A Hybrid Soft Solar Cell Based on the Mycobacterial Porin MspA Linked to a Sensitizer–Viologen Diad

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S *Supporting Information*

ABSTRACT: A prototype of a nano solar cell containing the mycobacterial channel protein MspA has been successfully designed. MspA, an octameric transmembrane channel protein from *Mycobacterium smegmatis*, is one of the most stable proteins known to date. Eight Ruthenium(II) aminophenanthroline–viologen maleimide Diads (Ru-Diads) have been successfully bound to the MspA mutant MspAA96C via cysteine–maleimide bonds. MspA is known to form double layers in which it acts as nanoscopic surfactant. The nanostructured layer that is formed by (Ru-Diad)$_8$MspA at the TiO$_2$ electrode is photochemically active. The resulting "protein nano solar cell" features an incident photon conversion efficiency of 1% at 400 nm. This can be regarded as a proof-of-principle that stable proteins can be successfully integrated into the design of solar cells.

Solar energy is the ultimate renewable source of energy, which has no match in abundance and availability. Solar cells have been in existence since 1887,[1,2] and they have great potential to be used as the world's leading sustainable energy source for the future. Photovoltaic cells have been developed and improved throughout their history and have evolved into many different types.[3,4] The introduction of dye-sensitized solar cells (DSSCs) by O'Regan and Grätzel in 1991 has revolutionized the scope and efficiency of solar cell applications.[5] The initially observed overall yield for conversion of visible light to current was 7−8%, and the device was proven to be stable for up to five million cycles.[6] Over the last two decades, DSSC technology has been developed and improved significantly.[6,7] An improved overall incident photon to current conversion efficiency (IPCE) of 12% was achieved using a porphyrin-sensitized solar cell with cobalt(II/III)-based redox electrolyte.[8] A second area of growing developmental interest is the incorporation of solid-state materials to replace electrolytes in DSSCs. Liquid electrolytes tend to decompose and fail under long-term exposure to light.[9] The incorporation of renewable materials into the solar cell design would lead to "greener" solar cells. At least a part of their components could be grown in plants, such as algae.[10] This approach has the potential of decreasing the production costs of solar cells. It also would decrease the demand for precious materials in manufacturing

solar cells. Consequently, a greener solar cell technology will make solar energy conversion more applicable in countries that do not have access to materials for solar cell production in the quantities required for their broad technical application.

Photosynthetic reaction centers are archetypal solar-harvesting aggregates comprising sensitizers, electron acceptors, and stabilizing proteins and membranes. They have to be incorporated into solar cell prototypes using either electrostatic forcers[11] or peptide surfactants.[12] However, their structural intricacies do not permit the construction of devices with long-term stability.

Metal complexes containing 2,2′-bipyridine subunits are known to act as efficient electron-transfer systems.[13−15] Ruthenium(II) complexes containing 2,2′-bipyridine units have proven to be significant as components of artificial photosynthetic systems.[16] Among these, Ruthenium(II)−polypyridyl-based Diads and triads have shown promising results as models for the photosynthetic reaction center because of their high efficiency in electron transfer and increased charge separation lifetimes.[14,15] Ruthenium(II)−phenanthroline-based dyes have also been recognized as promising candidates for light harvesting.[16] To investigate their applicability in solar cells, a novel Ruthenium(II) aminophenanthroline−viologen Diad featuring a maleimide group for attachment to cysteines (Ru-Diad 1) has been synthesized and tethered to the A96C mutant of the mycobacterial channel protein MspA[13] [see Scheme 1 and the Supporting Information (SI)]. MspA from *Mycobacterium smegmatis* is a homooctameric channel protein that exhibits extraordinary thermal stability.[17] Its size and shape (see Scheme 1) are very suitable for the formation of supramolecular aggregates. MspA behaves like a nanoscopic surfactant: it forms vesicles in aqueous solution[18] and structured layers on flat surfaces.[17] For the purpose of designing an MspA-based solar cell, eight Ru-Diad 1 moieties were attached through their maleimide groups via Michael addition to the cysteines in the MspAA96C mutant.[19]

One major objective of this study was to generate a nanostructured layer formed by the stable protein MspA at the TiO$_2$ interface. The supramolecular interactions of MspA to form the layer on TiO$_2$ cause the Ru-Diads to be located in defined positions (Scheme 2). This permits the study of the

