Electronic Supplementary Material (ESI) for Chemical Science.

Electronic Supplementary Information (ESI)

Ru-protein-Co biohybrids designed for solar hydrogen production: understanding electron transfer pathways related to photocatalytic function

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Fig. S1 Representative photocatalytic hydrogen production time course profiles for the Ru-Fd-CoPy and Ru-ApoFld-CoBF$_2$ biohybrid complexes during visible light illumination. The assay conditions for Ru-Fd-CoPy are 2 µM Ru-Fd-CoPy biohybrid (1.7 Co/Fd, 0.6 Ru/Fd) in 10 mM MES buffer, pH 6.3, 100 mM sodium ascorbate. The assay conditions for Ru-ApoFld-CoBF$_2$ are 1.7 µM Ru-ApoFd-Co biohybrid (1.0 Co/Fd, 0.9 Ru/Fd) in 10 mM MES buffer, pH 6.3, 100 mM sodium ascorbate.

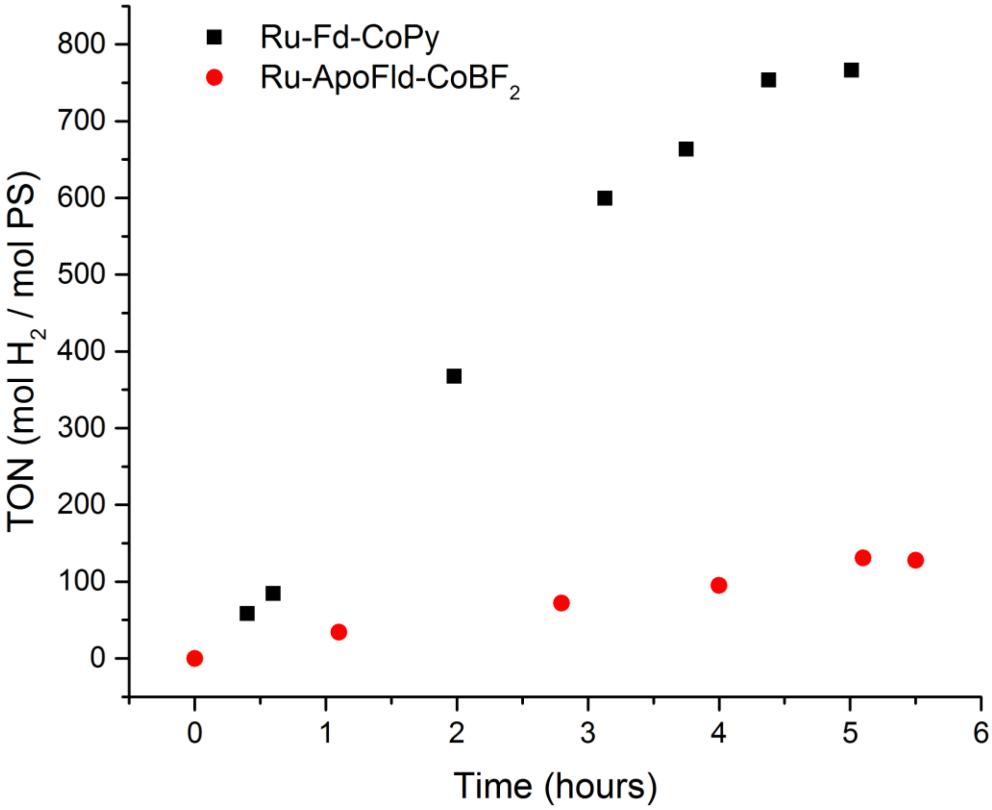


Fig. S2 CW X-band EPR spectra of Fd and Ru-Fd-CoPy biohybrid. All EPR spectra were collected at 10 K. Samples were illuminated for 2 s at 200 K or 293 K, followed by immersion in liquid N_2 while illuminated and placed in a pre-cooled (10 K) EPR cavity for measurement. An asterisk marks organic species omitted for clarity (g ~ 2.0). Ru-Fd-CoPy + asc, dark (red, 1); Ru-Fd-CoPy + asc, light at 200 K (green, 2); Ru-Fd-CoPy + asc, light at 293 K (blue, 3); Fd + dithionite (black, 4). Typical Co(II) EPR spectra for cobaloximes[1] are observed in all Ru-Fd-CoPy + asc samples. Ru-Fd-CoPy + asc illuminated at 200 K (green, 2) displays a Ru(III) intermediate (g = 2.9, marked with an arrow) while Ru-Fd-CoPy + as illuminated at 293 K (blue, 3) exhibits the same g-values (2.05, 1.96, 1.89) and similar linewidth as the reduced [2Fe-2S] cluster in the native Fd protein (black,4), suggesting reduction of the [2Fe-2S] cluster in Ru-Fd-CoPy during photocatalysis.

