Trends in Biotechnology

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REVIEWS

Review

Putting Photosystem I to Work: Truly Green Energy

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Meeting growing energy demands sustainably is one of the greatest challenges facing the world. The sun strikes the Earth with sufficient energy in 1.5 h to meet annual world energy demands, likely making solar energy conversion part of future sustainable energy production plans. Photosynthetic organisms have been evolving solar energy utilization strategies for nearly 3.5 billion years, making reaction centers including the remarkably stable Photosystem I (PSI) especially interesting for biophotovoltaic device integration. Although these biohybrid devices have steadily improved, their output remains low compared with traditional photovoltaics. We discuss strategies and methods to improve PSI-based biophotovoltaics, focusing on PSI-surface interaction enhancement, electrolytes, and light-harvesting enhancement capabilities. Desirable features and current drawbacks to PSI-based devices are also discussed.

PSI-Based Biophotovoltaics

The concentration of CO_2 in the atmosphere of the Earth recently passed 400 ppm – the highest in over 400 000 years. A major contributor to this increase in CO_2 levels is the combustion of carbon-based fuels to meet growing worldwide energy demands, and supplies of these fuels are by their nature limited [1]. There is a clear need for alternative, sustainable methods of energy production to meet growing demands. One such method is solar energy conversion, harnessing the nearly 175 000 TJ (terajoules) of photonic energy striking the Earth annually [2]. Phototrophs are the primary living organisms on Earth that utilize this energy production strategy through photosynthesis, and they have been evolving more efficient and specialized photosynthetic apparatus for nearly 3.5 billion years.

The emerging field of applied photosynthesis offers the potential to use the light-harvesting protein–pigment supercomplexes of photosynthesis as photosensitizers in **dye-sensitized solar cells** (**DSSCs**; see Glossary), thereby creating biohybrid solar cells [3]. More background on DSSCs is given in Box 1. These protein–pigment supercomplexes are also referred to as reaction centers. One of the most commonly utilized reaction centers in biophotovoltaic devices is Photosystem I (PSI), which is found in all oxygenic phototrophs [4,5], and PSI from the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 is especially commonly used. Further biological background on PSI is given in Box 2. PSI has several desirable characteristics for inclusion into biophotovoltaic devices, including an **internal quantum efficiency (IQE)** approaching 100%, the generation of the greatest reducing power found in nature at –1.2 V versus **standard hydrogen electrode (SHE)** the **standard hydrogen electrode (SHE)**, the ability for *T. elongatus* genetic transformation, the availability of high-quality 3D PSI crystal structures, and relative ease of purification. Furthermore, PSI from *T. elongatus* is thermostable, a highly favorable characteristic for integration into solar devices. Biological components have several advantages for developing photovoltaics technology: low energy input, little to no geopolitical resource limitation, abundant and/or diverse starting materials, and nontoxic materials or fabrication methods.

Highlights

Studies of alternative electrode and semiconductor materials have improved PSI orientation and activity on electroactive surfaces.

Stable, photoactive dense packing of PSI on electrode and semiconductor surfaces is difficult. Crosslinking, redox polymer hydrogels, 3D semiconductor architectures, and biocompatible electrolytes could improve PSI-based device stability and output.

Native PSI utilizes chlorophylls *a* and *b* for its light-harvesting antenna, leaving a 'green gap' in its absorption spectrum. Dyes and nanoparticles that can use this green gap and transfer their energy to PSI can enhance the photoactivity of PSI *in vitro*.

PSI reduction kinetics post-photoexcitation are slow and limiting, and novel redox mediators and modified proteins to improve kinetics could improve photosensitizer regeneration rates and enhance photocurrent densities in PSI-based devices.

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Box 1. Schematic and Workings of a Dye-Sensitized Solar Cell (DSSC)

DSSCs contain five primary components: a photoactive dye referred to as a photosensitizer, a transparent electrode enabling photoexcitation, a conductive semiconductor, one or more redox mediators in an electrolyte, and a counter-electrode (Figure I). All DSSCs, including biologically based DSSCs, perform in a similar manner. (A) First, the photosensitizer (PSI) absorbs a photon, and (B) the photosensitizer is then promoted to an excited state. Next, charge separation by the photoexcited sensitizer occurs, and (C) the generated electron is injected into the working electrode via the semiconductor. (D) The photosensitizer is then reduced by the redox mediator in the electrolyte, priming it for further photoexcitation events to generate more photocurrent. Finally, (E) the redox mediator is reduced in turn at the counter-electrode, completing the circuit.

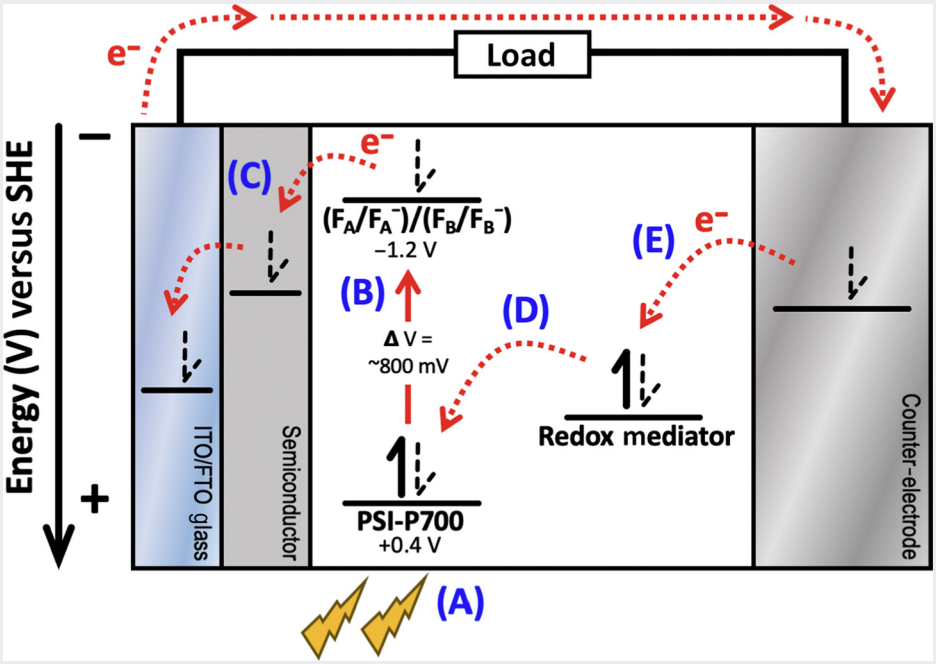

Figure I. Dye-Sensitized Solar Cells (DSSCs Are an Emerging Third-Generation Photovoltaic Technology. Solar photonic energy is converted into electronic energy that is capable of performing work in a manner similar to that of biological photosynthesis. Abbreviations: FTO, fluorine-doped tin oxide; ITO, indium-tin oxide; SHE, standard hydrogen electrode.

