

Light-driven Electron Injection from a Biotinylated Donor to Ru(α -diimine)₃²⁺- labeled Streptavidin

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Supporting Information

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1 Materials and Instruments

Recombinant cysteine-containing streptavidin isoforms were engineered, expressed, purified and quantified as previously described.¹ Other reagents, substrates and materials were purchased from Sigma-Aldrich, TCI and Acros. MV^{2+} was added as methylviologene dichloride hydrate purchased from Sigma-Aldrich and used as is. UV-Vis experiments were conducted on a Varian 50 Scan UV-Vis spectrophotometer. NMR experiments were performed on a Bruker Avance III NMR spectrometer operating at 400 MHz proton frequency and 101 MHz carbon frequency. Chemical shifts were referenced to residual d_6 -DMSO (2.50 ppm) for 1H spectra or d_6 -DMSO (39.52 ppm) for ^{13}C spectra. Signals are quoted as s (singlet), d (doublet), t (triplet) and m (multiplet). Flash column purification was performed on a Biotage Isolera One. Mass spectra were acquired on Bruker esquire 3000 plus and Bruker maxis 4G QTOF EDI spectrometers. An LP920-KS instrument from Edinburgh Instruments was used for transient absorption spectroscopy. The frequency doubled output of a Quantel Brilliant b laser served as an excitation source. The laser pulse duration was 10 ns and the pulse frequency was 10 Hz. The typical pulse energy used for transient absorption studies was 15 mJ. Detection of transient absorption spectra occurred on an ICCD camera from Andor. All optical spectroscopic experiments were performed under aerated conditions in MilliQ water.

2 Synthesis

The compounds TAA-NO₂,² TAA-NH₂,³ [Ru(bpy)₂(phenNH₂)](PF₆)₂⁴ and Biotin pentafluorophenyl ester⁵ were synthesized as previously reported.

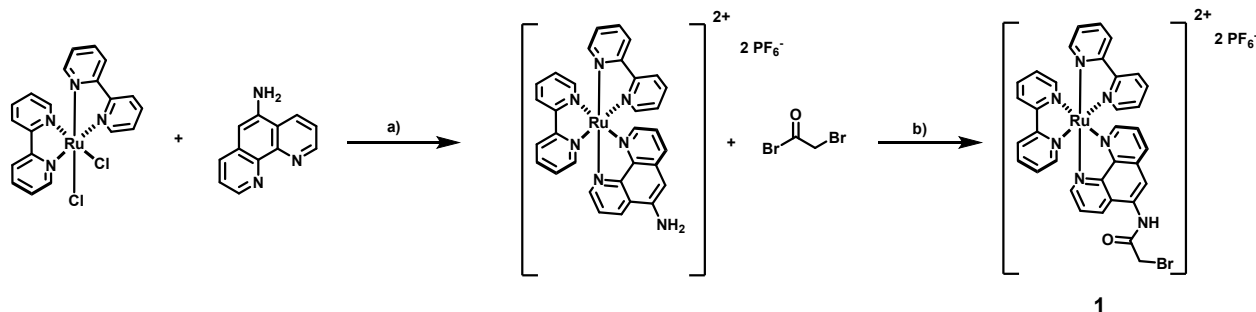


Figure S1. Synthesis of [Ru(bpy)₂(phenNHCOCH₂Br)](PF₆)₂ **1**: a) phenNH₂, [Ru(bpy)₂Cl₂], MeOH, reflux, overnight b) [Ru(bpy)₂(phenNH₂)](PF₆)₂, BrCH₂COBr, CH₃CN, r.t., overnight

2.1 Synthesis of [Ru(bpy)₂(phenNHCOCH₂Br)](PF₆)₂ (**1**)

Adapted from Fedorova *et al.*⁶ Under N₂, [Ru(bpy)₂(phenNH₂)](PF₆)₂ (30.0 mg, 0.033 mmol) was dissolved in dry acetonitrile (2.0 ml). Bromoacetyl bromide (13.0 μl, 0.15 mmol) was added and the reaction mixture was stirred overnight at room temperature in the dark. The solvent was removed *in vacuo* and the residue was dissolved in a minimal amount of MeOH. A saturated aqueous solution of NH₄PF₆ was added and the precipitate was collected, washed with water followed by Et₂O to yield the product as a red solid (0.027 mmol, 27.6 mg, 82%).

¹H (400MHz, d₆-DMSO): 10.87 (s, 1H), 8.93-8.76 (m, 6H), 8.64 (s, 1H), 8.21 (tt, *J* = 7.9, 1.5 Hz, 2H), 8.17 (dd, *J* = 5.3, 1.1 Hz, 1H), 8.11 (tdd, *J* = 7.8, 2.7, 1.5 Hz, 2H), 8.05 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.94 (dd, *J* = 8.6, 5.2 Hz, 1H), 7.87-7.79 (m, 3H), 7.62-7.54 (m, 4H), 7.35 (ddt, *J* = 7.4, 5.8, 1.5 Hz, 2H), 4.34 (d, *J* = 1.3 Hz, 2H). ¹³C (101 MHz, d₆-DMSO): 166.5, 156.8, 156.55, 156.52, 152.48, 151.5, 151.46, 147.24, 144.81, 137.97, 137.85, 136.47, 133.1, 132.28, 130.1, 127.84, 127.81, 127.74, 126.59, 126.3, 125.9, 124.47, 124.38, 29.89. HRMS (ESI⁺) calcd. for C₃₄H₂₆BrN₇ORu²⁺ 364.5207; found, 364.5213.

