Electronic Supplementary Information (ESI)

Z-Scheme Solar Water Splitting via Self-Assembly of Photosystem I-Catalyst Hybrids in Thylakoid Membranes

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Contents:

Chlorophyll Assays

The chlorophyll content of spinach thylakoid membranes was determined using the equation:[1]

[Chl] (mg/ml) = (0.0101 x A_{645}) + (0.0145 x A_{652}) + (0.00401 x A_{663}) x dilution factor

Samples for the H_2 measurements were measured prior to illumination; typically 50 µl sample was diluted in 3.0 ml cold 80% acetone and measured in a Beckman DU 640 spectrophotometer.

The chlorophyll content of cyanobacterial thylakoid membranes was determined using the equation:[1]

[Chl] (mg/ml) = (A_{665}/79.24) x dilution factor

Samples for the H_2 measurements were measured prior to illumination; typically 50 µl sample was diluted in 3.0 ml 100% methanol and measured in a Beckman DU 640 spectrophotometer.

O_2 Measurements in the presence of external electron acceptors to test viability of thylakoid preparations

PSII activity of the isolated thylakoid membranes was determined by the rate of O_2-evolution in the presence of artificial electron acceptor molecules. Measurements were performed in sealed 5.3 mL spectrophotometer cell with a path length of 1.0 cm. Thawed thylakoid membranes were added to 4.0 ml N_2-purged buffer in the dark. The solution contained the external electron acceptors ferricyanide (1 mM, from a 150 mM stock solution of potassium hexacyanoferrate (III) in Milli-Q water) and 2,5-dichloro-p-benzoquinone (DCBQ, 250 µM, from a 37 mM stock solution in 100% ethanol). 1 mM NH_4Cl (1.4 M stock in Milli-Q water) was added as an uncoupler. The sample was placed in front of the light-assembly, and a needle piercing Clark electrode (Unisense, OXY-NP sensor) was inserted into the septa cap of the cell. The sample was continuously stirred. The activity was measured in the dark for 30 -60 sec, followed by illumination with a 300 W xenon lamp using a 500 nm long-pass filter, a heat absorbing filter (KG-2, Schott) and a 29 cm water filter. The sample set-up is shown in Figure S7A. A typical O_2 plot is shown in Figure S7B. The inhibition of O_2 evolution by 1 mM DCMU is shown in Figure S7B. Note, the O_2 of thylakoids in the absence of added acceptor molecules, i.e. the O_2 evolution with only native PQ pool in the membrane, is well below the detection limit for our Clark electrode. For this reason, GC was used to determine the O_2 values in Fig. 3C, 3F and Fig. 6B.

Stock Solutions for H_2 measurements

Chemicals were purchased from Sigma-Aldrich. 1.2 M sodium ascorbate in 10 mM MES ((2-(N-morpholino)ethanesulfonic acid)) buffer, pH 6.2, was made fresh the day of the experiment. 78 mM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was prepared in DMSO. Stock solutions of purified *T. lividus* cyt c_6[2] were typically 0.6 – 2.0 mM in 10 mM MES, pH 6.2.

$P700^+$ Quantification

Four types of EPR samples were used to quantitate the PSI content in membranes: isolated cyanobacterial PSI, *S. leopoliensis*, spinach, and BBY membranes[3]. Each sample contained a final concentration of 1.6 mg/ml Chl, with 10 mM sodium ascorbate, 0.3 mM DCPIP, and 0.5 mM DCMU. These samples were dark-adapted for 30 min at room temperature prior to freezing in liquid nitrogen. The samples were transferred from liquid nitrogen to the pre-cooled resonator in a dark laboratory. cw X-band (9.5 GHz) EPR measurements were carried out with a Bruker ELEXSYS II E500 EPR spectrometer (Bruker Biospin Corp, Rheinstetten, Germany). For the quantification of PSI content using the $P700^+$ signal, the samples were illuminated *in situ* for 10 s at 40 K and measured immediately afterwards.

