Supporting Information

## Photochemical Tyrosine Oxidation with a Hydrogen-Bonded Proton Acceptor by Bidirectional Proton-Coupled Electron Transfer

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## **Experimental Methods**

Materials. L-tyrosine methyl ester (98%, Aldrich), isonicotinic acid (Aldrich), 1hydroxybenzatriazole hydrate (HOBt) (Advanced ChemTech), *N*-methylmorpholine (NMM) (Alfa-Aesar), dichloromethane (DCM) (99.5%, Sigma-Aldrich), L-phenylalanine methyl ester hydrochloride (98%, Aldrich), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC•HCl) (98%, Aldrich), dimethylformamide (DMF) (Anhydrous, 99.8%, Sigma-Aldrich), citric acid monohydrate (Mallinckrodt Chemicals), sodium bicarbonate (NaHCO<sub>3</sub>) (Sigma-Aldrich), magnesium sulfate (MgSO<sub>4</sub>) (anhydrous, Sigma-Aldrich), ethyl acetate (EtOAc) (99.9%, Sigma-Aldrich) pentacarbonylchlororhenium(I) (Re(CO)<sub>5</sub>Cl) (98%, Strem), 1,10phenanthroline (Phen) (99%, Aldrich), thallium(I) hexafluorophosphate(V) (TIPF<sub>6</sub>) (97%, Strem), acetonitrile (MeCN) (99.9%, Sigma-Aldrich), toluene (99.5%, Sigma-Aldrich), acetone (99.9%, Sigma-Aldrich), diethyl ether (Et<sub>2</sub>O) (99.0%, Sigma-Aldrich), d<sup>6</sup>-acetone ((CD<sub>3</sub>)<sub>2</sub>CO) (Cambridge Isotope Laboratories), d<sup>2</sup>-dichloromethane (CD<sub>2</sub>Cl<sub>2</sub>) (Cambridge Isotope Laboratories), pyridine (Anhydrous, 99.8%, Sigma-Aldrich), and imidazole (99.5%, Sigma-Aldrich) were used as received. Tricarbonyl (1,10-phenanthroline)(acetonitrile) rhenium(I) hexafluorophosphate ( $[Re(Phen)(CO)_3-(NCMe)]PF_6$ ) prepared was as previously reported.1

**General Methods.** <sup>1</sup>H NMR spectra were obtained using a Varian Inova-500 NMR spectrometer at the MIT Department of Chemistry Instrumentation Facility (DCIF) and internally referenced using the proteo impurity for the relevant deuterated solvent (d<sup>6</sup>-acetone or d<sup>2</sup>-dichloromethane). Chemical shifts are reported relative to tetramethylsilane (TMS). Elemental analysis data were obtained from Midwest Microlab, LLC (Indianapolis, IN). UV-vis absorption spectra were collected on a Varian Cary 5000 UV-vis-NIR spectrometer; steady-state emission data were collected using a PTI QM 4 Fluorometer equipped with a 150 W Xe-arc lamp for excitation and a photomultiplier tube (Hamamatsu R928) cooled to -78 °C for detection. Samples for both absorption and emission experiments were dilute solutions of the complexes as indicated (10 – 50  $\mu$ M) in quartz spectroscopy cells.

Synthesis 4-isonicotinoyl-L-tyrosine methyl ester (Py–Y). Isonicotinic acid (835.7 mg, 6.79 mmol, 1.0 eq), L-tyrosine methyl ester (1360.8 mg, 6.97 mmol, 1.0 eq), HOBt (994.3 mg, 7.36 mmol, 1.1 eq), EDC•HCl (1438.2 mg, 7.50 mmol, 1.1 eq), and NMM (3.4 mL, 31. mmol, 4.1 eq) were dissolved in 250 mL DMF/DCM (1:10 v/v) and stirred at room temperature for 3 days. The reaction mixture was washed sequentially with 1% citric acid (w/v in H<sub>2</sub>O, 100 mL), 1% NaHCO<sub>3</sub> (w/v in H<sub>2</sub>O, 100 mL), and H<sub>2</sub>O (4 × 100 mL). The organic layer was dried over MgSO<sub>4</sub> and the solvent removed by rotary evaporation. Crude product was separated by flash chromatography (silica, EtOAc) to yield pure Py–Y as a

<sup>&</sup>lt;sup>1</sup> S. Y. Reece and D. G. Nocera, *J. Am. Chem. Soc.*, 2005, **127**, 9448.

white solid (593.7 mg, 29%). <sup>1</sup>H NMR (500 MHz, CD<sub>2</sub>Cl<sub>2</sub>, 20 °C):  $\delta$  = 8.71 (m, 2H, Py-H), 7.56 (m, 2H, Py-H), 6.98 (m, 2H, Ar-H), 6.75 (m, 2H, Ar-H), 6.65 (d, 1H, N-H), 4.98 (m, 1H, C<sub>α</sub>-H), 3.77 (s, 3H, OCH<sub>3</sub>), 3.18 (m, 2H, C<sub>β</sub>-H); Anal. calcd. for C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>: C, 63.99; H, 5.37; N, 9.33; found: C, 64.08; H, 5.42; N, 9.32.

