

Intermolecular Electron-Transfer Catalyzed on Nanoparticle Surfaces

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Supporting Information

Reagents

Horse heart Cyt *c* was obtained from Sigma Aldrich. Sodium dithionite was used to reduce Cyt *c* and separated using a G-25 Sephadex column. When noted, solutions of Cyt *c* were made anaerobic by gently passing hydrated N₂ over their surfaces while stirring for 60 minutes. The ligands and **Au-TX** nanoparticles were prepared following previously published procedures (You, C. C.; De, M.; Han, G.; Rotello, V. M. *J. Am. Chem. Soc.*, **2005**, *127*, 12873-12881). Co(phen)₃(ClO₄)₃ was synthesized and recrystallized according to literature (Schilt, A. A.; Taylor, R. C., *J. Inorg. Nucl. Chem.*, **1959**, *9*, 211-221). Concentrations were determined using a Hewlett-Packard 8453 UV-Vis spectrophotometer.

Stopped-flow reactivity studies

Kinetic experiments were performed on an OLIS RSM-100 stopped-flow spectrophotometer. The OLIS scandisk collected absorbance data from 390 – 610 nm for several seconds with a 20 mm observation cell pathlength. The temperature of drive syringes and observation cell was maintained at 25.0° C by a Neslab RTE-211 recirculating water bath.

Co(phen)₃³⁺ reactivity experiments were derived from previous literature methods. All solutions were prepared using 10 mM Tris buffer (12 mM NaCl, pH 7.4), and were degassed with N₂ prior to loading drive syringes via 3-way stopcocks. One drive syringe contained the oxidant Co(phen)₃³⁺ in excess (0.1– 2.0 mM) while the other contained Cyt *c* (10.0 μM) pre-incubated with the appropriate **Au-TX** (0.2 – 1.0 μM). These solutions were rapidly mixed in a 1:1 ratio and the absorbance change at 550 nm monitored. For each data point, 4-5 individual shots were averaged for the reported *k*_{obs} and uncertainties (1σ).

Data collection and processing was handled using Globalworks software and subsequent fitting in Igor Pro. Absorbance change traces as a function of time at 550 nm were fit using single exponential decays to determine *k*_{obs}; global SVD fits using the A→B kinetic model concurred with these single-wavelength fits. The apparent rate of electron transfer, (*k*_{ET})_{app}, was determined using equation S1 where [Co]₀ is the initial concentration of Co(phen)₃³⁺ concentration and *K*_D is the dissociation constant of Cyt *c*:**Au-TX** and Co(phen)₃³⁺.

$$k_{obs} = (k_{ET})_{app} \frac{[Co]_0}{[Co]_0 + K_D} \quad (S1)$$

Au-TX concentration was varied for each individual particle over several experiments. Plotting k_{ET} versus **Au-TX** concentration gives a slope that represents a bimolecular rate constant for that particular Cyt *c*:**Au-TX** complex with $Co(phen)_3^{3+}$, $(k_{ET}/K_D)_{app}$.

The oxidation of Cyt *c* by $Co(phen)_3^{3+}$ alone was performed under the same conditions in 10 mM Tris buffer (pH 7.40, 12mM NaCl, 25.0 °C) as shown in Figure S1. The kinetic data in Figure S2 was fit according to equation S2 where k_{∞} and k_o represent the reaction rate at high and low $Co(phen)_3^{3+}$ concentrations respectively. The inhibition constant K_i was fixed at $1 \times 10^7 M^{-2}$.

$$k_{obs} = k_o - \frac{K_i [Co]_o^2 (k_o - k_{\infty})}{1 + K_i [Co]_o^2} \quad (S2)$$

