Supporting Information

A Cobalt Metallopeptide Electrocatalyst for the Selective Reduction of Nitrite to Ammonium

Yixing Guo,[†] Jesse R. Stroka,[†] Banu Kandemir,[†,‡] Claire E. Dickerson,[†] and Kara L. Bren[†,*]

[†]Department of Chemistry, University of Rochester, Rochester, NY 14627-0216

[‡]Department of Chemistry, Middle East Technical University Northern Cyprus Campus, Mersin 10 Turkey"

*Corresponding Author. E-mail: bren@chem.rochester.edu

CoGGH preparation and characterization

Cobalt-Gly-Gly-His (CoGGH) was prepared and purified as described previously, yielding Co(III)GGH.[1] Co(III)GGH in Millipore-purified water at pH 8.0 was characterized by electrospray ionization mass spectrometry (ESI-MS; Electrospray Thermo Scientific LTQ Velos ESI/ion-trap mass spectrometer). ESI-MS in positive and negative mode shows one major peak consistent with the calculated molecular weight of CoGGH (Figures S1, S2). UV-vis absorption spectroscopy (using a Shimadzu UV-2401PC) was performed on CoGGH in 50 mM MOPS, pH 8.0 (Figure S3). The diamagnetic Co(III)GGH complex displays UV-vis bands at 427 nm and 545 nm that are attributed to ligand-field transitions in a pseudo-octahedral ligand field (Figure S3).[1,2] For characterization of Co(II)GGH, a deoxygenated solution of Co(III)GGH in 50 mM MOPS, pH 8.0, was reduced with 20 equivalents of $Na_2S_2O_4$ (Sigma Aldrich) in a Vac Atmospheres glovebox under nitrogen. Co(II)GGH has a broad absorption feature centered at ~625 nm, attributed to ligand-field transitions.[3] The X-band EPR spectrum of Co(II)GGH was recorded at 5.8 K in 50 mM pH 8.0 MOPS buffer : glycerol (2:3) at 1 mW power. The spectrum is indicative of a high-spin (S = 3/2) species (Figure S4). CoGGH has been resistant to crystallization; its coordination sphere in different redox states will be deduced in future work using a suite of spectroscopic methods, aided by computation. For studies of substrate reduction by CoGGH, $NaNO_2$ (Sigma Aldrich) was used as the source of nitrite, NH_2OH*HCl (Sigma Aldrich) was used as a NH_2OH source. Nitric oxide gas (Airgas) was purified by passing through an Ascarite (Sigma-Aldrich) column with NaOH on silica; a −80 ºC cold trap was used to remove any remaining nitrogen oxide impurities. CuGGH and NiGGH used in control experiments were prepared as described.[1]

Electrochemical methods

Cyclic voltammetry (CV) measurements were performed on a CH Instruments electrochemical analyzer (CHI620D). A three-electrode setup was used, consisting of a platinum wire auxiliary electrode (Alfa Aesar), Ag/AgCl (1 M KCl) reference electrode (CH instruments), and a hanging mercury drop electrode, the HMDE WK2, from the Polish Academy of Sciences. An HMDE was used as the working electrode because mercury is also known to inhibit the activity of colloidal catalysts,[4,5] and forms an amalgam with cobalt.[6] Furthermore, a HMDE has low background in water. A rinse test demonstrated that CoGGH does not adsorb to the mercury.[1] Notably, the onset potential for proton reduction by CoGGH (−1.2 V vs. Ag/AgCl (1M

KCl)[1] is outside of the window for nitrite reduction by CoGGH. CV was performed in 50 mM MOPS buffer under nitrogen at a scan rate of 0.1 V/s and pH of 7.2 on 5-mL solutions except where noted otherwise.

Controlled potential electrolysis (CPE) experiments were performed in a custom electrolysis cell (Adams & Chittenden) with three equi-volume chambers of 1.5-cm diameter and 6-cm height. The center compartment contains the mercury pool electrode while the two other compartments contain a Pt coil auxiliary electrode and an Ag/AgCl (1 M KCl) reference electrode. For each experiment, 5 mL of solution was placed into each of the three cell chambers. The cell was purged with nitrogen gas (Airgas) for 20 minutes before each experiment, unless indicated otherwise. CPE of nitric oxide samples was conducted on deoxygenated solutions of pH 7.2 MOPS buffer under 1 atm of nitric oxide gas. CPE of hydroxylamine was performed using 1 M hydroxylamine hydrochloride as the source of NH_2OH in pH 7.2 MOPS buffer. After CPE, the solution was tested for ammonia using a standard colorimetric method.[7] In a typical experiment, 5 μL of CPE solution, 0.4 mL of 1 M phenol solution, 0.4 mL of 2 M nitroferricyanide solution, and 1 mL of oxidizing solution (25% Clorox bleach, 0.6 M sodium citrate, 0.2 M NaOH) were added and diluted with water to a final volume of 10 mL. Absorbance was measured at 630 nm. A standard calibration curve was constructed by plotting A(630 nm) vs. $[NH_4^+]$ using known amounts of $(NH_4)_2SO_4$ (Sigma Aldrich) (Figure S12 and S13). After the amount of ammonia generated was determined, turnover number (TON), turnover frequency (TOF), and Faradaic efficiency values were calculated using the following equations:

$$\text{TON} = n(NH_4^+) / n(\text{CoGGH})$$

$$\text{TOF} = \text{TON}/t$$

$$\text{Faradaic efficiency (\%)} = (n_e \cdot F \cdot 100\%)/Q_T$$

Where value $n(NH_4^+)$ indicates the moles of ammonium produced, $n(\text{CoGGH})$ indicates number of moles of catalyst in the reaction, t is the length of the CPE experiment, n_e is the moles of electrons needed to produce the observed amount of ammonium (see equation 1), F is the Faraday constant, and Q_T is the total amount of charge passed in the CPE experiment in Coulombs.

