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Photocatalytic Hydrogen Production by Protein Systems

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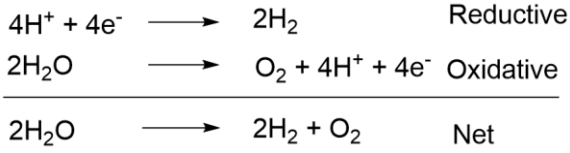
Abstract
Integration of synthetic molecular catalysts with protein structures has emerged as a new field of study that provides novel opportunities to understand and improve on catalytic processes. The use of proteins to develop photocatalytic biohybrid systems enhances this field by further enabling the development of direct donor-acceptor systems that utilize protein architectures to facilitate photocatalysis. This mini-review focusses primarily on current efforts to combine protein scaffolds with homogeneous synthetic molecular catalysts and photosensitizers for photocatalytic hydrogen (H_2) production, while other methods of H_2 production will be briefly introduced in context.

Introduction

The sun is a long lasting and abundant source of energy, capable of providing power for our planet's increasing energy needs (Lewis and Nocera 2006, Cook et al. 2010). Nature captures and converts the sun's energy into useful fuels through the process of photosynthesis. In photosynthesis, light induces a highly efficient charge-separation in reaction center (RC) proteins. Photo-excitation of the primary electron donor and subsequent electron transfer to a variety of cofactors produces a long-range charge-separation across a lipid bilayer. These light reactions generate the reducing power needed for fuel production, in the form of NADPH (ultimately used to produce sugars) within plants, algae, and photosynthetic bacteria (Blankenship 2002). The one of the current challenges facing the greater adoption of solar energy involve developing methods to efficiently capture the sun's energy and convert it to a useful fuel source that can be stored until needed. Using inspiration from nature's RC proteins has proven to be an interesting design strategy for the development of synthetic and protein-based assemblies which can capture and convert solar energy into H_2. Another major challenge to the adoption of solar fuels is the relative cost and efficiency of solar energy production. Using photosynthetic proteins which are already optimized to capture the sun's energy could reduce the costs and increase the overall efficiency of solar fuels in the long term.

H_2 Production by Synthetic Catalysts

H_2 is a desirable solar fuel source. It is a clean burning fuel, producing only water upon combustion, and has high energy storage capacity in its chemical bonds (140 MJ/kg) (Utschig et al. 2015). The electrolysis of water, **Scheme 1,** produces both H_2 and O_2, however, with the aid of sacrificial electron donors, either half reaction can be performed independently (McKone et al. 2014). The reductive half reaction efficiently produces H_2.



Scheme 1. Overall water splitting reaction. The reductive half reaction produces H_2, while the oxidative half reaction produces O_2.

In the last decade, a wide variety of homogeneous H_2 evolution reaction (HER) catalysts have been developed. In addition, there are many types of heterogeneous HER systems such as quantum dots, nanorods, and semiconductors linked to catalysts or proteins, however, these systems are beyond the scope of this review. New homogeneous catalysts have transitioned away from the use of noble metals, such as platinum, replacing them with

first row transition metals, Fe, Ni, or Co (Du and Eisenberg 2012). H_2 evolving catalysts have been extensively studied as electrocatalysts with electrocatalytic turnover frequencies (TOFs) reported as high as 100,000 s^{-1} (Helm et al. 2011). Recent efforts have also included replacing the first row transition metals with completely organic catalysts for H_2 production (Haddad et al. 2016). Electrocatalytic systems require an applied over-potential to provide driving force for catalysis and therefore cannot directly utilize the sun's energy unless incorporated in a photo-electrochemical cell or other device to convert sunlight into energy. This limits the practicality of electrocatalytic systems to provide a long term solution to our renewable energy needs.

