Electronic Supplementary Information (ESI)

Directed Assembly of Thylakoid Membrane on Nanostructured TiO_2 for a Photo-electrochemical cell

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(SI-1) Experimental Section:

Membrane Isolation:

The thylakoid membrane was extracted from cyanobacteria *Synechocystis* 7803. *Synechocystis sp*. PCC 6803 strain was grown in BG11 medium at 30˚C under 30 μmol photons $m^{-2}\cdot s^{-1}$ with air bubbling in 15 l carboys autotrophically. Cells were harvested at exponential growth phase and resuspended in Resuspension Buffer (RB, 50 mM MES-NaOH, pH 6.5, 10 mM $MgCl_2$, 5 mM $CaCl_2$, 25% glycerol). DNase and protease inhibitor cocktail (Sigma, St. Louis, MO, USA) were added according to manufacturer's protocol. The cell suspension was broken with four cycles of French Press at 1000 psi. After removing unbroken cells by centrifugation at 1,500xg for 15 min, membranes were pelleted by centrifugation at 35,000xg for 30 min. The pelleted thylakoid membrane was washed twice using RB buffer and finally resuspended in RB at 1 mg/ml of chlorophyll *a*.

TiO_2 and membrane deposition:

Columnar TiO_2 nanostructured films were deposited onto tin-doped indium oxide (ITO) coated aluminosilicate glass (Delta technologies, CO) using an aerosol chemical vapor deposition (ACVD) process described previously.[1] Briefly, titanium tetraisopropoxide (TTIP, 97% Sigma-Aldrich) was used as a precursor and loaded into a bubbler at 297 K. The N_2 carrier gas was kept at a constant flow rate of 0.475 L min^{-1} through the bubbler. Additionally, a dilution flow rate (N_2) of 0.475 L min^{-1} was

used. The TiO_2 formed as a result of the decomposition of the precursor, nucleates in the gas phase and forms particles. These particles are deposited onto ITO glass kept at a constant temperature of 550 ºC where they sinter to form columnar TiO_2 single crystal structures. The total deposition time of TiO_2 was fixed at 60 minutes. The morphology of the nanostructure titanium dioxide film was examined using field emission scanning electron microscopy (FESEM). Gold sputtering of the samples was performed for 30 seconds before FESEM analysis in order to improve the resolution of the images. The crystallinity of the film is analyzed using X-ray diffraction.

Thylakoid membrane modification and solution preparation:

For each of the three cell configuration cases, the membrane sample preparation is shown in the table S1 below. The membrane sample was diluted with water and surfactant, and centrifuged to remove glycerol and salts. The centrifuged membrane is diluted with ethanol, water ammonium acetate and surfactant. The concentration of surfactant varies according to the cell configuration and is mentioned in the table. The solution properties are given in the Table S2.

Table S1. Experimental procedure to prepare the electrospray solution including the solution composition and the state of membrane (in parenthesis and italic) for the three cases.

	Experimental Procedure				
Case 1-no surfactant addition	Membrane sample + Water *(Intact membrane)*	Centrifugation →	Intact Membrane	Electrospray solution preparation →	Membrane + 10% ethanol + 3mM ammonium acetate and water *(Intact membrane)*
Case 2- surfactant addition after centrifugation	Membrane sample + Water *(Intact membrane)*	Centrifugation →	Intact Membrane	Electrospray solution preparation →	Membrane + 10% ethanol + 3mM ammonium acetate + DDM solution (0.01% v/v in water) *(Broken membrane; PSI, PSII and cytochrome b_6f solubilized with lipid and surfactant)*
Case 3- surfactant addition before centrifugation	Membrane sample + DDM solution (0.01% v/v in water) *(Broken membrane; PSI, PSII and cytochrome b_6f solubilized with lipid and surfactant)*	Centrifugation →	PSI, PSII and cytochrome b_6f with very less concentration of lipid and surfactant (<0.007 % v/v)	Electrospray solution preparation →	Membrane + 10% ethanol + 3mM ammonium acetate + water *(Broken membrane; PSI, PSII and cytochrome b_6f solubilized with very low concentration of lipids and surfactant)*

Table S2. Electrospray solution properties and conditions for deposition.

