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## Review

# Biohybrid solar cells: Fundamentals, progress, and challenges

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## ABSTRACT

Over the last two decades many reports have been published on diverse types of biohybrid electrodes utilizing components of the photosynthetic apparatus. Currently, the development of such devices does not extend beyond laboratory research. In the future, these electrodes could be used in biosensors, solar cells, and as a new technique to investigate photosynthetic pigment-protein complexes. Efficiency of light-to-current conversion is particularly important for solar cell applications. Selection of a suitable substrate for special pigment-protein complexes is a significant challenge for building an inexpensive and efficient device. Various combinations of pigment-protein complexes and substrates, as well as different measurement conditions make it difficult to directly compare performance of various solar cells. However, it has been shown, that one of the possible substrate materials, namely nanostructured TiO<sub>2</sub>, is the most preferred material for the immobilization of pigment-protein complexes in terms of both cost and efficiency. The photocurrent values reaching several mA, were reported for TiO<sub>2</sub>-based biohybrid electrodes. However, the efficiency of TiO<sub>2</sub>-based biohybrid is still far from its potential maximum value due to fundamental challenges related to designing an optimum interface between TiO<sub>2</sub> nanostructure and pigment-protein complexes containing electron transferring cofactors. To date, counterproductive back reactions, also referred to as charge recombination, still dominate and lower internal quantum efficiency of these systems.

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**Abbreviation:** ACVD, aerosol chemistry vapor deposition; AM, air mass; ATP, adenosine triphosphate; CNT, carbon nanotubes; DCBQ, 2,5-dichloro-1,4-benzoquinone; DDM, *n*-dodecyl-β-D-maltoside; DET, direct electron transfer; DMCU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DSSC, Dye-sensitized solar cells; ETC, electron transport chain; FTO, fluorine doped tin oxide; ITO, indium tin oxide; LHCI, light harvesting complex I; LHCI, light harvesting complex II; MET, mediated electron transfer; MWCNT, multi-walled carbon nanotubes; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NTA, nitrilotriacetic acid; OEC, oxygen evolving complex; PBEC, photo-bioelectrochemical cells; PSI, photosystem I; PSII, photosystem II; RC, reaction center; SAM, self-assembled monolayer; TCO, transparent conductive oxide.

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## Contents

1. Introduction .....	136
2. Brief history of photovoltaics .....	137
3. Dye-sensitized solar cell .....	137
4. Photosynthesis .....	138
4.1. Photosynthetic pigments .....	139
4.2. Reaction centres .....	140
4.3. Antenna systems .....	140
4.4. Electron transport chains .....	140
4.5. Plant and cyanobacteria .....	141
5. Solar cell-based incorporating components of the photosynthetic apparatus .....	141
5.1. Basic principles .....	141
5.2. Solar cell or biohybrid electrode .....	142
5.3. Common sensitizers .....	143
5.3.1. Bacterial RC as sensitizer .....	143
5.3.2. PSI as sensitizer .....	143
5.3.3. PSII as sensitizer .....	143
5.3.4. Thylakoid as sensitizer .....	144
5.4. Main challenges .....	145
5.4.1. Increasing absorption cross-section of electrodes .....	145
5.4.2. Immobilization and orientation .....	147
5.4.3. Prolonged viability .....	147
5.4.4. Absorption range .....	147
6. TiO <sub>2</sub> -based solar cell .....	148
6.1. Reaction centres of purple bacteria as sensitizer .....	148
6.2. Photosystem II as photosensitizer .....	150
6.3. Photosystem I as sensitizer .....	150
6.4. Thylakoids as sensitizer .....	152
7. Conclusions .....	152
Conflicts of interest .....	153
Acknowledgements .....	153
References .....	153



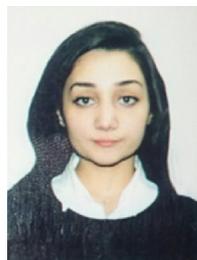
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## 1. Introduction

Worldwide energy consumption is slowly but steadily growing due to the growth in the world's population, increased utilization of power-consuming devices, and the rising demand of energy in developing countries [1,2]. Since fossil fuels remain the dominant energy source, increasing energy consumption will bring with it greater ecological problems regarding increased greenhouse gas emissions and depletion of a finite non-renewable resources. Therefore, one of the greatest problems facing the global community today is to replace inevitably diminishing fossil fuels with renewable sources of ecofriendly energy production [1]. The Earth receives almost 120 000 TW of solar energy, a value that greatly exceeds our current global demand of ~17 TW [1–3]. Thus, the research and development of novel devices for the conversion of photonic energy from sun light to electricity is a very attractive direction of contemporary research into alternative energy technologies. These devices are called solar or photovoltaic cells.

Currently it is customary to classify solar cells into the three generations [4,5]. The first generation consists of conventional wafer-based cells containing crystalline silicon. The second generation includes thin film solar cells composed of amorphous silicon, cadmium telluride and copper indium gallium selenide. The third emerging generation employs various architectures such as organic photovoltaics (OPV), dye sensitized solar cells (DSSC), and quantum dot solar cells. Each solar cell type has efficiency limits, design advantages and resource limitations. Collectively, current technologies have several drawbacks such as: inherently high energy input requirements, natural and geopolitical resource limitation, and environmental toxicity of certain components used in fabrication. Due to these obstacles, many researchers have begun to explore more natural strategies, such as the process of solar energy conversion *via* chlorophyll based photosynthesis [1].

Photosynthesis is arguably the most important natural process in existence, transforming our once lifeless planet into a living world. While primitive photosynthetic bacteria such as purple and green sulfur bacteria perform anoxygenic photosynthesis (producing elemental sulfur from hydrogen sulfide with the help of sunlight), cyanobacteria, algae and plants carry out oxygenic photosynthesis to convert water and carbon dioxide to sugars and release

oxygen as a by-product [6]. Light-induced charge separation and subsequent electron transfer precede the synthesis of the energy storage molecules resulting from photosynthesis [7,8]. Recently, focus has centered around the investigation of solar energy capture technologies based on natural photosynthesis, because the internal quantum efficiency of this charge separation event approaches 100%.

## 2. Brief history of photovoltaics

Photovoltaic refers to solar energy utilization by the direct conversion of light energy to electricity [9,10]. The first reports of the photovoltaic effect appeared in the middle of the 19th century, in an investigation using an electrolytic cell by Alexandre Becquerel. This cell consisted of two platinum electrodes immersed in the acid solution with silver chloride, which when illuminated generated a photocurrent [11]. In the first half of the twentieth century, breakthrough works were carried out in semiconductor theory and crystal growth. Combining these advances, Bell Laboratories (USA) developed the first silicon based solar cell in 1946 [12] and developed the first solar cell which could produce significant power by 1954 [13]. Continuing into the present, silicon-based solar cells dominate the photovoltaic market. Production of high-purity monocrystalline silicon requires high temperature and vacuum, which increase both energy input and cost [5,14,15]. This high cost restricted solar cells largely to the space industry for more than a decade. However, during the energy crisis in the 1970s, the need to decrease fossil fuel consumption pushed scientists and inventors to develop more cost effective solar cells [7,11,14–16]. This trend of decreasing cost has continued with the advent of new technologies, mass production, and government subsidies such that solid-state, Si-based solar panels are widespread. This review will focus on solar cells and photoelectrodes, based on components of the photosynthetic apparatus, which are more favorable due to their environmental friendliness, inexpensiveness, abundance and high quantum yield. We will consider photosynthetic processes, structure of the photosynthetic apparatus and its attractive features to applications in solar cell design. We will describe the most recent achievements in the design of solar cells or photoelectrodes based on photosystems and bacterial reaction centers. Similar to photosynthesis where evolution has added large light harvesting antennae structures, the performance of low band gap semiconductor solar cells can be enhanced by the addition of a substance with wide absorption spectra. The most impressive results in the field of bio-based solar cells to date have been achieved using TiO<sub>2</sub>-based biohybrid electrodes, like those used in the DSSC. Therefore, we will begin by outlining the structure and design principles of these devices.

## 3. Dye-sensitized solar cell

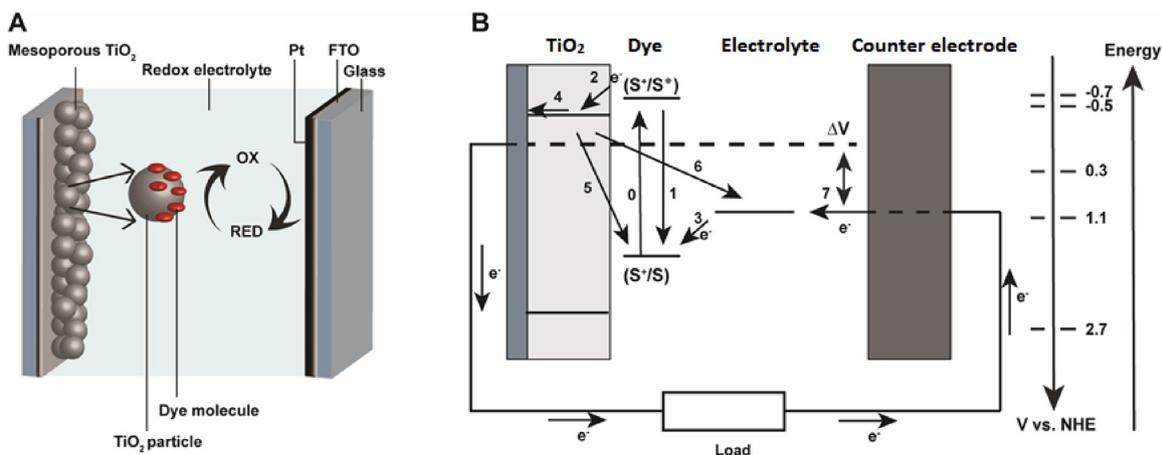
Dye-sensitized solar cells have recently attracted much interest as a promising energy harvesting technology. This is in part due to low-cost of fabrication, integration into a thin film format, high efficiency under low-light conditions, earth abundant composition and absorption over the visible light spectrum [11,17–19]. The most important feature of DSSCs is the use of mesoscopic semiconductor (most commonly TiO<sub>2</sub>) layer that has a very large effective surface area. This expansion of the surface increases the efficiency of the solar cell significantly [11]. The DSSC consists of two glass electrodes coated with a transparent conductive oxide (TCO) layer on one side. Fluorine doped tin oxide, SnO<sub>2</sub>: F (FTO) and indium tin oxide (ITO) are the most commonly used TCO substances. The photoanode is formed by the porous semiconductor layer deposited onto TCO-glass. The nanometer-sized particles of

semiconductor are bonded to each another to form a nanoporous three-dimensional structure. Dye molecules are attached to the surface of this film as the single layer. Aside from the nanocrystalline TiO<sub>2</sub>, ZnO and SnO<sub>2</sub> semiconductors are also used as the mesoscopic material [20,21]. However, TiO<sub>2</sub> gives the best results so far in terms of the DSSC efficiency. The advantages of TiO<sub>2</sub>, which include stability, environmental friendliness, and high refractive index, have also attracted significant interest in using this oxide as a white pigment in paint, toothpaste, sunscreen, self-cleaning materials, and food [17].

Dye molecules (referred to as sensitizer) are adsorbed onto the TiO<sub>2</sub> surface. Photoexcitation of this dye molecule results into the passage of an electron into the conduction band of the oxide material [22]. Sensitizers used in DSSCs are separated into two main groups regarding their nature: synthesized metal-complex dyes (including metal complexes, such as polypyridyl functionalized complexes of ruthenium, iridium and osmium) and organic dyes (natural and synthetic). The sensitizer in DSSCs fulfills a molecular electronic pump function. As visible light is absorbed by the sensitizer, it's raised to a higher energy level. The elevated energy electrons are then injected into the semiconductor. Electrons move in the semiconductor by diffusion and not by electric field. The oxidized dye molecule obtains electrons from the reduced form of an electrolyte which fills the gap between the electrodes, residing in the cavities of pores in particular. The most commonly used electrolyte for DSSC is iodide/triiodide (I<sup>-</sup>/I<sub>3</sub><sup>-</sup>) solution [18,23]. The dye is reduced by the I<sup>-</sup> species of the iodide/triiodide redox couple of electrolyte. Through this process, iodide is oxidized to triiodide, which migrates to the counter electrode and is reduced back by the electron from the external circuit. A single layer of catalytic platinum is overlaid onto another TCO-glass electrode to facilitate the electron transfer to the electrolyte from the external circuit. Open-circuit voltage of the solar cell is the difference between the Fermi level of the nanocrystalline film and electrolyte redox potential [24]. A schematic illustrating the principles of operation and structure of the DSSC is shown in Fig. 1.

The sensitizers used in organic DSSCs include polypyridyl complexes [19,25], porphyrins [26,27], phthalocyanines [28], coumarins [29,30], indolines [31], conjugated polymers [32] and perylenes [33]. In 1991, initial impressive results in the study of DSSC was reported for Polypyridyl complexes of ruthenium served as sensitizer [19]. Soon a new dye, N3, *cis*-di(thiocyanato)-bis(2,2'-bipyridyl)-4-4'-dicarboxylate ruthenium (II) complex was discovered by Nazeeruddin and colleagues in 1993 [34], and the yield of DSSC created with this dye has increased to 11% [11,26,35,36]. However, these dyes have several disadvantages including complex synthesis, high cost, and global resource limitation of ruthenium.

These limitations have pushed investigators for a source of natural dyes that are both more cost-effective yet also could serve as a low cost and sustainable resource. These natural dyes, could be readily extracted from fruits, flowers and leaves and may offer advantages over the ruthenium-complexes. The trade-offs between these natural dyes and Ru based dyes are often decreased conversion efficiency, durability or long-term stability [37]. As a result of these benefits, studies into the use of natural dyes remains a popular area of DSSC research. For instance, Cherapy et al. investigated in 1997 the photoelectrical properties of DSSC that used the pigments found in blackberries, anthocyanin, as the sensitizer and investigated its photovoltaic efficiency [38]. This natural dye from blackberries was extracted using a mixture of methanol/acetic acid/water and yielded an incident photon to electron conversion efficiency of 0.56% [38]. In 2006, Hao et al. established that the chemical structure of the dye substance directly influences the conversion efficiency of the DSSC. In their investigation, the best photovoltaic performance has been



**Fig 1. Structure and redox reactions of DSSC.** A. Structure of DSSC is depicted. Ox and Red denote reduced and oxidized species of electrolyte. B. Diagram of operational principles of DSSC is shown. Reactions of electron transfer, denoted by numerals 0,2,3,4,7—are the direct desirable reactions. Only they are realizing in ideal DSSC. The reactions, denoted by numerals 1, 5, 6 are the undesirable reactions of recombination. The potentials for a DSC based on the N3 dye,  $\text{TiO}_2$ , and the  $\text{I}^-/\text{I}_3^-$  redox couple is shown. Their redox-potential are represented against the normal hydrogen electrode (NHE) also called standard hydrogen electrode (SHE). Adapted from [17]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

noted in a DSSC fabricated using the natural dye from black rice. In this device, an open-circuit voltage of 0.551 V and short-circuit current of 1.142 mA/cm<sup>2</sup> were recorded [39]. They also conclude that the molecule of anthocyanins can be immobilized to the surface of the  $\text{TiO}_2$  semiconductor *via* hydroxyl and carbonyl groups, whereas other well-known natural pigments such as chlorophyll and carotene cannot be adhered to the semiconductor surface [39]. In 2008, Zhang et al. created a betalain-based DSSC using extracted red beet roots. This DSSC had a short-circuit current of 2.42 mA cm<sup>-2</sup> and open-circuit voltage of 0.44 V [40]. In 2013, new dyes were synthesized using modified phenothiazine with different chain length of alcohols. Although these dyes do not include metal atoms, and have yielded 10–11% efficiency [41,42].

