Supporting Information

Nature-Driven Photochemistry for Catalytic Solar Hydrogen Production: A Photosystem I – Transition Metal Catalyst Hybrid

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Contents:

Reference 14: Blankenship, R. E.; Tiede, D. M.; Barber, J.; Brudvig, G. W.; Fleming, G.; Ghirardi, M.; Gunner, M. R.; Junge, W.; Kramer, D. M.; Melis, A.; Moore, T. A.; Moser, C. C.; Nocera, D. G.; Nozik, A. J.; Ort, D. R.; Parson, W. W.; Prince, R. C.; Sayre, R. T. *Science* **2011**, *332*, 805-809.

Experimental

General. Reagent grade $CoCl_2 \cdot 6H_2O$ was obtained from JT Baker Chemical Company. All other ACS reagent grade chemicals were obtained from Aldrich or Sigma and used as received. $Co(dmgH)_2pyCl$ was prepared as previously described.[1] The compound was identified by 500 MHz ^1H NMR, ESI-MS, UV-Vis absorbance spectroscopy, and cyclic voltammetry and matched all previously reported characteristics.[2] PSI was isolated from cyanobacterial membranes of protonated *Synechococcus lividus* or *Synechococcus leopoliensis*.[3] Cytochrome c_6 was isolated from *Synechococcus lividus*.[4]

Preparation of PSI-cobaloxime hybrid. Dark-adapted 5 μM PSI monomer was incubated with 10 - 60 μM $Co(dmgH)_2pyCl$ (from a 5 mM stock solution of $Co(dmgH)_2pyCl$ in DMSO) in a buffer containing 20 mM Hepes pH 8.1, 10 mM $MgSO_4$, and 0.03% n-dodecyl β-D-maltopyranoside (*S. leopoliensis*) or 20 mM Hepes pH 6.9 and 0.03% n-dodecyl β-D-maltopyranoside (*S. lividus*). The PSI and $Co(dmgH)_2pyCl$ solution was tumbled (Labquake rotisserie) for 2 h at room temperature in the dark. The samples were concentrated with Amicon 50000 MWCO filtration devices and then repeatably diluted 8-fold with either 20 mM Hepes pH 8.1, 10 mM $MgSO_4$, and 0.03% n-dodecyl β-D-maltopyranoside (*S. leopoliensis*) or 20 mM Hepes pH 6.9 and 0.3 % n-dodecyl β-D-maltopyranoside buffer (*S. lividus*) and concentrated to wash away unbound cobaloxime.

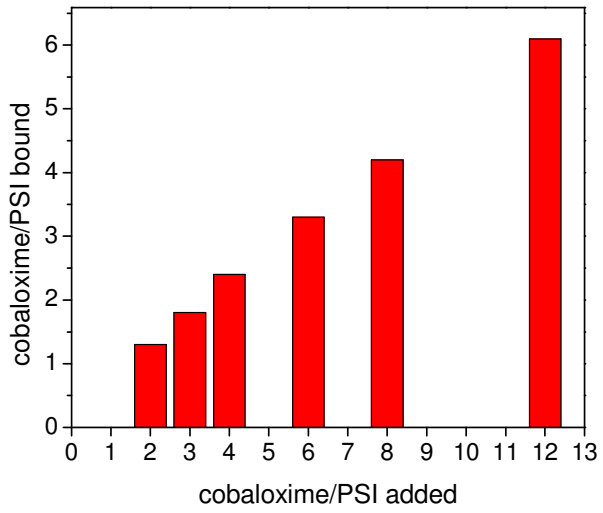

Figure S1. Metal binding studies of *S. leopoliensis* PSI.

