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Electronic Supporting Information for

Co-assembly of photosystem II in nanotubular indium-tin oxide multilayer films templated by cellulose substance for photocurrent generation

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Experimental Details

Materials

Indium(III) acetylacetonate (99.99% trace metals basis), 2,5-dichloro-1,4-benzoquinone (DCBQ, 98%), Vitamin C, bovine serum albumin (BSA) and betaine were bought from Sigma-Aldrich. Tin(IV) isopropoxide (99% metals basis, 10% w/v in isopropanol) was purchased from Alfa Aesar. Filter paper (quantitative ashless) derived from cotton was purchased from Hangzhou Xinhua Paper Industry Co., Ltd. (China). Organic solvents, such as ethanol, acetone and methanol, and sucrose, NaOH, $MgCl_2$ and NaCl were purchased from Sinopharm Chemical Reagent Co., Ltd. All the chemicals were of analytical grade and were used without further purification. The deionized water with resistivity higher than 18.2 MΩ cm was used in all related experiments. The ITO substrates and Au paste used in the photoanode system fabrication were bought from GSG Holding Co., Ltd. and Ted Pella, Inc., respectively.

Isolation of PSII enriched membranes from spinach leaves

PSII enriched membranes were isolated and purified from fresh spinach leaves, according to the previous methods with modifications.[1] About 200 g fresh spinach leaves were washed with deionized water and kept at 4 °C overnight. All the following steps were carried out at 4 °C. The stored spinach leaves were homogenized with 400 mL of buffer A (20 mM Tricine-NaOH pH 7.8, 0.4 M sucrose, 2 mM $MgCl_2$, 40 mM NaCl, 2 mM Vitamin C, 0.2% BSA) and then filtered with cotton gauze to obtain the filtrate. This filtrate was centrifuged at 2,000 rmp for 1 min to remove the precipitation, and the chloroplasts were then collected by centrifuging the supernatant at 6,000 rpm for 15 min. The precipitant of the chloroplasts was resuspended in hypotonic buffer B (20 mM Tricine-NaOH pH 7.8, 5 mM $MgCl_2$, 10 mM NaCl, 0.2% BSA) and stirred for 15 min to break the chloroplasts. The suspension was centrifuged at 1,600 rmp for 2 min to remove the water soluble proteins from the outer surface of the thylakoid membranes, and the thylakoid membranes were collected by centrifugation of the supernatant at 8,600 rmp for 15 min. After resuspending the precipitation of the thylakoid membranes in a high ionic strength buffer C (20 mM MES-NaOH pH 6.5, 0.4 M sucrose, 15 mM NaCl, 5 mM $MgCl_2$), the chlorophyll (Chl) concentration was determined and finally adjusted to 2.5 mg Chl mL^{-1}. Buffer D (buffer C with an additional 20 wt% TX-100) was added to the suspension slowly until the ratio of TX-100 to Chl (w/w) is 20:1 and then stirred gently for 10 min. Starch grains and debris were removed by centrifugation of the suspension at 10,300 rmp for 1 min, followed by centrifuging the supernatant at 24,000 rmp for 30 min to obtain the precipitate, which was then washed with buffer C repeatedly until the supernatant is colorless. The final PSII enriched membranes was resuspended in 5 mL buffer E (buffer C containing an additional 0.5 M betaine) and frozen in liquid nitrogen. The Chl concentration of the PSII was determined to be 4 mg Chl mL^{-1}.

