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Direct electron transfer from photosystem II to hematite in a hybrid photoelectrochemical cell

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A hybrid photoanode integrating cyanobacterial photosystem II (PSII) with a hematite film for water oxidation is constructed. Direct electron transfer from PSII to the excited Ti/Fe₂O₃ electrode occurs under light irradiation, resulting in a significant improvement of the photocurrent.

Photosynthetic protein/semiconductor hybrid systems have drawn intensive attentions for their potential advantages including electron and energy transfer between the enzyme proteins and artificial materials¹⁻³. The photosynthetic enzyme photosystem II (PSII) plays a crucial role in solar to chemical energy conversion via water oxidation reaction in high plant, algae and cyanobacteria^{4,5}. The hybrid assemblies of the photoelectrochemical and photocatalytic systems based on PSII enzyme to realize solar conversion have attracted more and more research efforts^{1,6-8}. Many strategies have been developed to couple PSII with the electrode substrates^{7,9}, nanoparticles¹⁰ and redox metal complexes¹¹. Recently, Reisner et al. have made a progress in the hybrid system by coupling PSII with indium tin oxide electrode materials to generate photocurrent and improve the photoresponse^{2,12}. Besides, much attention has been paid to the photobiofuel cells with the PSII-functionalized electrodes⁷. However, many challenges remain in understanding the electron transfer mechanism in such hybrid systems, as well as in constructing a controllable and stable hybrid system.

Semiconductors have many advantages such as tunable band structures, continuous spectral absorption and so on¹³. Therefore, exploring suitable semiconductor materials to construct a new hybrid system with PSII is of great importance. Interfacial electron

transfer from PSII to semiconductor photocatalyst is considered to be very difficult in the hybrid system due to the special electron transfer pathway. We have recently reported the assemblies of PSII and semiconductor photocatalysts for overall water splitting, in which the H₂ producing photocatalysts (e.g. Ru/SrTiO₃:Rh) and plant PSII membrane are integrated in an aqueous solution using Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ as a shuttle for electron transfer¹⁴. However, it remains an interest to study the direct electron transfer from water oxidation reaction by PSII to semiconductor photocatalyst without exogenous redox mediator and the binding linker. Hematite (α -Fe₂O₃) with a band gap of 2.1 eV is a prospective photoanode material owing to its low cost, chemical stability and biocompatibility¹⁵⁻¹⁷, despite of its short hole diffusion length. In the case of the bio-functionalized hybrid systems, assembly of the hematite with biological motifs is attractive to develop hybrid photoanode systems based on the biointerface concept¹⁸. The interface between inorganic and biological components is alternative for electron transfer. To date, no self-assembled bio-photoelectrochemical hybrid device integrating PSII protein with hematite photoanode that produces photocurrent under visible light irradiation is reported.

Herein we present a hybrid photoanode integrating PSII with a mesoporous, hematite material for solar water splitting. The biocatalyst, PSII core complex isolated from the thermophilic cyanobacterium *T. vulcanus*, is assembled on the surface of the Ti/Fe₂O₃ (surface Ti doped) electrode via a linker-free self-assembly approach. The photocurrent of the PSII-Ti/Fe₂O₃ hybrid anode is improved when compared with the bare Ti/Fe₂O₃ electrode due to the direct electron transfer from PSII to the excited Ti/Fe₂O₃ electrode. Effects of PSII on the charge transfer resistance at the interface are also discussed.