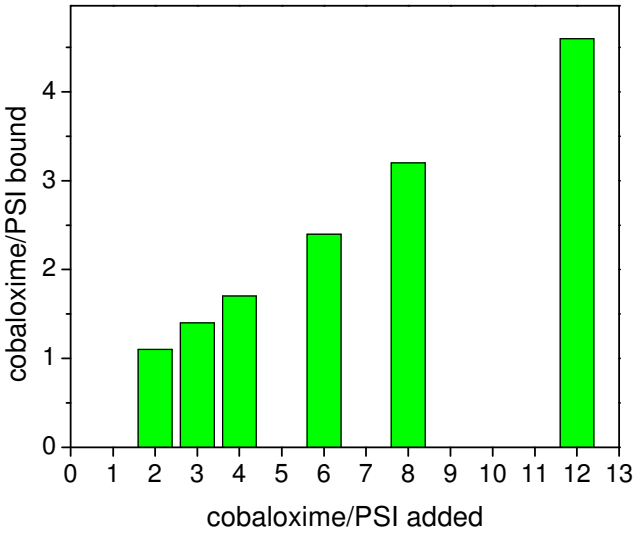

Figure S2. Metal binding studies of *S. lividus* PSI.

Metal analysis of the resultant complexes indicates that $Co(dmgH)_2pyCl$ binds readily to native PSI. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) on a Thermo Scientific iCAP 600 spectrometer was used to determine Co and Fe content for each sample. The PSI protein concentration was determined by Fe analysis assuming 12 Fe per PSI monomer. Figures S1 and S2 show metal binding results for *S. leopolinsis* and *S. lividus* protein samples that were incubated with 2 – 12 mol equivalents of $Co(dmgH)_2pyCl$ per PSI monomer in the buffer conditions detailed above. We found that samples containing 2-4 Co bound per PSI gave the highest H_2 production rates.

H_2 experimental sample preparation. The general procedure of sample preparation for a H_2 experiment is as follows. Each PSI-cobaloxime complex used for H_2 experiments was

prepared fresh, by the methods detailed above, the morning of the H_2 experiments. ~3.9 mL 20 mM MES buffer pH 6.3 and 100 mM sodium ascorbate (ascorbate stock solution ~ 1.5 M in MES buffer, prepared fresh the day of the experiment) is bubbled with N_2 in a N_2 box for at least 30 min. The detergent, necessary to keep PSI in solution, is added to a final concentration of 0.03 % n-dodecyl β-D-maltopyranoside from a 13% stock solution, followed by addition of cytochrome c_6 (from > 1 mM cytochrome c_6 solution) and the freshly prepared PSI-cobaloxime sample (typically 30 – 50 μM PSI) to final concentrations ~ 4-12 μM cytochrome c_6 and 60-100 nM PSI monomer. (Several specific conditions are detailed below.) Note, the sample can no longer be bubbled directly because of the detergent. Thus, N_2 is blown on top of the sample for at least 2 min before the sample is placed in the light.

H_2 measurements. H_2 photocatalysis experiments were performed in a N_2-purged sealed 5.3 mL spectrophotometer cell with a path length of 1.0 cm. The H_2 calibration curves were determined by GC analysis of aliquots from the 1.3 ml headspace following known addition of H_2 gas bubbled into 4.0 mL of buffer. The Varian CP-4900A GC is equipped with a 10m 5-angstrom molecular sieves column with a thermal conductivity detector and UHP N_2 carrier gas.

The samples were illuminated with a 300 W Xenon lamp (Perkin-Elmer). The light was extensively filtered using a 500 nm filter, a 29 cm water filter and a low-pass filter (KG-1, Schott). The intensity of light as measured behind the sample was 3000 μE m^{-2} s^{-1}.(MQ-100 Quantum meter, Apogee Instruments Inc.) Samples (50 μL – 100 μL) were taken from the headspace every 10 - 30 min and analyzed for H_2 by gas

chromatography (Varian CP-4900). Experimental controls for H_2 production can be found in Table S1.

Table S1. Hydrogen Production Control Experiments.[a]

Sample	Hydrogen NO[b]	Hydrogen YES
1 μM $Co(dmgH)_2pyCl$	X	
Cyt c_6, 1 μM $Co(dmgH)_2pyCl$	X	
PSI	X	
PSI-cobaloxime hybrid	X	
Cyt c_6, PSI	X	
Cyt c_6, PSI, 1 μM $Co(dmgH)_2pyCl$	X	
Cyt c_6, PSI-cobaloxime hybrid		X

[a] All samples contained 100 mM Na ascorbate. *S. lividus* PSI was used for the controls containing PSI.

