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Biological Components and Bioelectronic Interfaces of Water Splitting Photoelectrodes for Solar Hydrogen Production

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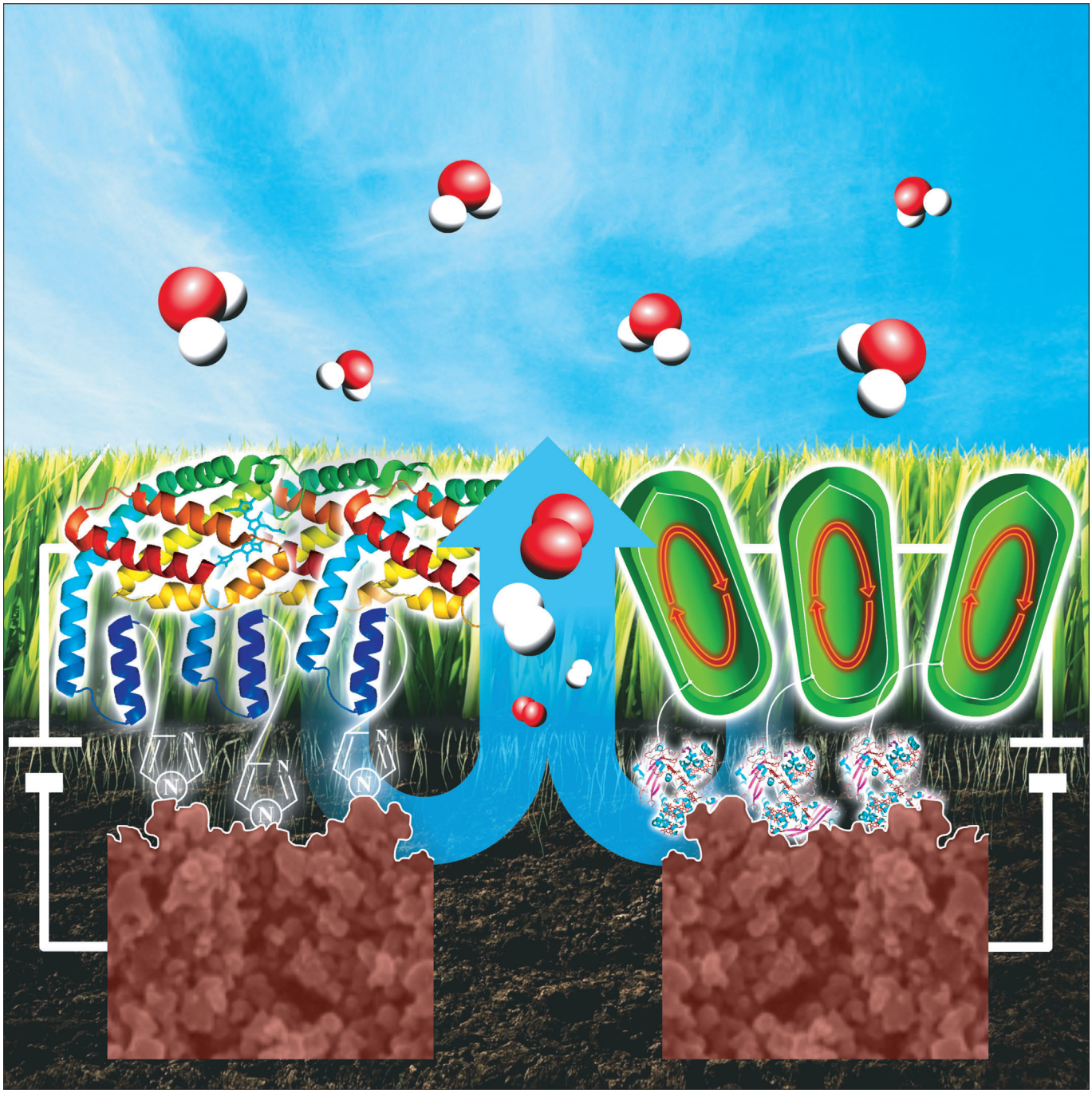

Abstract: Artificial photosynthesis (AP) is inspired by photosynthesis in nature. In AP, solar hydrogen can be produced by water splitting in photoelectrochemical cells (PEC). The necessary photoelectrodes are inorganic semiconductors. Light-harvesting proteins and biocatalysts can be coupled with these photoelectrodes and thus form bioelectronic interfaces. We expand this concept toward PEC devices with vital bio-organic components and interfaces, and their integration into the built environment.