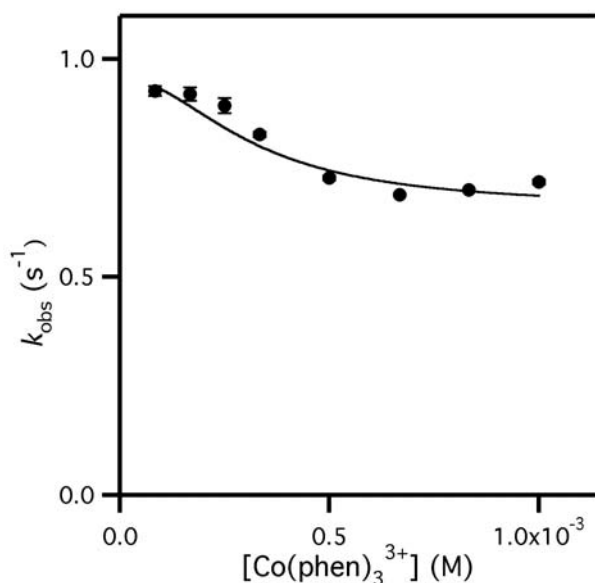


Figure S1. Cyt *c* (5 μM) oxidation by $Co(phen)_3^{3+}$ in 10 mM Tris buffer (pH 7.40, 12mM NaCl, $I = 20$ mM, 25.0 °C). The curve was fit according to equation S2 with the inhibition constant K_i fixed at $1 \times 10^7 M^{-2}$, resulting in $k_{\infty} = 0.66 \pm 0.0018$ and $k_o = 0.96 \pm 0.0076$.

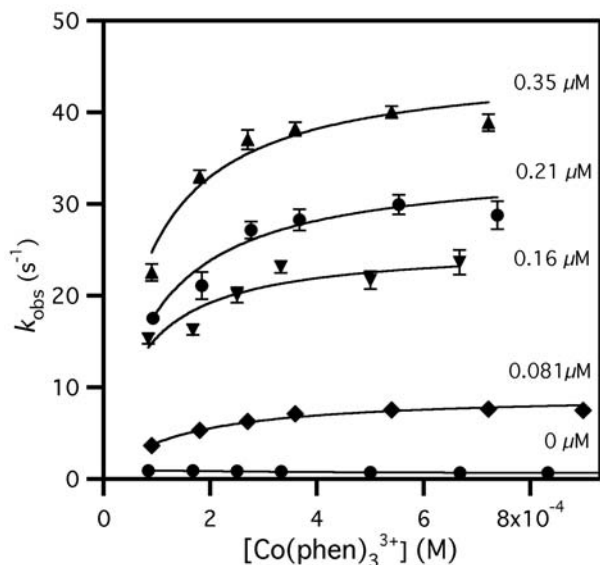


Figure S2. Cyt *c* (5 μM) oxidation by Co(phen)_3^{3+} in the presence of varied **Au-TPhe** (0 – 0.35 μM) in 10 mM Tris buffer (pH 7.40, 12mM NaCl, $I = 20$ mM, 25.0 $^\circ\text{C}$).

Table S1. Tabulated k_{obs} (s^{-1}) data for Cyt *c* oxidation by Co(phen)_3^{3+} in the presence of varied **Au-TX** concentrations.

	[Au-TX] (μM)	[Co(phen) $_3^{3+}$] (mM)	k_{obs} (s^{-1})	$\pm 1\sigma^a$
Cyt <i>c</i> :Au-TPhe	0.081	0.0899	3.65	0.0728
		0.180	5.32	0.0476
		0.270	6.30	0.131
		0.359	7.13	0.123
		0.540	7.55	0.165
		0.721	7.65	0.125
Cyt <i>c</i> :Au-TPhe	0.16	0.0832	15.3	0.534
		0.167	16.3	0.568
		0.250	20.1	0.851
		0.332	23.2	0.662
		0.500	21.7	0.950
		0.667	23.7	1.33
Cyt <i>c</i> :Au-TPhe	0.21	0.0920	17.5	0.0810
		0.184	21.1	1.46
		0.276	27.2	0.926
		0.367	28.3	1.18
		0.553	29.9	1.04
		0.738	28.8	1.53
Cyt <i>c</i> :Au-TPhe	0.35	0.0899	22.6	0.921
		0.180	33.0	0.679
		0.270	37.0	1.09
		0.359	38.2	0.733
		0.540	40.1	0.663
		0.721	38.9	0.905

