# Langmuir

# Photoelectrochemistry of Photosystem I Bound in Nafion

David R. Baker,<sup>†</sup> Richard F. Simmerman,<sup>‡</sup> James J. Sumner,<sup>†</sup> Barry D. Bruce,<sup>‡</sup> and Cynthia A. Lundgren<sup>\*,†</sup>

<sup>†</sup>U.S. Army Research Laboratory, Sensors and Electron Devices Directorate, Adelphi, Maryland 20783, United States

<sup>‡</sup>Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee-Knoxville, Knoxville, Tennessee 37996, United States

**Supporting Information** 

**ABSTRACT:** Developing a solid state Photosystem I (PSI) modified electrode is attractive for photoelectrochemical applications because of the quantum yield of PSI, which approaches unity in the visible spectrum. Electrodes are constructed using a Nafion film to encapsulate PSI as well as the hole-scavenging redox mediator  $Os(bpy)_2Cl_2$ . The photoactive electrodes generate photocurrents of 4  $\mu$ A/cm<sup>2</sup> when illuminated with 1.4 mW/cm<sup>2</sup> of 676 nm band-pass filtered light. Methyl viologen (MV<sup>2+</sup>) is present in the electrolyte to scavenge photoelectrons from PSI in the Nafion film and transport charges to the counter electrode. Because MV<sup>2+</sup> is positively charged in both reduced and oxidized states, it is able to diffuse through the cation permeable channels of Nafion. Photocurrent is produced when the working electrode is set to voltages negative of the Os<sup>3+</sup>/Os<sup>2+</sup> redox potential. Charge transfer through the Nafion film and photohole scavenging at



the PSI luminal surface by  $Os(bpy)_2Cl_2$  depends on the reduction of Os redox centers to  $Os^{2+}$  via hole scavenging from PSI. The optimal film densities of Nafion (10  $\mu$ g/cm<sup>2</sup> Nafion) and PSI (100  $\mu$ g/cm<sup>2</sup> PSI) are determined to provide the highest photocurrents. These optimal film densities force films to be thin to allow the majority of PSI to have productive electrical contact with the backing electrode.

## INTRODUCTION

Designing systems to incorporate electroactive biomolecules ex situ has been an active area of research for several decades.<sup>1,2</sup> A wide variety of proteins and enzymes incorporated within polymer matrices have been successfully cast onto electrode surfaces in high surface concentrations for electrochemical purposes, such as in sensors and fuel cell applications.<sup>3–9</sup> Initial research into the activity of isolated cytochromes deposited on electrodes in polymer films demonstrated that proteins could be directly accessed electrochemically while simultaneously protected from dissolution and denaturation.<sup>10</sup> Biosensors functionalized with polymer-encapsulated enzymes, especially glucose oxidase, have been used to monitor biochemistry on electrode surfaces and have contributed to the understanding of various biochemical mechanisms.<sup>11-13</sup> The stability and high activity of polymer-bound electrochemical macromolecules allows them to be employed commercially as cost-effective biosensors, such as in blood-sugar monitors for diabetic patients.<sup>14</sup> Recently, advancements in bioelectronics have incorporated redox molecules and other electron transfer additives, such as carbon nanotubes, to further enhance the signal output pushing detection limits below  $5 \times 10^{-8}$  M.<sup>15–18</sup>

Similarly, the goal for biomimetic solar devices is to increase electron transfer rates between isolated, light-harvesting complexes, and electrode surfaces. Recently, photosynthetic reaction centers, such as photosystem I (PSI), photosystem II (PSII), and others, have been incorporated onto electrode surfaces to harvest solar energy for either photovoltaic or solar fuel (H<sub>2</sub> evolution) purposes.<sup>19–27</sup> These photoactive proteins have been primarily activated electrochemically either by attachment to functionalized surfaces or encapsulation into

redox-polymer matrices. Just as in previously developed biosensing electrodes, the surface protein concentration can be increased beyond the monolayer coverage of functionalized surfaces by using conductive polymers to retain photoactive proteins in a 3D matrix, thereby increasing the amount of light that can be absorbed.