Supporting Figures

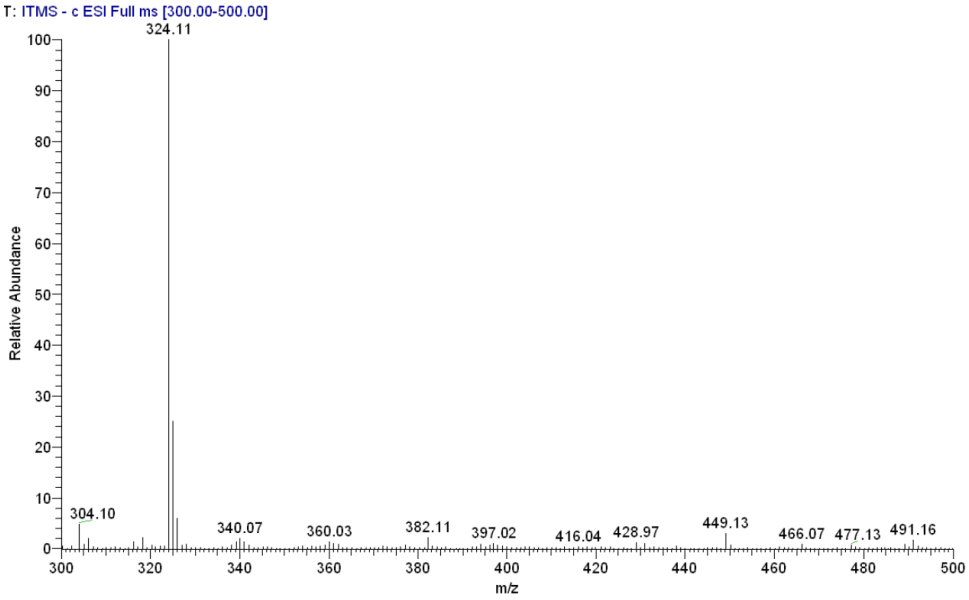

Figure S1. ESI-MS of CoGGH. CoGGH (10 mM) was prepared in nitrogen-purged water at pH 8.0 for injection. Data collected in negative mode. Observed: 324.11, calculated: 324.09.

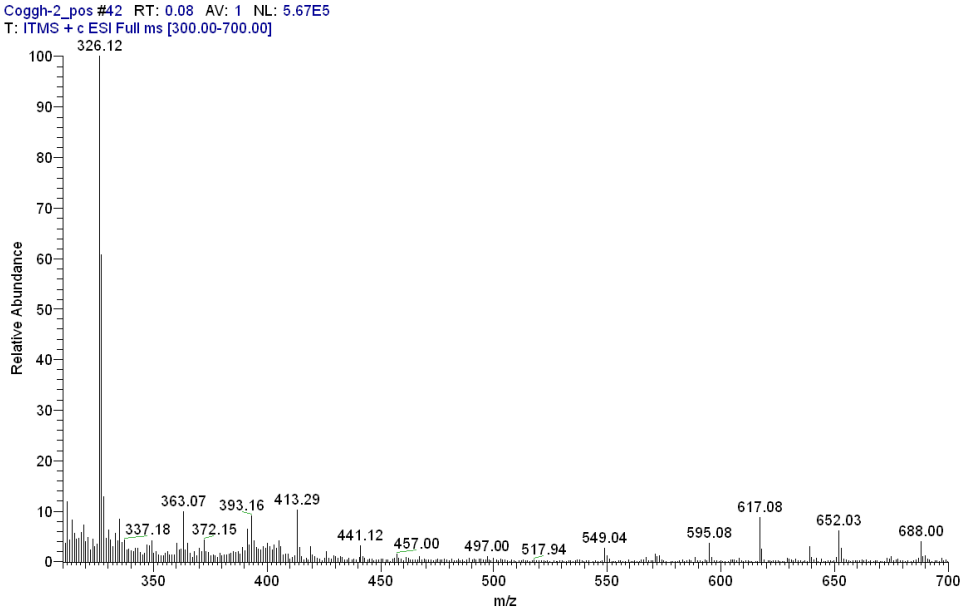

Figure S2. ESI-MS results of CoGGH. CoGGH (10 mM) was prepared in nitrogen-purged water at pH 8.0 for injection. Data collected in positive mode. Observed: 326.12. Calculated: 326.01.

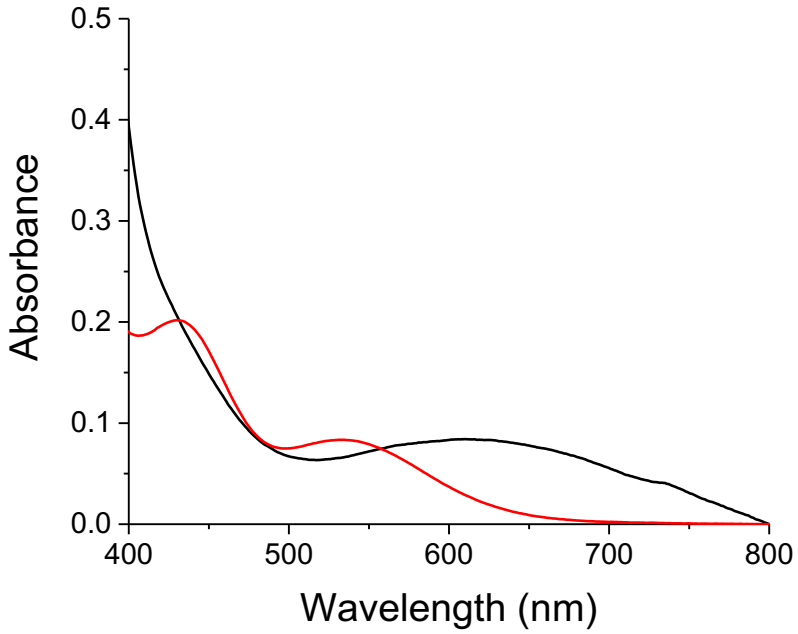

Figure S3. UV-vis spectrum of 1 mM Co(III)GGH in 50 mM MOPS buffer, pH 8.0 (red) and the same sample after reduction with $Na_2S_2O_4$ to yield Co(II)GGH (black).