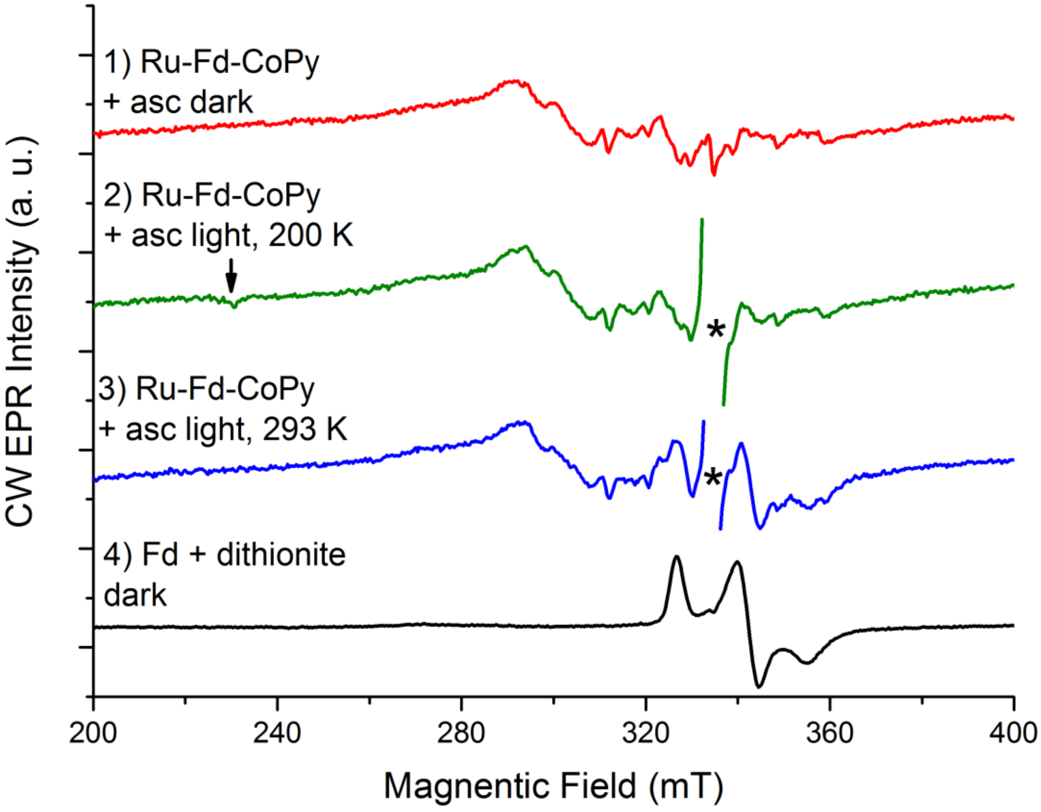


Fig. S3 CW X-band EPR spectra of Ru-ApoFld-CoBF$_2$ biohybrid. Data were collected at 10 K, with illumination for 2 s at 293 K, followed by immersion in liquid N$_2$ during illumination and placed in a pre-cooled EPR resonator for measurement. Ru-ApoFld-CoBF$_2$ (black, 1); Ru-ApoFld-CoBF$_2$, light (red, 2); Ru-ApoFld-CoBF$_2$ + asc, dark (blue, 3); Ru-ApoFld-CoBF$_2$ + asc, light at 293 K (green, 4).

