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Proton-Coupled Electron Transfer from Tryptophan: A Concerted Mechanism with Water as Proton Acceptor

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Supporting Information

ABSTRACT: The mechanism of proton-coupled electron transfer (PCET) from tyrosine in enzymes and synthetic model complexes is under intense discussion, in particular the pH dependence of the PCET rate with water as proton acceptor. Here we report on the intramolecular oxidation kinetics of tryptophan derivatives linked to $[Ru(bpy)_3]^{2+}$ units with water as proton acceptor, using laser flash-quench methods. It is shown that tryptophan oxidation can proceed not only via a stepwise electron−proton transfer (ETPT) mechanism that naturally shows a pH-independent rate, but also via another mechanism with a pH-dependent rate and higher kinetic isotope effect that is assigned to concerted electron−proton transfer (CEP). This is in contrast to current theoretical models, which predict that CEP from tryptophan with water as proton acceptor can never compete with ETPT because of the energetically unfavorable PT part ($pK_a(Trp^{\bullet}H^+) = 4.7 \gg pK_a(H_3O^+) \approx -1.5$). The moderate pH dependence we observe for CEP cannot be explained by first-order reactions with OH^- or the buffers and is similar to what has been demonstrated for intramolecular PCET in $[Ru(bpy)_3]^{3+}$−tyrosine complexes (Sjödin, M.; et al. *J. Am. Chem. Soc.* **2000**, *122*, 3932. Irebo, T.; et al. *J. Am. Chem. Soc.* **2007**, *129*, 15462). Our results suggest that CEP with water as the proton acceptor proves a general feature of amino acid oxidation, and provide further experimental support for understanding of the PCET process in detail.

Important to many significant energy conversion processes in chemistry and biology are oxidation−reduction reactions in which both electrons and protons are transferred. Changes in electron content and oxidation state can profoundly affect acid−base and other thermodynamic properties. Therefore, the thermodynamic coupling between electrons and protons is a universal phenomenon during the electron- and proton-transfer process, generally referred to as proton-coupled electron transfer (PCET).[1] A particularly important example is the PCET of water oxidation in Photosystem II, where a key reaction step is the oxidation of $Tyrosine_Z$ by the photo-oxidized primary donor (P_{680}^+).[2] $Tyrosine_Z$ oxidation is coupled to deprotonation to a nearby base, which may occur either as consecutive PT and ET reactions or as a single, concerted reaction step.[3] The latter mechanism avoids the energetic cost of charge formation but is less robust to structural changes. Insight into the mechanism of such reactions is necessary for further understanding and mimicking photosynthesis.

We previously reported intramolecular PCET from tyrosine to photo-generated $Ru^{III}(bpy)_3$ in a covalently linked complex as a concerted electron−proton transfer (CEP) reaction[4] (denoted CPET or EPT by others[1c,f]). When water was the proton acceptor, the rate constant showed an unusual pH dependence: a plot of $\log k_{CEP}$ increased by 0.5 per pH unit in the range pH 5−10. Direct reaction with the buffer or OH^- could be excluded, as it would give a normal first-order dependence (slope = 1 in a plot of $\log k_{CEP}$ vs pH). Thanks to the intramolecular nature of the reaction and direct time-resolved laser spectroscopic measurements, we could resolve the reaction from tyrosine without interference from the very reactive fraction of tyrosinate that dominates the pH dependence in bimolecular studies. These results generated an interesting discussion and controversy about the mechanism of Tyr_Z oxidation, specifically the pH dependence.[5]

