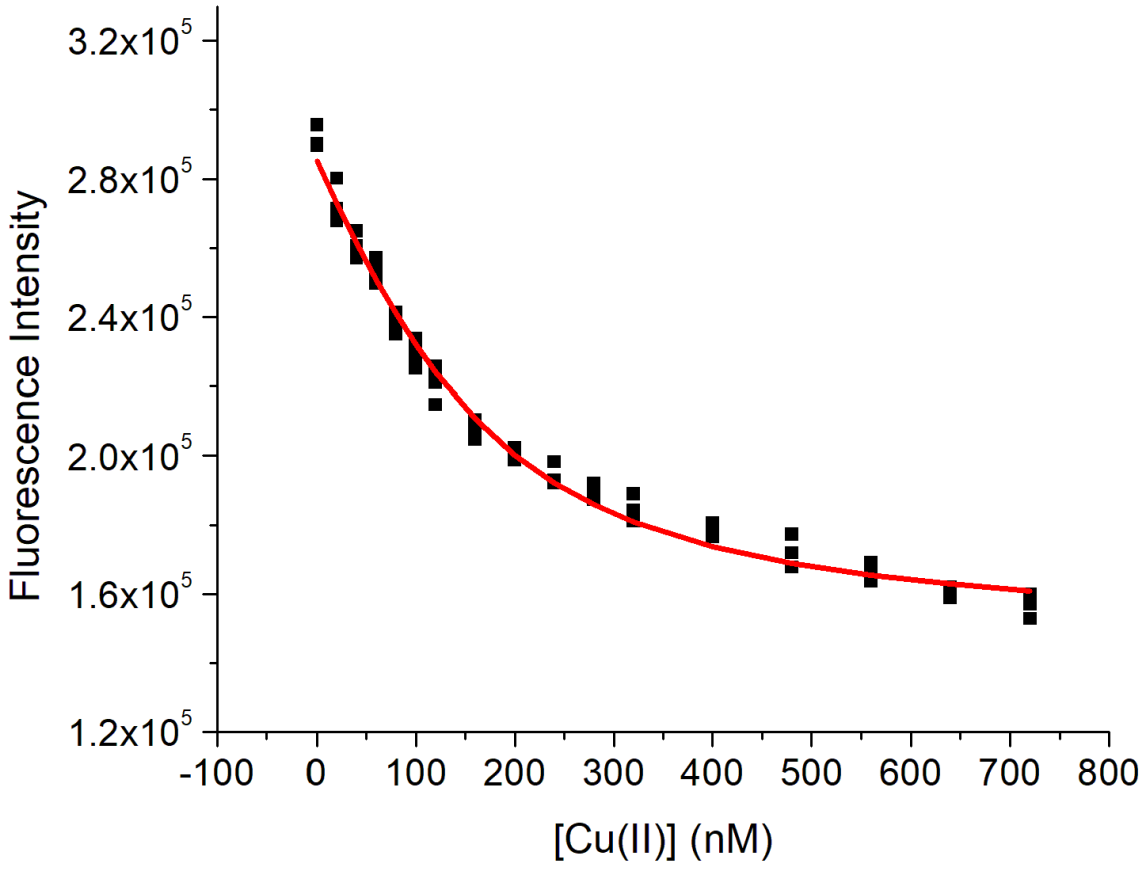

Figure S6. Titration of 140nM (TRIW-$_{\varepsilon m}$H)$_3$ in 50mM MES at pH 5.9 with Cu(II). Titration was monitored via tryptophan fluorescence quenching. Data points are depicted in black while the fit is depicted in red.