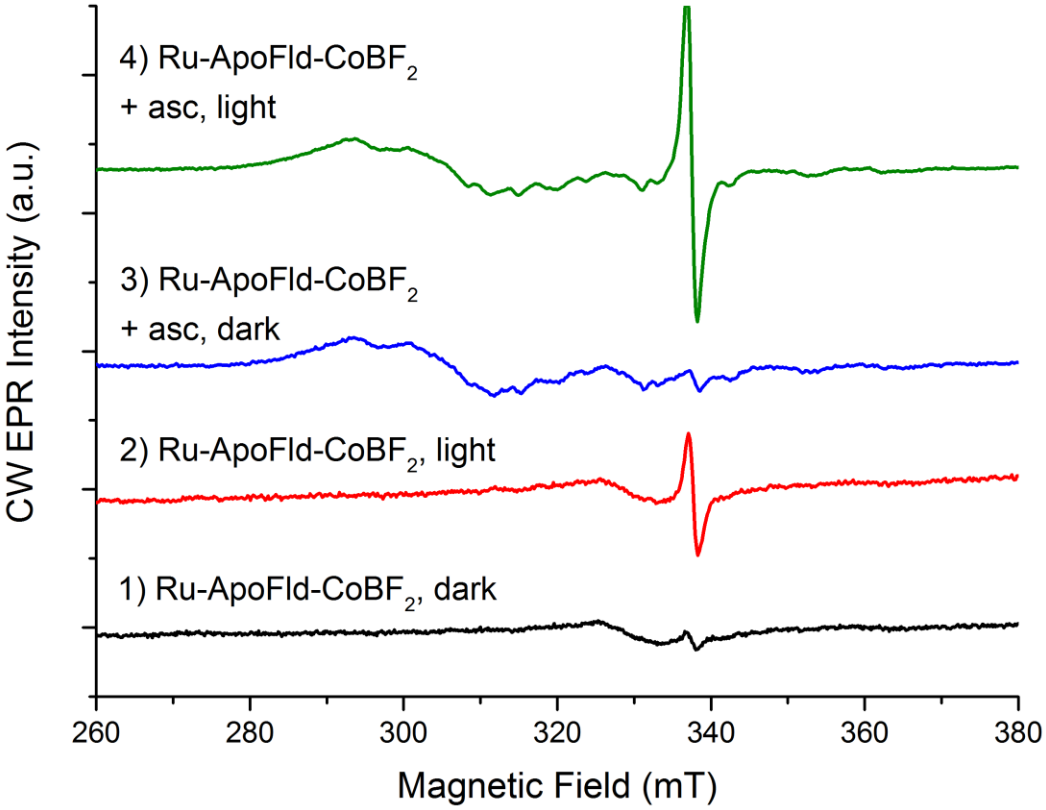


Fig. S4 Correlation plots of (A) g_y vs. g_x and (B) $|A_z|(^{59}Co)$ vs. g_x for the $CoBF_2$ catalyst in various solvents and protein environments. Ru-Fd-$CoBF_2$ and Ru-ApoFld-$CoBF_2$ parameters are new to this work. Other parameters are provided in previous work.[1, 2]