This study characterizes the embedding of cyanobacterial PSI, isolated from Thermosynechococcus elongatus (T. elongatus), in the ionomer Nafion by measuring the photoelectrochemical properties of electrodes. PSI is attractive for use in biomimetic solar devices because upon photon absorption, it produces a long-lived photoelectron that is spatially separated from its corresponding photohole by 7 nm, resulting in low backtransfer rates and an internal quantum yield near unity.<sup>28,29</sup> PSI has also shown an ability to remain stable over long time scales when employed in solar harvesting devices, with reports of retaining its activity months after device construction.<sup>30,31</sup> Nafion has long been incorporated in biosensors to house enzymes and proteins because it has a high retention for uncharged and negatively charged species, which prevents significant leaking of signal molecules.<sup>32–36</sup> Conversely, Nafion permits transport of positively charged species allowing for electrochemical connectivity if the mediator in the electrolyte is positively charged, such as a viologen species. In addition to these characteristics, Nafion does not absorb visible wavelengths of light. These properties make Nafion a widely used polymer matrix that can be employed to house photosynthetic

Received: August 5, 2014 Revised: October 17, 2014 Published: October 23, 2014



Figure 1. Scheme depicting operation of PSI-based Nafion-Os(bpy)<sub>2</sub>Cl<sub>2</sub> photoelectrochemical electrode. Photoelectrons ( $e^-$ ) are extracted by MV<sup>2+</sup> ions, which are turned over at the counter electrode (CE). Photoholes ( $h^+$ ) are scavenged by and transferred to the back electrode (Au) by Os(bpy)<sub>2</sub>Cl<sub>2</sub> molecules (red clovers).

protein complexes, such as PSI, for energy conversion applications.<sup>18</sup>

#### EXPERIMENTAL SECTION

Electrode substrates were fabricated with indium-doped tin oxide glass slides (15 $\Omega$ , Delta Technologies) coated with 300 nm Au by e-beam evaporation. An aqueous mixture of Nafion, Os(bpy)<sub>2</sub>Cl<sub>2</sub>, and PSI was drop-cast onto the Au surface and dried in air, resulting in a film  $\sim$ 3  $\mu$ m thick. Once dry, both Os(bpy)<sub>2</sub>Cl<sub>2</sub> and PSI were trapped in the polymer as a result of charge and size limitations. The surface concentration of Os(bpy)<sub>2</sub>Cl<sub>2</sub> in the dried film was held constant at 2 nmol/cm<sup>2</sup>, and the PSI surface density was 100  $\mu$ g/cm<sup>2</sup> PSI unless otherwise stated. The aqueous electrolyte of 0.1 M MgCl<sub>2</sub>, which contained 250  $\mu$ M methyl viologen dichloride (MV<sup>2+</sup>) as a redox mediator, was used to rehydrate the Nafion/PSI/Os(bpy)<sub>2</sub>Cl<sub>2</sub> film. MV<sup>2+</sup> acts to harvest photoelectrons from PSI at its stromal surface. MV<sup>2+</sup> is then theoretically oxidized at the counter electrode to act as a photovoltaic cell, though more likely is oxidized by dissolved oxygen in the electrolyte solution, as has been previously reported.<sup>25,37</sup> After the film was hydrated, the Nafion became a porous structure as a result of the charged sulfonate groups aligning with each other away from the hydrophobic fluorinated backbone.<sup>38</sup> These surface-charged pores allow cations, such as the  $MV^{2+}$  mediator, to move through the film while limiting the diffusion of anionic and uncharged species [PSI, Os(bpy)<sub>2</sub>Cl<sub>2</sub>]. Dissolution of PSI is also limited due to steric hindrances imposed by the Nafion matrix.

Samples were illuminated with 1.4 mW/cm<sup>2</sup> 676 nm bandpass filtered light. This wavelength targeted the red absorbance peak of chlorophyll within PSI. The system used a three-electrode configuration with an applied potential of -150 mV vs Ag/AgCl. This potential was applied for 5 min as a stabilization step to minimize unwanted transient dark currents. Measurements were made with a Gamry Reference 600 potentiostat. All photoelectrochemical experiments were performed under air.