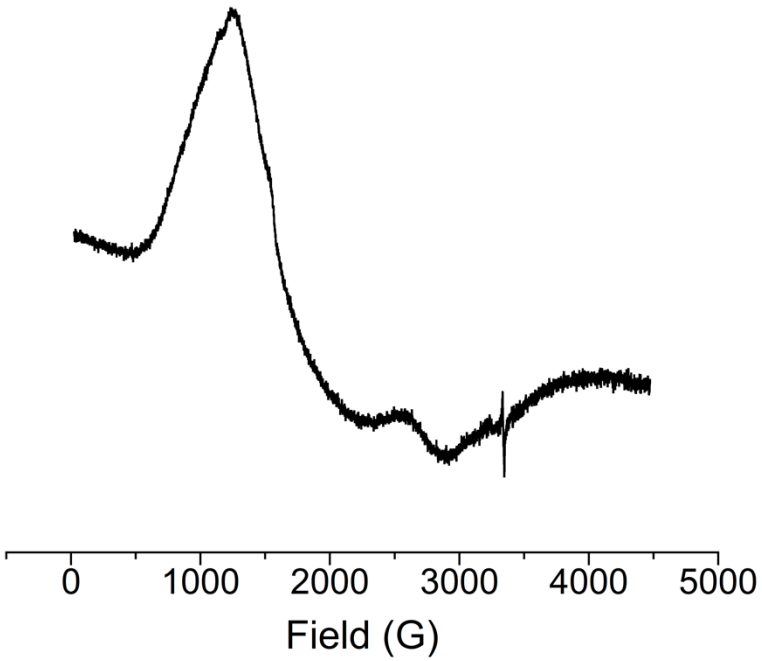

Figure S4. X-band EPR spectrum of 1 mM Co(II)GGH in 50 mM MOPS buffer, 60% glycerol, reduced by addition of $Na_2S_2O_4$. T = 5.8 K, microwave frequency 9.38 GHz, power: 1 mW.

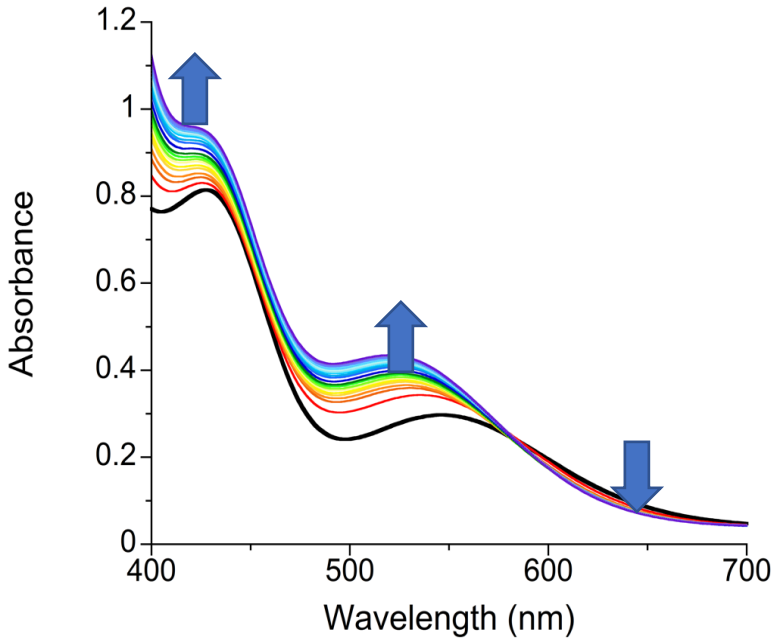

Figure S5. UV-vis spectra of 4.9 mM Co(III)GGH in the presence of variable amounts of $NaNO_2$, in 50 mM MOPS, pH 8.0. Arrows indicate the effect of increasing [$NaNO_2$] from 0 to 40 mM. The thick black line is the spectrum of Co(III)GGH before addition of sodium nitrite.

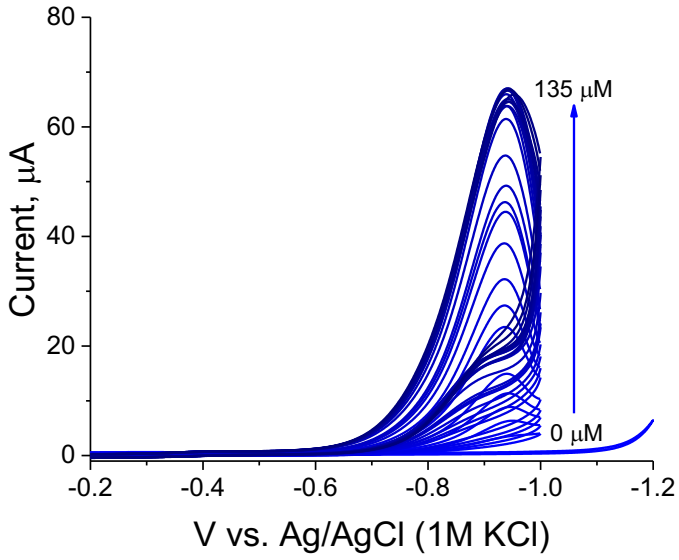

Figure S6. Cyclic voltammetry of 100 mM sodium nitrite in the presence of varied (0-135 µM) CoGGH, in 50 mM pH 7.2 MOPS buffer. Scan rate 100 mV/s. HMDE working electrode, Pt counter electrode, Ag/AgCl (1 M KCl) reference electrode.

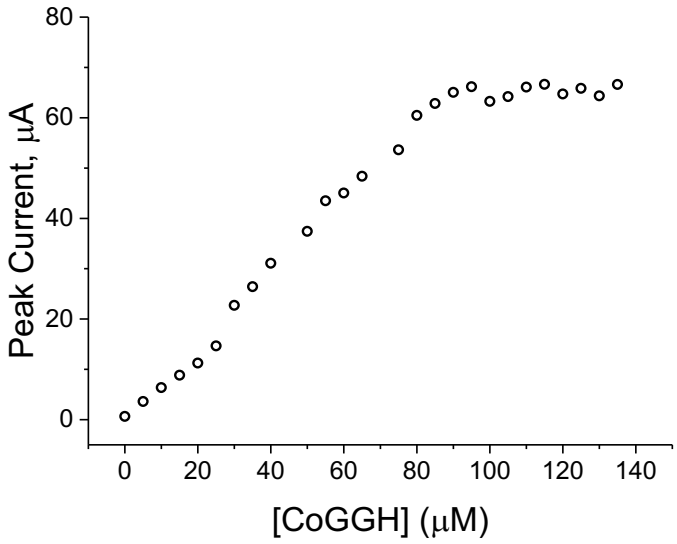

Figure S7. Peak current as a function of [CoGGH] for the data shown in Figure S6.