Synthesis of the nanotubular ITO films

Nanotubular ITO films were synthesized by means of layer-by-layer (LBL) self-assembly method using cellulose filter paper as a template, based on the reported method with modifications.[2] The precursors of In alkoxide and Sn alkoxide for ITO materials preparation in our research are different from the previously reported case, resulting in a better solubility and a shorter stirring time of the precursor solution. Indium(III) acetylacetonate was dissolved in methanol and acetone (1:1, v/v) and stirred for 1 h at 50 ºC. Tin(IV) isopropanol was then added dropwise into the indium alkoxide precursor solution and stirred for another 1 h to give a precursor solution for ITO thin film deposition, in which the total concentration of indium alkoxide and tin alkoxide was 10 mM, and the molar ratio of indium and tin was controlled to be 20:1. The whole LBL self-assembly process was proceeded at a room temperature without the need of a heating device, making the preparation method more facile to operate compared with that in the previous literature. The deposition cycle was repeated for n ($n = 5$, 10, 15) times to achieve ITO thin gel layer with a certain thickness on the cellulose. Then, the as-prepared ITO gel layer/filter paper composite sheet was dried at 60 ºC in oven overnight, and was afterwards subjected to calcination at 450 ºC (heating rate 1 ºC min^{-1}) in air for 6 h to remove the original filter paper, and the ITO gel layers coated on the cellulose nanofibers were then transformed into crystallized ITO nanotubes, which was denoted as $(\mathrm{ITO})_n$-NTs.

Measurements of oxygen evolution activity of PSII

The PSII O_2 evolution activity was measured by two approaches. For the PSII O_2 evolution activity detection with Clark-type oxygen electrode, a PSII suspension with Chl concentration of 10 µg mL^{-1} containing 5 mM DCBQ as electron acceptor, 20 mM NaCl, 3 mM $CaCl_2$, 20 mM MES (pH 6.0) was used as the reaction system under continuous white light illumination ($\lambda < 800$ nm, $P = 10$ mW cm^{-2}) for 1 h, and the O_2 evolution activity of PSII was measured to be ~213 µmol O_2 (mg Chl)$^{-1}$ h^{-1}. In addition, the O_2 evolution activity of PSII was also roughly evaluated by detection of the volume of the generated gas in a home-made reactor. The above PSII buffer solution was added in a quartz reactor under vigorous stirring. The generated gas was collected in a measuring cylinder filled with water to evaluate the gas volume, and then the PSII O_2 evolution activity was estimated to be about 179 µmol O_2 (mg Chl)$^{-1}$ h^{-1}, which was slightly lower than the value measured by the Clark-type oxygen electrode. This result might be due to that some oxygen dissolved in the buffer solution, causing the volume reduction.

Supplementary Figures

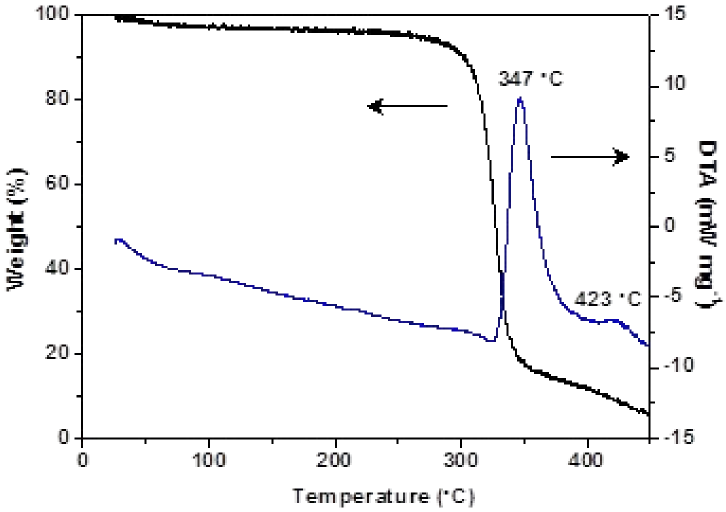

Fig. S1 DTA/TG curves of the as-deposited ITO gel layer/filter paper composite sheet. The composite sheet was calcined at a heating rate of 10 ºC min^{-1} from 25 ºC to 450 ºC in air.

