We report on a rational protocol to improve the orientation of a cyanobacterial PSII on a self-assembled monolayer (SAM)-functionalized mesoITO electrode resulting in improved interfacial electronic communication between ITO and PSII. Covalent immobilization of oriented PSII to the electrode surface further improved DET and the stability of the enzyme (Figure 1).

MesoITO electrodes with a geometrical surface area of 0.25 cm² were synthesized by annealing a paste of ITO nanoparticles (<50 nm diameter, 20 wt% in acetic acid/EtOH) on ITO-coated glass slides at 450 °C. The mesoITO electrodes (sheet conductivity and high PSII loading. Several redox enzymes with biotechnological relevance, such as hydrogenases and laccases, were previously immobilized on electrodes in a controlled orientation to improve DET and the electrostability of the enzyme in protein film electrochemistry. Compared to these enzymes, the controlled immobilization of PSII on electrodes is not straightforward: PSII is photoactive and relatively huge (a molecular weight of ~700 kDa and a size of 10.5 × 20.5 × 11.0 nm³ for cyanobacterial PSII dimers), and its electron donation sites to electrodes are placed on one region at the stromal side of PSII (Figure 1).

Figure 1. Schematic representation for the assembly of (A) mesoITO|SAM-CO$_2$$_-$-, (B) electrostatic immobilization of PSII (c-mesoITO|SAM-CO$_2$$_-$|PSII), and (C) covalent bonding (c-mesoITO|SAM-CO$_2$$_-$|PSII). (D) Red-light-driven DET and 2,6-dichloro-1,4-benzoquinone (BQ) MET resulting in photocurrents with c-mesoITO|SAM-CO$_2$$_-$|PSII.

Solar light driven splitting of water into its elements is a sustainable route to generate hydrogen-based fuels. A major challenge in water splitting is the thermodynamically uphill reaction of water oxidation to O₂, which involves a four-electron and four-proton transfer process. Photosystem II (PSII) is the natural water oxidation enzyme managing light absorption, charge separation, and the extraction of electrons and protons through the oxidation of water in a pH neutral aqueous solution. PSII operates at a high rate; it oxidizes ~100 water molecules per second during full sunlight irradiation with an upper turnover number estimated to be ~1 × 10⁹ within the lifetime of the [Mn$_4$Ca] water oxidation catalyst. PSII therefore serves as the unchallenged benchmark and an inspiration in photocatalytic water oxidation.

Protein film photoelectrochemistry (PF-PEC), the photochemical analogue to protein film electrochemistry, is currently in its infancy as a probe to study the function of photosynthetic enzymes, such as photosystem I and PSII. PF-PEC is thereby a promising technique to study the photocatalytic rates and performance of photosynthetic enzymes under different conditions. We have recently reported on direct electron transfer (DET) from PSII to a mesoporous indium tin oxide (mesoITO) electrode. PSII was adsorbed to the bare ITO surface in a random orientation and the three-dimensional and transparent mesoITO electrode allowed for good electrical conductivity and high PSII loading. Several redox enzymes, such as photosystem I and PSII, were previously immobilized on electrodes in a controlled orientation to improve DET and the electrostability of the enzyme in protein film electrochemistry. Compared to these enzymes, the controlled immobilization of PSI on electrodes is not straightforward: PSI is photoactive and relatively huge (a molecular weight of ~700 kDa and a size of 10.5 × 20.5 × 11.0 nm³ for cyanobacterial PSI dimers), and its electron donation sites to electrodes are placed on one region at the stromal side of PSI (Figure 1).
resistance of ~8 kΩ sq⁻¹, sheet thickness of ~3 μm)⁶⁻⁹ were modified with different phosphonic acid-linked SAMs (meso-ITO SAMs). Phosphonic acids are commonly used to modify inorganic oxide surfaces including ITO with high surface coverage and stability in aqueous solutions.⁹,¹⁰ The phosphonic acid compounds (1 mM, Chart 1) were self-assembled on mesoITO from an ethanolic solution containing NET₃ (2 mM) for 24 h, followed by heating at 140 °C under N₂ for 24 h and rinsing with NET₃ (5% v/v) in EtOH (see Supporting Information, SI). The dangling carboxylic acid and amine functionality resulted in negatively and positively charged SAMs under pH neutral conditions, respectively.