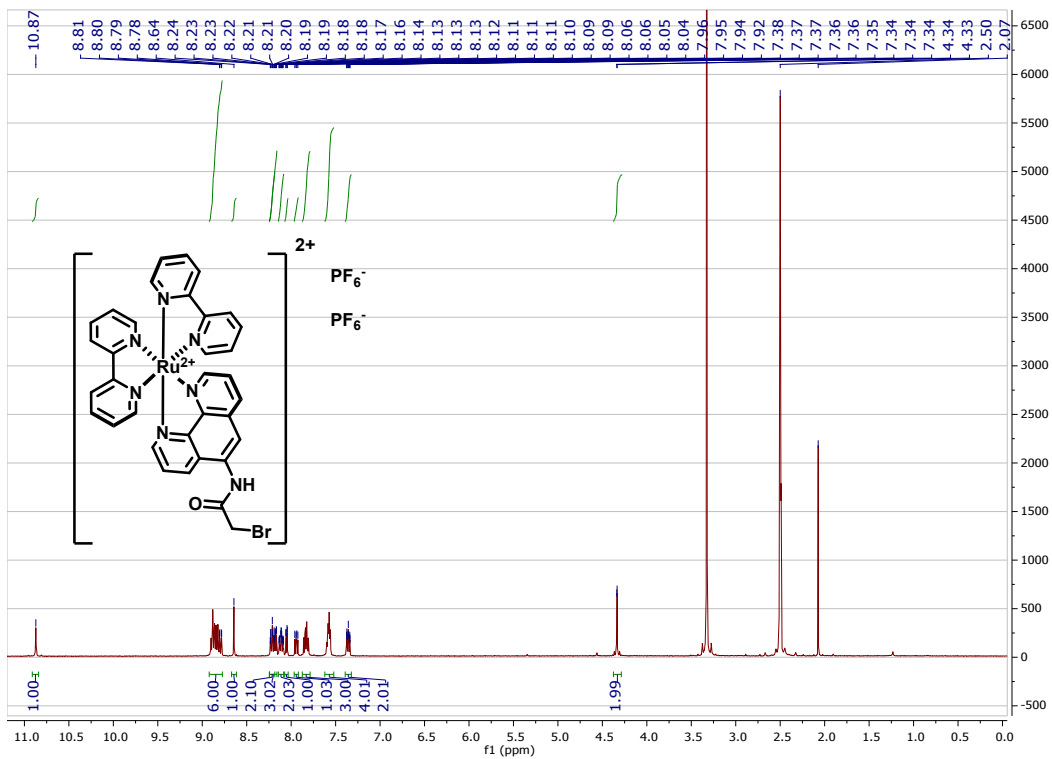


Figure S2. ¹H-NMR spectrum of **1** in *d*₆-DMSO

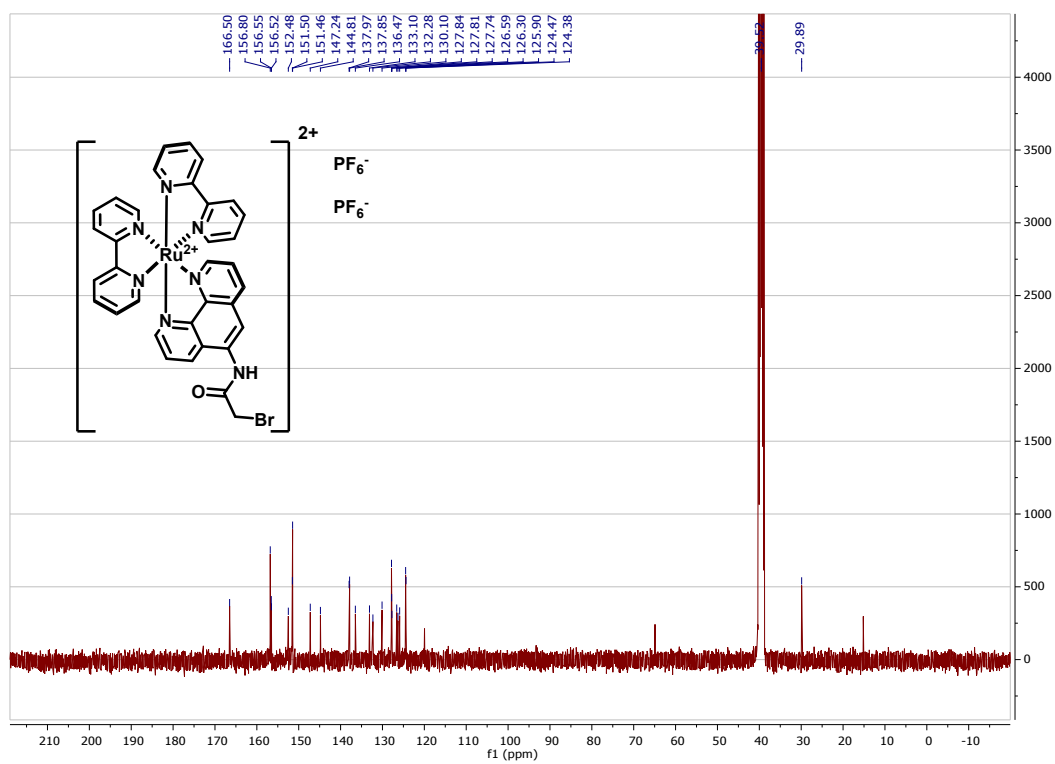


Figure S3. ¹³C-NMR spectrum of **1** in *d*₆-DMSO

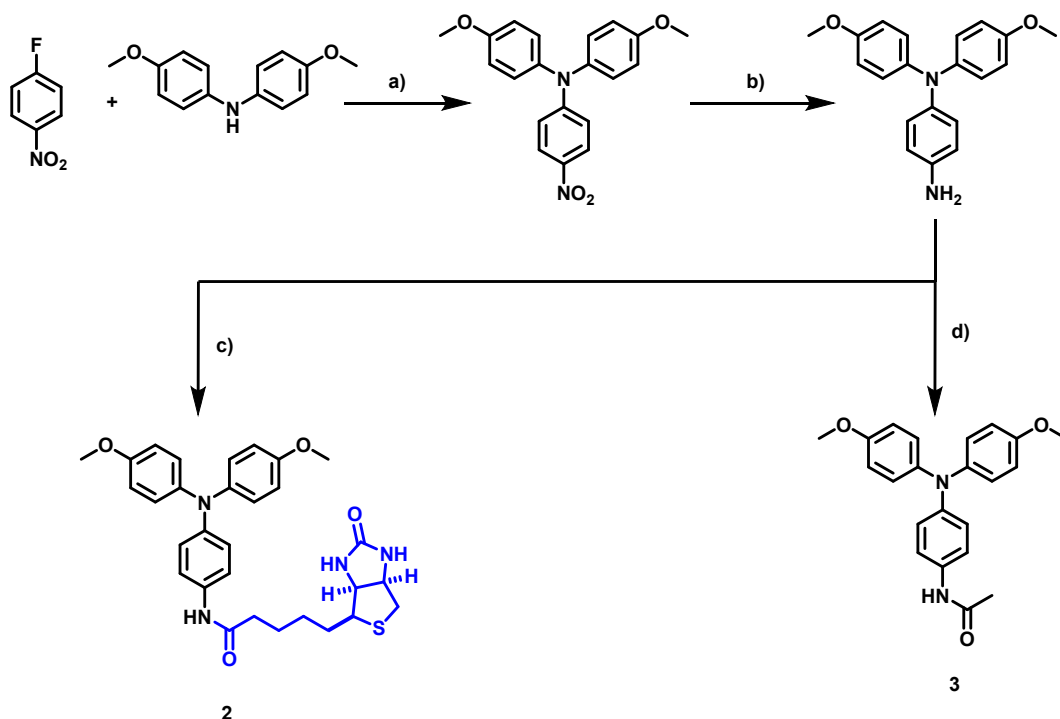


Figure S4. Synthesis of **Biot-TAA 2** and **TAA-Ac 3**: a) *p*-fluoronitrobenzene, 4,4'-dimethoxydiphenylamine, NaH, DMAc, 100°C, 3 h b) H₄N₂, Pd/C, EtOH, 75°C, overnight. c) Biotin-PFP, TAA-NH₂, DMF, r.t., overnight d) AcOCl, Et₃N, TAA-NH₂, DCM, r.t., overnight

2.2 Synthesis of Biot-TAA (2)

Biotin pentafluorophenyl ester (37.4 mg, 0.09 mmol) was dissolved in dry DMF (1.0 ml) under N₂. TAA-NH₂ (29.2 mg, 0.09 mmol), dissolved in dry DMF (1.0 ml), was added dropwise at 0°C. After addition, the reaction mixture was stirred overnight at room temperature. Dry DCM (3.0 ml) was added and the organic phase was washed with degassed H₂O (3 x 10.0 ml). The combined organic phases were dried over Na₂SO₄ and the solvents were removed under reduced pressure. To remove residual DMF, the compound was dissolved in a minimal amount of dry DCM and precipitated with cold Et₂O to yield the product as an off-white solid (0.06 mmol, 33.5 mg, 68%).