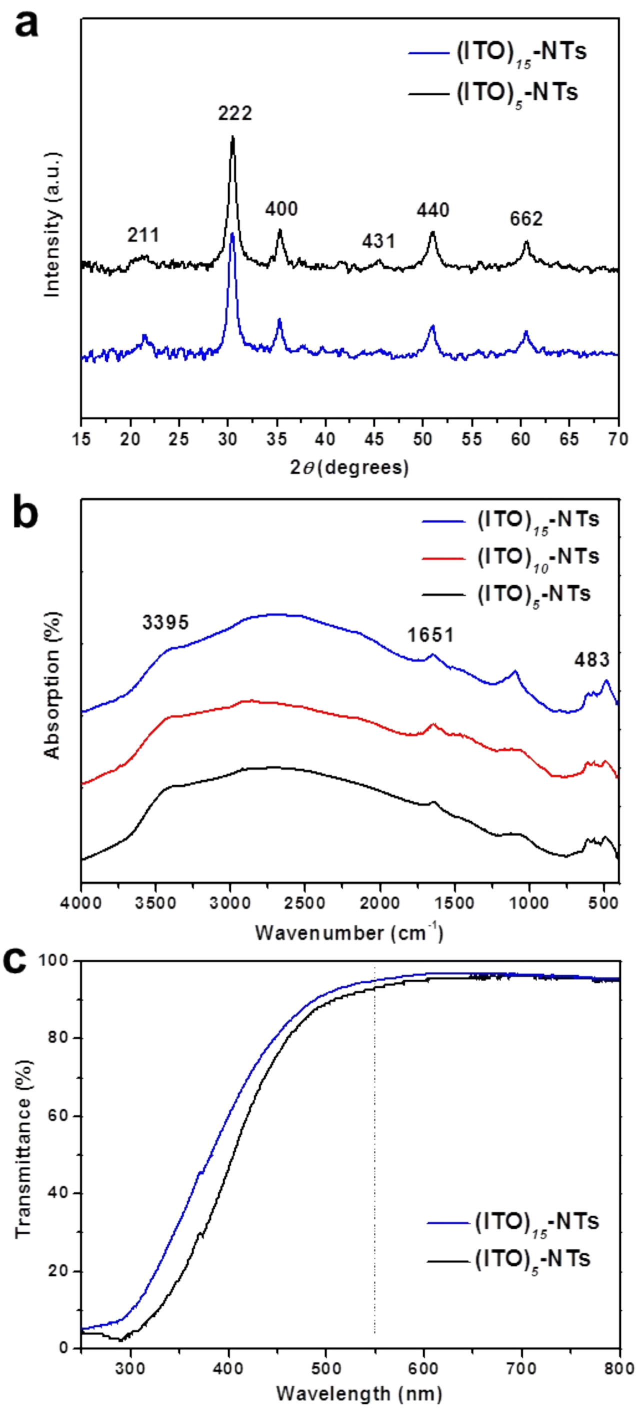

Fig. S2 Spectral characterizations of the samples (ITO)$_n$-NTs. (a) XRD patterns (n = *5*, *15*), (b) FTIR spectra (n = *5*, *10*, *15*), (c) UV−vis spectra (n = *5*, *15*).

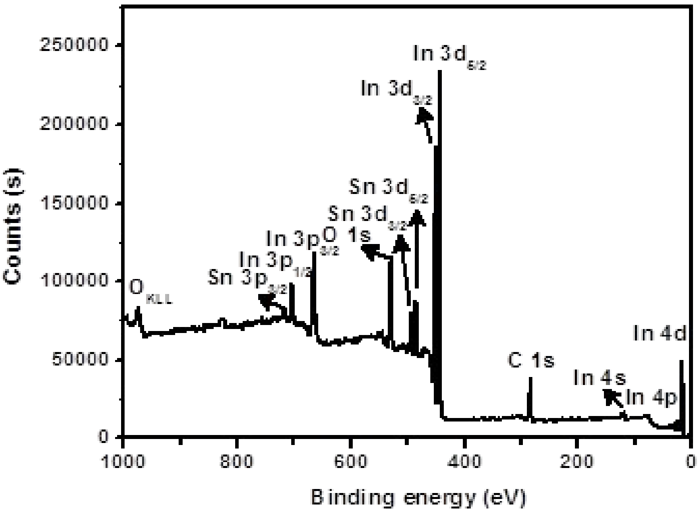

Fig. S3 XPS wide scan spectrum of the sample (ITO)$_{10}$-NTs.

