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Filling the Green Gap of a Megadalton Photosystem I Complex by Conjugation of Organic Dyes

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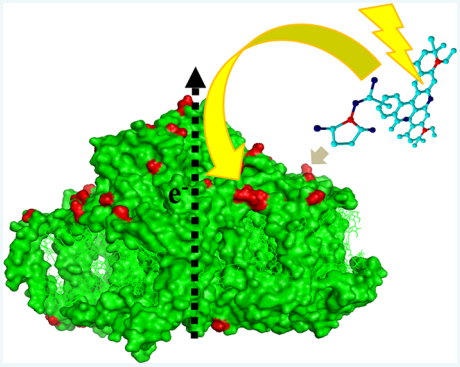
S *Supporting Information*

ABSTRACT: Photosynthesis is Nature's major process for converting solar into chemical energy. One of the key players in this process is the multiprotein complex photosystem I (PSI) that through absorption of incident photons enables electron transfer, which makes this protein attractive for applications in bioinspired photoactive hybrid materials. However, the efficiency of PSI is still limited by its poor absorption in the green part of the solar spectrum. Inspired by the existence of natural phycobilisome light-harvesting antennae, we have widened the absorption spectrum of PSI by covalent attachment of synthetic dyes to the protein backbone. Steady-state and time-resolved photoluminescence reveal that energy transfer occurs from these dyes to PSI. It is shown by oxygen-consumption measurements that subsequent charge generation is substantially enhanced under broad and narrow band excitation. Ultimately, surface photovoltage (SPV) experiments prove the enhanced activity of dye-modified PSI even in the solid state.



INTRODUCTION

During photosynthesis, absorbed sunlight is efficiently converted into chemical energy. The membrane multiprotein complex PSI plays a key role in this process, as upon light irradiation it induces electron transfer over a 1 V potential difference at near 100% quantum efficiency.[1] The two most important features of PSI are charge separation and light harvesting, which have been a great inspiration for researchers to mimic these processes with artificial systems.[2−9] Charge separation takes place in the core of the complex within the reaction center at a special pair of chlorophylls named P700.[10] From there, the excited electrons migrate through the chain of primary electron acceptors Chla, phylloquinone, and three Fe_4S_4 clusters, thereby traversing almost the entire volume of PSI.[11] The resulting positive charge at P700 is refilled by the electron carrier cytochrome c_6 from the lumen side resetting PSI for the next photoexcitation. For the second important function, i.e., light harvesting, a network of pigment antennae surrounding the reaction center is responsible.[12−14]

However, an essential part of the solar energy is not exploited by PSI because these photosynthetic pigments (chlorophylls and carotenoids) do not absorb light in the wavelength range of 450−600 nm, which is termed the "green gap". Although the intensity of the sunlight reaches its maximum in this spectral region, PSI harvests the light poorly at these wavelengths. The lack of absorption in the green gap is a consequence of the chlorophyll absorption spectrum with its characteristic Soret and Q bands with maxima at 430 and 665 nm, respectively. The low absorbance between these two peaks significantly reduces the energy conversion efficiency of the multiprotein complex. In Nature, partial closing of this gap is realized with the help of other protein complexes, such as phycobilisomes, which are found in cyanobacteria and red algae.[15] The protein assemblies absorb light in the range between 500 to 650 nm with the help

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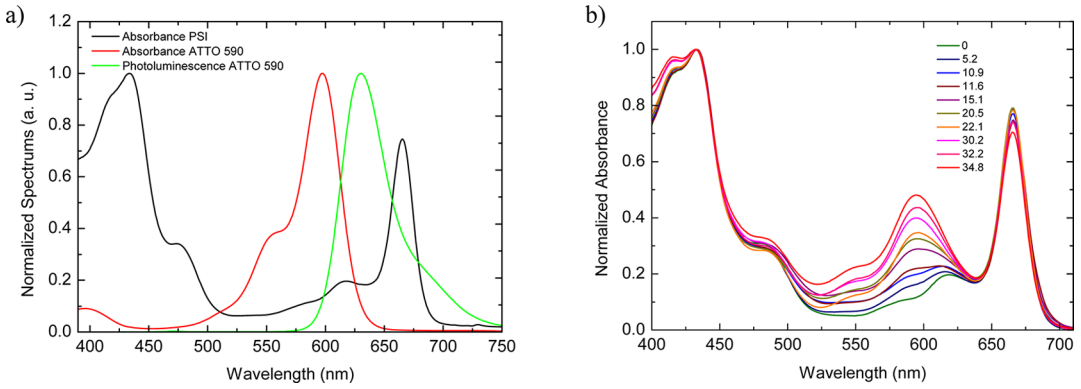