Spray solution properties				Electrospray conditions			Droplet properties
Solvent	Dielectric constant	Ammonium Acetate Concentration	Conductivity	Flow rate	Cone-jet Voltage	Current	Mean droplet size[i]
	-	mM	$\mu S\ cm^{-1}$	$\mu L\ min^{-1}$	kV	nA	nm
10% Ethanol in water (v/v)	72.3	3	235	1±0.01	5.85-6.2	650-700	702

[i]Using scaling law equation[2]

Electrospray Deposition

An in-house electrospray deposition was used as described in previous study[3] The membrane solution was pumped at a flow rate of 1 $\mu L\ min^{-1}$ through a syringe connected to a needle using a syringe pump. The needle had an inner diameter of 125 μm and was tapered at the machine shop at Washington University in St. Louis. High voltage was applied to the needle and the TiO_2 deposited substrate was grounded. CO_2 sheath flow rate of 20 $cc\ min^{-1}$ was supplied in the chamber to prevent the corona discharge of surrounding fluid. The voltage is adjusted to 5.85-6.2 kV using high voltage source with the current of 650-700 nA to get a cone-jet mode for electrospray deposition. The tip of the needle is monitored using a camera. The deposition was carried out for 10 min, 20 min, and 40 min for all the three cases. Absorption spectra of the membrane are measured in solution and after deposition using Shimadzu UV 2600 Spectrophotometer.

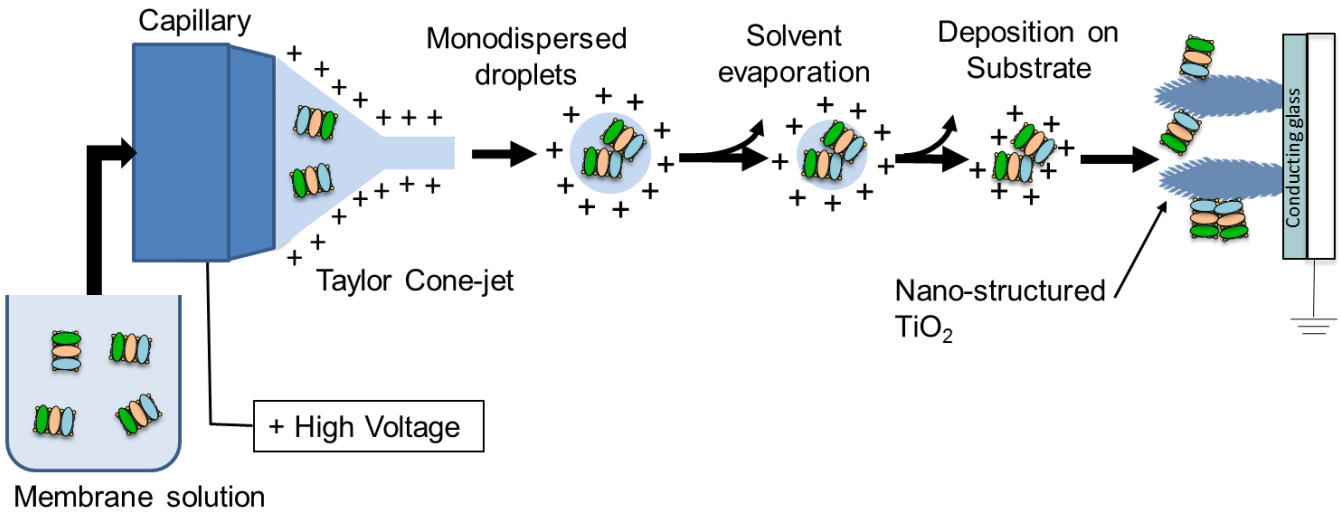

Fig. S1 Overview of linker-free deposition of thylakoid membrane on nanostructured TiO_2 columns using electrospray

Photo-electrochemical Characterization:

Three electrode photo-electrochemical characterization of the membrane sensitized TiO_2 working electrode was performed using an in-house electrochemical set up. Pt wire was used as counter electrode and Ag/AgCl as reference electrode. Light source used was 450 W Xe arc lamp (Newport Corporation, CA). The electrode was tested with 0.1 M KCl solution and linear sweep voltammetry was performed using a VersaStat 4 (Princeton Applied Research, TN) potentiostat. No electron donor or acceptor was added to the electrolyte. Water filter was used to block infrared wavelength and 400 nm cut-off filter (Newport Corporation, CA) is used to block UV wavelengths where needed. Incident photo-to-current conversion efficiency (IPCE) was measured in visible range (400-750nm) using a monochromator in front of the xenon lamp to select the wavelength of the light. IPCE was calculated from the equation given in reference[4]

(SI-2) SEM image of 1-D columnar structure of TiO_2

SEM image of the 1-D, single crystal, nanostructured TiO_2 columns shows the average column height of 1.6 μm. A previous study[1] has shown this to be the optimal morphology and height for PEC characterization.