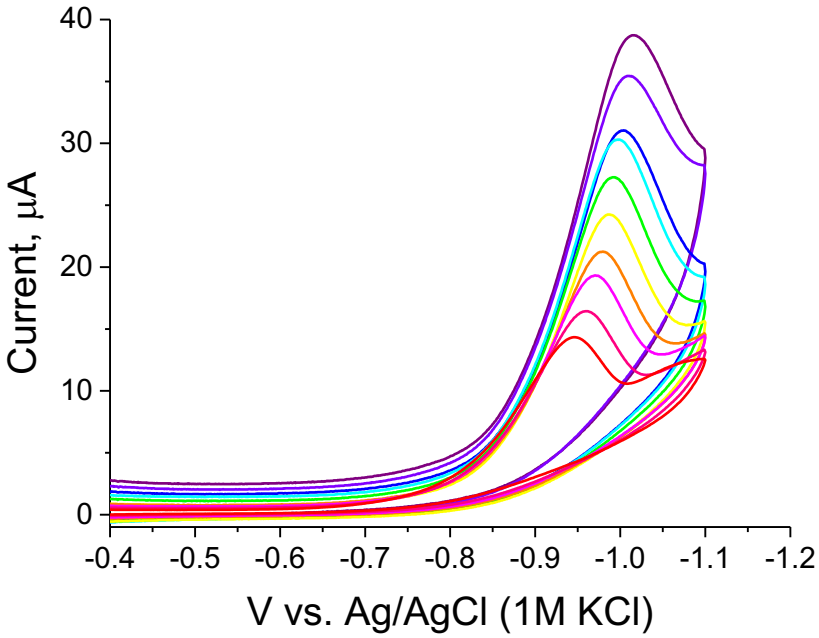

Figure S8. Cyclic voltammetry of 25 μM CoGGH in 100 mM $NaNO_2$, 50 mM pH 7.2 MOPS buffer solution as a function of scan rate (100 mV/s to 1 V/s in 100 mV/s increments, with peak current increasing as scan rate is increased).

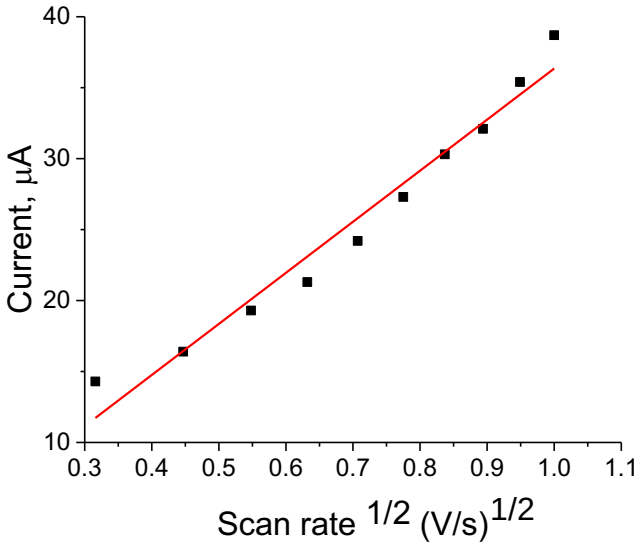

Figure S9. Peak current as a function of square root of scan rate $(V/s)^{1/2}$. 25 µM CoGGH in 100 mM $NaNO_2$, 50 mM MOPS buffer solution at pH 7.2.

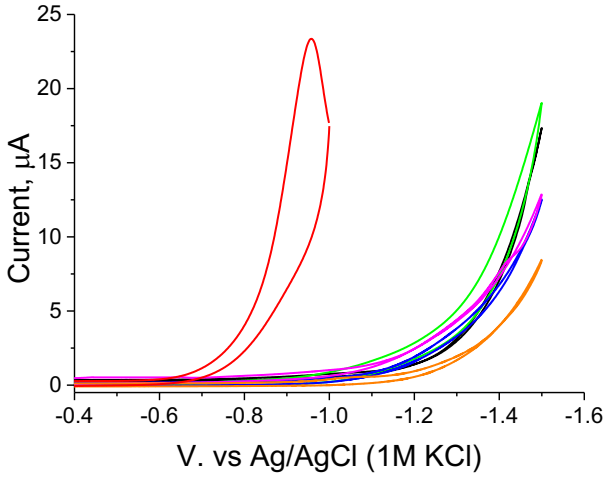

Figure S10. Cyclic voltammograms of 50 µM CoGGH (red), $CoCl_2$ (green), NiGGH (pink), CuGGH (blue), free GGH (orange) and no added catalyst (black), with 100 mM $NaNO_2$ 50 mM MOPS, pH 7.2.

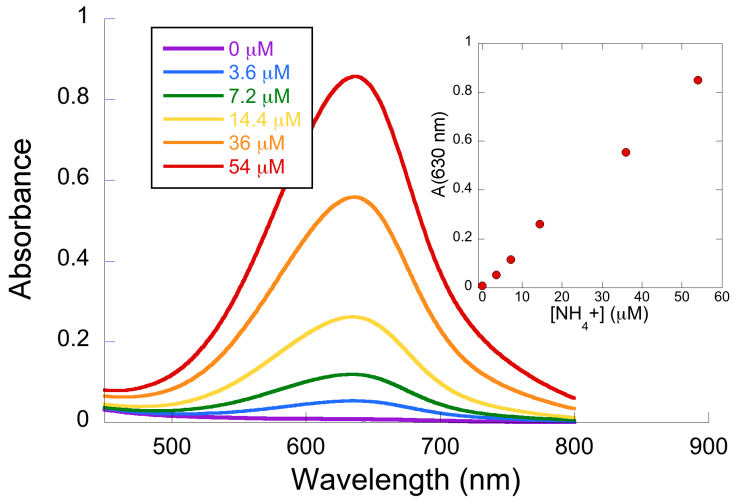

Figure S11. UV/Vis absorption spectra of NH_4^+ in phenol-hypochlorite solution. Spectra shown are at [NH_4^+] of 0 μM (violet), 3.6 μM (blue), 7.2 μM (green), 14.4 μM (yellow), 36 μM (orange), 54 μM (red). Inset: resulting calibration curve.