Introduction

The energy supply of the developed world depends on fossil fuels, a finite resource, and faces frequently recurring energy crises. Artificial photosynthesis (AP) is one approach to sustainable energy production. AP is the harvesting, conversion and storage of solar energy into the chemical bonds of fuel molecules. It is inspired by naturally occurring photosynthesis in which solar energy is converted to chemical energy and stored in the form of carbohydrates. In this overview, we look at approaches in which proteins and pigments involved in photosynthesis are combined with inorganic structural and functional support materials to enhance photoelectrochemical water splitting and thus solar H_2 production. A brief overview on natural photosynthesis and hydrolysis in photoelectrochemical cells (PECs), also referred to as AP, is followed by an in-depth discussion of protein attachment to electrodes. Such systems have bioelectric interfaces at which charge transfer is an essential feature. The issue of protein denaturation is preceded by an insight into the use of whole organisms and the inherent challenges this entails. We present our approach to enhance photocurrents on semiconductor photoelectrodes by using cyanobacteria extracts, isolated cyanobacterial protein, and intact cyanobacteria. These approaches are multidisciplinary, extending from the conventional device disciplines, such as condensed matter physics and inorganic chemistry to electrochemistry, to the life sciences including biophysics, bacteriology, and microbiology.

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Brief Overview of Natural and Artificial Photosynthesis

For 100 years, natural photosynthesis has been considered a promising starting blueprint for the development of renewable solar fuels.[1,2] As Rabinovich mentioned over 50 years ago, there would be no reason that man would never discover inexpensive photochemical systems permitting the conversion of 20% solar energy into chemical energy.[3] In 2008, Tributsch pointed to the positive role of energy crises for the development of solar energy technology and economy.[4] In a very recent paper, artificial photosynthesis was identified as a route to meet the objectives of the Strategic Energy Technology-Plan for the European Union, but the need for scientific breakthroughs was emphasized.[5]

In pioneering papers of the late 50s and early 60s, Melvin Calvin[6–8] demonstrated how photosynthetic components from shredded plants such as spinach can be sublimated over interdigital electrodes and provide a bioelectronic interface where the functionalities of proteins can be directly measured with electric signals upon light exposure (Figure 1). Calvin was one of the first who rationalized the photosynthetic apparatus as a molecular machine, or even as a molecular industrial complex. He explains how the components of the cell cooperate as functional units in photosynthesis and they depend on their interconnectivity for electron and ion transport.

The principle of photosynthesis is the hydrolysis (or photolysis) of an electron donor (H_2O in oxygenic photosynthesis) into H^+ and an electron acceptor (O_2 in oxygenic photosynthesis).[9,10] Oxygenic photosynthesis occurs in plants, cyanobacteria, and algae. These organisms contain complexes of proteins, metals, and chromophores that are organized in photosystems (PS) that are integrated in the cell membrane (cyanobacteria) or intracellular membranes (eukaryotes, that is, plants and algae). PS have evolved to use photons to excite an electron of the chromophore chlorophyll and funnel it through a chain of electron-transport proteins to generate chemically bound energy (ATP and NADPH). In oxygenic photosynthesis, the electron hole in chlorophyll is filled by the hydrolysis of H_2O in the oxygen evolving complex. Two PS have been described, PSI and PSII, composed of around 35 and 22 proteins, respectively, and over 110 and roughly 100 cofactors, respectively, such as lipids, chromophores, and metals. So-called light-harvesting complexes (LHC) composed of chromophores and proteins serve to increase the number of absorbed photons.[11] Cyanobacteria and some algae contain specific water-soluble protein–chromophore complexes called phycobilliproteins. These are organized in phycobillisomes associated with the photosynthetic membranes.

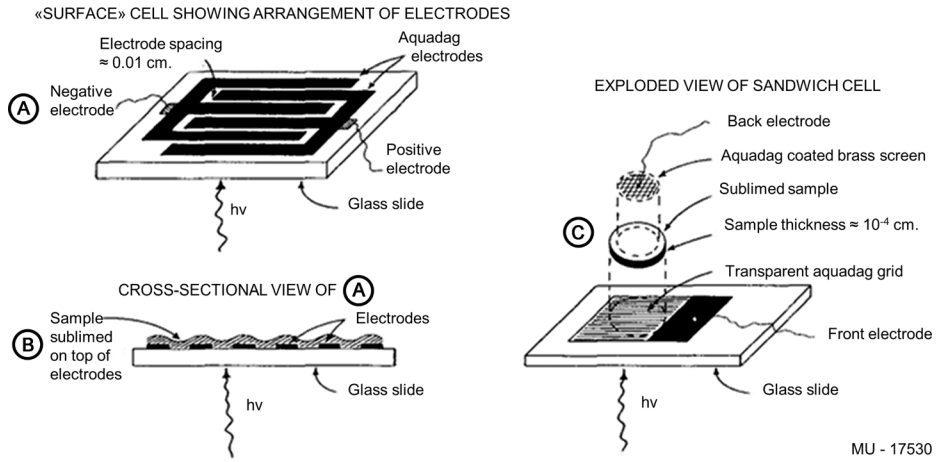

Figure 1. Historical sketch of simple conductivity cells by Calvin et al.[8] A) Electrodes are laterally assembled on a glass slide and are covered by a layer of the pigment phtalocyanin (cross section in B). Light is provided from the bottom through the glass-side. C) Sketch of a stacked bioelectrode assembly. Reproduced with permission from Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley California.