Although the efficiency of PSI-based biophotovoltaic devices has been increasing steadily [5,6], they remain relatively low compared to traditional photovoltaics. Several factors contribute to this, including low electrode surface coverage of PSI, slow rates of **electron injection** into the photoelectrode, limited absorbance in the UV/visible (UV-Vis) spectrum, rapid relaxation of the blue photon-excited state, and competition from rapid internal back reactions. All of these properties lower the overall internal and external quantum efficiencies of PSI-based biophotovoltaic devices.

This review covers multiple advances in strategies for bioengineering of PSI as well as new approaches for integration with inorganic materials that are used in the applied photosynthesis field, including advances in electrode and **semiconductor** materials and wiring strategies, enhancing the **optical cross-section** for enhanced PSI photoexcitation, and biocompatibility improvements to electrolytes and electron transfer kinetics. We aim specifically to discuss the biocompatibility aspects of these different approaches and methods of enhancing the photoactivity of PSI, rather than focusing on improvements on the inorganic side of these

Glossary

Charge recombination: a process by which the electron in the photosensitizer that was promoted to a higher energy state falls back to its ground state, causing the charge separated (+/–) state to no longer exist.
Dichlorophenolindophenol (DCPIP): a commonly used synthetic dye for measuring rates of electron transfer by Photosystem I.
Dye-sensitized solar cell (DSSC): a type of solar cell belonging to the newest third-generation of solar cells, invented in 1991. DSSCs are also commonly known as Grätzel cells after their creator, Michael Grätzel. There are multiple attractive features of DSSCs as compared to traditional silicon *p-n* junction type cells: ease of fabrication, generally low cost, and ability to make semi-flexible and semi-transparent devices.
Electron injection: the emission of electrons from one solid to another.
Internal quantum efficiency (IQE): the percentage of electrons generated per photon absorbed by the sample of interest. A high IQE percentage describes an efficient conversion rate of absorbed photons into electrons, by either a photosensitive dye or a solar cell.
Midpoint potential: the thermodynamic point at which a chemical species switches its tendency from donating to accepting electrons – in other words, when it becomes thermodynamically more likely for the chemical species to be reduced or oxidized. Midpoint potentials, or E_m, are reported in the standard measurement unit of volts (V) relative to a standardized reference electrode. It is thermodynamically favorable for chemical species with a more negative midpoint potential to donate their electrons to a chemical species with a more positive midpoint potential.
Optical cross-section: a description of the amount of the UV-Vis spectrum that is absorbed by a photosensitizer such as PSI. An increased optical cross-section in either wavelengths absorbed, or intensity of absorption, should both in theory yield increased photoactivity of the photosensitizer.
Photocurrent density: the magnitude of photocurrent generated per unit of active electrode area; a method to standardize the electrochemical output and efficiency of devices.

Box 2. PSI Is a Cytochrome c_6–Ferredoxin Transmembrane Oxidoreductase *In Vivo*

In vivo, PSI is a membrane-bound protein–pigment complex that is embedded in thylakoid membranes where the other light reactions of oxygenic photosynthesis are catalyzed (Figure I). Monomeric cyanobacterial PSI is composed of 12 protein subunits [99] (~16 in algae and higher plants [15]), ~100 chlorophyll *a* molecules comprising a light-harvesting antenna, and an internal electron transfer chain composed of six chlorophylls, two phylloquinones, and three [4Fe4S] clusters (see Figure 1 in main text). In cyanobacteria, PSI trimerizes into a supercomplex ~1.2 MDa in size. Upon photoexcitation, PSI performs charge separation and generates unidirectional charge transfer using a special pair of chlorophyll molecules (denoted P700) with an internal quantum efficiency approaching 100%, generating a reducing potential of approximately –1.2 V versus the standard hydrogen electrode (SHE). This greatest reducing potential found in nature, as well as the high degree of stability of PSI post-extraction (>90 days for cyanobacterial [53] and >280 days for higher plant PSI [52]) have led to great interest in further bioengineering and integration of PSI into hybrid biophotovoltaic devices to improve their output. The typical PSI used in the creation of biophotovoltaic devices comes from the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1, although the higher plant PSI from *Spinacia oleracea* is also commonly used. Timescales of forward and reverse electron transfer steps from PSI internal cofactors to the special pair are shown, as well as their **midpoint potentials** and the midpoint potentials of common PSI electron donors and acceptors and semiconductor materials.

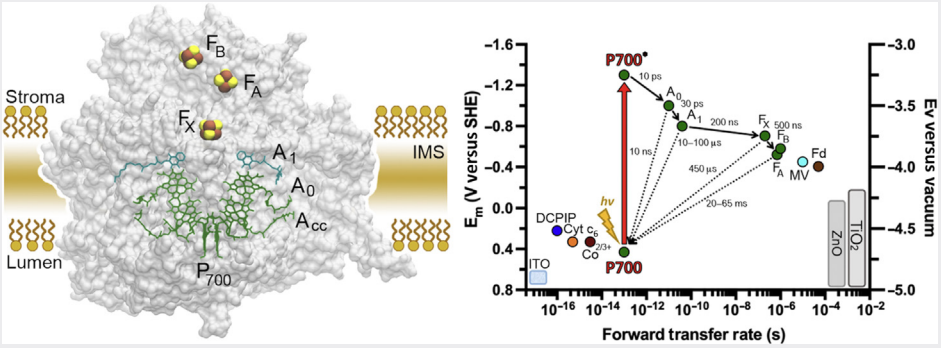

Figure I. Photosystem I (PSI) Is a Light-Activated Cytochrome c_6–Ferredoxin Oxidoreductase *In Vivo*. PSI is remarkably robust and performs its enzymatic activity with an efficiency approaching one electron generated per every one photon absorbed, nearing 100% efficiency. Abbreviations: Cyt c_6, cytochrome c_6; **DCPIP, dichlorophenolindophenol**; IMS, inter-membrane space; Ito, indium-tin oxide; SHE, standard hydrogen electrode.