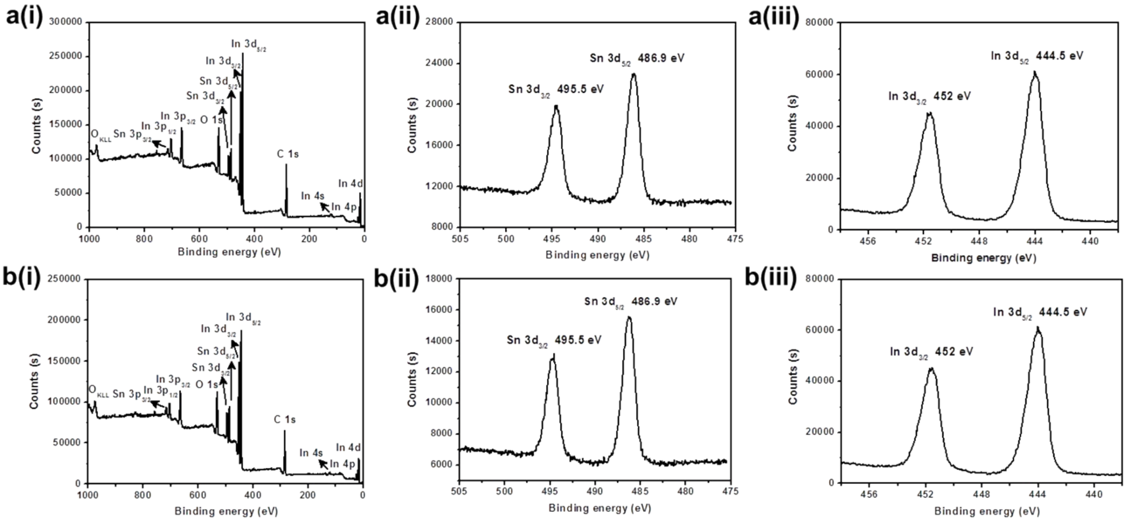

Fig. S4 XPS spectra of the samples (ITO)$_n$-NTs. XPS spectra of the samples (a) (ITO)$_5$-NTs and (b) (ITO)$_{15}$-NTs. (i) Wide scan spectra; (ii) Sn 3d spectra and (iii) In 3d spectra.

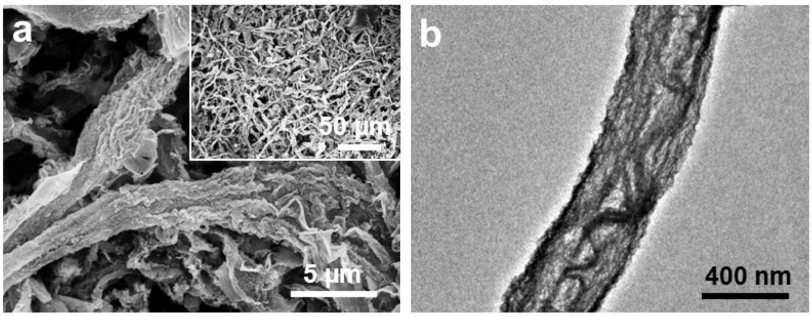

Fig. S5 Electron microscope images of the (ITO)$_{10}$-NTs film pasted on the Au photoanode. (a) Overview FE-SEM image; inset of (a) shows a hierarchical irregular porous nanotube networks of the (ITO)$_{10}$-NTs film, and (b) overview TEM image of an individual ITO nanotube.