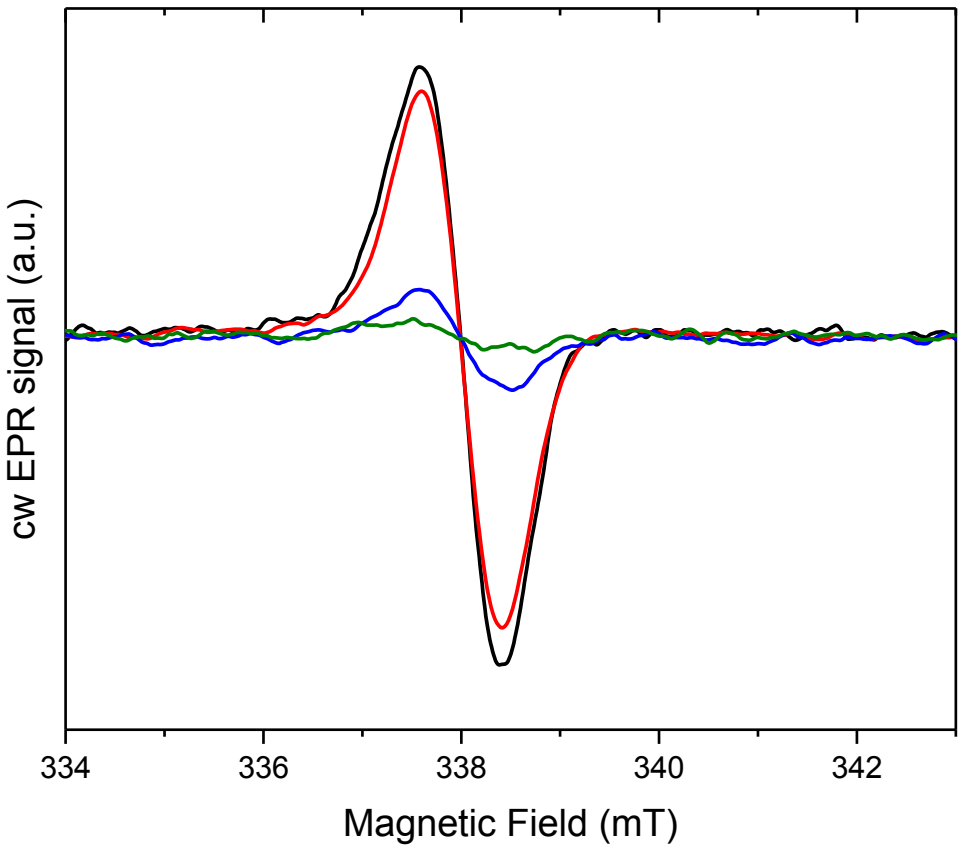

Figure S1. cw X-band EPR spectra $P700^+$ formation were obtained to estimate the number of PSI RC proteins in thylakoid membranes. The samples were dark-adapted at room temperature for 30 min prior to freezing in liquid N_2 and placement in the pre-cooled resonator at 40 K. For each sample, a dark spectrum was collected, followed by illumination of the sample in the resonator at 40 K. The spectra shown are light-minus-dark difference spectra which show the light-induced formation of $P700^+$ at low temperature. Each sample contained 1.6 mg/ml total chlorophyll concentration. Spectra are shown for isolated PSI (*S. leopoliensis*, black), *S. leopoliensis* thylakoids (red), spinach thylakoids (blue), and BBY membranes[3] (green). Comparison of the signals resulted in Chl/PSI ratios of 120 Chl/ PSI in *S. leopoliensis* thylakoid, >600 Chl/ PSI in spinach, and >2000 Chl/PSI in BBY. These ratios were used to calculate the TOF (mol H_2 (mol PSI)$^{-1}h^{-1}$) listed in Table 1.