Despite these advances there are still several problems with DSSC technology that require further development [43]. In the DSSC, electrolyte solution can permeate into the  $\text{TiO}_2/\text{TCO}$  interface through the cavities in the mesoporous layer creating an electrical short between the liquid electrolyte and the TCO substrate [44] allowing recombination reactions between the electron passed to the TCO electrode and oxidized redox species of electrolyte [45] (Fig. 2). Thus a barrier is needed to prevent this reaction, yet the barrier should not hinder the electron transfer from  $\text{TiO}_2$  to the TCO [46]. Due to limitations of exciton migration, semiconductor thicknesses in DSSCs are limited to the micron scale (5–20  $\mu\text{m}$ ), which reduces the optical density. However, the optical cross-section can be increased by adding a scattering layer of appropriate sized nanoparticles and significant improvements during the last two decades by various new architectures of  $\text{TiO}_2$  nanostructures [47,48].

Studies have shown that the liquid electrolyte may also limit DSSC performance. For example, recombination reactions can occur within the electrolyte causing a decrease in photocurrent produced. In addition, aqueous electrolyte solutions evaporate over time and many strong electrolytes can result in corrosion of the electrodes. For these reasons, polymer based electrolytes have been deployed for use in DSSCs to overcome the issues of leaking and sealing, flammability, shape flexibility and electrochemical stability faced by liquid electrolytes. However, due to lower ionic conductivity of polymer electrolytes, liquid electrolytes remain the dominant choice in the DSSC field [58,59].

Together it is clear that continuing success of DSSCs will drive commercialization of its components. There are, large firms on the solar market, which produce and sell different components of the

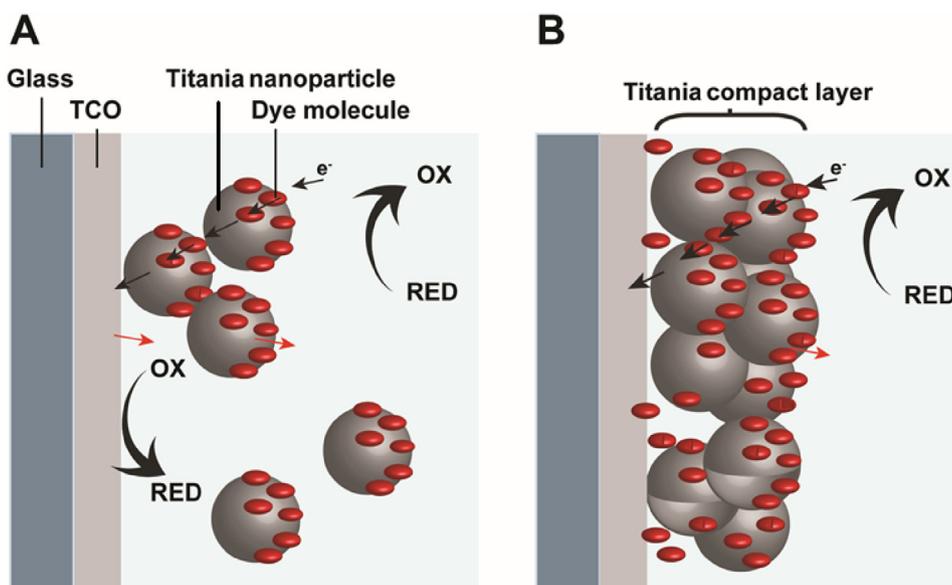
DSSC: electrolytes,  $\text{TiO}_2$  pastes with varying particle size, sensitizers and TCO-glass electrodes [49]. Therefore, it's easy to purchase these components to build solar cells and investigate improvements. In turn, this easy access increases interest and stimulates the intensive development of this field. Hence, there are many different techniques reported in the literature for in-lab preparation of  $\text{TiO}_2$  nanopowders. They include sol-gel, hydrothermal, anodic oxidation, chemical vapor deposition and electrodeposition techniques [17].

The use of natural photosynthetic pigments vs. high-cost, synthesized ruthenium complexes has offered the DSSC field a more sustainable technology and has been recently extended to include photosynthetic complexes as natural dyes. Exploiting photosynthetic pigment-protein complexes in solar cells is a natural and exciting convergence of two research areas. Components of the intricately arranged photosynthetic apparatus achieve light induced charge separation with remarkably high quantum efficiency. In addition to energy convertors, biohybrid solar cells can be used to investigate fundamental processes of the photosynthetic apparatus [24]. For a more complete understanding of the implications and possibilities of photosynthetic protein activity for energy conversion in such devices, we will outline the natural process of photosynthesis in brief.

#### 4. Photosynthesis

Photosynthesis is the major solar energy capture and storage process on Earth. Through storage as energy dense fossil fuels, photosynthesis provides nearly all energy resources to humanity [50–53]. Current progress in agriculture and bioenergy is largely due to ongoing research into phototrophic organisms [16,54]. Researchers have achieved meaningful and corroborative results using *in vitro* systems to mimic the natural processes of photosynthesis that occurs *in vivo*. They create artificial systems based on the principles, and utilizing the proteins involved in natural photosynthesis, approximating the same functions in a manufactured system [27,55–57].

All members of the plant kingdom, algae and many different bacteria are phototrophs. Several species of archaea can generate a transmembrane potential difference under light that necessary for ATP synthesis, but archaea with photoinduced assimilation of  $\text{CO}_2$  are unknown [50]. The ability to do photosynthesis is widespread, encompassing six different photosynthetic phyla of the bacterial



**Fig. 2. Principles of functionalization of compact layer.** A. There are oxidized species of electrolyte can be reduced by electrons from the TCO, in the absence of compact layer. B. Electrons from mesoscopic layer are transferred to compact layer from the same material without some obstacles and electrolyte cannot penetrate through this layer to TCO.

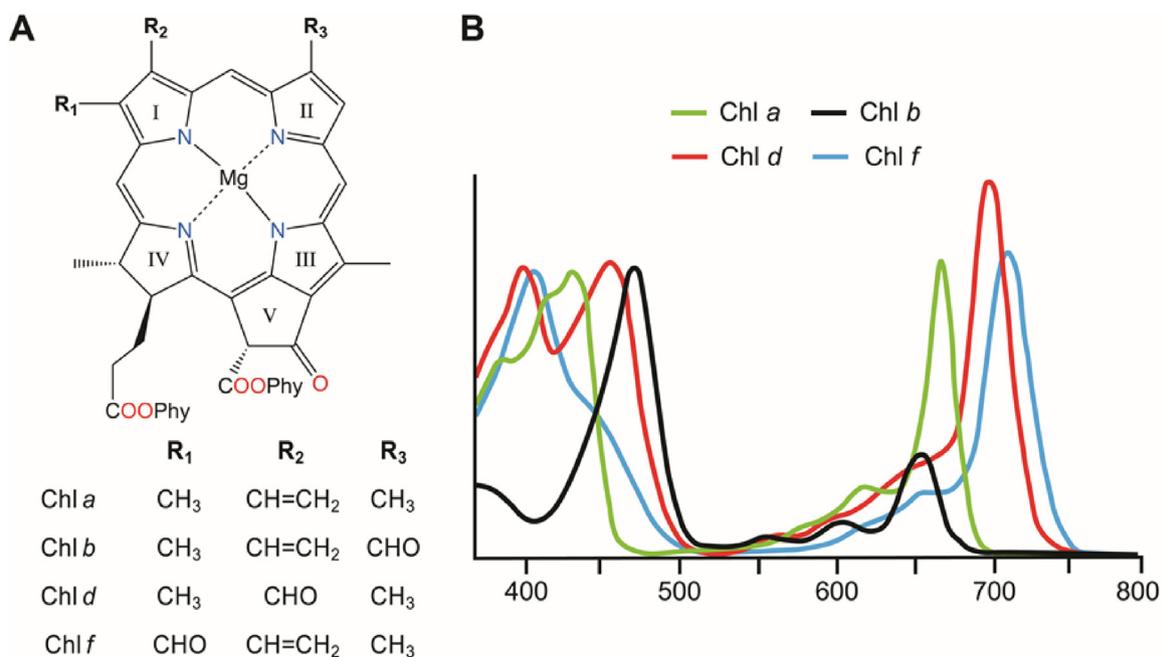
kingdom that includes the cyanobacteria, proteobacteria (purple bacteria), green sulphur bacteria (GSB), firmicutes (heliobacteria), filamentous anoxygenic phototrophs (FAPs, also frequently called the green non-sulphur bacteria), and acidobacteria [50,58]. Depending on the electron donor and remaining oxidant, two types of photosynthesis are considered: oxygenic photosynthesis (which can split  $H_2O$  and yield  $O_2$ ) and anoxygenic photosynthesis (which use other electron donors). All prokaryotic photosynthetic organisms except cyanobacteria perform anoxygenic photosynthesis. Plants (vascular and non-vascular), and cyanobacteria all perform oxygenic photosynthesis. They utilize water as their primary electron source and molecular oxygen is released as a by-product of water oxidation.

The photochemical and electron/proton transfer steps of photosynthesis occur primarily within membranes. In many photosynthetic organisms, photosynthesis occurs in specialized membranes called thylakoid membranes [59]. In some prokaryotic organisms, the cytoplasmic membrane serves as the photosynthetic membrane [60]. The minimal, functional and structural unit of photosynthesis is called a photosystem [8,14]. Photosystems are all multi-subunit transmembrane pigment-protein complexes containing antenna subunits, a reaction centre, and accessory stabilized subunits. Often the photosystem is associated with one or more peripheral light-harvesting complexes. The nature of these antennae complexes is quite diverse and varies with different organisms. Most antennae complexes are also membrane proteins with the exception of the cyanobacterial phycobilisomes [61,62]. Antenna pigments absorb photons, and the resulting excitation energy migrates through the funnel of pigments to the reaction centre. Upon receipt of this excitation energy from the antennae, charge separation occurs in the reaction centre. Alternatively, energy in antenna can be absorbed by specialized quenching molecules, mainly carotenoids, and dissipated into heat. This is a photoprotection strategy employed by photosynthetic organisms in times of stress due to high light irradiance.

#### 4.1. Photosynthetic pigments

Chlorophylls are the most widespread and diverse group of photo-pigments in nature. Their biosynthesis is a complex

pathway with 17 or more steps [50,63]. Chlorophylls are the pigments utilized in oxygenic photosynthetic organisms; plants and cyanobacteria with bacteriochlorophyll found in anoxygenic phototrophs. Five variants of chlorophyll are now known, these include: chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*), chlorophyll *c* (chl *c*), chlorophyll *d* (chl *d*) and chlorophyll *f* (chl *f*) [64,65]. There are, virtually, several kinds of chlorophyll *c* (chl *c*<sub>1</sub>, chl *c*<sub>2</sub>, chl *c*<sub>3</sub>, and others) with minor differences between them. Various kinds of chlorophylls differ from each other by radicals (Fig. 3A). Structural differences impart differences in absorption spectra (Fig. 3B). Chlorophyll *a* is the main pigment of cyanobacteria and plants. Chl *d* and Chl *f* both absorb longer wavelength light in comparison with other chlorophylls. Chl *d* is the only known chlorophyll that can replace all or approximately all the functions of chlorophylls in oxygenic photosynthesis: antenna energy carrier, pigment of the special pair of the reaction centre and participant in the electron-transport chain [64]. The chemical structure of Chl *d* is different from Chl *a* in only the C3 position, where a formyl group in Chl *d* replaces the vinyl group in Chl *a* (Fig. 3A) [66,67]. Chlorophyll *f* is the most red-shifted chlorophyll [68]. We will not delve further into this topic here, sufficed to say that extensive literature exists which outlines distinct kinds of chlorophylls [65,69,70]. The main pigments of anoxygenic photosynthesis are the bacteriochlorophylls. Bacteriochlorophylls differ from chlorophylls by the degree of unsaturation within the macrocyclic ring [69]. As mentioned briefly before, there are carotenoids in all photosynthetic organisms, that are necessary for photoprotection. They generally play the role of accessory pigments, and, in many cases, serve as key regulatory molecules. Carotenoids, unlike chlorophylls, are also found in many other types of organisms, so their evolutionary history may reflect many other functions in addition to photosynthesis [63,71]. The cyanobacterial light-harvesting structures called phycobilisomes, contain phycobiliproteins that function in a similar fashion to previously described antennae complexes, capturing photonic energy and funneling it to the photosystem. Absorption spectra of phycobiliproteins have maxima in the green, yellow and red regions. So, many of cyanobacteria reflect blue light, leading to their early designation as blue-green algae [62].



**Fig. 3. Chlorophylls in oxygenic photosynthesis.** A. Chemical structure of chlorophylls: Chl a, Chl b, Chl d, Chl f. They differ each other by presence and location of formyl radical CHO. B. Absorbance spectra of Chl a, Chl b, Chl d, Chl f. Chlorophyll f is more longwave absorbed chlorophyll.

#### 4.2. Reaction centres

The reaction centre (RC) is at the heart protein complex of photosynthesis [72]. RCs contain protein-associated dimer of specialized chlorophyll molecules termed the special pair and protein-associated cofactors mainly to perform the transfer of electrons. The special pair receives light energy from the antenna and reaches the excited state. The excited special pair donates the electron fulfilling the role as primary donor, and is subsequently reduced, acting as the primary acceptor. After that, special pair can again absorb energy from antenna pigments [73]. Owing to a very tight arrangement of cofactors in the RC, reverse reactions in that scheme are very unlikely, and the quantum yield of charge separation has a value approaching one. This is a very attractive feature for researchers in the solar cell field [74]. Two types of RCs are known, and they differ from each other by the electron carriers on the acceptor side. Type I RCs have FeS compounds at the end of its electron-transport chain and type II RCs have pheophytin/quinone complexes in this position [50]. Anoxygenic phototrophs have just one type, either type I or II, however, all oxygenic phototrophs have RCs of both types (Fig. 4). The photosystem of oxygenic photosynthetic organisms that contains type I RC is called photosystem I. Another one, contained type II RC, is called photosystem II (PSII) [8,50].

#### 4.3. Antenna systems

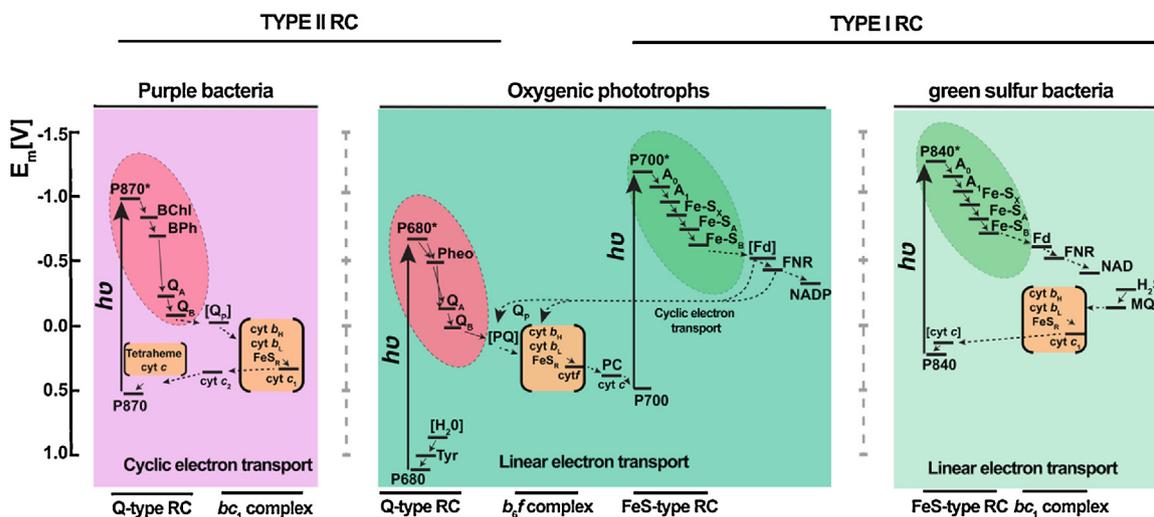
Typical light adsorption cross-section of RC pigments is very low. Without the associated light collectors, sunlight conversion would be very ineffective. All photosynthetic organisms have a light-harvesting antenna system, that functions to collect sunlight energy and transfer it to the RC where the excited state energy is used to drive photochemistry [50,62]. While the presence of an antenna is universal, the structure of the antenna complexes and even the types of pigments used in them is remarkably diverse in distinct types of photosynthetic organisms [4].