¹H (400 MHz, *d*₆-DMSO): 9.79 (s, 1H), 7.40 (d, *J* = 9.0 Hz, 2H), 6.94-6.84 (m, 8H), 6.79 (d, *J* = 8.9 Hz, 2H), 6.44 (s, 1H), 6.36 (s, 1H), 4.30 (ddt, *J* = 7.6, 5.1, 1.1 Hz, 1H), 4.13 (ddd, *J* = 7.8, 4.4, 1.8 Hz, 1H), 3.72 (s, 6H), 3.12 (ddd, *J* = 8.5, 6.1, 4.4 Hz, 1H), 2.82 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.58 (d, *J* = 12.5 Hz, 1H), 2.26 (t, *J* = 7.3 Hz, 2H), 1.71-1.27 (m, 6H). ¹³C (101 MHz, *d*₆-DMSO): 170.66, 162.68, 154.99, 143.54, 140.84, 133.24, 125.27, 121.85, 120.31, 114.79, 61.03, 59.18, 55.40, 55.21, 39.85, 36.10, 28.23, 28.09, 25.22. HRMS (ESI⁺) calcd. for C₃₀H₃₄N₄O₄S⁺ 546.2301; found, 546.2296.

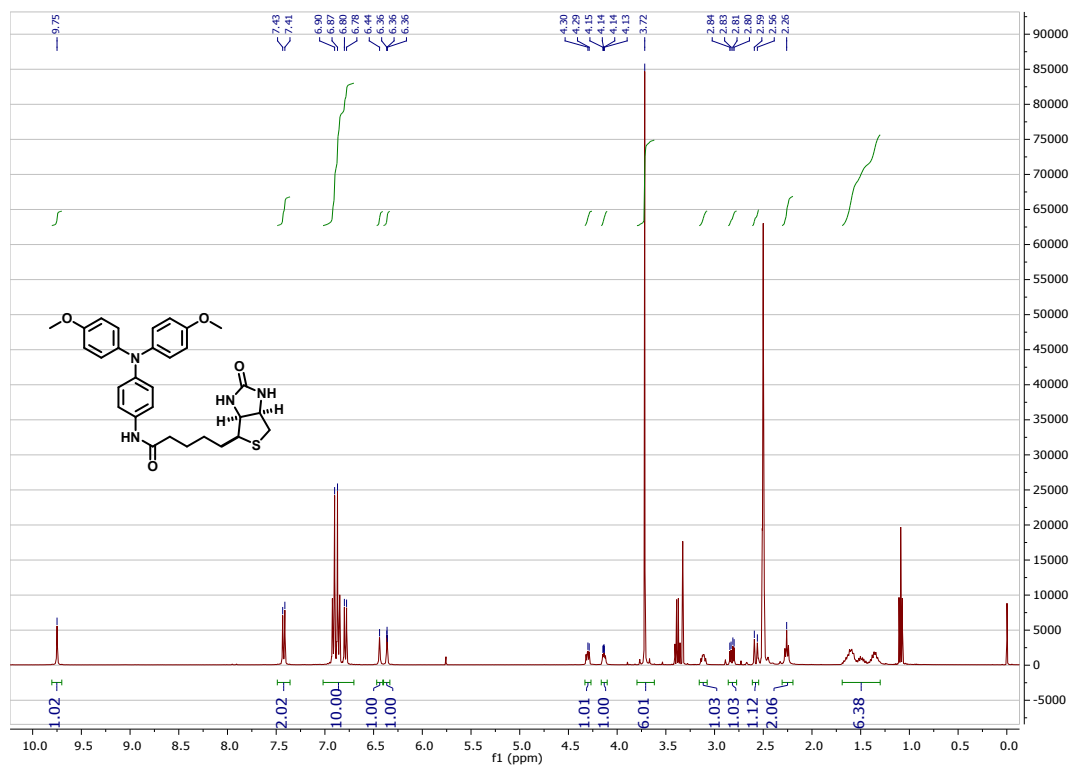


Figure S5. ^1H -NMR spectrum of **2** in d_6 -DMSO

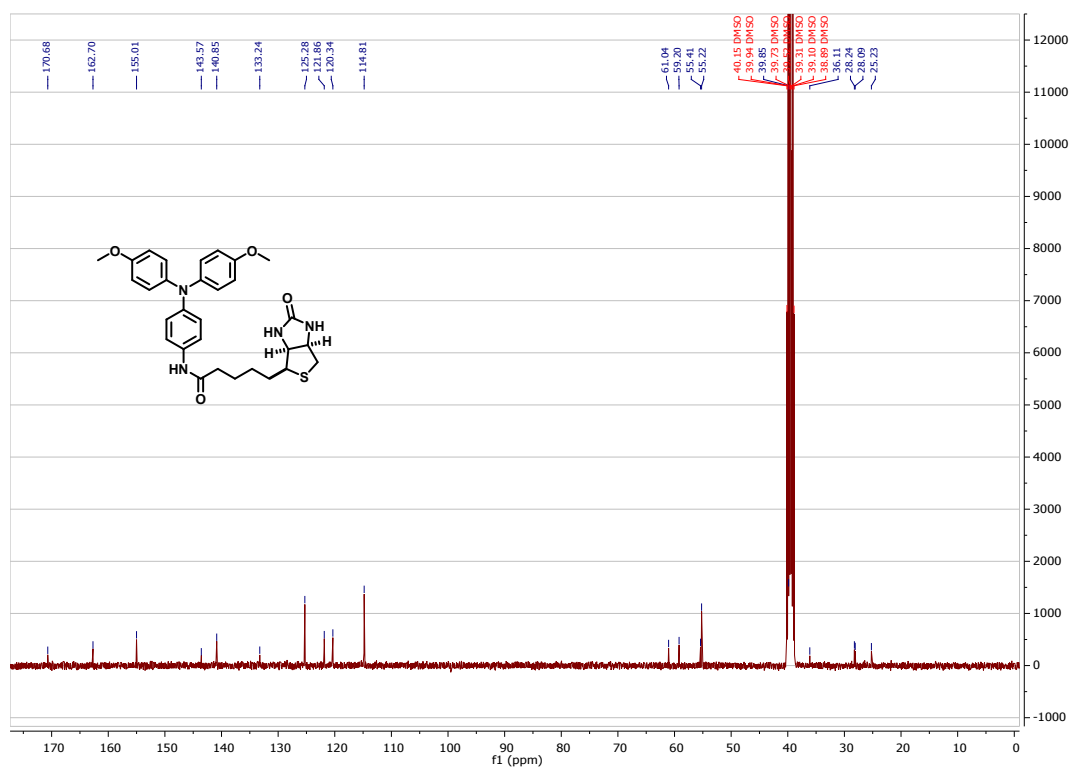


Figure S6. ^{13}C -NMR spectrum of **2** in d_6 -DMSO

2.3 Synthesis of TAA-Ac (3)

A solution containing of TAA-NH₂ (89.0 mg, 0.28 mmol) and Et₃N (39 μl, 0.53 mmol) in dry DCM (4.0 ml) was added dropwise to a cooled solution of acetylchloride (20.0 μl, 0.28 mmol) in dry DCM (1.0 ml) at 0°C under an inert atmosphere. After addition, the reaction mixture and stirred overnight at room temperature. Dry diethyl ether (10.0 ml) was added and the reaction mixture was washed with NH₄Cl sat. (2 x 10.0 ml) and brine (10.0 ml), dried over Na₂SO₄ and concentrated under reduced pressure. Further purification of the crude mixture by automated column chromatography (cyclohexane / EtOAc, 0%→50%) afforded the product as an off-white solid (0.14 mmol, 51 mg, 50%).