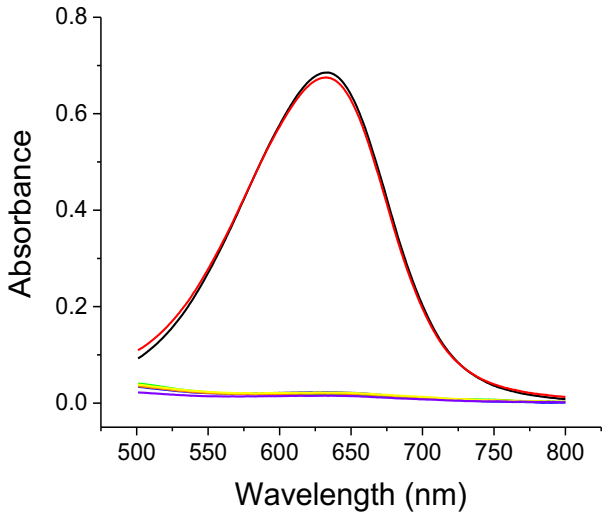

Figure S12. UV-vis absorption spectra of solutions after 5.5 hours of CPE at –0.9 V of 1.0 M $NaNO_2$ in 1.0 M pH 7.2 MOPS buffer, diluted by a factor of 2000 in phenol-hypochlorite solution. Spectra are shown from the following CPE experiments (Data were collected under N_2 unless indicated otherwise): 25 μM CoGGH (red), 25 μM CoGGH under air (black), rinse test (blue), 25 μM NiGGH (green), 25 μM GGH (orange), 25 μM $CoCl_2$ (yellow), no added metal/peptide (violet). The only experiments to yield absorbance above the baseline (~0.01 absorbance units given the limits of our spectrometer) are those with CoGGH present (black and red lines).

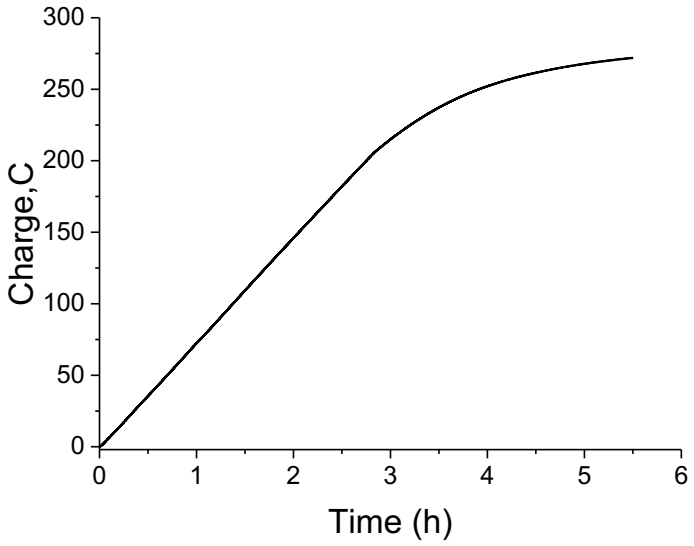

Figure S13. Controlled potential electrolysis under air of 1.0 M aqueous nitrite solution with 1 M pH 7.2 MOPS buffer and 25 μM CoGGH showing charge build up over time with an applied potential of –0.9 V vs Ag/AgCl (1M KCl).

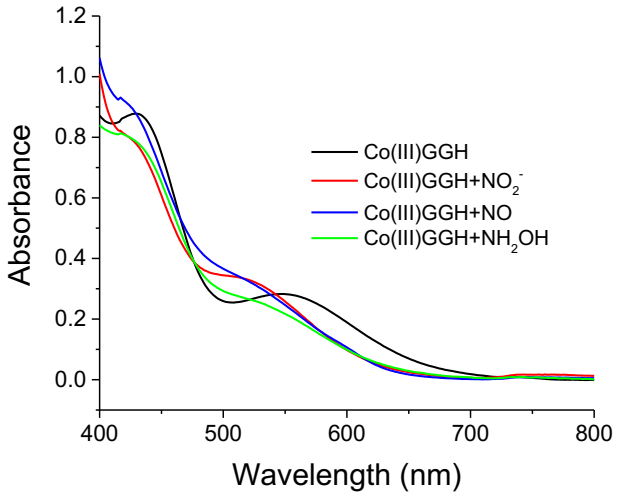

Figure S14. UV/Vis of 5 mM Co(III)GGH alone (black) and in the presence of: 50 mM $NaNO_2$ (red), purified NO(*g*) at 1 atm (blue), and 50 mM NH_2OH*HCl (green). Samples are in 50 mM pH 8.0 MOPS buffer.

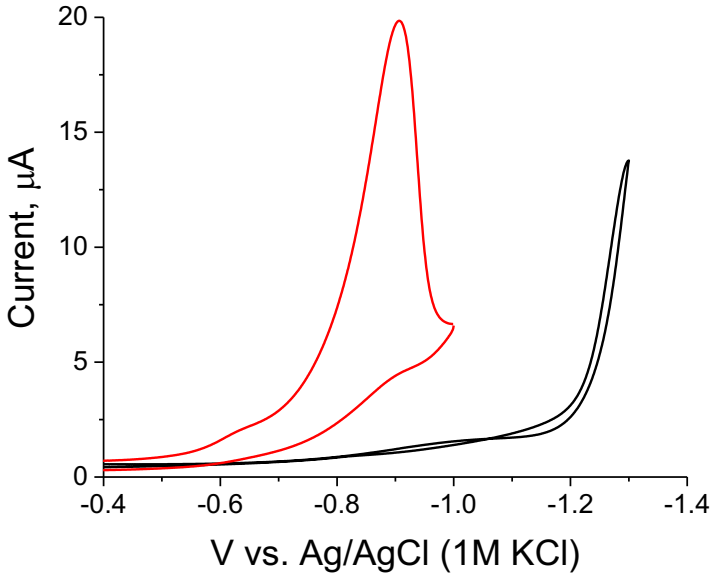

Figure S15. Cyclic voltammograms of 50 mM MOPS, pH 7.2, saturated with NO(*g*) (black) and the same solution with 25 µM CoGGH (red). The scan rate is 100 mV/s at an HMDE working electrode.