#### 4.4. Electron transport chains

Electron carriers form an electron transport chain (ETC) in the thylakoid membrane. Electron transfer is activated when the electron donor, with very high negative reduction potential, passes an electron to a more positive carrier, facilitating “downhill” transfer in the ETC. The excited special pair of RC is such donor. The ETC includes fixed electron carriers and mobile electron carriers. The last operate within the membrane (*i.e.* plastoquinol) or outside the membrane (*i.e.* plastocyanin) from one pigment-protein complex to another or to an enzyme in plasma [7,56,75].

Electron transport can be cyclic or linear. The ETC for cyclic electron transport is a closed loop, with the final acceptor donating an electron to the primary donor [76]. This transport does not conclude to the production of a redox equivalent [50]. But cyclic electron transport conjugates with transmembrane proton transfer, terminating with the generation of a transmembrane proton gradient, resulting in the subsequent synthesis of ATP. During linear electron transport, an electron from the special pair is used to reduce NADP (or NAD in bacteria) [77]. Both, ATP and NADPH (NADH), result from these photochemical reactions. Linear electron transport requires an exogenous electron donor.

In anoxygenic bacteria compounds such as H<sub>2</sub>S, H<sub>2</sub>, or other organic substrates serve as exogenous electron donors. In contrast, oxygenic organisms use water as their main electron source. The reduction potential of water is  $E^\circ(2\text{H}_2\text{O}/\text{O}_2) = +0.82\text{ V}$ , and that of NADP is  $E^\circ(\text{NADPH}/\text{NAD}^+) = -0.32\text{ V}$  [8] (Fig. 4). For electron transfer from one to another, the energy of one quantum of visible light is not sufficient. To overcome this, oxygenic organisms utilize two photosystems with different types of RCs [8,50]. Photosystem II adsorbs light quanta with shorter wavelength and contains a manganese rich, metalloenzyme subunit called the oxygen-evolving complex (OEC), which is responsible for the oxidation of water [75,78]. The functionalized core of the OEC consists of a tetranuclear manganese complex that facilitates a five-step oxidation process, involving two water molecules. Four of these five steps are light-dependent. Electrons from H<sub>2</sub>O are passed to the special pair that is



**Fig. 4. Electron transfer components in photosynthetic reaction centers.** Diagram of electron transport in oxygenic phototrophs and anoxygenic bacteria. Reaction centers are shown on left and include the purple bacteria and PSII in oxygenic organisms. Type II are enclosed in pink color; reaction centers of type I are highlighted by green color. The redox potentials of cofactors are represented on the left in volts (V) against the normal hydrogen electrode. Adapted from [50]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oxidized following a photoexcitation event. In one cycle of the OEC, four liberated protons from water remain in the lumen (in plants and cyanobacteria) or periplasm (cyanobacteria). At the terminal, light-independent step in this process  $O_2$  is evolved as by-product [7,79].

Lending to the conceptual path of the electron flow through the ETC in oxygenic organisms during linear electron flow, this path is often visualized as the so-called Z-scheme [55]. In addition to the photosystems, the ETC includes the membrane bound cytochrome  $bc_1$  or  $b_6/f$  complex. These complexes oxidize quinols produced by photochemistry in type II RCs or during cyclic electron transfer involving type I RCs. The second function of these proteins involves the pumping of protons across the thylakoid membrane, lowering the pH of the lumen and generating a proton gradient. Almost all phototrophic organisms have a cytochrome  $bc_1$  or  $b_6/f$  type complex of generally similar architecture [80].

Composition of the extracted pigment-protein complexes depends on the isolation technique, relating mainly to PSII. dependent on the purity of the extract, one can obtain: BBY particles (fragments of thylakoid membranes enriched by PSII complexes without PSI complexes core-complexes), core complexes including the RC, intrinsic antenna subunits and subunits of the oxygen evolving complex, and single reaction centres without any antenna subunit or oxygen evolving complex [81–84].

The term photosystem does not apply to pigment-protein complexes of anoxygenic photosynthetic organisms. For example, in purple bacteria, isolated complexes are referred to as RC, RC-LHI or RC-LHI-LHII [50]. The first contains only reaction centre, the last contain RC and both antenna complexes (referred to as “light harvesting complexes,” hence the LH abbreviation). Isolation of RCs from bacterial cells is easier than from cyanobacteria or, surely, from higher plants [85].

#### 4.5. Plant and cyanobacteria

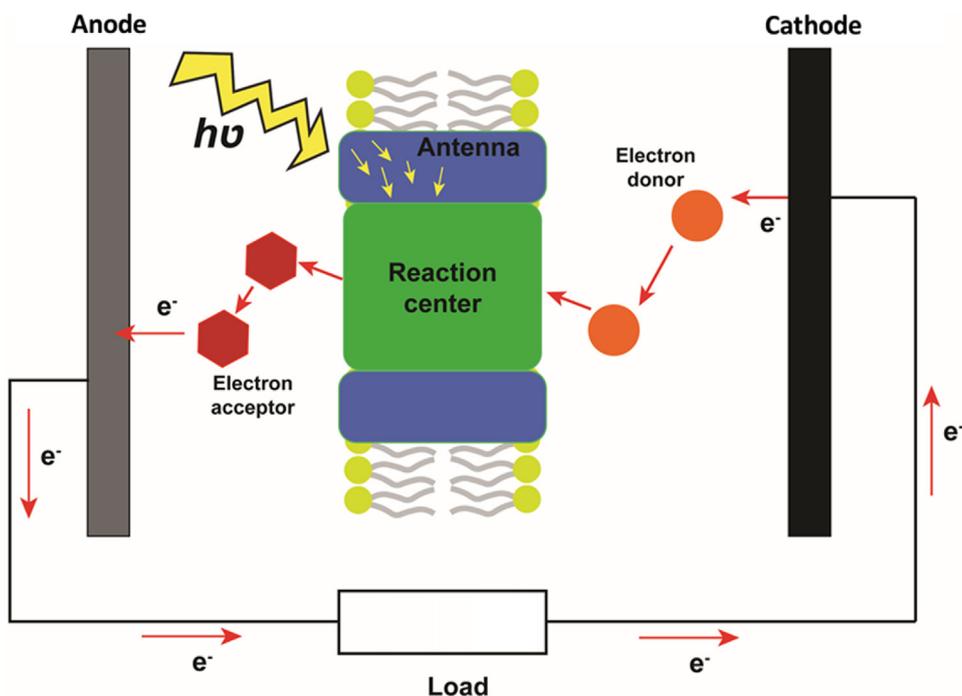
The photosynthetic apparatus of plants and bacteria are very similar. Both types of phototrophs undergo oxygenic photosynthesis. Therefore, the thylakoid membranes of each contain PSI, PSII and cytochrome  $b_6/f$  complexes [8]. Though differences exist between cyanobacteria, and high plants, the protein subunits of photosystems I and II are remarkably highly conserved across

the two groups. One distinction between them, is the structure of the external antenna. Cyanobacteria contain phycobilisomes as antenna complexes. The phycobilisome is a peripheral membrane protein incorporates phycobilins as their main pigment. Phycobilisomes are docked to PSII in the normal state, the most optimum and occupied state, allowing for linear electron transfer and photosynthesis to occur. Plant thylakoids contain light harvesting complex I (LHCI) connected with PSI and light harvesting complex II (LHCII) antenna complexes, primarily bound to PSII [62,86]. LHCII can migrate from PSII to PSI under high light stress, this action is referred to as state transition. LHCI and LHCII are pigment-protein transmembrane complexes containing chlorophylls and carotenoids [62,86]. Another key distinction between plants and cyanobacteria concerns the oligomeric state and soluble electron donor proteins associated with PSI. PSI exists only in monomeric form in plants and can be found in trimeric form in cyanobacteria. Plastocyanin, a copper based soluble electron carrier protein, is the only electron donor for PSI in plants [87]. Whereas in cyanobacteria, both plastocyanin and cytochrome c may act as electron donors to PSI.

## 5. Solar cell-based incorporating components of the photosynthetic apparatus

### 5.1. Basic principles

The ETC is a principal component of the photosynthetic apparatus. An initial point of the electron transport in the photosynthetic membrane is the charge separation event in the RC stimulated by light energy. This separation has a very large quantum yield due to the highly complex, precisely ordered system of pigments and cofactors arranged within the reaction center and adjacent antennae complex. This effectively represents a ready-made nanomachine, built to convert light energy into electrochemical energy, in a naturally abundant manner. To harness and institute this strategy for our own uses, we need to isolate the membrane proteins involved in the collection and conversion of photons to electrons and siphon those electrons directly into an electrical circuit. We then need to engineer a system to facilitate the reduction of RC pigments from the returning electrons of the external circuit, in



**Fig. 5. Schematic of a general biohybrid solar cell.** Common scheme of operation of solar cell based on components of photosynthetic apparatus. Electron transport medium between photosynthetic complex and electrode is not depicted.

other words optimize an electron mediator that will be functional with the RC. A schematic illustrating the resulting device principles can be seen in Fig. 5.

In the last two decades, many reports have appeared in the literature regarding the direct conversion of light energy to electricity, or the light-induced production of molecular hydrogen owing to the biomolecules. These technologies, in so-called photo-bioelectrochemical cells (PBECs), use plant or bacterial photosynthetic complexes as biocatalysts [6]. Regarding this review, both the terms PBEC and solar cell based on complexes of pigment proteins denote the same thing. Biohybrid systems in these cells were built by immobilizing these catalysts immediately onto the electrode surface [88–91] or via linker molecule [92–100].

## 5.2. Solar cell or biohybrid electrode

The three main challenges for building PBECs are 1) assembling the biohybrid photoelectrode. 2) utilization of an artificial cost-effective and rapid electron donor to enable reduction the oxidized RC pigment, and 3) designing a counter-electrode to enable electron transfer from the external circuit to the mediating system. The distinguishing feature of PBECs is the biohybrid photoelectrode. There are components of the photosynthetic apparatus immobilized on the surface of the conductive or semiconductive material in this photoelectrode. The immobilization of pigment protein complexes with optimal orientation, minimal loss of activity, and long-term stability, may be the primary challenge for PBEC building. Narrowing the investigation to only one electrode, rather than the whole PBEC, can help address these questions. As previously noted, the utilization of pigment-protein complexes as sensitizers not only occurs in the field of electricity generation. There exists a sizeable group of researchers focused on building devices geared toward hydrogen production, using biomolecules [55,101,102]. In addition to energy production, biohybrid electrodes can be used in biosensor applications, or as an anode in fuel cells [103–105]. Therefore,

there is a significant and growing body of work dedicated to the study of only photoelectrodes, rather than whole PBECs.

Efficiency measurements of photoelectrodes are distinct from that of a complete solar cell. In PBECs it is common to compare efficiency with other photovoltaic conversion strategies. Four parameters are often used for the characterization of various solar cells. These parameters include: short-circuit current  $I_{sc}$ , open-circuit voltage  $V_{oc}$ , fill-factor FF and efficiency  $\eta$  [15]. Methods of assessment of these parameters is widely described in the literature [1,15,106]. Additionally, a unified set of standard test conditions for the characterization of solar cells are used, these include: air mass of AM 1.5, light intensity of  $1 \text{ kW m}^{-2}$ , and a temperature of 298 K [15]. Special solar simulators provide light with accurate solar spectra and variable intensity [107–109]. This characterization allows one to define suitability of a device under field simulated conditions. For analyses of complete solar cells, scientists implement voltammetry measurements using a power source and ammeter. The resulting curve of current dependence on applied voltage at constant light condition provides you with all needed parameters, assuming the light intensity is known.

Here, we will spend more attention to the hybrid electrodes rather than the whole solar cell, because there are more reports detailing the construction of single electrodes rather than whole solar cells in the literature. For characterization of a biohybrid electrode, three-electrode polarographic experiments are implemented [99,110]. Commonly, this characterization is more specific than previously described for whole solar cells, as it provides more information about the electrode. It does not, however, provide integral information about whole cell. Characterization of a biohybrid electrode does not provide information about ionic diffusion in electrolyte or cathodic reactions, which are both very important processes in solar cells. Investigations into the solar cell are a logical continuation of the study of biohybrid electrodes, being that the latter are base components of the solar cell system [97]. Characterization of the light activity of the pigment-proteins complexes immobilized onto the electrode by treating this hybrid electrode

as the working electrode in the typical three-electrode scheme, is also referred to as protein film photoelectrochemistry or PF-PEC [78]. There remains some difficulty in the direct comparison of efficiency between biohybrid electrodes from different works, as thus far there are no standard test conditions applied to investigations of biohybrid electrodes. It is possible, that such guidelines cannot exist, because researchers focus on different goals, therefore designing different conditions from which to conduct their experiments. Such conditions often include distinct variations in temperature, light condition (wavelength and/or intensity) and applied potential (also referred to as bias) to the solar devices being tested.

### 5.3. Common sensitizers

The components of the photosynthetic apparatus most commonly used as sensitizers are the following:

Reaction centers of purple bacteria [74,95,111–114] (Fig. 6);

Monomeric core-complexes of PSI from plant chloroplasts [95,115–118] (Fig. 6);

Core-complexes of PSI from cyanobacteria (in both trimeric and monomeric forms) [90,93,100,107,119–121] (Fig. 6);

PSII complexes (from plants and cyanobacteria) [92,104,122–125];

Thylakoid membranes or combinations of both PSI and PSII [94,126–128].

Both the size of the pigment-protein complexes, and the redox-potentials of the associated primary donor and acceptor of its ETC are critical parameters of the sensitizer (Fig. 6). Accurate determination of size is needed to assess the total amount of sensitizer molecules that can be deposited onto an electrode with fixed surface area. The values of redox potentials are necessary to choose the appropriate electron mediator (Fig. 4).

#### 5.3.1. Bacterial RC as sensitizer

The first RC complex discovered, which has subsequently been biochemically characterized for more than 20 years, belongs to the photosynthetic purple sulfur bacteria. Following the previously stated conventions, this is a type II RC. All purple bacterial RCs have a core domain made up from the H-, L- and M-polypeptides. L- and M-polypeptides encase the cofactors that participate in some manner of energy or electron transfer [95,129]. These cofactors include: bacteriochlorophyll *a* special pair (P840 or just P), two bacteriochlorophyll *a* (Bchl) monomers, two bacteriopheophytin *a* (BPhe), two ubiquinone molecules  $Q_A$  and  $Q_B$ , and carotenoids. The H subunit is more peripheral, containing a large cytoplasmic domain [85]. The special pair of bacteriochlorophyll is designated as P870 due to its longwave absorption maximum, spanning approximately 10 nm. Redox potential of this special pair is  $E^\circ(\text{P870}^+/\text{P870}) \cong +0.45 \text{ V}$ , with the redox potential of  $Q_B$  in the purple bacterial RC of about 0 V vs NHE. Therefore, a steady-state redox difference of about  $-0.5$  to  $-0.4 \text{ V}$  results from charge separation [74]. Regarding biohybrid electrode fabrication, the RC of purple bacteria exhibit two main advantages. First, they are the smallest known complexes to perform light-induced charge separation. Thus, one can immobilize more active complexes of purple bacterial RCs, than that of other photosystem complexes, on the same area. Secondly, the extraction of purple bacteria RCs is easier than photosystem complexes from oxygenic organisms [74].