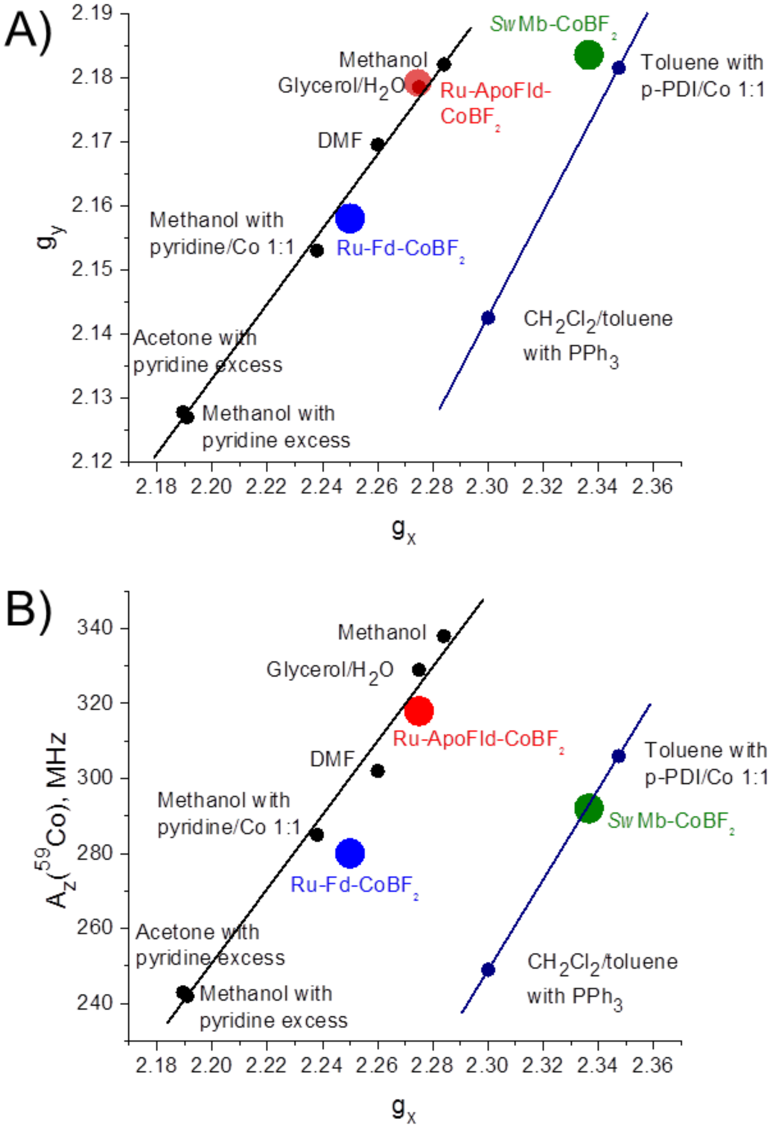


Fig. S5 UV-visible absorbance spectra of 50 µM Ru(II) PS (Ru^{2+}) in 20 mM HEPES pH 7.9, 1 mM $Ru(III)(bpy)_3$ in water (Ru^{3+}), 50 µM $CoBF_2$ catalyst (Co^{2+}) in 20 mM HEPES pH 7.9,50 µM Fd ($[2Fe\text{-}2S]^{3+/3+}$) in 20 mM HEPES pH 7.9, and 50 µM Fd ($[2Fe\text{-}2S]^{2+/3+}$) in 20 mM CAPS pH 10.0 with 10 mM sodium dithionite. Multiple overlapping features in the 300-450 nm range make it difficult to resolve individual kinetic effects within this region. Reduction of the [2Fe-2S] cluster with sodium dithionite decreases the absorbance of the protein in the 400-500 nm region, however, it contributes signal in the 500-650 nm region.