**Synthesis of 4-isonicotinoyl-L-phenylalanine methyl ester (Py–F).** Isonicotinic acid (423.0 mg, 3.44 mmol, 1.0 eq), L-phenylalanine methyl ester hydrochloride (745.5 mg, 3.46 mmol, 1.0 eq), HOBt (520.3 mg, 3.85 mmol, 1.1 eq), EDC•HCl (723.5 mg, 3.77 mmol, 1.1 eq), and NMM (1.5 mL, 14. mmol, 4.1 eq) were dissolved in 250 mL DMF/DCM (1:10 v/v) and stirred at room temperature for 3 days. The reaction mixture was washed sequentially with 1% citric acid (w/v in H<sub>2</sub>O, 100 mL), 1% NaHCO<sub>3</sub> (w/v in H<sub>2</sub>O, 100 mL), and H<sub>2</sub>O (4 × 100 mL). The organic layer was dried over MgSO<sub>4</sub> and the solvent removed by rotary evaporation. Crude product was separated by flash chromatography (silica, EtOAc) to yield pure Py–F as a waxy white solid (656.3 mg, 67%). <sup>1</sup>H NMR (500 MHz, CD<sub>2</sub>Cl<sub>2</sub>, 20 °C): δ = 8.71 (m, 2H, Py-H), 7.56 (m, 2H, Py-H), 7.28 (m, 3H, Ar-H), 7.14 (m, 2H, Ar-H), 6.71 (d, 1H, N-H), 5.03 (m, 1H, C<sub>α</sub>-H), 3.77 (s, 3H, OCH<sub>3</sub>), 3.25 (m, 2H, C<sub>β</sub>-H); Anal. calcd. for C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>: C, 67.59; H, 5.67; N, 9.85; found: C, 67.98; H, 5.73; N, 9.89.

Synthesis of tricarbonyl (1,10-phenanthroline)(Py-Y)rhenium(I) hexafluorophosphate ([Re]-Y-OH). [Re(Phen)(CO)<sub>3</sub>(NCMe)]PF<sub>6</sub> (212.9 mg, 0.335 mmol, 1.0 eq) and Py-Y (130.7 mg, 0.435 mmol, 1.3 eq) were dissolved in 20 mL acetone and heated to reflux with stirring overnight. The solvent was removed by rotary evaporation and the resulting yellow oil was redissolved in 10 mL DCM, to which 200 mL Et<sub>2</sub>O was added to precipitate the desired product. The resulting solid was isolated by vacuum filtration to yield [Re]-Y-OH as a pale yellow powder (264.2 mg, 88%). <sup>1</sup>H NMR (500 MHz, CD<sub>2</sub>Cl<sub>2</sub>, 20 °C):  $\delta$  = 9.57 (m, 2H, Ar-H), 8.76 (s, 2H, Ar-H), 8.34 (m, 2H, Ar-H), 8.13 (m, 4H, Ar-H), 7.40 (m, 2H, Ar-H), 6.83 (m, 2H, Ar-H), 6.63 (d, 1H, N-H), 6.56 (m, 2H, Ar-H), 4.76 (m, 1H, C<sub>\alpha</sub>-H), 3.69 (s, 3H, OCH<sub>3</sub>) , 3.01 (m, 2H, C<sub>\beta</sub>-H). Anal. calcd. for C<sub>31</sub>H<sub>24</sub>F<sub>6</sub>N<sub>4</sub>O<sub>7</sub>PRe: C, 41.57; H, 2.70; N, 6.25; found: C, 41.36; H, 2.80; N, 6.17.

