# II.K.19 A Hybrid Biological/Organic Photochemical Half-Cell for Generating Dihydrogen

John H. Golbeck[1,2,*] (Principal Investigator),
Donald A. Bryant[1] (Co-Principal Investigator)
[1]Department of Biochemistry and Molecular Biology;
[2]Department of Chemistry
The Pennsylvania State University
University Park, PA 16802
Phone: (814) 865-1163; Fax: (814) 863-7024
E-mail: jhg5@psu.edu; dab14@psu.edu

DOE Program Manager: Dr. Michael Markowitz
Phone: (301) 903-6779
E-mail: Mike.Markowitz@science.doe.gov

## Objectives

The objective of this work is to engineer a hybrid biological/organic half-cell that couples Photosystem I to [FeFe]-hydrogenase using molecular wire technology. The construct will carry out the light-driven half-cell reaction $2H^+ + 2e^- + 2\ h\nu \rightarrow H_2$. The methodology involves constructing a Photosystem I variant and a [FeFe]-hydrogenase variant so that a molecular wire can be attached directly to their surface-located iron-sulfur clusters. The objectives include optimizing the coupling chemistry between the two proteins; measuring the quantum yield and rate of dihydrogen production; and engineering long-term stability of the Photosystem I–molecular wire–[FeFe]-hydrogenase half-cell.

## Technical Barriers

Three preconditions must be met for the proposed hybrid photochemical half-cell to function: (i) Photosystem I must be engineered so that a cysteine ligand to the terminal [4Fe-4S] cluster on the PsaC subunit is replaced with a glycine residue. (ii) An [FeFe]-hydrogenase variant must be engineered so that a cysteine ligand to one of the [4Fe-4S] clusters is similarly replaced with a glycine residue. These changes will expose iron atoms in Photosystem I and in [FeFe]-hydrogenase to the medium, allowing the -SH 'rescue ligand' from the molecular wire to form a strong coordination bond. (iii) A 'molecular wire' must be synthesized, which contains an electron transfer cofactor and a –SH group at each end so that the latter can serve as 'rescue' ligands to the modified [4Fe-4S] clusters on Photosystem I and on the hydrogenase. When all of these conditions are met, and in the presence of a sacrificial donor, Photosystem I will convert two photons into energetic electrons; these electrons will be transferred through the molecular wire to the tethered hydrogenase, which will reduce two protons to hydrogen.

## Abstract

A molecular wire is used to connect two proteins through their physiologically relevant redox cofactors to facilitate direct electron transfer. Photosystem I (PS I) and an [FeFe]-hydrogenase serve as the test bed for this new technology. By tethering a photosensitizer module with a hydrogen-evolving catalyst, attached by Fe/S coordination bonds to the $F_B$ iron-sulfur cluster of PS I and the distal iron-sulfur cluster of $H_2$ase, efficient electron transfer between the two components can be assayed via light-induced hydrogen evolution. These hydrogen producing nanoconstructs additionally self-assemble when the PS I variant, the hydrogenase variant and the molecular wire are combined.

## Progress Report

We have recently reported the assembly of biological/organic hybrid nanoconstructs that generate hydrogen in the light. In these nanoconstructs, electrons are transferred directly from a photochemical module, Photosystem I (PS I), to a catalytic module, either a Pt nanoparticle (NP) or an [FeFe]-hydrogenase via a covalently attached molecular wire. In neither case are any spectroscopic changes visible that would allow electron transfer to be monitored between the photochemical and catalytic modules. In this

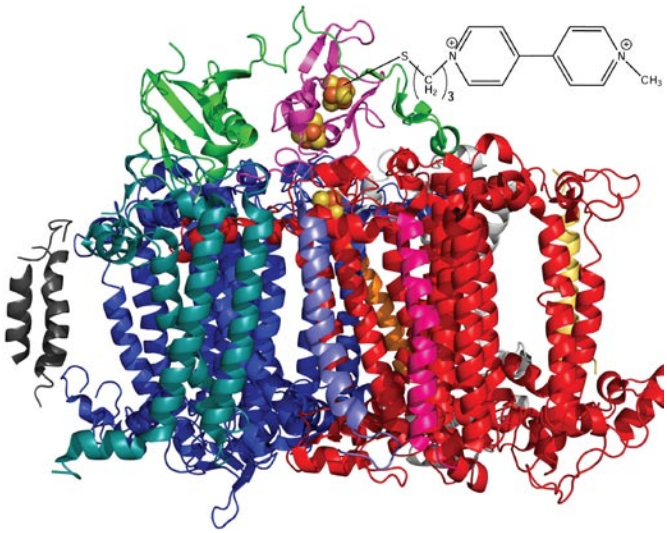

**FIGURE 1.** Depiction of the PS I—1-(3-thiopropyl)-1′-(methyl)-4,4′-bipyridinium construct. The electron transfer cofactors $F_X$, $F_A$, and $F_B$ (from bottom to top) are shown as spheres within the protein scaffold. The molecular wire is attached at the $F_B$ cluster, located inside the PsaC subunit (pink). The PsaD subunit (green) resides on top of the membrane spanning portion and adjacent PsaC. The TPMBP moiety is not drawn to scale.

study, the catalytic module was replaced with an organic cofactor consisting of 1-(3-thiopropyl)-1′-(methyl)-4,4′-bipyridinium chloride that allowed electron transfer to be measured to a spectroscopically observable marker. EPR and optical spectroscopy showed that the tethered redox cofactor was attached to PS I through the $F_B$ cluster of PsaC. Under steady-state illumination, the rate of reduction of the 4,4′-bipyridinium cofactor was comparable to the rate of $H_2$ evolution observed for the PS I–molecular wire–Pt-NP and PS I–molecular wire–[FeFe]-hydrogenase nanoconstructs. These observations provide proof-of-concept for incorporating a redox cofactor in the molecular wire, thereby setting the stage for monitoring the rate and yield of electron transfer between PS I and the tethered [FeFe]-hydrogenase.