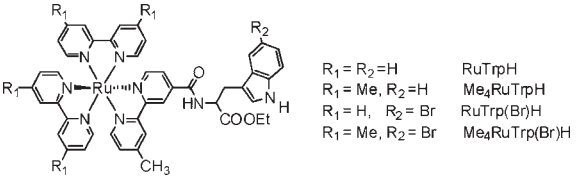
The tyrosine redox potential $E^{o\prime}(TyrO^{\bullet}/TyrOH)$ is pH-dependent from pH −2 to 10, so the driving force for the overall PCET process ($-\Delta G^{o\prime}_{PCET}$) is also pH-dependent. However, this equilibrium potential includes the entropy of proton release to the bulk. For the Ru−tyrosine reactions it was suggested that the rate dependence on pH followed a Marcus-type dependence on $-\Delta G^{o\prime}_{PCET}$,[4] but it was also noted that the connection between the bulk equilibrium potential $E^{o\prime}$ and the relevant energetics of the CEP step is not clear.[4b,c] As noted in Krishtalik's fundamentally sound analysis of PCET[6] and emphasized by others,[1e,5b−5f] the relevant driving force in such a case will indeed be independent of pH if a water molecule or small water cluster is the primary proton acceptor. Proton dilution to the bulk is a subsequent process, and the observed k_{CEP} should then be independent of pH. When pH-dependent PCET rate laws do appear, they may have a variety of origins such as buffer-assisted PCET or PTET with initial proton transfer to OH^-.[6b] However, all these possibilities were ruled out for the Ru−tyrosine reactions.[4c] Another interesting prediction of Krishtalik's analysis[6a] is that CEP can never compete with stepwise electron−proton transfer (ETPT) unless the intermediate radical cation has $pK_a < 0$, as for tyrosine. The argument is that proton transfer from an oxidized amino acid to the accepting water cluster is uphill, unless the former is at least equally as acidic as H_3O^+ ($pK_a \approx -1.5$). Because CEP has additional proton-coupling constraints compared to the ET step of an ETPT reaction, CEP would not be able to compete if the driving force is also lower.

We reasoned that tryptophan linked to $Ru^{II}(bpy)_3$ units would be interesting for further study of PCET with water as acceptor. First, tryptophan is an amino acid that is frequently

Received: February 18, 2011
Published: April 18, 2011

dx.doi.org/10.1021/ja201536b | *J. Am. Chem. Soc.* 2011, 133, 8806–8809

Scheme 1



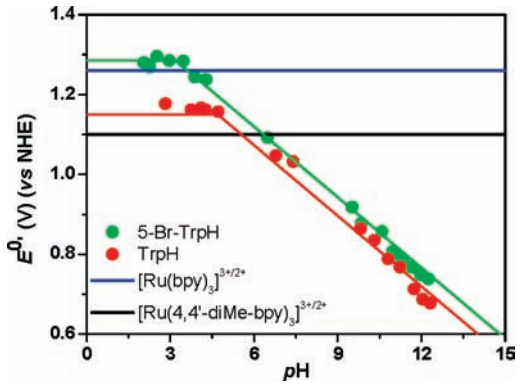


Figure 1. DPV peak potentials for oxidation of the tryptophan moieties in **RuTrpH** and **RuTrp(Br)H**. The lines show the theoretically expected pH dependence of the potentials. The $Ru^{III/II}$ potentials are $E^{o\prime}$ = 1.26 and 1.10 V for $Ru(bpy)_3^{III/II}$ and $Ru(Me\text{-}bpy)_3^{III/II}$, respectively.

involved in PCET under physiological conditions.[7] Second, the pK_a of oxidized tryptophan is ~4.7,[4b] much higher than for H_3O^+. Third, its radical cation ($Trp^{\bullet}H^+$) and neutral radical ($Trp^{\bullet}$) have absorption maxima around 570 and 510 nm, respectively (Figure S2). Thus, it may be possible to observe and even distinguish directly the ETPT and CEP mechanisms. We designed the complexes in Scheme 1 for further studies of bidirectional PCET in water, with special attention to the proton acceptor in this PCET oxidation process. **RuTrpH** was investigated in a previous study and found to undergo a pH-dependent PCET assigned to CEP, but only in a narrow range at high pH.[4b] Following the strategy used for Ru−tyrosine complexes, we designed the new complexes to have a lower driving force for the ET step, which suppresses the ETPT rate, with the aim to establish CEP over a wider pH range.

The redox potentials for **RuTrpH** and **RuTrp(Br)H** were determined by differential pulse voltammetry (DPV, Figure 1). While the $Ru^{III/II}$ couple is pH-independent,[4b] the tryptophan potential shows the pH dependence expected for a 1 e^-/1 H^+ couple above the pK_a of the tryptophan radical cation, in agreement with previous reports.[8] From our data we determined pK_a ≈ 4.7 ($Trp^{\bullet}H^+$) and 3.5 ($Trp^{\bullet}(Br)H^+$). The difference between the one-electron tryptophan potentials at low pH is ~135 mV, but the difference in the proton-coupled potentials is only ~65 mV because the electron-withdrawing bromide increases the one-electron oxidation potential but decreases the pK_a value. The reaction free energies for intramolecular one-electron oxidation (ΔG°_{ET}) and PCET oxidation ($\Delta G^{\circ\prime}_{PCET}$, see above) of the tryptophan by Ru^{III} are listed in Table 1.

