Riboflavin As Bioorthogonal Photocatalyst For The Activation Of A PtIV Prodrug

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[Rf] = 50 µM

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[Rf] = 0.13 µM

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Figure S15. Plot of Fe(II) mol vs. irradiation time at 460 nm. The ferrioxalate actinometer (K₃[Fe(C₂O₄)₃]) (0.15 M) was irradiated with a 460-nm light source in a plate reader and a phenatroline-based developing solution was then added to determine spectrophotometrically the amount of Fe(II) ions generated by irradiation, as described by S. L. Hopkins et al. Data were fitted with the equation \( y = (3.12 \cdot 10^{-9} \pm 0.17 \cdot 10^{-9})x + (1.60 \cdot 10^{-8} \pm 4.52 \cdot 10^{-9}) \). R-Square = 0.998. Being the absolute quantum yield \( \Phi_{Fe(II)} \) for (K₃[Fe(C₂O₄)₃]) (0.15 M) at 460 nm equal to 0.65, a photon flux of \( 8.09 \cdot 10^{-9} \pm 0.94 \cdot 10^{-9} \) mol photon·sec\(^{-1} \) was obtained for the light source using the equation:

\[
\Phi_{Fe(II)} = \frac{\eta_{Fe(II)}}{I_{abs}}
\]

where \( \eta_{Fe(II)} \) the moles of Fe(II) produced photochemically and \( I_{abs} \) the absorbed photon dose by the Fe-oxalate complex.
Figure S16. Moles of photodissociated succinate ligand vs. irradiation time at 460 nm. MES (18 mM) solutions of \(\text{I} (1.00 \text{ mM})\) and \(\text{Rf} (50 \mu \text{M})\) placed in a plate reader were irradiated at 460 nm for different time intervals and using the same setup employed for the ferrioxalate actinometry. The amount of photodissociated succinate was quantified by \(^1\)H NMR spectroscopy and data were fitted with the equation 

\[
y = (-5.26 \cdot 10^{-7} \pm 4.2 \cdot 10^{-8})e^{32.89 \pm 6.45} + (-5.44 \cdot 10^{-7} \pm 2.9 \cdot 10^{-8}).
\]

R-Square = 0.969.

By employing the equation

\[
\phi = \frac{\text{number of molecules (or moles) consumed or produced per unit time}}{\text{number of photons adsorbed per unit time}} = \frac{d [\text{Succinate}]}{dt} \frac{2}{\text{Lamp Intensity}}
\]