¹H (400 MHz, *d*₆-DMSO): 9.75 (s, 1H), 7.42 (d, *J* = 8.9 Hz, 2H), 6.94-6.84 (m, 8H), 6.79 (d, *J* = 8.9 Hz, 2H), 3.72 (s, 6H), 1.99 (s, 3H). ¹³C (101 MHz, *d*₆-DMSO): 167.72, 155.01, 143.56, 140.82, 133.23, 125.29, 121.75, 120.25, 114.79, 55.20, 23.80. HRMS (ESI⁺) calcd. for C₂₂H₂₂N₂O₃⁺ 362.1630; found, 362.1624.

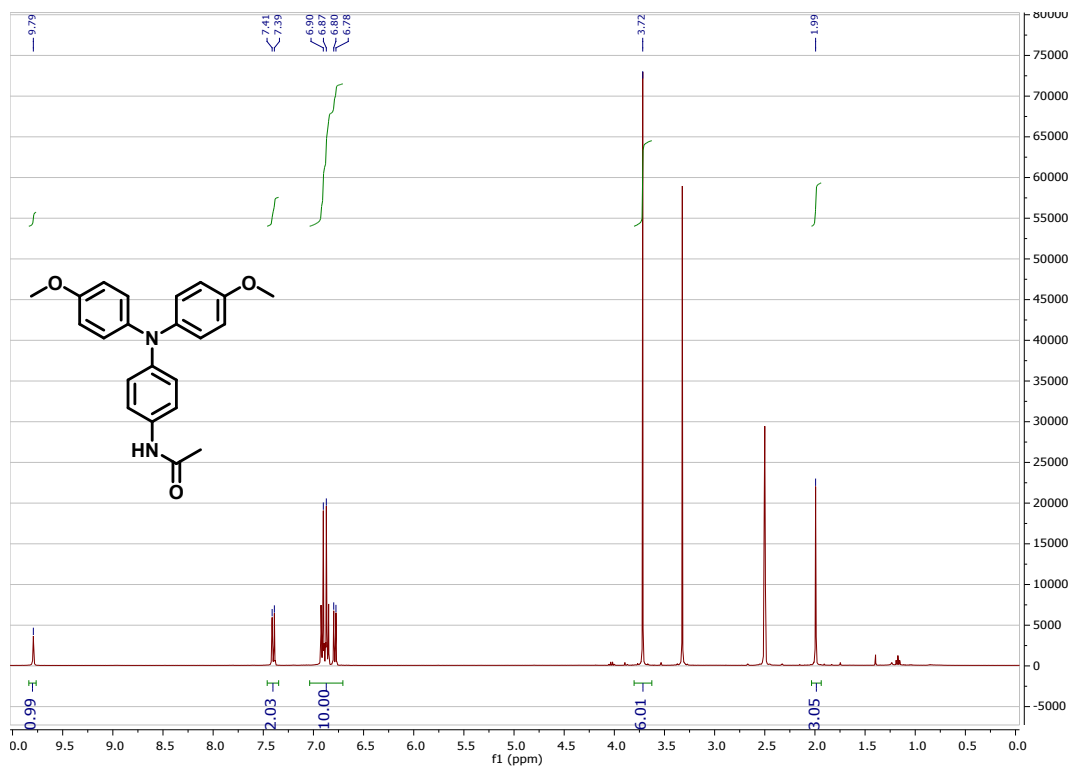


Figure S7. ¹H-NMR spectrum of 3 in *d*₆-DMSO

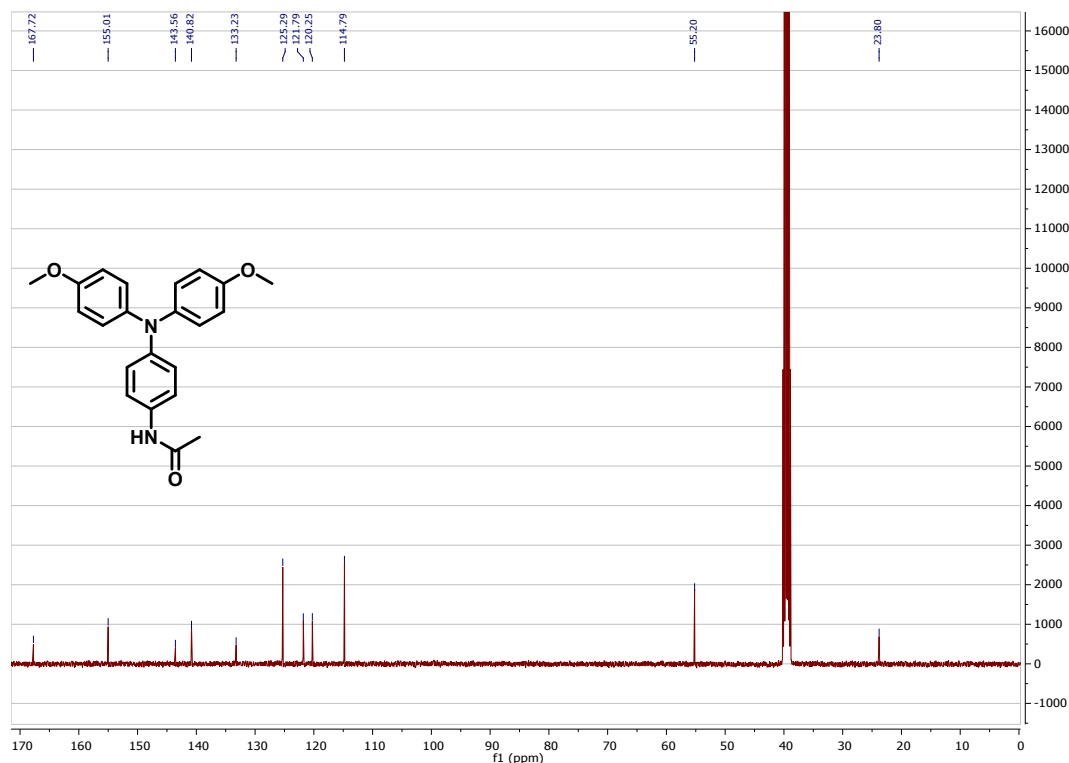


Figure S8. ^{13}C -NMR spectrum of **3** in d_6 -DMSO

3 Manipulation of the streptavidin mutants

3.1 Bioconjugation of $[\text{Ru}(\text{bpy})_2(\text{phenNHCOCH}_2\text{Br})](\text{PF}_6)_2$ (**1**) to the cysteine-containing streptavidin mutants

According to a modified protocol of Roelfes *et al.*⁶

The Sav mutants (1.2 μmol) were dissolved in NaH_2PO_4 buffer (6.0 ml, 50 mM, pH 7.6) and $[\text{Ru}(\text{bpy})_2(\text{phenNHCOCH}_2\text{Br})](\text{PF}_6)_2$ **1** (9.6 μmol , 9.8 mg) in 50 μl DMSO were added. The mixture was agitated in the dark at 4°C, 600 rpm for 24 h. The solution was then centrifuged and transferred to a Spectra/Por[®] 1 Dialysis Membrane Standard RC Tubing (MWCO: 6-8 kD) and dialyzed against MilliQ water in the dark for 5 days at 4°C, changing the water twice a day. After 5 days, the dialyzed water was colorless. The solution was filtered through a Filtropur S 0.2 μm and lyophilized for 2 days to yield a yellow fluorescent protein sample.