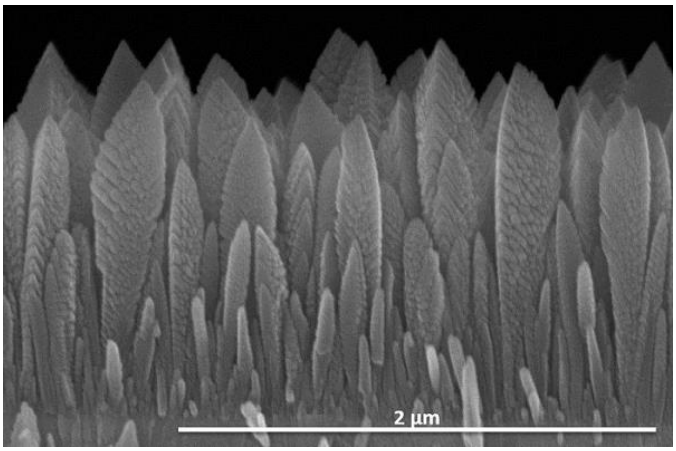

Fig. S2 SEM image of 1-D single crystal nanostructured TiO_2

(SI-3) XRD results of TiO_2

XRD spectra of the TiO_2 nanostructures presented in Figure S2 shows the highest peak for (112) planes and few small peaks for other planes, confirming the anatase phase of TiO_2. The highest intensity of the peak for (112) validate the absence of grain boundaries thus confirms the single crystal structure of TiO_2.

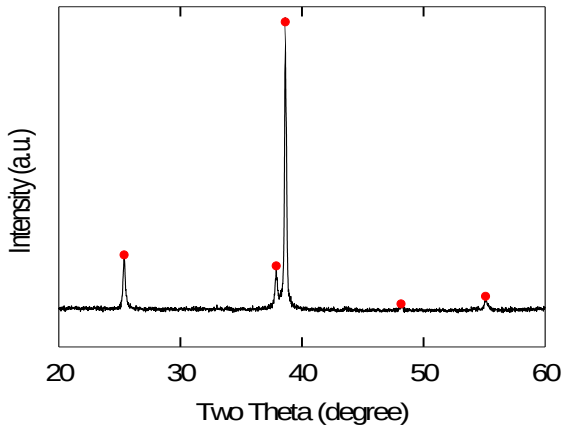

Fig. S3 XRD spectra of 1-D single crystal nanostructured TiO_2

(SI-4) Calculation of surfactant concentration profile with time and distance travelled by PSI, PSII and cytochrome b_6f[5]

Calculation of the surfactant concentration profile with time can be solved by the diffusion equation:

$$c(t) = \frac{N}{A}\frac{1}{2\sqrt{\pi Dt}}\exp(\frac{-x^2}{4Dt}) \quad \text{[Eq. (1)]}$$

Where, c(t) is the concentration of surfactant at a distance x away from the surface, t is the time, D is the diffusion coefficient, N is the amount of DDM deposited and A is the surface area. Although the surfactant is deposited over columnar TiO_2, all of the surfactant is assumed to be located on the surface (at x = 0). The concentration of surfactant with time can be calculated by:

$$c_{CMC} = \frac{N/_A}{2\sqrt{\pi Dt}} \quad \text{[Eq. (2)]}$$

Table (3a) shows the detailed parameters used in the calculation. [Eq.(2)] is used to solve for the time required to reach a concentration below CMC. During that time, PSI, PSII and cytochrome b_6f may diffuse into the electrolyte. The diffusion length of these molecules can be calculated by the root mean square distance

$$L = \sqrt{2Dt} \quad \text{[Eq. (3)]}$$

where D is the diffusion coefficient and t is time. The diffusion coefficient of one molecule of PSI, PSII and cytochrome b_6f is calculated by Stoke-Einstein equation:

$$D = \frac{kT}{3\pi\mu d} \quad \text{[Eq. (4)]}$$

Where: μ is the viscosity of solution, k is Boltzmann constant, d is the size of the spherical particle. Using the diffusion coefficient from [Eq. (4)] and time from [Eq. (2)], the distance travelled by individual PSI, PSII and cytochrome b_6f are calculated and listed in Table 1 in the paper. Table S3 (a) provides the value of the parameter used in this calculation and Table S3 (b) shows the calculation of diffusion coefficient of PSI, PSII and cytochrome b_6f.