Photoelectrochemical cells (PEC), on the other hand, make use of solar energy for the production of electricity and hydrogen without using biological components. They are based on photoinduced charge separation[12,13] similar to that observed in photosynthesis.[14] Thus, the process is frequently referred to as artificial photosynthesis (AP). In particular, electrolysis of water leading to the production of O_2 and H_2 is induced by photons that excite electrons at the so-called photoanode generating a photocurrent between photoanode and a counter electrode, at which the hydrogen gas can evolve. Improvement of the electrode materials by finding the right absorber properties and electrocatalytic properties has been a major task in materials research for many decades. Progress in nanoparticle synthesis has been the material for some of the recent success in this context.[15–18]

Application of Proteins to Photoelectrodes

To enhance photocurrents, proteins have been investigated for the functionalization of the photoanode, and for the cathode, at which O_2 evolution and H_2 production take place, respectively. Whereas light-harvesting proteins and pigments, such as the ones forming the antenna of photosystem I (PSI) and II (PSII), are generally exploited at the anode, proteins with hydrogen evolving complexes are immobilized at the cathode.

The development of hybrid photoanodes dates back to 1971, when Tributsch and Calvin studied biosemiconductor electrodes for the photoelectrochemical reactions of excited chlorophyll molecules at the surface of a single-crystal wide band gap ZnO semiconductor electrode.[19] They found that the excited chlorophyll molecule injected electrons into the conduction band of ZnO and thus gave rise to the anodic photocurrent. The presence of electron donors such as hydroquinone or phenylhydrazine in the electrolyte helped the chlorophyll molecules in mediating the transfer of electrons from energy levels (reference redox potential) of the reducing agents into the conduction band (reference Fermi energy) of the semiconductor that is the electron acceptor. When an appropriate electrochemical gradient was provided, the electron capture by the semiconductor electrode was irreversible. The excited chlorophyll molecules at the semiconductor electrodes showed similarity in properties to that of chlorophyll molecules in the photosynthetic reaction center such as unidirectional charge transfer or charge separation. However, this system has no galvanic separation. The photogalvanic effect in the organic–inorganic system was first observed by Rabinowitch in 1940 in the thionine–iron system.[20] The photogalvanic effect of chlorophyll molecules was studied by attaching chlorophyll molecules to a metal electrode, such as Pt, in series with a quinhydrone half-cell.[21] Due to the illumination with visible light, the "chlorophyll *a*" aggregate underwent a charge-transfer interaction with the subsequent formation of a *p*-type semiconductor film. This gave rise to a photocurrent with a positive photopotential at the platinum–chlorophyll bioelectrode. A reverse current was observed when the light was turned off. This reversible photogalvanic reaction was only observed when light was incident on the chlorophyll molecule. The effect was absent on the chlorophyll-free side of the electrode. The observed photocurrent at 700 nm wavelength matched well with that of the chlorophyll molecule as evident from its photoaction spectrum.[21]

Recently, Kato and co-workers built a hybrid photoanode for water oxidation from cyanobacterial PSII deposited on a mesoporous indium tin oxide (ITO) electrode.[22] The photocurrent density upon illumination with red light (635 nm) at pH 6.5 at $+0.5$ V vs. NHE (normal hydrogen electrode) bias voltage was $1.6 \pm 0.3\ \mu A\,cm^{-2}$. The charge transfer at the interface occurred by two competing pathways to the metal oxide interface with the application of minimum bias $+0.2$ V vs. NHE during red light illumination. Recently, an "artificial leaf" device based on the co-immobilization of a chlorophyll derivative and a formate dehydrogenase (an enzyme that catalyzes the oxidation of HCO_2^- to CO_2) on silica gel based thin film was fabricated for the reduction of CO_2 to formic acid.[23] These coating strategies are based on the chemical modification of the electrode surface by electrostatic adsorption. We have recently immobilized the β-subunit of C-phycocyanin (CPC, phycobilliprotein from cyanobacteria, consisting of α- and two β-protein subunits carrying one and two chromophores, respectively) on porous iron oxide electrodes.[24,25] The immobilization process required diimidazole, a zero length crosslinking molecule. This was sufficient for the immobilization and covalent attachment of the protein to the hematite. Hematite is a low cost, abundant, and environmental benign semiconductor with band-gap energy of

2.1 eV, which makes it a suitable solar light absorber. Its promising theoretical solar-to-hydrogen efficiency of almost 15%[12] has practically never been realized so far. The CPC antenna protein extends the natural optical absorption range of iron oxide and yields a significant photocurrent enhancement by a factor of three[25] and an increase of the hydrogen production by a factor of six.[26] In this case, the C-phycocyanin film on the electrode had denatured due to exposure to the strongly caustic KOH with pH 13. Still, the chromophores of CPC remained integer and functional in this harsh environment and helped increase the photocurrent density.