Synthesis of tricarbonyl (1,10-phenanthroline)(Py-Y)rhenium(I) hexafluorophosphate ([Re]-F). [Re(Phen)(CO)<sub>3</sub>(NCMe)]PF<sub>6</sub> (233.6 mg, 0.367 mmol, 1.0 eq) and Py–F (134.2 mg, 0.472 mmol, 1.3 eq) were dissolved in 20 mL acetone and heated to reflux with stirring overnight. The solvent was removed by rotary evaporation and the resulting yellow oil was redissolved in 10 mL DCM, to which 200 mL Et<sub>2</sub>O was added to precipitate the desired product. The resulting solid was isolated by vacuum filtration to yield [Re]–F as a pale yellow powder (280.3 mg, 87%). <sup>1</sup>H NMR (500 MHz, CD<sub>2</sub>Cl<sub>2</sub>, 20 °C):  $\delta$  = 9.56 (m, 2H, Ar-H), 8.78 (s, 2H, Ar-H), 8.30 (m, 2H, Ar-H), 8.14 (m, 4H, Ar-H), 7.45 (m, 2H, Ar-H), 7.20 (m, 3H, Ar-H), 7.09 (m, 2H, Ar-H), 6.82 (d, 1H, N-H), 4.79 (m, 1H, C<sub>\alpha</sub>-H), 3.66 (s, 3H, OCH<sub>3</sub>), 3.04 (m, 2H, C<sub>\beta</sub>-H). Anal. calcd. for C<sub>31</sub>H<sub>24</sub>F<sub>6</sub>N<sub>4</sub>O<sub>6</sub>PRe: C, 42.32; H, 2.75; N, 6.37; found: C, 42.55; H, 2.68; N, 6.20. **Emission Quenching Titrations.** Emission titrations were performed by adding sequential volumes of pyridine (neat) or imidazole (1.0 M in DCM) to a sample of [Re]–Y–OH (50  $\mu$ M in DCM). Emission spectra were collected using a fluorometer and rescaled to account for for changes in [Re]–Y–OH concentration using the formula  $I_r = I_c \times V_0 / (V_0 + \Delta V)$ , where  $I_r$  is the rescaled emission spectrum,  $I_c$  is the corrected emission spectrum obtained from the fluorometer directly,  $V_0$  is the initial volume of the sample, and  $\Delta V$  is the total additional volume added to the sample to reach the indicated concentration. The rescaled emission spectra were integrated; the integrated emission intensity, I, was used in subsequent analysis.

**Nanosecond Laser Flash Photolysis.** Nanosecond timescale laser flash photolysis experiments utilized a system that has previously been reported,<sup>2,3</sup> with a number of modifications. For this report, samples were flowed without recirculation to prevent interference from decomposition products. Within the system previously described and referenced above, one of two diffraction gratings was used for each of these experiments. All reported transient absorption experiments (full spectra and single wavelength kinetics) were performed using the 250 nm blaze grating (300 grooves/mm); emission kinetics studies employed the 500 nm blaze grating (300 grooves/mm). Transient absorption spectra reported are an average of 1000 four-spectrum sequences; for time-resolved emission experiments, individual traces are an average of 200 sweeps.

**Equilibrium Association Constant Determination.** Equilibrium constants were determined for the association of [Re]–Y–OH and pyridine or imidazole by monitoring emission quenching as a function of base added. The titration was performed in accordance with the procedure described above in the experimental section to yield corrected emission spectra as shown in the main text (Figure 3). For each concentration, the integrated emission intensity was computed by summing the individual intensity values over the range of wavelengths for the emission spectrum. Fitting to determine the equilibrium association constants for [Re]–Y–OH binding to bases was performed according to published methods.<sup>4</sup>

**Base-Dependent Quenching Kinetics.** Emission lifetimes were measured for both [Re]–F and [Re]–Y–OH as a function of pyridine and imidazole concentrations. For each of the four possible combinations, six different concentrations were measured. For each independent sample, five transient emission kinetics traces were collected.

<sup>&</sup>lt;sup>2</sup> Z.-H. Loh, S. E. Miller, C. J. Chang, S. D. Carpenter and D. G. Nocera, *J. Phys. Chem. A*, 2002, 106, 11700.

<sup>&</sup>lt;sup>3</sup> S. Y. Reece, M. R. Seyedsayamdost, J. Stubbe and D. G. Nocera, D. G. *J. Am. Chem. Soc.*, 2007, **129**, 13828.

<sup>&</sup>lt;sup>4</sup> J. J. Concepcion, M. K. Brennaman, J. R. Deyton, N. V. Lebedeva, M. D. E. Forbes, J. M. Papanikolas and T. J. Meyer, *J. Am. Chem. Soc.*, 2007, **129**, 6968.

In order to extract excited state lifetimes, each trace is fit to an exponential decay function in OriginPro (v. 8.0, OriginLab, Northampton, MA). Each fit yields an exponential decay constant, taken to be the measured excited-state lifetime for the given trace. Emission kinetics were monitored at 550 nm.