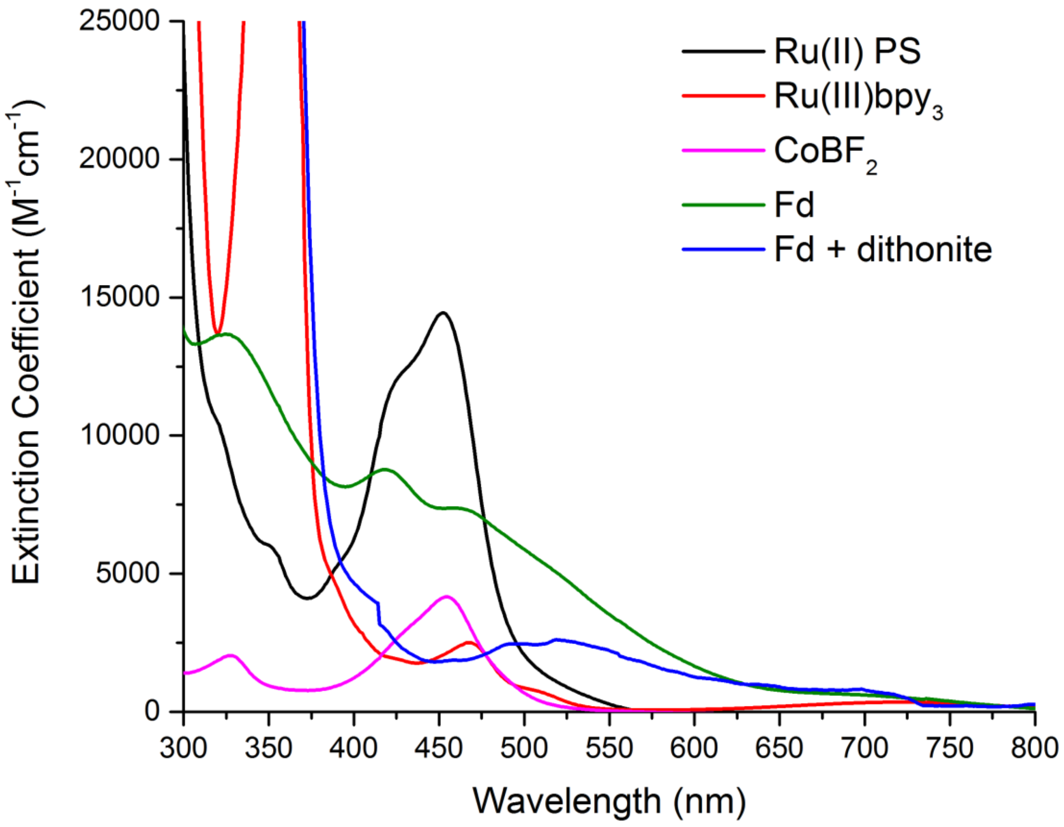


Fig. S6 Transient optical absorption spectra at selected times for the Ru-Fd biohybrid with 200 mM sodium ascorbate (left panel) and without ascorbate (right panel). Samples were excited at 460 nm and detected with a fiber laser. In the absence of ascorbate, oxidatively quenched Ru(III) is formed within 5 ns, and electron transfer from Ru(III) to the [2Fe-2S] with reduction of the cluster from $[2Fe\text{-}2S]^{3+/3+}$ to $[2Fe\text{-}2S]^{2+/3+}$ occurs in $\tau_1 = 300 \pm 40$ ns. Recovery of the Ru(II) ground state occurs in $\tau_2 = 13 \pm 2$ ms (Kinetic traces, **Fig. S7**). In the presence of sodium ascorbate, reductive quenching occurs more rapidly ($\tau_1 = 140 \pm 10$ ns). The reductively quenched Ru(I) species centered at 520 nm decays in $\tau_2 = 4.8 \pm 0.3$ µs. Subsequently, there is formation of a bleach feature from 475-575 nm consistent with the reduction of the [2Fe-2S] cluster in $\tau_3 = 700 \pm 300$ µs. Full recovery of the ground state Ru(II) species occurs in $\tau_4 = 7.8 + 1.1$ ms (Kinetic Traces, **Fig. S8**).

