Electronic Supplementary Information

Solar energy conversion by thionine-capped thylakoid membranes embedded in indium tin oxide film on a graphite surface

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1. Electrode preparation

1) Isolation of thylakoid membrane

For this work, thylakoid membrane was extracted from fresh spinach leaf purchased at the local market and its isolation process was based on Danielsson et al..[1,2] Firstly, the purchased spinach was washed in a clean water to remove dirt onto spinach leaf and then rinsed with distilled water. This process was done in a frigid condition at 4 °C to prevent the rapid decrease in activity of isolated thylakoid. Spinach leaf of 100 g was homogeneously blended in 50 mM phosphate buffer [pH 7.4] containing 5 mM $MgCl_2$ and 300 mM sucrose for a sufficient time. Thus obtained homogeneous substance was filtered using nylon fabrics (pore size 25 μm) and subject to centrifugation in the same buffer. This process of washing and centrifugation was repeat two times in tricine buffer. Then the osmosis was conducted for acquiring isolated thylakoid membrane in prepared 5mM MgCl solution. Finally, isolated thylakoid membrane was washed with low salted buffer and its chlorophyll content was checked by spectrophotometry based on Arnon.[3] Eventually, the isolated thylakoid membrane was stored at −80°C whose chlorophyll content was adjusted to 3~ 4 mg Chl mL^{-1}.

The activity measurement of the isolated thylakoid membrane was conducted by measuring evolved oxygen from TMs with an oxygen electrode (Hansatech Instruments Ltd., Norfolk, UK) under illumination of light intensity of 100 mW cm^{-2}. The measuring solution was 15 mM MES(2-(Nmorpholino) ethanesulfonic acid) buffer at pH 6.5 containing 2 mM ferricyanide, 0.5 mM phenyl-p-benzoquinone which serves as an electron acceptor. The concentration of chlorophyll of the isolated thylakoid membrane was 20 μg Chl mL^{-1}.

2) Electrode preparation with a TM/TH/ITO composite thin film

For the preparation of a working electrode, a porous graphite rod mounted in a Teflon frame was used. The graphite surface was polished with a sandpaper, then subsequently sonicated in ethanol and distilled

water for 15 min and finally rinsed carefully with distilled water and dried in an oven at 80 ℃ for 30 min. ITO NPs were used as received. 25 mg of ITO NPs were suspended in 1 mL of pH 7.5 HEPES buffer (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) containing 15 mM sucrose and 5 mM NaCl and sonicated for 30 min for dispersion. Three steps were taken to form a TM/TH/ITO NP film on the graphite electrode. Firstly, 50 μg mL^{-1} of TM solution and 20 μM thionine solution were mixed incubated for 30 min at 4 ℃. This mixture was subject to centrifugation at 10,000 rpm for 15 min followed by the removal of the supernatant. The TM/TH aggregate pellet was carefully rinsed with buffer solution and subject to centrifugation at 10,000 rpm once again followed by the removal of supernatant. Pre-prepared 200 μL of ITO NPs solution was added to the mixture. After Finally, the mixture solution was suspended. A 10 μL of an aqueous mixture of thylakoid, thionine, and ITO NPs was drop cast onto the graphite electrode surface and incubated in a vacuum chamber for 10 h.

2. Electrochemical and photoelectrochemical measurements

Cyclic voltammetry and chronoamperometry were employed to examine electrochemical behaviors of thionine (free and bound state to TMs) and thylakoid membranes. A typical three-electrode system was used with Pt wire and Ag/AgCl as counter and reference electrodes, respectively, using a potentiostat (CompactStat, Ivium Technologies, Netherlands) in 10 mM HEPES buffer of pH 7.5 containing 100 mM KCl at 20 ℃. Three independent experiments were done.

For photocurrent measurements, a solar simulator with a Xenon lamp (McScience, Korea) was used at one sun illumination (100 mW cm^{-2}).

3. Calculation of quantum efficiency (QE)

We assume that average wavelength of incident light is 500 nm. Then,
100 mW cm^{-2} corresponds to 2.77 x 10^{17} photons per cm^2. Therefore, QE at the maximum photocurrent of 41.4 μA cm^{-2} is given by Eq. S1.

QE(%)

$$= \frac{generated\ electrons/s}{number\ of\ incident\ photons/s} \times 100 = \frac{(41.4\ \mu A\ cm^{-2}) \times (1\ mol\ e^{-}/96485\ C) \times}{2.51\ \times\ 10^{17} photons\ s^{-1}} \times 100 = 0.10\% \quad \text{(S1)}$$

4. Calculation of turnover frequency (TOF) for water oxidation

The mass of chlorophylls in the 1:20 TM/TH/ITO NP film was determined by spectrophotometry to 1.5 μg.

Number of Chl = (1.85 x 10^{-6} g) / (893 g mol^{-1}) x (6.022 x 10^{23} mol^{-1}) = 1.25 x 10^{15} Chl

Number of PSII in spinach is 105 and the ratio PSI/PSII is known to be 1.13. In thylakoid membranes, the ratio of chlorophylls in PSI and PSII is 0.88.

From this information, the number of PSII in the sample is calculated.