The hematite phase Ti/Fe₂O₃ films (Fig. S1, ESI[†]) we prepared for the hybrid photoanode have a three-dimensional porous structure with a pore diameter of about 200 nm and thickness of 300 nm (Fig. 1a and b). The porous structure is suitable to accommodate the PSII with a size of 10.5 nm in depth, 20.5 nm in length and 11.0 nm in width¹⁹. The PSII-Ti/Fe₂O₃ hybrid anode was prepared via a self-assembly approach under mild condition without adding any molecular linker for cross-linking PSII. PSII was

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[†] Electronic Supplementary Information (ESI) available: full experimental details, XRD patterns (S1), fluorescence intensity imaging (S2) and UV-vis absorption spectrum (S3). See DOI: 10.1039/x0xx00000x

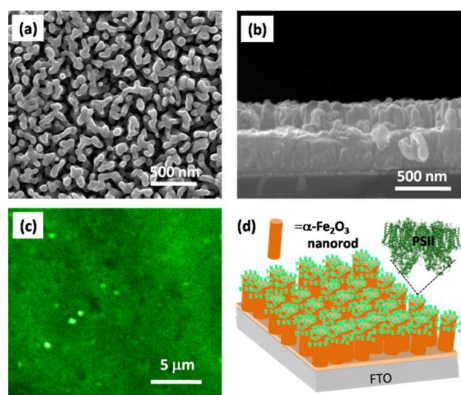


Fig. 1 SEM of the Ti/Fe₂O₃ film: (a) top view and (b) side view. (c) The fluorescence intensity image of PSII-Ti/Fe₂O₃ hybrid electrode. (d) Proposed Schematic configuration of the PSII-Ti/Fe₂O₃ hybrid electrode.

dropped on the Ti/Fe₂O₃ film, and the PSII core complexes were expected to bind within the pores and channels of the electrode. The unbounded PSII was removed by rinsing with the buffer solution before characterization and photoelectrochemical measurements. Fluorescence intensity image is used to monitor the dispersion of PSII on the hybrid electrode on the basis of the characteristic fluorescence emission from chlorophylls of PSII. Fig. 1c shows the PSII-Ti/Fe₂O₃ hybrid electrode presents an almost even fluorescence distribution on the whole surface of the electrode. The bare Ti/Fe₂O₃ has no fluorescence signal (Fig. S2a, ESI[†]), but a strong signal can be detected from the PSII buffer on the glass slide (Fig. S2b, ESI[†]). Thus, these imaging results indicate that the PSII complexes are well-distributed on the surface of the Ti/Fe₂O₃ film. In addition, the fluorescence intensity of the hybrid anode after photoelectrochemical measurements is approximately equivalent with that before the measurements (Fig. S2c, ESI[†]). The content of PSII on the Ti/Fe₂O₃ was ca. 2.224 ± 0.093 pmol and its thickness is calculated to be 12 layers by assuming that the PSII is an ideal two-dimensional size²⁰. PSII enzymes were multilayered coverage on the Ti/Fe₂O₃ film (Fig. 1d). These results indicate the assembly of the PSII and Ti/Fe₂O₃ forms bio-inorganic junction in the PSII-Ti/Fe₂O₃ hybrid photoanode.

Solar water oxidation photocurrents over the PSII-Ti/Fe₂O₃ and bare Ti/Fe₂O₃ photoanodes were measured. After assembling with PSII complex, the onset potential of the electrode was negatively shifted by about 100 mV (Fig. S3, ESI[†]), indicating the decrease of overpotential for water oxidation. Fig. 2a shows the amperometric I-t curves of the hybrid electrodes for 120 seconds at 0.4 and 0.5 V vs. SCE under chopped light illumination. Obvious photocurrent is observed at 0.4 V vs. SCE under irradiation. For comparison, we also prepared the hybrid electrode with PSII particles depleted of the Mn-cluster by NH₂OH treatment, which showed a remarkably decreased photoresponse upon illumination. This reveals that the photocurrent is generated from light-induced water oxidation by PSII but not from the PSII itself. We also tested the PSII-FTO electrode prepared by the same method as that used for the hybrid anode, but observed no photocurrent upon light illumination. We didn't observe fluorescence signals for PSII on the smooth surface of FTO glass (Fig. S4, ESI[†]). Therefore, it is reasonable to suppose that PSII will shed from the FTO surface. This reflects the important