Cyt <i>c</i> : Au-TAsp	0.18	0.0797	4.68	0.118
		0.160	6.59	0.0497
		0.239	8.33	0.0788
		0.318	9.20	0.136
		0.478	9.94	0.116
		0.639	10.9	0.411
		0.797	10.9	0.227
Cyt <i>c</i> : Au-TAsp	0.25	0.0798	14.2	0.286
		0.160	20.5	0.340
		0.240	27.5	1.01
		0.319	29.1	0.530
		0.479	31.6	0.564
		0.640	33.2	1.00
		0.798	32.7	0.473
Cyt <i>c</i> : Au-TAsp	0.38	0.0797	18.1	0.480
		0.160	30.1	0.719
		0.239	36.4	0.758
		0.318	40.7	0.404
		0.478	40.8	1.66
		0.639	45.0	1.09
		0.797	44.3	1.47
Cyt <i>c</i> : Au-TAsp	0.52	0.0798	29.9	2.01
		0.160	44.3	0.670
		0.240	52.9	1.78
		0.319	61.8	1.24
		0.479	66.4	3.22
		0.640	66.3	1.26
		0.798	65.7	1.06
		0.958	64.9	0.896

a. Estimated uncertainty from fits to a single exponential decay, reported as $\pm 1\sigma$.

Table S2. Data fits for the oxidation of Cyt *c*:**Au-TX** by $\text{Co}(\text{phen})_3^{3+}$ as determined from $k_{\text{obs}} = k_{\text{ET}(\text{app})}[\text{Co}]_0/([\text{Co}]_0 + K_{\text{D}})$.

	[Au-TX] (μM)	$(k_{\text{ET}(\text{app})}$ (s^{-1})	K_{D} ($\times 10^4 \text{M}^{-1}$)
Cyt <i>c</i>:Au-TAsp			
	0.18	13 ± 0.31	1.4 ± 0.12
	0.25	39 ± 1.3	1.3 ± 0.17
	0.38	52 ± 2.1	1.2 ± 0.19
	0.52	77 ± 3.1	1.1 ± 0.19
Cyt <i>c</i>:Au-TPhe			
	0.081	8.9 ± 0.32	1.2 ± 0.17
	0.16	26 ± 1.7	0.66 ± 0.21
	0.21	34 ± 1.8	0.88 ± 0.20
	0.35	45 ± 2.0	0.77 ± 0.16

Isothermal Titration Calorimetry

An isothermal calorimeter purchased from Microcal Inc. (Northampton, MA), was used in all isothermal titration calorimetric (ITC) experiments, operated at 25 °C. Each microcalorimetric titration experiment consisted of 45 successive injections of a constant volume (6 μL /injection) of $\text{Co}(\text{phen})_3^{3+}$ complex and Cyt *c* solution into the reaction cell (1.4 mL) charged with different **Au-TX** solutions in the same buffer (10 mM Tris, 12 mM NaCl, pH 7.40). The concentrations of the **Au-TX** solutions and titrants were used were as follows,

Au-TAsp/ $\text{Co}(\text{phen})_3^{3+}$: 7.5 μM / 1250 μM

Au-Phe/ $\text{Co}(\text{phen})_3^{3+}$: 5 μM / 500 μM

Au-Asp/ Cyt *c*: 5 μM / 500 μM .

Titration of the $\text{Co}(\text{phen})_3^{3+}$ complex and Cyt *c* at the same concentrations with the buffer only was also carried out as the control experiment. The final titration curves were obtained by subtracting the control enthalpies from the enthalpies measured in the titration experiments. The Origin program supplied by Microcal Inc. was used to calculate the binding constant (K_S), binding ratios (n) and molar enthalpy change (ΔH) from the titration curve. The molar Gibbs free energy changes (ΔG) and entropies (ΔS) of reaction were calculated from the experimentally determined K_S and ΔH values.

