Light-Driven Electron Injection from a Biotinylated Triarylamine Donor to $[Ru(diimine)_3]^{2+}$-Labeled Streptavidin

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID	OB-COM-06-2016-001273.R1
Article Type:	Communication
Date Submitted by the Author:	06-Jul-2016
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Light-Driven Electron Injection from a Biotinylated Triarylamine Donor to $[Ru(diimine)_3]^{2+}$-Labeled Streptavidin

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Abstract

Electron transfer from a biotinylated electron donor to photochemically generated Ru(III) complexes covalently anchored to streptavidin is demonstrated by means of time-resolved laser spectroscopy. Through site-selective mutagenesis, a single cysteine residue was engineered at four different positions on streptavidin, and a Ru(II) tris-diimine complex was then bioconjugated to the exposed cysteines. A biotinylated triarylamine electron donor was added to the Ru(II)-modified streptavidins to afford dyads localized within a streptavidin host. The resulting systems were subjected to electron transfer studies. In some of the explored mutants, the phototriggered electron transfer between triarylamine and Ru(III) is complete within 10 ns, thus highlighting the potential of such artificial metalloenzymes to perform photoredox catalysis.

Introduction

Streptavidin (hereafter Sav) is an exquisitely robust protein of ca. 64 kDa comprised of four homologous sub-units, each of which can bind a biotin molecule with an association constant of $\sim 10^{14}$ M^{-1} under physiological conditions.[1] Sav can readily be over-expressed and purified from the bacterium *Escherichia coli*. Owing to this combination of favorable properties, biotin-streptavidin systems have been exploited in various contexts where host-guest recognition is desirable, ranging from targeted drug delivery, to live cell imaging and new applications in catalysis.[2]

By biotinylation of small metal catalysts, novel catalytic functions can be conferred to the resulting biotin-streptavidin assemblies, resulting in artificial metalloenzymes.[3] Their function can be tailored either chemically, i. e., through variation of the biotin-linker-catalyst moiety, or genetically through site-specific mutagenesis of Sav. In the best case, such chemogenetic optimization can lead to catalytic performance which is superior to that observed for the related small metal catalyst outside the protected environment of the biotin binding pockets of Sav, manifesting for example in unusually high enantioselectivity.[4]

In principle, such artificial metalloenzymes should also be amenable to the emerging field of redox photocatalysis,[5] and for this purpose it would be desirable to equip the biotin-streptavidin systems with redox photosensitizers. Complexes of d^6 metal ions such as ruthenium(II) and iridium(III) are good candidates in this regard.

There have been several studies of the luminescence properties of biotinylated d^6 metal complexes, including ruthenium(II) polypyridines,[6] rhenium(I) tricarbonyl diimines,[7] and cyclometalated iridium(III) complexes.[8] In most cases, binding to streptavidin leads to enhanced luminescence properties, and this is important for example for cell imaging purposes.[9] The above mentioned classes of metal complexes are well suited for photosensitization of electron transfer reactions,[10] but, to the best of our knowledge, this has not yet been realized within the context of the biotin-streptavidin technology.[11] However, ruthenium(II) and rhenium(I) complexes have been employed frequently for investigation of phototriggered electron-transfer in other proteins (e. g., azurin, cytochrome c, or plastocyanin), mostly for distance-dependence studies and for elucidating electron tunneling pathways.[12] The ruthenium(II) and rhenium(I) metal centers were commonly ligated to histidine ligands for this purpose, and the natural redox-active groups of these proteins (e. g., blue copper centers or heme groups) served as electron-transfer reaction partners.

Typical photoredox systems usually require a photosensitizer combined with a distinct catalytic moiety. As the binding of biotinylated probes to Sav is non-cooperative, introducing two distinct dyads within a streptavidin tetramer remains an unmet challenge.[13] To assemble such dyads using a Sav scaffold, we thus opted to bioconjugate the photosensitizer to an engineered cysteine residue, thus leaving the four biotin-binding sites of Sav free to introduce either a catalytic or an electron-donor moiety.