Table S3 (a) Parameters used for the calculation of diffusion time of surfactant and diffusion length of PSI, PSII and cytochrome b_6f**. (b)** Diffusion coefficient of PSI, PSII and cytochrome b_6f using Stoke-Einstein equation**.**

(a)

DDM added	0.01 % v/v of water
Water added	180 µL
DDM concentration	0.009 % v/v
Flow rate	1 µL min^{-1}
Deposition area	0.1 cm^2
Diffusion coefficient of DDM[6]	5.4×10^{-10} m^2 s^{-1}
CMC of DDM	0.007 % v/v
Temperature	293.15 K
Solution Viscosity	0.00089 Pa.s
k_b (Boltzmann constant)	1.38×10^{-23} J K^{-1}

(b)

	Volume equivalent diameter (Å)	**Diffusion coefficient (m^2 s^{-1})**
PSI[5]	220	2.19×10^{-11}
PSII[7]	100.30	4.81×10^{-11}
Cytochrome *b_6f*[7]	74.81	6.45×10^{-11}

(SI-5) Absorption Spectra of the membrane in solution and after deposition

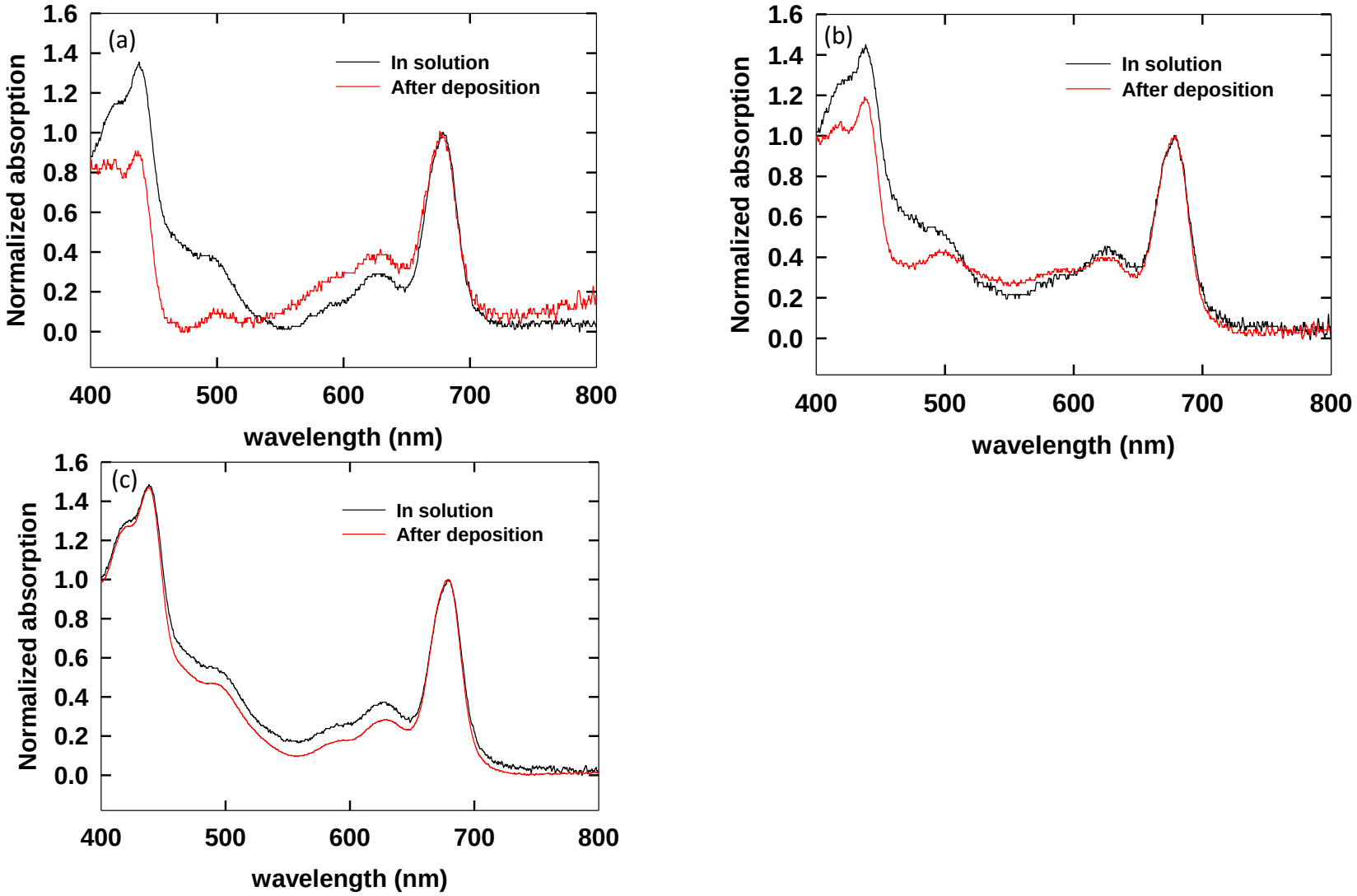

Figure S4. Absorption Spectra of the membrane in solution and after deposition ITO slide measured for (a) Case 1 –no surfactant addition, (b) Case 2 -surfactant addition after centrifugation and, (c) Case 3 -surfactant addition before centrifugation