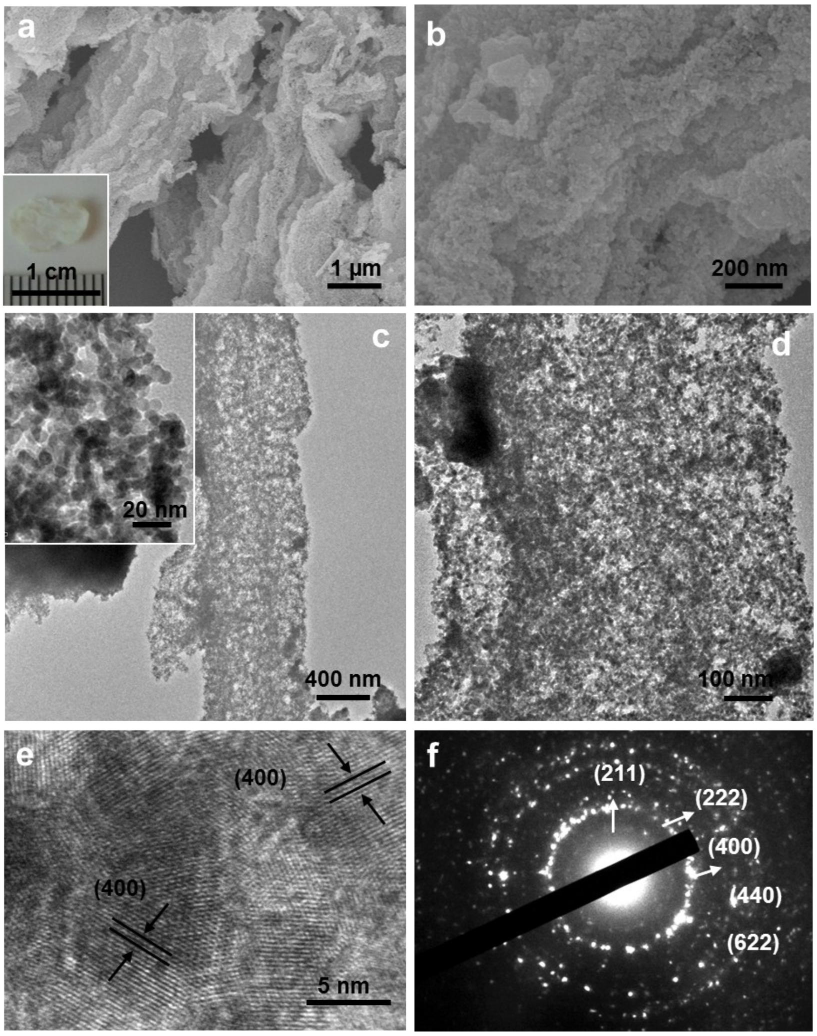

Fig. S6 Electron microscope images of the sample (ITO)$_5$-NTs. (a) Overview and (b) enlarged FE-SEM images of the sample (ITO)$_5$-NTs; inset of (a) shows the photograph of the (ITO)$_5$-NTs film. (c) Overview and (d) enlarged TEM images of an individual ITO nanotube; inset of (c) shows the average size of ITO nanoparticle is about 6 nm. (e) HR-TEM micrograph and (f) SAED pattern of the sample (ITO)$_5$-NTs.