Protein film electrochemistry requires only a small amount of enzyme sample to give good quality electrochemical data.² We minimized the amount of sample required in this study, and 2 μL of a PSII solution (0.067 mg Chlorophyll a mL⁻¹, 1.1 pmol PSII) were drop-cast on mesoITO SAM. After 30 min in the dark, the electrode was rinsed with a buffered solution (10 mM MES, pH 6) to remove weakly bound PSII from the surface before immersing the electrode into the electrolyte buffer. This procedure allowed for electrostatic immobilization of PSII on the charged mesoITO SAM surface (e-mesoITO SAM/PSII). The optical transparency of ITO⁹,¹⁰ allowed us to record electronic absorption spectra in transmission mode of e-mesoITO SAM/PSII, which showed the expected features of PSII on ITO with an absorbance maximum at 675 nm (see Figure S1).¹¹ The amount of PSII on the electrode was quantified by recording UV–vis absorption spectra of chlorophyll a in PSII in solution after desorption of the enzyme from ITO (see SI) for mesoITO PSI without SAM (0.27 ± 0.07 pmol), e-mesoITO SAM-C₂NH₃⁺ PSI (0.35 ± 0.05 pmol), and e-mesoITO SAM-C₂CO₂⁻ PSI (0.39 ± 0.03 pmol, Table 1). Photocurrent responses of mesoITO PSI (in the absence of SAM, unmodified mesoITO), e-mesoITO SAM-C₂CO₂⁻ PSI, and e-mesoITO SAM-C₂NH₃⁺ PSI were recorded under red-light irradiation (679 nm, 10 mW cm⁻²) at pH 6.5 at 25 °C (Figure 2). DET occurred from PSII to ITO in all cases, but the carboxylic acid SAM resulted in the highest photocurrent response (0.28 ± 0.07 μA cm⁻², Table 1). A 3-fold increase in initial DET photocurrent was observed for e-mesoITO SAM-C₂CO₂⁻ PSI compared to mesoITO PSI without SAM and e-mesoITO SAM-C₂NH₃⁺ PSI. No significant difference was observed in photocurrent responses of mediated electron transfer (MET) between mesoITO PSI without SAM, e-mesoITO SAM-C₂CO₂⁻ PSI, and e-mesoITO SAM-C₂NH₃⁺ PSI (0.9–1.4 μA cm⁻²), where a differential redox mediator, 2,6-dichloro-1,4-benzoquinone, was used. This observation suggests that the negatively charged mesoITO SAM-C₂CO₂⁻ surface resulted in improved orientation of PSII on ITO and, consequently, enhanced interfacial electronic communication between PSII and ITO. No photocurrent was observed in the absence of PSII for mesoITO SAM-C₂CO₂⁻ PSI in the absence of the [Mn,Ca] water oxidation catalyst (Mn-depleted PSI)¹¹ on e-mesoITO SAM-C₂CO₂⁻ PSI, resulting in only a minor photocurrent (<0.02 μA cm⁻²), demonstrating that the photocurrent response resulted from water oxidation.

Table 1. Summary of DET and MET photocurrent responses (j, μA cm⁻²), amounts of PSII immobilized on mesoITO (pmol) and turnover frequencies (TOFs, mol O₂ (mol PSII)⁻¹ s⁻¹) of e- and c-mesoITO SAM/PSII electrodes

| e-mesoITO SAM/PSII | j, μA cm⁻² | PSII, pmol | DET, mol O₂ (mol PSII)⁻¹ s⁻¹ | MET, mol O₂ (mol PSII)⁻¹ s⁻¹ |
|-------------------|------------|------------|-----------------------------|-----------------------------|
| SAM-C₂CO₂⁻        | 0.28 ± 0.07 | 1.4 ± 0.6   | 0.39 ± 0.03                  | 0.33 ± 0.07                  |
| SAM-C₂NH₃⁺        | 0.09 ± 0.02 | 1.1 ± 0.1   | 0.35 ± 0.05                  | 0.09 ± 0.02                  |
| unmodified         | 0.08 ± 0.01 | 0.9 ± 0.1   | 0.27 ± 0.07                  | 0.18 ± 0.02                  |
| e-mesoITO SAM/PSII|             |            |                             |                             |
| SAM-C₂CO₂⁻        | 0.43 ± 0.03 | 4.5 ± 0.2   | 0.50 ± 0.06                  | 0.61 ± 0.12                  |
| SAM-C₂CO₂⁻        | 0.25 ± 0.03 | 3.4 ± 0.8   | 0.38 ± 0.02                  | 0.37 ± 0.10                  |
| <0.02⁴           |            |            |                             |                             |