Semiconductor: a material with an electrical conductivity value somewhere between that of a conductor and an insulator. Semiconductors can help with collection of charge and have more unidirectional current conduction. Some semiconductors are also thermally conductive and can assist in dissipation of excess heat.

Standard hydrogen electrode (SHE): a redox electrode whose relative oxidation–reduction midpoint potential is set as zero volts at any temperature T. This redox electrode is based on the following half-cell reaction $2H^+(aq) + 2e^- \rightarrow H_2(g)$, and sets the zero point for the thermodynamic scale of oxidation–reduction midpoint potentials.

biohybrid devices, to offer a novel perspective on advances in the applied photosynthesis field [6,7].

Advances in Electrode Materials, Integration Strategies, and Device Architecture

There are two different methods by which electron transfer between an electrode and a biological electron transfer complex such as PSI can occur: (i) direct electron transfer (DET) between the two, and (ii) mediated electron transfer (MET) where electron transfer between the electrode and the biological enzyme is performed by a mediator [8]. Because the electron transfer that PSI catalyzes is unidirectional from the lumenal to the stromal face of the complex, and PSI readily undergoes **charge recombination**, rational design and bioengineering to optimize PSI–electrode interactions are especially critical for enhancing device performance. This can be achieved in several ways: modification/biofunctionalization of electrode surfaces, utilization of different semiconductor materials and architectures, and enhancement of PSI coverage and orientation on the electrode surface.

Electrode Materials

The choice of electrode and semiconductor materials is of paramount importance for any electrochemical experiment or device, including photobioelectrochemical devices such as PSI-based biophotovoltaics, because proper orientation of redox-active enzymes without perturbing their structure or activity is crucial [9] for achieving optimal activity. For this reason, one area of recent

interest has been the effect on PSI of immobilization onto conductive surfaces, and how to improve PSI photoactivity on these surfaces. The most commonly used electrode materials in PSI-based biophotovoltaics for the photoactive electrode are either Au or indium-tin oxide (ITO)/fluorine-doped tin oxide (FTO)-coated optically transparent glass, owing to the optically transparent nature of the conductive glass and the highly conductive and biologically inert nature of gold [10]. Several desirable properties are necessary for PSI-based biophotoelectrode materials, including optical transparency to allow photoexcitation, high electroactivity for collection of photocurrent, biocompatibility, and ideally the potential to reduce charge recombination.

Several advances have been made in electrode and semiconductor material choices, as outlined below. Compared with being free in solution, cyanobacterial PSI immobilized on a conductive glass surface displays acceleration of the excitation decay of its antenna chlorophyll [11–13] from 16 to 11 ps, and this was suggested to result from a high packing density of PSI on the surface and not from effects due to protein hydration. This was corroborated by studies on a different photosynthetic protein–pigment complex from algae and higher plants, light-harvesting complex BII (LHCBII), where both accelerated excitation decay and red-shifted absorption spectra of a subsection of the chlorophyll antenna were seen [14]. To our knowledge, there are currently no reports on the effects of hydration states on energy transfer in PSI, although a dense network of hydrogen bonds through water molecules in the crystal structure of PSI is involved in antenna chlorophyll and protein subunit coordination, suggesting that proper hydration is necessary for optimal PSI activity [15].

Carbon-based materials for electrodes are one of the newest emerging areas for improving biohybrid device performance. These include the potential of graphene as an alternative material for PSI photobioelectrodes [16,17]. The desirable properties of graphene include high electrical conductivity, optical transparency, and mechanical strength, although graphene affinity for proteins is low. It has been shown to be possible to obtain significant improvements in the generated photocurrents specifically by utilizing covalent binding modifications on graphene, and pyrene-based functionalization yielded an exclusively unidirectional cathodic photocurrent. Another electrode material of interest is p-doped silicon [18,19]. Utilization of p-doped Si electrode material has reduced charge recombination, a highly desirable characteristic. Isolated *Spinacia oleracea* PSI immobilized using an Os-modified poly(1-vinylimidazole-co-allylamine) polymer to integrate PSI in a hydrogel on a p-doped Si electrode yielded further decreases in PSI charge recombination and generated **photocurrent densities** in excess of 0.3 mA/cm^2 [20].

Optimizing Directional Orientation of PSI on Nonbiological Surfaces

Because PSI catalyzes unidirectional electron transfer from its lumenal to its stromal side, it is necessary to develop methods for directional binding of the protein to electrode and semiconductor surfaces, as well as to enhance its affinity for interaction at the biological–inorganic interface. Examples of some of the different methods of PSI interaction with the electrode are shown in Figure 1. Possibly the simplest method of orientation has been to use the asymmetry of the PSI structure itself [4,15]. PSI has a strong dipole moment due to the differential charge of its lumenal and stromal surfaces, especially PSI from higher plants. Utilization of this dipole results in oriented attachment of a PSI self-assembled monolayer (SAM)-coated gold surface [16,17]. In this work it was clear that the surface properties of the SAM were crucial, and carboxy- or hydroxy-terminated thiols performed best [21]. Earlier work also used bioengineering of PSI to add exposed thiols to attach directly to an Au surface without the need for SAMs [22]. These efforts have largely resulted in attachment of the lumenal surface to the electrode surface, as shown in Figure 1. Another method involves the use of small affinity tags of short amino acid chains that can selectively bind to metals. These short peptides can be selected using phage display and