Another recent example is based on TiO_2 as photocatalyst combined with a hydrogenase (an enzyme that catalyzes the oxidation of H_2).[27] In this case, [NiFe] hydrogenase was immobilized on TiO_2 nanoparticles, which had previously been functionalized with a ruthenium sensitizer. This system shows good hydrogen evolution in the presence of a sacrificial electron donor. Hydrogenases are among the most well-studied biological catalysts. While hydrogenases are traditionally considered unstable and prone to oxidation and denaturation, their adsorption on electrodes turns out to be a viable route for chemical stabilization.

Different types of hybrid photoanode systems have been developed recently for energy conversion. TiO_2 nanotubes combined with bacteriorhodopsin protein showed enhanced photocurrent efficiency due to the proton pumping character of the protein in continuous light illumination.[28,29] The performance of this biomimetic light-harvesting component is believed to be based on the interaction of dyes and chlorosomes.[30]

A system in which a whole LHC was immobilized on amino terminated ITO showed an enhanced photocurrent at 880 nm which is the absorption maximum of LHC.[31] Reisner et al. developed a hybrid photoanode for water photooxidation made of cyanobacterial PS II deposited on a mesoporous ITO electrode.[22] The photocurrent (1.6 ± 0.3 μA cm^{-2} at $+0.5$ V vs NHE) was recorded by illumination with red light (635 nm) at pH 6.5. The charge transfer at the interface occurred by two competing pathways to the metal oxide interface with the application of minimum bias $+0.2$ V vs. NHE during red light illumination. These strategies are based on the chemical modification of the electrode surface by electrostatic adsorption. To improve attachment of the biomolecules to the electrode, covalent functionalization strategies are preferred over electrostatic adsorption.

In view of these examples, we can anticipate that the efficiency of hybrid photoanodes can be increased further by tuning the right surface functionalization chemistry followed by an increased charge transport across the biomolecule–semiconductor interface.

Concerning the enhancement of H_2 production at photocathodes, a recent technological example is TiO_2 nanotubes that were functionalized with hydrogenase and then used as photocathodes in KOH for solar hydrogen production by water splitting in PEC.[32] This biohybrid cell assembly supposedly produced 140 μmol H_2 cm^{-2} h^{-1} and was reportedly stable for at least three weeks exposure to air. In this case, the hydrogen evolution rate was carefully optimized for a better performance. Depending on the enzyme concentration, the hydrogen evolution rate varies from 140 to 115 μmol H_2 cm^{-2} h^{-1}. They found that a lower hydrogenase concentration yields more hydrogen, possibly due to a more stable contact to the electrode, which slightly deviated from a slurry based system.

Metalloproteins such as hydrogenases are not the only proteins that can be employed in artificial photosynthesis. For example, microperoxidase-11 is a small protein composed of 11 amino acids that carries one heme molecule and iron which act as a molecular catalyst operating under neutral conditions. Substitution of iron with cobalt could make this complex a promising alternative to most oxygen-sensitive hydrogenases.[33,34]

Industrial PEC reactors must be designed to perform over a long time. Covalent immobilization of the biomolecules to the electrodes might thus be a preferred strategy over electrostatic adsorption to overcome the possible instability under the extended working conditions. In an extension of our previous studies on CPC coating of hematite, we explored refined coating technologies against simpler alternatives. In this context, co-polymerization of CPC with melanin, a natural bio-organic, previously classified amorphous Mott–Davis-type semiconductor[35] and after reassessment classified "electronic–ionic hybrid conductor",[36] turns out to be a process strategy for enhancing the photocurrent density. However, melanin dissolves in KOH, which is a preferred electrolyte for hematite photoanodes. Through its pH, KOH produces an additional Nernst voltage that counts towards the necessary DC (direct current) bias, which is necessary in hematite-based PEC systems. However, the CPC + melanin system is efficient in pH 7 phosphate buffer saline (PBS). This is a clear advantage for applications in the built environment where a pH neutral and environmental benign electrolyte is likely more accepted by the public than a strongly caustic solution.