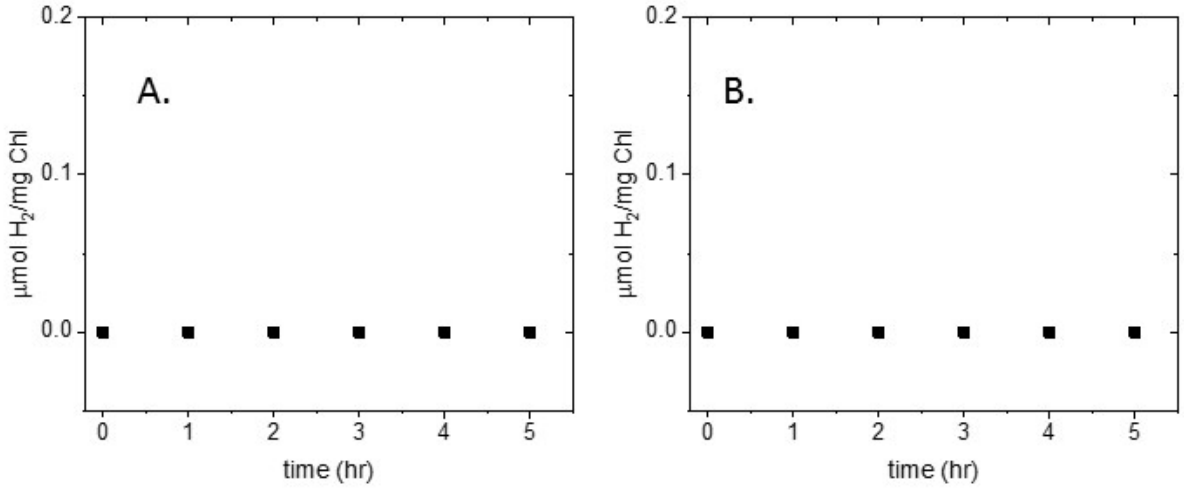

Figure S2. H_2 production from isolated thylakoid membranes with no catalysts attached. (A) 0.030 mg/ml [Chl] *S. leopoliensis* thylakoid membranes in 10 mM MES, pH 6.1, 100 mM sodium ascorbate, 4 µM cyt c_6, and 1 mM DCMU. (B) 0.022 mg/ml [Chl] spinach thylakoid membranes in 10 mM MES, pH 6.1, 100 mM sodium ascorbate, and 1 mM DCMU. Samples were illuminated and 100 µl aliquots were removed from the headspace every 60 min and measured for H_2 content with GC as detailed in the experimental procedures section.

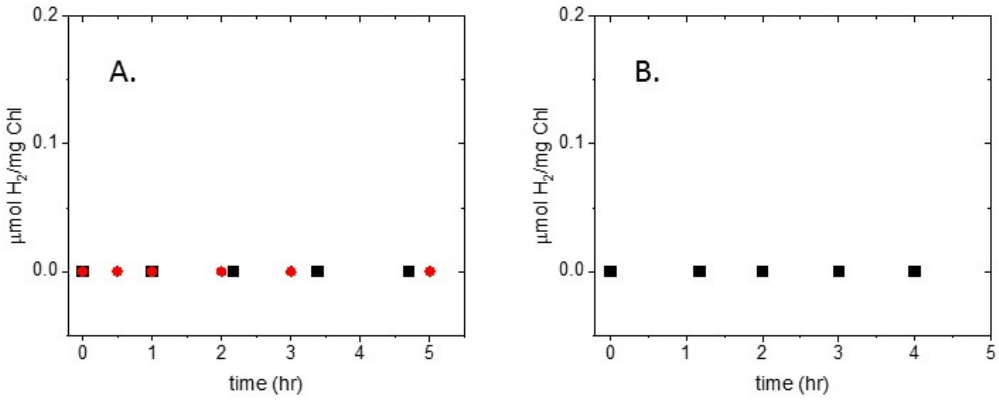

Figure S3. Control experiments showing that MES buffer (2-(N-Morpholino)ethanesulfonic acid) does not act as a SED in H_2 production in the thylakoid-catalyst experimental system. (A) 0.047 mg/ml [Chl] *S. leopoliensis* thylakoid/Pt nanoparticle complex (black squares) and 10 µM cyt c_6 or 0.066 mg/ml [Chl] spinach thylakoid/Pt nanoparticle complex (red circles) in 10 mM MES pH 6.1 and 1 mM DCMU. (B) 0.062 mg/ml [Chl] spinach thylakoid/Pt nanoparticle complex in 100 mM MES pH 6.1 and 1 mM DCMU.