Intramolecular PCET between the tryptophan and Ru^{III} units was triggered by the "flash-quench method" which was used extensively in our previous work.[4] Excitation of $[Ru(bpy)_3]^{2+}$

Table 1. Free Energies of Tryptophan Oxidation

	RuTrpH	RuTrp(Br)H	Me_4RuTrpH	Me_4RuTrp(Br)H
ΔG°_{ET} (eV)[a]	−0.100	+0.035	+0.060	+0.185
$\Delta G^{\circ\prime}_{PCET}$ (eV)[b]	−0.235	−0.170	−0.075	−0.010

[a] $\Delta G^{\circ}_{ET} = -e(E^{\circ}(Ru^{III/II}) - E^{\circ}(Trp^{\bullet}H^+/TrpH))$; work term neglected. [b] At pH 7, $\Delta G^{\circ\prime}_{PCET} = -e(E^{\circ}(Ru^{III/II}) - E^{\circ}(Trp^{\bullet}/TrpH))$.

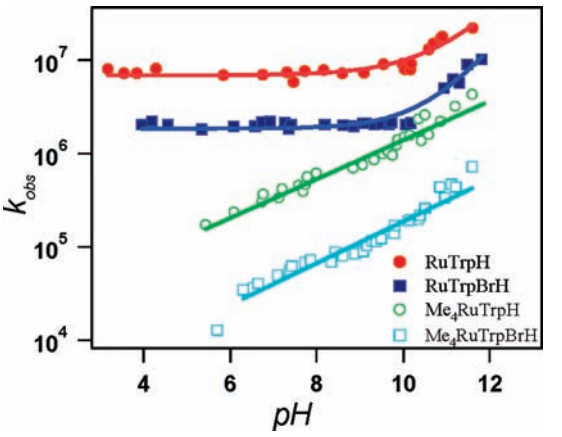


Figure 2. pH dependence of the observed rate constants for intramolecular oxidation of tryptophan to the flash-quench-generated Ru^{III} in 0.5 mM phosphate/borate buffer. The solid lines are linear fits to the data using one pH-dependent (linear) term; for the first two complexes a pH-independent term is also used. Note that for **RuTrpH** the subsequent deprotonation of $TrpH^{+\bullet}$ ($\tau \approx 450$ ns) is slower than the initial ET step (Figure S4).

with a 5 ns, 460 nm laser pulse followed by oxidative quenching with methyl viologen (MV^{2+}) gave the corresponding Ru^{III} complex, seen from the rapid appearance of $MV^{\bullet+}$ absorption around 390 and 600 nm and the bleach of the Ru^{II} ground state around 450 nm (Figure S1). Subsequent intramolecular PCET between the tryptophan and Ru^{III} units could be monitored using the Ru^{II} absorption recovery at 450 nm at different pH values (Figure S1). Single-exponential fits gave a first-order rate constant for PCET from tryptophan to Ru^{III} as plotted in Figure 2. For the slowest reactions, a mixed first-/second-order fit was used to account for the competing second-order recombination of Ru^{III} with $MV^{\bullet+}$ (see SI). Separate experiments with the irreversible acceptor $[Co(NH_3)_5Cl]^{2+}$, when there was no recombination, gave the same PCET rate constants. The resulting $Trp^{\bullet}$ product, with an absorption around 510 nm, could be detected using $Ru^{III}(NH_3)_6$ as acceptor instead of MV^{2+} (Figures 3 and S6). The formation of $Trp^{\bullet}$ from Ru^{III} was in all cases quantitative, as judged from the transient absorption changes and known extinction coefficients (Figure S2).