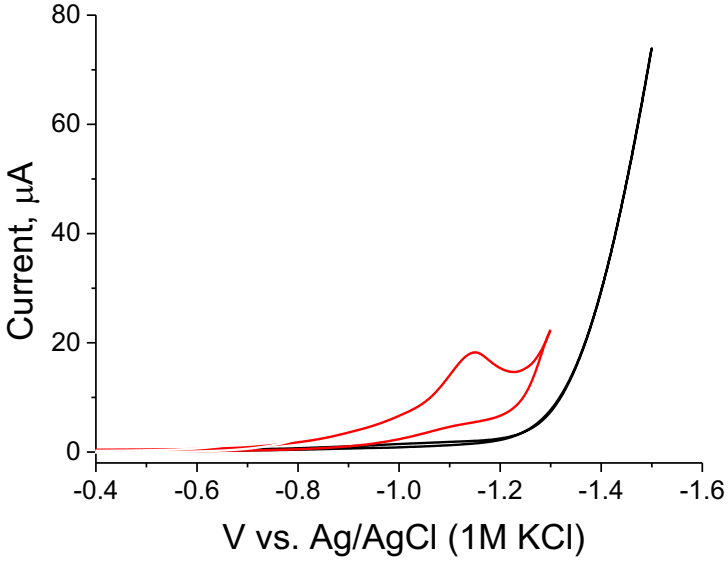

Figure S16. Cyclic voltammograms of 100 mM NH_2OH*HCl in 50 mM pH 7.2 MOPS buffer (black) and in the presence of 25 µM CoGGH (red). The scan rate 100 mV/s at an HMDE working electrode.

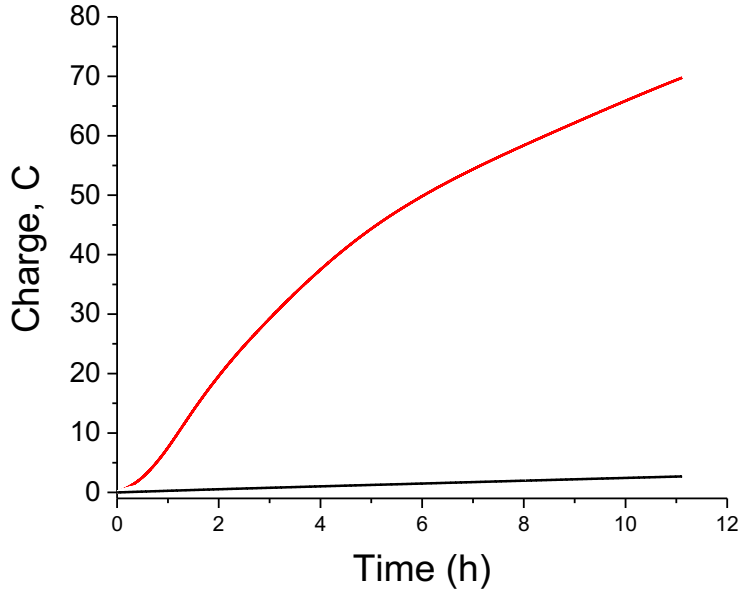

Figure S17. Controlled potential electrolysis of 1.0 M MOPS buffer at pH 7.2 saturated with purified NO(*g*) (black) and with added 25 μM CoGGH (red). Data was collected at an applied potential of –0.9 V vs Ag/AgCl (1M KCl).

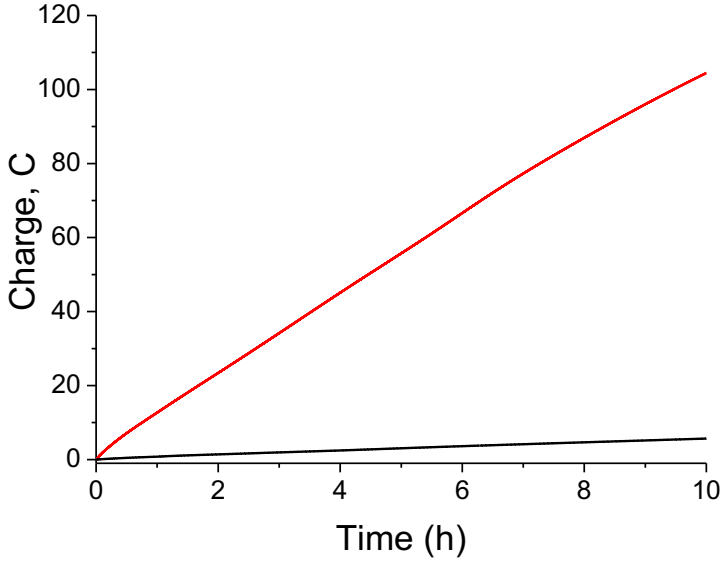

Figure S18. Controlled potential electrolysis of aqueous 1.0 M NH_2OH*HCl in 1.0 M MOPS buffer at pH 7.2 (black) and with added 25 μM CoGGH (red). Data was collected under nitrogen at an applied potential of –1.2 V vs Ag/AgCl (1M KCl).

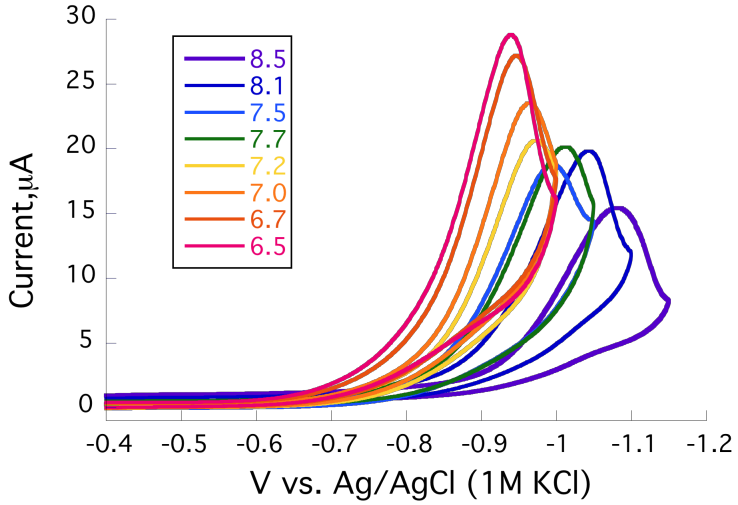

Figure S19. Cyclic voltammetry of 25 μM CoGGH and 100 mM $NaNO_2$ in 50 mM MOPS buffer as a function of pH (from 6.5 to 8.5). The peak current and peak potential both decrease as pH increases.