#### RESULTS/DISCUSSION

 $Os(bpy)_2Cl_2$  was dispersed in the film to act as a hole transport species through the polymer.<sup>6,23,39</sup> After chlorophyll in PSI absorbed a photon, the energy was efficiently transferred to the

reaction center ( $P_{700}$ ) where an electron-hole pair was created. In nature, the photogenerated hole is scavenged by the charge transfer protein cytochrome  $c_6$  (for *T. elongatus*).<sup>40</sup> In this study,  $Os(bpy)_2Cl_2$  acted as a surrogate for cytochrome  $c_6$  by donating an electron to the  $P_{700}$  site and also conducted charges between PSI and the electrode surface via a hopping mechanism with other  $Os(bpy)_2Cl_2$  molecules in the film. A hopping mechanism for charge transfer is needed because of diffusion being slowed by the Nafion on the uncharged  $Os(bpy)_2Cl_2$ .<sup>38</sup> MV<sup>2+</sup> acted to mediate the transfer of electrons, most probably from the [4Fe–4S] center  $F_B$  of PSI to the counter electrode or dissolved  $O_2$ , creating an electron transfer pathway by which light provided the driving force, and the redox mediators shuttled charges from PSI to electrodes as depicted in Figure 1.

Photocurrent arose after the electrodes were illuminated and reached a steady current level within 15 s, seen in Figure 2 with the dark currents subtracted for clarity. When the light was turned off, the current in the system returned to background levels within 2 min. These slow rise and decay times were likely caused by the limited diffusional transport of mediators through the Nafion matrix.

The ability of the system to generate photocurrent was tested with various controls to confirm that current was arising solely from PSI. Without  $Os(bpy)_2Cl_2$  in the polymer film, there was no photocurrent observed, proving that some charge mediator is needed to extract photoholes from PSI, as has been suggested in previous studies.<sup>25,41</sup> The need for a surrogate holescavenging redox mediator has been shown to be unnecessary if PSI is isolated from plant or algal sources because  $P_{700}$ reduction is performed by a different charge transfer protein (cytochrome  $c_6$  or plastocyanin) and because of differences in the lumenal surface of the PsaF subunit near the binding site of the soluble charge transfer protein.<sup>29,31,42</sup> Without PSI, the Nafion/Os(bpy)\_2Cl\_2 film also showed no activity in the MV<sup>2+</sup> electrolyte, demonstrating that Os(bpy)\_2Cl\_2 is not photoactive Langmuir





Figure 2. Photocurrent of Nafion/PSI/Os(bpy)<sub>2</sub>Cl<sub>2</sub> electrode (a), the controls of no  $Os(bpy)_2Cl_2$  (b), no PSI (c), and no  $MV^{2+}$  in solution (d). Incident light was 1.4 mW/cm<sup>2</sup> bandpass filtered light (676 nm), and the applied potential was -0.15 V vs Ag/AgCl.

under 676 nm light. When the Nafion/PSI/Os(bpy)<sub>2</sub>Cl<sub>2</sub> film was illuminated in an electrolyte solution devoid of MV<sup>2+</sup>, a small current was observed that was <20% that of the fully assembled system, represented by trace d in Figure 2. Without MV<sup>2+</sup> to extract photoelectrons the Os<sup>3+</sup> redox centers, present in the film after scavenging photoholes, may be able to accept excited electrons from PSI. The slow rise time to achieve maximum current levels makes this hypothesis difficult to test, although efforts are currently underway. Photoaction spectra were compared with PSI's absorbance spectrum used to confirm that PSI was the sole source of photocurrent (data shown in Figure S1 of the Supporting Information). In addition, no difference in photocurrent was observed when the system was purged with N<sub>2</sub>, indicating the current does not occur by the formation of superoxide (results not shown).

The use of redox mediators to regulate electron transport in the system required investigation of the proper potentials to apply at the working electrode. Current-voltage (I-V) curves shown in Figure 3 demonstrate that no photocurrent was observed in the oxidative region positive of 25 mV vs Ag/AgCl. After this onset potential, the cathodic photocurrent was seen to grow as further reducing potentials were applied until a saturation photocurrent was attained at approximately -100 mV vs Ag/AgCl. Application of further reducing potentials resulted in no increase of photocurrent, indicating that access to  $Os(bpy)_2Cl_2$  in the 2<sup>+</sup> state was no longer limiting. The onset of 25 mV vs Ag/AgCl corresponded to the redox potential of  $Os^{3+}/Os^{2+}$  in  $Os(bpy)_2Cl_2$  as determined by cyclic voltammetry (CV). This corroborates the suggested mechanism described above in which hole transport occurs by Os(bpy)<sub>2</sub>Cl<sub>2</sub> redox molecules scavenging photoholes from excited P700\* reaction centers and transferring them to the electrode surface. This process should not be viable until the working electrode is at a sufficiently reducing potential, that is, the reduction potential of  $Os^{3+}/Os^{2+}$ .