The next approach we investigate is aimed at the direct interaction of light-harvesting proteins with the hematite surface by means of genetic engineering. Several CPC variants have been developed using the bacterial host *E. coli*. The α-subunit of CPC from the cyanobacterium *Synechocystis* PCC6803[37] is recombinantly produced in a form carrying a six-histidine tag (Histag, HisCPC). HisCPC is an interesting alternative to the natural isolate from *Arthrospira* sp. (*Spirulina*) that we used in the initial studies, because the histidine tag binds specifically to cations on a substrate and thus can enhance attachment of the protein on an electrode. In addition, the entire process technologies such as the coating should be low-cost. A clear advantage of using iron oxide as photoanode is the abundance and low-cost of this mineral, despite some of its shortcomings such as short hole diffusion length, for example. Hematite photoanodes can easily be coated and with low-cost processes on large scale panels.[38] Concomitantly, we use algal CPC because it is a low-cost resource directly harvested from algae.

Assessment of Charge Transfer between Proteins and Photoelectrodes

As mentioned above, the efficiency of the charge transfer between proteins and electrode is of crucial importance. An established method for the quantitative assessment of the electrical properties of biophysical systems is electrochemical impedance spectroscopy (EIS).[39,40] Properties and processes in the bulk of the electrode, on the electrode surface, and in the electrolyte (electrochemical double layer) are characterized by frequency dependent (dispersive) resistors, capacitors, and inductivities, which are organized in serial or parallel arrangement, where current and voltages follow Kirchhoff's rules. EIS can be utilized to study the consecutive build up and assembly of protein-based architectures on inorganic supports, such as in biosensors.[41] Figure 2 illustrates a photoanode assembly

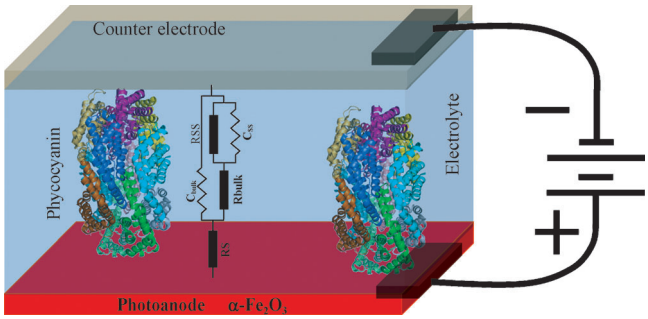

Figure 2. Schematic representation of an iron oxide photoelectrode with attached protein molecules (here CPC, Protein Database ID: 4F0T),[45] along with an electric circuit representing the bioelectrochemical, electrochemical, and solid-state processes taking place during water splitting.

from a solid hematite electrode with attached CPC molecules immersed in an electrolyte, and facing a platinum counter electrode. The water oxidation reaction takes place at the photoanode, at which the oxygen gas evolves: $4h^+ + 2H_2O \rightarrow O_2 + 4H^+$. The necessary electron holes (h^+) are generated as electron hole pairs in the semiconductor photoanode upon light excitation: $h\nu \rightarrow e^- + h^+$, which must then diffuse through the electrode to the electrolyte. At the counter electrode, which can be a photocathode, protons are reduced and form hydrogen gas: $4H^+ + 4e^- \rightarrow 2H_2$. We assume an electrolyte serial resistance R_s, an electrochemical double-layer capacitance C_{EDL}, a charge-transfer resistance R_{CT} in series with Warburg impedance Z_W (this is the parallel circuit of a capacitance and a resistor).

In line with Tributsch' suggestion, the response of a bioelectrochemical cell assembly to an AC perturbation can be mathematically modeled with only a handful of electric components.[42] Caution must be exercised, however, as to which processes take place at the molecular scale. For artificial photosynthesis and in the case of whole-cell utilization, the electronic transport across bilayer lipid membranes is a relevant process[43] that can be studied in detail with EIS. We have to point out, however, that electrochemical and electroanalytic methods can be invasive and even destructive.[44]