Figure 1. (a) Absorption spectra of PSI (black line) and ATTO 590 (red line). The emission spectrum of the dye is shown in green (excitation 590 nm). (b) Absorption spectra of PSI modified with a different number of ATTO 590 dyes acquired after purification. Up to 35 chromophores are attached to PSI on average.

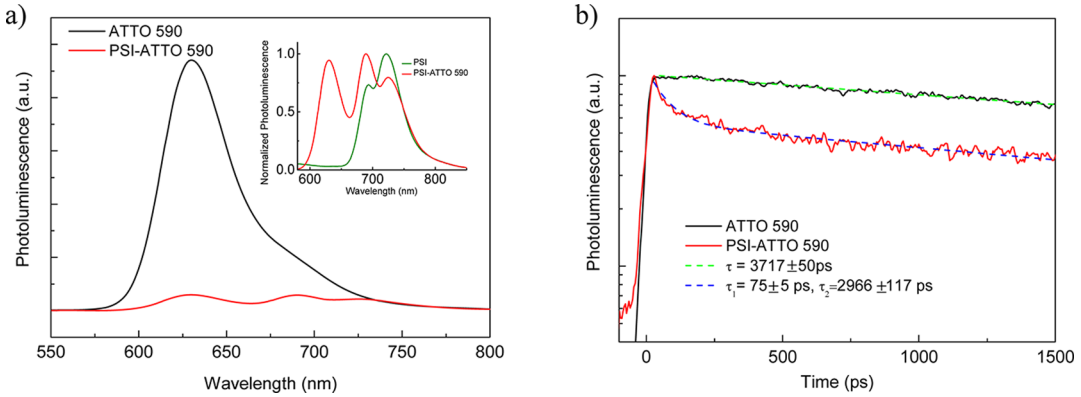

Figure 2. Steady-state (a) and time-resolved fluorescence (b) measurements of dye-modified PSI. (a) When ATTO 590 dyes are covalently connected to PSI, their emission is almost completely quenched (solid red line, excitation wavelength 380 nm), while in the absence of PSI, a clear emission peak is detected for the pristine dyes (solid black line, excitation wavelength 380 nm). The inset shows normalized PL of PSI and PSI-ATTO 590 dyes at fixed PSI concentration (50 μM). (b) Fluorescence decay of ATTO 590-PSI conjugate (red line) and of ATTO 590 dyes in the absence of PSI (black line). The energy dissipation from the organic dyes upon excitation at 380 nm in the PSI complex is substantially faster compared to the free dyes. The experiments were performed at the same concentration of dyes (5 μM) being either dissolved in the buffer or attached to PSI. The ATTO 590-PSI conjugates had an average number of 6 dyes per PSI complex.

of phycobilin pigments that are contained in special molecular aggregates called phycoerythrin, phycocyanin, and allophycocyanin.

These complexes funnel absorbed light energy to PSI and PSII for photosynthetic reactions.[16,17] To date, the energy transfer mechanisms between these proteins and charge separation centers at a distance of more than 20 Å is not fully understood.[18,19] Another light-harvesting system where energy transfer over a long distance occurs is the Fenna-Matthews-Olson (FMO) complex in green sulfur bacteria, which live in deep lakes where only a minor fraction of the sunlight is available.[16,20] These examples demonstrate that utilizing sunlight energy outside the main absorption region of photosynthetic complexes is a viable way to increase the efficiency of photosynthetic energy conversion for life in habitats with low sun intensities.

In this study, we follow a chemical approach to increase light absorption in the green gap to improve the electron transfer efficiency of PSI by conjugation of organic dyes. Such bio-organic hybrid systems might be incorporated into biosolar or biofuel cells containing PSI as active material.[21−23] We demonstrate energy transfer from the organic dyes to the PSI and an up to 4 times improved PSI functionality under white-light irradiation in water and in the solid state.

