
Sean C. Marguet, Michael J. Stevenson, and Hannah S. Shafaat*

The Ohio State University, 100 W. 18th Ave., Columbus, Ohio 43210
Supporting Information

Supplemental Figures .................................................................................................................. S3-S29
S1. UV-Vis spectra of TA samples prior to and following photolysis
S2. UV-Vis spectra of TA samples prior to and following photolysis with Asc present
S3. UV-Vis of RuZn^{II}Rd during TA experiment
S4. UV-Vis of RuFe^{III}Rd during TA experiment
S5. UV-Vis of RuNi^{II}Rd during TA experiment
S6. GC calibration curve for H_{2} detection
S7. Global fit of TCSPC kinetic traces for RuZn^{II}Rd, RuFe^{III}Rd, and RuNi^{II}Rd
S8. TA spectra of RuZn^{II}Rd, RuFe^{III}Rd, RuNi^{II}Rd, and [Ru(bpy)_{3}]^{2+}
S9. Global fit of RuZn^{II}Rd TA kinetic traces
S10. Global fit of RuFe^{III}Rd TA kinetic traces
S11. Global fit of RuNi^{II}Rd TA kinetic traces
S12. TA kinetic trace of RuNi^{II}Rd at 520 nm
S13. TA kinetic traces of RuFe^{II}Rd and RuFe^{III}Rd at 520 nm
S14. Modified Latimer diagrams for RuFe^{II}Rd and RuFe^{III}Rd
S15. TA kinetic trace of RuFe^{III}Rd at 410 nm
S16. Global fit of RuFe^{II}Rd TA kinetic traces
S17. Stern-Volmer quenching kinetics for RuZn^{II}Rd (TCPSC)
S18. Stern-Volmer quenching kinetics for RuFe^{III}Rd (TCPSC)
S19. Stern-Volmer quenching kinetics for RuNi^{II}Rd (TCPSC)
S20. Stern-Volmer quenching kinetics for RuZn^{II}Rd (TA)
S21. Stern-Volmer quenching kinetics for RuFe^{III}Rd (TA)
S22. Stern-Volmer quenching kinetics for RuNi^{II}Rd (TA)
S23. TA spectra of RuZn^{II}Rd, RuFe^{III}Rd, and RuNi^{II}Rd with Asc present
S24. TA kinetic traces showing Ru^{I} formation at 520 nm in RuZn^{II}Rd, RuFe^{III}Rd, and RuNi^{II}Rd
S25. UV-Vis spectra of FeRd + 100 mM Asc at pH 6.5 and pH 8
S26. Modified Latimer diagram of competing pathways for RuNiRd
S27. Modified Latimer diagram of the second photocycle for RuNiRd

Supplemental Tables .................................................................................................................. S30-S33
ST1. TCSPC global fit parameters for RuZn^{II}Rd, RuFe^{III}Rd, and RuNi^{II}Rd
ST2. TA global fit parameters for RuZn^{II}Rd, RuFe^{III}Rd, RuFe^{II}Rd, and RuNi^{II}Rd
ST2. Stern-Volmer quenching constants (k_{Q}) obtained from TCSPC, emission, and TA measurements
ST4. GC results following injection of headspace from TA experiments