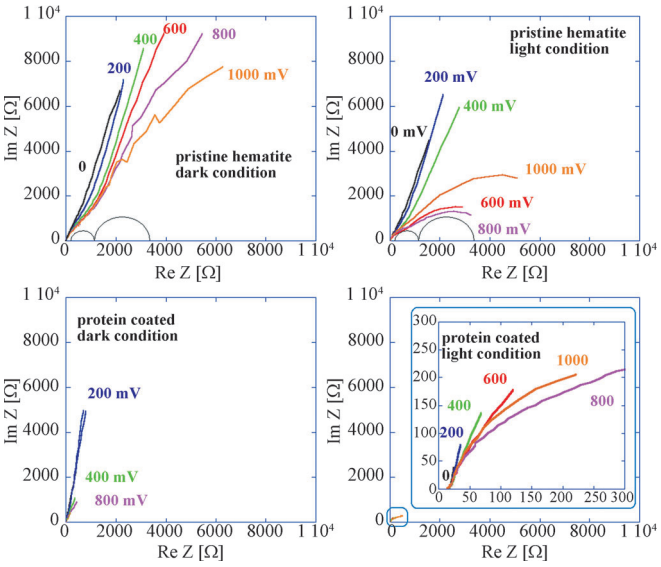

Figure 3. Top: Nyquist plots of impedance spectra of porous pristine hematite film under dark (left) and under illuminated (right) condition with DC bias from 0 to 1000 mV (indicated with bold numerals 0, 200, 400, 600, 800, 1000 mV). The two thin black semicircles with radii of 1000 and 2200 Ω drawn on the Re Z axis indicate the electrode resistance and the charge-transfer resistance, which becomes particularly obvious for 600, 800, and 1000 mV under light condition. Bottom: Nyquist plots of impedance spectra of protein (C-phycocyanin) coated porous hematite films under dark (left) and under illuminated (right) condition, recorded with DC bias ranging from 0 to 1000 mV (bias numbers are shown in bold in the plots). For all plots, the data window corresponds to 10^4 Ω. The inset in the right panel shows a magnification of the impedance range of the protein coated film under illumination (data window size 300 Ω).

The impedance spectra of a pristine porous hematite electrodes are shown in Figure 3 in Nyquist representation for DC bias potentials ranging from 0 to 1000 mV. The impedance is under dark conditions for the low frequencies in the 10 kΩ range. Under illumination (top right panel, Figure 3), the impedance decreases by a factor of five to ten as the bias potential approaches the water-splitting potential, which is around 500 mV vs. the Ag^+/AgCl reference electrode in the KOH electrolyte. At about this bias, the emergence of two semicircles in the spectra is clearly visible.

When the hematite electrode is functionalized with C-phycocyanin (Figure 3, bottom), the electrical impedance of this assembly is considerably smaller than under dark conditions. This holds particularly for the real part. Upon illumination, the protein-coated electrode experiences a decrease of its impedance.

We have modelled the impedance spectra of such a bioelectrode assembly with an electric circuit as shown in the upper part of Figure 4. While this model is likely an oversimplification of the complex reality, the least-square fit to the model fits the experimental spectra well.

The least-square fit of the spectra shows a serial resistance around 14 Ω and around 11.5 Ω for the pristine electrode. The charge-transfer resistance R_{ct} of the pristine electrode decreases from 2377 to 1271 Ω upon illumination, whereas the R_{ct} for the protein-coated film decreases from 4070 to 443 Ω upon illumination. The fit parameters for these two samples are summarized in Table 1.

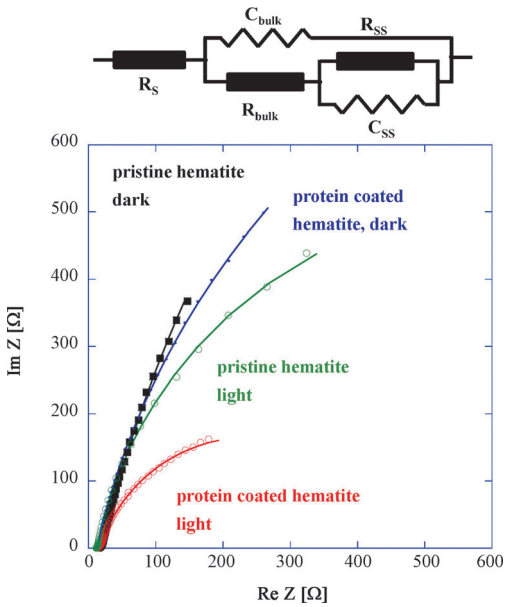

Figure 4. Electric impedance circuit (top) with serial resistance R_S, bulk resistance R_{bulk}, bulk capacitance C_{bulk}, and a parallel circuit from surface state capacitance C_{SS} and resistance R_{SS}, originating from surface states. The capacitances are interpreted as constant phase elements. Nyquist plots of four impedance spectra of porous pristine and protein (C-phycocyanin) coated hematite film under dark and under illuminated (light) condition with DC bias of 1000 mV. The frequency range for the least square fit of the above electric circuit (solid lines) extends from 63291 to 10 Hz.

Table 1. Fit parameters for the impedance spectra of pristine hematite and protein (C-phycocyanin) coated hematite in dark and illuminated condition. The fit model is based on the electric circuit shown in Figure 4.