(SI-6) Onset potential values

Table S4 Onset potential values (in volts) for bare TiO_2 and the three cases from the Linear scan voltammetry measurements under (a) UV and visible light (250-900 nm) illumination (b) Visible light (400-900 nm) for all the deposition time considered for

(a)

In UV and visible light			
Deposition time	10 min	20 min	40 min
bare TiO_2	0.866		
Case 1	0.896	0.940	0.931
Case 2	0.920	0.928	0.906
Case 3	0.870	0.954	0.952

(b)

In Visible light			
Deposition time	10 min	20 min	40 min
bare TiO_2	0.366		
Case 1	0.482	0.488	0.470
Case 2	0.460	0.471	0.452
Case 3	0.438	0.460	0.458

(SI-7) Linear sweep voltammetry results for case 2

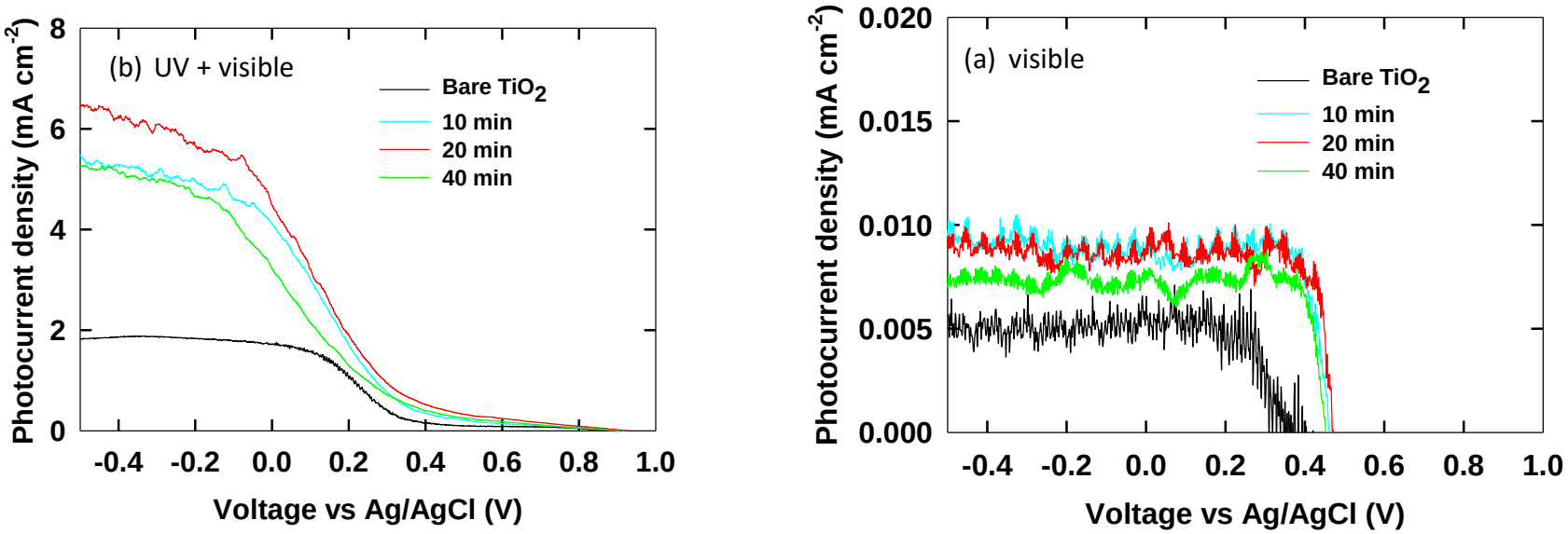

Fig. S5 Linear sweep voltammetry under (a) UV and visible light illumination (b) only visible light illumination for bare TiO_2 (black), sensitized TiO_2 for different deposition time 10 min (cyan), 20 min (red) and 40 min (green) for the case 2-when surfactant is added after centrifugation