Photocatalytic H_2 production directly produces fuel from sunlight. Photocatalytic systems use photosensitizers (PSs), such as $Ru(bpy)_3^{2+}$ (bpy = 2,2'-bipyridine) derivatives or organic dyes, to capture photons. Many photocatalytic systems are multimolecular; they contain an HER catalyst, a PS, and a sacrificial electron donor which interact through diffusion (**Figure 1a**) (Du and Eisenberg 2012). Multimolecular photocatalytic systems have been developed with a wide diversity of first-row transition metal catalysts (particularly Fe, Ni, and Co), and have been extensively reviewed elsewhere (Du and Eisenberg 2012, Berardi et al. 2014, McKone, Marinescu et al. 2014). Photocatalytic experiments of Co and Ni HER catalysts have produced more modest TOFs than electrocatalytic systems (3,400 h^{-1} and 460 h^{-1} respectively) (McNamara et al. 2012, Gross et al. 2014); however, these systems are the starting point for understanding PS – catalyst electron transfer in order to develop practical systems for directed H_2 production with controlled electron transfer relays.

Supramolecular complexes, which contain a photosensitizer directly linked to a catalyst (**Figure 1b),** have been developed using cobaloximes linked axially or equatorially to a $Ru(bpy)_3^{2+}$ photosensitizer. Cobaloximes were originally used as mimic of vitamin B12 and are pseudomacrocyclic bis(dialkylglyoximato)cobalt complexes (**Figure 2e**) (Schrauzer 1968, Razavet et al. 2005). Axially-linked supramolecular complexes produce less H_2 than multimolecular systems (32 h^{-1}), and are hampered by instability in solution (Fihri et al. 2008, Mulfort and Tiede 2010, Mulfort et al. 2013). In equatorially-linked complexes catalytic intermediates have been observed; however, these complexes suffered from extremely short excited state lifetimes (ps) and back charge recombination and therefore were unable to produce any measurable H_2 (Mukherjee et al. 2013). Supramolecular systems represent a first step to understanding direct donor-acceptor interactions between PSs and catalysts leading to the development of new motifs for enhanced photocatalysis.

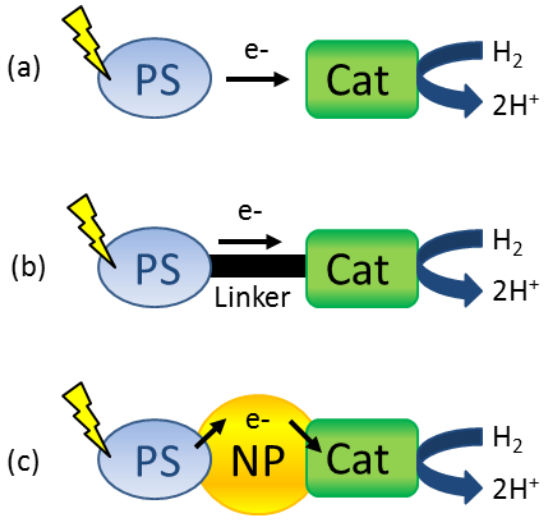


Figure 1. Types of Photocatalytic HER Systems Using Synthetic Catalysts and PSs. (a) multimolecular, (b) supramolecular, (c) nanoparticle based systems for HER photocataylsis.

Nanoparticle based systems also serve as promising mediums for photocatalytic H_2 production as they offer mechanisms to prevent the back recombination issues encountered by supramolecular complexes. In these systems, electrons can be shuttled from the PS through the nanoparticle to the catalyst with nanoparticle serving as a location to collect electrons until needed for catalysis (**Figure 1c**)

(Mulfort and Utschig 2016). This architecture has the ability to use particular anchoring groups on the PS and catalyst to facilitate binding to the nanoparticle. The Reisner group has characterized the lifetimes and reaction mechanisms of RuP-TiO_2-Co and RuP-TiO_2-Ni complexes (where RuP is a Ru complex decorated with phosphonate groups, [Ru^{II}(2,2'-bipyridine)$_2$(2,2'-bipyridine-4,4'-diylbisphosphonic acid)]Br_2). They demonstrated that electron transfer through the nanoparticle uses an oxidative quenching mechanism for the Ru PS while multimolecular catalysis uses a reductive quenching mechanism (Gross, Reynal et al. 2014, Reynal et al. 2015, Willkomm et al. 2015, Willkomm et al. 2016). These efforts currently are at the proof of concept stages and optimization of these efforts will enable rapid photocatalytic hydrogen production from much more stable systems than currently exist. Future efforts to improve nanoparticle based systems could provide opportunities to scale up photocatalytic H_2 production for industrial needs through coupling to electrodes or molecular wires.