For **RuTrpH** the rate was constant over a large range and became pH-dependent above pH 10, in agreement with the previous report.[4b] The data can be fitted by a constant term and a pH-dependent term with a slope ≈ 0.5 (log k vs pH). **RuTrp(Br)H** showed a closely parallel behavior, with a consistently slower rate, except that the reaction stopped below the pK_a of $Trp(Br)H^{\bullet+}$ because the overall reaction was endergonic when the radical did not deprotonate.[9] Interestingly, **Me_4RuTrpH** and **Me_4RuTrp(Br)H** showed very different behavior. For both complexes the rate was pH-dependent in the entire range and

dx.doi.org/10.1021/ja201536b | J. Am. Chem. Soc. 2011, 133, 8806–8809

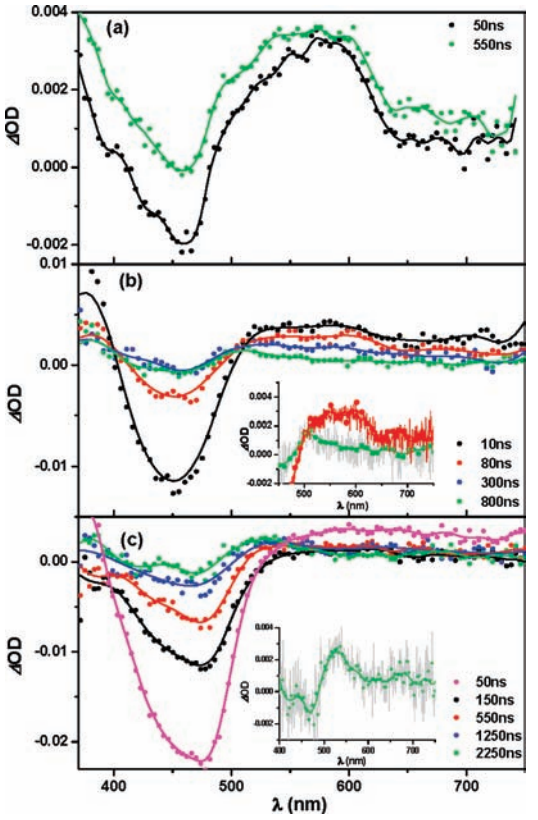


Figure 3. Transient absorption spectra after 460 nm laser flash-quench generation of the Ru^{III} species using 30−50 mM $[Ru(NH_3)_6]^{3+}$ acceptor, in 0.5 mM phosphate:borate buffer. (a) **RuTrpH** at pH 3: The product is tryptophan radical cation (570 nm). (b) **RuTrpH** at pH 9: The 10 ns spectrum shows mainly the Ru excited state. The initial product (80 ns spectrum) is the protonated radical (570 nm) that deprotonates to give the neutral radical with absorption maximum at 510 nm. (Inset: Magnification of the 80 and 800 ns spectra.) (c) **Me_4RuTrpH** at pH 9: The product is tryptophan radical formed directly. (Inset: Magnification of the 2250 ns spectrum.)

could be fitted by a straight line of slope ≈ 0.3 (log k vs pH). With this weaker Ru^{III} oxidant there was no reaction at pH <5.5 because of the unfavorable driving force. Note that the data were obtained in 0.5 mM phosphate:borate (1:1) buffer, and it was checked that the buffer concentration did not affect the observed PCET rate in this range (Figure S5).

The kinetic isotope effects (KIEs) were also very different for the four complexes: for **RuTrpH** and **RuTrp(Br)H** we found only small isotope effects in the pH-independent region at pH <9, $k_H/k_D \approx 1.0$ and 1.6, respectively. In contrast, for the pH-dependent reaction of **Me_4RuTrpH** and **Me_4RuTrp(Br)H** we found a significant isotope effect: $k_H/k_D \approx 3.5$ for both complexes at pH <9. For the former complexes there was a similarly large effect in the pH-dependent region at pH >10: $k_H/k_D \approx 2.4$ for **RuTrpH** and 4.0 for **RuTrp(Br)H**.

It is clear that replacement of the Ru^{III} oxidant unit resulted in a quite different kinetic behavior, which we propose is due to a switch from a stepwise to a concerted mechanism. The arguments for this are developed in the following paragraphs.