RESULTS AND DISCUSSION

The protein complex of PSI used in this study was extracted from the thermophilic cyanobacterium *Thermosynechococcus elongatus*.[10] Under the conditions of extraction, PSI exists nearly exclusively as a trimeric complex, with a size of a = 281.0 Å, b = 281.0 Å, and c = 165.2 Å and a mass of about 1.05 MDa (Figure S1a,b). Moreover, a large number of light harvesting cofactors are incorporated within PSI, i.e., 96 chlorophylls and 22 carotenoids per monomer. Importantly, the PSI monomer employed in this study contains approximately 70 surface-exposed lysine residues that are available for attachment of dyes. The commercially available fluorescent dye ATTO 590 was chosen because of its large absorption in the green region, its high fluorescence quantum yield of 80%, and the lack of overlap with the PSI main absorption peak at 665 nm. The calculated Förster resonance energy transfer radius $R_{\mathrm{ATTO590\text{-}Chla}}$ of 57.3 Å[24,25] may allow efficient energy transfer from the artificial emitter to PSI chlorophylls from any attachment point on the protein scaffold.

The lysine residues of PSI were modified with the *N*-hydroxysuccinimide ester derivative of ATTO 590. During the coupling reaction, various dye-to-protein ratios were selected in order to synthesize PSI conjugates exhibiting different amounts of bound organic dyes. The hybrids were purified by extensive

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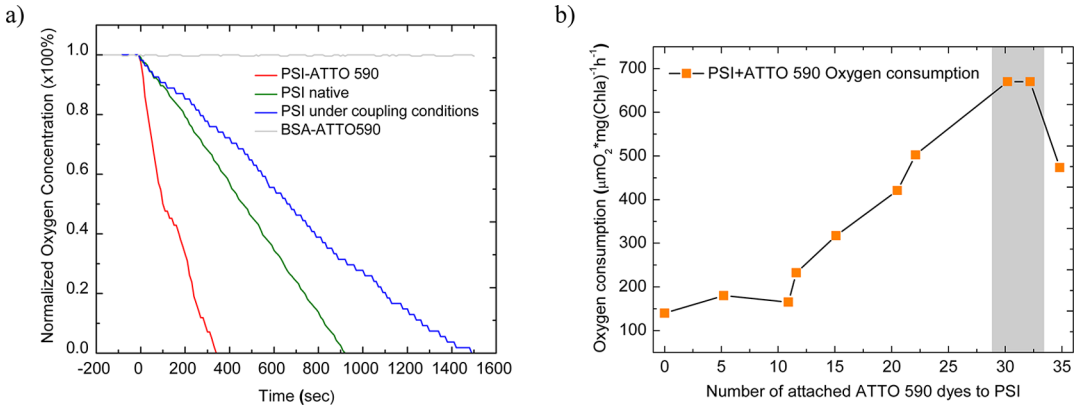

Figure 3. Oxygen consumption experiments of PSI covalently functionalized with ATTO 590 dyes using the procedure described in reference.[27] (a) Faster oxygen consumption was observed for PSI that was on average modified with 32.2 ATTO 590 dyes on average (red) compared to the native PSI protein that was exposed to the same coupling conditions but without dye attachment (blue). PSI that is not exposed to basic coupling conditions exhibits increased functionality (green) compared to PSI that was in incubated in a high pH solution (blue). When ATTO 590 dyes are attached to BSA, which is not photoactive, no reaction was observed (gray). (b) Average number of coupled dyes per PSI protein was increased from 5 to 34. The maximum oxygen consumption activity was observed for 32 coupled dyes per PSI monomer. White-light illumination (Figure S5) was used as a source with an intensity of 36 mW/cm^2.

dialysis prior to calculation of the amount of coupled dyes. The main absorption peaks of the ATTO 590 dye (590 nm) and PSI (665 nm) do not overlap (Figure 1a), which allowed us to calculate the precise number of attached dyes by taking into account the absorption spectrum of the modified PSI and using reported extinction coefficients.[26]

The average number of dyes attached to PSI was ranging on average from 5.2 to 34.8 dyes per PSI monomer for different coupling ratios, as depicted in Figure 1b. To achieve these different degrees of modification of PSI, from 1 up to 9 equiv of activated dye molecules per surface exposed lysine were chosen during the conjugation reaction. The chemical modification did not affect the trimeric structure of the PSI complex, which was revealed by transmission electron microscopy (TEM) (Figure S2). Due to the fact that we identified the surface-exposed lysine residues from a reported crystal structure we expected a homogeneous distribution of the dyes at the available positions on the top and bottom of PSI. To exclude unspecific binding, PSI was treated with the nonreactive carboxylic acid derivative of ATTO 590 following the same coupling and purification procedures. No absorption in the green gap was observed (Figure S3). This proves the presence of a covalent bond between PSI and ATTO 590 and that the increased adsorption is not due to unspecific adsorption of dyes to PSI.