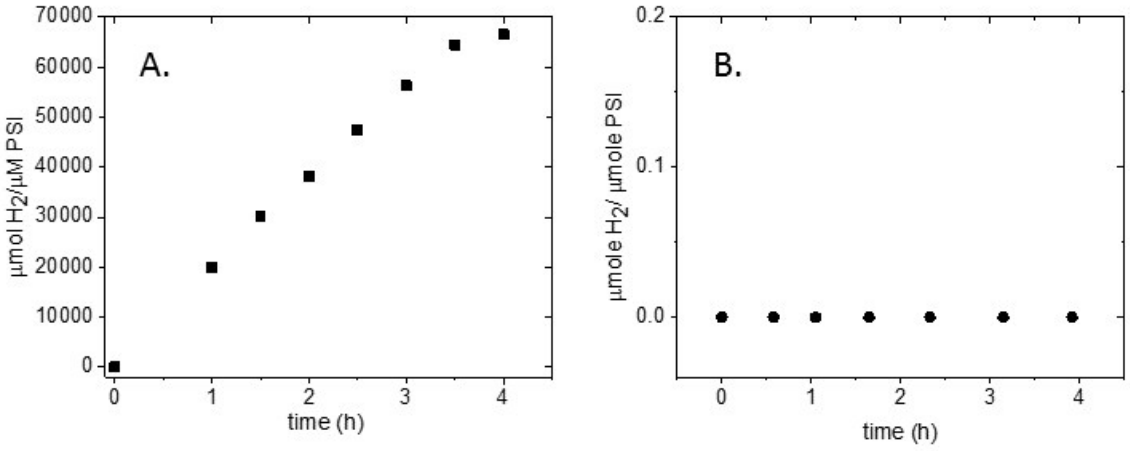

Figure S4. Control experiments showing that MES buffer (2-(N-Morpholino)ethanesulfonic acid) does not act as a SED in H_2 production observed for isolated PSI-Pt nanoparticle hybrids. (A) H_2 production from a solution containing 30 nM PSI-Pt nanoparticle hybrid (PSI isolated from *S. leopoliensis*, hybrid complex contains 1 PSI: 1 PtNP ratio as determined by ICP-AES analysis) with 4 µM cyt c_6 in 40 mM MES pH 6.2, 0.03 % n-dodecyl β-D-maltopyranoside, and 100 mM sodium ascorbate. (B) No observable H_2 production was observed by GC analysis for a solution containing 50 nM *S. leopoliensis* PSI-Pt nanoparticle hybrids (a 1 PSI: 1 PtNP ratio was determined by ICP-AES analysis) with 12 µM cyt c_6 in 10 mM MES pH 6.3 and 0.03 % n-dodecyl β-D-maltopyranoside. This control experiment shows that cyt c_6 alone does not suffice as a donor to rereduce P_{700}^+ in a manner such that two sequential light-generated electrons can be obtained rapidly from PSI for efficient H_2 production.

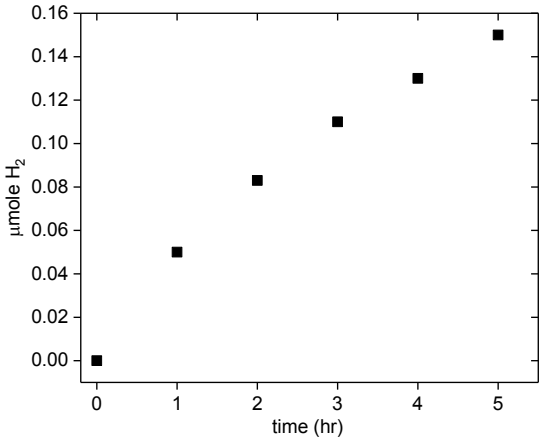

Figure S5. H_2 production from *T. lividus* thylakoid/Pt nanoparticle complex with 100 mM sodium ascorbate, 1 mM DCMU, 10 µM cyt c_6, 10 mM MES, pH 6.1. The sample (0.17 mg Chl) was illuminated and 100 µl aliquots were removed from the headspace every 60 min and measured for H_2 content with GC as detailed in the experimental procedures section.