5. Example Cu(I) Titration for K_D determination

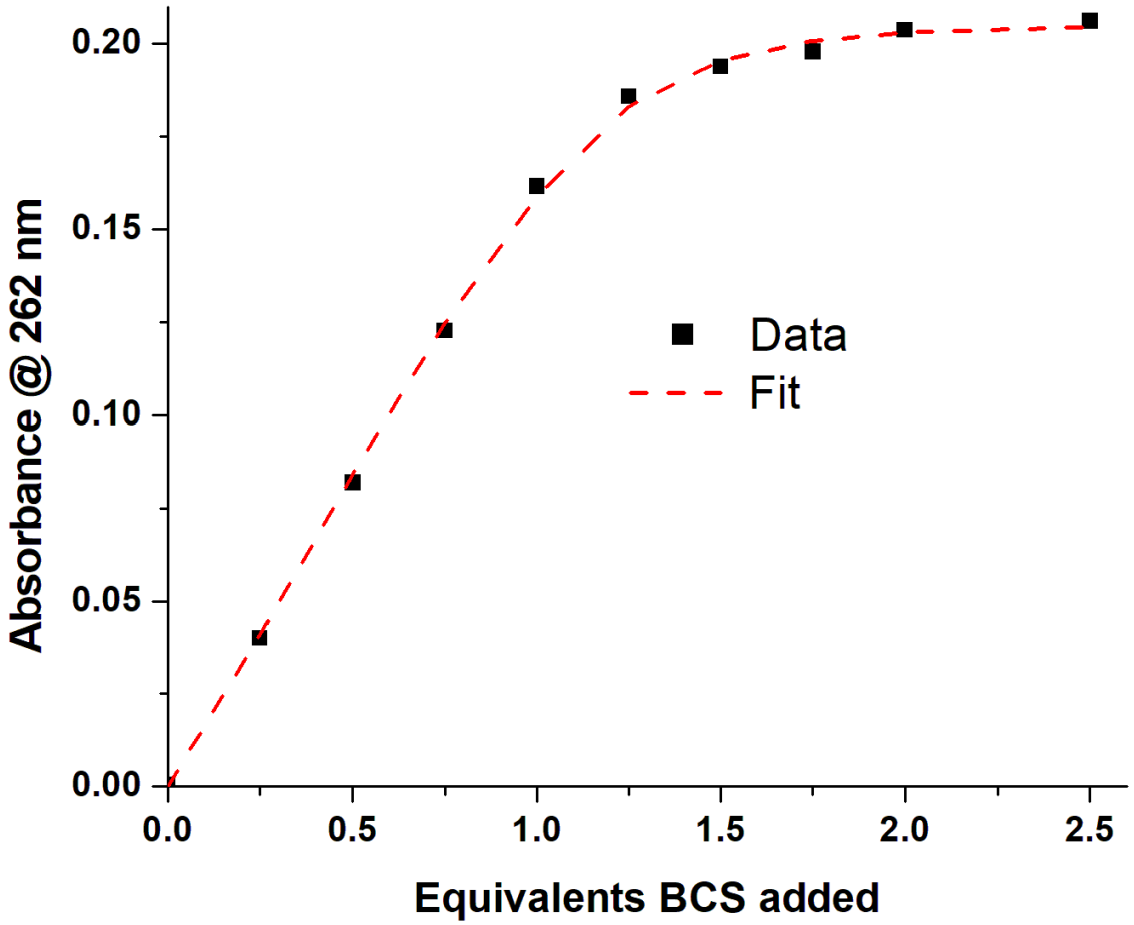

Figure S7. Titration of 100 μM (TRIW-$_{\varepsilon m}$H)$_3$ and 50 μM Cu(I) in 50mM MES at pH 5.9 with bathocuproine disulfonate.