"Initial photocurrents measured with a bias potential of +0.5 V vs NHE in an aqueous electrolyte buffer solution (pH 6.5) under red-light illumination (679 nm, 10 mW cm⁻²) at 25 °C. Photocurrent responses for MET were recorded in the presence of 2,6-dichloro-1,4-benzoquinone (1 mM) in the electrolyte solution. "Amount of PSII on a mesoITO electrode (0.25 cm² geometrical surface area). "TOFs were calculated based on photocurrent densities obtained during 30 s red-light illumination with four electrons per O₂. "No photocurrent detected.
Photoinduced DET from PSII to an electrode can only take place through one region at the stromal side of PSII (Figure 1), and the results with electrostatically immobilized PSII can be explained by taking into consideration the dipole moment of PSII. Its positive dipole vector is located on the stromal side of the thylakoid membrane, where the potential electron donor sites, in particular the quinones QA and Qb, are located (Figure 1). Analysis of the crystal structures of PSII from Thermosynechococcus elongatus and Thermosynechococcus vulcanus showed strong dipole moments of 17357 and 16513 D, respectively, from the luminal surface to the stromal surface (Figure S2).13 PSII can therefore orient itself on negatively charged surfaces in a favorable orientation during the adsorption process, which is in agreement with the 3-fold enhanced interfacial DET using the mesoITO-SAM-C2CO2− electrode.

Subsequently, we investigated the effect of covalent immobilization of oriented PSII on carboxylate-functionalized mesoITO. Treatment of c-mesoITO-SAM-C2CO2−|PSII with 6 µL of the coupling agents EDC (36 mM) and NHS (17 mM) in MES solution (10 mM, pH 6.5) for 2 h yielded covalently amide-bonded PSII electrodes (c-mesoITO-SAM|PSII, Figure 1). The covalent bonding method led to the immobilization of 0.50 ± 0.06 pmol of PSII on c-mesoITO-SAM-C2CO2−|PSII.

The covalently bonded PSII shows further enhanced photocurrents (0.43 ± 0.03 µA cm−2) compared to electrostatically adsorbed PSII on mesoITO (Table 1). The covalent immobilization of PSII on ITO also resulted in an increased stability of the photocurrent responses compared to electrostatically adsorbed PSII (Figure 2B). c-mesoITO-SAM-C2CO2−|PSII showed a significantly slower photocurrent decay (half-life time ∼12 min) compared to the electrostatically bonded PSII on the SAM-functionalized and SAM-free mesoITO electrodes (both have half-life times of <5 min) during continuous red-light illumination. About 20% and 10% of the initial photocurrent remained after 1 h of continuous light illumination for covalently and electrostatically bonded PSII, respectively (Figure 2B). A positively charged ITO surface (isoelectric point of ITO: 7.4)14 and a positive bias potential (+0.5 V vs NHE) result presumably in electrostatic repulsion from PSII to ITO with a short alkyl linker, but only MET (no DET) with the insulating long alkyl chain SAM.6c,11,16

Turnover frequencies (TOFs) for DET on c-mesoITO-SAM-C2CO2−|PSII were determined to be 0.61 ± 0.12 and 4.6 ± 0.6 (mol O2) (mol PSII)−1 s−1 for DET and MET, respectively, where we assumed all PSII dimers on the electrode were photoactive (see SI). The TOF for DET with c-mesoITO-SAM-C2CO2−|PSII, 0.61 ± 0.12 s−1, is higher than for c-mesoITO SAM-C15CO2−|PSII (0.53 ± 0.07 s−1) and mesoITO|PSII (0.18 ± 0.02 s−1), supporting that immobilization of oriented PSII was achieved by covalent linkage after electrostatic preorientation. The photon-to-current conversion efficiency of c-mesoITO-SAM-C2CO2−|PSII was determined to be ∼0.01% and 0.1% for DET and MET, respectively (see SI). This result is a lower estimate, because it was assumed that the electrode absorbs all incident photons, and the quantum efficiency of PSII also increases with a lower light intensity.6c

In conclusion, we demonstrated covalent immobilization of preoriented PSII on a carboxylate-functionalized mesoITO electrode for visible light-driven water oxidation. Electrostatic interactions between PSII and the negatively charged mesoITO electrode orient PSII via its dipole moment in its favorable orientation for interfacial electron transfer. The carboxylates from the SAM can be covalently coupled with EDC and NHS to amine residues from amino acids in PSII. PF-PEC of PSII allowed us to determine a TOF for O2 evolution of 0.61 ± 0.12 s−1 for DET using c-mesoITO-SAM-C2CO2−|PSII. The reported approach provides access to biophysical studies, such as mechanistic PF-PEC of PSII, and can be extended to other photosynthetic enzymes.

ASSOCIATED CONTENT

Supporting Information
Experimental details, figures, and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

dx.doi.org/10.1021/jacs.3b04699
J. Am. Chem. Soc. 2013, 135, 10610−10613