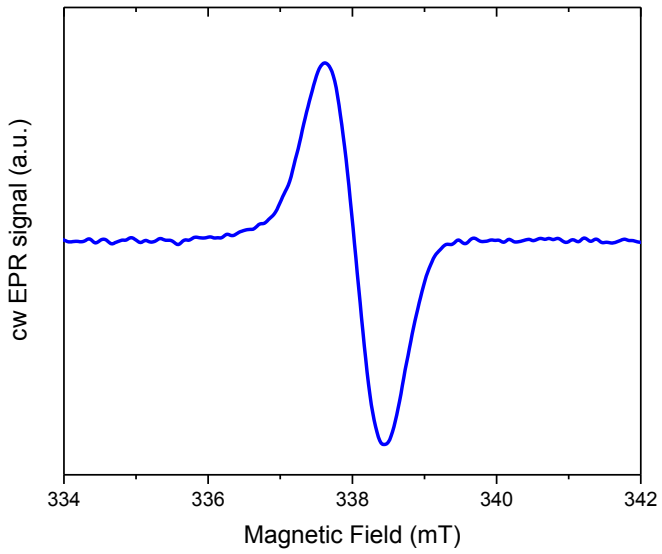

Figure S6. cw X-band EPR spectrum of *S. leopoliensis* thylakoid membrane/Pt nanoparticle complexes. The sample was dark-adapted at room temperature for 20 min prior to freezing in liquid N_2 and placement in the pre-cooled resonator at 10 K. A dark spectrum was collected, followed by illumination of the sample in the resonator at 10 K. The spectrum shown is the after light-dark difference spectrum which shows the light-induced formation of $P700^+$ at low temperature.

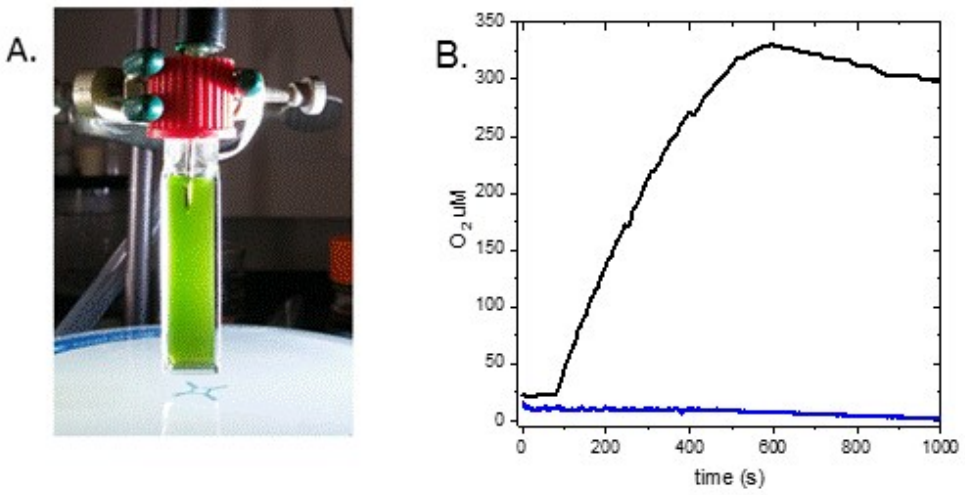

Figure S7. (A) Experimental cell used for O_2 and H_2 measurements. (B) O_2 trace for thylakoid membranes isolated from baby spinach leaves. The activity of this sample was measured to be 100 µmol O_2/ mg Chl/ hr when 1 mM ferricyanide and 250 µM DCBQ were used as external electron acceptors for PSII (black trace). O_2 evolution is not observed in the presence of 1 mM DCMU (blue trace). The buffer contained 20 mM MES, pH 6.2, and 1 mM NH_4Cl. Samples with Pt nanoparticle bound exhibit nearly the same O_2 evolution profiles as the native membranes.

References:

1. Lichtenthaler, H. K. (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes, *Meth. Enzymol. 148*, 350-382.
2. Crespi, H. L., Smith, U., Gajda, L., Tisue, T., Ammeraal, R. M. (1972) Extraction and purification of ^{1}H, ^{2}H, and isotope hybrid algal cytochrome, ferredoxin, and flavoprotein, *Biochim. Biophys. Acta 256*, 611-618.
3. Berthold, D. A., Babcock, G. T., Yocum, C. F. (1981) A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes, *FEBS Lett. 134*, 231-234.