Depending on the orientation of these RCs onto the substrate, RC-biohybrid electrodes can realize cathodic or anodic current in electrochemical cells [111]. In 2006, Trammel et al. report building two types of RC-based solar cells with carbon based electrodes. The first such device, produced light-induced anodic current with *N*-(1-pyrene)-iodoacetamide as a linker, and the second producing light-induced cathode current using

$\text{His}_6\text{-Ni}^{2+}$ -nitrilotriacetic acid (NTA) as the linker. These electrodes differ in terms of distinct orientation of RC complexes to the carbon substrate [111]. The following artificial electron carriers, ubiquinone-10 (2,3-dimethoxy-5-methyl-6-geranio-1,4-benzoquinone), and cytochrome *c* (reduced by  $\text{Na}_2\text{S}_2\text{O}_4$ ) were used as the donor to produce anodic and cathodic current, respectively (Fig. 7 A). The device which produced cathodic current was more efficient in these experiments. This is –due to the distance between P840 and carbon in this device being less than the distance between quinone and the electrode in the anodic current producing case. The distance is calculated as the sum of the length of the linker with the size of the RC complex [111].

#### 5.3.2. PSI as sensitizer

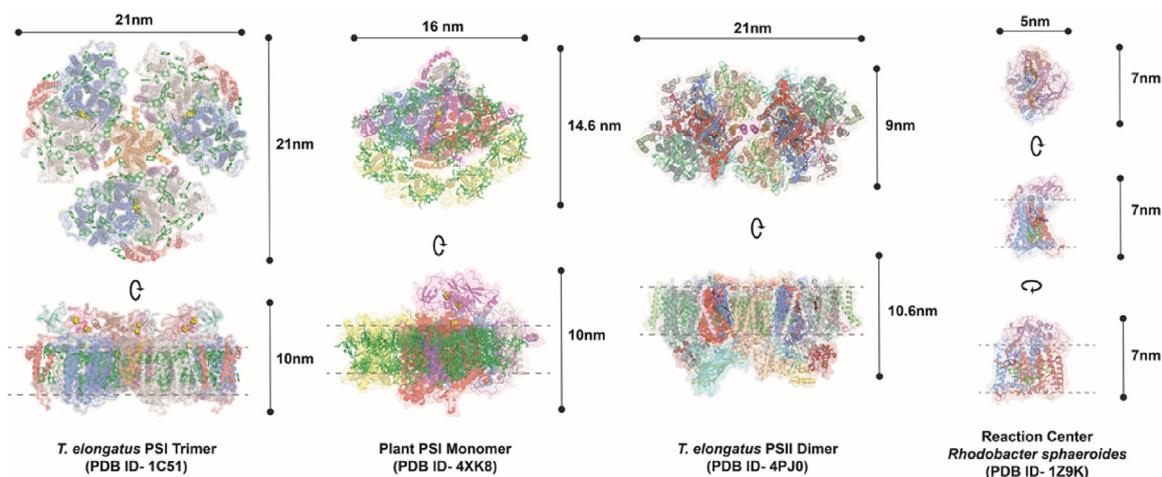
PSI is a transmembrane, multi-subunit protein-chlorophyll complex, that mediates light-induced electron transfer from hydrophilic mediators plastocyanin or cytochrome in the thylakoid lumen, to another soluble carrier protein, ferredoxin, located in the stroma or cytoplasm in plants and cyanobacteria respectively [107].

The ETC within PSI includes a specialized chlorophyll dimer (the special pair, P700), a monomeric chlorophyll *a*, two phylloquinones, and three [4Fe-4S] iron-sulfur centers. Maximal size of trimeric and monomeric PSI are about 25 nm and 13 nm respectively [119]. The redox potential of the primary donor, P700, is  $+0.43 \text{ V}$  and that of the final iron-sulfur acceptor,  $F_B$ , is  $-0.53 \text{ V}$ , producing a net redox difference of  $-1.0 \text{ V}$  [87]. For this reason, PSI has drawn widespread attention for use in biomimetically inspired energy conversion devices [130]. The oligomeric state of PSI in plants is monomeric, while PSI exists primarily as a trimer in cyanobacteria. Faulkner et al. demonstrated that a considerably dense monolayer of spinach-extracted PSI complexes must be formed on an electrode surface to obtain optimal photocurrents. They went on to introduce a new method for preparing these dense PSI monolayers, that was 80 times faster than the method of photosystem precipitation from solution [115]. This study discovered that the precipitation of the isolated PSI complexes onto gold electrode at vacuum condition with following rinsing with water produces a dense monolayer of PSI that yields  $\sim 100 \text{ nA cm}^{-2}$  of photocurrent in the presence of suitable intermediaries. The orientation of soaked PSI layers on an electrode is of critical concern because PSI acts as a photodiode, allowing electron transfer from the P700 reaction center to the  $F_A/F_B$  sites once a photon of light is captured [115].

In similar fashion, Frolov et al. designed a PBEC based on a gold electrode modified by PSI complexes. This cell could generate a voltage of  $0.498 \pm 0.02 \text{ V}$  [87]. Frolov chose the robust PSI reaction centers from the cyanobacteria *Synechocystis* sp. PCC6803. The main reason for the structural stability of this PSI, is the fact that all chlorophyll and carotenoid molecules are integrated into the complex of core subunits, while, in plant and bacterial reaction centers, the antenna chlorophylls are connected to chlorophyll-protein complexes that are bound to the core subunits. In fact, there was no requirement to use peptide surfactants, that were necessary for stabilization of plant PSI and the bacterial reaction centers [87]. An attentive selection of the amino acids, that were replaced to cysteines for covalent attachment of the PSI to the gold surface, was the second factor that ensured the structural and functional stability of the self-assembled, oriented PSI [87]. Included in the references here is a comprehensive review that covers recent progress in PSI-based photovoltaic cells [130].

#### 5.3.3. PSII as sensitizer

Like PSI, PSII is also a membrane bound, multisubunit pigment-protein complex. However, PSII exists primarily as a dimer. Each monomer contains about 20 subunits, including a reaction center, antenna and accessory subunits as well as the OEC previously



**Fig. 6. Structure of the known reactions centers.** Proteins and cofactors in different reaction centers and their approximate dimensions in nm: (From left to right) Trimeric PSI from *T. elongatus* (PDB ID-1C51), plant PSI-LHCI complex (PDB ID-4XK8), PSII dimer from *T. elongatus* (PDB ID- 4PJ0), bacterial reaction center from *Rhodobacter sphaeroides* (PDB ID- 129K).

described. As PSII isolated complexes retain the OEC, they can use water as their primary electron donor. This functional asset reveals the most favorable advantage of using these complexes. The internal ETC of PSII consists of the following cofactors: 6 Chl *a* (the special pair, P680 being among them), 2 pheophytin *a* (Pheo *a*) molecules, 2 plastoquinones, a non-heme iron, redox active tyrosine residues (YZ and YD) and the inorganic ions previously described of the OEC [8]. Maximal size of the dimeric PSII complex is about 21 nm [131]. Redox potentials of special pair and final acceptor are  $E^\circ(\text{P680}^+/\text{P680}) \cong +1.12\text{ V}$  and  $E^\circ(\text{Q}_B/\text{Q}_B^-) \cong +0.03\text{ V}$ , respectively. The resulting redox difference is about  $-1.1\text{ V}$  [8].

PSII complexes are less stable than the PSI *in vitro*. This limitation can be overcome by exploiting suitable immobilization techniques and stabilizing species. Vittadello et al. reported the application of histidine-tagged PSII protein complexes, extracted from *Synechococcus elongatus*, covalently bonded to a gold electrode functionalized with  $\text{Ni}^{2+}$ -NTA [132]. The current density of the resulting PBEC reached  $43\ \mu\text{A cm}^{-2}$  [44,14]. While the photochemical energy conversion efficiency of the freshly isolated PSII was 0.7, the same parameter for the PSII immobilized on gold was 0.53. This clearly shows that the PSII complexes were photochemically stable even after immobilization [132]. Correspondingly, Israeli scientists developed a PBEC based on bacterial PSII complexes isolated from the thermophilic cyanobacterium *Mastigocladus laminosus*. The photoanode of this PBEC consists of a matrix of 2-mercapto-1,4-benzoquinone electropolymerized onto the gold surface, and a layer of the PSII complexes immobilized into this matrix. The anode was electrically connected to the cathode by bilirubin oxidase/carbon nanotubes. It is argued The photo-induced quinone-mediated electron transfer is argued to drive to the generation of photocurrent, with an output power of 0.1W [99]. Recently, the design principles stated above were implemented in a slightly separate way by Noji et al., where a nanodevice developed for the artificial separation of water controlled by light. They used a conjugation of PSII complexes with gold nanoparticles (GNP) [123]. This report marks the conjugation of GNPs with PSII complexes have been employed in an artificial system for light-driven water splitting, yielding  $\text{O}_2$  and  $\text{H}_2$  gases. PSII core complexes extracted from the thermophilic cyanobacterium *Thermosynechococcus elongatus*, in which a His-tag was introduced into the C-terminus of CP47, were immobilized on GNPs with a 20-nm diameter *via*  $\text{Ni}^{2+}$ -NTA. PSII was oriented such that the acceptor side was directed toward the GNP surface. Attributed to their high thermal stability, the PSII

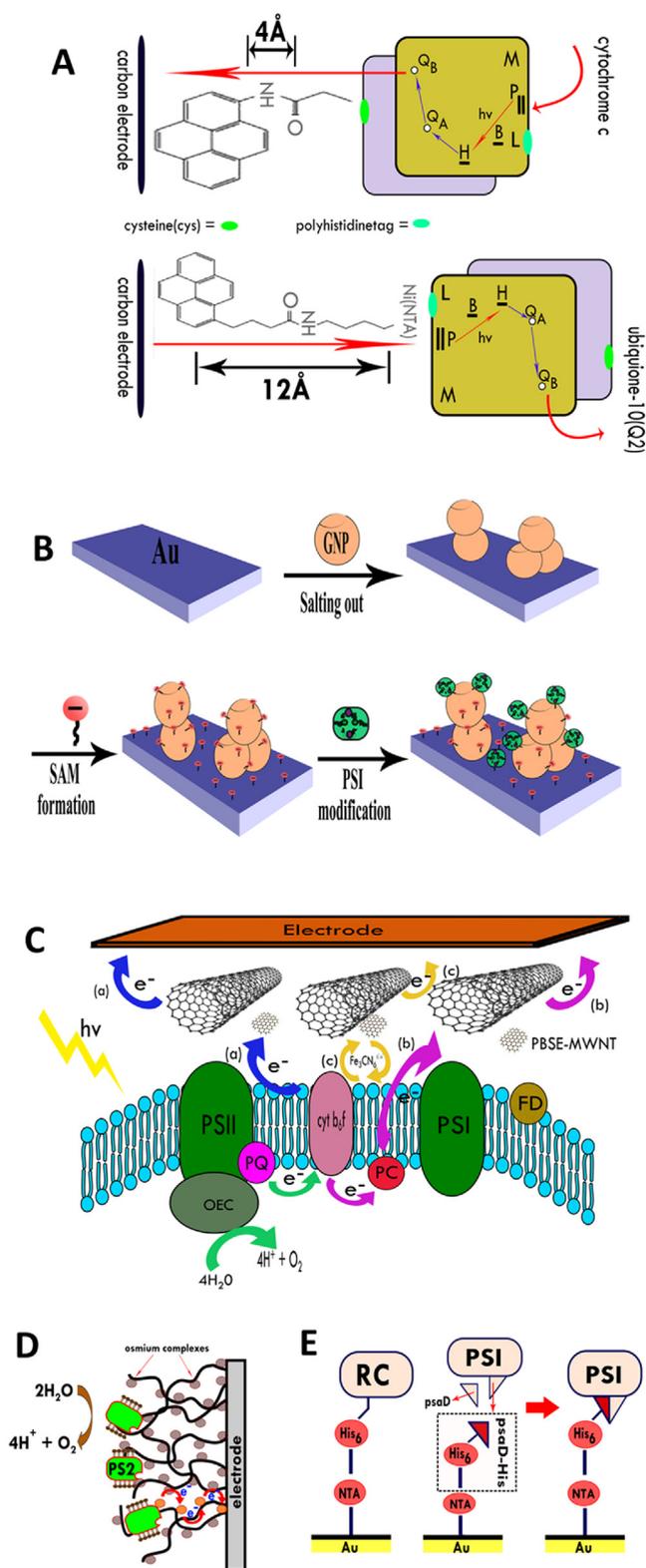
core complexes from *T. elongatus* are an appropriate material for making artificial systems that are intended to experience full light conditions [123]. Optical analysis indicated that four to five PSII dimers are bonded to a single GNP, and that PSII immobilized on the planar or nanostructured surface of a gold electrode keeps  $\text{O}_2$  evolution comparable to free PSII. The  $\text{O}_2$  evolution activities of the PSII-GNP conjugate was measured at  $25^\circ\text{C}$  using a Clark-type  $\text{O}_2$  electrode in a 20 mM MES buffer (pH 6.5) [123]. Kato et al. offer an in-depth review that examines various different types of PSII functionalized electrodes by protein film photoelectrochemistry [78].

#### 5.3.4. Thylakoid as sensitizer

Thylakoid membranes contain the entirety of the photosynthetic proteins, including both PSI and PSII. Thylakoids can efficiently actualize light induced electron transfer along the Z-scheme, following extraction from their native environment, in the presence of an external electron acceptor. Being that the OEC is retained, thylakoid containing PBECs do not require any exogenous electron donor [15]. The redox potential of PSI acceptors is more negative than that of PSII acceptors, therefore devices of this type can utilize semiconductor substrates with wider bandgaps. Further, it has long been supposed that PSII and PSI complexes are more stable within their native thylakoid lipid membranes.

A team of researchers led by Robert Carpentier, are cited as the first to use thylakoid membranes isolated from spinach leaves as a photosensitizer, immobilized on an electrode to generate photocurrent. In their work, a platinum electrode was used as the final acceptor. Measurements were conducted in light and in dark, in the presence and absence of potassium ferrocyanide ( $\text{K}_4[\text{Fe}(\text{CN})_6]$ ), as an intermediary. Native thylakoids generated a photocurrent of up to 6–9  $\mu\text{A}$  without a mediator, and four times more current in the presence of potassium ferrocyanide [133]. The generation of photocurrent without mediator was associated with either direct transfer of electrons from membrane proteins to the electrode surface, or the presence of molecules in the buffer that can function as mediators.

On the other hand, thylakoid membrane preparation includes sufficient part of photoelectrochemically silent components such as lipids. It is lowered the efficient cross section of absorption of sensitizer. In general, the smaller components of photosynthetic apparatus we use, the larger cross section of absorption we obtain. In this key, reaction center is the more preferential sensitizer. It is



**Fig. 7. Different examples of biohybrid electrodes.** A. Biohybrid electrode based on RC from *R. sphaeroides*. Two types of binding RC to carbon substrate realize anodic current (1) and cathodic current (2). M, L, H – refer to M-, L- and H-subunits respectively; P – special pair, Bacteriochlorophyll monomer, H (on green background, not on grey one) – bacteriopheophytin. Adapted from [111]. B. PSI-based biohybrid electrode. PSI complexes were extracted from *T. Elongatus*. Preparation procedure of PSI– gold nanoparticle hybrid is shown. SAM of 3-mercapto-1-propanesulfonic acid sodium salt used for PSI binding. Adapted from [120]. C. Spinach thylakoid-based biohybrid electrode. 1-Pyrenebutanoic acid succinimidyl ester (PBSE) is used as the molecular tethering agent to attach thylakoids onto multi-walled carbon nanotubes (MWCNT).

because the RC is smallest pigment-protein complex yet capable to the light induced charge-separation.