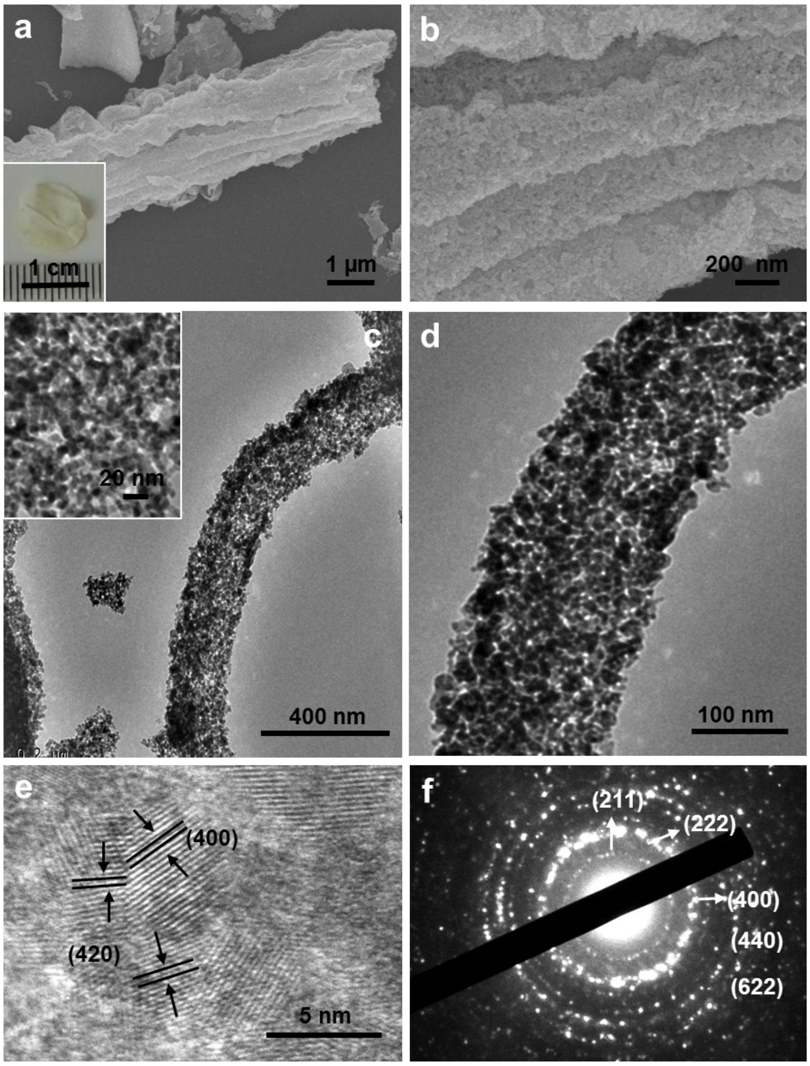

Fig. S7 Electron microscope images of the sample (ITO)$_{15}$-NTs. (a) Overview and (b) enlarged FE-SEM images of the sample (ITO)$_{15}$-NTs; inset of (a) shows the photograph of the (ITO)$_{15}$-NTs film. (c) Overview and (d) enlarged TEM images of an individual ITO nanotube; inset of (c) shows the average size of ITO nanoparticle is about 6 nm. (e) HR-TEM micrograph and (f) SAED pattern of the sample (ITO)$_{15}$-NTs.