Protein Based H_2 Production

Another direction for H_2 production involves taking inspiration from nature. Hydrogenases perform natural H_2 production. They are metalloenzymes which directly convert protons and electrons to H_2 or oxidize H_2 back to protons and electrons (eq. 1):

$$2H^+ + 2e^- \leftrightarrows 2H_2 \quad (1)$$

Most hydrogenases are found in archaea or bacteria, with a few found in eukarya (Vignais et al. 2001, Tamagnini et al. 2002, Thauer et al. 2010). Hydrogenases have several types of metal centers, [NiFe], [FeFe], and [Fe] (only one metal center). [FeFe]-hydrogenases are extremely sensitive to oxygen, while [NiFe]-hydrogenases are more tolerant to oxygen. Both [NiFe]-hydrogenases and [FeFe]-hydrogenases have cofactors containing inorganic CO and CN ligands (**Figure 2a-b**). These are large, multisubunit enzymes which require complex multiprotein systems to prepare mature cofactors for catalysis (Blokesch et al. 2001, Hube et al. 2002, Posewitz et al. 2004, Butland et al. 2006, McGlynn et al. 2007, Mulder et al. 2009, Lubitz et al. 2014). Inspiration from the general design scaffold of [NiFe] and [FeFe]-hydrogenase active sites has been used to develop several biomimetic or bioinspired catalysts for H_2 production (**Figure 2c-d**) (Lyon et al. 1999, Helm, Stewart et al. 2011). These bioinspired catalysts have been combined with other natural or semi-synthetic proteins in order to create "artificial hydrogenases" that perform similar chemistry under photocatalytic conditions or other conditions that are easier to work with in the laboratory such as an oxygenic atmosphere.

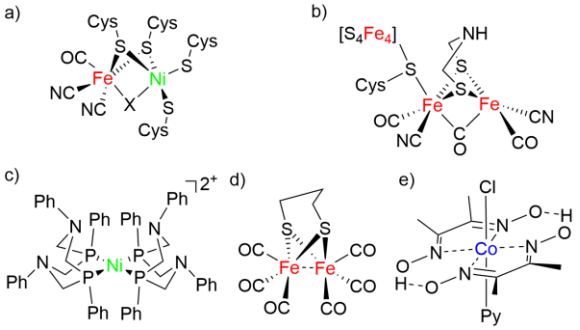


Figure 2. Structure of Active Sites of [NiFe] and [FeFe]-Hydrogenases and HER Catalysts Inspired by the Enzymes. (a) [NiFe]-hydrogenase, (b) [FeFe]-hydrogenase, (c) DuBois' Ni[bis(diphosphine)] catalyst, (d) Darensbourg's [(μ-SCH_2-CH_2-CH_2-S)$Fe_2(CO)_6$] catalyst, referred to as a [FeFe] catalyst, (e) Cobaloxime [Co(dmgH)$_2$pyCl] catalyst used in many artificial hydrogenase systems.

Photosystem I-Biohybrids for H_2 Production

One approach to use sunlight to drive hydrogenase-based activity takes advantage of the optimized photochemical reactions that occur in RC proteins. In photosynthesis, RC proteins capture and convert sunlight with near unity quantum efficiency. Photosystem I (PSI) catalyzes the reductive side of photosynthesis, where the electrons generated from splitting water by Photosystem II (PSII) are ultimately directed to the reduction of $NADP^+$ to NADPH (a biological reductant) for use in the Calvin cycle's CO_2 fixation process (Kuhlbrandt and Wang 1991, Fork and Herbert 1993, Brettel and Leibl

2001, Vassiliev et al. 2001). PSI contains many chlorophyll and carotenoid molecules for built-in light harvesting. Oxidation of the primary electron donor of PSI, P700, is concomitant with rapid, sequential electron transfers that terminate in an electron transfer relay through three terminal Fe-S clusters, and result in formation of a stabilized charge-separated state $P700^+[Fe\text{-}S]^-$ (lifetime ~60 ms between P700 and F_B) (Jordan et al. 2001, Vassiliev, Antonkine et al. 2001, Blankenship 2002). This environment is optimal for creating a direct electron transfer pathway for H_2 production by redirecting PSI's photogenerated electrons from use in $NADP^+$ reduction to an abiotic HER catalyst thereby using PSI's optimal light capture/conversion capabilities in lieu of a synthetic PS. These systems still require the addition of sacrificial electron donor to continue catalysis, and frequently need a redox mediator protein to bring electrons to the acceptor side of PSI.