As Sav possesses no cysteine residue, site-directed mutagenesis was used to engineer this highly nucleophilic residue at selected positions. The resulting single-point mutants were subsequently bioconjugated with a $[Ru(bpy)_2(phen)]^{2+}$-photosensitizer **1** (Figure 1, bpy = 2,2'-bipyridine, phen = 1,10-phenanthroline). The triarylamine (TAA) unit was equipped with a biotin group to yield **Biot-TAA** (see SI for details, **2** displayed in Figure 1). The resulting **Biot-TAA** / Ru(II)-streptavidin adducts are donor-acceptor systems in which phototriggered electron transfer can be investigated. The goal of this work was to explore whether phototriggered electron injection from a biotinylated donor to ruthenium(II)-labeled streptavidin is possible and if so, what position on the surface of Sav is most suitable for the covalent anchoring of the photosensitizer.

Results and discussion

Structural and synthetic aspects. The ruthenium(II) complex (*rac*-**1** displayed in Figure 1) was covalently tethered to streptavidin through nucleophilic attack by a cysteine residue with concomitant elimination of HBr.[14] Single cysteine residues were introduced by site directed mutagenesis on each of the four homologous units of streptavidin, leading to a fourfold Ru(II)-labeling of each streptavidin tetramer. The biotinylated triarylamine unit **Biot-TAA** was synthesized as a binding partner. The acetyl-substituted triarylamine (**TAA-Ac**) **3** served as a reference compound (Figure 1). Syntheses and mutagenesis procedures are presented in the Supporting Information.

Inspection of the X-ray structure of S112A Sav (pdb code: 3PK2)[15] led us to select four positions for the introduction of a cysteine residue by site-directed mutagenesis. The following four mutants were produced using a previously published method and purified by affinity chromatography[16] and characterized by ESI-MS: Sav T66C, R84C, S112C,

and K121C (Figure 2). Subsequent bioconjugation was achieved by mixing the mutant protein overnight with an eightfold excess of Ru-complex **1** at 4°C in phosphate buffer (50 mM, pH 7) in the dark. The excess of the unbound Ru-complex **1** was removed by dialysis and the resulting Ru_4Sav bioconjugates (Ru_4Sav T66C, Ru_4Sav R84C, Ru_4Sav S112C, and Ru_4Sav K121C respectively) were characterized by ESI-MS, revealing a quantitative conversion to the Ru-containing proteins (See SI for details). The biotin-binding capacity of the Ru_4Sav bioconjugates was assessed relying on a displacement titration using HABA (2-(4-hydroxyphenylazo)benzoic acid). Upon incorporation within Sav, HABA displays an absorption at λ_{max} 506 nm. Upon addition of biotinylated probes, the HABA is displaced, leading to a disappearance of the absorption at 506 nm.[4a, 17] This simple procedure revealed that all four biotin-binding sites can accommodate the biotinylated cofactor **Biot-TAA**, and the affinity is similar to that of pure unmodified biotin (see Figure3, more details in the Supporting Information). This confirms that the bulky **Biot-TAA** moieties bind to Ru_4Sav isoforms, despite the presence of the bulky $Ru(bpy)_2(phenNHCOCH_2)$ moieties which, in the S112C and K121C mutants, lie in the proximity of the biotin-binding vestibule.

Since no crystal structure could be obtained so far, through-space distances were estimated based on a previously published crystal structure of an Ir-loaded streptavidin mutant (pdb code: 3PK2).[15] The arylated biotin moiety bearing a *para*-sulfur atom was used for distance estimation. This latter sulfur atom was selected as a surrogate for the nitrogen atom of the **Biot-TAA** moiety (Figure S16). The shortest through-space distances from the sulfur atom to the α-carbon of the amino acids targeted for mutagenesis ranges from 7.1 to 22.1 Å (Table 1). As, by symmetry four cysteine residues result from a single point mutation, the mean value of the two closest residues was also determined: it increases from K121 (7.9 Å) < S112 (9.5 Å) < R84 (18.3 Å) < T66 (20.0 Å) .