Supplemental References ............................................................................................................. S34
Figure S1. UV-Vis spectra of RuZn$^{II}$Rd (black), RuFe$^{III}$Rd (red), and RuNi$^{II}$Rd (green) before (dashed lines) and after (solid lines) the TA experiment in 1 M phosphate buffer, pH 6.5. (Inset) Zoomed-in view of the low-energy region of each spectrum showing the d→d transitions of RuFe$^{III}$Rd and RuNi$^{II}$Rd.
Figure S2. UV-Vis spectra of RuZn^{II}Rd (black), RuFe^{III}Rd (red), and RuNi^{II}Rd (green) before (dashed lines) and after (solid lines) the TA experiment with 100 mM Asc in 1 M phosphate buffer, pH 6.5. (Inset) Zoomed-in view of the low-energy region of each spectrum showing the d→d transitions of RuFe^{III}Rd and RuNi^{II}Rd.
Figure S3. UV-Vis spectra of RuZn\textsuperscript{II}Rd with 100 mM Asc in 1 M phosphate buffer, pH 6.5, during a TA experiment. Spectra were measured after 0, 250, 500, 750, 1000, and 1250 pulses of 532 nm light at 40 mW (as indicated).
Figure S4. UV-Vis spectra of RuFe$^{III}$Rd with 100 mM Asc in 1 M phosphate buffer, pH 6.5, during a TA experiment. Spectra were measured after 0, 250, 500, 750, 1000, and 1250 pulses of 532 nm light at 40 mW (as indicated).
Figure S5. UV-Vis spectra of RuNi$^{II}$Rd with 100 mM Asc in 1 M phosphate buffer, pH 6.5, during a TA experiment. Spectra were measured after 0, 250, 500, 750, 1000, and 1250 pulses of 532 nm light at 40 mW (as indicated).
Figure S6. Calibration curve for gas chromatography assays using Scotty Analyzed Gases (A0908910) standard (100 ppm H₂). Data were fit to the given equation.