		R_S [Ω]	R_{tr+} [Ω]	$R_{ct,tr+}$ [Ω]	
protein coated	dark	14.8	7.66	4070	
	light	13.9	5.99	443	
pristine	dark	11.5	9.81	2377	
	light	11.6	10.3	1271	
		CPE1T (±)	CPE1P (+)	CPE2-T(+)	CPE2-P(X)
protein coated	dark	5.0E-05	0.86	2.38E-05	0.832
	light	7.3E-05	0.82	2.98E-05	0.8
pristine	dark	7.1E-06	0.97	4.24E-05	0.815
	light	5.1E-06	1	3.83E-05	0.831

Electron holes are necessary at the electrode surface for water oxidation. They are generated in hematite when under bias and illumination, and they do have actually a split origin. The rational for this suggestion is an observable disagreement[46] between the optical absorption coefficients measured in optical and photoelectrochemical experiments. This had stirred speculation that the latter corresponds to a ligand-to-metal charge transfer $O^{2-} \rightarrow Fe^{3+}$. The former could also originate from a metal-to-metal charge transfer according to a photon-induced disproportionation reaction $2\,Fe^{3+}$ $[h\nu] \rightarrow Fe^{2+} + Fe^{4+}$. Here, Fe^{2+} stands for $Fe^{3+} - e^-$ and Fe^{4+} for $Fe^{3+} - h^+$. This implies that the second hole is linked to an iron site (Fe3d) but with different energy than the hole in the oxygen valence band.[46]

EIS is so far being used for rudimentary assessment of the electronic structure of electrodes. The aforementioned two electron holes h^+ (one O 2p type hole and on Fe 3d type hole), which emerge in iron oxide during PEC water splitting,[46] actually show up in the X-ray absorption spectra of the oxygen K-shell.[47] Moreover, the variation of their spectral weight during change of the DC bias potential is paralleled by so-called surface trap states, which contribute with a capacitive but not directly visible signature in the impedance spectra.[48] EIS spectra recorded at different bias potentials allow for identification of such capacitive surface trap states.

Based on our experience with combining EIS and X-ray spectroscopy in PEC experiments, we were inspired to apply both methods to biohybrid electrodes. We have immobilized the CPC molecule on hematite using three different methods. We used hematite single-crystal substrates to rule out any effects that could result from porosity or roughness. A first hematite film was coated with CPC by adsorption (physisorption), a second one by co-polymerization with tyrosinase-produced melanin, and the third one with the purified HisCPC. Photosynthetic proteins previously used were complex natural isolates. We aimed at assessing the effect of HisCPC from *Synechocystis* PCC8603 single monomeric small (186 aa, 20 kDa) protein with specific absorbance at 626 nm. As described in Tooley et al.[37] HisCPC was produced in *E. coli*, purified and immobilized on the hematite thin film by direct adsorption (calculated isolectric point pI 5.85) in PBS pH 7.4 or in a melanin-assisted manner. HisCPC showed features similar to the ones of the commercial CPC from *Spirulina*, which is also composed of a β-subunit.

To investigate potential changes in the electronic structures depending on the coating methods, valence band X-ray photoelectron spectroscopy (VB-XPS, Figure 5) was applied. Because of the chemical complexity of the interface formed by hematite and protein-film, we chose to excite the samples with monochromatic X-ray photons ranging from 50 to 60 eV. This allows us to be resonant to the Fe3p excitation threshold of the iron in the hematite substrate. This spectroscopic approach requires tunable X-ray photon energy from synchrotron radiation and allows for the enhancement of the valence band spectrum with chemical contrast from the iron.[49] The spectra were recorded at Beamline 8–1 at Stanford Synchrotron Radiation Lightsource (SSRL), Menlo Park, CA under ultra-high vacuum conditions as typical for conventional XPS experiments. A solar simulator was used to shine visible light on the samples while VB-XPS were recorded.

The photon excitation energy was tuned to the Fe3p absorption threshold, which resulted in minor spectral differences in the his-tag CPC system, yet considerable differences in the dark spectra of the PC + melanin system (Figure 5). Also, significant differences were observed for chemisorbed CPC when measured in dark or under illumination. The relative shift of the VB spectrum toward the Fermi energy (binding energy BE = 0) is virtually 0 for the his-tag CPC sample, but increasingly larger for the two other coating strategies.