Next, photoluminescence (PL) measurements were performed to characterize the dye-modified PSI complex, containing 6 dyes per PSI monomer on average. The fluorescence of ATTO 590 when attached to PSI was quenched, which could indicate energy transfer from the dye to PSI or to other dyes. Additionally, time-resolved measurements at 590 nm showed a biexponential PL decay with 75 ± 5 ps and 2966 ± 117 ps, respectively, when ATTO 590 is connected to the protein scaffold. The control experiment employing free ATTO 590 showed a monoexponential decay with a lifetime of 3717 ± 50 ps (Figure 2b). In order to exclude an effect of the protein backbone on the fluorescence behavior of the dye, bovine serum albumin (BSA), a protein lacking any photosynthetic activity, was modified with ATTO 590. Only a small change in steady-state fluorescence spectrum was detected and the emission decay was unaffected (see Figure S4). These control experiments confirm that energy transfer from dye to PSI indeed occurs within the dye−PSI conjugate.

To investigate if energy transferred from the dye to PSI leads to an improved functionality, oxygen consumption experiments were carried out. The rate of electron transfer under illumination from the P700 reaction center to the iron−sulfur cluster via the cascade electron-transfer chain can be measured directly by oxygen consumption experiments based on the Mehler reaction.[27] These measurements were performed with native PSI and PSI modified with covalently attached ATTO 590 under white-light illumination (for spectrum see Figure S5) as shown in Figure 3a. The time required for full oxygen consumption was reduced by a factor of 4 for PSI coupled with 32 dyes as compared to pristine PSI that was exposed to the same coupling conditions as the dye-modified PSI. The alkaline pH of 9.2 reduced the activity of PSI as compared to PSI at neutral pH (Figure 3a, green graph). In previous work, the proton concentration of the solution affected the activity of PSI.[28] Moreover, it was observed that with an increasing number of dyes attached to PSI, the oxygen consumption rate increased (Figure 3b). The maximum charge separation activity of the dye-modified PSI complex was observed when an average of 32 dyes per PSI monomer were attached. An additional increase in the number of attached dyes did not lead to an increase in the functionality of the complex. This observation requires a more detailed study, but might originate from internal PSI structural changes when overloaded with attached dyes, or protein aggregation due to an increase in hydrophobicity of the protein surface.

To verify that the increased oxygen consumption of PSI−dye complexes is indeed due to energy transfer from covalently coupled dyes to PSI, followed by subsequent charge separation, two control experiments were carried out. In the first one, the dye was covalently attached to a nonphotoactive BSA scaffold following the same coupling procedure as for PSI (see Experimental Section). In this control experiment, no oxygen consumption was detected, showing that the dyes by themselves do not lead to the oxygen consumption (Figure 3a). In the second experiment, carboxylic acid-functionalized ATTO 590 dyes (instead of the activated ester derivatives) were present together with PSI in the reaction buffer, ensuring