Electron transfer studies. To explore the electron transfer in the **Biot-TAA** / Ru(II)-streptavidin dyads a flash quench technique was applied (Figure 4).[18] Therefore, the ruthenium(II) photosensitizer was excited selectively at 532 nm with laser pulses of ~10 ns duration. In the presence of 120 mM methylviologen dichloride (MV^{2+}) added to the aqueous solution, the luminescent ^{3}MLCT excited state of the photosensitizer *Ru(II) is quenched oxidatively with diffusion-limited kinetics, i. e., initial excitation ("flash") is followed by oxidation of *Ru(II) to Ru(III) ("quench") within the ~10 ns duration of the laser pulse.

In a sample containing 50 μM Ru(II)-streptavidin (K121C mutant) and 120 mM MV^{2+} but no **Biot-TAA** in MilliQ water at 25 °C, one observes the typical spectroscopic signatures of the $MV^{\bullet+}$ radical (green trace in Figure 5a).[19] Specifically, there is a sharp and intense absorption at 393 nm and a broader, less intense band with a maximum at 605 nm which can be attributed unambiguously to $MV^{\bullet+}$.[19-20] The oxidation product Ru(III) manifests as a bleach at ~450 nm. When adding 25·μM **Biot-TAA** to the same solution, an additional band at ~760 nm becomes observable (black trace in Figure 5a) in addition to the $MV^{\bullet+}$ bands. The band at 760 nm is typical for TAA^+,[21] and hence this is clear evidence for electron transfer from TAA to Ru(III), which (based on the relevant redox potentials)[21] is expected to be associated with a reaction free energy (ΔG_{ET}^0) of ca. -0.5 eV. In the reference experiment in which 50·μM Ru(II)-streptavidin and 120 mM MV^{2+} were measured in the presence of 25·μM **TAA-Ac**, the band at 760 nm does not

appear (blue trace in Figure 5a), confirming that in the Ru(II)-streptavidin (K121C mutant) / TAA-biotin system, the phototriggered electron transfer from TAA to Ru(III) does indeed rely on the tight association between the streptavidin host and the **Biot-TAA** guest.

The TAA^+ signal at 760 nm rises with instrumentally limited kinetics (black trace in Figure 5b), indicating that phototriggered electron transfer from TAA-biotin to Ru(III)-streptavidin (K121C mutant) occurs with a rate constant $k_{ET} \geq 10^8\ s^{-1}$. The signal then decays on a timescale > 10 μs, due to bimolecular electron transfer between **Biot-TAA$^+$** and $MV^{\bullet+}$ (Figure 5b). $MV^{\bullet+}$ has non-negligible absorbance at 760 nm hence the observation of small signals even in absence of **Biot-TAA** (blue and green traces in Figure 5b).

For the S112C mutant, qualitatively similar results are obtained (Supporting Information). There is clear indication for rapid ($k_{ET} \geq 10^8\ s^{-1}$) electron transfer from **Biot-TAA** to Ru(III)-streptavidin, and the resulting photoproduct is again very long-lived (> 10 μs). However, for the R84C and T66C mutants, no such evidence was obtained. We conclude that these two ruthenium(II)-labeled mutants are not well suited for electron transfer with biotinylated triarylamine guests. This findings correlate with the increasing distances between the photosensitizer and the electron donating triarylamine. It is possible that electron transfer between photoexcited Ru(II) and MV^{2+} is less efficient in these cases even though they seem to be even better exposed as the Ru(II) complexes in the other two mutants.

Summary and conclusions

Rapid phototriggered electron transfer from biotinylated guests to ruthenium photosensitizers which are covalently attached to cysteine residues of streptavidin is possible in carefully selected mutants. This is an important finding in view of photoredox catalysis which makes use of catalysts which are embedded in the protected environment of a biotin binding pocket of streptavidin and photosensitizers which are bound to the surface of streptavidin. Catalysts in the biotin binding pocket can exhibit markedly better performance than outside streptavidin,[3a, 4b] and our study paves the way to photoredox catalysis in specifically engineered artificial metalloenzymes.

Acknowledgments

This work was supported by the Swiss National Science Foundation as part of the NCCR Molecular Systems Engineering as well as the SNI (PhD scholarship awarded to Sascha Keller).