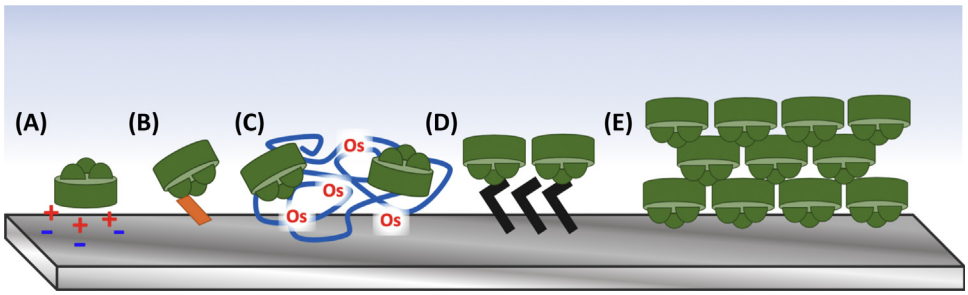

Figure 1. Photosystem I (PSI) Electrode and Semiconductor Wiring Strategies. Different strategies to improve PSI activity with electrode and semiconductor surfaces are shown. (A) Use of native electrostatic regions on the stromal or lumenal faces of modified graphene electrodes. (B) Use of metal-binding peptides fused to subunits of PSI to orient and noncovalently bond PSI to the surface. (C) Redox polymer hydrogels, such as those utilizing Os-based complexes with PSI embedded withifn. (D) Thiol-modified surfaces on gold electrodes to covalently link proteins such as PSI to the electrode surface. (E) Thin-film dried monolayers of PSI to generate multilayers and improve PSI total coverage.

are specific for different metal oxides including ZnO and TiO_2. These peptides can then be bioengineered as a fusion protein onto exposed surfaces of the PSI stromal surface. Specific TiO_2- and ZnO-binding peptides have been recombinantly fused onto either the stromal subunits of PSI (PsaD and PsaE) or onto the native electron acceptor protein from PSI, ferredoxin (Fd) [23]. Although this could be performed *in vivo,* the ability to 'rebuild' the stromal surface of PSI *in vitro* using *Escherichia coli*-expressed proteins offers a simpler and better controlled path for bioengineering [24,25]. This replacement of native PSI subunits with the recombinant peptide-tagged subunits has yielded incorporation efficiencies of up to 90%, high affinities for nanostructured semiconductors [23], and has been utilized in a device yielding one of the highest photocurrent densities to date by orienting the stromal face of PSI towards the photoelectrode.

3D TiO_2 Semiconductor Architectures

On ITO/FTO electrode surfaces, semiconductor layers are often utilized for multiple reasons. These include collection of photosensitizer-derived charge, improved electron injection into the electrode and thus through the cell circuit, and the ability to create 3D architectures to enhance photosensitizer coverage. The most commonly used semiconductor material in biologically based solar cells is TiO_2 because of its abundance, nontoxicity, and stability [26]. The dye or plant pigment such as PSI utilized as the photosensitizer is then typically adsorbed onto the semiconductor surface to create a photobioelectrode [26,27].

The development of 3D semiconductor architectures has attracted interest for improving device performance. These novel architectures can dramatically increase the surface area of the electrode and permit several orders of magnitude improvement in coverage of the photosensitizer dye (PSI). In addition to increasing the surface area, contact with redox electrolytes in wet electrochemical cells will also be improved [26]. Increased PSI coverage would yield more available photosensitizer to generate larger photocurrents in a biophotovoltaic device. 3D structures used include nanotubes, nanoparticles, and nanopyramids, and most recently several papers with impressive photocurrent densities have been published that utilize an inverse opal design [28–31]. Inverse opal designs involve the use of self-assembling monodispersed colloidal spherical particles embedded in the TiO_2; these are then removed, creating a 3D ordered porous structure with a very large surface area and adjustable pore structure and size [32]. These different 3D architectures are shown schematically in Figure 2 [33–35]. One particularly interesting new study utilized *Spinacia oleracea* photosystem II (PSII) and a tungsten trioxide (WO_3) nanoparticle film on an FTO semiconductor surface, and yielded high photocurrents and enhanced electron

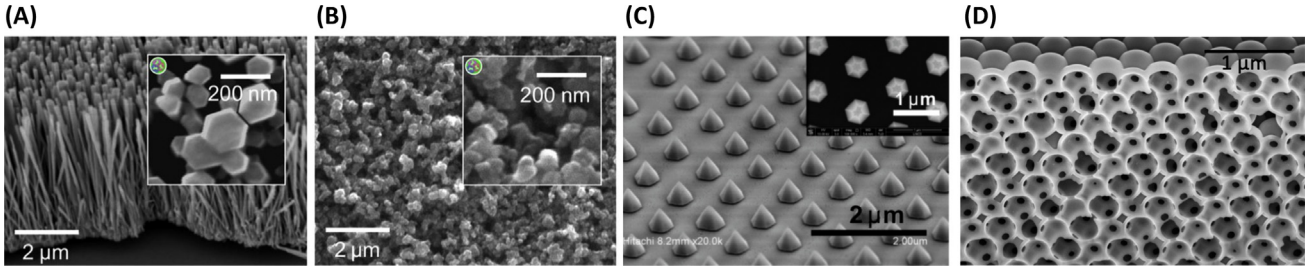

Figure 2. 3D Semicosnductor Architectures and Structures. 3D architectures used to improve photosensitizer (photosystem I, PSI) coverage on working electrode surfaces are shown. (A) nanotubes, (B) nanoparticles, (C) nanopyramids, and (D) inverse opal designs. These 3D architectures are also compatible with the different approaches shown in Figure 1 for improved PSI wiring. Panels (A) and (B) are reproduced from Mershin *et al.* under license (https://creativecommons.org/licenses/by-nc-sa/3.0/#). Panel (C) was adapted, with permission, from [34]. Panel (D) was reproduced from [35] under a CC-BY license.

transfer through the FTO layer into the electrode. Beyond 3D architectures, multiple methods have recently been employed to enhance PSI coverage, including deposition of multiple PSI thin films [36], crosslinking to stabilize PSI films on the electrode surfaces [37], and the use of redox polymers to create a hydrogel with embedded PSI [20,31]. Redox polymers have the added benefit not only of stabilizing multilayers, allowing greater concentrations of PSI deposition, and reducing charge recombination, but also of electronically connecting the reaction centers while allowing less stringent unidirectional binding. The use of redox polymers in PSI-based biophotovoltaic devices is likely to increase in future studies because of their simplicity and improved protein stability and activity when embedded in these redox hydrogels.