3.2 Mass Spectrometry of the Ru-Sav adducts

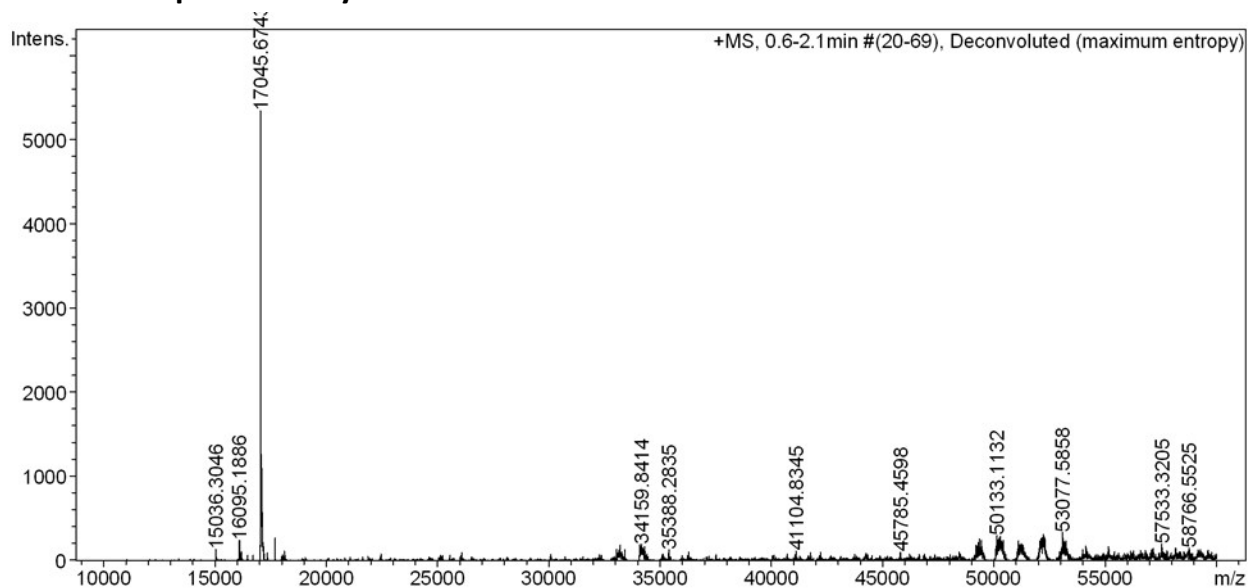


Figure S9. Mass spectrum of K121C-Ru modified Sav mutant. K121C unmodified mass: 16400 Da, expected for bioconjugated with Ru complex 17049 Da, found 17046 Da.

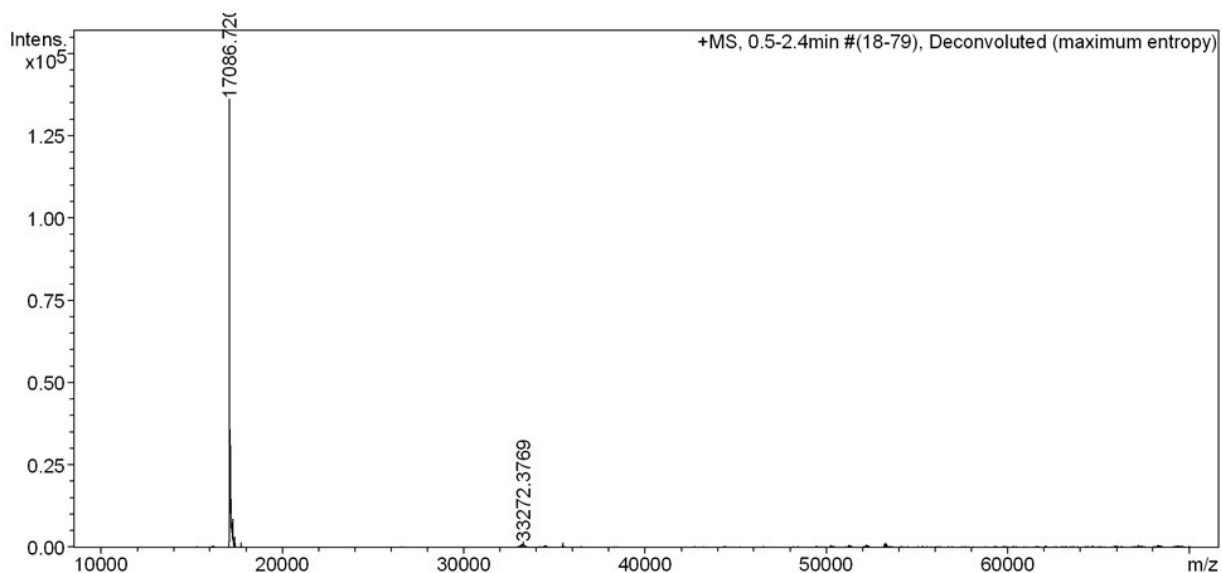


Figure S10. Mass spectrum of S112C-Ru modified Sav mutant. S112C unmodified mass: 16441 Da, expected for bioconjugated with Ru complex 17090 Da, found 17087 Da.

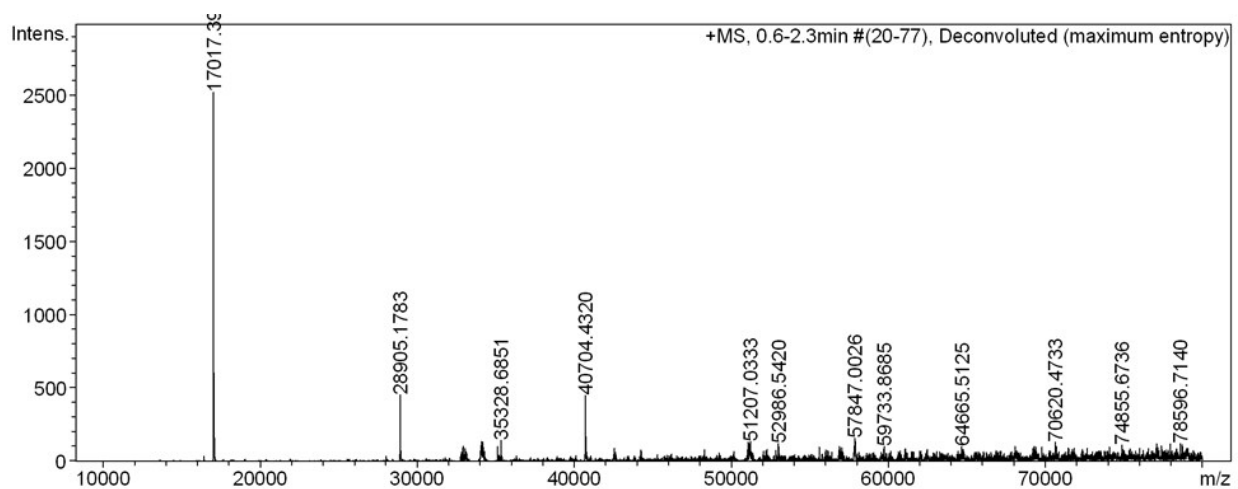


Figure S11. Mass spectrum of R84C-Ru modified Sav mutant. R84C unmodified mass: 16372 Da, expected for bioconjugated with Ru complex 17021 Da , found 17017 Da.