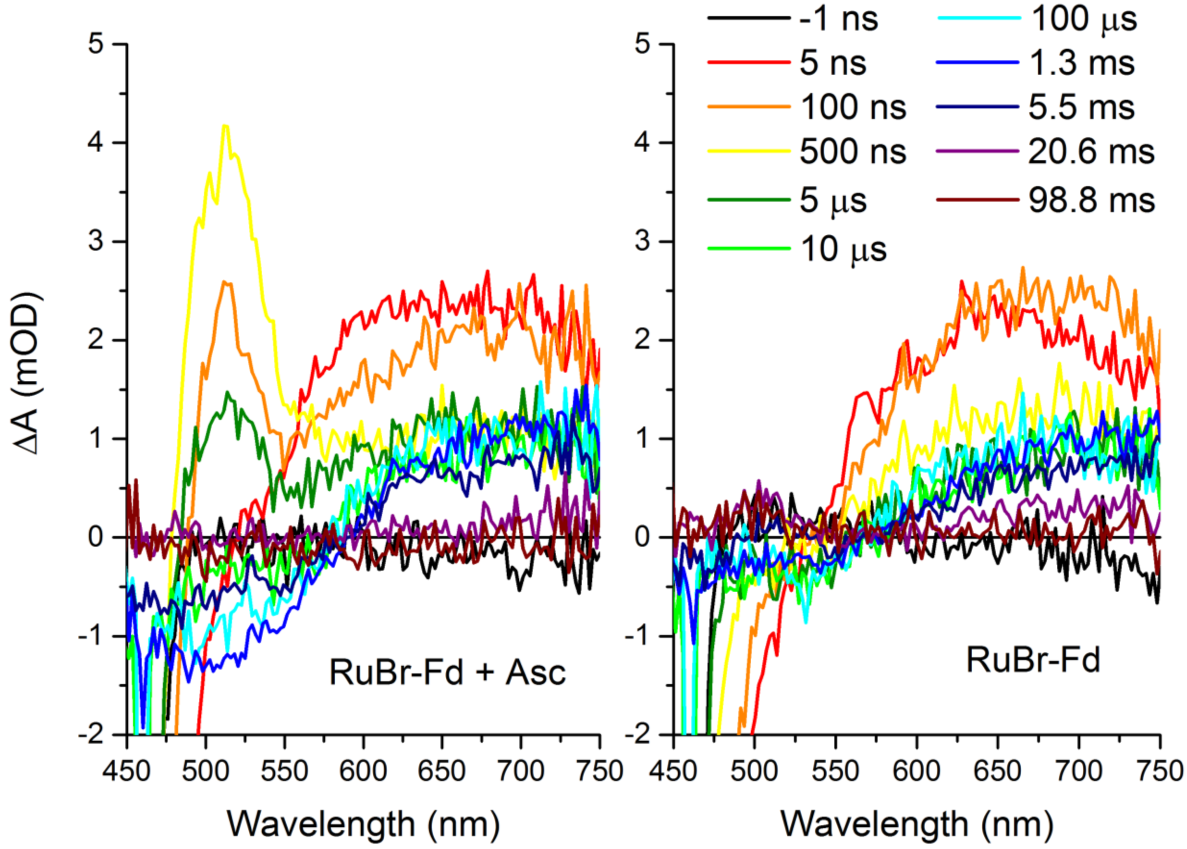


Fig. S7 Kinetic fits of transient optical kinetic data for the Ru-Fd biohybrid using a global fitting routine at three wavelengths (520 nm (black), 620 nm (red), 736 nm (blue)) in Origin Pro 9.1. The data set was fit with a bi-exponential decay function from 10 ns – 100 ms.

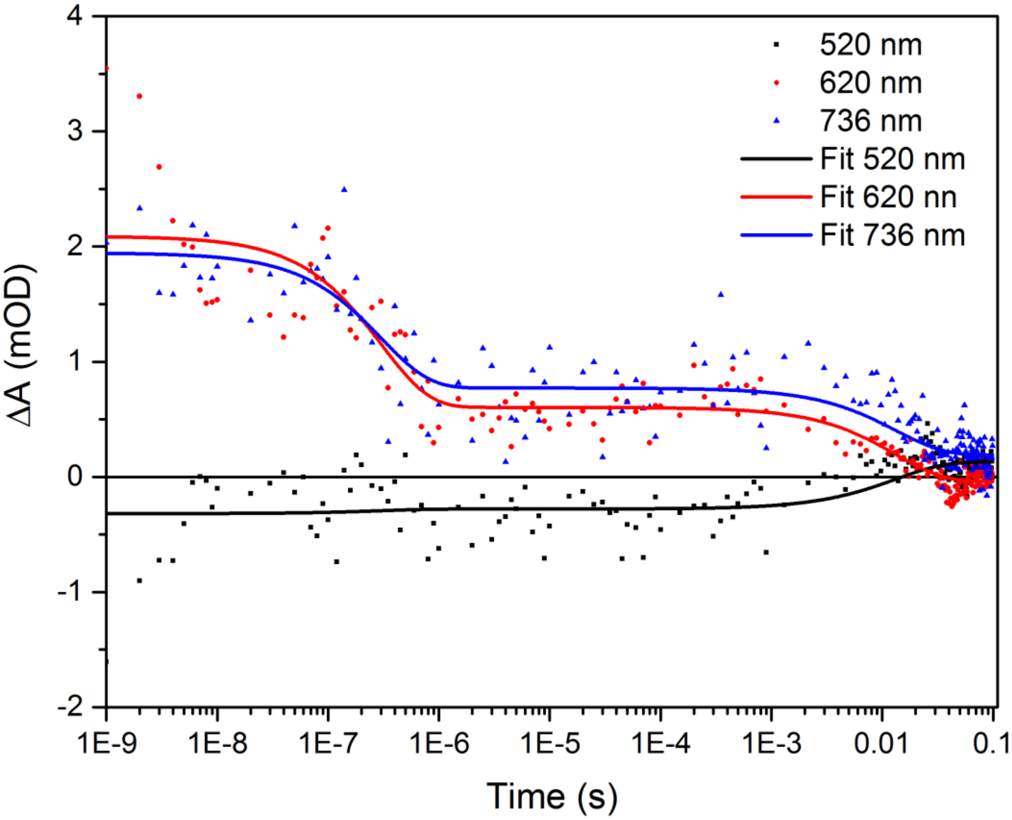


Fig. S8 Kinetic fits of transient optical kinetic data for the Ru-Fd biohybrid with 200 mM sodium ascorbate using a global kinetic fitting routine at three wavelengths (520 nm (black), 620 nm (red), 736 nm (blue)) in Origin Pro 9.1. The data set was fit with a four-exponential decay function from 10 ns – 100 ms.