Several systems were developed that link PSI light-driven chemistry to the H_2 production capabilities of hydrogenases (**Figure 3**). A [NiFe]-hydrogenase genetically fused to PSI (Ihara et al. 2006) and a multi-protein complex containing an [FeFe]-hydrogenase, PSI, and ferredoxin (Fd) (Yacoby et al. 2011) both produced H_2 at modest TOFs (>1,200 h^{-1}). A PSI-molecular wire-[FeFe]-hydrogenase was exceptionally effective with continuous H_2 production for four hours until the sacrificial electron donor was exhausted. This system has the highest reported TOF for any photocatalytic system (190,000 h^{-1}) (Lubner et al. 2011). While combination of the native hydrogenase and native photosystem works well in the laboratory, there are practical limitations to building H_2 production devices with such massive proteins that have limited oxygen tolerance and require multiple protein complexes for catalytic activity. Due to these concerns, recent endeavors have used PSI with smaller synthetic catalysts, such as those studied for molecular systems.

Incorporation of synthetic catalysts with PSI for solar H_2 production initially required Pt as a highly effective and rapid HER catalyst. Photo-platinized PSI was able to produce H_2 for >85 days, although at a low TOF (7.2 h^{-1}) (Iwuchukwau et al. 2010). PSI directly linked to a Pt-nanoparticle through a molecular wire (Grimme et al. 2008) and noncovalent electrostatic attachment of Pt-nanoparticles to the stromal side of PSI (Utschig et al. 2011a) both demonstrated much higher TOFs (4,200 h^{-1} and 21,000 h^{-1} respectively). In addition, EPR studies of the PSI-Pt-nanoparticle complex demonstrated blocking of electron transfer to the native electron transfer protein, flavodoxin (Fld), in the presence of Pt (Utschig, Dimitrijevic et al. 2011a). These studies provide insights into the capability of RC proteins to produce H_2; however, implementation of these systems on a large scale is limited by the scarcity and high expense of Pt.

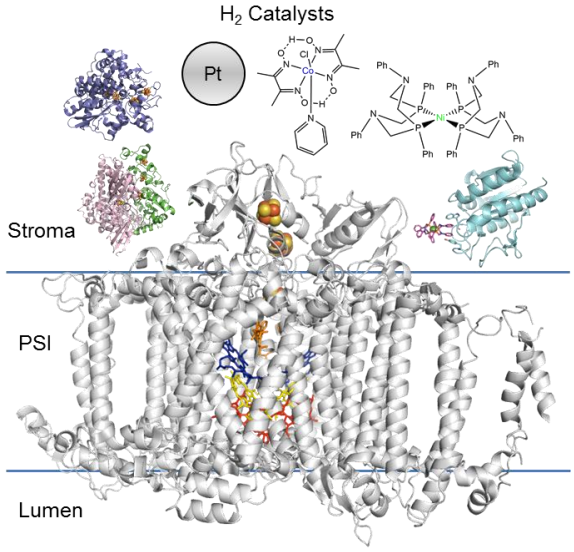


Figure 3. PSI-Biohybrid Catalysts. Chlorophyll molecules surrounding PSI (gray, 1JB0) capture photon energy and transfer energy to the primary electron donor P700. Electrons donated by electron transfer proteins plastocyanin or cytochrome c or artificial electron donors on the luminal side of PSI initiate electron transfer from P700 (red) to three terminal Fe-S clusters on the stromal side of the lipid membrane. Various protein, nanoparticle and molecular H_2 production catalysts have been incorporated with PSI including (from left) [NiFe]-

hydrogenases (1H2A), [FeFe]-hydrogenases (1HFE), Pt nanoparticles, cobaloxime catalysts, nickel bis(diphosphine) catalysts, and protein delivered catalysts (1CZL).