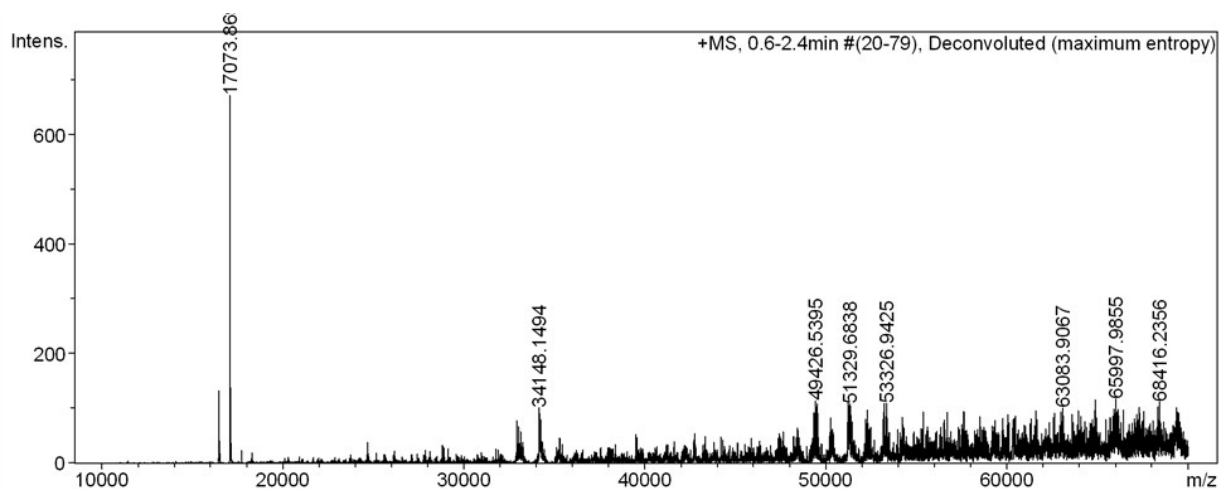


Figure S12. Mass spectrum of T66C-Ru modified Sav mutant. T66C unmodified mass: 16427 Da, expected for bioconjugated with Ru complex 17076 Da , found 17074 Da.

4 Photoexcitation experiments

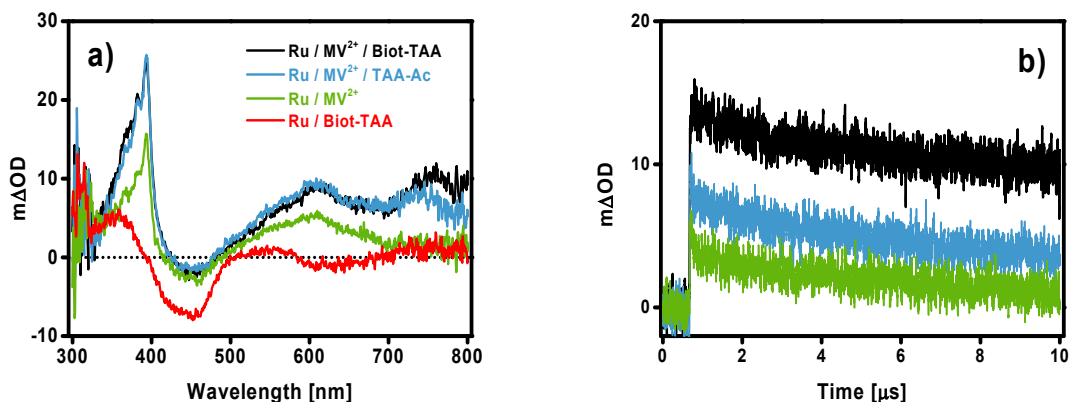


Figure S13. (a) Transient absorption spectra of S112C-Ru measured after excitation at 532 nm with laser pulses of ~ 10 ns duration. The spectra were time-integrated over 200 ns immediately after excitation. Sample concentrations were: $5 \cdot 10^{-5}$ M Ru(II)-streptavidin, 120 mM MV^{2+} , $2.5 \cdot 10^{-5}$ M **Biot-TAA** or **TAA-Ac**, where applicable. The solvent was MilliQ water at 25 °C. (b) Temporal evolution of the transient absorption signal at 760 nm for 3 of the 4 samples from (a).

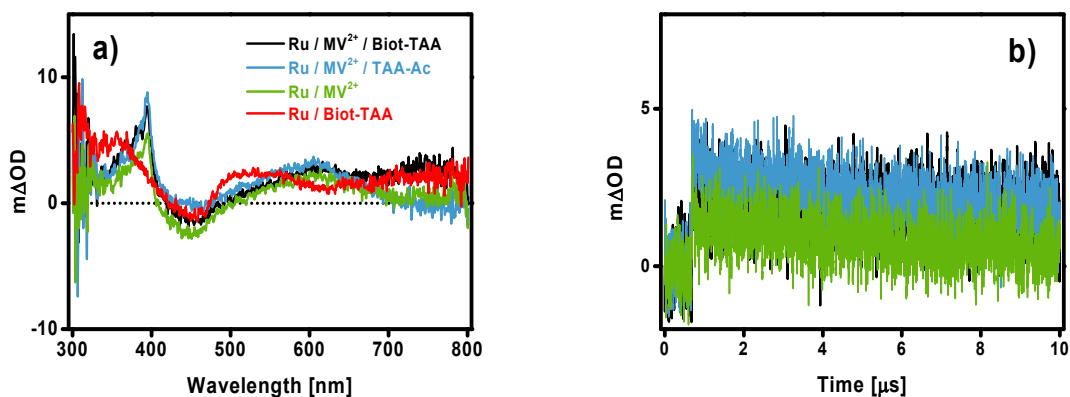


Figure S14. (a) Transient absorption spectra of R84C-Ru measured after excitation at 532 nm with laser pulses of ~ 10 ns duration. The spectra were time-integrated over 200 ns immediately after excitation. Sample concentrations were: $5 \cdot 10^{-5}$ M Ru(II)-streptavidin, 120 mM MV^{2+} , $2.5 \cdot 10^{-5}$ M **Biot-TAA** or **TAA-Ac**, where applicable. The solvent was MilliQ water at 25 °C. (b) Temporal evolution of the transient absorption signal at 760 nm for 3 of the 4 samples from (a).