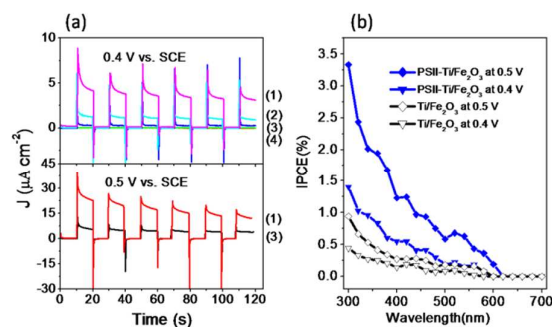


Fig. 2 (a) Amperometric j-t curves of PSII-Ti/Fe₂O₃ (1), Mn depleted PSII-Ti/Fe₂O₃ (2), Ti/Fe₂O₃ (3) and PSII-FTO (4) electrodes at different potentials under AM 1.5G simulated solar light (100 mW cm^{-2}) in 50 mM phosphate sodium buffer solution (pH 6.0). (b) IPCE of Ti/Fe₂O₃ and PSII-Ti/Fe₂O₃ electrodes at different potentials in 50 mM phosphate sodium buffer solution (pH 6.0).

role of porous Fe₂O₃ electrode as a host of PSII. The photocurrent of the PSII-Ti/Fe₂O₃ hybrid anode is enhanced up to $24 \mu\text{A cm}^{-2}$, almost 4 times higher than that of the bare Ti/Fe₂O₃ at 0.5 V vs. SCE under AM 1.5G simulated sunlight irradiation (Fig. 2a). The enhancement of the photocurrent implies that the stromal side of PSII is close to the hematite surface, resulting in the direct electron transfer from PSII to the Ti/Fe₂O₃ electrode under light irradiation. It should be noted that the system was not stable due to the photodamage of PSII under continuous light irradiation (Fig. S5, ESI[†]).

Insight into the interaction between hematite and PSII in the PSII-Ti/Fe₂O₃ hybrid photoanode was provided by measuring the photoresponse as a function of the wavelength of incident light. The Ti/Fe₂O₃ shows a broad light absorption range with the wavelength below 600 nm and the PSII has two intrinsic absorption bands which are originated from the chlorophylls²¹ (Fig. S6, ESI[†]). Fig. 2b shows that the incident photo-to-current conversion efficiency (IPCE) of the bare Ti/Fe₂O₃ and PSII-Ti/Fe₂O₃ hybrid anode at different applied potentials. The IPCE curve of the PSII-Ti/Fe₂O₃ hybrid electrode was much higher than that of the bare Ti/Fe₂O₃ sample at wavelengths from 300 to 600 nm in accordance with UV-visible absorption spectrum of the Ti/Fe₂O₃. However, no photoresponse of the PSII-Ti/Fe₂O₃ samples was observed when the wavelength of the incident light is in the range of 600 to 700 nm, even though PSII can absorb and utilize light for water oxidation in this range (Fig. S6, ESI[†]). This suggests that the electron transfer

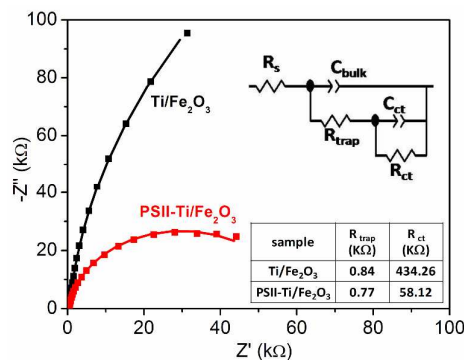


Fig. 3 EIS of Ti/Fe₂O₃ and PSII-Ti/Fe₂O₃ electrodes under AM 1.5G simulated solar light (100 mW cm^{-2}) in 50 mM phosphate sodium buffer solution (pH 6.0). The inserts are the equivalent circuit and the fitted values of the R_{ct} .

from PSII to Ti/Fe₂O₃ stops when the Ti/Fe₂O₃ substrate is not excited. Thus, charge separation and transfer at the interface is of importance in the PSII-functionalized hematite electrode.