Let N_{PSII} and N_{PSI} represent the number of PSII and PSI, respectively. Then the total number of chlorophylls in the sample is given by the following equations.

$$Chl_{PSI} + Chl_{PSII} = 1.25 \times 10^{15} \text{ Chl} \quad \text{(S2)}$$

$$Chl_{PSII} = 105 \times N_{PSII}$$

$$N_{PSI} = 1.13 \times N_{PSII}$$

$$Chl_{PSI} = 105 \times 0.88 \times N_{PSI} = 105 \times 0.88 \times 1.13 \times N_{PSII} = 104 \times N_{PSII} \quad \text{(S3)}$$

From S2 and S3, N_{PSII} = 6.0 x 10^{12} chlorophylls in the sample.

In the unit area, there are 8.4 x 10^{13} (= 6.0 x 10^{12} / 0.0706 cm^2) PSII units per unit area.

In the meantime, the number of oxidized water molecules per second is calculated from photocurrent. 41.4 μA cm^{-2} corresponds to 1.3 x 10^{14} H_2O molecules per second. Therefore, the turnover frequency of water oxidation is

TOF = 1.3 x 10^{14} H_2O s^{-1}/ 8.4 x 10^{13} PSII = 1.5 H_2O molecules per second

Supporting Figures

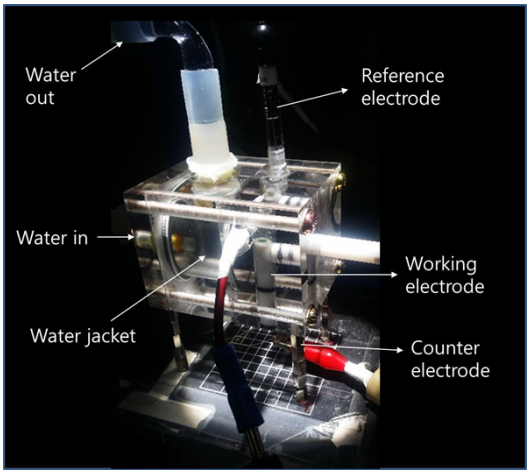

Figure S1. Electrochemical setup for photocurrent measurements. Light impinges the top of the working electrode from a solar simulator located above. Temperature of the cell was maintained at room temperature by circulating water.

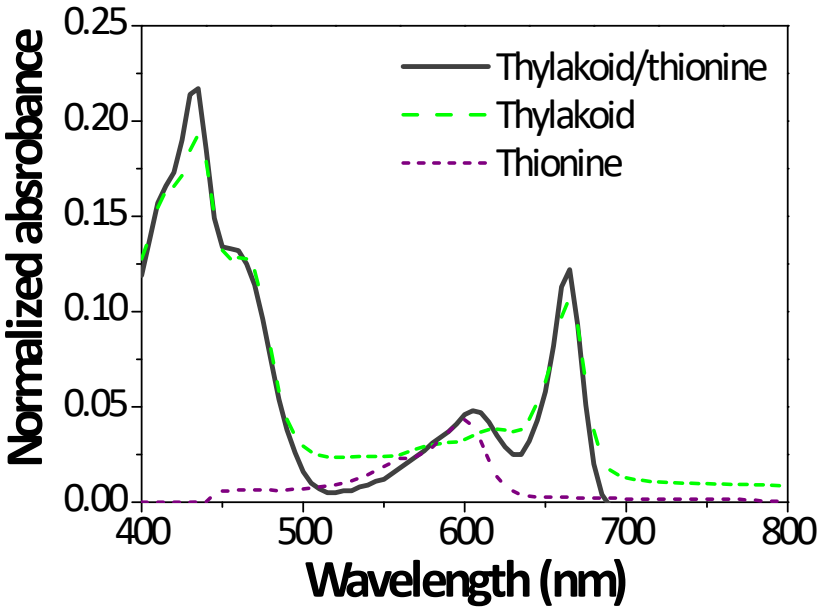

Figure S2. Absorption spectra of thionine, thylakoid membrane, and a TM/TH thin film. Characteristic absorption peaks of thionine and thylakoid membranes are clearly identified in a TM/TH film.

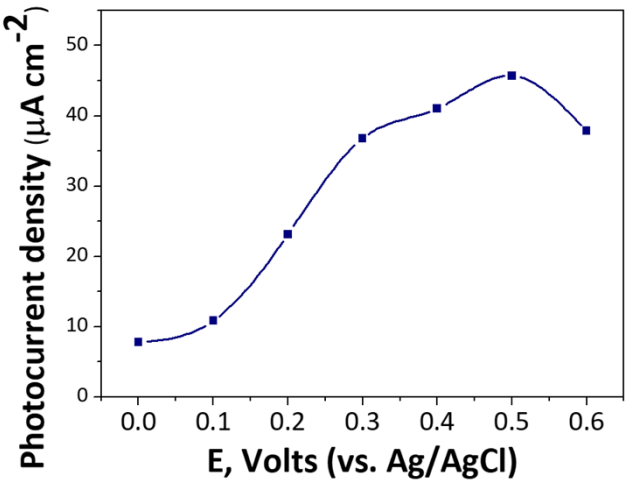

Figure S3. Effect of applied potential on the photocurrent from a TM/TH/ITO NP thin film formed on the graphite surface.

References

1. R. Danielsson, P. A. Albertsson, F. Mamedov and S. Styring, *Bba-Bioenergetics*, 2004, **1608**, 53-61.
2. R. Danielsson and P. A. Albertsson, *Bba-Bioenergetics*, 2009, **1787**, 25-36.
3. D. I. Arnon, *Plant physiology*, 1949, **24**, 1-15.