Table 1. Estimated through-space distances between α-carbon atom of the targeted mutagenesis positions and the sulfur atom of a biotinylated cofactor based on the crystal structure of streptavidin (pdb code: 3PK2)[15]. The two closest Cα are listed as well as their mean values (Figure S16-S20 in the Supporting Information for details).

mutant	R_1 [Å]	R_2 [Å]	mean [Å]
K121C	7.1	8.6	7.9
S112C	6.9	12.1	9.5
R84C	14.8	21.7	18.3
T66C	17.8	22.1	20.0

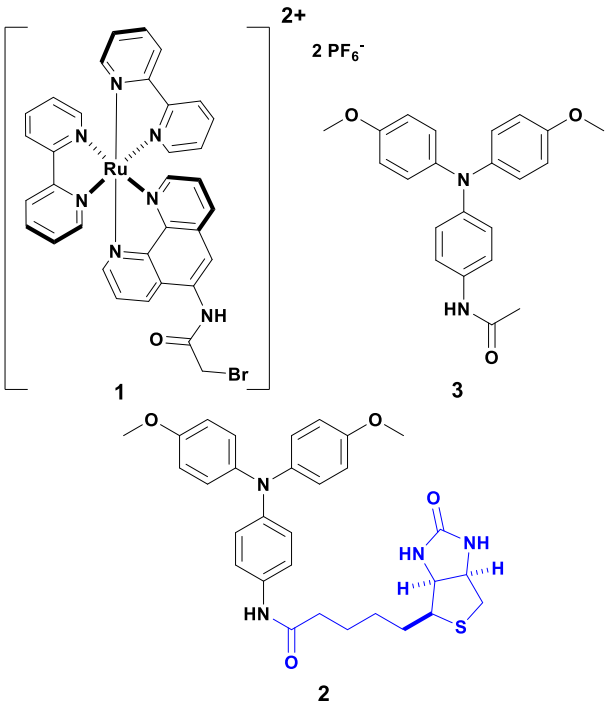


Figure 1. Chemical structures of the ruthenium(II)-label for streptavidin **1**, the **Biot-TAA** moiety **2** (biotin is displayed in blue) and the **TAA-Ac** reference molecule **3**. See Supporting Information for syntheses.

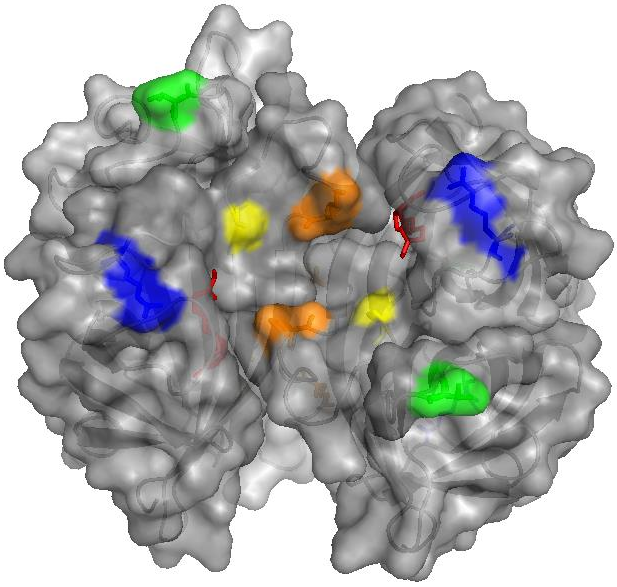


Figure 2. Surface display representation of homotetrameric streptavidin, highlighting the symmetry related positions selected for introducing cysteine residues: K121 (orange), S112 (yellow), R84 (blue), T66 (green) and biotin (red stick representation); (pdb code: 3PK2).[15]