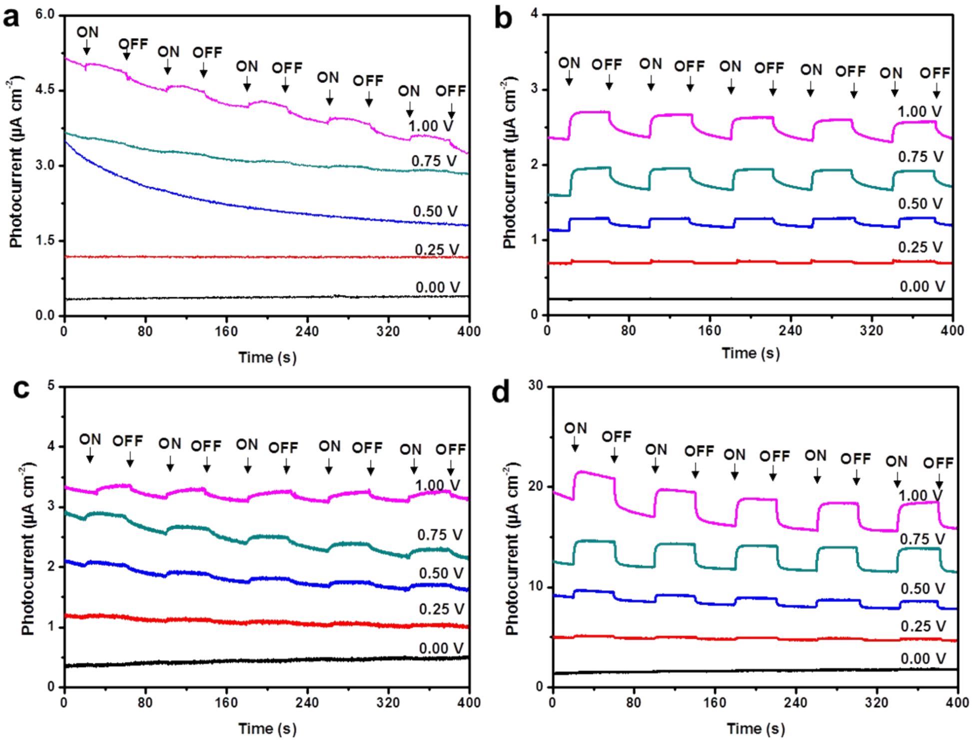

Fig. S8 DET photocurrent responses of the fabricated photoanodes at varied bias potentials. DET photocurrent responses of the (a) Au, (b) PSII, (c) PSII/Au and (d) $(ITO)_{10}$/Au photoanodes to repetitive illumination of white light and darkness cycles. A series of bias potentials of 0.00, +0.25, +0.50, +0.75 and +1.00 V *vs.* SCE were applied at 25 ºC.

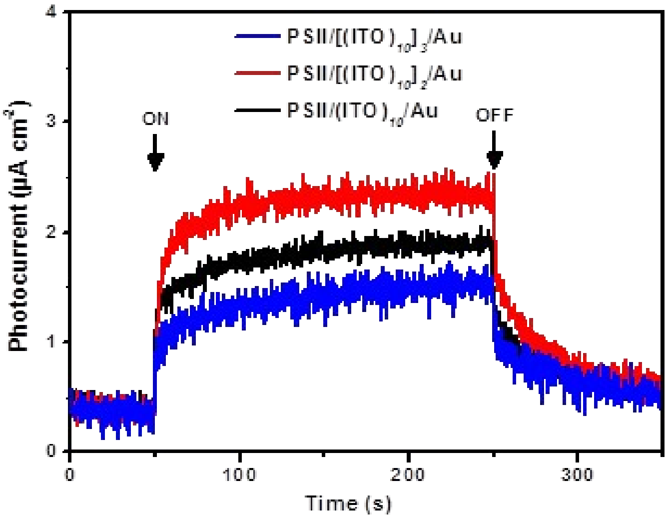

Fig. S9 DET photocurrent responses of the PSII/$(ITO)_{10}$/Au, PSII/$[(ITO)_{10}]_2$/Au and PSII/$[(ITO)_{10}]_3$/Au photoanodes. A bias potential of +0.25 V *vs.* SCE was applied at 25 ºC.

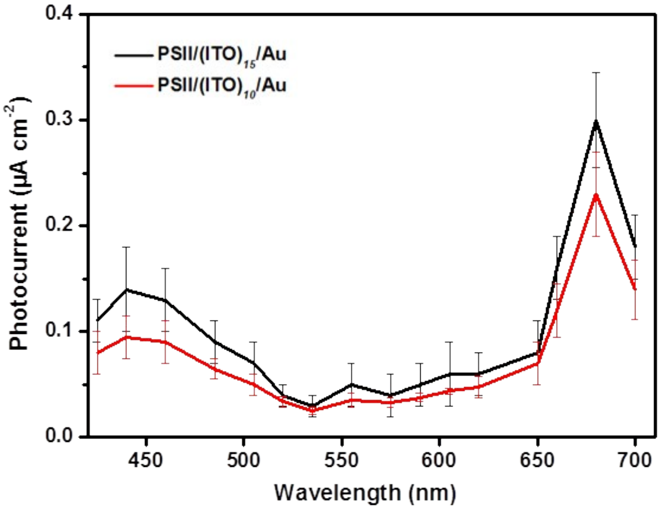

Fig. S10 Photocurrent action spectra of the PSII/ITO/Au photoanodes.

References

[1] B. Hankamer, J. Nield, D. Zheleva, E. Boekema, S. Jansson and J. Barber, *Eur. J. Biochem.*, 1997, **243,** 422−429.

[2] Y. Aoki, J. Huang and T. Kunitake, *J. Mater. Chem.*, 2006, **16,** 292−297.