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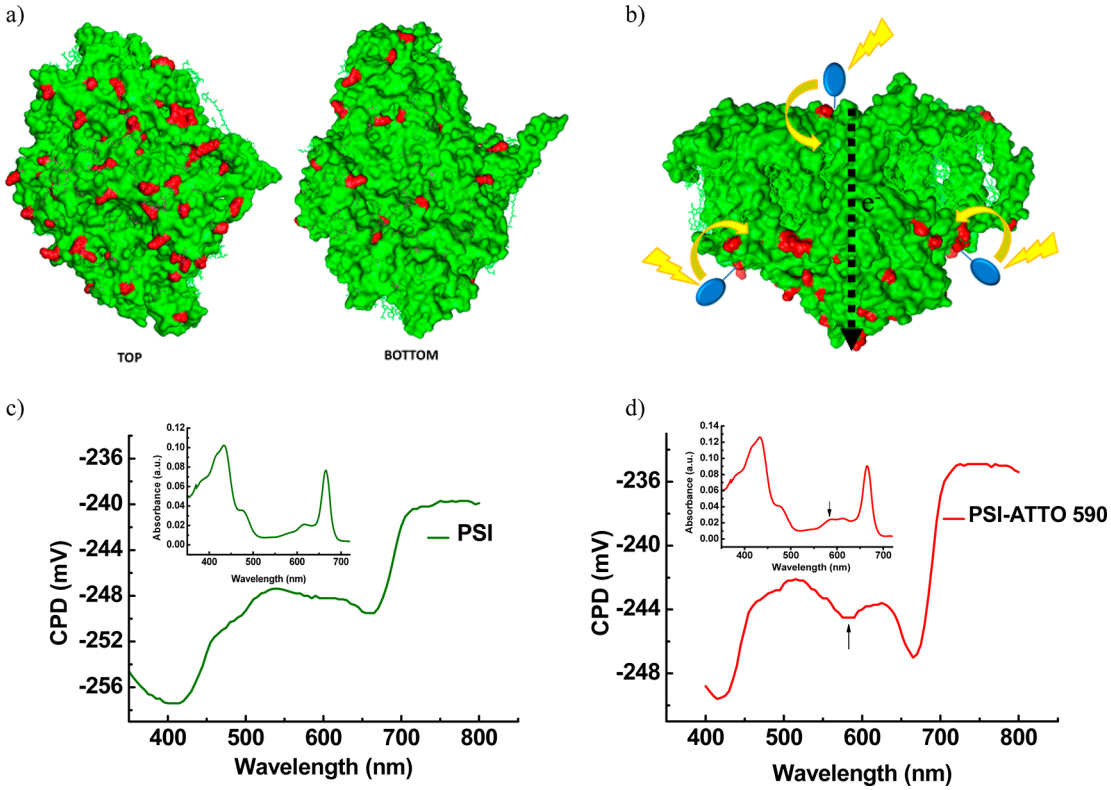

Figure 4. (a) 3D structure of PSI-monomer according to a crystal structure analysis.[10] Available amino groups of lysine residues for dye coupling on the top and bottom sides of PSI surface are indicated in red. The picture was generated with PyMOL software and ACD/ChemSketch. (b) Schematic picture of light induced charge separation in PSI-monomer with attached dyes (blue circles). (c) SPV spectra of native PSI monolayer on gold surface. The contact potential difference (CPD) under illumination of unmodified PSI showed negative values (green line), which coincide with the absorption spectrum of the unmodified multiprotein complex (inserted graph). (d) SPV spectra of PSI-ATTO 590 monolayer whereas the modified PSI spectrum (red line) showed the appearance of a new peak in CPD at 590 nm (indicated by arrow), corresponding to the absorption spectrum of the dye-modified protein complex (inserted graph). PSI was modified with 6 ATTO 590 dyes on average per PSI monomer.

that no covalent bonds could be formed with the PSI backbone. In this control experiment, no change in the oxygen consumption was detected with respect to pristine PSI (Figure S6). These control experiments prove that the boost in functionality of the dye-modified PSI results from additional energy transfer from the organic dyes to PSI. This transfer does only occur if the dyes are covalently attached to the multiprotein scaffold.

To further investigate the origin of the increased oxygen consumption, experiments with selective excitation were performed (Figure S7). Therefore, optical filters were introduced into the measurement setup. With a 660 nm band-pass filter, thereby exciting the photosystem directly (ATTO 590 dyes are not excited), the oxygen-consumption rate of dye-modified PSI was similar to the rate of pristine PSI, which indicates that the chemical modification did not affect the PSI functionality and that the increased activity is not due to direct photoreduction of MV^{+2} from the ATTO 590 dyes. In contrast, when a 580 nm band-pass filter was introduced, dye-modified PSI showed significantly increased activity in relation to native PSI under the same conditions. Interestingly, the oxygen-consumption rate for modified PSI excited at 580 nm (dye absorption) was measured to be faster than native PSI excited at 660 nm (main absorption peak of PSI). This again indicates the occurrence of efficient energy transfer from the dyes to the main absorption peak of PSI. The higher activity at 580 nm excitation can be explained by a nonsaturating light intensity at both wavelengths, which results from the use of filters at 580 and 660 nm.