**Detailed Analysis of Excited-State Decay Pathways.** The observed lifetimes,  $\tau_{obs}$ , for both [Re]–F and [Re]–Y–OH correspond to the excited-state lifetime and rate constant for excited-state decay,  $k_{obs}$ , according to eq. 1.

$$\frac{d[\operatorname{Re}^*-\operatorname{AA}]}{dt} = -k_{obs}[\operatorname{Re}^*-\operatorname{AA}] = -\frac{1}{\tau_{obs}}[\operatorname{Re}^*-\operatorname{AA}]$$
(1)

Figure 5 illustrates the proposed pathways for [Re]–Y–OH excited-state decay. There are both static and dynamic quenching components, which correspond to reactions of bound and free [Re]–Y–OH, respectively. To construct a rate law, each of these decay pathways must be incorporated. Each of the terms below, and their associated rate constants, is associated with a particular excited-state decay pathway. The overall rate law for [Re]–Y–OH excited-state decay is therefore composed of four terms.

The first term is an intrinsic unimolecular rate of excited state decay which depends on [Re<sup>\*</sup>–Y], the total concentration of <sup>3</sup>[Re]<sup>\*</sup> MLCT excited-state and  $k_0$  is the intrinsic rate constant for excited state decay in the absence of any charge-transfer processes. The second decay pathway involves intramolecular oxidation of tyrosine and depends upon  $k_{\text{ET}}$ , the rate constant for intramolecular oxidation of the tyrosine phenol without proton transfer to base.

The bimolecular terms include a rate for intramolecular base oxidation, where  $k_q$  is the rate constant for bimolecular oxidation of base in solution by the [Re] excited state and [B] is the concentration of base in solution. The final term shown corresponds to intramolecular tyrosine oxidation with associated proton transfer to a hydrogen bonded base, where  $k_{PCET}$  is the rate constant for tyrosine oxidation with concomitant proton transfer to base, and [Re\*-Y]<sub>B</sub> is the concentration of [Re]-Y-OH molecules that are bound to base.

$$\frac{d[\text{Re}^*-\text{Y}]}{dt} = -k_0[\text{Re}^*-\text{Y}] - k_{ET}[\text{Re}^*-\text{Y}] - k_q[\text{Re}^*-\text{Y}][\text{B}] - k_{PCET}[\text{Re}^*-\text{Y}]_B$$
(2)

In order to define a raw law only in terms of only measured paramters and experimental variables, it would be useful to replace the term for bound [Re]–Y–OH molecules only,  $[Re^*-Y]_B$ , in terms of the total concentration,  $[Re^*-Y]_C$  The concentration of bound [Re]-Y-OH is related to the concentration of base in solution, the total concentration of [Re]-Y-OH in solution, the equilibrium constant for this association reaction,  $K_A$ , which can be

measured by steady-state emission quenching.  $[Re^*-Y]_U$  is the concentration of [Re]-Y-OH molecules that are not bound to base.

$$K_{A} = \frac{[\text{Re}^{*}-\text{Y}]_{B}}{[B][\text{Re}^{*}-\text{Y}]_{U}}$$
(3)

By definition,  $[Re^*-Y]_B$  and  $[Re^*-Y]$  are related by  $\chi_B$ .

$$[\operatorname{Re}^* - Y]_B = \chi_B [\operatorname{Re}^* - Y]$$
(4)

Similarly,  $[Re^*-Y]_U$  and  $[Re^*-Y]$  are related by  $\chi_B$ .

$$[\text{Re}^* - Y]_U = (1 - \chi_B)[\text{Re}^* - Y]$$
(5)

Substituting for  $[Re^*-Y]_U$  and  $[Re^*-Y]_B$  in terms of  $\chi_B$ , the fraction of bound [Re]-Y-OH.

$$K_A = \frac{\chi_B}{[B](1 - \chi_B)} \tag{6}$$

Rearranging,  $\chi_B$  is as follows.

$$\chi_B = \frac{K_A[B]}{1 + K_A[B]} \tag{7}$$

Substituting:

$$[\operatorname{Re}^{*}-\operatorname{Y}]_{B} = \frac{K_{A}[B]}{1+K_{A}[B]}[\operatorname{Re}^{*}-\operatorname{Y}]$$
(8)

Therefore, the overall rate law is:

$$\frac{d[\text{Re}^*-\text{Y}]}{dt} = -(k_0 + k_{ET} + k_q[\text{B}] + k_{PCET} \frac{K_A[B]}{1 + K_A[B]})[\text{Re}^*-\text{Y}]$$
(9)