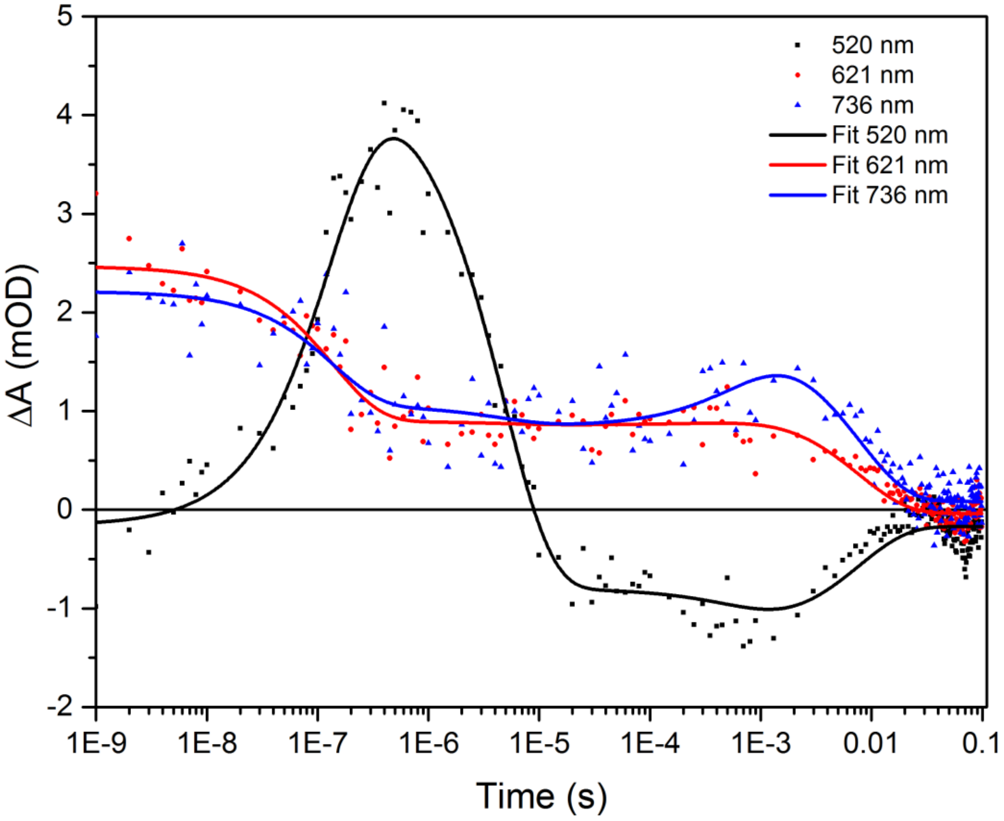
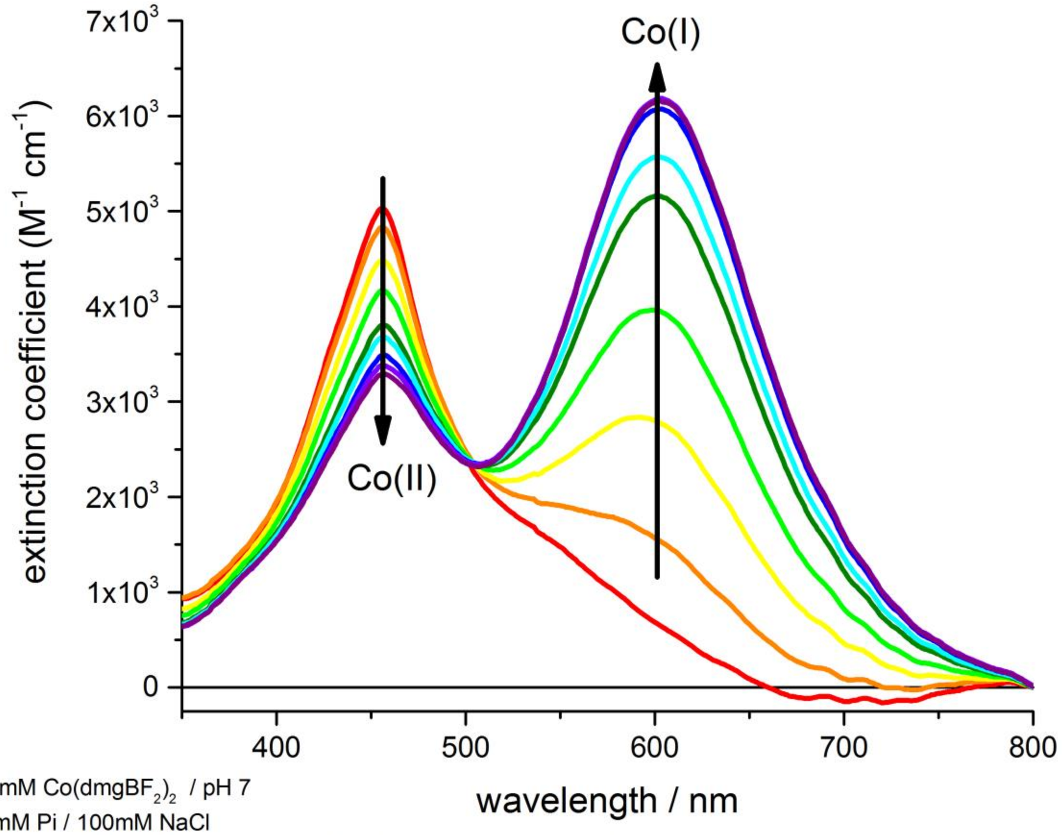
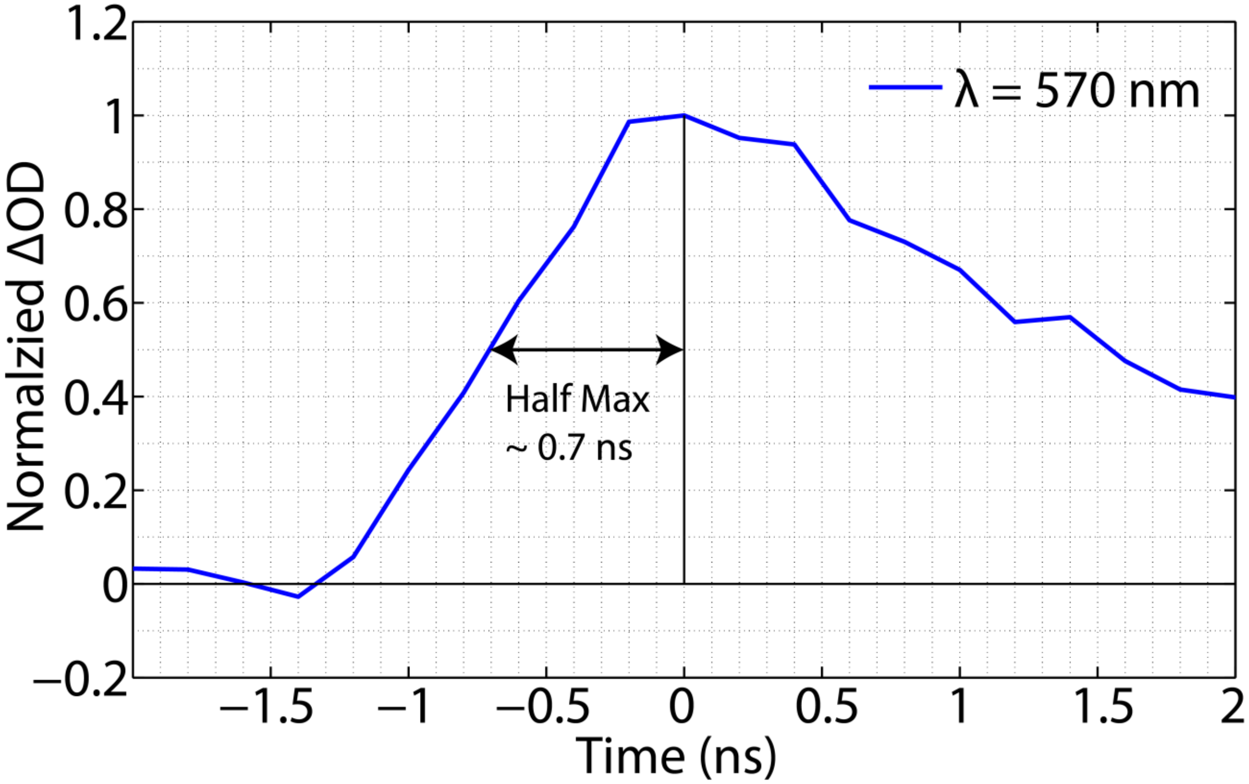


Fig. S9 Spectroelectrochemistry of 0.4 mM $CoBF_2$ catalyst in 10 mM phosphate buffer, pH 7.0 with 100 mM sodium chloride as an electrolyte. The Co(I) species has a maximal absorbance at 600-610 nm. Spectroelectrochemistry experiments were performed as previously reported.[3]



0.4mM $Co(dmgBF_2)_2$ / pH 7
10mM Pi / 100mM NaCl
Pt mesh working / Pt wire auxiliary / Ag/AgCl reference / -700mV

Fig. S10 Instrument response function for homebuilt transient absorption instrument as described in the Experimental section. Measurements were taken using 460 nm excitation of [Cu(mesphen)(dimethylphen)]·PF_6 in acetonitrile.[4]



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