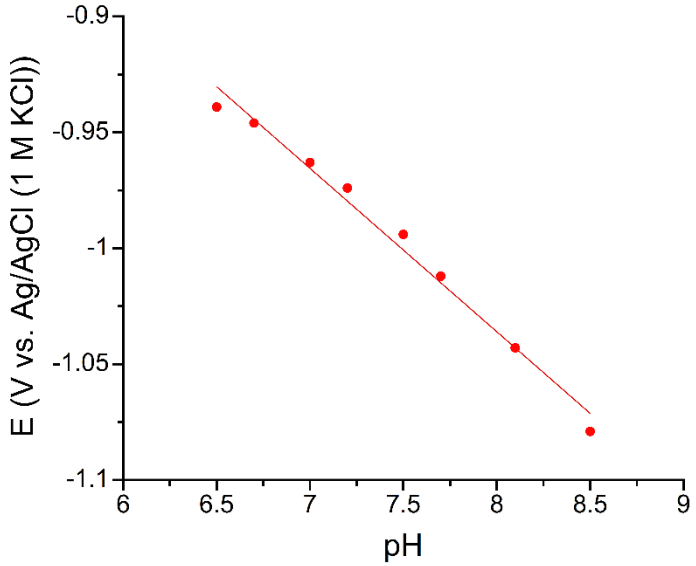

Figure S20. Catalytic peak potential of 25 μM CoGGH in 100 mM $NaNO_2$, 50 mM pH 7.2 MOPS buffer as a function of pH (plot of Figure S19 data). The slope of the best-fit line shown is -70.5 mV/pH.

References

(1) Kandemir, B.; Kubie, L.; Guo, Y.; Sheldon, B.; Bren, K. L. Hydrogen Evolution from Water under Aerobic Conditions Catalyzed by a Cobalt ATCUN Metallopeptide. *Inorg. Chem.* **2016**, *55*, 1355-1357.
(2) Hawkins, C. J.; Martin, J. Cobalt(III) Complex of Glycylglycyl-L-Histidine - Preparation, Charcterization, and Conformation. *Inorg. Chem.* **1983**, *22*, 3879-3883.
(3) Bertini, I.; Luchinat, C. High Spin Cobalt(II) as a Probe for the Investigation of Metalloproteins. *Adv. Inorg. Biochem.* **1984**, *6*, 71-111.
(4) Anton, D. R.; Crabtree, R. H. Dibenzo[a,E]Cyclooctatetraene in a Proposed Test for Heterogeneity in Catalysts Formed from Soluble Platinum Group Metal-Complexes. *Organometallics* **1983**, *2*, 855-859.
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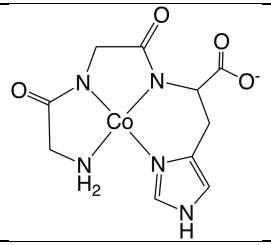
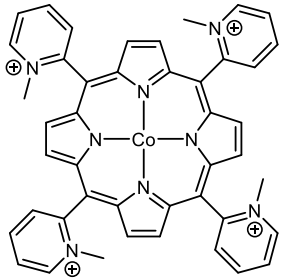
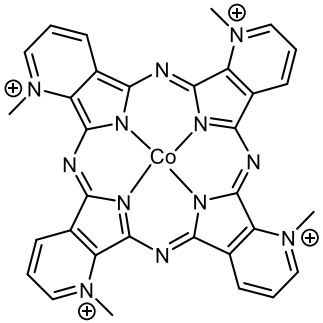
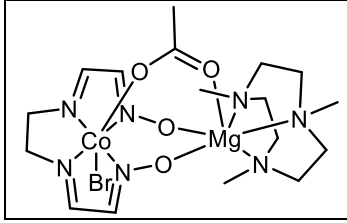
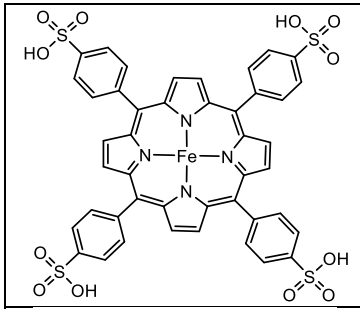
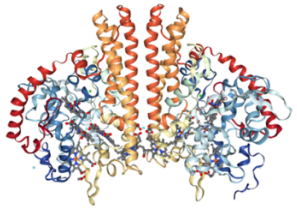
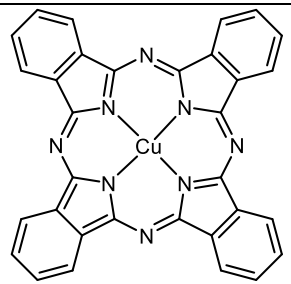
(7) Weatherburn, M. W. Phenol-Hypochlorite Reaction for Determination of Ammonia. *Anal. Chem.* **1967**, *39*, 971.