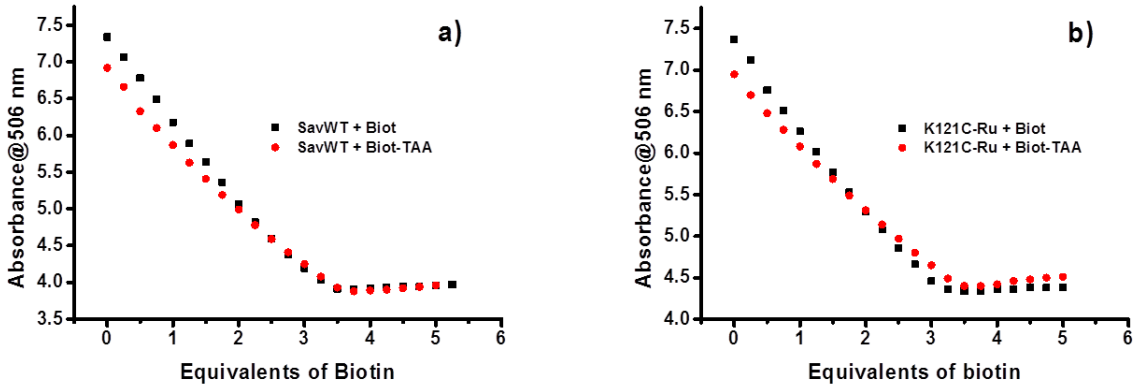


Figure 3. HABA displacement titrations of biotin (black symbols) and the biotinylated triarylamine **Biot-TAA** (red symbols) for a) WT streptavidin and b) K121C-Ru (see Supporting Information for experimental details).

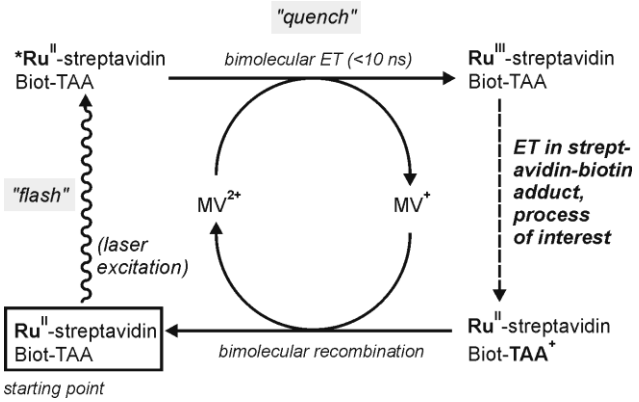


Figure 4. Illustration of the flash-quench procedure used for studying electron transfer in the **Biot-TAA** / Ru(II)-streptavidin adducts.[12] MV^{2+} stands for methylviologene.

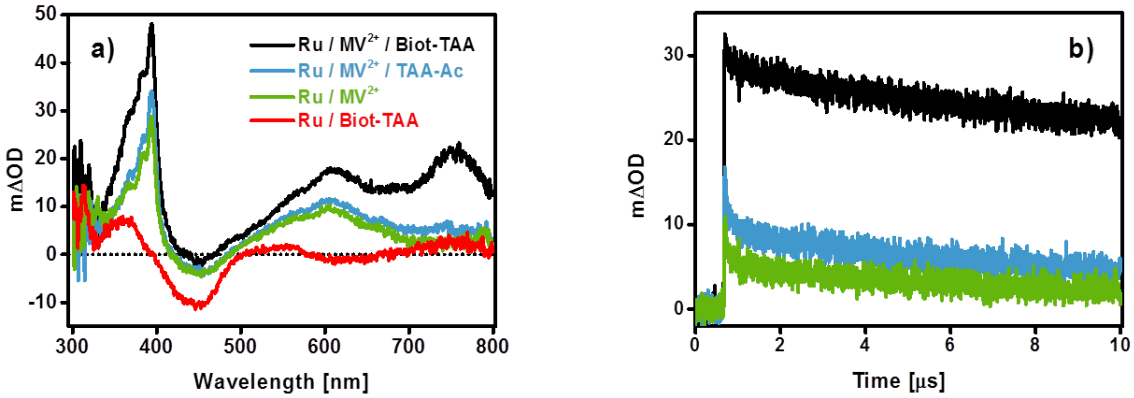
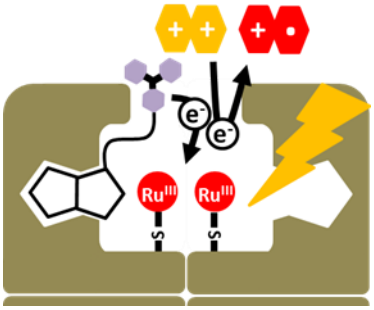


Figure 5. (a) Transient absorption spectra of K121C-Ru measured after excitation at 532 nm with laser pulses of ~10 ns duration. The spectra were time-integrated over 200 ns immediately after excitation. Sample concentrations were: 50·µM Ru(II)-streptavidin, 120 mM MV^{2+}, 25·µM **Biot-TAA** or **TAA-Ac**, 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).