The reduction potential of Os(bpy)<sub>2</sub>Cl<sub>2</sub> was measured to be 0 V vs Ag/AgCl, and  $MV^{2+}$  was determined to be -0.7 V vs Ag/AgCl, both measured by CV. These values have appropriate band-matching with the corresponding potentials of the photohole (-0.3 V vs Ag/AgCl) and photoelectron (-0.7 V



Figure 3. Photocurrent response versus the applied potential of Nafion/Os(bpy)<sub>2</sub>Cl<sub>2</sub>/PSI films (a), control experiments without MV<sup>2+</sup> present in the electrolyte (b), and Os(bpy)<sub>2</sub>Cl<sub>2</sub> absent from the Nafion film (c).

vs Ag/AgCl) to direct charges in the desired cathodic direction. 43,44 As mentioned previously, the benefit to using MV<sup>2+</sup> as the electron scavenger is that the reduced species  $(MV^{+\bullet})$  is still positively charged. It is therefore able to diffuse through the Nafion film and oxidize at the counter electrode. When oxidized by PSI, the Os center of Os(bpy)<sub>2</sub>Cl<sub>2</sub> changes from an oxidation state of Os<sup>2+</sup> to Os<sup>3+</sup>, and the mediator becomes positively charged. The Os<sup>3+</sup> state will last only until it transfers charges to other Os centers or reaches the backing electrode, which is held at a reducing potential primarily to keep the Os population in an Os<sup>2+</sup> state unless reacting with PSI. It should be noted that some Os(bpy)<sub>2</sub>Cl<sub>2</sub> could possibly diffuse out of the film over time when in its positively charged state.

Interestingly, Figure 3b shows photocurrent without MV<sup>2+</sup> in solution to scavenge photoelectrons, which supports results observed in Figure 2d. Although the amount of current passed through the system was again significantly less without MV<sup>2+</sup> than with  $MV^{2+}$ , the presence of any photocurrent implies that electrons are still scavenged from PSI by another species in the system. As described above, the spatial and electrochemical confines within Nafion may allow the oxidized form of Os(bpy)<sub>2</sub>Cl<sub>2</sub> to accept photoelectrons from the stromal side of PSI after scavenging a photohole from the luminal P700 site.

Because diffusion of MV<sup>2+</sup> was the primary pathway for electron movement within the cell, the density of Nafion was extremely important for performance of the system and needed to be characterized. Photocurrent, after 5 min of hydration in the electrolyte solution, was measured at various Nafion concentrations (Figure 4). Photocurrent was seen to be negligible for Nafion surface concentrations of 100  $\mu g/cm^2$ and larger. Current peaked at 10  $\mu$ g/cm<sup>2</sup> Nafion and was effectively zero without Nafion present on the electrode surface. In the experiments without Nafion, there was no binder to hold PSI or  $Os(bpy)_2Cl_2$  to the electrode surface, and the drop-cast films were observed to simply dissolve, resulting in no current when exposed to light after the 5 min of equilibration time in the solution prior to testing. As the polymer became denser, the ability of redox mediators to transport through the film



**Figure 4.** Photocurrent response as a function of Nafion concentration. Films were constructed with a PSI concentration of 100  $\mu$ g/cm<sup>2</sup> PSI. Applied potential was -0.15 V vs Ag/AgCl. Error bars are 95% confidence intervals, n = 4.

therefore appeared to become limited, thereby generating less photocurrent. The use of any polymer to contain PSI will likely experience concentration-dependent effects.

The concentration of PSI in the Nafion film was also examined to determine how the addition of more lightabsorbing species could affect the performance of the film. Figure 5 illustrates how photocurrent responded to increasing



**Figure 5.** Photocurrent and electron turnover rate of the Nafion/ $Os(bpy)_2Cl_2/PSI$  electrodes at various PSI concentrations. Applied potential was -0.15 V vs Ag/AgCl and was under 1.4 mW/cm<sup>2</sup> of 676 nm bandpass filtered light. Error bars are 95% confidence intervals, n = 4.