Further efforts to use PSI for solar H_2 production integrate inexpensive molecular catalysts used in synthetic H_2 production catalysis systems discussed above. Cobaloxime catalysts are one of the best studied HER catalysts with detailed mechanistic and spectroscopic characterization of catalysis (Eckenhoff et al. 2013). Cobaloxime catalysts have been incorporated with PSI via self-assembly and these hybrids rapidly produce H_2 at rates similar to the PSI-Pt-nanoparticle systems (10,200 h^{-1}) (Utschig et al. 2011b). The cobaloxime catalyst is able to collect electrons from the Fe-S clusters on the stromal side of PSI in a similar manner to the Pt-nanoparticles or native electron transfer proteins. This system has great capacity to be useful in industrial systems, although it is hampered by instability of the cobaloxime catalyst on PSI, and catalysis stops after about 1.5 h (Utschig, Silver et al. 2011b). Increasing the stability of the catalyst or PSI/catalyst interactions could provide a very useful catalytic system.

A further extension of this work includes a PSI-Ni-bis(diphosphine) catalyst biohybrid. This system also self-assembles with PSI to photocatalytically produce H. In addition, the system can also initiate self-repair through delivery of the Ni catalyst by the protein Fld, which has a docking site on the stromal side of PSI. Protein directed delivery of the nickel catalyst extends the time of catalysis by 30% and significantly increases TOF (2630 h^{-1} and 4500 h^{-1} respectively). This study provides the first spectroscopic evidence of molecular catalysts in a protein environment, using EPR to demonstrate that the Ni catalyst binds to both PSI and to the FMN binding pocket of Fld (Silver et al. 2013). Additional characterization of the electron transfer processes in the PSI-biohybrids continue to inhibit further implementation of these systems due to their large size and many cofactors with overlapping spectroscopic features. Using a small electron transfer protein like Fld to deliver a non-native catalyst to PSI provides insight in the placement of inorganic catalysts inside protein environments as these smaller proteins are easier to observe spectroscopically. Using insights from spectroscopy, it is possible to design better binding sites for catalysts where they can directly bring catalysts to the appropriate locations on PSI to enable faster catalysis or self-repair. This insight may allow creation of artificial hydrogenases which can been combined with light harvesting modules to bypass the spectroscopic challenges of the large PSI biohybrids.

Photocatalytic Artificial Hydrogenases

In protein systems, just as with synthetic molecular systems, some research effort has focused on developing electrocatalytic protein systems for H_2 production. These systems span from peptides as short as three amino acids to longer helical peptides and small proteins. The Bren group has published several of these systems. A Gly-Gly-His tri-peptide which has a Co nitrogen coordinated to the center of the peptide produced 275-475 TON H_2 depending on reaction conditions, with TOF <200 h^{-1} (**Table 1**) (Kandemir et al. 2016b). Peptide fragments of larger proteins which protect the catalyst from degradation have much faster rates of electrocatalysis. Microperoxidase-11, an 11-amino acid fragment of cytochrome c containing a bound Co-porphyrin catalyst, produces 25,000 turnovers (TON) of H_2 with a TOF of >24,000 h^{-1} for the first 10 minutes of catalysis. Catalysis appears to stop with porphyrin degradation at extended time (Kleingardner et al. 2014). The same structural motif has also been extended into a full protein environment through engineering of the heme protein, cytochrome c_{552} (Ht-cyt c_{552}) from *Hydrogenobacter thermophillus*. This work mutated the native Met ligand to an Ala residue, enabling a Co-porphyrin to bind in the native heme binding site. The Co-porphyrin is ligated by the proximal His residue and contains an open coordination site for substrate access

(Kandemir et al. 2016a). Electrochemical assays demonstrate TON for H_2 evolution of >270,000 for the protein based catalyst where the protein protects the porphyrin from degradation enabling catalysis for longer than 6 h (Kandemir, Chakraborty et al. 2016a). While these systems provide a good framework for understanding integration of molecular catalysts in protein environments, they do not extend our understanding of how to take photons to fuels.

Table 1. Relative Activity of Artificial Hydrogenase Systems.