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References

(1) M. Wilchek and E. A. Bayer, *Avidin-Biotin Technology*, Academic Press, INC., San Diego, 1990.

(2) C. M. Dundas, D. Demonte and S. Park, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 9343.

(3) (a) T. R. Ward, *Acc. Chem. Res.*, 2011, **44**, 47; (b) J. C. Lewis, *ACS Catal.,* 2013, **3**, 2954; (c) J. Bos and G. Roelfes, *Curr. Opin. Chem. Biol.,* 2014, **19**, 135.

(4) (a) M. Skander, N. Humbert, J. Collot, J. Gradinaru, G. Klein, A. Loosli, J. Sauser, A. Zocchi, F. Gilardoni and T. R. Ward, *J. Am. Chem. Soc.,* 2004, **126**, 14411; (b) T. K. Hyster, L. Knörr, T. R. Ward and T. Rovis, *Science,* 2012, **338**, 500.

(5) (a) C. K. Prier, D. A. Rankic and D. W. C. MacMillan, *Chem. Rev.,* 2013, **113**, 5322; (b) J. M. R. Narayanam and C. R. J. Stephenson, *Chem. Soc. Rev.,* 2011, **40**, 102; (c) M. D. Levin, S. Kim and F. D. Toste, *ACS Cent. Sci.,* 2016, **2**, 293.

(6) (a) K. K. W. Lo, W. K. Hui, C. K. Chung, K. H. K. Tsang, T. K. M. Lee, C. K. Li, J. S. Y. Lau and D. C. M. Ng, *Coord. Chem. Rev.,* 2006, **250**, 1724; (b) K. K. W. Lo and T. K. M Lee, *Inorg. Chem.,* 2004, **43**, 5275; (c) T. Soller, M. Ringler, M. Wunderlich, T. A. Klar, J. Feldmann, H. P. Josel, J. Koci, Y. Markert, A. Nichtl and K. Kürzinger, *J. Phys. Chem. B,* 2008, **112**, 12824; (d) S. Delahaye, C. Loosli, S.-X. Liu, S. Decurtins, G. Labat, A. Neels, A. Loosli, T. R. Ward and A. Hauser, *Adv. Funct. Mater.,* 2006, **16**, 286.

(7) (a) K. K. W. Lo, W. K. Hui, C. K. Chung, K. H. K. Tsang, D. C. M. Ng, N. Y. Zhu and K. K. Cheung, *Coord. Chem. Rev.,* 2005, **249**, 1434; (b) K. K. W. Lo and W. K. Hui, *Inorg. Chem.,* 2005, **44**, 1992; (c) K. K. W. Lo, K. H. K. Tsang and K. S. Sze, *Inorg. Chem.,* 2006, **45**, 1714; (d) K. K. W. Lo, M. W. Louie, K. S. Sze and J. S. Y Lau, *Inorg. Chem.,* 2008, **47**, 602; (e) K. K. W. Lo, W. K. Hui and D. C. M. Ng, *J. Am. Chem. Soc.,* 2002, **124**, 9344.

(8) (a) K. K. W. Lo, K. Y. Zhang, S. K. Leung and M. C. Tang, *Angew. Chem. Int. Ed.,* 2008, **47**, 2213; (b) K. K. W. Lo, K. H. K. Tsang, K. S. Sze, C. K. Chung, T. K. M. Lee, K. Y. Zhang, W. K. Hui, C. K. Li, J. S. Y. Lau, D. C. M. Ng and N. Zhu, *Coord. Chem. Rev.,* 2007, **251**, 2292; (c) K. K. W. Lo and J. S. Y. Lau, *Inorg. Chem.,* 2007, **46**, 700; (d) K. Y. Zhang and K. K. W. Lo, *Inorg. Chem.,* 2009, **48**, 6011; (e) K. K. W. Lo, J. S. W. Chan, L. H. Lui and C. K. Chung, *Organometallics,* 2004, **23**, 3108; (f) K. K. W. Lo, C. K. Li and J. S. Y. Lau, *Organometallics,* 2005, **24**, 4594.