#### 5.4. Main challenges

In Table 1 we listed information of several distinct biohybrid electrodes which does not contained any TiO<sub>2</sub>. The comparison of them is difficulty for distinct measurement condition. But one can see, that no one from presented electrodes did not generate current more than one milliampere.

One must consider follows questions when designing biohybrid electrodes

- 1) Provide large absorption cross-section [87,93,107];
- 2) Robust anchoring of biomolecules onto the substrate [95,107];
- 3) Minimization of resistance between photosynthetic RCs and substrate [74,119,121,122];
- 4) Stabilization of susceptible biomolecules onto the electrode [93];
- 5) Extent of the adsorption band of the sensitizer [134];
- 6) Minimization of undesired reactions of recombination [91,102,130].

##### 5.4.1. Increasing absorption cross-section of electrodes

**5.4.1.1. Nanostructured materials.** The employment of mesoporous, nanostructured materials widely used and developing technique to increase the absorption cross-section of biohybrid electrodes. These 3-dimensional, porous materials have a larger effective surface area than that of a flat glass or metal electrode. The increase in surface area allows for the immobilization of more molecules of sensitizer at the same geometrical surface area. Another advantage of nanostructured electrode materials is that due to large efficient surface area, the effective surface of contact between protein and substrate is extended. Gold nanoparticles, carbon nanotubes and mesoscopic TiO<sub>2</sub> are commonly applied as the nanostructured material [94,107,120,122,123]. In fact, such a PBEC can be called a dye-sensitized solar cell, where photosynthetic complexes serve in place of dyes. In the several iterations of these devices, TiO<sub>2</sub> is used as a substrate, typically on the photoanode. But generally, PBECs are not referred to as DSSCs. One main difference between the technologies, is that the charge separation in DSSC typically occurs at the TiO<sub>2</sub>/sensitizer interface, while in PBECs this crucial step occurs in the photosynthetic reaction center. Again, this distinction becomes increasingly blurred as photosynthetic complexes become more widely integrated into DSSC designs.

The Terasaki group in 2006 used nanostructured gold as a substrate for cyanobacterial PSI complex (Fig. 7B). Before PSI immobilization, a nanostructured gold electrode was modified by a self-assembled monolayer (SAM) of 3-mercapto-1-propanesulfonic acid. PSI was isolated from the thermophilic cyanobacteria, *Thermosynechococcus elongatus*. They compared the efficiency of biohybrid electrodes with various surface areas. Maximum photocurrent, totaling 1.6 μA cm<sup>-2</sup>, was generated on the electrode with the largest surface area [120].

Adapted from [94]. D. PSII-based biohybrid electrode. PSII complexes were extracted from *T. Elongatus*. PSII complexes have been immobilized in copolymer of vinylimidazole and allylamine contained [Os(bipy)2Cl<sub>2</sub>]. Osmium ions served as electron carriers. The Electrode realized cathodic current. Adapted from [92]. E. RC- and PSI-based biohybrid electrodes with Ni<sup>2+</sup>-NTA as binding agent. (1) Bacterial reaction centers are immobilized using a His<sub>6</sub> tag at the C-terminal end of the M subunit. (2) Oriented PSI assembly is achieved when native psaD is exchanged with immobilized psaD-His, previously genetically modified with a His<sub>6</sub> tag. Adapted from [95]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**  
Examples of different biohybrid electrodes which did not base on the TiO<sub>2</sub>.

Source	Sensitizer type	Electrode	Light condition	Current, $\mu\text{A cm}^{-2}$
[92]	PSII ( <i>T. Elongatus</i> )	Gold/Os-polymer/PSII	2.65 mW cm <sup>-2</sup> , 675 nm	45
[93]	PSI-trimers ( <i>T. elongatus</i> )	Gold/Os-polymer/PSI	1.8 mW cm <sup>-2</sup> 680 nm	29
[97]	PSI and PSII ( <i>T. elongatus</i> )	Gold/Os-polymer/PSII anode Gold/Os-polymer/PSI cathode	34.9 mW cm <sup>-2</sup> 685 nm	2.0
[128]	PSI and PSII ( <i>Mastigocladus Laminsus</i> )	ITO/PBV <sup>2</sup> /PSI/PBV <sup>2+</sup> /PSII	0.28 mW cm <sup>-2</sup> 680 nm	1.2
[100]	PSI of ( <i>M. Laminsus</i> )	Gold/Bis-aniline-Cross-Linked/Pt NP/Ferredoxin/PSI Composite	1.13 mW 437 nm	1.38
[138]	PSII, ( <i>T. elongatus</i> )	Mesoscopic-ITO/COO-NHC(or EDC)/PSII	10 mW cm <sup>-2</sup> 679 nm,	4.5
[89]	PSI, spinach	P-doped Silicon/Gold/PSI	190 mW cm <sup>-2</sup> > 633 nm	875
[113]	RC with a polyhistidine tag ( <i>Rb. Sphaeroides</i> )	CNT/Ni <sup>2+</sup> -NTA/RC	5 mW cm <sup>-2</sup> < 700 nm	1.41
[94]	Thylakoid membrane (Spinach)	Gold-thylakoid/MWNT composite electrode	80 mW cm <sup>-2</sup>	38
[120]	PSI ( <i>T. elongatus</i> )	Gold nanoparticles-MPS-PSI	3.3 mW 680 nm	1.6
[122]	PSII ( <i>T. vulcanus</i> )	Gold nanoparticles/Ni <sup>2+</sup> -NTA/his-tagged PSI	3.3 mW 680 nm	2.44

Carbon nanotubes (CNTs) are very interesting nanomaterials that are size-compatible and physically accessible to proteins on both the exterior and interior surfaces of the tube wall. They are known to exhibit superior and unique electron transfer (ET), as well as thermal and mechanical properties. The Lebedev group was the first to introduce arrays composed of CNTs and RCs, linked together through a pyrene-functionalized Ni<sup>2+</sup>-NTA, and their application for generating photocurrents [113]. In 2011, Bedford et al. immobilized thylakoids on conductive nanofibers by using the electrospinning technique. In this work, the so-called electrospinning method is used to create nanofibers from a solution of thylakoids and poly(3,4-ethylenedioxythiophene)/poly(styrene sulfonate) (PEDOT), and poly(ethylene oxide) (PEO) [127]. By immobilizing thylakoids into electrically conductive PEDOT/PEO nanofibers, one can measure changes with response to light *via* electronic properties of the system [127]. The required minimum chlorophyll concentration to produce photocurrent was  $\sim 2 \text{ mg mL}^{-1}$ . At power densities lower than  $1 \text{ mW cm}^{-2}$ , the change in photocurrent was insignificant. Thus, to produce a measurable photocurrent, a power density of more than  $1 \text{ mW cm}^{-2}$  must be used on fibers electrospinning from a solution that contains a thylakoid preparation with at least  $2 \text{ mg/mL}$  of chlorophyll. The maximum electric power generated by the cell was  $24 \text{ mW cm}^{-2}$  upon illumination by red light with a wavelength of  $625 \text{ nm}$  [127]. Further, Calkins et al. created solar cells by using thylakoids isolated from spinach. Thylakoids were immobilized on an anode modified with multi-walled carbon nanotubes (MWCNT) by using a molecular tethering chemistry (Fig. 7C). A glass electrode modified with a laccase/MWCNT system was used as the cathode. This study presented a maximum current density of  $68 \mu\text{A cm}^{-2}$  and a maximum power density of  $5.3 \text{ mW cm}^{-2}$ . The compound electrode based on thylakoid/MWCNT produced a constant state current density of  $38 \mu\text{A cm}^{-2}$ , two times higher than previously reported for like systems [94].

CNTs allow for more than immobilizing thylakoid membranes or purified photosystem proteins. In their works, Ramasamy et al. have constructed PBECs using whole cells of *Nostoc* sp. (NOS) connected with CNTs for the anode, as well as CNTs in conjugation with an enzyme called laccase as the cathode [135]. Upon illumination (intensity  $76 \text{ mW cm}^{-2}$ ), this cell generated a photocurrent density of  $30 \text{ mA m}^{-2}$  at  $0.2 \text{ V}$  (vs. Ag/AgCl). The cell generated a maximum current density of  $250 \text{ mA m}^{-2}$  and a top power density of  $35 \text{ mW m}^{-2}$  without any intermediary. By the addition of 1,4-benzoquinone as a redox mediator, the electricity genera-

tion capacity was remarkably increased with a current density of  $2300 \text{ mA m}^{-2}$  and a power density of  $100 \text{ mW m}^{-2}$ . The top power densities were  $35 \text{ mW m}^{-2}$  and  $24 \text{ mW m}^{-2}$  under light and dark conditions, respectively, without the addition of any mediators [135].

**5.4.1.2. Redox polymer.** Another method to increase absorption cross-section, is by using polymers with integrated redox pair both as a gel matrix for bio-molecule immobilization and as mediating medium between the photosynthetic protein and conductive solid electrode. In this gel, increased concentrations of RCs can be packed per cm<sup>2</sup> of geometrical surface area. This has been supported by multiple researchers, by immobilizing PSII in an osmium-including redox polymer based on poly(1-vinylimidazole) and a matrix of 2-mercapto-1,4-benzoquinone (MBQ). These systems have also been shown to be electropolymerized on the surface of gold electrodes [92,93,99]. These polymers serve as a stabilizer, linker and electron mediator in these devices (Fig. 7D).

**5.4.1.3. Multilayer.** Expanding upon the previously outlined consideration regarding maintenance of high absorption cross section of the sensitizer/electrode system, formation of photosystem multilayers onto solid electrodes have been tested. In 2008, the Frolov group built a PBEC consisting of a multilayer of cyanobacterial PSI complexes. PSI complexes were platinized on the stromal side. This allowed the investigators to connect the luminal side of one complex to stromal platinized side of another complex. Devices based on two layers, generated photovoltage about  $0.330 \text{ V}$ , where three layers generated photovoltage of about  $0.386 \text{ V}$  [136]. A method suggested by Ciesielski et al. did neither require the use of photosystems isolated from mutated cyanobacteria, nor the use of a high vacuum, so it was more economical and less time-consuming. In their study, a plate of gold (thickness of  $\sim 125 \text{ nm}$ ) immobilized on a silicon substrate, served as the cathode. A working surface of transparent plastic plate coated with lead oxide, doped with indium, served as the anode of the photoelement [116]. A space between them was filled in half with a liquid composed of  $5 \text{ mM}$  2,6-dichlorophenolindophenol,  $100 \text{ mM}$  ascorbic acid, and  $100 \text{ mM}$  NaCl in  $5 \text{ mM}$  phosphate buffer at pH 7.0. In the other half, there was a buffer solution containing PSI complexes ( $\sim 9 \mu\text{M}$ ), Triton-X100 (0.05% w/v),  $0.14 \text{ M}$  NaH<sub>2</sub>PO<sub>4</sub> in  $0.2 \text{ M}$  phosphate buffer at pH 7.0. The PSI complexes were precipitated on a gold electrode for seven days. As a result, a multilayer of the PSI complexes with a thickness of  $1\text{--}2 \mu\text{m}$  was obtained. The solar cell generated a pho-

tocurrent about  $2 \text{ mA cm}^{-2}$  under illumination by a standard light intensity (cloudless sky at noon). The device demonstrated a considerable stability and retained activity under ambient conditions for at least 280 days. The multilayer has two distinct disadvantages. First, the transfer of electrons is difficult for those RCs that are not in direct contact with the substrate. Second, reduction of oxidized pigments in the RCs connected directly with the substrate is slowed due to restricted access of reducing agents to reach them [90]. The first problem can be solved by using an additional mediator; the oxidized mediator molecules receiving electrons from the excited pigments and transferring them to the electrode.

#### 5.4.2. Immobilization and orientation

Mutant pigment-protein complexes are often needed for the successful immobilization or for the making of multilayer structure. In addition to the engineering of pigment protein complexes, solid substrates often also need modification. For example, in many electrodes, biomolecules are fixed on the gold substrate due to the action of the self-assembled monolayer (SAM). Gold is a very useful material for electrodes due to its corrosive resistance and high conductivity. However, gold has an inherent hydrophobic nature and does not interact favorably with proteins [78]. A SAM is a layer of molecules on the surface of a specific substrate that spontaneously forms when the substrate is immersed in a solution of the active surfactant in an organic solvent. On the surface of gold, the formation of a SAM from thiols and disulfides due to the Au-S bonds is known [78]. There are two ways to prepare protein-modified gold electrodes. In the first method, pigment-protein complexes are engineered in such a way as to have a large amount of cysteine groups on the surface, and thus they themselves can form a SAM on the gold surface [87]. In the second strategy, pigment-protein complexes are chemically combined with a previously formed SAM. One of the widely used techniques in this area is the use of mutant pigment-protein complexes, to which a polyhistidine tag is attached [95]. It is well known from the practice of chromatography that  $\text{Ni}^{2+}$ -NTA selectively binds to this tag [137]. Therefore, a SAM can be adhered to the gold surface due to Au-S binding, and at the next step, the  $\text{Ni}^{2+}$ -NTA can be bound to the SAM. SAMs can provide both electrostatic interactions and covalent interactions between the protein and electrode [138]. Connection of biomolecules to the electrode would be more robust using the covalent interaction as opposed to one purely electrostatic in nature. Therefore covalent interactions may have a more substantial stabilization effect than less strong electrostatic interactions [138].

In 2004, Das et al. reported work comparing photoelectrodes with purple bacteria RCs and with PSI core-complexes from spinach [95].  $\text{His}_6$ - $\text{Ni}^{2+}$ -NTA-SAM-structures were used for immobilizing pigment-protein complexes on the gold substrate, as with many other works concerning bio-hybrid gold electrodes (Fig. 7E). The electrode with the RCs generated a cathodic current (electrons from the electrode through the RCs were fed to the electrolyte), and the electrode with PSI generated an anodic current (electrons from the electrolyte reduced P700, and the electrons from the PSI ETC moved to the gold substrate). This is due to the different orientations of the dipole  $\text{P}^+/\text{A}^-$  relative to the gold electrode for different complexes. In addition, the stability of PSI on the gold electrode was significantly lower. To maintain PSI stability, required the use of peptide surfactants, which was not needed for the RC based electrode [95].

When using a non-hydrophobic mesoscopic surface with appropriate pore size, modification can be avoided. However, orientation specific immobilization is necessary for successful electron transfer. Protein can be deposited onto the electrode in both productive and nonproductive configurations, and greater proportions of molecules in favorable conformations will correlate with higher cell

efficiencies. Distance is also a factor; if the acceptor side of the photosynthetic ETC is far from the electrode surface, electron transfer will be hindered. Engineering of the photosystem can therefore promote optimal orientation onto the electrode, which will also ensure optimal distance of the acceptor side of the ETC to the electrode [139]. Another consideration related to orientation is the technique employed to deposit biomolecules onto the electrode. Under the most common evaporation-based technique of immobilization, uneven deposition does occur. Aerosol techniques of electrospray deposition can allow the layer to be deposited in a controlled manner. Recently, studies have come out positing special reconstitution of photosystems to maximize the rate of electron transfer from pigment-protein complexes to the electrode. In such a way, one of the acceptor cofactors in the photosynthetic pathway exchanges to another cofactor with a protruding domain to the connect with the SAM [120,124]. One can modify the surface of electrodes with specific molecules, providing covalent bonds between sensitizer and substrate; such molecules are called linkers. So, various SAMs act as linkers. One linker provides fixation of a specific protein onto the substrate, while another linkers, serve as a molecular wire [124]. In 2009, scientists working on this technology connected PSI complexes to a gold electrode *via* molecular nanowires [121]. Nanowires are a unique linker, which serve also as an electron mediator. One part of the nanowire was incorporated in the vitamin K-site in place of the native vitamin K, and the other side was connected to the SAM on the gold electrode. In such a system, electrons from RC were transferred to the electrode through the nanowire [121]. Further, currently engineered redox centers in redox polymers provide for the successful acceptance of electrons from photosystems. Substantial connection between redox-polymers and solid electrodes provides high rate of electron transfer between them, as well as produces a stabilizing effect itself [93].