We have recently optimized our biohybrid biological/organic nanoconstruct that uses a covalently bonded molecular wire to connect the $F_B$ cluster of the Photosystem I (PS I) from *Synechococcus* sp. PCC 7002 directly with the distal [4Fe-4S] cluster of the [FeFe]-hydrogenase I from *Clostridium acetobutylicum*. These studies show that in the presence of cross-linked Cyt $c_6$, with 1,8-octanedithiol as the molecular wire, in potassium phosphate buffer at pH 6.5, the PS I–molecular wire–[FeFe]- hydrogenase nanoconstruct evolves $H_2$ at a rate of 2850 µmoles mg $Chl^{-1}$ $h^{-1}$, which is equivalent to an electron transfer throughput of 5700 µmoles mg $Chl^{-1}$ $h^{-1}$, or 142 µmoles $e^-$ PS $I^{-1}$ $s^{-1}$.

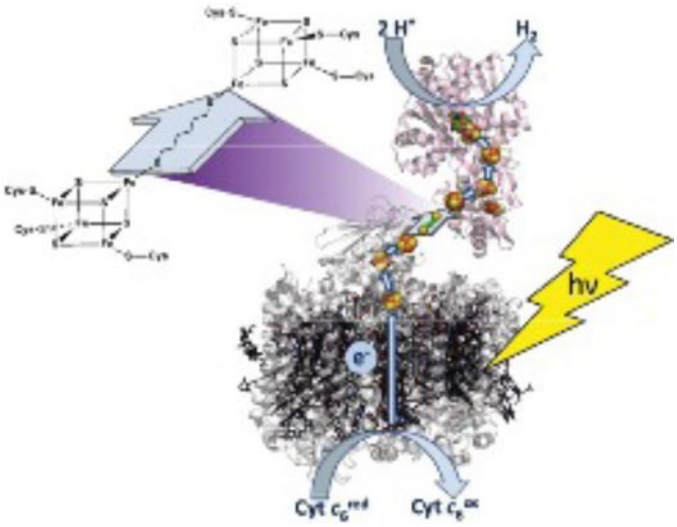


**FIGURE 2.** Schematic of the PS I-molecular wire-[FeFe]-hydrogenase nanoconstruct. Assembled photosynthetic nanoconstructs where the connecting wire is 1,6-hexanedithiol. Soluble electron donors include cytochrome $c_6$, ascorbate and PMS. Arrows indicate electron transfer through the system, including reduction of protons to $H_2$.

Putting this into perspective, cyanobacteria evolve $O_2$ at a rate of ~400 µmoles mg $Chl^{-1}$ $h^{-1}$, which is equivalent to an electron transfer throughput of 1600 µmoles mg $Chl^{-1}$ $h^{-1}$, or 46 $e^-$ PS $I^{-1}$ $s^{-1}$, assuming a PS I to PS II ratio of 1.8 as occurs in the cyanobacterium *Synechococcus* sp. PCC 7002. The significantly greater electron throughput by our hybrid biological/organic nanoconstruct over *in vivo* oxygenic photosynthesis validates our concept of tethering proteins through their physiologically relevant redox cofactors to overcome diffusion-based rate limitations on electron transfer.

## Future Directions

Our plans for the future include (i) measuring the quantum yield of hydrogen evolution in the PS I–molecular wire–[FeFe]-hydrogenase nanoconstruct; (ii) docking the PS I–molecular wire–[FeFe]-hydrogenase nanoconstruct on either a graphene or gold electrode so that it functions as a cathode of an electrochemical cell; (iii) tethering a variant of an oxygen-tolerant [NiFe]-hydrogenase on the PS I–molecular wire module; (iv) investigating other entry portals for the molecular wire, including [2Fe-2S] clusters and cysteine residues placed near organic or inorganic cofactors of redox enzymes; (v) developing methods to produce these nanoconstructs biologically.

## Publication list (including patents) acknowledging the DOE grant or contract

**1.** Grimme, R.A., Lubner, C.E., and Golbeck, J. (2009) Maximizing $H_2$ Production in Photosystem I/Dithiol Molecular Wire/Platinum Nanoparticle Bioconjugates, *Dalton Trans.*, 10106-10113.

**2.** Lubner, C.E., Grimme, R., Bryant, D.A., and Golbeck, J.H. (2010) Wiring photosystem I for direct solar hydrogen production, *Biochemistry 49*, 404-414.

**3.** Lubner, C.E., Knorzer, P., Silva, P.J.N., Vincent, K.A., Happe, T., Bryant, D.A., and Golbeck, J.H. (2010) Wiring an [FeFe]-Hydrogenase with Photosystem I for Light-Induced Hydrogen Production, *Biochemistry 49*, 10264-10266.

**4.** Lubner, C., Heinnickel, M., Bryant, D.A., and Golbeck, J.H. (2011) Wiring Photosystem I for Electron Transfer to a Tethered Redox Dye, *Energy Environ. Sci. (in press)*.

**5.** Lubner, C., Bryant, D.A., and Golbeck, J.H. (2011) Wired Reaction Centers, In *Molecular Solar Fuels* (Hillier, W., and Wydrzynski, T., Eds.), Royal Society of Chemistry, London.

**6.** Lubner, C., Applegate, A., Knörzer, P., Happe, T., Bryant, D.A., and Golbeck, J.H. (2011) Optimizing Light-Induced $H_2$ Evolution in Photosystem I-Molecular Wire-[FeFe]-$H_2$ase Constructs, *(in preparation)*.