(SI-8) Photocurrent density values

Table S5. Photocurrent densities values from the Linear scan voltammetry measurements under (a) UV and visible light (250-900 nm) illumination (b) Visible light (400-900 nm) for all the deposition time considered for bare TiO_2 and for the three cases.

(a)

Photocurrent density in UV and visible light (mA cm^{-2})			
Deposition time	10 min	20 min	40 min
bare TiO_2	1.833		
Case 1	2.55± 0.08	6 ± 0.17	4.05 ± 0.25
Case 2	5.25 ± 0.1	6.25 ± 0.15	4.8 ± 0.4
Case 3	4.4 ± 0.4	6.7 ± 0.15	6 ± 0.07

(b)

Photocurrent density in Visible light (µA cm^{-2})			
Deposition time	10 min	20 min	40 min
bare TiO_2	0.005		
Case 1	6.65	7.72	7.49
Case 2	9.1	8.22	7.22
Case 3	6.64	11.52	8.88

(SI-9) Photocurrent action spectra for case 1 and case 2

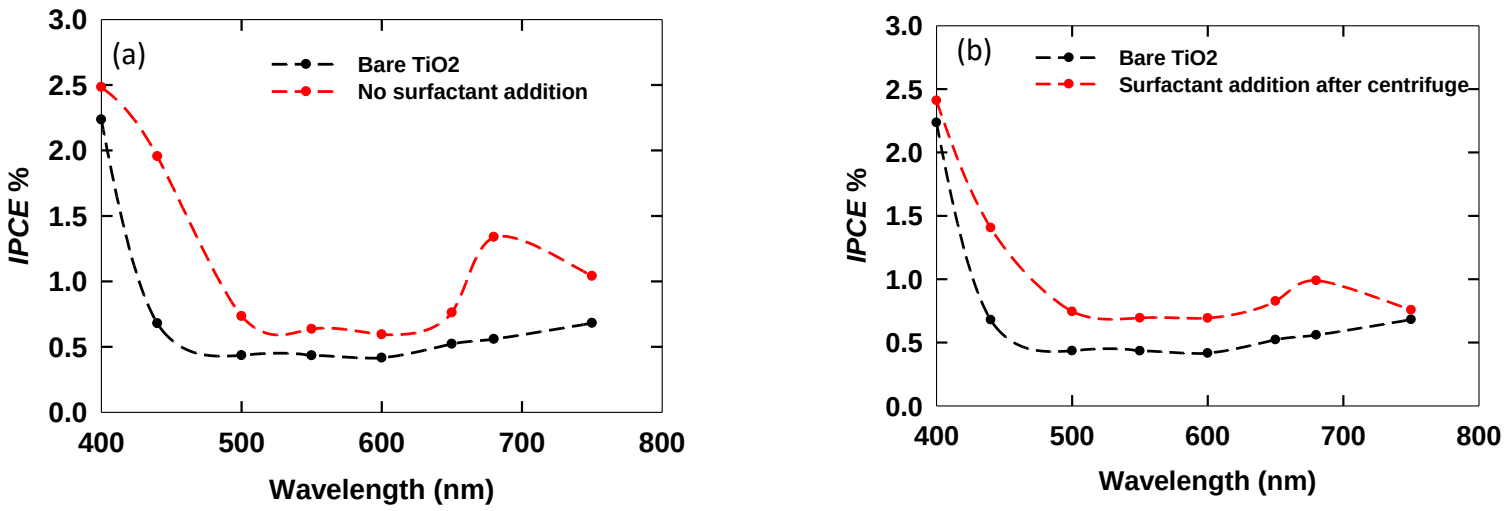

Fig. S6 Photocurrent action spectra for bare TiO_2 (black) and sensitized TiO_2 (red) for (a) Case 1- no surfactant addition and (b) Case 2- surfactant addition after centrifugation.

(SI-10) References

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