Alternative Carbon-Based Semiconductor Materials

In addition to the commonly studied metal oxides (TiO_2 and ZnO) as common semiconductor materials for DSSCs, there has been interest in studying alternative semiconductor materials. Specifically, carbon-based semiconductor materials have been a burgeoning area of interest for enhancing the output and efficiency of PSI-based biophotovoltaics. Multiple studies have been published on pi-system modified graphene as a semiconductor for PSI-based devices as well as enhancing photocurrent production [16,17]. Carboxy-modified C_{70} fullerenes can improve electrical interaction of a PSI monolayer with an Au electrode, generating photocurrent densities up to 15 μA/cm^2. Carbon-based semiconductor materials are attractive for multiple reasons, including the abundance and sustainability of carbon-based systems, attractive strength/mechanical properties, and the ability to design nanostructures with the increased surface area of various carbon architectures [38]. Further work may improve the protein affinity and optical properties of these alternative carbon-based semiconductor materials and enable PSI interaction in the proper orientation relative to the electroactive surface, greatly facilitating electron injection to the electrode.

PSI Stability Enhancement on Surfaces and in Solution

Although PSI immobilization and interaction with the electrode are of great importance for enhancing device performance, the effects of both purification and device integration on PSI stability must also be considered [39]. The effect of the microenvironment on the stability of proteins, especially membrane proteins, is well documented [40,41]. Both the structure and function/activity of proteins are known to be affected by environmental factors such as pH, ionic strength, and lipid environment for membrane proteins. Further, the immobilization of membrane proteins such as PSI on surfaces has proved to be difficult owing to issues such as aggregation that lead to lower protein activity. Multiple methods have been studied to improve protein stability, including electrospray deposition [42], the use of peptide surfactants and lipids to stabilize detergent

micelles [33,43,44], encapsulation of PSI in microparticles, and novel nondetergent-based purification methods.

One emerging method for the purification of membrane-bound proteins is the use of styrene–maleic acid (SMA) copolymers. These are theorized to act by wrapping around the membrane protein of interest as well as around a portion of the native lipid environment region surrounding the protein during solubilization of the protein out of the membrane, forming a SMA lipid particle (SMALP). Cyanobacterial PSI from *T. elongatus* is to date the largest protein complex to be purified using this method [45], and faster initial photochemistry leading to charge separation has been seen in this SMALP-PSI [46]. However, it has been shown that subunits of PSI are lost during this purification method, and this may be an issue for PSI isolated from other sources such as higher plants, and the long-term stability and viability remain unknown.

Nano- and microparticle encapsulation is a commonly used method for drug delivery and improved biocargo stability *in vivo*. Recently, the encapsulation of PSI in polymer-based microparticles has been studied as a way to potentially stabilize PSI in a protective environment [47]. Upon encapsulation in poly(lactic-co-glycolic acid) (PLGA) microparticles, both the stability and photoactivity of PSI were improved post-lyophilization as compared with purely detergent-purified PSI. The ability to make larger micron-sized structures also would likely simplify handling for use in device fabrication. Designer peptide surfactants, specifically those with increased positive charges, have been shown to similarly stabilize and enhance the photoactivity of cyanobacterial PSI [43]. A different approach that has also been used focuses instead on reducing the concentration of detergent or lipid, and takes advantage of the hydrophobic regions of PSI for linker-free deposition of the protein on TiO_2 semiconductor surfaces, yielding even coverage over the semiconductor surface and decreased aggregation [42].

One final issue covered in this review that affects the stability of PSI-based biophotovoltaics concerns the potential generation of reactive oxygen species (ROS). Photoexcitation of chlorophyll can yield a triplet chlorophyll excited state, which readily reacts with molecular oxygen to form ROS. ROS are highly damaging to proteins, and will lead to degradation of the device over time. It has been shown that buildup of ROS and other highly oxidizing species does in fact occur on PSI-based electrodes [20,48–50]. The incorporation of either biological or synthetic oxygen radical scavengers, or the development of anaerobic cells [51], is likely to help with improving the long-term stability of devices and lowering photocurrent density drops over time [49]. However, several studies already that indicate that PSI can remain photochemically active for many months, suggesting that device lifetime may be promising [52,53].

Enhancing the Light-Harvesting Capabilities of PSI

PSI has an impressive internal quantum efficiency (IQE) nearing 100% [54], but there is a 'green gap' in the chlorophyll *a* and chlorophyll *b* absorption spectra shown in Figure 3 where these pigments do not significantly absorb photonic energy, yielding wasted opportunities for PSI photoexcitation [4]. Two main approaches to increase the optical cross-section of PSI will be discussed: integration of biological light-harvesting antenna complexes, and incorporation of organic dyes/synthetic compounds. These biological and synthetic conjugators need to be biologically compatible and must be able to constructively interact with the PSI antenna chlorophyll, while not affecting photoexcitation or stability of PSI. In this section we discuss advances using synthetic dyes and nanoparticles. Figure 3 shows the absorption spectra of the synthetic dyes mentioned as well as the green gap that is present in the spectra of chlorophyll *a* and *b*. If the optical cross-section of PSI can be extended to fill in this green gap, more photoexcitation of PSI leading to higher photocurrent densities can hopefully be achieved.