a quantum yield \(\phi\) of 1.4 ± 0.1 was determined.
Figure S17. Photostability of Rf in MES buffer. $^1$H NMR spectra of a MES/D$_2$O (9:1, MES 18 mM, pH 6.0) solution of 240 µM Rf under 460-nm light irradiation (2.5 mW·cm$^{-2}$) for $t_{irr} = 0$, 30 and 60 min. $^1$H NMR signal labelling: ● methyl groups of Rf isoalloxazine ring.
Figure S18. Photostability of Rf in MES buffer and water. UV-Vis spectrum of Rf (50 µM) in MES buffer (20 mM, pH 6) and in water (pH 7) at different irradiation times ($\lambda_{irr} = 460$ nm, 2.5 mW·cm$^{-2}$; MES: violet $t_{irr} = 0$, magenta $t_{irr} = 2.5$ min, and pink $t_{irr} = 5$ min; Water: dashed-blue $t_{irr} = 1$ min). The absorption profile obtained after light irradiation in water (dashed blue line) corresponds to lumichrome, a common photoproduct of Rf photolysis.
Figure S19. Photostability of Rf in Phosphate Buffer (PB). $^1$H NMR spectra of a PB/D$_2$O (9:1, 100 mM, pH 5.5) solution of 240 µM Rf under 460-nm light irradiation (2.5 mW·cm$^{-2}$) for $t_{irr}$ = 0, 30 and 60 min. Changes in the $^1$H NMR signal at 3.5–3.8 ppm indicate the ribityl side chain is undergoing intramolecular photodegradation. Appearance of the peak at at 2.33 ppm (●) is consistent with the formation of 2,3-butanedione, a photoproduct obtained by O$_2$-oxidation of the isoalloxazine ring.
Figure S20. Photolysis of 1 in HEPES buffer in the presence of 50 µM Rf. $^1$H NMR spectra of a HEPES/D$_2$O solution (9:1, HEPES 18 mM, pH 6.0) of 120 µM 1 and 50 µM Rf under 460-nm light irradiation (2.5 mW·cm$^{-2}$) for $t_{\text{irr}} = 0$ sec, 30 sec, 1 min. $^1$H NMR signal labelling: ● Pt–OCOCH$_2$CH$_2$CO$_2$–, ● Pt–OCOCH$_2$CH$_2$CO$_2$–, ● methyl groups of Rf isoalloxazine ring, ● free $^-$ O$_2$CCH$_2$CH$_2$CO$_2$–.
Figure S21. Stability control in the dark for Rf/1 in HEPES buffer. $^1$H NMR spectra of HEPES/D$_2$O solution (9:1, HEPES 18 mM, pH 6.0) containing 120 μM 1 and 50 μM Rf in the dark for t = 0 and 3 h. $^1$H NMR signal labelling: ● Pt–OCOCH$_2$CH$_2$CO$_2^-$, ● Pt–OCOCH$_2$CH$_2$CO$_2^-$ and ● methyl groups of Rf isoalloxazine ring.
Figure S22. Photolysis of 1 in the presence of Rf and NaN₃ in water. (a) ¹H NMR spectra of a H₂O/D₂O (9:1, pH 3) solution of Rf/1 (50/120 µM) with 3 mM NaN₃ under 460-nm light irradiation (2.5 mW·cm⁻²) for tᵣᵣ = 0 and 30 min; (b) ¹H NMR spectra of a H₂O/D₂O (9:1, pH 2.7) solution of Rf/1 (240/120 µM) without NaN₃ irradiated under the same conditions. In all experiments, HCOOH was added (3 mM and 24 mM for a and b respectively) to improve the photoconversion of 1 and slightly reduce the photodecomposition of Rf. ¹H NMR signal labelling: ● Pt–OCOCH₂CH₂CO₂⁻, ● Pt–OCOCH₂CH₂CO₂⁻, ● methyl groups of Rf isoalloxazine ring, ● free O₂CCH₂CH₂CO₂⁻.
Figure S23. Photolysis of 1 in the presence of Rf and NaN₃ in Phosphate Buffer (PB). ^1H NMR spectra of a PB/D₂O (9:1, 100 mM, pH 5.5) solution of Rf/1 (240/120 µM), with (a) or without (b) 1 mM NaN₃ under 460-nm light irradiation (2.5 mW·cm⁻²) for t_irr = 0 and 30 min. In all experiments, 24 mM HCOOH was added to improve the photoconversion of 1 and slightly reduce the photodecomposition of Rf. ^1H NMR signal labelling: ● Pt–OCOCH₂CH₂CO₂⁻, □ Pt–OCOCH₂CH₂CO₂⁻, ● methyl groups of Rf isoalloxazine ring, ● free ‘O₂CCH₂CH₂CO₂⁻’.
Figure S24. Photolysis of 1 in MES buffer in the presence of Rf and NaN₃. ¹H NMR spectra of a MES/D₂O (9:1, 18 mM, pH 6.0) solution of Rf/1 (50/120 µM) with 18 mM NaN₃ under 460-nm light irradiation (2.5 mW·cm⁻²) for tᵣᵣ = 0, 2.5, 5 and 10 min. ¹H NMR signal labelling: ● Pt–OCOCH₂CH₂CO₂⁻, ● Pt–OCOCH₂CH₂CO₂⁻, ● methyl groups of Rf isoalloxazine ring, ● free − O₂CCH₂CH₂CO₂⁻.
Figure S25. Photolysis of 1 in MES buffer in the presence of Rf under Ar atmosphere or in air. $^1$H NMR spectra of a MES/D$_2$O (9:1, 18 mM, pH 6.0) solution of Rf/1 (50/200 µM) under 460-nm light irradiation (6 mW·cm$^{-2}$) for $t_{irr} = 0$ and 30 sec. $^1$H NMR signal labelling: ● Pt–OCOCH$_3$CH$_2$CO$^-$, ● Pt–OCOCH$_3$CH$_2$CO$^-$, ● methyl groups of Rf isosalloxazine ring, ● free O$_2$CCH$_3$CH$_2$CO$^-$.
**Figure S26.** Lifetime decay profile for **Rf**. Fluorescence lifetime measurement for a 5 μM **Rf** solution in MES buffer (10 mM) measured in a time-correlated single photon counting (TCSPC) setup. **Rf** exhibits a mono-exponential decay with a lifetime of 4.60 nm (green dots). IRF (grey dots) = instrument response function (prompt).
Figure S27. Lifetime decay profile for Rf in the presence of 1. Fluorescence lifetime measurement for a 5 μM Rf solution in MES buffer (10 mM) measured in the presence of 1.8 mM of 1 in a time-correlated single photon counting (TCSPC) setup. Rf exhibits a mono-exponential decay with a lifetime of 4.63 nm (blue dots). IRF (grey dots) = instrument response function (prompt).