Scheme 1. Structure of (Ru-Diad)$_8$MspA Containing Eight Ru-Diad 1 Units Attached to MspAA96C via Maleimide–Cysteine Linkages; (Ru-Diad)$_8$MspA Was Used as a Nanoscopic Surfactant Forming a Double Layer on TiO$_2$
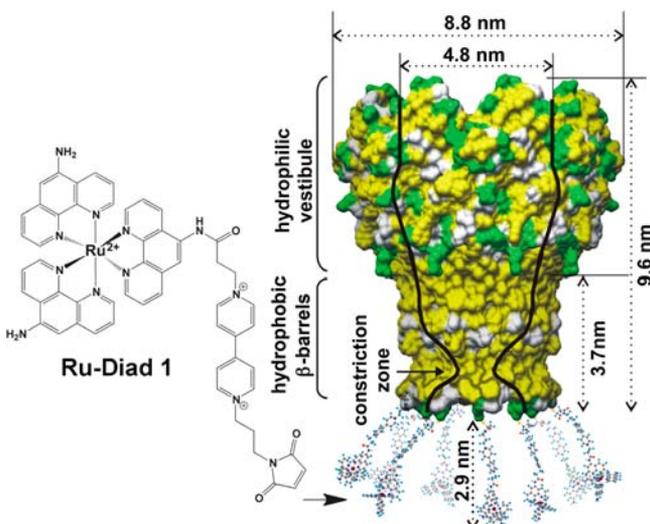
Scheme 2. Design and Operating Principle of the Prototype of an MspA-Based "Hybrid Soft Cell" Consisting of an (Ru-Diad)$_8$MspA Double Layer Adsorbed onto Nanosized TiO$_2$
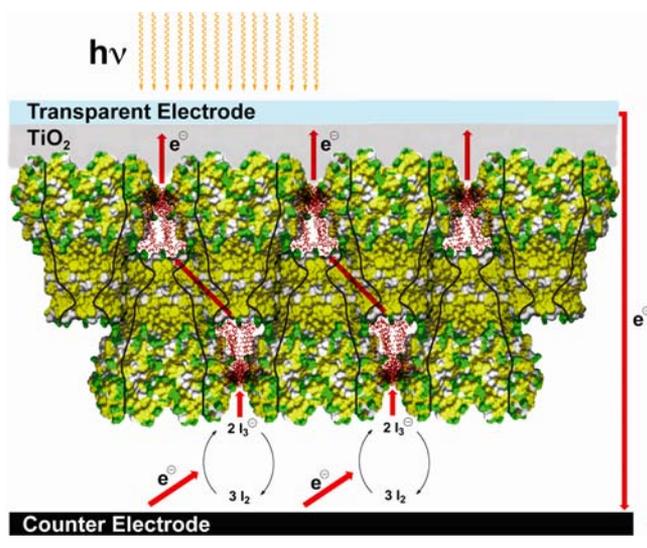
distance dependence of electron-transfer processes in a solar cell. Iodolyte solution[20] was used to mediate the thermal electron transfer between the counter electrode and the nanostructured MspA layer. The double layer of MspA featuring covalently tethered Diads can be regarded as a biomimetic model for studying the effect of the membrane in photosynthesis.

As depicted in Scheme 2, the novel protein-incorporating cell consisted of a transparent electrode onto which a nanocrystal-line TiO$_2$ layer was deposited. The semiconductor was coated with Ru-Diad 1 that was chemically attached to the periplasmatic region of MspAA96C (see the SI for experimental details). The specific contact between the dye and protein can principally be altered by changing the nature of dye, the length and physical properties of the tether, and the type of protein (wild-type or mutant). A platinum electrode was used as the

counter electrode and a commercial Iodolyte solution[20] as the contact electrolyte.

Single-photon counting experiments. Time-resolved emission measurements were performed using a single-photon counter (HORIBA, λ_{EX} = 404 nm, λ_{EM} = 550–700 nm). As anticipated, the novel Ru-Diad 1 exhibited a complex time-resolved emission behavior that could be satisfactorily fitted by assuming three principal exponential components [τ_1 = 0.57 ns (7.0%); τ_2 = 8.4 ns (13.3%); τ_3 = 283.8 ns (79.7%)]. The reference compound tris(1,10-phenanthrolin-5-amine)Ruthenium(II) di-chloride has a monoexponential lifetime of 387 ns in aerated phosphate-buffered saline (PBS). The corresponding intra-molecular electron-transfer quenching rate constants are k_1 = 1.75 × 10^9 s^{-1}, k_2 = 1.16 × 10^8 s^{-1}, and k_3 = 9.45 × 10^5 s^{-1}, as obtained from the expression $k_i = 1/\tau_i - 1/\tau_{ref}$.[21]