PSI concentration. Initially, the overall photocurrent increased with PSI concentration, but a maximum was found around at  $\sim 100 \ \mu g/cm^2$  PSI ( $\sim 400 \ \mu g/cm^2$  chlorophyll). Higher concentrations of PSI caused a reduction in overall cell current. The peak in photocurrent was expected due to the geometry of the cell. Because of frontside illumination, PSI complexes at the surface absorb incident light before PSI closer to the electrode surface. Photoholes generated by these initial light absorbers

have many more hopping steps to reach the backing electrode, increasing the likelihood that they could be lost due to cumulative hopping efficiencies, resulting in a reduction of photocurrent. By converting photocurrents into electron turnover rates (MW =  $356\,000$  g/mol, 1/3 of the PSI trimer complex<sup>29</sup>), it is evident that with additional PSI added to the Nafion film, each individual PSI contributed fewer electrons. The turnover rates for PSI, even at low concentrations, were approximately an order of magnitude slower than in other polymer systems<sup>23,24</sup> and much slower than electrodes in which PSI is bound to functionalized surfaces.<sup>41</sup> The theoretical rate is dependent on the lifetime of the charge separated state in PSI, which is typically on millisecond time scales.<sup>45</sup> These time scales indicate that the turnover rate in the Nafion system still has massive room for improvement. The speed is likely limited by the interactions with redox mediators and the connectivity of each PSI complex. Efforts to understand and improve mediator-PSI kinetics are currently underway.

#### CONCLUSION

The use of Nafion as a scaffold to encapsulate PSI was shown to be an effective means of electrode construction that allowed for PSI to be both electrochemically active and stable. PSI was accessed electrochemically using the redox mediator Os-(bpy)<sub>2</sub>Cl<sub>2</sub> as the photohole scavenger and MV<sup>2+</sup> as the photoelectron scavenger. Characterization of the film showed that photocurrent is obtained when the applied potential is negative of the Os<sup>3+</sup>/Os<sup>2+</sup> reduction potential (0 mV vs Ag/ AgCl), which drives charge migration in the polymer film. The surface density of Nafion was found to have a trade-off behavior because lower concentrations provided high diffusivity for redox mediators, although higher density films are needed to prevent dissolution. A surface density of 10  $\mu$ g/cm<sup>2</sup> Nafion provided peak photocurrents of  $-4 \ \mu A/cm^2$ . The PSI optimization process also found that excess protein inhibits light-activated charge separation and charge transfer. In the current study, the concentration of PSI that generated peak photocurrent was determined to be 100  $\mu$ g/cm<sup>2</sup> PSI. Therefore, when constructing photoactive films of PSI in Nafion, many factors must be addressed, including selection of mediators and the loading density of both polymer and photoactive protein. The use of Nafion as a support matrix for PSI electrodes has here been shown to be a simple and useful platform from which future studies of solid state PSI electrodes can be investigated.

#### ASSOCIATED CONTENT

#### **S** Supporting Information

Photoaction spectra and absorbance spectra for electrodes with and without PSI. This material is available free of charge via the Internet at http://pubs.acs.org.

#### AUTHOR INFORMATION

#### **Corresponding Author**

\* Address: 2800 Powder Mill Rd. Adelphi, MD 20783, USA. Phone: (301) 394-2541. E-mail: cynthia.a.lundgren2.civ@mail. mil.

#### Notes

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

Financial support from the U.S. Department of the Army and U.S. Army Materiel Command are gratefully acknowledged.

Research was supported, in part, by a contractual appointment to the U.S. Army Research Laboratory Postdoctoral Fellowship Program administered by the Oak Ridge Associated Universities (D.R.B.). B.D.B. acknowledges support from TN-SCORE, a multidisciplinary research program sponsored by NSF-EPSCOR (EPS-1004083), and support from the Gibson Family Foundation. R.F.S. was supported as an IGERT fellow from the National Science Foundation IGERT program (DGE-0801470). We also acknowledge support from the Directors Strategic Initiative, "Understanding Photosystem I as a Biomolecular Reactor for Energy Conversion" at the Army Research Laboratory, Adelphi, MD, USA (ARL Contract #W911NF-11-2-0029).

#### REFERENCES

(1) Santhanam, K. S. V.; Jespersen, N.; Bard, A. J. Application of a Novel Thermistor Mercury Electrode to the Study of Changes of Activity of an Adsorbed Enzyme on Electrochemical Reduction and Oxidation. J. Am. Chem. Soc. **1977**, 99 (1), 274–276.