The electrochemical impedance spectroscopy (EIS) was measured to clarify the charge transfer resistance of the hybrid system²². Fig. 3 shows the EIS results of the bare Ti/Fe₂O₃ and PSII-Ti/Fe₂O₃ hybrid anode at the applied potential of 0.4 V vs. SCE. The insert presents the corresponding equivalent circuit and the values of the charge transfer resistance (R_{ct}) related to the semicircle fitted from the Nyquist plots. The fitted results indicate that when PSII protein is adsorbed on the hematite surface, the R_{ct} of the PSII-Ti/Fe₂O₃ anode is much smaller than that of the bare Ti/Fe₂O₃ electrode. The PSII is responsible for the low R_{ct} value which promotes the charge transfer across the interface between the electrode surface and electrolyte. The R_{trap} represents the electrical impedance at the bio-inorganic interface between PSII and the hematite surface. Therefore, the formation of the bio-inorganic junction between PSII and Ti/Fe₂O₃ is important for promoting charge separation and transfer.

The electron transfer pathway proposed for the PSII-Ti/Fe₂O₃ hybrid PEC system for water splitting is shown in Fig. 4. Under light irradiation, the initial charge separated state of the PSII is formed within few tens of picoseconds after excitation and then the photogenerated electrons and holes move apart toward opposite directions, resulting in their spatial separation²³. The holes are transferred to the Mn₄CaO₅ cluster for water oxidation and the electrons move to the plastoquinone Q_A and subsequently to the Q_B on the stromal side of the PSII²⁴. Thus, the stromal side of PSII close to the surface of Ti/Fe₂O₃ substrate is important for the interfacial electron transfer. It appears that electrons from PSII are prone to be transferred to the conduction band of the hematite, which is similar to the way of dye-sensitised solar cells²⁵ in terms of the energy diagram. However, the results of IPCE in our system have revealed that the photocurrent can be generated when both PSII and Ti/Fe₂O₃ are excited simultaneously under the light with the wavelength shorter than 600 nm, and there was no photoresponse when only PSII is excited. Thus, the pathway of interfacial electron transfer is from Q_B of the PSII into the valence band of the excited Ti/Fe₂O₃. The electrons accepted by Ti/Fe₂O₃ then flow to the counter electrode for hydrogen production.

The present work has demonstrated a hybrid photoanode integrating PSII with hematite film via a self-assembly approach. Photoelectrochemical water splitting is achieved in a nearly neutral aqueous solution by the hybrid PSII-Ti/Fe₂O₃ electrode under AM 1.5G simulated sunlight irradiation, as evidenced by significantly

enhanced photocurrent generation in the presence of the PSII enzymes. The electrons generated from water oxidation by PSII are injected into the valence band of the excited Ti/Fe₂O₃. Decrease in the charge transfer resistance was observed, indicating the attachment of oriented PSII enzymes to the porous surface of the nanostructured Ti/Fe₂O₃ electrode for improving the photoelectrochemical efficiency. The PSII-Ti/Fe₂O₃ hybrid system presents new opportunities for generating solar fuels via merging natural photosynthetic system with artificial tunable catalyst.

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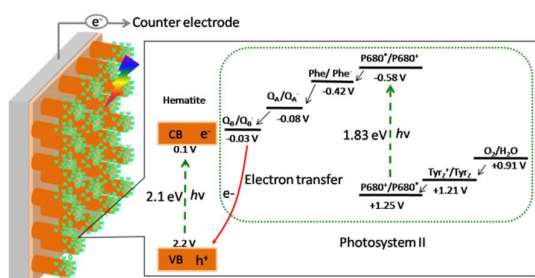


Fig. 4 Schematic illustration of the electron transport pathway from PSII to Ti/Fe₂O₃ in the PSII-Ti/Fe₂O₃ hybrid photoanode.