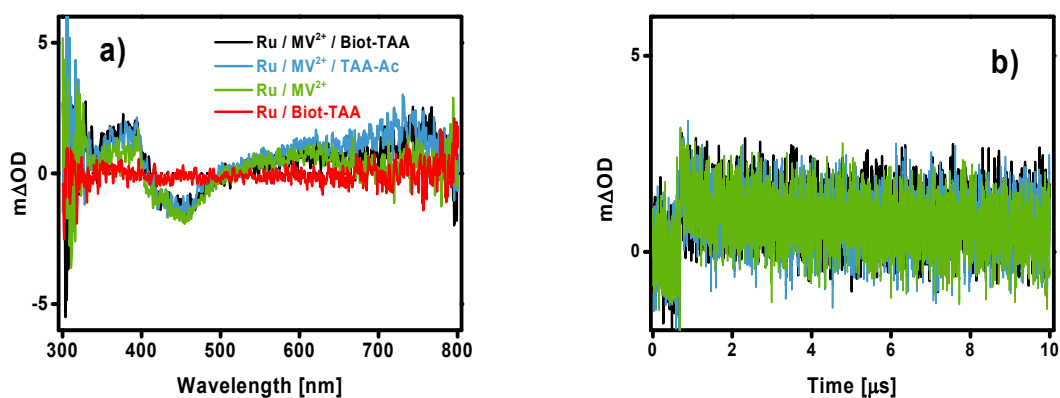


Figure S15. (a) Transient absorption spectra of T66C-Ru measured after excitation at 532 nm with laser pulses of ~ 10 ns duration. The spectra were time-integrated over 200 ns immediately after excitation. Sample concentrations were: $5 \cdot 10^{-5}$ M Ru(II)-streptavidin, 120 mM MV^{2+} , $2.5 \cdot 10^{-5}$ M **Biot-TAA** or **TAA-Ac**, where applicable. The solvent was MilliQ water at 25 °C. (b) Temporal evolution of the transient absorption signal at 760 nm for 3 of the 4 samples from (a).

5 Distance estimations

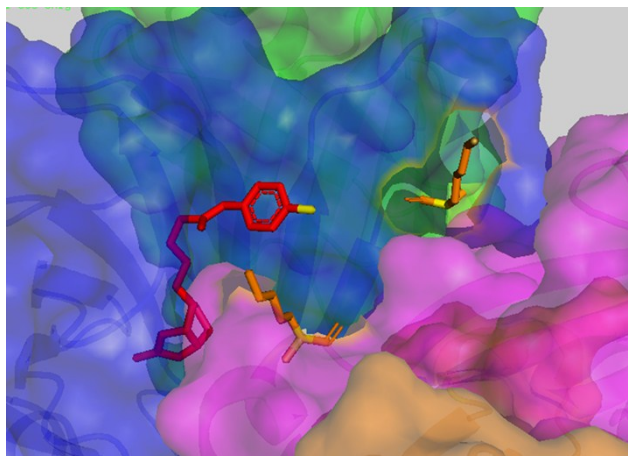


Figure S16. Close-up view of the biotin binding vestibule bearing a single arylated biotin from pdb code: 3PK2. The distances were measured through space from the sulfur atom (yellow) of the cofactor (red) to the α -carbon atoms (yellow) of the amino acids of interest (here K121 displayed in orange). The iridium-Cp*-complex was omitted for clarity.

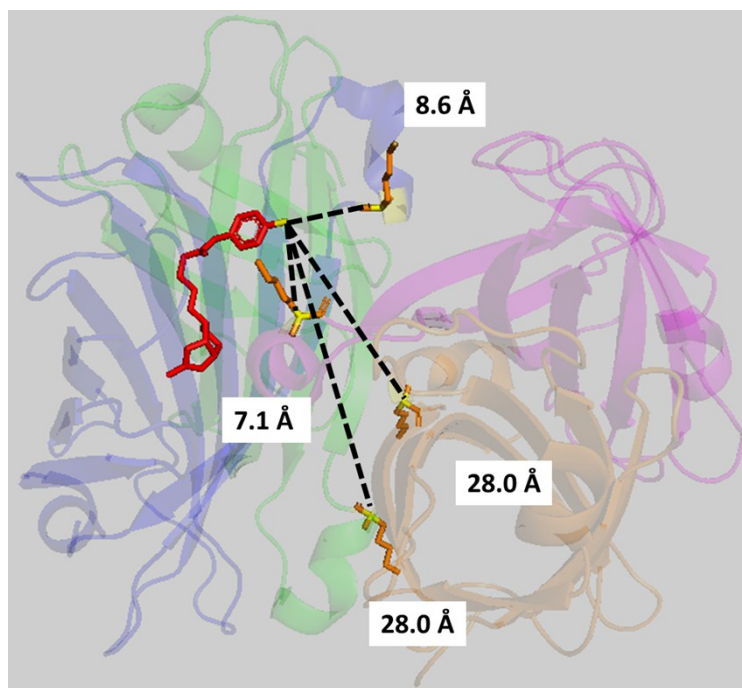


Figure S17. Estimated through-space distances between the sulfur atom of a biotinylated cofactor (pdb code 3PK2) and the α -carbon atoms of the K121 residue of all four monomers of homotetrameric Sav.

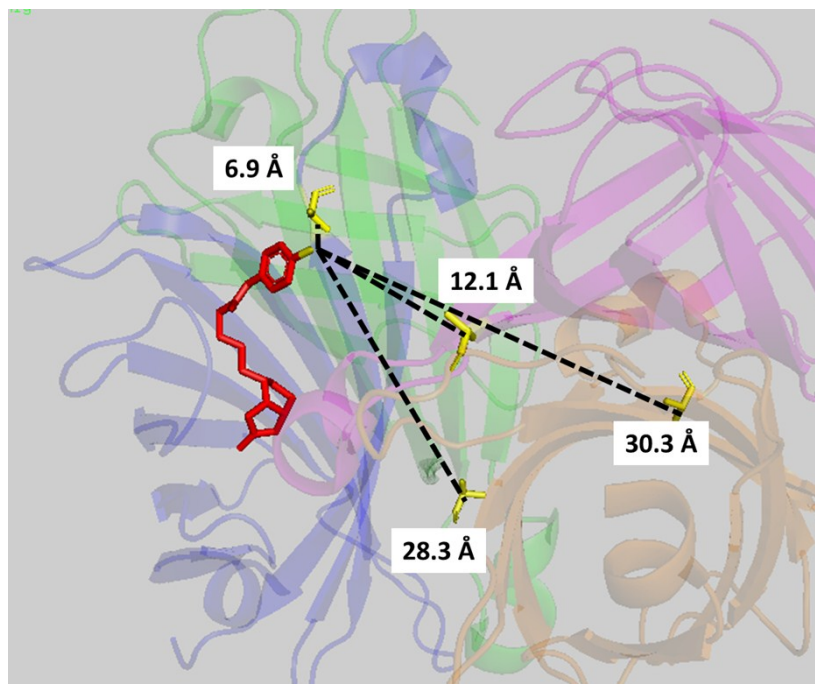


Figure S18. Estimated through-space distances between the sulfur atom of a biotinylated cofactor (pdb code 3PK2) and the α -carbon atoms of the S112A residue of all four monomers of homotetrameric Sav.