(2) Guilbault, G. G.; Kramer, D. N.; Cannon, P. L. A New, General Electrochemical Method of Determining Enzyme Kinetics. Kinetics of the Enzymic Hydrolysis of Thiocholine Iodide Esters. *Anal. Biochem.* **1963**, 5 (3), 208–216.

(3) Osman, M. H.; Shah, A. A.; Walsh, F. C. Recent Progress and Continuing Challenges in Bio-Fuel Cells. Part I: Enzymatic Cells. *Biosens. Bioelectron.* **2011**, *26* (7), 3087–3102.

(4) Guo, L. H.; Hill, H. A. O. Direct Electrochemistry of Proteins and Enzymes. *Adv. Inorg. Chem.* **1991**, *36*, 341–375.

(5) Habermuller, K.; Ramanavicius, A.; Laurinavicius, V.; Schuhmann, W. An Oxygen-Insensitive Reagentless Glucose Biosensor Based on Osmium-Complex Modified Polypyrrole. *Electroanalysis* **2000**, *12* (17), 1383–1389.

(6) Gregg, B. A.; Heller, A. Redox Polymer Films Containing Enzymes. 1. A Redox-Conducting Epoxy Cement – Synthesis, Characterization, and Electrocatalytic Oxidation of Hydroquinone. *J. Phys. Chem.* **1991**, 95 (15), 5970–5975.

(7) Ohara, T. J.; Rajagopalan, R.; Heller, A. Wired Enzyme Electrodes for Amperometric Determination of Glucose or Lactate in the Presence of Interfering Substances. *Anal. Chem.* **1994**, *66* (15), 2451–2457.

(8) Ohara, T. J. Osmium Bipyridyl Redox Polymers Used in Enzyme Electrodes. *Platinum Met. Rev.* **1995**, *39* (2), 54–62.

(9) Foulds, N. C.; Lowe, C. R. Enzyme Entrapment in Electrically Conducting Polymers – Immobilization of Glucose-Oxidase in Polypyrrole and Its Application in Amperometric Glucose Sensors. J. Chem. Soc., Faraday Trans. 1 1986, 82, 1259–1264.

(10) Bianco, P.; Haladjian, J. Electrochemistry of Ferredoxin and C-Type Cytochromes at Surfactant Film-Modified Pyrolytic Graphite Electrodes. *Electrochim. Acta* **1997**, *42* (4), 587–594.

(11) Nakabayashi, Y.; Omayu, A.; Yagi, S.; Nakamura, K. Evaluation of Osmium(II) Complexes as Electron Transfer Mediators Accessible for Amperometric Glucose Sensors. *Anal. Sci.* **2001**, *17* (8), 945–950.

(12) Guschin, D. A.; Castillo, J.; Dimcheva, N.; Schuhmann, W. Redox Electrodeposition Polymers: Adaptation of the Redox Potential of Polymer-Bound Os Complexes for Bioanalytical Applications. *Anal. Bioanal. Chem.* **2010**, 398 (4), 1661–1673.

(13) Calvo, E. J.; Etchenique, R.; Pietrasanta, L.; Wolosiuk, A.; Danilowicz, C. Layer-By-Layer Self-Assembly of Glucose Oxidase and  $Os(Bpy)_2CIPyCH_2NH-poly(Allylamine)$  Bioelectrode. *Anal. Chem.* **2001**, 73 (6), 1161–1168.

(14) Vashist, S. K.; Zheng, D.; Al-Rubeaan, K.; Luong, J. H. T.; Sheu, F.-S. Technology Behind Commercial Devices for Blood Glucose Monitoring in Diabetes Management: A Review. *Anal. Chim. Acta* **2011**, 703 (2), 124–136.

(15) Wooten, M.; Karra, S.; Zhang, M.; Gorski, W. On the Direct Electron Transfer, Sensing, and Enzyme Activity in the Glucose

Oxidase/Carbon Nanotubes System. Anal. Chem. 2013, 86 (1), 752-757.

(16) Ricci, F.; Amine, A.; Palleschi, G.; Moscone, D. Prussian Blue Based Screen Printed Biosensors with Improved Characteristics of Long-Term Lifetime and pH Stability. *Biosens. Bioelectron.* **2003**, *18* (2–3), 165–174.

(17) Lee, J. M.; Xu, G. R.; Kim, B. K.; Choi, H. N.; Lee, W. Y. Amperometric Tyrosinase Biosensor Based on Carbon Nanotube-Doped Sol-Gel-Derived Zinc Oxide-Nafion Composite Films. *Electroanalysis* **2011**, *23* (4), 962–970.