For **RuTrpH** at pH <4.7 the reaction is a single ET process because the oxidized tryptophan is not deprotonated ($pK_a = 4.7$); the transient spectra in Figure 3a show the clear signature of the $Trp^{\bullet}H^+$ product. At pH >4.7 the transient spectra give direct evidence for an ETPT mechanism: Figure 3b shows the formation of a $Trp^{\bullet}H^+$ intermediate concomitant with Ru^{II} recovery ($\tau \approx$ 130 ns), which then deprotonates to form $Trp^{\bullet}$ with $\tau \approx 490$ ns (Figure S3). The oxidation rate remains constant at pH 3−10, which is expected for an ETPT mechanism where the first step is effectively irreversible ($\Delta G°_{ET} = -100$ meV). The driving force and the rate for the ET step do not depend on pH, so the observed oxidation rate is the same as for single ET at pH <4, and the KIE is negligible. At pH >10 the rate instead becomes pH-dependent, with a significant isotope effect, showing that the mechanism is different (see below).

For **RuTrp(Br)H** the data are entirely analogous to those for **RuTrpH** except that for kinetic reasons the radical cation intermediate of the ETPT reaction cannot be detected (Figure S6a). This is because the observed oxidation is slower than for **RuTrpH** and the subsequent deprotonation is expected to be much faster ($pK_a = 3.5$ instead of 4.7), so very little intermediate builds up. The relatively slow initial ET rate is consistent with the higher potential for oxidation of the Trp-(Br)H unit. In fact, at pH < $pK_a(Trp^{\bullet}(Br)H^+)$, when the radical cation does not deprotonate, the overall reaction is slightly endergonic ($\Delta G°_{ET} = +0.035$ eV; Table 1) and does not occur to a detectable degree.[9] In the ETPT region (pH 4−10), however, the initial ET is stabilized by the following exergonic deprotonation that drives the overall reaction.

For **Me_4RuTrpH** and **Me_4RuTrp(Br)H** the mechanism is apparently different, showing a continuously pH-dependent rate and a significant KIE. We assign this to a concerted mechanism because the stepwise mechanism can be excluded:

Stepwise, proton-first mechanisms (PTET) with water, OH^-, or buffer species as proton acceptor are too slow to explain the observed rates, as the pK_a of TrpH is ∼17[10] (∼16 for Trp-(Br)H). Thus, even deprotonation with OH^- is uphill and therefore slower than diffusion-controlled: with $\Delta pK_a(TrpH - H_2O) \approx 2$, the pseudo-first-order constant is $k_p[OH^-] \approx 10^4\ s^{-1}$ at pH 10, which is much slower than the observed values. Also, all PTET mechanisms should be first order in concentration of base and thus give a much stronger pH dependence (slope = 1 in Figure 2) than observed (slope = 0.3). Finally, the rates would be faster with brominated tryptophans because of the lower pK_a value, but we instead observe lower rates. We do not exclude contribution from PTET with OH^- for the data at pH >11 for **Me_4RuTrpH** and **Me_4RuTrp(Br)H**, and there is some positive deviation from the straight line fit in Figure 2. However, we conclude that PTET mechanisms can be excluded as an explanation for our data below pH 11.

Step-wise, electron-first mechanisms (ETPT) cannot be faster than the initial ET step. For **RuTrpH** and **RuTrp(Br)H** we measure this rate constant at pH <10. The observed PCET rate constant increases at high pH and the KIE increases, which is not consistent with rate-limiting ET in this pH region. For **Me_4RuTrpH** and **Me_4RuTrp(Br)H** both the pH dependence and KIE are inconsistent with an ETPT mechanism with rate-limiting ET.

Finally, reversible ET followed by rate-limiting deprotonation can also be excluded for **Me_4RuTrpH** and **Me_4RuTrp(Br)H** because neither of the reaction steps would give rise to the observed pH dependence. The potentials for $Ru^{III/II}$ and $Trp^{\bullet}H^+$/TrpH are independent of pH, and if the electronic coupling or other parameters governing the pure ET rate would for some unknown reason depend on pH in the range 3−10, this would have been reflected in the observed rates for **RuTrpH** and **RuTrp(Br)H**. Also the rate of the subsequent $Trp^{\bullet}H^+$ deprotonation is pH-independent in this range, as expected for an Eigen acid with water as

acceptor; this is also confirmed by our direct measurements on **RuTrpH** (Figure S4).[11] Note that buffer species and OH^- can be excluded as primary proton acceptors under these conditions, with the same arguments as for the CEP mechanism discussed next.