Recalling eq. 1:

$$\frac{1}{\tau_{\rm Y}} = k_0 + k_{ET} + k_q [B] + k_{PCET} \frac{K_A[B]}{1 + K_A[B]}$$
(10)

In order to calculate the desired rate constant,  $k_{PCET}$ , the other four parameters ( $k_0$ ,  $k_{ET}$ ,  $k_q$ , and  $K_A$ ) must be determined. Fitting the data to eq. 10 should theoretically yield values for  $k_{PCET}$ ,  $k_q$ ,  $K_A$ , and the sum  $k_0 + k_{ET}$ ; however, the relatively small number of data points makes accurately determining all of these constants infeasible. By simplifying the system to systematically exclude individual effects, each rate constant can be determined with greater accuracy.

Returning to the basic rate law and recalling the inability of [Re]–F to undergo phenol oxidation reactions or participate in association with base, we obtain a much-simplified expression.

$$\frac{d[\operatorname{Re}^*-\mathrm{F}]}{dt} = -k_0[\operatorname{Re}^*-\mathrm{F}] - k_q[\operatorname{Re}^*-\mathrm{F}][\mathrm{B}]$$
(11)

Again substituting with eq. 1, we obtain a similar relationship for lifetimes of the [Re]–F, which as expected, is equivalent to the Stern-Volmer relationship.

$$\frac{1}{\tau_{\rm F}} = k_0 + k_q [B] \tag{12}$$

Using a typical Stern-Volmer analysis, the base dependence of [Re]–F lifetimes yields values for  $k_0$  and  $k_q$ . As described above, analysis of a steady-state emission titration yields values for  $K_A$ . With these values in hand, values for  $k_{ET}$  and  $k_{PCET}$  can be extracted from a fit where previously determined values for  $k_0$ ,  $k_q$ , and  $K_A$  are used.

Although the data in this paper are analyzed as above, an alternative approach is to directly use the difference in excited-state lifetimes between [Re]–F and [Re]–Y–OH, a quenching rate constant, to determine the overall rate of tyrosine oxidation. This approach relies on the fact all processes except tyrosine oxidation that occur for [Re]–Y–OH must necessarily occur for [Re]–F as well. The quenching rate constant,  $k_{obs}$  is therefore, in terms of the observed rate constants, defined by eq. 12.

$$k_{obs} = \frac{1}{\tau_{\rm Y}} - \frac{1}{\tau_{\rm F}} \tag{13}$$

At a given base concentration, subtracting eq. 11 from eq. 9,

$$\frac{1}{\tau_{\rm Y}} - \frac{1}{\tau_{\rm F}} = k_{ET} + k_{PCET} \frac{K_A[B]}{1 + K_A[B]}$$
(14)

Finally, substituting into 13:

$$k_{obs} = k_{ET} + k_{PCET} \frac{K_A[B]}{1 + K_A[B]}$$
(15)

This data can be fit to equation (15) leaving all three parameters ( $K_A$ ,  $k_{ET}$ , and  $k_{PCET}$ ) free; however, having previously determined the equilibrium association constant in a steadystate titration experiment,  $K_A$  can be taken as known, leaving only  $k_{PCET}$  and  $k_{ET}$  free.



**Figure S1.** Excited-state quenching titrations monitored by emission lifetime. Dilute solutions of Re–F and Re–Y (50  $\mu$ M in dichloromethane) were excited at  $\lambda_{exc} = 355$  nm and time resolved emission kinetics were monitored at  $\lambda_{obs} = 550$  nm on the ns to  $\mu$ s timescale. The observed rate constants for excited state quenching ( $k_{obs}$ ) were calculated from the emission lifetimes for Re–AA ( $k_{obs} = 1/\tau_{AA}$ , AA = F, Y), measured as a function of base concentration. Data for Re–F (bottom) are shown with a linear fit (gold) while data for Re–Y (top) are fit (blue) to Eq. 10.



**Figure S2.** Transient absorption spectra ( $\lambda_{exc} = 355 \text{ nm}$ ) of [Re]–F (gold) and [Re]–Y–OH (blue) (50 µM in dichloromethane) in the presence of 1.0 M pyridine. Colored spectra are collected immediately after the laser pulse; early time points are shown above (collected every 1000 ns for [Re]–F and every 500 ns for [Re]–Y–OH) while later time points are shown below (collected every 20 µs for [Re]–F and every 50 µs for [Re]–Y–OH). Lighter shades of gray represent earlier time points than darker shades.