6. Electron Paramagnetic Resonance Spectra and Fits

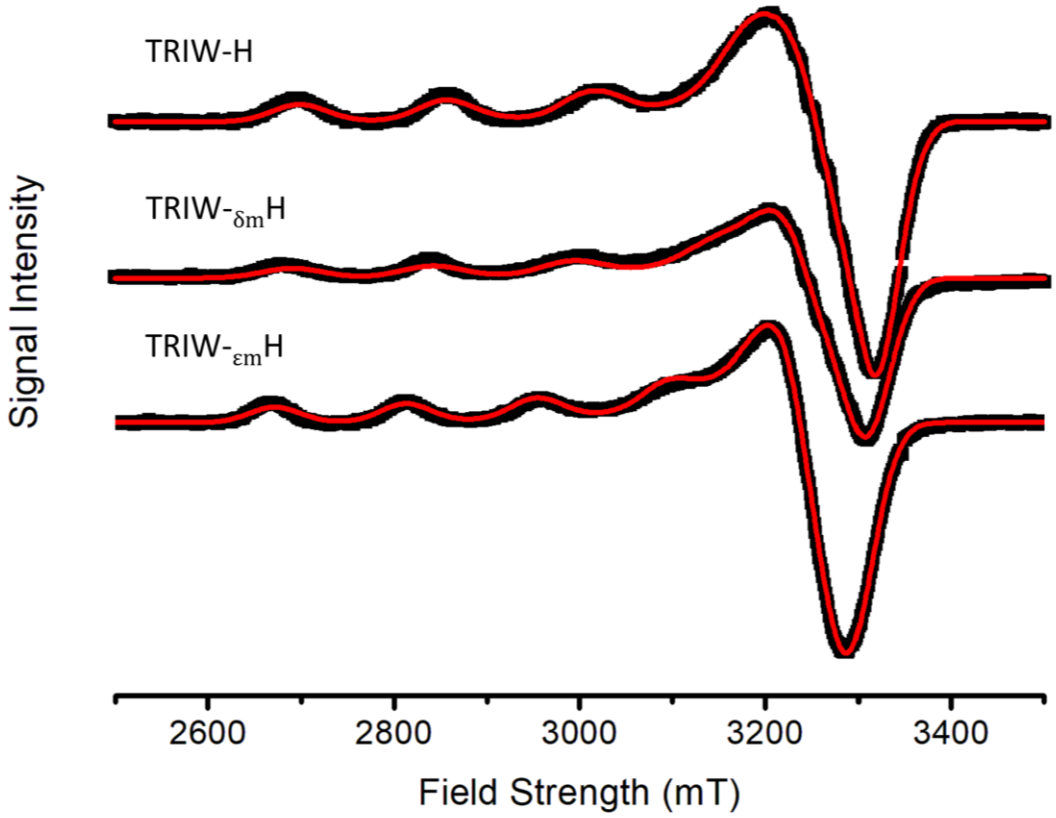

Figure S8. EPR spectra and fits for Cu(II)(TRIW-H)$_3$, Cu(II)(TRIW-δ_mH)$_3$, and Cu(II)(TRIW-ε_mH)$_3$ at pH 5.9. Fits were done using SpinCount.

7. UV-Vis Spectra of Cu(II)(TRIW-$_{\varepsilon m}$H)$_3$ and Cu(II)(TRIW-$_{\delta m}$H)$_3$

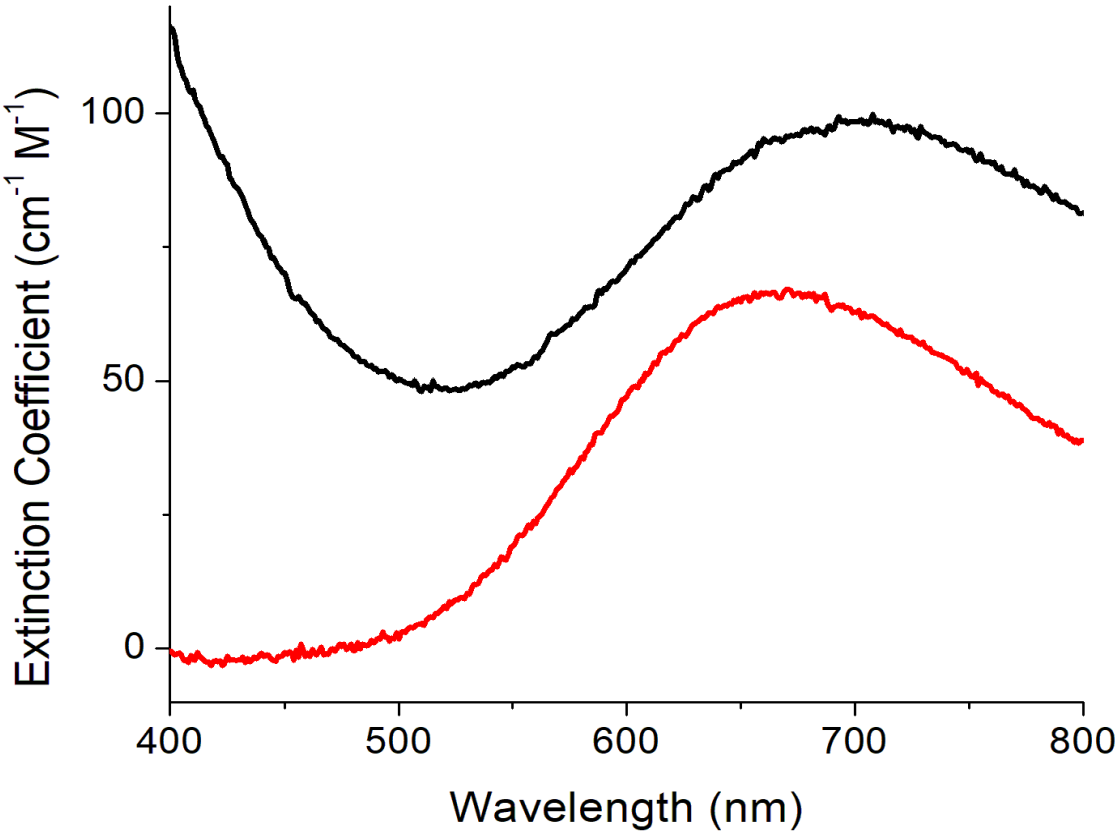

Figure S9. Electronic absorption spectra at pH 5.8 of 300 µM Cu(II) in the presence of 300 µM (Black) (TRIW-$_{\varepsilon m}$H)$_3$ or (red) (TRIW-$_{\delta m}$H)$_3$.