It is noteworthy that under these conditions, (Ru-Diad)$_8$MspA forms vesicles, as indicated by dynamic light scattering (DLS) measurements (see the SI). To evaluate whether photoinduced electron-transfer processes between the photoexcited Diads and TiO$_2$ are possible if the Diads are incoporated into a supramolecular structure at the TiO$_2$–Iodolyte interface (Scheme 2), we measured their time-resolved emission pattern in the presence of increasing TiO$_2$ concentrations in PBS dispersions. The corresponding quenching rate constants were calculated to be k_{q1} = 5.25 × 10^{11} L g^{-1} s^{-1}, k_{q2} = 8.65 × 10^9 L g^{-1} s^{-1}, and k_{q3} = 4.16 × 10^8 L g^{-1} s^{-1}, following the classic Stern–Volmer approach.[22] It is noteworthy that all three components were quenched by TiO$_2$. The component with the shortest luminescence lifetime was quenched the most by TiO$_2$, whereas the longest was quenched the least (Figure 1). It is our hypothesis that Ru-Diads that are
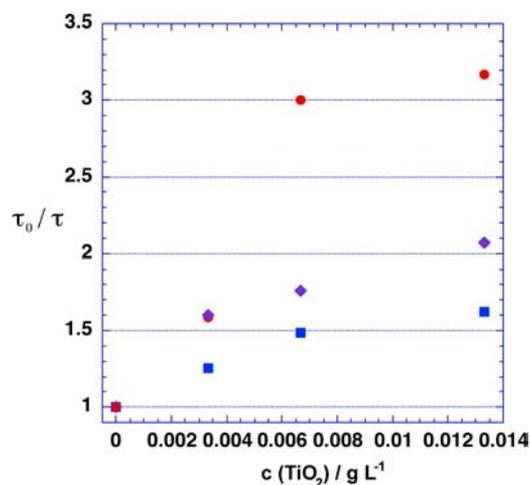
Figure 1. Time-resolved quenching of (Ru-Diad)$_8$MspA as a function of the dispersed amount of TiO$_2$ (Degussa P25). Red, short-lived component (1); purple, medium-lived component (2); blue, long-lived component (3).

closer to the TiO$_2$ surface are quenched more strongly. However, MspA is able to support electron-transfer processes between all of the photoexcited Ru-Diads and the TiO$_2$ surface, in agreement with earlier findings.[23] This makes MspA a very suitable protein for the design of solar cells and functional electrodes.

Fabrication and operation of a Grätzel-type MspA-incorporating nano solar cell prototype. Ru-Diad 1 was synthesized, purified, and characterized as described in the SI. The semiconductor

electrodes were prepared as follows: Commercial fluorine-doped tin oxide (FTO) glass plates were used for the base of the prototype cells. These were cut into 1.5 in. × 1 in. pieces and cleaned using detergent (sodium dodecyl sulfate) by gentle rubbing over microcloth pads (BAS). The detergent was washed off with deionized water, and the plates were further cleaned using 0.10 M HCl in ethanol, acetone, and 2-propanol solutions in an ultrasonic bath for 15 min sequentially. Finally, the plates were treated with plasma cleaning to remove all of the organic impurities. Thin films of TiO_2 were deposited on the cleaned FTO plates by adapted literature procedures.[24,25] The exact procedure and instrumentation is described in detail in the SI.

The current switching behaviors of TiO_2 and TiO_2 covered with a layer formed by $(Ru\text{-}Diad)_8MspA$ (Scheme 2) were tested to determine whether the protein-dye assembly is stable under working voltaic conditions. As expected, TiO_2 alone did not show any evidence of a current. However, the $(Ru\text{-}Diad)_8MspA$ assembly showed clear evidence of photo-conductance at 0.1 V, and the effect was reproducible throughout the experimental time (Figure 2).
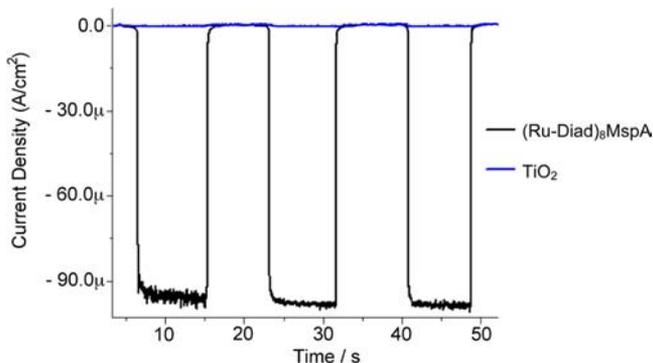
Figure 2. Current switching behavior of $(Ru\text{-}Diad)_8MspA$ on TiO_2. See Figure 3 caption for experimental details

The conductance of the complexes was probed at varying voltages, and the results are shown in Figure 3 as a plot of current density versus potential. In accordance with results obtained above, the MspA−Ru-Diad 1 assembly showed substantial and stable current over the applied voltage range. In the dark, the observed current was close to zero, but under simulated solar light, a steady current of 100 $\mu A/cm^2$ was observed over the −0.10 to 0.20 V range. Beyond 0.20 V, the current started to increase exponentially, and by 0.40 V we observed open-current conditions.