#### 5.4.3. Prolonged viability

Pigment-protein complexes are very susceptible to degradation in the ambient environment [91,107]. After extraction, they lack their native environment and regeneration/repair mechanisms. They can be stored frozen ( $-80^\circ\text{C}$ ) with cryoprotectant without losing activity [140,141]. But at elevated temperature and in solution, they will denature gradually, but irreversibly [91]. The lipid bilayer of extracted thylakoid membranes also deteriorate during the exposure to the elevated temperature [127]. It is suggested, that stability of photosynthetic macro-molecules, immobilized onto the substrate, increases after desiccation, owing to a stabilizing interaction with the substrate. However, the drying of photosynthetic macro-molecules with substrate is not applicable to long-term working conditions of the device SC. To increase their efficiency, different stabilizer agents are added, including peptide detergents or osmolytes [142,143].

#### 5.4.4. Absorption range

Chlorophyll absorption maxima are well defined, but restricted to two narrow bands at the edges of the visible spectrum. Antenna carotenoids broaden the absorption spectra of photosystems, but electron transfer with the participation of carotenoids are less effective. To broaden this absorption spectrum of photosynthetic macro-molecules incorporated into biohybrid electrodes, adding antenna complexes, both biological and artificial, can be employed. Beneficial properties of artificial complexes for this purpose are their biocompatibility and ability to very effectively transfer energy to the RC. It has been shown that the CdSe/ZnS and CdTe quantum dots are suitable for this function [134]. Quantum dots consist of a semiconductor nanocrystal with a diameter on the order of several nanometers.

## 6. TiO<sub>2</sub>-based solar cell

The most actively investigated substrate for photo bioelectrode building is nanostructured titanium dioxide. TiO<sub>2</sub> is biocompatible, can easily chelate the COOH groups and its bond strength is enhanced by its porous structure (the contact surface of TiO<sub>2</sub>/protein is larger in nanostructured titanium dioxide than in a flat plate) [119]. The TiO<sub>2</sub> industry is rapidly expanding due to the success of DSSCs. There are many different kinds of TiO<sub>2</sub> nanostructures being developed [17]. This growth provides the ability to choose appropriate structures dependent on which pigment-protein complexes are used. In most cases, immobilization of protein complexes onto TiO<sub>2</sub> does not require any linker molecules. As previously stated, the direct comparison of different results is not always representative due to differences in testing parameters and equipment, we nevertheless have paid special attention to the TiO<sub>2</sub>-based PBEC because the milliampere-range photocurrent output obtained by these devices (Table 2) [90,126]. Therefore, we have dedicated a section to the TiO<sub>2</sub>-based PBECs, utilizing components of photosynthetic apparatus. Here are included both studies about solar cells and biohybrid electrodes. TiO<sub>2</sub>-based PBECs are very similar to DSSCs. The main distinguishing feature separating them is the sensitizer. In classical DSSCs the sensitizer is comprised of a single molecule, that donates electrons to the TiO<sub>2</sub> conduction band following excitation. Thus, charge separation in the DSSC occurs at the sensitizer/TiO<sub>2</sub> interface. In the TiO<sub>2</sub>-based PBEC, charge separation occurs in the RC. The electron is then transferred from the final acceptor of ETC into the conduction band of the TiO<sub>2</sub>.

However the use of photosynthetic macromolecules for the sensitization TiO<sub>2</sub> suffers 3 limitations: 1) large size and limited functional surface area for TiO<sub>2</sub> contact, 2) susceptibility to degradation or proteolysis, and 3) limited rates of electron transfer due to inherent insulating properties of lipids/detergents and potential redox mismatch with the conduction band of the TiO<sub>2</sub>.

Nevertheless, isolated photosystem complexes may be orders of magnitude less expensive and renewable than the synthetic ruthenium-based sensitizers and do not contain any heavy metals. They are also more wide-spread and biologically abundant in biology in comparison to anthocyanins. Chlorophyll contained RCs are in every photosynthetic organism. Chlorophylls are probably the most abundant and certainly the most obvious biological pigments [65,69]. So, obtaining photosystems does not require the use of rare plants. Furthermore, it has been shown that performance of biohybrid electrodes depends not only on the type of sensitizer, but also from the TiO<sub>2</sub> nanostructure used, the method of its deposition into the electrode and the method of sensitizer immobilization. The deposition of mesoporous TiO<sub>2</sub> film typically follows these three steps:

- 1) Obtaining TiO<sub>2</sub> nanopowder (nanoparticle, nanotubes etc.) as a starting material;
- 2) Preparation TiO<sub>2</sub> paste;
- 3) Preparation of a mesoporous film.

As mentioned above, commercial nanopowder is not always suitable for use with pigment-protein complexes. Commercial TiO<sub>2</sub> nanopowder or pre-made TiO<sub>2</sub> paste were used in four of the eight studies reviewed here. Sol-gel or hydrothermal techniques are commonly used to prepare nanocrystalline TiO<sub>2</sub> from a titanium alkoxide precursor [144]. Titanium alkoxide suffers from peptization or hydrolyzation in these techniques. For the creation of mesoporous material, special organic or inorganic templates have been used [145,146]. This template is an organic polymer, which is later removed by calcination. The type of the template determines the form of the resulting TiO<sub>2</sub> nanocrystal. Depending on template, one can obtain nanotubes, nanoparticles or other morphologies.

Nanocrystalline titania material is solvated in polar solvent with some additive to permit the control of TiO<sub>2</sub> aggregation [147]. TCO glass is covered with TiO<sub>2</sub> paste by doctor-blading, spin-coating or screen-printing techniques [91,119,148]. Some techniques allow titania film to be obtained on the TCO-glass from precursor immediately. They include anodic deposition or aerosol chemistry vapor deposition (ACVD) [149,150]. Most photocurrent from biohybrid electrodes functionalized with photosynthetic pigment-protein complexes were reported for the electrodes utilizing a ACVD technique of TiO<sub>2</sub> film preparation. In this technique, titanium tetraisopropoxide is deposited onto the TCO under nitrogen purging. The TiO<sub>2</sub> is formed due to the decomposition of the titanium tetraisopropoxide, nucleation occurs in the gas phase and forms particles. These particles are deposited onto ITO coated glass electrodes, and kept at a constant temperature of 773 K where they sinter to form columnar TiO<sub>2</sub> single crystal structures [90].

The most common method to cover TiO<sub>2</sub> by sensitizer is the incubation of electrode into the solution with sensitizer, over long periods of time, allowing for the gradual evaporation of solvent from the electrode [91,104,107]. However, electrodes with pigment-protein coating completed by electrodeposition techniques demonstrate better performance [90,126]. In this technique, a solution of proteins with surfactant is sprayed under high voltage from a specialized needle to the TiO<sub>2</sub> surface.

TiO<sub>2</sub> is itself photoactive material. It absorbs ultraviolet light and, to a lesser extent, far blue light [11]. It is difficult to analyze the contribution of the sensitizer alone in a TiO<sub>2</sub>-based hybrid electrode because TiO<sub>2</sub> is responsible for a significant part of photocurrent if ultraviolet light is present in light spectrum used for irradiating the cell during testing. Again, differences in experiment conditions (or absence in some cases) make comparison of results difficult between devices. For instance, it has been shown, that photocurrent depends not only on light, but also on temperature [91]. Therefore, the number of parameters that impact the efficiency of these devices needs to be further studied and accounted for as this burgeoning field moves forward.

### 6.1. Reaction centres of purple bacteria as sensitizer

Lu et al. used a WO<sub>3</sub>-TiO<sub>2</sub> mesoporous film with tailored pore size as a substrate for the reaction center of purple bacteria [148]. Their design was based on reports positing that the TiO<sub>2</sub> doped amorphous WO<sub>3</sub> and nanocluster WO<sub>3</sub>-TiO<sub>2</sub> show higher performance of light-induced charge separation compared to that of pure TiO<sub>2</sub> or WO<sub>3</sub> alone. WO<sub>3</sub>-TiO<sub>2</sub> paste was synthesized via a sol-gel method, with titanium butoxide and the tungsten hexachloride being the reactants. An even layer of the paste was deposited onto ITO-glass using the spin coating method, and then high temperature sintering was applied to the electrode. The authors offer a comparison between the structures WO<sub>3</sub>-TiO<sub>2</sub> and a just TiO<sub>2</sub> film prepared by anodic electrodeposition.

Protein immobilization was achieved by immersing the freshly prepared WO<sub>3</sub>-TiO<sub>2</sub> films in pH 8.0 Tris-HCl buffer solution containing RCs (at 4 °C) for 2–3 days. The substrate, with pore size of about 7.1 nm, shows the best loading of RCs. The investigators propose the reason for this result of better RC loading onto the WO<sub>3</sub>-TiO<sub>2</sub> compared to TiO<sub>2</sub> film is the increased hydrophilicity of WO<sub>3</sub>-TiO<sub>2</sub>. Prior to the addition of this hydrophobic semiconductor, the most electrostatically stable orientation of the RC was such that the more hydrophilic H-subunit domains of RC faced the substrate. The maximum loading of RC on the this WO<sub>3</sub>-TiO<sub>2</sub> films, calculated from the differential absorption spectra, reaches 0.63 μmol/g. Authors used the three-electrode scheme to assess the photoelectric response. Electrode bias was about -0.3 V vs Ag/AgCl electrode during these experiments. The buffer in the electrochemical cell contained 8 mM sodium dithionite. The elec-

**Table 2**  
Examples of different TiO<sub>2</sub> biohybrid electrodes and comparing of their performance with the same bare electrode.

*	Sensitizer	Mesoporous film	Mesoporous film preparation	Sensitizer coating method	Photoelectrochemical study	Light	Current, $\mu\text{A cm}^{-2}$	Control current, $\mu\text{A cm}^{-2}$
[148]	RC ( <i>Rb. Sphaeroides</i> )	3D worm-like WO <sub>3</sub> -TiO <sub>2</sub> on ITO. Thickness: 150 nm Porous diameter 7.3 $\mu\text{m}$ , Surface area 150m <sup>2</sup> /g	Sol-gel of WCl <sub>6</sub> and Ti(OBu) <sub>4</sub> in solution of polymer P123 in ethanol Spin-coating of paste on ITO calcination	Soaking electrode in solution of RC in Tris-HCl (pH 8.0) at 4 °C for 2 days with following rinsing.	PF-PEC Electrode bias about -0.3 V vs SHE 8 mM sodium dithionite in buffer	1) white light 5 mW cm <sup>-2</sup>  2) $\lambda > 600 \text{ nm}$ , 0.1 mW cm <sup>-2</sup>	~30  ~0.9	Bare TiO <sub>2</sub> , without sensitizer ~6.7  <0.05
[74]	RC ( <i>Rb. Sphaeroides</i> )	Mesoporous film of TiO <sub>2</sub> on ITO; Thickness: 4 $\mu\text{m}$ ; Particle diameter 6 nm, Surface area 300m <sup>2</sup> /g	Rolling of homogenized paste with TiO <sub>2</sub> nanopowder, PEG, Triton and different acids with followed by calcination	Soaking electrode in solution of RC (~5.5 $\mu\text{M}$ ) in Tris-HCl (pH 8.0) at 4 °C for 2 days with following rinsing.	PF-PEC Electrode bias about -0.3 V vs Ag-AgCl for TiO <sub>2</sub> without RC and about -0.25 V for TiO <sub>2</sub> -RC Electrode working surface about 0.8 cm <sup>2</sup> 10 mM sodium dithionite; T = 5 °C	White light 80 mW cm <sup>-2</sup>  $\lambda > 680 \text{ nm}$ , 45 mW cm <sup>-2</sup> ~0.150	~50  ~2.3 TiO <sub>2</sub> -PSII without DCBQ ~0.050	Bare TiO <sub>2</sub> , without sensitizer ~25  0
[104]	PSII core complexes (CP47 His-tagged mutant from <i>T. elongatus</i> )	Commercialized ITO-glass with TiO <sub>2</sub> covering (Solaronix, Switzerland); Thickness: 250–500 nm; Particle size: 20 nm; Calcination before using	Electrode soaking in solution of PSII and DDM (0.03%) at 4 °C overnight For monolayer: 4 $\mu\text{g Chl ml}^{-1}$ For multilayer: 400 $\mu\text{g Chl ml}^{-1}$	PF-PEC Electrode bias about +0.644 V vs SHE; Sequence of short illumination intervals (10–20s); T = 25 °C; 100 $\mu\text{M}$ of DCBQ	$\lambda \approx 590 \text{ nm}$ 800 $\mu\text{E m}^{-2} \text{ s}^{-1}$ Or about 16 mW cm <sup>-2</sup>			
[125]	BBY particle (Spinach)	Mesoporous film of TiO <sub>2</sub> on ITO; Thickness: 2.5 nm; Particle size: 11 ± 1 nm	Sol-gel of Ti(OBu) <sub>4</sub> on cellulose paper with following calcination Following preparation of TiO <sub>2</sub> -ethanole paste and transferring it onto ITO with following drying in air	PSII solution in 20 mM MES (pH 6.5) with about 1 mg Chl ml <sup>-1</sup> was dropped onto TiO <sub>2</sub> and left at 4°C in the dark for 1 h followed drying with nitrogen without sintering.	PF-PEC Electrode bias about +0.25 V vs SCE; T = 25 °C; 1 mM of DCBQ	White light Intensity is not signed  light not exciting TiO <sub>2</sub> (550–800 nm), 10 mW cm <sup>-2</sup>	~10.6  ~8.8	Bare TiO <sub>2</sub> , without sensitizer ~5.8  0
[107]	PSI Trimer ( <i>T. elongatus</i> )	Nanocrystalline TiO <sub>2</sub> film on ITO; Thickness: 3.8 nm; Pore diameter 60nm roughness factor $r_{\text{TiO}_2} \approx 200$	Paste of TiO <sub>2</sub> nanopowder, ethyl cellulose and terpineol was screen-printed on the ITO-glass followed incubation in ethanol and drying at 125 °C.	PSI at 0.4 mg/mL was mixed with in a 1:1 ratio with 0.1% (w/v) of the cationic peptide surfactant Ac-AAAAAAK-NH <sub>2</sub> (AGK) 40 mL drop of PS-I solution to air dried at room temperature on nanostructured semiconducting electrode.	Voltammetry of completed solar cell DSSC was prepare with FTO/TiO <sub>2</sub> (ZnO)/PSI anode, Pt/ITO cathode and Co(II)/Co(III) ion containing electrolyte Z813.	solar simulator with neutral density filters was used 100 mW cm <sup>-2</sup>	~362	Bare TiO <sub>2</sub> , without sensitizer ~277
	PSI Trimer ( <i>T. elongatus</i> ) with modification	ZnO nanowires grown on Zn-nanoparticle-seeded ITO-glass Height of wire: 3 $\mu\text{m}$ roughness factor $r_{\text{ZnO}} \approx 30$	Wires were grown onto FTO from ZnO quantum dots nuclei through the thermal decomposition of a zinc complex				~20	Is not signed
[119]	PSI monomer and trimer ( <i>A. Platensis</i> )	Double TiO <sub>2</sub> layer: Bottom film: Thick $\approx 2 \mu\text{m}$ Particle size $\approx 20 \text{ nm}$ Upper film: Thick $\approx 2 \mu\text{m}$ Particle size $\approx 200 \text{ nm}$	Commercialized pastes of TiO <sub>2</sub> (for both films) were used. Pastes were printed on FTO glass by layer-by-layer screen printing. TiO <sub>2</sub> /FTO electrodes were sintering.	Cooled TiO <sub>2</sub> /FTO electrodes was soaked into solution of PSI, 80 $\mu\text{g Chl ml}^{-1}$ , with 1,0 mM DDM in 20 mM MES (pH 6.5).	DSSC was prepare with FTO/TiO <sub>2</sub> /PSI anode, FTO/Pt cathode and I <sup>-</sup> /I <sup>2-</sup> ion containing electrolyte The current-voltage characteristics of each cell were recorded with a Keithley 2400 source/meter	white light 100 mW cm <sup>-2</sup>	~1 310	Is not signed
[90]	PSI trimer (HT3 cells)	columnar TiO <sub>2</sub> film on ITO Height 1.6 $\mu\text{m}$	ACVD used to deposit columnar TiO <sub>2</sub> nanostructured films on ITO-glass	PSI mixed with DDM. This solution was sprayed on TiO <sub>2</sub> by electrospray deposition methods.	PF-PEC The PSI – TiO <sub>2</sub> electrochemical cells were tested with a 20 mM sodium ascorbate, 250 $\mu\text{M}$ DCPIP and 0.1 M KCl electrolyte solution. Time deposition about 5 min.	220 mW cm <sup>-2</sup> UV + visible light (250–900 nm)	~2 510 (~4150 for 10 min deposition)	Bare TiO <sub>2</sub> , without sensitizer ~1 380
						visible light only (400–900 nm) UV + visible light (250–900 nm), visible light only (400–900 nm),	~1 050 6700 ± 150 ~11.5	~270 ~1 833 ~0.005
[126]	Thylakoid membrane ( <i>Synechocystis</i> sp. 6803)	columnar TiO <sub>2</sub> film on ITO Height 1.6 $\mu\text{m}$	ACVD used to deposit columnar TiO <sub>2</sub> nanostructured films on ITO-glass	Thylakoid solution with 1 mg Chl ml <sup>-1</sup> was diluted with water and surfactant and centrifuged. This solution was sprayed on TiO <sub>2</sub> by electrospray deposition methods.	PF-PEC The PSI – TiO <sub>2</sub> electrochemical cells were tested with 0.1 M KCl electrolyte solution without any donors or acceptors. Time deposition about 20 min.			