(18) Huang, Q. D.; Lu, Z. Q.; Rusling, J. F. Composite Films of Surfactants, Nafion, and Proteins with Electrochemical and Enzyme Activity. *Langmuir* **1996**, *12* (22), 5472–5480.

(19) Hartmann, V.; Kothe, T.; Poller, S.; El-Mohsnawy, E.; Nowaczyk, M. M.; Plumere, N.; Schuhmann, W.; Rogner, M. Redox Hydrogels with Adjusted Redox Potential for Improved Efficiency in Z-Scheme Inspired Biophotovoltaic Cells. *Phys. Chem. Chem. Phys.* **2014**, *16* (24), 11936–11941.

(20) Kothe, T.; Plumeré, N.; Badura, A.; Nowaczyk, M. M.; Guschin, D. A.; Rögner, M.; Schuhmann, W. Combination of a Photosystem 1-Based Photocathode and a Photosystem 2-Based Photoanode to a Z-Scheme Mimic for Biophotovoltaic Applications. *Angew. Chem., Int. Ed.* **2013**, 52 (52), 14233–14236.

(21) Yehezkeli, O.; Tel-Vered, R.; Wasserman, J.; Trifonov, A.; Michaeli, D.; Nechushtai, R.; Willner, I. Integrated photosystem IIbased photo-bioelectrochemical cells. *Nat. Commun.* **2012**, *3*, 742.

(22) Wang, F.; Liu, X. Q.; Willner, I. Integration of Photoswitchable Proteins, Photosynthetic Reaction Centers and Semiconductor/ Biomolecule Hybrids with Electrode Supports for Optobioelectronic Applications. *Adv. Mater.* **2013**, *25* (3), 349–377.

(23) Baker, D. R.; Manocchi, A. K.; Lamicq, M. L.; Li, M.; Nguyen, K.; Sumner, J. J.; Bruce, B. D.; Lundgren, C. A. Comparative Photoactivity and Stability of Isolated Cyanobacterial Monomeric and Trimeric Photosystem I. J. Phys. Chem. B 2014, 118 (10), 2703–2711.

(24) Badura, A.; Guschin, D.; Kothe, T.; Kopczak, M. J.; Schuhmann, W.; Rogner, M. Photocurrent Generation by Photosystem 1 Integrated in Crosslinked Redox Hydrogels. *Energy Environ. Sci.* **2011**, *4* (7), 2435–2440.

(25) Badura, A.; Kothe, T.; Schuhmann, W.; Rogner, M. Wiring Photosynthetic Enzymes to Electrodes. *Energy Environ. Sci.* 2011, 4 (9), 3263–3274.

(26) Efrati, A.; Tel-Vered, R.; Michaeli, D.; Nechushtai, R.; Willner, I. Cytochrome *c*-Coupled Photosystem I and Photosystem II (PSI/PSII) Photo-Bioelectrochemical Cells. *Energy Environ. Sci.* **2013**, *6* (10), 2950–2956.

(27) Magnuson, A.; Anderlund, M.; Johansson, O.; Lindblad, P.; Lomoth, R.; Polivka, T.; Ott, S.; Stensjö, K.; Styring, S.; Sundström, V.; Hammarström, L. Biomimetic and Microbial Approaches to Solar Fuel Generation. *Acc. Chem. Res.* **2009**, *42* (12), 1899–1909.

(28) Hogewoning, S. W.; Wientjes, E.; Douwstra, P.; Trouwborst, G.; van Ieperen, W.; Croce, R.; Harbinson, J. Photosynthetic Quantum Yield Dynamics: From Photosystems to Leaves. *Plant Cell* **2012**, *24* (5), 1921–1935.

(29) Grotjohann, I.; Fromme, P. Structure of Cyanobacterial Photosystem I. *Photosynth. Res.* **2005**, *85* (1), 51–72.

(30) Iwuchukwu, I. J.; Vaughn, M.; Myers, N.; O'Neill, H.; Frymier, P.; Bruce, B. D. Self-Organized Photosynthetic Nanoparticle for Cell-Free Hydrogen Production. *Nat. Nanotechnol.* **2010**, *5* (1), 73–79.