System	TON	TOF (h^{-1})	Duration of Catalysis	Type of Catalysis	Reference
Co-GlyGlyHis	475	200	2.5 h	Electrocatalysis	(Kandemir, Kubie et al. 2016b)
Co-Microperoxidase-11	25,000	24,000	10 m	Electrocatalysis	(Kleingardner, Kandemir et al. 2014)
Co-porphyrin-Hy-cyt c_{552}	>270,000	NR[a]	6-24 h	Electrocatalysis	(Kandemir, Chakraborty et al. 2016a)
[FeFe]-Peptide	84	37	2.3 h	Photocatalysis, Free $[Ru(bpy)_3]^{2+}$ PS	(Roy et al. 2012)
[FeFe]-apocyt c	80	126	2 h	Photocatalysis, Free $[Ru(bpy)_3]^{2+}$ PS	(Sano et al. 2011)
[FeFe]-Nitrobindin	130	138	6 h	Photocatalysis, Free $[Ru(bpy)_3]^{2+}$ PS	(Onoda et al. 2014)
Ni-Rd	>100	30	8 h	Photocatalysis, Free $[Ru(bpy)_3]^{2+}$ PS	(Slater and Shafaat 2015)
Co-porphryin-Mb	520	88	12 h	Photocatalysis, Free $[Ru(bpy)_3]^{2+}$ PS	(Sommer et al. 2014)
Co-porphryin-cyt b_{562}	305	80	8 h	Photocatalysis, Free $[Ru(bpy)_3]^{2+}$ PS	(Sommer et al. 2015)
Cobaloxime-Mb	5	NR[a]	5 m	Photocatalysis, Free DAF[b] PS	(Bacchi et al. 2014)
Cobaloxime-Heme oxygenase	15	NR[a]	15 m	Photocatalysis, Free DAF[b] PS	(Bacchi et al. 2016)
[FeFe][Ru]-Pep18	9	11.4	2 h	Photocatalysis, Bound Ru PS	(Sano et al. 2012)
Ru-Fd-$CoBF_2$ (cobaloxime)	320	60	6 h	Photocataylsis, Bound Ru PS	(Soltau et al. 2015)
Ru-Fd-CoPy (cobaloxime)	650	170	6 h	Photocataylsis, Bound Ru PS	(Soltau et al. 2016)
Ru-ApoFld-$CoBF_2$ (cobaloxime)	85	30	6 h	Photocataylsis, Bound Ru PS	(Soltau, Dahlberg et al. 2016)

[a]NR = Not Reported, [b]DAF = Deazaflavin

Most efforts to build a photocatalytic artificial hydrogenase, have focused on developing protein – catalyst architectures that are effective HER catalysts, without adding a light absorbing molecule to the protein – catalyst scaffold. These systems use a free PS in solution and perform catalysis similar to the multimolecular catalytic scheme used for evaluating synthetic catalysts (**Figure 1a**). Fe, Ni, and Co catalysts have all been incorporated into protein systems using protein ligation strategies or metal substitution reactions. Mimics of the [FeFe]-hydrogenase, evolving from the Darensbourg catalyst described above (**Figure**

2d, referred to as a [Fe-Fe] catalyst) (Lyon, Georgakaki et al. 1999) were the first to be inserted into helical peptides (Jones et al. 2007). Addition of a $Ru(bpy)_3^{2+}$ PS to a helical [FeFe]-peptide with a nearby Lys residue to stabilize the catalyst yielded 84 TON (Roy, Madden et al. 2012), while incorporating the [FeFe] catalyst into the heme Fe pocket of apocyt c achieved 80 TON with $Ru(bpy)_3^{2+}$ as a PS (Sano, Onoda et al. 2011). Another system used a maleimide derivative of the synthetic [FeFe] catalyst and inserted the catalyst internally inside the cavity of the ß-barrel protein, nitrobindin. Using free $Ru(bpy)_3^{2+}$ as a PS, the system achieved 130 TON with an initial TOF of 138 h^{-1} (Onoda, Kihara et al. 2014).

Ni has also been used in artificial hydrogenases. The metal center of a rubredoxin (Rd) protein, which natively contains an active site with an Fe center ligated by four Cys residues, was replaced with Ni. This Ni-Rd was used a minimal model of a hydrogenase active site and photochemical H_2 production using $Ru(bpy)_3^{2+}$ as a free PS produced >100 TON of H_2 with a TOF of 30 h^{-1}. (Slater and Shafaat 2015).