Figure S28. DFT-optimized structure and frontier orbitals of a selected 1-RfH₂ adduct. (a) Conformation of a selected 1-RfH₂ adduct optimized by DFT at the PBE0/def2-SVP¹,² level (H-bond contacts highlighted with violet lines). (b–d) Frontier orbitals for the optimized conformation of the 1-RfH₂ adduct (isodensity surfaces plotted with the isovalue of 0.02 e⁻·bohr⁻³). The stabilization energy for 1-RfH₂ is – 52.0 kcal·mol⁻¹ and is calculated using the formula ΔE = E₁-RfH₂ – (E₁ + E_RfH₂).
Figure S29. DFT-optimized structure and frontier orbitals of a selected RfH⁻ adduct (N5). (a) Conformation of a selected 1-RfH⁻ adduct (N5-protonated) optimized by DFT at the PBE0/def2-SVP¹² level (H-bond contacts highlighted with violet lines). (b–d) Frontier orbitals for the optimized conformation of the 1-RfH⁻ adduct (isodensity surfaces plotted with the isovalue of 0.02 e⁻·bohr⁻³). The stabilization energy for 1-RfH⁻ is −68.8 kcal·mol⁻¹ and is calculated using the formula ΔE = E₁-RfH⁻ − (E₁ + E_RfH⁻).
Figure S30. DFT-optimized structure and frontier orbitals of a selected RfH⁻ adduct (N1). (a) Conformation of a selected 1-RfH⁻ adduct (N1-protonated) optimized by DFT at the PBE0/def2-SVP¹,² level (H-bond contacts highlighted with violet lines). (b–d) Frontier orbitals for the optimized conformation of the 1-RfH⁻ adduct (isodensity surfaces plotted with the isovalue of 0.02 e⁻·bohr⁻³). The stabilization energy for 1-RfH⁻ is –61.2 kcal·mol⁻¹ and is calculated using the formula ΔE = E₁-RfH − (E₁ + E_RfH).
Figure S31. Photolysis of 1 in MES buffer in the presence of 50 µM FMN. $^1$H NMR spectra of a MES/D$_2$O solution (9:1, MES 18 mM, pH 6.0) of 120 µM 1 and 50 µM FMN under 460-nm light irradiation (2.5 mW·cm$^{-2}$) for $t_{irr} = 0$ sec, 30 sec and 1 min. $^1$H NMR signal labelling: ● Pt–OCOCH$_3$CH$_2$CO$_2^-$, ● Pt–OCOCH$_3$CH$_2$CO$_2^-$, ● methyl groups of FMN isoalloxazine ring and ● free $^-$O$_2$CCH$_2$CH$_2$CO$_2^-$.
Figure S32. Stability of 1 in MES buffer in the presence of 50 µM FMN. $^1$H NMR control spectra of a MES/D$_2$O solution (9:1, MES 18 mM, pH 6.0) of 120 µM 1 and 50 µM FMN in the dark for 3 h. $^1$H NMR signal labelling: ● Pt–OCOCH$_2$CH$_2$CO$_2^-$, ○ Pt–OCOCH$_2$CH$_2$CO$_2^-$, ● methyl groups of FMN isoalloxazine ring.
Figure S33. Photolysis of 1 in MES buffer in the presence of 50 µM FAD. \(^1\)H NMR spectra of a MES/D\(_2\)O solution (9:1, MES 18 mM, pH 6.0) of 120 µM 1 and 50 µM FAD under 460-nm light irradiation (2.5 mW·cm\(^{-2}\)) for \(t_{irr} = 0\) sec, 30 sec and 2.5 min. \(^1\)H NMR signal labelling: ● Pt–O\(\text{COCH}_2\text{CH}_2\text{CO}_2^-\), ● Pt–O\(\text{COCH}_2\text{CH}_2\text{CO}_2^-\), ● methyl groups of FAD isoalloxazine ring and ● free –O\(\text{O}_2\text{CCH}_2\text{CH}_2\text{CO}_2^-\).
Figure S34. Stability of 1 in MES buffer in the presence of 50 µM FAD. $^1$H NMR control spectra of a MES/D$_2$O solution (9:1, MES 18 mM, pH 6.0) of 120 µM 1 and 50 µM FAD in the dark for 3 h. $^1$H NMR signal labelling: ● Pt–OCOCH$_2$CH$_2$CO$_2^-$, ● Pt–OCOCH$_2$CH$_2$CO$_2^-$, methyl groups of FAD isoalloxazine ring.
Figure S35. Dependency of photocatalytic prodrug activation on pH. Photocatalytic activation of 1 at different pHs (4-10) and at a fixed concentration of Rf (50 µM), 1 (120 µM) and MES (18 mM). An irradiation time of 2 min and 30 sec was set for all the samples ($\lambda_{exc} = 460$ nm, 2.5 mW·cm$^{-2}$).
Figure S36. Photostability of cell culture medium. $^1$H NMR spectra of cell culture medium (Ham's F-12K supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin) before and after 1 min of irradiation with 460-nm light (6 mW·cm$^{-2}$).
Figure S37. Photostability of Rf in cell culture medium. UV-Vis spectra of Rf (50 μM) in cell culture medium (Ham's F-12K supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin) with 3 mM MES in the dark (violet line), after 1 min of irradiation (pink line) at 460 nm (6 mW·cm$^{-2}$) and after 1 min of irradiation in the absence of MES (blue line) at 460 nm (6 mW·cm$^{-2}$).
Figure S38. Photolysis of 1 in cell culture medium in the presence of Rf and MES. $^1$H NMR spectra of cell culture medium/MES/D$_2$O (7.5:1.5:1, 3 mM MES) solution containing 1.92 mM 1 and 50 µM Rf under 460-nm light irradiation (6 mW·cm$^{-2}$) for $t_{irr} = 0$, 30 sec, 2.5 min and 3 min. Cell culture medium corresponds to Ham’s F-12K medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. $^1$H NMR signal labelling: ● Pt–OCOCH$_2$CH$_2$CO$_2$-, ● Pt–OCOCH$_3$CH$_2$CO$_2$- ● free O$_2$CCCH$_2$CH$_2$CO$_2$. 

