

Supporting information for:

Intermolecular Proton-Coupled Electron Transfer Oxidation from Tryptophan with Water as Proton Acceptor

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Experimental procedures

O-Acetate-Tryptophan (***O*-Et-TrpH**) and *N*-Acetyl-Tryptophan (***N*-Ac-TrpH**) (structures given in Scheme 1) were commercially available from Sigma-Aldrich and used directly without further purification. [Ru(bpy)₃]Cl₂ and [Ru(dmb)₃]Cl₂ (bpy = 2,2'-bipyridine; dmb = 4,4'-dimethyl-2,2'-bipyridine) were synthesized. The structures were confirmed by ¹HNMR and ESI-MS.

The solution was buffered with 0.5 mM Na₂HPO₄ (SigmaUltra 99%) and 0.5 mM H₃BO₃ (SigmaUltra 99.5%), and the pH adjusted with concentrated NaOH (Elektrokemiska Aktiebolaget, Pro Analysis) or HCl (P-H TAMM). *O*-Acetate-Tryptophan (***O*-Et-TrpH**) or *N*-Acetyl-Tryptophan (***N*-Ac-TrpH**) was dissolved in the buffer solution to a concentration of 1 to 5 mM, and the pH was measured. [Ru(bpy)₃]Cl₂ or [Ru(diMe**bpy**)₃]Cl₂ and methyl viologen (Sigma, highest grade commercially available) were added to the analyte solution to a concentration of 40–60 μM and 30–40 mM, respectively.

The bimolecular electron transfer from tryptophan to [Ru(bpy)₃]³⁺ was investigated using a flash-quench method described earlier (ref. 8 of main paper). Sample excitation was provided by an OPO (Opotek) pumped by a frequency tripled Q-switched Nd:YAG laser (Quantel Brilliant B), delivering c.a. 7 ns pulses at 460 nm (c.a. 20 mJ/pulse). A pulsed Xenon lamp of an Applied photophysics LKS60 setup provided analyzing light that was passed through 1×1 cm quartz sample cuvette in a right-angle configuration and through a monochromator before hitting the P928 type photomultiplier. The PMT signal was converted and digitized using a HP Infiniium digital oscilloscope (2G samples/s). Transient absorption traces were generated from the raw data used the LKS60 software.

[Ru(bpy)₃]²⁺ was excited with a <10 ns 460 nm laser pulse, and the excited state was oxidatively quenched by the methyl viologen MV²⁺ giving [Ru(bpy)₃]³⁺ and MV^{•+}. The concomitant bimolecular electron transfer from the tryptophan to [Ru(bpy)₃]³⁺ was followed by the recovery of the [Ru(bpy)₃]²⁺ signal at 450 nm. Recombination between MV^{•+} and [Ru(bpy)₃]³⁺ or the oxidized tryptophan was controlled by monitoring the disappearance of the MV^{•+} absorption at 600 nm. The analyzing light was produced by a pulsed xenon lamp, and after passing the sample the light was detected as a function of time with a Hamamatsu R928 photomultiplier. Electron transfer from the tryptophan was in most cases kept rapid, by the use of a high

tryptophan concentration, compared to the recombination reaction with MV^{*+} , making MV^{*+} recombination insignificant for the recovery of $[Ru(bpy)_3]^{2+}$. The pseudo-first order rate constant for the electron transfer between $[Ru(bpy)_3]^{3+}$ and tryptophan was determined by fitting the 450 nm transients to a single-exponential function, and the second-order rate constant was extracted from the pseudo-first-order rate constant by division of the tryptophan concentration. For the case of with slow rate constants, however, the pseudo-first order reactions for the Ru(II) recovery were corrected for the recombination reaction with viologen radical, using

$$[Ru(III)] = [Ru(III)]_{t=0} e^{-kt} [MV^{*+}] / (1 + k_{2nd}t[MV^{*+}]) \quad (S1)$$

where k is the rate constant for intramolecular electron transfer from tryptophan and k_{2nd} is the second-order rate constant for the decay of MV^{*+} .

In all measurements the temperature was kept at room temperature and purged with Argon gas for at least 10 minutes before and during the measurement. The kinetic deuterium isotope effect was measured using D_2O (Aldrich 99.9% atom purity) as solvent assuming that the exchange with the protons in the buffer and compounds are faster than the sample preparation.

For the reaction between **O-Et-TrpH** and $[Ru(bpy)_3]^{3+}$ a fit to the data of rate constant as a function of pH could be made according to eq. S2, with $k_{CEP} \propto 10^{0.56 \times \text{pH}}$ before saturation (Figure 2),

$$k_{\text{obs}} = k_{\text{ETPT}} + k_{\text{CEP}} \quad (S2)$$

$$k_{\text{CEP}} = k_d k_{\text{CEP}}^{EC} / (k_d + k_{\text{CEP}}^{EC}) \quad (S2b)$$

$$k_{\text{CEP}}^{EC} = k^0 \times 10^{\alpha \text{pH}} \quad (S2c)$$

where k_{obs} is the observed second order rate constant, k_{ETPT} is the pH-independent rate constant at low pH, and k_{CEP} is the rate constant for the pH-dependent reaction ascribed to CEP. k_{CEP} saturates as the diffusion controlled limit is reached. A conventional steady state treatment with reversible formation of the encounter complex (k_d and k_{-d}) followed by the CEP reaction in the encounter complex (k_{CEP}^{EC}) yields eq. S2b. The pH-dependence of k_{CEP} was then described by eq. S2c, in order to account for the observed pH-dependence of k_{obs} . The fit gave $\alpha = 0.56$. Note that a first-order dependence on $[OH^-]$ would instead have given $\alpha = 1$