Cobalt porphyrins have been inserted in myoglobin (Mb) and cytochrome b_{562} (cyt b_{562}) in a similar manner to the electrocatalytic work with Ht-cyt c_{552}. Using excess $Ru(bpy)_3^{2+}$ as a PS, Co-porphyrin-Mb produces up to 520 TON of H_2, with a TOF of 88 h^{-1} and mutations to the amino acids of the porphyrin binding site modulate TON (Sommer, Vaughn et al. 2014). Similar photocatalytic studies using Co-porphyrin-cyt b_{562} demonstrated lower TON with the native active site (125 TON); however, site-directed mutagenesis revealed that the active site could be tuned to improve catalysis. Replacement of the native Met residue with either Ala or Asp more than doubled the TON for the system (Sommer, Vaughn et al. 2015).

Cobaloximes have been used extensively as H_2 catalysts in multimolecular, supramolecular, and nanoparticle catalysis, and have also been extended into protein environments. Recent work extensively characterized the binding of a cobaloxime axially to a His residue in the heme pocket of Mb by UV-vis, EPR, and XAFS measurements. Photochemical H_2 production with the cobaloxime-Mb hybrid using multiple PS and sacrificial electron donor combinations led to a maximum of 5 TON. Structural studies predicted that H_2 production was limited by the rigidity of Mb binding pocket, which prevented catalyst reorganization needed for catalysis (Bacchi, Berggren et al. 2014). A very recent follow up inserts the cobaloxime catalyst into the substrate (hemin) binding pocket of heme oxygenase reports up to 15 TON of H_2 with free photosensitizer in solution. UV-vis and EPR studies indicate the presence of multiple binding modes for the cobaloxime in the protein suggesting that the catalyst environment changes during catalysis (Bacchi, Veinberg et al. 2016).

All of these efforts demonstrate divergent methods to engineer non-native cofactors into protein environments, and develop catalytic active sites at novel locations. These results significantly advance the field of protein design and modification, they have not succeeded at coupling photons to fuels. Successful photon-to-fuel systems must directly link a light absorbing molecule (added PS or native protein chromophore) to a catalyst in protein environment. This type of environment more closely resembles the supramolecular and nanoparticle based synthetic systems that are able to perform direct charge transfer of photons to catalysts. Currently few examples of these integrated systems exist. One example uses an 18-amino acid peptide fragment of apocyt c and binds both an [FeFe] catalyst and a Ru PS to Cys and His residues respectively within the peptide (**Figure 4a**). This system directly transfers electrons from PS to catalyst via the peptide in a supramolecular-like motif and is proposed to follow a reductive quenching mechanism for the Ru PS. While this system developed a novel PS attachment mechanism, the peptide structure did not stabilize the [FeFe] catalyst, which only achieved 9 TON before decomposition of the catalyst (Sano,

Onoda et al. 2012). This rate of degradation is more rapid than for the protein with a free PS in solution which achieved 80 TON (Sano, Onoda et al. 2011).

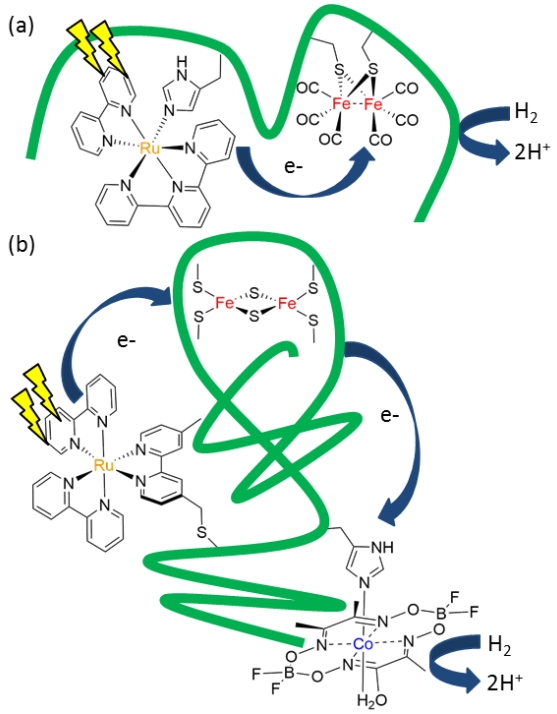


Figure 4. Schemes for Direct Photocatalytic H_2 Production in Peptides and Proteins. Photocatalytic H_2 production in an (a) [FeFe][Ru]Pep-18 peptide (Sano, Onoda et al. 2012) and in a (b) Ru-Fd-$CoBF_2$ biohybrid (Soltau, Niklas et al. 2015).