(9) (a) V. Fernandez-Moreira, F. L. Thorp-Greenwood and M. P. Coogan, *Chem. Commun.,* 2010, **46**, 186; (b) S. James, K. P. Maresca, J. W. Babich, J. F. Valliant, L. Doering and J. Zubieta, *Bioconjugate Chem.,* 2006, **17**, 590.

(10) O. S. Wenger, *Coord. Chem. Rev.,* 2009, **253**, 1439.

(11) A. Fürstenberg, O. Kel, J. Gradinaru, T. R. Ward, D. Emery, G. Bollot, J. Mareda and E. Vauthey, *ChemPhysChem,* 2009, **10**, 1517.

(12) (a) J. N. Onuchic, D. N. Beratan, J. R. Winkler and H. B. Gray, *Annu. Rev. Biophys. Biomol. Struct.,* 1992, **21**, 349; (b) H. B. Gray and J. R. Winkler, *Ann. Rev. Biochem.,* 1996, **65**, 537; (c) D. S. Wuttke, M. J. Bjerrum, J. R. Winkler and H. B. Gray, *Science,* 1992, **256**, 1007; (d) B. R. Crane, A. J. Di Bilio, J. R. Winkler and H. B. Gray, *J. Am. Chem. Soc.,* 2001, **123**, 11623; (e) C. Fehl and B. G. Davis, *Proc. R. Soc. A,* 2016, doi: 10.1098/rspa.2016.0078.

(13) A. Loosli, U. E. Rusbandi, J. Gradinaru, K. Bernauer, C. W. Schlaepfer, M. Meyer, S. Mazurek, M. Novic and T. R. Ward, *Inorg. Chem.,* 2006, **45**, 660.

(14) J. Bos, F. Fusetti, J. M. Arnold, A. J. M. Driessen and G. Roelfes, *Angew. Chem. Int. Ed.,* 2012, **51**, 7472.

(15) M. Dürrenberger, T. Heinisch, Y. M. Wilson, T. Rossel, E. Nogueira, L. Knörr, A. Mutschler, K. Kersten, M. J. Zimbron, J. Pierron, T. Schirmer and T. R. Ward, *Angew. Chem. Int. Ed.,* 2011, **50**, 3026.

(16) G. Klein, N. Humbert, J. Gradinaru, A. Ivanova, F. Gilardoni, U. E. Rusbandi and T. R. Ward, *Angew. Chem. Int. Ed*., 2005, **44**, 7764.

(17) O. Livnah, E. A. Bayer, M. Wilchek and J. L. Sussman, *FEBS Lett.,* 1993, **328**, 165.

(18) (a) M. J. Bjerrum, D. R. Casimiro, I.-J. Chang, A. J. Di Bilio, H. B. Gray, M. G. Hill, R. Langen, G. A. Mines, L. K. Skov, J. R. Winkler and D. S. Wuttke, *J. Bioenerg. Biomembr.,* 1995, **27**, 295; (b) D. Hanss and O. S. Wenger, *Eur. J. Inorg. Chem.,* 2009, **25**, 3778; (c) D. Hanss and O. S. Wenger, *Inorg. Chem.,* 2009, **48**, 671.

(19) P. S. Braterman and J. I. Song, *J. Org. Chem.,* 1991, **56**, 4678.

(20) A. Pannwitz and O. S. Wenger, *Phys. Chem. Chem. Phys.,* 2016, **18**, 11374.

(21) (a) K. Sreenath, T. G. Thomas and K. R. Gopidas, *Org. Lett.,* 2011, **13**, 1134; (b) V. Coropceanu, N. E. Gruhn, S. Barlow, C. Lambert, J. C. Durivage, T. G. Bill, G. Nöll, S. R. Marder, J. L. Brédas, *J. Am. Chem. Soc.,* 2004, **126**, 2727; (c) J. Hankache and O. S. Wenger, *Chem. Commun.,* 2011, **47**, 10145; (d) J. Hankache, M. Niemi, H. Lemmetyinen and O. S. Wenger, *Inorg. Chem.,* 2012, **51**, 6333.

(22) J. R. Winkler and H. B. Gray, *J. Am. Chem. Soc.,* 2014, **136**, 2930.