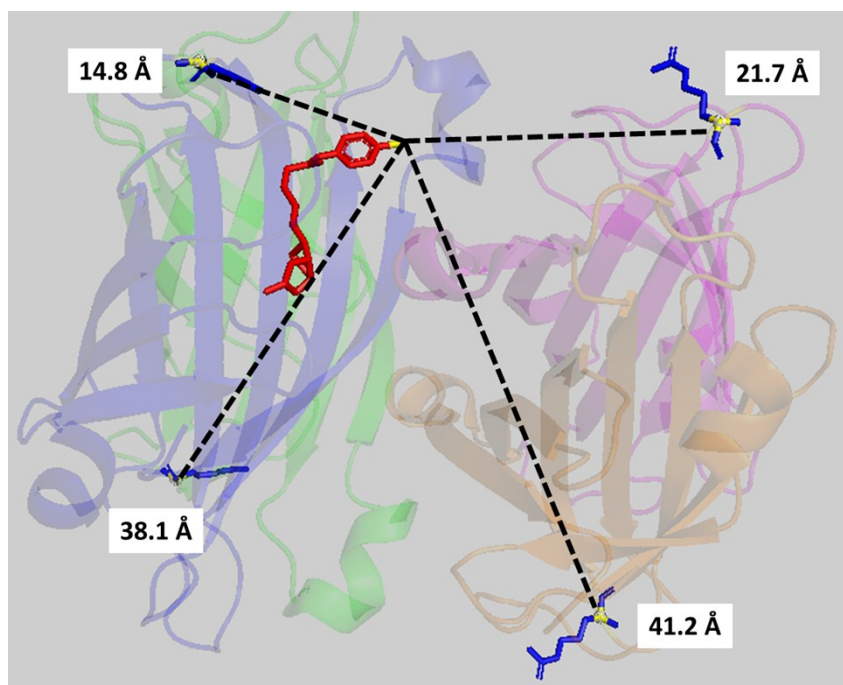


Figure S19. Estimated through-space distances between the sulfur atom of a biotinylated cofactor (pdb code 3PK2) and the α -carbon atoms of the R84 residue of all four monomers of homotetrameric Sav.

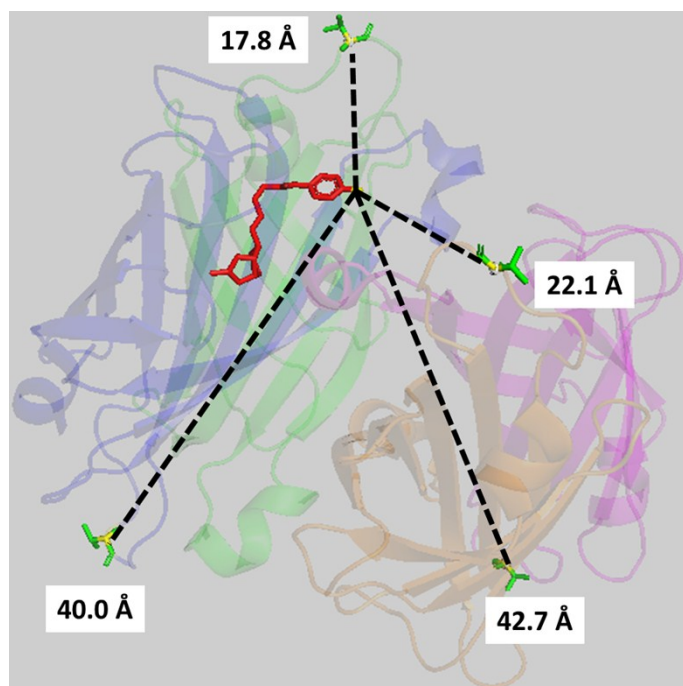


Figure S20. Estimated through-space distances between the sulfur atom of a biotinylated cofactor (pdb code 3PK2) and the α -carbon atoms of the T66 residue of all four monomers of homotetrameric Sav.

6 Biotin-binding site determination⁷

The Sav mutants (76.8 nmol) were dissolved in NaH_2PO_4 buffer (2.4 ml, 20 mM, pH 7.0). The solutions were treated with a 2-(4-hydroxyphenylazo)benzoic acid (HABA) solution (300 μl , 9.6 mM) in NaH_2PO_4 buffer (20 mM, pH 7.0) filled into a cuvette and left standing for 5 minutes. A blank of the buffer was measured at 506 nm in a Varian 50 Scan UV-Vis spectrophotometer. The resulting sample was analyzed and aliquots (5.0 μl) of a biotin (or **Biot-TAA**) solution (0.96 mM) in DMSO were added after each spectroscopic determination. The data points were plotted with Origin Pro 9.0.0 to determine the number of biotin-binding sites and the stoichiometry for the Sav embedded dyads.

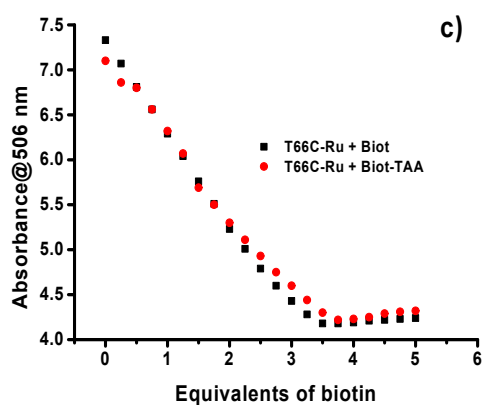
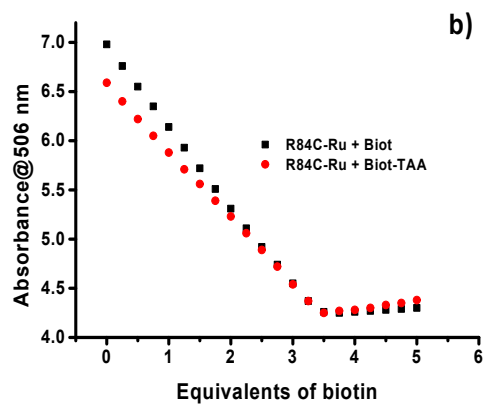
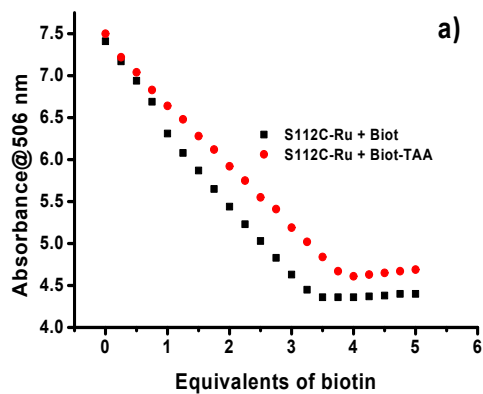


Figure S21. HABA displacement titrations of biotin (black symbols) and the biotinylated triarylamine **Biot-TAA** (red symbols) of a) S112C-Ru b) R84C-Ru c) T66C-Ru.

7 References

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