[b]No measurable H_2 detected via GC analysis

General advice and optimized experimental conditions. The H_2 reactions were found to be sensitive to several parameters. To achieve the high rate of catalysis, we first optimized the experimental conditions using our PSI-Pt nanoparticle system.[5] The experimental conditions were then re-optimized for the PSI-cobaloxime system. Note,

we find the H_2 production to be sensitive to the donor conditions, as has been previously reported.[6,7,8] In particular, the ratio of cytochrome c_6 /PSI, salt and pH were found to influence the H_2 production, and we interpret this sensitivity to be due to the influence of these factors on the cytochrome – PSI interaction.[9-12] That said, we have repeatedly observed H_2 production from PSI-cobaloxime complexes prepared with PSI isolated from two species of cyanobacteria, *S. lividus* and *S. leopoliensis*, and multiple purifications of each. Also, we have observed H_2 production to last anytime from 5 min to 4 h and are currently investigating if this is the result of the catalyst dissociating from PSI. We have tested H_2 production at a variety of pH values ranging from pH 5.7 – 8.3. The best H_2 rates were observed for reactions run at pH 6.13 – 6.32.

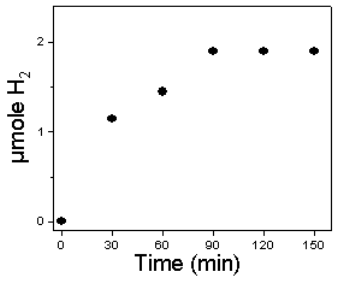

Figure S3. Time course for H_2 production from a 80 nM *S. leopliensis* PSI-cobaloxime solution.

The exact experimental conditions for Figure 2B and Figure S3 and the video are: 80 nM *S. leopoliensis* PSI-cobaloxime (3.6 $Co(dmgH)_2pyCl$/PSI monomer bound), 4 µM *S. lividus* cytochrome c_6, 100 mM ascorbate, 20 mM MES pH 6.3, 0.03 % n-dodecyl β-D-maltopyranoside at a final volume of 4.0 ml. The sample in 2C: 61 nM *S. lividus* PSI-cobaloxime (2.5 $Co(dmgH)_2pyCl$/PSI monomer bound), 8 µM *S. lividus* cytochrome c_6, 5 mM $MgSO_4$, 100 mM ascorbate, 20 mM MES pH 6.3, 0.03 % n-dodecyl β-D-maltopyranoside at a final volume of 4.0 ml.

PSI-Pt nanoparticle hybrid. Figure S4 shows data for the ~80000 mol H_2/mol PSI (TON) as mentioned in the text. 9.8 nM *S. leopoliensis* PSI-Pt nanoparticle (prepared as detailed in Ref. 5), 4 µM *S. lividus* cytochrome c_6, 100 mM ascorbate, 20 mM MES pH

6.2, 0.03 % n-dodecyl β-D-maltopyranoside at a final volume of 3.0 ml. The sample was illuminated as detailed above.

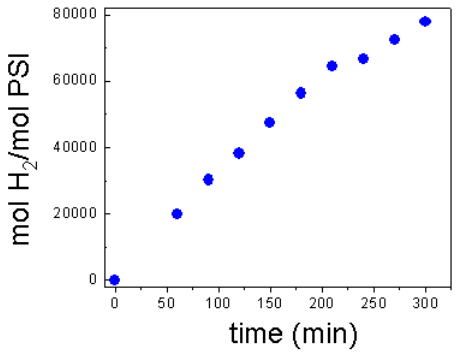

Figure S4. Time profile of H_2 production from PSI/Pt-nanoparticle biohybrid complex[5] showing sustained high rates of catalysis for 5 h.

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