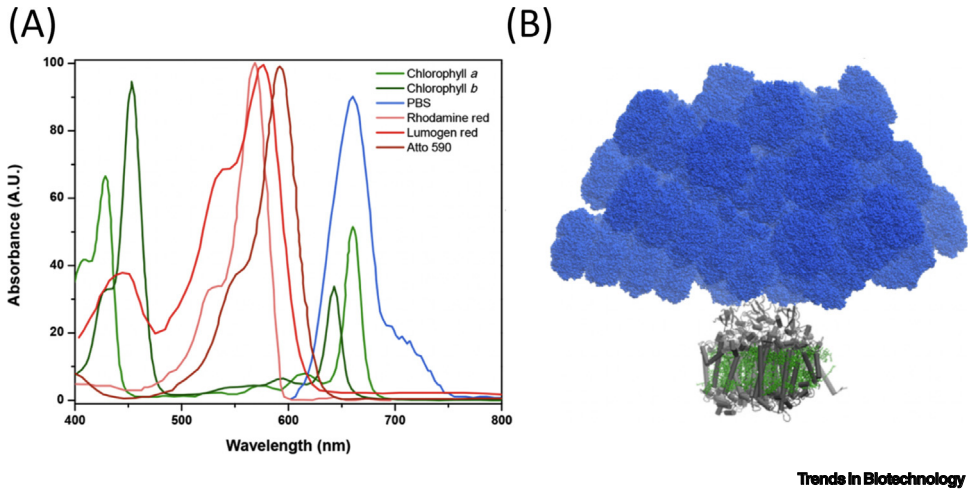

Figure 3. Spectra of Biological Light-Harvesting Antennas and Organic Dyes Used to Increase Photosystem I (PSI) Optical Cross-Section. (A) The absorption spectra of the dyes Atto 590, rhodamine red, and Lumogen red are shown together with the absorption spectra of chlorophyll *a,* chlorophyll *b*, and a generic cyanobacterial phycobilisome (PBS). The 'green gap' at ~500–600 nm in the UV–visible absorption spectra of chlorophyll *a* and *b* is easily noted. (B) A depiction of PSI shown in grey with its chlorophyll a antenna in green. Shown above in blue is a depiction of the native light harvesting antenna PBS complex, which is significantly bigger than the reaction center in size. Abbreviation: A.U., arbitrary units.

One important note is that PSI is intensely absorbing, and ~100 molecules of chlorophyll are bound per cyanobacterial monomer. The use of PSI multilayers to enhance photocurrent in devices can prevent light from penetrating fully through the multilayer, leading to reduced PSI photoexcitation. Attempts to attenuate this effect include reducing the chlorophyll antenna size of PSI [55]. This would permit photoexcitation deeper into the multilayers by reducing the amount of light absorbed by surface PSI layers. Modulation of antenna size *in vivo* has also been studied, and reducing chlorophyll content *in vivo* does enhance photosynthetic efficiency, potentially by modulating photoexcitation rates [56,57].

Conjugation of Organic Dyes and Synthetic Compounds to Enhance Optical Cross-Section

Another method for expanding the photonic energy that is capable of PSI photoexcitation, that has expanded in the past decade, is the conjugation of organic dyes, nanoparticles, and other synthetic compounds. This allows the creation and utilization of compounds with specific spectral properties to fill in the absorption gaps of biological photosensitizers such as PSI [6]. Advances in the incorporation of two different types of compounds, organic dyes and nanoparticles, are discussed below.

The use of organic dyes to fill in the green gap of chlorophyll-based protein–pigment complexes has been studied for the past decade. One of the earlier reports on modulating optical cross-sections assessed synthetic porphyrin and chlorin dyes with a variety of side groups and their spectral properties and ability to self-assemble into biomimetic light-harvesting antenna systems [58]. The highly ordered nature of these dyes, similar to the order of pigments in biological protein–pigment complexes, allowed photoexcitation even when aggregated and did not affect the photochemical efficiency [59]. The ability to couple together different dyes in synthetic biomimetic systems to enhance light-harvesting capabilities led to interest in dye conjugation to biological reaction centers such as PSI to similarly supplement their optical cross-section.

One report on dyes conjugated with a biological protein–pigment complex used recombinant LHC II and either one or three Rhodamine dyes covalently attached via cysteine residues on the protein [60]. Dye addition did not affect the assembly, stability, or activity of LHC II. Although

labeling efficiencies were low, electron transfer efficiency to LHC II was nearly 100%. Other dyes that have been similarly utilized include Alexa Fluor 660, 647, and 750 dyes, and these yielded similarly improved photogeneration of charge separated species while maintaining protein stability [61]. To date, the dyes Lumogen Red and ATTO 590 have been use to fill in the green gap of PSI. Coupling of ~30 ATTO 590 dye molecules to PSI increased the oxygen consumption activity of PSI by over fourfold, and the addition of Lumogen Red allowed greater energy transfer to PSI via Förster resonance energy transfer. Expanding the light-harvesting capabilities of PSI through dye conjugation [62,63] is likely to be an area of further focus for improving the outputs of PSI-based biophotovoltaic devices. The spectral features of PSI and the dyes previously mentioned are shown in Figure 3.

Conjugation of Nanoparticles to Enhance Optical Cross-Section

The second method discussed for enhancing the optical cross-section of PSI involves the incorporation of nanoparticles with photosynthetic reaction centers – specifically, quantum dots (QDs) and metal nanoparticles (NPs). There are a few potential drawbacks to note, namely the bulky size of QDs compared with dyes, and the nonspecific electrostatic interactions between QDs and reaction centers may be limiting for the activity of the conjugated system. Regardless, the incorporation of nanoparticles has proved to be an intriguing method for enhancing the optical cross-section of reaction centers such as PSI to enhance photoactivity for biophotovoltaic device improvement.

The optoelectronic properties of QDs are as a function of both size and shape [64], allowing fine-tuning to enhance their optical cross-section. QDs have previously been shown to be capable of transferring their photoexcitation energy to reaction centers, including PSI [65,66]. Reaction center charge separation has been found to increase anywhere between three- and fivefold upon QD conjugation. To date, more work on the use of NPs for optical cross-section enhancement has been done for other reaction centers than for PSI. A study assessing the interaction of CdTe QDs with the *Rhodobacter sphaeroides* reaction center found that a polyhistidine tag on the reaction center allowed targeted binding of the QD to the tag, and up to ~95% QD labeling efficiency was achieved [67]. The ability to now fine-tune the optoelectronic as well as binding properties of QDs makes this an area with great potential for further biophotovoltaic device improvement.

Metal NPs have also been utilized with PSI, specifically Pt NPs for H_2 evolution directly from the photogenerated electrons [53]. However, other metals including Ag and Au have been used. Ag NPs were found to enhance chlorophyll fluorescence up to 18-fold in peridinin–chlorophyll–protein, and 5–20-fold for thin films of cyanobacterial PSI [68,69]. Similarly, incorporation of Au NPs into leaves and chloroplasts *in vivo* increased reaction center reduction rates [70]. Although metal NPs may not directly expand optical cross-sections, the enhanced photochemical activity of reaction centers, including PSI, should improve the photocurrent outputs of devices. The use of metal nanostructures such as nanopyramids [30] and NPs have been suggested to enhance photochemical activity through plasmonic coupling, and further engineering to enhance interactions in this biotic–abiotic interface is likely to produce further biophotovoltaic device improvement [71].