\[ \text{TCD peak area} = (383.17 \pm 17.5) \times \text{n mole H}_2 \]
Figure S7. Global fit of the TCSPC kinetic traces for (A) RuZn$^{II}$Rd, (B) RuFe$^{III}$Rd, and (C) RuNi$^{II}$Rd (n=3). Samples were measured in 1 M phosphate buffer, pH 6.5. See Material and Methods in the main text for description of the global fit procedure.
Figure S8. TA spectra of RuZn$^{II}$Rd (black), RuFe$^{III}$Rd (red), RuNi$^{II}$Rd (green), and [Ru(bpy)$_3$]$^{2+}$ (orange) in 1 M phosphate buffer, pH 6.5, measured 10 ns after excitation.
Figure S9. Global fit of the TA kinetic traces at (A) 370 nm, (B) 450 nm, and (C) 650 nm for RuZn\textsuperscript{II}Rd (n=4). Samples were measured in 1 M phosphate buffer, pH 6.5. See Material and Methods in the main text for description of the global fit procedure.
Figure S10. Global fit of the TA kinetic traces at (A) 370 nm, (B) 450 nm, and (C) 650 nm for RuFe$^{	ext{III}}$Rd (n=4). Samples were measured in 1 M phosphate buffer, pH 6.5. See Material and Methods in the main text for description of the global fit procedure.
**Figure S11.** Global fit of the TA kinetic traces at (A) 370 nm, (B) 450 nm, and (C) 650 nm for RuNi\textsuperscript{II}Rd (n=4). Samples were measured in 1 M phosphate buffer, pH 6.5. See Material and Methods in the main text for description of the global fit procedure.
Figure S12. TA kinetic trace of RuNiII Rd (green) at 520 nm in 1 M phosphate, pH 6.5.
Figure S13. TA kinetic traces of [Ru(bpy)$_3$]$^{2+}$ (orange), RuFe$^{II}$Rd (grey), and RuFe$^{III}$Rd (red) at 520 nm in 1 M phosphate, pH 6.5.
Figure S14. Modified Latimer diagrams for (A) reductive and (B) oxidative quenching of *Ru\textsuperscript{II} by RuFe\textsuperscript{III}Rd and RuFe\textsuperscript{III}Rd, respectively. Reduction potentials are defined versus NHE and $\varepsilon^o$*Ru(II) = 2.12 eV.\textsuperscript{1-3}
Figure S15. TA kinetic trace of RuFe$^{III}$Rd at 410 nm in 1 M phosphate, pH 6.5
Figure S16. Global fit of TA kinetic traces at (A) 370 nm, (B) 450 nm, and (C) 650 nm for RuFe^{II}Rd (n=4). Samples were measured in 1 M phosphate buffer, pH 6.5. See Material and Methods in the main text for description of the global fit procedure.
Figure S17. Stern-Volmer quenching analysis of RuZn\textsuperscript{II}Rd with ascorbate using (A) TCSPC decays and (inset) corresponding luminescence intensities. (B) Stern-Volmer plot of RuZn\textsuperscript{II}Rd quenching by ascorbate using $\tau_0/\tau_q$ (circles) and $I_0/I_q$ (squares) ratios from TCSPC time constants and luminescence, respectively. Samples contained ~5 µM RuZn\textsuperscript{II}Rd in 1 M phosphate buffer at pH 6.5, in the presence of 0, 1, 3, 10, 15, 30, and 100 mM ascorbate. All experiments performed under a N\textsubscript{2} atmosphere. Luminescence spectral intensities were normalized to Ru concentration.
Figure S18. Stern-Volmer quenching analysis of RuFe$^{III}$Rd with Asc using (A) TCSPC decays and (inset) corresponding luminescence intensities. (B) Stern-Volmer plot of RuFe$^{III}$Rd quenching by Asc using $\tau_0/\tau_q$ ratios using TCSPC time constants. Samples contained ~5 μM RuFe$^{III}$Rd in 1 M phosphate buffer at pH 6.5, in the presence of 0, 1, 3, 10, 15, 30, and 100 mM Asc. All experiments performed under a N$_2$ atmosphere. Luminescence spectral intensities were normalized to Ru concentration.
Figure S19. Stern-Volmer quenching analysis of RuNi^{II}Rd with Asc using (A) TCSPC decay and (inset) corresponding luminescence intensities. (B) Stern-Volmer plot of RuNi^{II}Rd quenching by Asc using $\tau_0/\tau_q$ ratios using TCSPC time constants. Samples contained ~5 µM RuNi^{II}Rd in 1 M phosphate buffer at pH 6.5, in the presence of 0, 1, 3, 10, 15, 30, and 100 mM Asc. All experiments performed under a N$_2$ atmosphere. Luminescence spectral intensities were normalized to Ru concentration.
Figure S20. Stern-Volmer quenching analysis of RuZn\textsuperscript{II}Rd with Asc using TA kinetic traces at (A) 370 nm, (B) 450 nm, and (C) 650 nm. (D) Stern-Volmer plot of RuZn\textsuperscript{II}Rd using $\tau_0/\tau_q$ ratios at 370 nm (circles), 450 nm (squares), and 650 nm (triangles) for analysis. Samples contained 50 μM RuZn\textsuperscript{II}Rd in 1 M phosphate buffer at pH 6.5, in the presence of 0, 1, 3, 10, 15, 30, and 100 mM Asc. All experiments performed under a N\textsubscript{2} atmosphere.
Figure S21. Stern-Volmer quenching analysis of RuFe$^{III}$Rd with Asc using TA kinetic traces at (A) 370 nm, (B) 450 nm, and (C) 650 nm. (D) Stern-Volmer plot of RuFe$^{III}$Rd using $\tau_0/\tau_q$ ratios at 370 nm (circles), 450 nm (squares), and 650 nm (triangles) for analysis. Samples contained 50 $\mu$M RuFe$^{III}$Rd in 1 M phosphate buffer at pH 6.5, in the presence of 0, 1, 3, 10, 15, 30, and 100 mM Asc. All experiments performed under a N$_2$ atmosphere.
Figure S22. Stern-Volmer quenching analysis of RuNiII Rd with Asc using TA kinetic traces at (A) 370 nm, (B) 450 nm, and (C) 650 nm. (D) Stern-Volmer plot of RuNiII Rd using $\tau_0/\tau_q$ ratios at 370 nm (circles), 450 nm (squares), and 650 nm (triangles) for analysis. Samples contained 50 μM RuNiII Rd in 1 M phosphate buffer at pH 6.5, in the presence of 0, 1, 3, 10, 15, 30, and 100 mM Asc. All experiments performed under a N$_2$ atmosphere.
Figure S23. TA difference spectra of (A) RuZn$^{II}$Rd, (B) RuFe$^{III}$Rd, and (C) RuNi$^{II}$Rd in 1 M phosphate, pH 6.5, with 100 mM Asc at indicated timepoints following excitation. TA spectra were smoothed using a 5-pt Savitzky-Golay algorithm.
Figure S24. TA kinetic traces of RuZn$^{II}$Rd (black), RuFe$^{III}$Rd (red), and RuNi$^{II}$Rd (green) at 520 nm. Samples were contained in 1 M phosphate, pH 6.5, with 100 mM Asc.
Figure S25. UV-Vis spectra of 30 µM FeRd protein sample at pH 6.5 (red) and pH 8 (grey) in 1 M phosphate buffer with 100 mM Asc.
Figure S26. Modified Latimer diagram showing both intra- and intermolecular electron transfer in RuNiRd in the presence of Asc. Reduction potentials are for free [Ru(bpy)]²⁺, Asc, and NiRd are reported versus NHE; ε°(*RuI) = 2.12 eV. "k_{r+nr}" is the amplitude-weighted rate constant from the RuZnRd TCSPC data.
Figure S27. Modified Latimer diagram for RuNiRd showing the second photocycle. Reduction potentials are for free [Ru(bpy)$_3$]$^{2+}$, Asc, and NiRd are reported versus NHE; $\varepsilon^0$(*Ru$^{II}$) = 2.12 eV.$^{1-6}$ $k_{r+nr}$ is the amplitude-weighted rate constant from the RuZnRd TCSPC data.
Table ST1. Global fit time constants of TCSPC kinetic traces of RuZn^{II}Rd, RuFe^{III}Rd, and RuNi^{II}Rd without quencher.