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Figure S39. MES toxicity in PC-3 cells. Control experiments performed treating PC-3 cells with MES (2 mM) under light irradiation, using two different incubation conditions. Cells were plated in 96-well plates with a density of 4000 cells per well supplemented with serum and antibiotics and left to grow for 24 h at 37 °C with 5% CO₂ and 90% humidity. MES was then dissolved in the cell culture medium to reach a 2 mM concentration and cells were incubated for 1 h and then irradiated for 1 min with 460-nm light (light dose 0.36 J·cm⁻²). Afterwards, cells were either incubated for other i) 6 hours or ii) 24 h before medium was replaced and cells grown of a total of 48 h. The SRB assay was employed for both incubation conditions to evaluate cell density.
Figure S40. Assessment of the presence of 1 in cell culture medium without MES buffer. $^1$H NMR spectra of cell culture medium/D$_2$O (9:1) solution containing 120 µM 1. Cell culture medium corresponds to Ham's F-12K medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. $^1$H NMR signal labelling: ● Pt–OCOCH$_2$CH$_2$CO$_2^–$. 
Figure S41. Photolysis of 1 in cell culture medium in the presence of Rf and MES. $^1$H NMR spectra of cell culture medium/MES/D$_2$O (7.5:1.5:1) solution containing 120 µM 1 and 40 µM Rf under 460-nm light irradiation (6 mW·cm$^{-2}$) for $t_{irr} = 0$ and 1 min. Cell culture medium corresponds to Ham’s F-12K medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. $^1$H NMR signal labelling: ● free $^\text{O}_2$CCH$_2$CH$_2$CO$_2^-$ (compared to Figure S30, the triplet signal of 1 at 2.55 ppm is here buried under one of the MES signals).
Figure S42. Photolysis of 1 in cell culture medium in the presence of Rf. $^1$H NMR spectra of cell culture medium/H$_2$O/D$_2$O (7.5:1.5:1) solution containing 120 μM 1 and 40 μM Rf under 460-nm light irradiation (6 mW·cm$^{-2}$) for $t_{irr} = 0$ and 1 min. Cell culture medium corresponds to Ham's F-12K medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. $^1$H NMR signal labelling: ● Pt–OCOCH$_2$CH$_2$CO$_2^-$, ● free $^\circ$O$_2$CCH$_2$CH$_2$CO$_2^-$.
Figure S43. Light-activated platination of 5′-guanosin monophosphate (GMP) by Rf/1. $^1$H NMR spectra of a MES/D$_2$O (9:1) solution (1.5 mM, pH 6.0) of 120 µM 1, 50 µM Rf after 460-nm light irradiation (1 min, 2.5 mW·cm$^{-2}$) and incubation with GMP (0.5 mM) for t = 0, 12 and 24 h. $^1$H NMR signal labelling: ■ mono-adduct cis-[Pt(NH$_3$)$_2$(N7-GMP)$_2$]$^2^+$. 
Figure S44. Platination of 5′-guanosin monophosphate (GMP) by cisplatin. $^1$H NMR spectra of a MES/D$_2$O (9:1) solution (2 mM, pH 6.0) of 120 µM cisplatin and incubation with GMP (0.5 mM) for $t =$ 0, 12 and 24 h. $^1$H NMR signal labelling: ■ mono-adduct $cis$-[Pt(NH$_3$)$_2$(N7-GMP)$_2$]$^{2-}$.
Figure S45. Inhibition of the polymerase chain reaction (PCR) using as template pET28b incubated with Rf (2.5 µM), Rf/1 (2.5:10 µM) and cisplatin (10 µM) in the dark and under 30 s of light irradiation at 460 nm (2.5 mW·cm⁻², 1.5 mM MES and pH 6). Values below the gel indicate intensities of amplified DNA fragment normalized by that of the DNA control in the dark.
Figure S46. Morphological analysis of PC-3 cells. Merged DIC (Differential Interference Contrast) and fluorescence microscopy images showing the effects of Rf/1 on PC-3 cells upon light irradiation. (A) untreated PC-3 cells, (B) Rf/1 (30:120 µM) in the dark, (C) Rf/1 (30:120 µM) activated by 460-nm light (light dose 0.36 J·cm⁻²) and (D) cisplatin (120 µM) in the dark. All samples were treated in the presence of 2 mM of MES. Cells were stained at the end of the incubation period (48 h) using the dye SYTOX® AADvanced™ (Invitrogen™) for dead cells (red channel).
Figure S47. LED setup for cell work and emission profile. (a) Custom-made array of blue emitting LEDs used for irradiation of 96-well plates. (b) Emission profile of the LED array (460 nm, 6 mW·cm$^{-2}$) employed in the cell work experiment.
Experimental details