Table S1. Nitrite reduction catalysts[a]

Catalyst	Confirmed Product(s)	Conditions	$E_{applied}$ vs. SHE[b]	TON (TOF)	Selectivity	Homogeneous or Heterogeneous catalysis	Ref.
	NH_4^+	In water, 25 μM catalyst, 1.0 M $NaNO_2$, 1.0 M MOPS, pH 7.2. Hg pool cathode.	-0.66 V	3550 ($645h^{-1}$)	90% FE for NH_4^+.	homogeneous	*This work*
	NH_3OH^+ NH_4^+	In water, 31.3 μM catalyst, 2.0 mM $NaNO_2$, 0.3 M of pH 4.0 "buffer solution." Vitreous carbon cathode	-0.50 V	N.R.[c]	Mixture of NH_2OH and NH_4^+ obtained with 33% yield for NH_4^+ * and 60% yield for NH_2OH.*	homogeneous	1
	NH_4^+	In water, 2.8 mM $NaNO_2$, 0.5 M NaOH, 1 μM catalyst. Carbon rod cathode	-1.39 V	N.R.	97% faradaic efficiency	heterogeneous	2
	N_2O	In MeCN, 20 mM [n-Bu_4N][NO_2], 20 mM Et_3NHCl, 100 mM [n-Bu_4N][ClO_4], 0.5 mM catalyst. Glassy carbon plate cathode	-0.96 V	N.R.	selective	homogeneous	3

	NH_4^+	In water, 20 mM NO_2^-, 1 mM catalyst, pH 6.7. Hg pool cathode	-0.66 V	N.R.	97% faradaic efficiency	homogeneous	4
 CcNiR PDB: 2E80	NR	In water, 1 mM nitrite, ~0.08 pmol of CcNiR adsorbed onto rotating pyrolytic graphite edge electrode, pH 7	-0.65 V	($1.9 * 10^6$ h^{-1})	N.R.	heterogeneous	5
	NH_2OH NH_3	In water, 0.1 M KOH, in the presence of nitrite, with a catalyst-coated carbon rod cathode	-1.3 V	N.R.	78% FE for NH_3.	heterogeneous	6

[a]Conditions shown exhibit the greatest selectivity for the most reduced product. Asterisks indicate values that have been calculated from data in the associated reference.
[b]Values used for reference electrode potentials when converting to SHE were obtained from: Daniel C. Harris. *Quantitative Chemical Analysis*, 7th ed.; W. H. Freeman and Company: New York, NY, 2007. pp 299-301.
[c]not reported
[d]not applicable

References to Table S1

(1) Cheng, S. H.; Su, Y. O. Electrocatalysis of Nitric-Oxide Reduction by Water-Soluble Cobalt Porphyrin - Spectral and Electrochemical Studies. *Inorg. Chem.* **1994**, *33*, 5847-5854.
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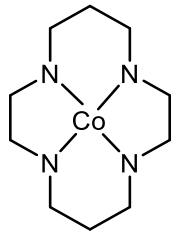
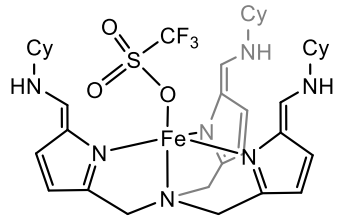
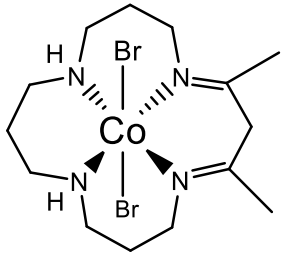
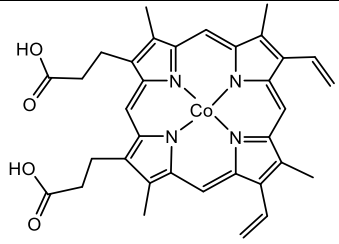
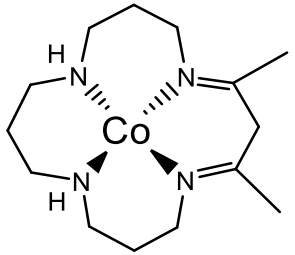
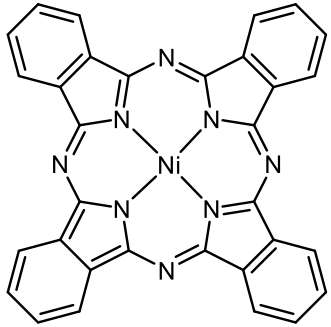
Table S2. Nitrate reduction catalysts[a]

Catalyst	Confirmed Product(s)	Conditions	$E_{applied}$ versus SHE[b]	TON (TOF)	Selectivity	homogeneity	Ref.
	NH_2OH NH_4^+	In water, 20 μM catalyst with 100 mM KNO_3. Hg pool cathode	-1.26 V	904 * (569 h^{-1}) for NH_2OH. 24 for NH_4^+.*	87% FE for NH_2OH, 3% FE for NH_4^+. (NH_4^+ is obtained in greater yield when an Ag, Pb, or Cu cathode is used).	heterogeneous	1
	NO	In a 7:4 mixture of MeCN:benzene, 3.5 eqv of $NaNO_3$, 6 eqv. of 1,2-diphenylhydrazine, $MgSO_4$, and 1 eqv. of catalyst for nitrate reduction	N.A.[c]	3.5 (0.083h^{-1})*	selective	homogeneous	2
	NH_4^+	In water, 0.5 mM catalyst, 0.1 M $NaNO_3$, pH = 3.5. Carbon rod cathode	-0.81 V	3.4 (1.7 h^{-1})*	97% FE for NH_4^+	homogeneous	3
	NO N_2O NH_3OH^+ NH_4^+	In water, 0.1 M $HClO_4$, 0.1 M $LiNO_3$ or 4 mM $NaNO_2$, pH = 1-2. Pyrolytic graphite cathode	-0.5 to -1.5 V	N.R.[d]	Selective for NH_2OH at -0.5 V vs RHE. Mixture of NH_3OH^+ and NH_4^+ at -0.75 V vs RHE and lower.	heterogeneous	4

	NH_4^+	In water, 0.1 M $NaNO_3$, 0.5 mM catalyst. Carbon felt cathode	N.R.	N.R.	selective	homogeneous	5
	NH_3	In water, 0.1 M KOH, in the presence of nitrate, with a catalyst-coated carbon rod cathode	-1.1 V	N.R.	85% FE for NH_3.	heterogeneous	6

[a]Conditions shown exhibit the greatest selectivity for the most reduced product. Asterisks indicate values that have been calculated from data in the associated reference.
[b]Values used for reference electrode potentials when converting to SHE were obtained from: Daniel C. Harris. *Quantitative Chemical Analysis*, 7th ed.; W. H. Freeman and Company: New York, NY, 2007. pp 299-301.
[c]not applicable
[d]not reported

References to Table S2

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