<table>
<thead>
<tr>
<th></th>
<th>$A_1$ (%)</th>
<th>$\tau_1$ (ns)</th>
<th>$A_2$ (%)</th>
<th>$\tau_2$ (ns)</th>
<th>$A_3$ (%)</th>
<th>$\tau_3$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RuZn^{II}Rd</td>
<td>56 ± 0.9</td>
<td>331 ± 7</td>
<td>44 ± 1.2</td>
<td>794 ± 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RuFe^{III}Rd</td>
<td>64 ± 1.8</td>
<td>3.5 ± 0.4</td>
<td>15 ± 1.4</td>
<td>205 ± 31</td>
<td>21 ± 1.4</td>
<td>924 ± 68</td>
</tr>
<tr>
<td>RuNi^{II}Rd</td>
<td>45 ± 0.6</td>
<td>17 ± 0.9</td>
<td>27 ± 1.1</td>
<td>243 ± 20</td>
<td>28 ± 1.3</td>
<td>937 ± 45</td>
</tr>
</tbody>
</table>
Table ST2. Global fit time constants (ns) and amplitudes (%) from TA kinetic traces of RuZn\textsuperscript{II}Rd, RuFe\textsuperscript{III}Rd, RuFe\textsuperscript{II}Rd, and RuNi\textsuperscript{II}Rd measured in the absence of Asc.