trode produced  $\sim 30 \mu\text{A cm}^{-2}$  at white light of  $5 \text{ mW cm}^{-2}$ , and did  $\sim 0.9 \mu\text{A cm}^{-2}$  at red light of  $0.1 \text{ mW cm}^{-2}$  ( $\lambda > 600 \text{ nm}$ ). The electrode with bare  $\text{WO}_3\text{-TiO}_2$  films produced  $6.7 \mu\text{A cm}^{-2}$  under white light, and almost did not produce any current at the red light ( $< 0.05 \mu\text{A cm}^{-2}$ ). Thus, addition of sensitizer (RC) to the  $\text{WO}_3\text{-TiO}_2$  films increased the photocurrent nearly fourfold. Stability of the  $\text{ITO/WO}_3\text{-TiO}_2\text{/RC}$  electrode was also characterized, and they display a photocurrent decrease  $\sim 15\%$  during 1 h of continuous illumination [148].

A group of Russian scientists, nearly two years after the Lu group, performed a very similar study, publishing a technique of RC immobilization onto nanostructured  $\text{TiO}_2$  [74]. In this study, RC complexes were fixed in porous substrate of  $\text{TiO}_2$  alone, due to the hydrophobic interactions, devoid of tungsten (VI) oxide. In this work, the H subunit is expected to face the buffer and not contact the  $\text{TiO}_2$  substrate. These researchers compared different  $\text{TiO}_2$  nanopowder types as the precursors for mesoporous  $\text{TiO}_2$  layer. They distinct from one another by particle size.  $\text{TiO}_2$  paste preparation was the same for every nanopowder.  $\text{TiO}_2$  paste had been obtained by mixing of  $\text{TiO}_2$  nanopowder, polyethylene glycol and detergent Triton X-100 in aqueous solution. Various acids were added due to prevent aggregation of  $\text{TiO}_2$  nanoparticles. Films were layered on the ITO-glass by rolling on the paste, forming a layer of  $\sim 4 \mu\text{m}$ . From the nanopowder with smallest nanoparticles, a mesoporous layer with the most effective surface is obtained, and therefore this film could adsorb the most RC particles. Acetic acid was used as an anti-aggregation agent in the best film preparation. The three-electrode scheme was again used for the photocurrent measurement. The bias applied to the electrode was about  $-0.3 \text{ V}$  vs  $\text{Ag/AgCl}$  for  $\text{TiO}_2$  without RC and about  $-0.25 \text{ V}$  for  $\text{TiO}_2\text{-RC}$ . The  $\text{TiO}_2\text{-RC}$  generated about  $50 \mu\text{A cm}^{-2}$  with incident white light intensity of about  $80 \text{ mW cm}^{-2}$ . In the same light condition, bare  $\text{TiO}_2$  electrodes generated a current just two times smaller. Under red-light illumination, with  $45 \text{ mW cm}^{-2}$  intensity, bare  $\text{TiO}_2$  did not generate any current, and  $\text{TiO}_2\text{-RC}$  generated  $\sim 1.125 \mu\text{A cm}^{-2}$ . This ETC has quinone (Q) as the final acceptor, and electron transfer from Q to  $\text{TiO}_2$  is energetically unfavorable. For successful electron transfer to the electrode, researchers artificially reduced all Qs by sodium dithionite. As a result, plastocyanin served as the electron transducer from protein to electrode. These authors went on to conclude from these results, that the excited triplet form of the P840 could donate electrons to the conductive band of  $\text{TiO}_2$  (Fig. 8A) [74].

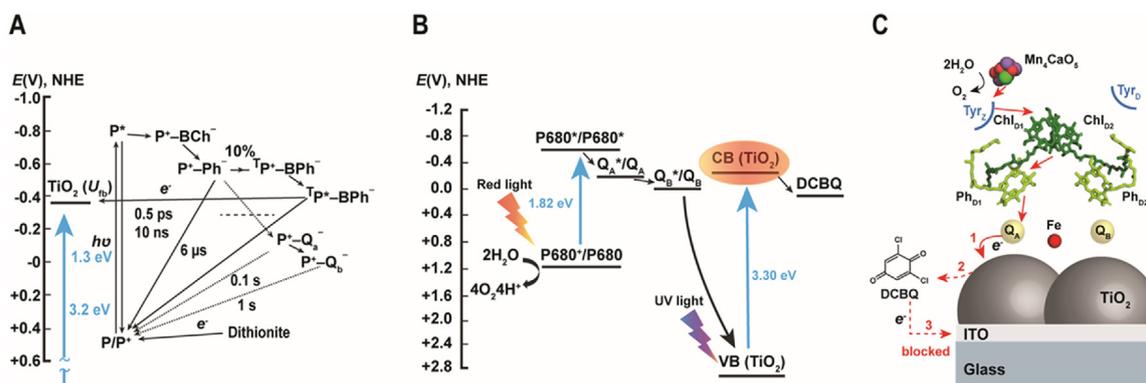
## 6.2. Photosystem II as photosensitizer

In 2016, the Li group integrated photosystem II from spinach thylakoid membranes into a  $\text{TiO}_2$  nanotube network [125]. They investigated two things both the relation between the light-to-current conversion efficiency and  $\text{TiO}_2$  structure and also compared direct electron transfer (DET) and mediated electron transfer (MET).  $\text{TiO}_2$  nanotubes had been synthesized by a surface sol-gel method from Titanium (IV) *n*-butoxide solution. Cellulose was used as the scaffold for surface sol-gel technique. The source of cellulose fibers was filter paper. Thereafter, each cellulose nanofiber of the filter paper was coated with a thin titania gel layer of  $\sim 0.5 \text{ nm}$  by one deposition cycle. Different numbers of deposition cycles were being applied to obtained layers with different thicknesses. This film was calcinated to remove the cellulose. To prepare the paste, commercialized  $\text{TiO}_2$  nanopowder or in-lab prepared  $\text{TiO}_2$  nanotubes were dissolved in ethanol and sonicated. Paste was deposited onto ITO-glass and was air dried, without any sintering. The investigators also measured electrodes with bare  $\text{TiO}_2$  (lacking PSII complexes), and electrodes without  $\text{TiO}_2$  for their controls. In the latter, PSII complexes were immobilized onto the ITO surface. Both, white

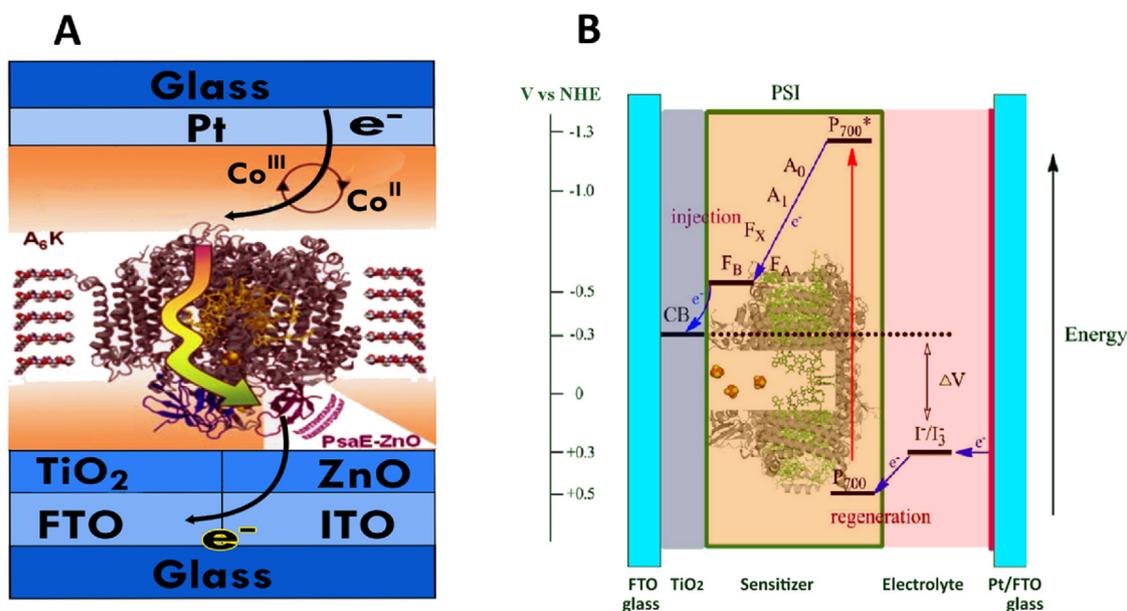
light and red light illumination was tested, and 2,5-dichloro-1,4-benzoquinone (DCBQ) was used as the mediator. Electrodes with bare  $\text{TiO}_2$  do not generate any current under red light in both DET and MET cases. The current from PSII/ $\text{TiO}_2$ /ITO-glass increased with  $\text{TiO}_2$  layer thickness under red light without any mediator. But for all other cases (white light and red light with MET), it was shown that the thicker the  $\text{TiO}_2$  layer, the less the current produced. The effect of the mediator on the current from the bare  $\text{TiO}_2$  electrode is very interesting. Their data suggests that DCBQ can interact with  $\text{TiO}_2$ . In all cases, MET was significantly more effective than DET. These results were explained by the rationale that DCBQ-mediated electron transfer from PSII to the ITO directly, bypassed the  $\text{TiO}_2$ . Photocurrent from PSII/ $\text{TiO}_2$ /ITO-glass was more than sum of current values of PSII/ITO and bare  $\text{TiO}_2$ /ITO. It was suggested, that electrons from PSII transfer to the valence band of  $\text{TiO}_2$  faster, and from there they move to the conductive band due to ultraviolet light. DCBQ, if present, can accept an electron from the conduction band of  $\text{TiO}_2$  and transfer it to the ITO (Fig. 8B). So, there are two ways of electron transfer from PSII to the electrode, direct from  $Q_A$  to the  $\text{TiO}_2$ , and mediated by the DCBQ. The best current value for PSII/ $\text{TiO}_2$ /ITO-glass systems was about  $10.6 \mu\text{A cm}^{-2}$ , just less than twice as much than from bare  $\text{TiO}_2$ . The  $\text{TiO}_2$  paste from commercial nanopowder provided less current than  $\text{TiO}_2$  nanotube paste [125]. Brinkert et al. also investigated the electron transfer pathways in a PSII/ $\text{TiO}_2$  hybrid system. In this experiment, PSII was extracted from *T. elongatus*. They compared the multilayers and monolayers of the PSII complexes as well as the effect of the mediators (DCBQ and 2-phenyl-*p*-benzoquinone, PpBQ) and inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DMCU) on the electron transfer in the electrode. The results correlate strongly with the results of the previous work. To confirm the DCBQ-mediated electron transfer immediately to ITO, the compact  $\text{TiO}_2$  layer between sensitized  $\text{TiO}_2$  and ITO was applied (Fig. 8C). The electrode with this layer did not demonstrate photocurrent increase in the presence of DCBQ. This was expected, due to the impermeability of the compact layer to the mediator species. Therefore, the following pathway of electron transfer is proposed: PSII quinone –  $\text{TiO}_2$  – DCBQ – ITO. This scheme is faster than DET due to the slow electron transfer in  $\text{TiO}_2$ . This may be due to the electrons from quinone PSII having insufficient energy to move into the  $\text{TiO}_2$  conduction band, so they remain close to the surface of the  $\text{TiO}_2$  in lower energy states, available for back interactions with mediators, and slow to migrate to the conducting electrode. It was shown by inhibitor analysis that direct electron transfer occurs not only from the  $Q_B$  site but also from  $Q_A$  site, which was unexpected. DCBQ can accept electrons from the acceptor side of PSII for complexes, which are not in direct contact with  $\text{TiO}_2$ . The electron diffusion in  $\text{TiO}_2$  is the rate limiting step in current generation for PSII/ $\text{TiO}_2$  hybrid electrodes, due to the energetic discrepancy of the PSII acceptor side and conduction band edge of  $\text{TiO}_2$  [104]. Photosystem I as sensitizer

## 6.3. Photosystem I as sensitizer

In their work in 2013, Mershin et al. report that dry PSI complexes self-assembled on nanostructured semiconductors [107]. These complexes were stabilized by a 2.4 nm long, cationic peptide surfactant Ac-AAAAAAK-NH<sub>2</sub> (A6K), consisting of six alanine residues and a lysine at the amidated C-terminus [142]. PSI mixed with this peptide in solution demonstrated enhanced stability. Researchers built the whole solar cell like the dye-sensitized solar cell with PSI as sensitizer. In solar cell, PSI complexes served as both the light harvester and charge separator. They used nanocrystalline  $\text{TiO}_2$  and ZnO nanowires to provide a large effective surface area for PSI adsorption and light harvesting (Fig. 9A). For the ZnO substrate, mutant PSI complexes are required for successful immobilization.  $\text{TiO}_2$  did not require the engineering of native PSI complexes.