Another catalyst-PS integrated protein system uses a protein, Fd, which has a Ru PS covalently attached to a Cys residue in the protein. It also covalently binds a cobaloxime catalyst to a His residue on the opposite side of the protein. The Ru PS and and Co catalyst are separated by a native [2Fe-2S] cluster in the protein matrix (**Figure 4b**). The Ru-Fd-$CoBF_2$ biohybrid system performs direct photocatalytic H_2 production, producing 320 TON with a TOF of 60 h^{-1}. Light driven electron transfer within the Ru-Fd-$CoBF_2$ biohybrid was characterized by EPR and transient optical spectroscopy. With light and ascorbate present, an oxidatively quenched Ru^{3+} species was observed by EPR. Additional EPR experiments demonstrated light-driven reduction of the [2Fe-2S] cluster in the absence of the Co catalyst. Transient optical spectroscopy experiments indicate the presence of a long-lived (> 1.5 ms) Ru(III)-Fd-Co(I) charge separated state that enables photocatalysis to occur, and is not observed in the absence of the [2Fe-2S] cluster. In the Ru-Fd-$CoBF_2$ system, the Ru PS and Co catalyst are spatially separated by protein and electron transfer occurs via a relay through the [2Fe-2S] cluster, both attributes which mimic the environment in native RC proteins and facilitate charge separation and photocatalysis (Soltau, Niklas et al. 2015).

Very recently, two follow up systems have been reported (Soltau, Dahlberg et al. 2016). One of these systems, Ru-Fd-CoPy, replaced the cobaloxime catalyst, with a different cobaloxime (**Figure 2e**) reported to produce faster catalysis (McCormick et al. 2010). The Ru-Fd-CoPy system produces 650 ± 150 TON of H_2 with a TOF of 170 ± 10 h^{-1}. This hybrid is the best reported system to date of a small protein architecture that incorporates both synthetic PS and catalyst moieties for photocatalytic H_2 production (Soltau, Dahlberg et al. 2016). The other follow up system uses the protein ApoFld, which does not contain an internal electron transfer moiety, and must perform direct electron transfer from PS to catalyst. This system, Ru-ApoFld-$CoBF_2$, produces less H_2 (TON 85 ± 35, TOF 30 ± 10 h^{-1}) and utilizes a reductive quenching pathway for catalysis as observed by EPR and transient optical spectroscopies (Soltau, Dahlberg et al. 2016). This work demonstrates the possibilities for using protein architectures to spectroscopically delineate important mechanistic features involved in coupling of photons to fuels in regards to understanding how to make and maintain charge separated states and redirect electrons to desired catalytic functions.

Future Outlook for Protein Based H_2 Production

Future efforts in photocatalytic artificial hydrogenases rely on semi-synthetic protein designs that are inspired by native RC

chemistry. Nature has optimized the process of photon capture and charge-separation over billions of years. In order to develop technologies that will efficiently use sunlight, we need to take advantage of the natural systems that are already available. Using inspiration from nature, in the form of RC proteins, such as PSI, or by re-creating those architectures in smaller semi-synthetic proteins, will provide many opportunities to refine and develop the field of photon capture and conversion and facilitate the use of solar fuels in modern technologies. Protein based H_2 production systems are subject to the same problems that effect natural proteins, such as protein stability and denaturation, as well as the stability of the catalyst both during catalysis and in the protein environment. Synthetic efforts to design better catalysts have greatly increased the lifetime of catalysts in the last few years, so now biochemists much develop better technologies to integrate catalysts into proteins. In addition, these architectures may provide opportunities for the development of new Z-scheme systems to perform water electrolysis (Scheme 1) while providing a renewable fuel source.

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