Materials
(-)-Riboflavin (Rf), formic acid, 2-(N-morpholino)ethanesulfonic acid (MES), sodium phosphate monobasic monohydrate, guanosine 5'-monophosphate disodium salt hydrate (GMP), cisplatin were purchased from Sigma Aldrich, sodium phosphate dibasic from PANREAC and K₂PtCl₄ from Precious Metals Online. All chemicals were used as received without additional purification. Ham's F-12K (Kaighn's) medium nutrient mixture and fetal bovine serum (FBS) were purchased from Invitrogen. Penicillin – Streptomycin was purchased from Teknovas. The pET28b plasmid was purchased from Novagen, DNA primers from Sigma and the Dream Taq polymerase and SYBR Safe dye from Thermo-Fisher.

Synthesis of cis,cis,trans-[Pt(NH₃)₂(Cl)₂(O₂CCH₂CH₂CO₂H)] (1)
The platinum complex was synthesized by following the procedure described by M. Reithofer et al.⁵

Instrumentation
Nuclear Magnetic Resonance (NMR). ¹H NMR spectra of the various samples were recorded on an AVANCE III Bruker 500 NMR spectrometer using standard pulse programs. Chemical shifts were reported in parts-per-million (δ, ppm) and referenced to the residual solvent peak.
UV–Vis absorption spectroscopy (UV-vis). All spectra of 1 and Rf were acquired in aqueous solution or buffers using a Varian Cary 5000 spectrophotometer.

Photoirradiation experiments
Photoirradiation experiments were performed on aqueous and buffer solutions obtained dissolving 1 and Rf at different concentrations in a 1 mL glass vial and irradiating the whole volume with a LED light source (λIRR = 460 nm, 2.5 mW·cm⁻², Prizmatix LED Multi-Wavelength MWLLS-11). In the case of cell experiments, black 96-well plates were photoirradiated using the blue LED array shown in Figure S3 (λIRR = 460 nm, 6 mW·cm⁻²). Power densities were measured with a Ophir photonics power meter.