Electrolyte and Redox Mediator Improvements for Enhanced PSI Turnover Rates

In all DSSCs, a redox mediator is present as a conductive electrolyte. This electrolyte/redox mediator solution is an important factor for numerous device parameters [26,72] including the fill factor and photocurrent density. The redox mediator shuttles electrons between the photosensitizer (PSI) and the counter-electrode, readying the photosensitizer for further photoexcitation

events. The canonical iodide/triiodide (I^-/I_3^-) redox pair [73] has been utilized in many DSSCs; however, it is incompatible with biophotovoltaics because of corrosivity and the generation of radical species [73]. Redox mediator choice is especially important for PSI-based biophotovoltaics because cyanobacterial PSI has very slow reduction kinetics – on the order of hundreds of milliseconds [74,75]. To date, comparatively little research has been done in these two areas. Advances in electrolytes and redox mediators for PSI-based biophotovoltaics will be crucial for further device improvement, and current approaches are shown in Figure 4.

Development of Redox Mediators and Enhancement of PSI Reduction Kinetics

In vivo, PSI is reduced by the metalloproteins cytochrome c_6 or plastocyanin [4]. However, PSI reduction by its native partners is relatively slow, necessitating either protein modification or the

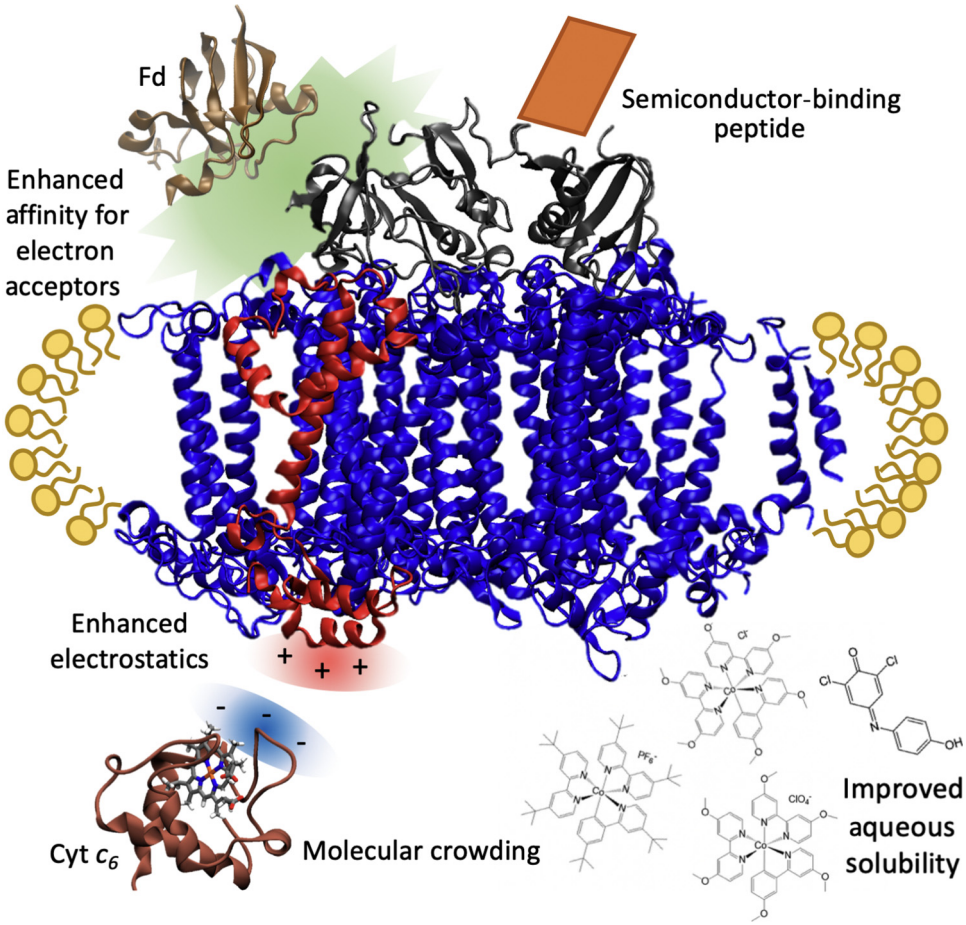

Figure 4. Modifications and Point Mutations of Photosystem I (PSI) and Redox Mediators To Enhance Affinities and PSI Reduction Kinetics. The various strategies to enhance PSI reduction kinetics are shown. On the stromal side, improved electron acceptor affinity and use of semiconductor-binding peptides reduce charge recombination and promote forward flow of electrons into the electrode and through the circuit. Point mutations in the PsaF subunit of PSI that enhance electrostatics on the lumenal face have been shown to correlate with increased negative charge on native electron donors such as cytochrome c_6. Increased molecular crowding, as well as increased ionic strength, have been shown to moderately increase PSI reduction kinetics. Finally, a variety of Co-based synthetic donors in conjunction with a traditional donor (dichlorophenolindophenol) are shown, and improving the aqueous solubility of synthetic donors has been shown to enhance PSI reduction kinetics while preserving protein stability. Abbreviations: Cyt, cytochrome; Fd, ferredoxin.

development of synthetic redox mediators to increase PSI reduction rates [5,75]. The interaction between PSI and its native redox mediators is partially driven by electrostatics, although determining a reaction mechanism has proved to be difficult – point mutations have led to only moderate increases in PSI reduction rates [74,76]. However, recent work has suggested that inclusion of the native cyanobacterial electron donor to PSI, cytochrome c_6, can improve device performance [77–79].

Owing to difficulties in achieving significant improvements by utilizing protein redox mediators, the study of synthetic redox mediators for more efficient PSI reduction has been an area of increased interest [80]. A report on polyviologen conductive solid polymer incorporation yielded a >10-fold increase in photocurrent densities compared with PSI-only devices, and there was no drop in photocurrent density over 30 days post-fabrication, likely because of the highly conductive properties of polyviologen [81]. One of the greatest photocurrent densities reported for a PSI-based device utilized synthetic Co-based complex redox mediators [33], and recent work has suggested that similar aqueous-compatible Co complexes have PSI reduction rates up to 38-fold greater than inorganic–soluble complexes [82]. Integration of aqueous-compatible mediators would likely lead to significant improvements in photocurrent densities, as well as the ability to fine-tune properties to enhance affinities and electron transfer rates to improve device performance.