To estimate the overall efficiency of the $(Ru\text{-}Diad)_8MspA$ solar cell prototype, the IPCE was calculated. The current generated in the visible region (i.e., 400−800 nm) was tested for this purpose, and an IPCE of 1% was achieved at 400 nm (Figure 4). Furthermore, control experiments (Figures S12 and S13 in the SI) clearly demonstrated that $(Ru\text{-}Diad)_8MspA$ on TiO_2 leads to significantly higher photoconductance (by a factor of 1.57) and photovoltage (by a factor of 1.47) compared with Ru-Diad on TiO_2 and MspA on TiO_2. The data obtained above constitute the first evidence for a protein-incorporating DSSC prototype.

To summarize, the development of a prototype for a new class of sustainable and "greener" solar cells using the highly stable mycobacterial porin MspA has been described. A novel Ruthenium(II) aminophenanthroline−viologen Diad (Ru-Diad
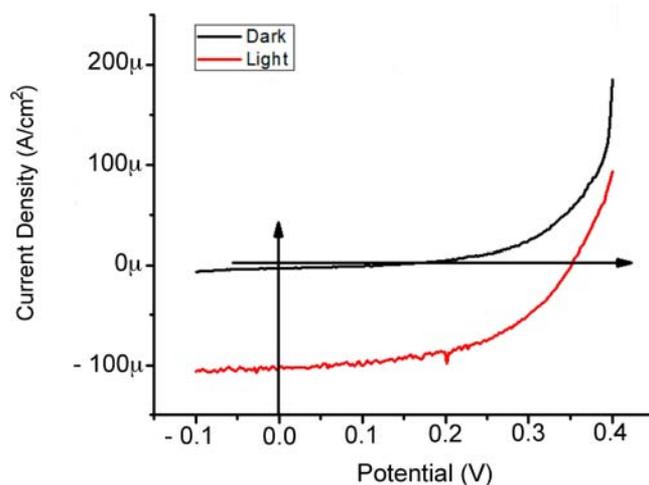
Figure 3. Current density vs potential graph for the solar cell prototype utilizing $(Ru\text{-}Diad)_8MspA$ on TiO_2. The experiment was performed under irradiation of AM1.5 simulated solar light in the presence of I-/I3- (0.6 M PMII, 0.1 M LiI, 0.05 M I2) redox mediator in acetonitrile.
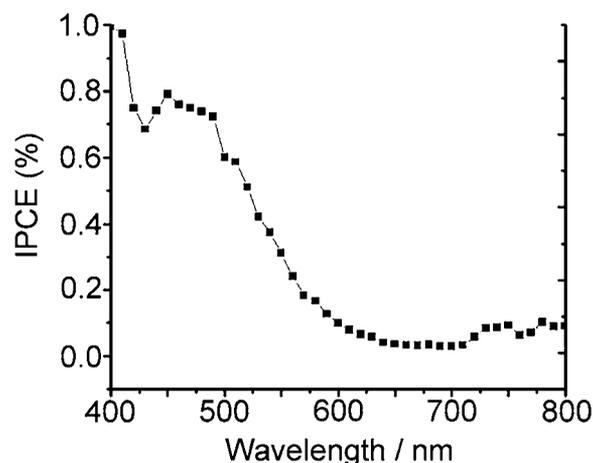
Figure 4. Plot of IPCE vs wavelength for $(Ru\text{-}Diad)_8MspA$ on TiO_2. See Figure 3 caption for experimental details.

1) featuring a maleimide group for attachment to cysteines in MspA mutants (here MspAA96C) was synthesized. The homooctameric MspA is able to bind eight Ru-Diads, affording $(Ru\text{-}Diad)_8MspA$. A Grätzel-type nanocystalline TiO_2-based DSSC prototype was developed utilizing $(Ru\text{-}Diad)_8MspA$ on TiO_2. This $(Ru\text{-}Diad)_8MspA/TiO_2$ cell conducts a steady, reproducible current with a maximum IPCE of 1% at 400 nm. Although 1% is relatively small, this result nevertheless provides the first evidence of a photovoltaic device that is based on a stable protein. We propose this technology as a new class of solar cells classified as "hybrid soft" solar cells.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis of Ru-Diad 1, procedure for extraction of MspAA96C, synthesis of the $(Ru\text{-}Diad)_8MspA$ assembly, DLS characterization of $(Ru\text{-}Diad)_8MspA$ vesicles, details of the the single-photon-counting quenching experiments summarized in Figure 1, solar cell fabrication procedure, and solar cell control experiments. 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.

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