Quantum yield determination by actinometry.
Ferrioxalate actinometry was employed to determine the photon flux of the blue LED array (λIRR = 460 nm, 6 mW·cm⁻²) and the yield of the photochemical activation for the Rf/1 system. Samples were irradiated in 96-well plates as described in the modified method of Bonnet and co-worker.³ ¹H NMR was employed to quantify the concentration of succinate ligand photoreleased and determine the yield of the photochemical reaction. Actinometry experiments were repeated three times.

Computational details
All calculations were performed with the Gaussian 09 program.⁶ The systems were analyzed with Density Functional Theory, using the PBE0/def2-SVP combination, which was previously used in similar studies.⁷ Solvent was considered by means of the polarized continuum model (PCM) with water as implicit solvent, and dispersion interactions were taken into account using Grimme’s dispersion correction with Becke and Johnson’s damping.⁸ The geometries were optimized and frequency calculations were run to ensure the lack of imaginary modes. Several isomers were found for each of the complexes presented;
only the global minima are presented in this work. The binding energy was calculated as the electronic energy balance of the reaction \( \text{Rf} + 1 \rightarrow \text{Rf}/1 \).

**Polymerase chain reaction (PCR)-inhibition assay using Rf/1-treated DNA.** 2 ng/µL pET28b was light irradiated at 460 nm for 30 s (2.5 mW·cm\(^{-2}\)) in presence of 2.5 µM of Rf and 10 µM of 1 in 1.5 mM MES at pH 6. As negative controls, DNA and DNA incubated with 2.5 µM Rf were also irradiated. Moreover, non-irradiated controls were run for each sample. As positive control, DNA was also incubated with 10 µM cisplatin. After incubating irradiated and non-irradiated mixtures for 24 h in the dark, 2 ng of plasmid DNA were used as template for the amplification of 1342 bps fragment by using the following primers: AACTTAATGGGCCCCTAACAG (primer forward) and CGTCCCCATTCGCCATCC (primer reverse). PCR was performed using the Dream Taq polymerase according to the manufacturer’s protocol. PCR was run for 30 cycles and its products were analysed by DNA electrophoresis using 1% agarose gels and SYBR Safe as dye to visualize DNA. The DNA bands were quantified using Image J.

**Photocatalysis kinetic model**

Assuming the reaction scheme reported below in which Rf acts as a photocatalyst, we have adopted a simplified kinetic model in which the reaction rate depends on both 1 and MES Eq. (1) (Figure S10 and S11). Nevertheless, such rate can be approximated with as a pseudo-first order reaction considering that the initial concentration of MES is much larger than the initial concentration of 1. Thus, the concentration of MES remains constant during the chemical reaction. Within this approach, a pseudo-first order reaction constant \( k_{\text{obs}} = 10.0 \pm 0.1 \cdot 10^{-3} \text{ s}^{-1} \) can be calculated fitting experimental data to Eq (2) (Figure S12) or to Eq (3) (Figure S10). Moreover, a second order reaction constant \( k = 1.3 \pm 0.1 \text{ M}^{-1} \text{s}^{-1} \) can be calculated using Eq (4) by fitting the experimental data for \( k_{\text{obs}} \) at different \([\text{MES}]_o\) (Figure S13).

**Reaction scheme**

\[
2[\text{MES}] + [\text{Rf}] \rightarrow 2[\text{MES}^*] + [\text{RfH}_2/\text{RfH}^-]
\]

\[
[\text{RfH}_2/\text{RfH}^-] + [1] \rightarrow [\text{Rf} + [\text{Pt}^{II}]]
\]

\[
2[\text{MES}] + [1] \rightarrow 2[\text{MES}^*] + [\text{Pt}^{II}]
\]

**Kinetic Model**

rate\((v) = k[\text{MES}][1] \quad \text{(1)}\)

\[ [1] = [1]_0 e^{-k_{\text{obs}}t} \quad \text{(2)}\]

\[ v = k_{\text{obs}} [1] \quad \text{[MES]}_o >> [1]_o \quad \text{(3)}\]

\[ k_{\text{obs}} = k[\text{MES}]_o \quad \text{(4)}\]
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