Because we can exclude the ETPT and PTET mechanisms for **Me_4RuTrpH** and **Me_4RuTrp(Br)H**, this leaves the CEP mechanism that we propose is responsible for the pH-dependent PCET reactions in Figure 2. The KIEs are consistent with CEP where the proton coupling is dependent on the differences in vibrational wave function overlap for the two isotopes. It is also consistent with the lack of a detectable $Trp^{\bullet}H^+$ intermediate for **Me_4RuTrpH**. A key point is the identity of the proton acceptor in the CEP process, and there are three possibilities: OH^-, base forms of the buffer, or water. Our control experiments varying the buffer concentration (Figure S5) show that the rate is independent of buffer in the range employed in Figure 2. With OH^- as acceptor, even a diffusion-controlled reaction cannot be faster than $k[OH^-] \approx 1 \times 10^{-3}\ s^{-1}$ at pH 7, which is much slower than the observed rate constants. Also, for OH^- or buffer as acceptor the pH dependence in Figure 2 would have been first-order in $[OH^-]$ or [base], with slope = 1 instead of 0.3 (or 0.5 as for **RuTrpH** and **RuTrp(Br)H** at pH >10). This leaves water as the primary acceptor for CEP.

A pH dependence similar to that shown here was reported for intramolecular PCET in analogous Ru−tyrosine complexes with water as acceptor (slope ≈ 0.5).[4] Also in that case the stepwise mechanisms could be excluded. Lowering the $Ru^{III/II}$ potential decreases the rate of ETPT, so the pH-dependent mechanism can compete. However, a key point of contention is the relevant driving force for the observed CEP, which should also be affected by the lower $Ru^{III/II}$ potential. According to Krishtalik's analysis, the driving force for CEP with a small cluster of water as proton acceptor is even 0.3−0.4 eV more endergonic than the pure ET oxidation of tryptophan, and it is difficult to explain why this mechanism would be able to compete with ETPT. The model with irreversible CEP and a small, pH-independent water cluster as primary proton acceptor therefore seems unable to explain the observed data. It is thus important to consider alternative models, such as reversible primary CEP followed by proton migration away from the initial cluster as part of the rate-determining step. However, it should be noted that straightforward reversible reaction schemes invariably give zeroth- or first-order dependence on OH^- or other bases, i.e. slope = 0 or = 1 in Figure 2, which is inconsistent with the present observations.

To conclude, tryptophan oxidation in aqueous solution is a paradigm of PCET and a good model for further discussion of how electrons and protons are coupled in PCET. In redox proteins tryptophan tends to react via pure ET or stepwise ETPT, and likewise when water is available as proton acceptor,[7] while tyrosine more often undergoes CEP.[2] The competition between concerted and stepwise reactions may be particularly intricate in cases like ribonucleotide reductase, where a chain of several tyrosines and tryptophans are believed to be responsible for long-range (35 Å) radical transfer.[7b] Our present data on model complexes strongly suggest that PCET from tryptophan with water as primary acceptor can be tuned to occur via either ETPT or CEP. Our data also establish an unusual dependence on pH for CEP, with slope = 0.3−0.5 in a plot of log k vs pH, similar to previous results for Ru−tyrosine complexes.[4] This is in contrast to the expected zeroth- or first-order dependences for common alternative mechanisms. Our results suggest that further revision of the models for PCET with water as proton acceptor may be necessary.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details and kinetic data for buffer dependence. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank Drs. Tania Irebo and Todd Markle for helpful discussions. This work was supported by the Swedish Energy Agency, The Swedish Research Council, and The Knut and Alice Wallenberg Foundation.

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(9) Very slow ($\tau \approx 2$ ms) recovery of the bleach at 450 nm observed with $[Co^{III}(NH_3)_5Cl]Cl$ as sacrificial quencher was attributed to an irreversible reaction of the radical fraction in equilibrium with Ru^{III}.

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(11) The deprotonation is pH-independent and slower than that previously reported (130 ns), which was measured under higher buffer concentration (10 mM). According to our buffer experiment (Figure S5), the basic form of the buffer is a significant proton acceptor at high buffer concentration.