<table>
<thead>
<tr>
<th></th>
<th>(\lambda)</th>
<th>(A_1)</th>
<th>(\tau_1)</th>
<th>(A_2)</th>
<th>(\tau_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RuZn\textsuperscript{II}Rd</td>
<td>370 nm</td>
<td>68 ± 3.5</td>
<td>324 ± 58</td>
<td>32 ± 3.5</td>
<td>776 ± 68</td>
</tr>
<tr>
<td></td>
<td>450 nm</td>
<td>59 ± 1.7</td>
<td>338 ± 14</td>
<td>41 ± 1.7</td>
<td>812 ± 17</td>
</tr>
<tr>
<td></td>
<td>650 nm</td>
<td>48 ± 2.9</td>
<td>324 ± 73</td>
<td>52 ± 2.9</td>
<td>776 ± 36</td>
</tr>
<tr>
<td>RuFe\textsuperscript{III}Rd</td>
<td>370 nm</td>
<td>13 ± 1.1</td>
<td>230 ± 31</td>
<td>87 ± 1.1</td>
<td>711 ± 64</td>
</tr>
<tr>
<td></td>
<td>450 nm</td>
<td>43 ± 1.7</td>
<td>163 ± 21</td>
<td>57 ± 1.7</td>
<td>905 ± 30</td>
</tr>
<tr>
<td></td>
<td>650 nm</td>
<td>63 ± 4.9</td>
<td>331 ± 99</td>
<td>37 ± 4.9</td>
<td>1072 ± 45</td>
</tr>
<tr>
<td>RuNi\textsuperscript{II}Rd</td>
<td>370 nm</td>
<td>54 ± 0.6</td>
<td>258 ± 7</td>
<td>46 ± 0.6</td>
<td>1078 ± 13</td>
</tr>
<tr>
<td></td>
<td>450 nm</td>
<td>53 ± 0.8</td>
<td>306 ± 14</td>
<td>47 ± 0.8</td>
<td>1234 ± 18</td>
</tr>
<tr>
<td></td>
<td>650 nm</td>
<td>51 ± 1.0</td>
<td>425 ± 15</td>
<td>49 ± 1.0</td>
<td>1287 ± 15</td>
</tr>
<tr>
<td>RuFe\textsuperscript{II}Rd</td>
<td>370 nm</td>
<td>48 ± 1.8</td>
<td>67 ± 9</td>
<td>52 ± 1.8</td>
<td>602 ± 15</td>
</tr>
<tr>
<td></td>
<td>450 nm</td>
<td>40 ± 2.0</td>
<td>228 ± 24</td>
<td>60 ± 2.0</td>
<td>1038 ± 42</td>
</tr>
<tr>
<td></td>
<td>650 nm</td>
<td>35 ± 1.1</td>
<td>246 ± 10</td>
<td>65 ± 1.1</td>
<td>884 ± 14</td>
</tr>
</tbody>
</table>
Table ST3. Stern-Volmer quenching rate constants ($k_Q$) for RuZn$^{II}$Rd, RuFe$^{III}$Rd, and RuNi$^{II}$Rd at pH 6.5 in 1 M phosphate buffer taken from TCSPC, emission, and TA measurements.

<table>
<thead>
<tr>
<th></th>
<th>RuZn$^{II}$Rd (M$^{-1}$s$^{-1}$)</th>
<th>RuFe$^{III}$Rd (M$^{-1}$s$^{-1}$)</th>
<th>RuNi$^{II}$Rd (M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emission</td>
<td>2.4 ± 0.42 x 10$^7$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCSPC</td>
<td>6.5 ± 3.2 x 10$^7$</td>
<td>1.6 ± 0.25 x 10$^8$</td>
<td>8.8 ± 3.6 x 10$^7$</td>
</tr>
<tr>
<td>TA$^a$</td>
<td>3.6 ± 0.50 x 10$^7$</td>
<td>6.0 ± 0.81 x 10$^7$</td>
<td>5.2 ± 0.86 x 10$^7$</td>
</tr>
</tbody>
</table>

$^a$ Average of 370, 450, and 650 nm quenching constants.
Table ST4. Gas chromatograph (GC) injections of the TA headspace with 50 µM RuZn$^{II}$Rd, 50 µM RuFe$^{III}$Rd, and 50 µM RuNi$^{II}$Rd in 100 mM Asc buffered with 1 M phosphate pH 6.5 after 50 min. of irradiation.

<table>
<thead>
<tr>
<th></th>
<th>nmols H$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RuZn$^{II}$Rd</td>
<td>0.158</td>
</tr>
<tr>
<td>RuFe$^{III}$Rd</td>
<td>0.117</td>
</tr>
<tr>
<td>RuNi$^{II}$Rd</td>
<td>0.640</td>
</tr>
</tbody>
</table>
REFERENCES