(31) Ciestelski, P. N.; Hijazi, F. M.; Scott, A. M.; Faulkner, C. J.; Beard, L.; Emmett, K.; Rosenthal, S. J.; Cliffel, D.; Jennings, G. K. Photosystem I - Based Biohybrid Photoelectrochemical Cells. *Bioresour. Technol.* **2010**, *101* (9), 3047–3053.

(32) Rubinstein, I.; Bard, A. J. Polymer Films on Electrodes. 4. Nafion-Coated Electrodes and Electrogenerated Chemiluminescence of Surface-Attached  $Ru(bpy)_3^{2^+}$ . J. Am. Chem. Soc. **1980**, 102 (21), 6641–6642.

(33) Karyakin, A. A.; Karyakina, E. E.; Gorton, L.; Bobrova, O. A.; Lukachova, L. V.; Gladilin, A. K.; Levashov, A. V. Improvement of Electrochemical Biosensors Using Enzyme Immobilization from Water-Organic Mixtures with a High Content of Organic Solvent. Anal. Chem. 1996, 68 (24), 4335-4341.

(34) Fortier, G.; Vaillancourt, M.; Belanger, D. Evaluation of Nafion as Media for Glucose-Oxidase Immobilization for the Development of an Amperometric Glucose Biosensor. *Electroanalysis* **1992**, *4* (3), 275–283.

(35) Hahn, O. E. W.; Hill, H. A. O.; Ritchie, M. D.; Sear, J. W. The Electrochemistry of Proteins Entrapped in Nafion. *J. Chem. Soc., Chem. Commun.* **1990**, *2*, 125–126.

(36) Liu, H. Y.; Deng, J. Q. An Amperometric Lactate Sensor Employing Tetrathiafulvalene in Nafion Film as Electron Shuttle. *Electrochim. Acta* **1995**, 40 (12), 1845–1849.

(37) Kothe, T.; Poller, S.; Zhao, F. Y.; Fortgang, P.; Rogner, M.; Schuhmann, W.; Plumere, N. Engineered Electron-Transfer Chain in Photosystem 1 Based Photocathodes Outperforms Electron-Transfer Rates in Natural Photosynthesis. *Chem.—Eur. J.* **2014**, *20* (35), 11029–11034.

(38) Hsu, W. Y.; Gierke, T. D. Ion Transport and Clustering in Nafion Perfluorinated Membranes. *J. Membr. Sci.* **1983**, *13* (3), 307–326.

(39) Gregg, B. A.; Heller, A. Redox Polymer-Films Containing Enzymes. 2. Glucose-Oxidase Containing Enzyme Electrodes. J. Phys. Chem. 1991, 95 (15), 5976–5980.

(40) Fromme, P.; Melkozernov, A.; Jordan, P.; Krauss, N. Structure and Function of Photosystem I: Interaction with its Soluble Electron Carriers and External Antenna Systems. *FEBS Lett.* **2003**, *555* (1), 40–44.

(41) Manocchi, A. K.; Baker, D. R.; Pendley, S. S.; Nguyen, K.; Hurley, M. M.; Bruce, B. D.; Sumner, J. J.; Lundgren, C. A. Photocurrent Generation from Surface Assembled Photosystem I on Alkanethiol Modified Electrodes. *Langmuir* **2013**, *29* (7), 2412–2419.

(42) Sommer, F.; Drepper, F.; Haehnel, W.; Hippler, M. The Hydrophobic Recognition Site Formed by Residues PsaA-Trp<sup>651</sup> and PsaB-Trp<sup>627</sup> of Photosystem I in Chlamydomonas Reinhardtii Confers Distinct Selectivity for Binding of Plastocyanin and Cytochrome  $c_6$ . J. Biol. Chem. **2004**, 279 (19), 20009–20017.

(43) Chen, G. P.; LeBlanc, G.; Jennings, G. K.; Cliffel, D. E. Effect of Redox Mediator on the Photo-Induced Current of a Photosystem I Modified Electrode. *J. Electrochem. Soc.* **2013**, *160* (6), H315–H320.

(44) Kievit, O.; Brudvig, G. W. Direct Electrochemistry of Photosystem I. J. Electroanal. Chem. 2001, 497 (1-2), 139–149.

(45) Parson, W. W. Photosynthetic Bacterial Reaction Centers – Interactions Among the Bacteriochlorophylls and Bacteriopheophytins. *Annu. Rev. Biophys. Bioeng.* **1982**, *11*, 57–80.