**Fig. 8.** Electron transfer in biohybrid  $\text{TiO}_2$ -based electrodes with a Type II RC. A. RC of purple bacteria onto  $\text{TiO}_2$ . Dashed lines denote the electron transfer events that did not take place in the RC due to a dark reduction of the quinone acceptors by dithionite. The potential is shown relative to the standard hydrogen electrode;  $U_b$  is the conduction band potential of semiconductor. Electron transfer from excited triplet state of special pair is most possible, because bacteriochlorophyll is deeper in protein than the P840. Adapted from [74]. B. BBY particles of spinach onto  $\text{TiO}_2$ . DCBQ accept electron from conduction band of  $\text{TiO}_2$  rather than from  $Q_A$  or  $Q_B$ . Electron from acceptor side of PSII injected into valence band of  $\text{TiO}_2$  that got excess holes owing to ultraviolet light excited the  $\text{TiO}_2$ . Adapted from [125]. C. PSII from *T. elongatus* onto  $\text{TiO}_2$ . Electron transfer from  $Q_A$  to  $\text{TiO}_2$  is possible. It is ensured, that DCBQ mediates electron transfer from  $\text{TiO}_2$  to ITO-glass. Adapted from [104].



**Fig. 9.** Whole solar cells based on PSI. A. Two variants of PSI sensitized solar cell with both  $\text{TiO}_2$  and ZnO substrates. ZnO in contrast to  $\text{TiO}_2$  requires engineered PSI complexes. PSI was extracted from *T. Elongatus*. Cobalt-based electrolyte was exploited. Adapted from [107]. B. PSI from *A. Platensis* onto  $\text{TiO}_2$ -electrode in the solar cell.  $\Gamma^-/\Gamma_3^-$ -based electrolyte was exploited. The primary free-energy losses ( $\Delta V$ ) are associated with electron injection from the excited sensitizer into the  $\text{TiO}_2$  conduction band (CB) and regeneration of the dye by the redox couple. Adapted from [119].

$\text{TiO}_2$  paste was deposited *via* the screen-printing method. Under standard sunlight the  $\text{TiO}_2$ -based BPEC reached electrical power outputs of up to  $81 \mu\text{W cm}^{-2}$  and area-normalized short-circuit current densities  $I_{sc}$  (normalized) of up to  $362 \mu\text{A cm}^{-2}$ . Efficiency of the ZnO-based BPEC was less than seen for the former device. Light absorption results for excitation of the primary electron donor (P700), electron transfer to primary and further to secondary electron acceptors are presented. In their BPEC, the role of plastocyanin was played by the  $\text{Co}(\text{II})/\text{Co}(\text{III})$  redox couple electrolyte, termed Z813 [151], and ferredoxin was replaced by either nanocrystalline  $\text{TiO}_2$  or ZnO-nanowires to supply a wide surface area for accepting electrons [107].

In 2015, Yu et al. report the most effective PSI and LHClI-based solar cells to date [119]. In their work, several solar cells were built with various structures of the mesoporous layer. The impact of a scattering layer was also studied. The transparent mesoporous layer contains nanoparticles of about 22 nm size, whereas the scat-

tering layer contains nanoparticles around 200 nm size.  $\text{TiO}_2$  pastes were printed on the conductive FTO-glass with a layer-by-layer screen printing method followed by sintering of the coated glass at  $500^\circ\text{C}$ . The transparent mesoporous layer was deposited first. The scattering layer of  $\text{TiO}_2$  was then deposited atop the transparent layer. The surfactant *n*-dodecyl- $\beta$ -D-maltoside (DDM) was used to keep PSI from *A. platensis* or LHClI from spinach suspended in solution, and to inhibit the formation of multilayers on the  $\text{TiO}_2$  surface. The electrode was soaked in the solution of the pigment-protein complexes and DDM in MES buffer. PSI complexes could permeate only into the scattering layer, while the LHClI complex, which is smaller, can permeate both into the scattering layer and the transparent layer. Thus, the overall loading of the  $\text{TiO}_2$  substrate by LHClI was more than by PSI complexes alone. This watermark achievement solar cells performed as follows:  $J_{sc} = 1.31 \text{ mA cm}^{-2}$  and  $\eta = 0.47\%$  for PSI-based and for the LHClI-based solar cell these values are  $J_{sc} = 1.51 \text{ mA cm}^{-2}$  and  $\eta = 0.52\%$ . But the data shows that

electron transfer from PSI to the TiO<sub>2</sub> was more efficient than from the LHCII. Iodine based electrolyte and FTO glass were used in the construction of these solar cells (Fig. 9B). Further, an AM1.5 filter was used to provide the specific light condition in these experiments [119].

In 2014, Shah et al. report a potentiometric investigation of PSI/TiO<sub>2</sub> a biohybrid electrode [90]. In this work, columnar TiO<sub>2</sub> nanostructured films were deposited onto ITO-glass using an aerosol-chemical vapor deposition (ACVD) process. The authors claimed that the conventional evaporation-based techniques of PSI immobilization on TiO<sub>2</sub> can result in uneven deposition over nanostructures that decreases the performance of biohybrid electrode. In this process, titanium tetraisopropoxide was purged by nitrogen, and PSI complexes were deposited onto the TiO<sub>2</sub> surface using an electrospray deposition technique. Briefly, a solution of PSI and DDM in ethanol was deposited *via* syringe to the electrode surface under an applied voltage. After which, the electrode was placed in the electrolyte solution, the surfactant was washed away, and the complexes were retained on the surface. One can control the thickness of the sensitizer layer by the regulation of deposition time. It is important, that the concentration of DDM is not too high, for rapid removal of surfactant from electrode with electrolyte solution. The faster the DDM concentration decreased below the critical micelle concentration, the greater the number of PSI complexes immobilized onto the electrode. On other hand, sufficient DDM is needed to prevent aggregation of the PSI complexes. Sodium ascorbate and dichlorophenolindophenol served as electron donors in the buffer. The electrode was exposed to visible light and UV + visible light to assess contribution of titania in photocurrent generation. Under the UV + visible light, photocurrent was almost twice as high than with visible light only for the PSI/TiO<sub>2</sub> electrode, and more than three times greater for the bare TiO<sub>2</sub>. Compared against each other, the photocurrent from PSI/TiO<sub>2</sub> was about two-fold greater than that for bare TiO<sub>2</sub>. The best result for this work is about 4.15 mA cm<sup>-2</sup> for PSI/TiO<sub>2</sub> at UV + visible light, with an optimal deposition time of 10 min [90].

#### 6.4. Thylakoids as sensitizer

The highest value of photocurrent from a PBEC was obtained from biohybrid electrodes utilizing thylakoid membranes as the sensitizer [139]. In fact, the associated membrane photosynthetic complexes PSII, b6f complex and PSI served as sensitizer without the intact native lipid bilayer. In this work, an environmentally friendly, linker, electron donor, and mediator-free PBEC was successfully fabricated by re-engineering the natural photosynthesis circuit using single step aerosol techniques. The substrate employed for the membrane preparation was nanostructured TiO<sub>2</sub>. The primary donor was water; no exogenic electron donors were added. Thylakoid membranes were processed with DDM to solubilize the membrane proteins. The resulting solution was applied to the TiO<sub>2</sub> surface by the electrospinning method. After the sensitized TiO<sub>2</sub> was exposed to the electrolyte, PSI, PSII and cytochrome b<sub>6</sub>/f aggregate on the surface and adhere to the TiO<sub>2</sub>, whereas the surfactant diffuses into the electrolyte. Technically, this is not a thylakoid-based solar cell, but it is a solar cell with a complete working Z-scheme. In their study, Kavadiya et al. observed the maximum photocurrent density 6.7 mA cm<sup>-2</sup> under UV and visible light, and 12 μA cm<sup>-2</sup> under visible light illumination [139].

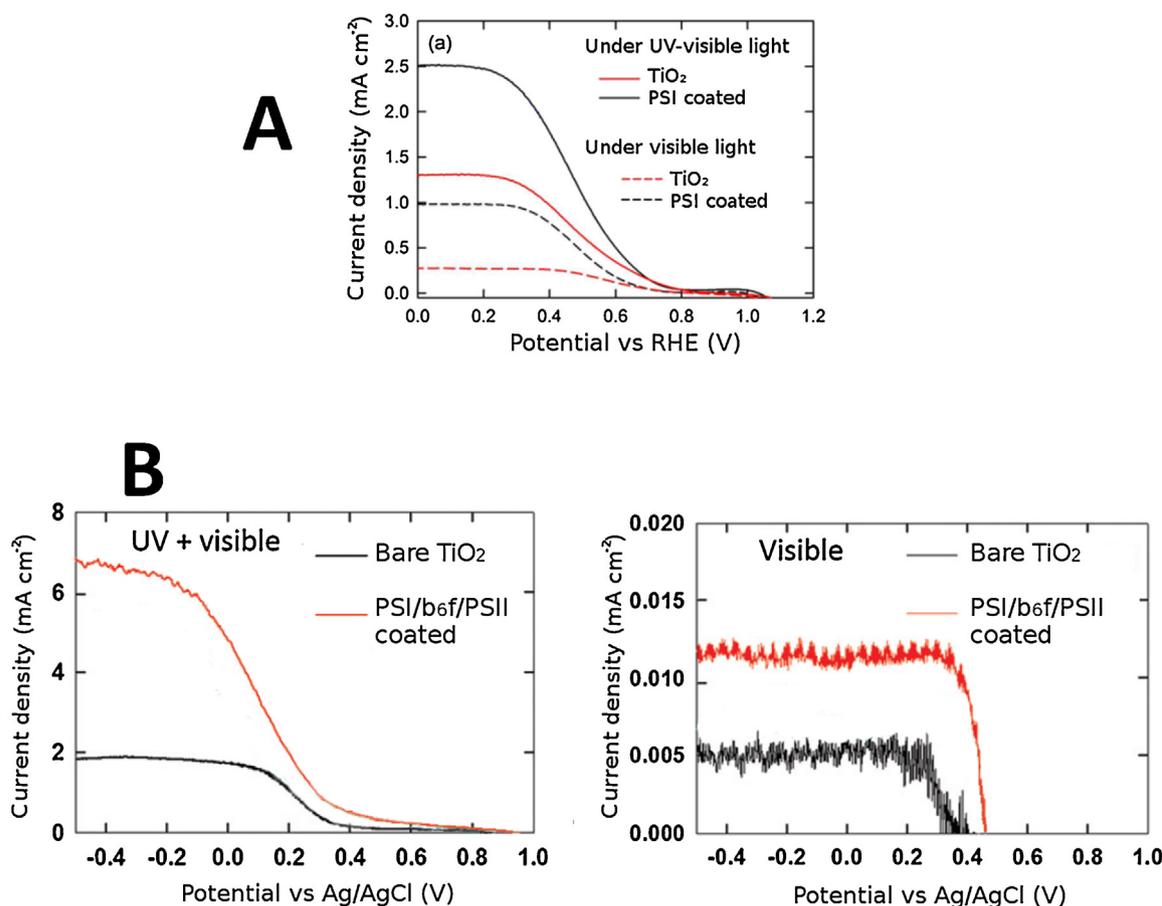
## 7. Conclusions

At this time, the Z-scheme and PSI-based biohybrid electrodes show more favorable results than those based on PSII complexes and bacterial RCs (Table 2). One can conclude, that type II RCs are

not as well suited for immobilization onto TiO<sub>2</sub>, at least without the presence of type I RCs. This is due to the energy discrepancy between the redox potential of quinones in the acceptor side of type II RCs and the conduction band of TiO<sub>2</sub> (Fig. 8A) [104,126]. Further, certain cofactors of PSII locate deep in the protein complex, hindering their access [74]. Possibly a material with a more appropriate conduction band can be used with PSII to facilitate more successful electron transport [104]. As previously discussed, the presence of DCBQ significantly increased electron transfer rates in PSII containing devices. Again, the DCBQ is proposed to transfer the electron directly to the TCO (Fig. 8B and C). Thus, a compact layer cannot be used in such cases, which will give rise to an increase in undesirable recombination reactions, significantly decreasing the efficiency of such bioelectrodes. In comparison to PSII containing devices, bacterial RCs demonstrate slightly better results. This can be attributed to the smaller size, and therefore greater permeability of bacterial RC onto the mesoporous semiconductor surface. Also, the sodium dithionite addition may enhance the electron transport to the TiO<sub>2</sub>. However, at this point, neither of these designs compete with the efficiency of current devices utilizing PSI or intact thylakoid membranes. PSI has a more suitable acceptor side to donate an electron to the TiO<sub>2</sub> conduction band. It has also been shown in the literature that PSI is more robust than PSII *in vitro* [130]. PSI is the only photosynthetic protein complex, that achieves electron separation, and has been used as a sensitizer in a complete TiO<sub>2</sub>-based solar cell (Fig. 9). All other species have only yet been used in biohybrid electrode studies. Peptide surfactants or detergent DDM have been used in all PSI/TiO<sub>2</sub> electrode studies so far, to stabilize protein complexes and prevent them from aggregation [107,119]. The choice of appropriate nanostructure is very important to PSI-based biohybrid electrodes [119]. The best result, 4.15 mA cm<sup>-2</sup> was achieved for an electro-deposited PSI coating on the ACVD-obtained film of columnar TiO<sub>2</sub> [90]. PSI requires a special donor for electron transport. The ability of PSII to split water, and the suitability of PSI acceptor side for electron transfer to TiO<sub>2</sub> attracts attention to exploit both PSI and PSII onto the electrode. To date, the largest photocurrent realized was from a biohybrid electrode with all Z-scheme complexes present [126].

There are two studies we consider to be the most exciting, both utilizing TiO<sub>2</sub> films prepared by ACVD, and sensitizer spread by electrochemical deposition. One of them presents a PSI coated biohybrid electrode and the other investigates a PSII/b<sub>6</sub>f/PSI coated electrode [90,126]. Both studies demonstrate superior results compared to other methods outlined in this review (Fig. 10). However, this is not due to the merit of the sensitizer alone. It is shown in Table 2 that the photocurrent generated by the control (a bare TiO<sub>2</sub> electrode), is also higher than reported in other studies. Moreover, these devices generate very small current with only visible light lacking UV. The sum of the current values from bare TiO<sub>2</sub> under UV + visible light and sintered TiO<sub>2</sub> under only visible light, is less than current from sintered TiO<sub>2</sub> under UV + visible light (Fig. 10A and B). Unfortunately, all information regarding light conditions in these studies are not presented, making the comparison of these studies to the larger body of literature difficult. One can suggest, that the interaction between sensitizer and TiO<sub>2</sub> during exposure to variable light conditions requires further investigation.

Further successes in the PBEC field are contingent upon the optimization of substrate material pairing with pigment-protein complexes. Much remains unclear about the processes occurring in such hybrid structures. To address this knowledge gap, a more complete understanding of what is happening on the molecular, atomic and subatomic levels are necessary to drive the selection of ideally compatible materials. Discoveries in these directions may well reveal for titania based electrodes, that there may not be a universal TiO<sub>2</sub> nanostructure for every kind of sensitizer. However, based on this review of the current literature, we can conclude that



**Fig. 10.** Linear scan voltammetry data for biohybrid electrode under various light conditions. Electrodes were made by ACVD techniques of obtaining the mesoporous TiO<sub>2</sub> film and electrodeposition technique of sensitizer. A. PSI from modified *Synechocystis* sp. PCC 6803 mutant strain served as sensitizer, adapted from [90]. B. Thylakoid membrane from *Synechocystis* sp. PCC 6803 strain served as sensitizer, adapted from [126].

TiO<sub>2</sub> is the most promising substrate material to be used photo-synthetic proteins. The performances of PBECs is significantly less than that of DSSCs based on single pigments for the moment, but the relentless rate of efficiency increase in the field PBEC research is encouraging.

#### Conflicts of interest

There are no conflicts to declare.

#### Acknowledgements

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