Effect of Electrolytes and Solvents on PSI Reduction Kinetics and Stability

An area that is only recently being assessed is the effect of electrolyte and solvent choice on biophotovoltaic device performance. Organic solvents such as acetonitrile have been used, although the volatility and environmental impact of these solvents as well as concerns regarding effects on protein stability and activity have led to interest in the use of fully aqueous systems [83]. Acetonitrile, diethyl ether, glycerol, and polyvinyl alcohol (PVA) have been shown to negatively affect both the photochemical activity and the conformation of PSI [82,84,85]. Recent work has also shown that acetonitrile can leach chlorophyll out from the PSI light-harvesting antenna [82]. One particularly interesting set of reports tested the effects of 50% diethyl ether versus a fully aqueous buffer system and found a ~20-fold increase in photocurrent densities from the aqueous PSI-based biophotovoltaic device [86]. Clearly, moving towards fully aqueous electrolyte systems is likely to be of great interest for enhancing the performance of PSI-based biophotovoltaic devices [83]. The effects of pH, salt concentration, and molecular crowding on PSI reduction kinetics have been studied in aqueous solution, although their effects on photocurrent densities in devices have not been tested [75,87]. The addition of lipids in aqueous electrolytes has been found to help to stabilize the charge separation and photoactivity of the *Rhodopsedomonas palustris* reaction center on an electrode [44], and the effects of lipid incorporation would be intriguing for future studies on PSI-based devices because synthetic peptide surfactants were previously shown to enhance PSI stability [43,88].

Concluding Remarks

The development of PSI-based biophotovoltaics to take advantage of the biological process of photosynthesis and to develop more carbon-neutral, sustainable energy production is of interest for meeting growing worldwide demands. Although the outputs of these PSI-based biophotovoltaics have increased significantly since their initial development, there are still multiple avenues for further improvement. We have outlined distinct roadblocks as well as recent advances for increasing the output of PSI-biophotovoltaic devices. The many unique properties of PSI including its nearly 100% quantum efficiency and remarkable stability post-purification have made it a well-studied system of continuing research in the fields of applied photosynthesis and bioelectrochemistry.

Outstanding Questions

Much of the work done to date on improving PSI-based biophotovoltaic devices has focused on enhancing the inorganic side of the bio–inorganic interface. Can studies on enhancing the stability of PSI and other reaction centers *in vitro* be applied to improve the long-term activity and stability of devices?

PSI has very slow reduction kinetics post-photoexcitation, which reduces the levels of PSI that are available for photoexcitation in a biophotovoltaic device. By enhancing PSI reduction rates, is it possible to enhance the concentration of available PSI to generate more photocurrent in the same amount of time? Is it possible to develop synthetic or protein redox mediators for PSI with enhanced affinities and electron transfer rates to improve device performance?

Organic solvents commonly used in device electrolytes have been shown to be detrimental to the stability and activity of PSI *in vitro*. However, they are often used in electrolytes for improved conductivity and redox mediator solubility. Could modification with aqueous-soluble functional groups increase the biocompatibility with synthetic redox mediators?

The conjugation of dyes and nanoparticles to improve optical cross-sections of reaction centers has been studied in several systems including PSI. Recent work suggests that it is possible to target these nanoparticles and dyes to specific residues of a protein. Could specific targeting closer to special pairs lead to enhanced PSI photoactivity?

Biological redox-active proteins such as PSI are currently being incorporated into many types of devices beyond solar cells. One such device is an e-skin – a wearable electrode designed to mimic the ability of human skin to transduce environmental cues to the brain via electrical signals, and these are being developed as a class of wearable biomedical devices for tracking patient health and delivery of medicines [89]. Biomolecules have also been incorporated into biohybrid photodiodes to generate biologically based photosensors [90–92], and may have potential to be incorporated into more advanced light detectors and cameras [86,93–95]. Other photo-based sensors have been published that incorporate PSI specifically, and their use ranges from UV-detection [96] to solid-state photosensors [97] and photoactivatable electronic switches in devices [98]. These diverse applications show the potential of photoactive redox proteins to make environmentally and biologically friendly versatile photoelectrochemical devices beyond solar cells.

The unidirectionality of PSI charge transfer and the difficulty in achieving strong interactions between membrane proteins and electrodes have necessitated research into alternative electrode and semiconductor materials, as well as various modifications to enhance affinities, directionality, and activity. Improvements to enhance the stability and long-term activity of PSI bioelectrodes and biophotovoltaic devices through electrolyte and redox mediator choice, in conjunction with redox polymers, crosslinking, and the addition of lipids, are likely to remain an area of increasing focus for improvement. The high absorption of PSI as well as the green gap in its UV-Vis absorption spectrum have hindered attempts to obtain denser active packing of PSI on electrode surfaces. Manipulating the optical cross-section to enhance PSI photoexcitation by incorporating biological light-harvesting antennas, synthetic dyes, and nanoparticles has yielded moderate improvements in photocurrent densities of devices to date. Finally, studies on the effects of electrolytes and redox mediators to improve PSI reduction kinetics are likely to provide improvements to device performance through enhanced photosensitizer regeneration rates (see Outstanding Questions).

Although the overall efficiencies and outputs of PSI-based biophotovoltaic devices remain low compared with nonbiologically based DSSCs and other emerging solar cell technologies, the ability to construct devices at relatively low cost and with relatively high ease using sustainable materials, as well as the high degree of modification and functionalization that is possible in DSSCs, continue to make them an attractive option for further research and optimization.

Acknowledgments

A.H.T. and B.D.B are supported by the Gibson Family Foundation, the Tennessee Plant Research Center, a University of Tennessee at Knoxville (UTK)/*Oak Ridge National Laboratory* (ORNL) Science Alliance Joint Directed Research Development (JDRD) award to B.D.B., a Dr Donald L. Akers Faculty Enrichment Fellowship to B.D.B., and National Science Foundation support to B.D.B. (DGE-0801470 and EPS-1004083). A.H.T. was supported by an R25 Fellowship from the National Institutes of Health (R25GM086761).

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