Next, the activity of PSI was assessed in the solid state by means of surface photovoltage (SPV) spectroscopy on a gold surface functionalized with 2-mercaptoethanol as directing layer (see SI for sample preparation). We estimated that 2/3 of all charged amino acids are located on the side of the iron−sulfur clusters of the PSI; therefore, the majority of complexes are oriented with the iron−sulfur clusters close to the gold surface, as shown earlier (Figure 4a).[29,30] In this solid state configuration the photoinduced charge cannot be compensated by electron-accepting and -donating molecules, as in the oxygen-consumption experiment; therefore, charge separation along the peptide backbone should result in the formation of a dipole pointing toward the surface.[31] This light-induced dipole was measured as an SPV signal using a Kelvin probe.[31]

The SPV spectrum of a native PSI shows the same spectral feature as the PSI absorption spectrum (Figure 4c). This confirms that charge separation indeed occurs upon excitation of the self-assembled PSI monolayer. The positive sign of the SPV signal indicates the formation of a net dipole pointing toward the surface, as anticipated. A positive SPV signal was observed for the dye-modified PSI (6 dyes attached, Figure 4b), as well. An additional peak at a wavelength of 590 nm, consistent with the absorption maximum of the coupled dye, appeared in the SPV spectrum of the modified PSI (Figure 4d). The appearance of this peak clearly indicates that the energy absorbed by the dyes is transferred to PSI and subsequently

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results in charge separation. A control experiment with dye-modified BSA monolayer (Figure S8) did not show any discernible features in the SPV spectrum (Figure S9), confirming again that the dye should be coupled to a photoactive protein scaffold in order to facilitate the charge separation process. These experiments prove that energy transfer and subsequent charge separation in PSI can occur even in solid state conditions demonstrating the potential use of these complexes in, for example, solid state biosolar cells.[29]

In previous studies, chemical coupling of organic dyes to the small reaction center of the purple photosynthetic bacterium *Rhodobacter sphaeroides* containing only four bacterio-chlorophylls[32] has been investigated.[33,34] The attachment of synthetic chromophores only resulted in improved activity when exciting the hybrid at a single wavelength coinciding with the absorption maxima of the attached dyes but improved functionality under more realistic broad band illumination remains elusive. We achieved this goal and have demonstrated up to 4-fold improved functionality under white-light irradiation within a molecular complex that is much larger (trimer contains 288 chlorophylls) than the reaction center. We show that energy transfer can be easily accomplished within significantly larger dimensions between synthetic dyes and the natural antenna system. Broadening the absorption of a similar photosynthetic complex has been realized by conjugation of metal nanoparticles or incorporation into plasmonic structures.[35,36] However, the effect of the inorganic nano-objects on electron transfer has not been investigated.

CONCLUSIONS

In conclusion, we have increased the activity of PSI by covalently coupling fluorescent dyes. The attached chromophores significantly enhance the absorption of the protein complex in the green region, where PSI does not absorb efficiently. Energy transfer from the organic dyes to PSI was confirmed by steady-state and time-resolved photoluminescence measurements. The dye-modified PSI showed a more than 4-fold enhancement in the oxygen-consumption rate under white-light excitation, indicating that the biochemical activity of PSI is significantly improved by covalent dye conjugation. The oxygen-consumption rate additionally showed a clear dependence on the number of attached dyes, reaching saturation for around 32 coupled chromophores per monomer of PSI. Ultimately, charge separation was observed for a PSI monolayer on a solid metal surface upon excitation of the coupled dye by means of the formation of an SPV. Our study shows that covalently coupled dyes can be used to fill the green gap in PSI and enhance the charge generation in this large megadalton protein complex both in solution and in dry conditions. As such, the hybrid systems consisting of PSI and organic chromophores reported herein represent important conjugates because they serve as models to study energy transfer processes to and within PSI and they might improve the photovoltaic conversion efficiency of PSI as active component in biophotovoltaic devices and hydrogen production cells.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.5b00583.

Experimental details and additional measurements of AFM, TEM, absorption spectra, steady-state and time-resolved measurements, oxygen consumption experiments and SPV (PDF)

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Notes

The authors declare no competing financial interest.

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