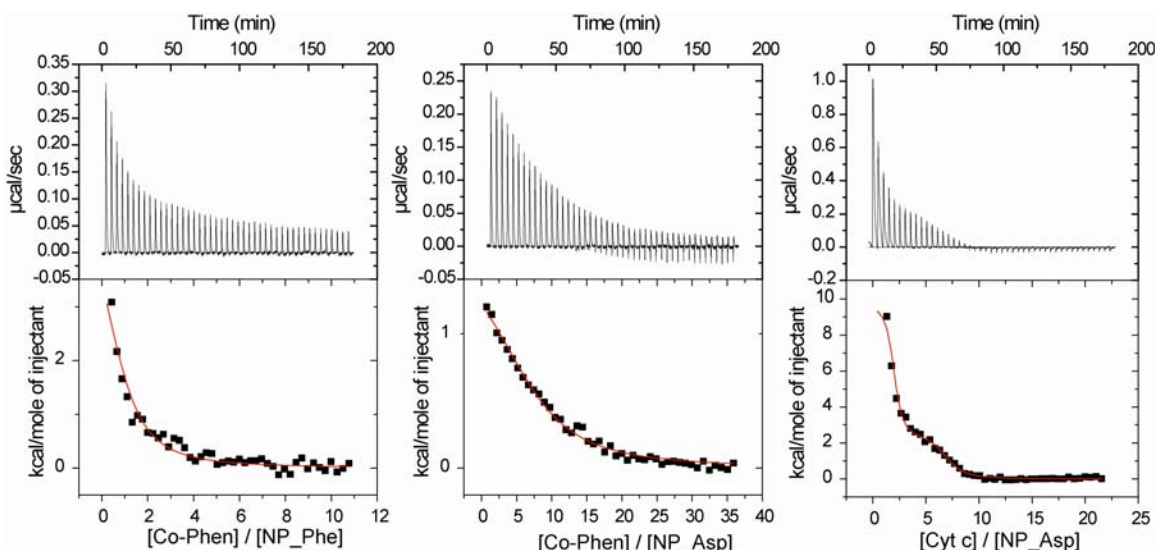


Figure S3. ITC analyses for the complexation of $\text{Co}(\text{phen})_3^{3+}$ with **Au-TPhe**; $\text{Co}(\text{phen})_3^{3+}$ with **Au-TAsp**; and Cyt *c* with **Au-TAsp** in 10 mM Tris, 12 mM NaCl buffer (pH = 7.4) at 25 °C.

Table S3. Complex stability constants (K_S), Gibbs free energy changes (ΔG), enthalpy changes (ΔH), entropy changes ($T\Delta S$), and binding stoichiometries (n) for the complexation of $\text{Co}(\text{phen})_3^{3+}$ complex with **Au-TPhe** and **Au-TAsp** and Cyt *c* with **Au-TAsp** in 10 mM Tris, 12 mM NaCl buffer (pH = 7.4) at 25 °C.

Complexation	$K_{S1}/$ M^{-1}	$-ΔG /$ $kcal\ mol^{-1}$	$ΔH /$ $kcal\ mol^{-1}$	$TΔS /$ $kkal\ mol^{-1}$	n					
Au-TAsp/ Co- Phen	$4.46 \pm 0.48 \times 10^4$	6.35	1.695 ± 0.09	8.05	7.14					
Au-TPhe/ Co- Phen	$1.65 \pm 0.23 \times 10^5$	7.12	5.494 ± 0.25	64.05	0.90					
Au-TAsp/ Cyt <i>c</i>	K_{S1}	K_{S2}	$-ΔG_1$	$-ΔG_2$	$ΔH_1$	$ΔH_2$	$TΔS_1$	$TΔS_2$	n_1	n_2
	$8.36 \pm$ 3.51×10^7	$1.33 \pm$ 0.62×10^6	10.79	8.34	9.72	2.45	20.51	10.79	1.80	5.00
					\pm 0.42	\pm 0.25				