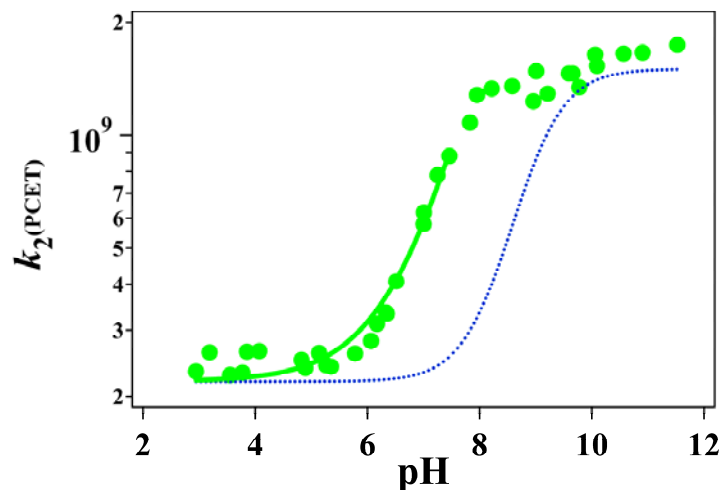
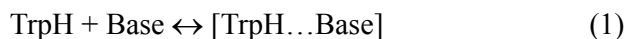


Figure S1. pH dependence of the observed rate constants for intermolecular oxidation of tryptophan to the flash-quench-generated Ru^{III} in 0.5 mM phosphate/borate buffer: *O*-Et-TrpH & $\text{Ru}(\text{bpy}_3)^{3+}$. The solid green line was fitted to the data using one pH-dependent (linear) term and one pH independent term together (see text); the blue dashed line was fitted according to the titration of amine group: $k_2 = \alpha k_{\text{NH}_2} + (1-\alpha)k_{\text{NH}_3^+}$, where $\alpha = (1+10^{\text{pH}-\text{pKa}})^{-1}$ and $\text{pKa} \approx 9.3$.

Exclusion of buffer dependence on the PCET rate: PCET with buffer base as the primary proton acceptor can be excluded under the present experimental conditions, for the following reasons: Firstly, the slope of $\log k$ vs. pH in Figure 2 at $\text{pH} < 7$ would have been unity ($\text{pKa} = 7.2$ and 9.2 for $\text{H}_2\text{PO}_4^{2-}$ and HBO_3 , respectively; $\log [\text{base}]$ increases linearly with pH also for a mixed buffer at pH below the pKa of the most acidic buffer). Instead $\log k$ increases only by a factor of three from pH 5 – 7. Secondly, experiments on intramolecular PCET in Ru-TrpH at different pH and varying buffer concentrations (including unbuffered solution) showed no rate dependence on buffer concentration below a few mM (ref. 8d of main manuscript). Thirdly, the kinetic analysis below shows that 0.5 mM buffer cannot give the high rates observed. The reaction would proceed via a precursor complex of either TrpH and buffer (case I) or TrpH and Ru^{III} (case II):

(I): Oxidation of a TrpH – Buffer complex by Ru^{III} :

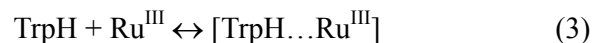


$$d[\text{Ru}^{\text{III}}]/dt = (k_1/k_{-1})k_2 [\text{Ru}^{\text{III}}][\text{TrpH}][\text{Base}]$$

However, in the buffer dependent control experiments (ref. 8d) no saturation was observed showing that the equilibrium constant for reaction 1 ($K_1 = k_1/k_{-1}$) is $K_1 < 10 \text{ M}^{-1}$. Thus at 0.5 mM buffer the equilibrium fraction of TrpH is $< 5 \times 10^{-3}$. This would give second-order rate constants that are at least 5×10^{-3} smaller than diffusion

controlled ($k \approx 3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$). In contrast we observe values up to $2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and $2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for **O-Et-TrpH** with $[\text{Ru}(\text{dmb})_3]^{3+}$ and $[\text{Ru}(\text{bpy})_3]^{3+}$, respectively.

(II): Reaction encounter of a TrpH – Ru^{III} complex with a buffer base:



$$d[\text{Ru}^{\text{III}}]/dt = (k_3/k_{-3})k_4 [\text{Ru}^{\text{III}}][\text{TrpH}][\text{Base}]$$

As there are no specific interactions between the TrpH and Ru^{III} species one can safely assume that the equilibrium constant for reaction 3 ($K_3 = k_3/k_{-3}$) is $K_3 < 10 \text{ M}^{-1}$. At 0.5 mM buffer and 1 mM TrpH, even a diffusion-controlled reaction 4 ($k \approx 3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) would never give a more rapid pseudo-first order reaction of Ru^{III} than $\tau = 50 \text{ } \mu\text{s}$. In contrast, in Figure 1 of the main manuscript we observe down to $\tau < 1 \text{ } \mu\text{s}$ at the highest pH-values.