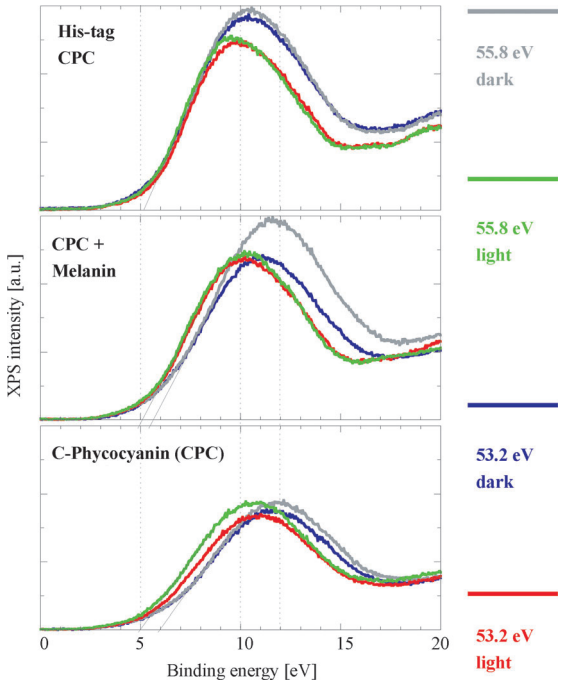

Figure 5. Valence band XPS spectra of three different protein coatings (CPC adsorbed, CPC coated with melanin, CPC with his-tag) on hematite single crystals, recorded with Fe3p resonant synchrotron radiation in UHV. In each dark and illuminated condition, excitation energies of 53.2 eV for Fe3p off-resonance; and 55.8 eV for Fe3p on-resonance, are applied.

The exact interpretation of the resonant VB-XPS spectra would require theoretical considerations with subsequent calculation of spectra. Yet, valid information can be drawn with the analysis of general trends on how the VB behaves, particularly on the edge movements upon light illumination. All tested systems showed a systematic shift to the lower binding side when exposed to light. Our interpretation of this observation is that the density of states (DOS) moves toward the Fermi level, which can be compared to the p-doped semiconductor DOS state. The electron-hole pairs are created by the excitation and generate the hole-doped states on the surface.[50] Since we are dealing here with a biohybrid system, it is important to distinguish between the photoactivity of the electrode and the photoactivity of the light antenna protein, and the charge transport and energy transport among them. There are not many reports in the public literature about biohybrid interfaces that are investigated with XPS or similar spectroscopy methods. To the best of the authors' knowledge, this is the first information where such a biohybrid electrode was tested with a resonant XPS in the dark and in the illuminated state.

Denaturation of Proteins

The limited chemical stability of proteins has led to the common perception that for example hydrogenases are of little practical use for energy conversion technology. However, hydrogenases from phototrophic bacteria can be relatively stable against degradation by denaturation[51] and, more importantly, an increasing number of oxygen-tolerant hydrogenases are being identified.[52] In addition, it appears that proteins are more robust against photodegradation when they are interacting with electrically conducting substrates as charges can be conducted away from the protein. This is not withstanding that during photocurrent measurements at high bias potentials (1.5 V vs. Ag/AgCl), foam is frequently found on the electrolyte surface, and agglomerates are found on the electrode surface. In this case it is important that sufficient electric linkage between protein and electrode is warranted.

An alternative to hydrogenases are oxygen evolving and hydrogen-evolving complexes of, for example, cyanobacteria.[53] These are known to be more stable against denaturation than hydrogenases. As mentioned above, we have functionalized iron oxide photoelectrodes with the light-harvesting antenna protein C-phycocyanin and found that the photocurrent could increase by a factor of two to three due to this processing. The standard electrolyte for photoelectrochemical water splitting on hematite is 1 M KOH, which has a pH of around 13 and thus poses a very harsh environment to proteins. Consequently, we found that C-phycocyanin denatures. However, the chromophores of phycocyanin survive this harsh alkaline environment and harvest light and pass on electric energy to the hematite.[26]

Ultimately, the systemic degradation of organic components produced by cyanobacteria can be countered by employing living cyanobacteria instead of isolated phycocyanin or other biological motifs. The organisms provide an automatic source of phycocyanin by regeneration and self-reproduction. This introduces a number of technical challenges, for example, sufficient charge transport between the bacterial cell and the metal oxide surface. Moreover, the extent of colonization by bacteria needs to be controlled against overpopulation to avoid passivation of the photoelectrode by growing too thick biofilms. Furthermore, bacteria need a specific electrolyte environment, which should be provided by industrial, agricultural, or residential waste water depending on the organism.

Benefits of Combining Living Organisms with Electrodes

As described above, photosynthetic organisms carry a whole range of proteins and pigments with specialized light absorbing properties. Incident light drives the light reaction but also leads to the formation of reactive oxygen species (ROS) that can destroy components of the PS and associated complexes. Apart from various mechanisms to deplete ROS, subunits of the multiprotein complexes PSI and PSII are constantly regenerated over time and the whole complexes are renewed over 30–75 h for PSI and $<$1–11 h for PSII.[54]

The use of whole cells might thus be beneficial for long-term applications. Furthermore, the capability of living organism to reproduce is a premise to employ them in photoelectrochemical devices and thus overcome or counteract the denaturation and degradation of proteins.

As a starting point, we have coated hematite electrodes with fragments from whole dry, and freeze-thawed *Spirulina* (*Arthrospira* sp.) cells and determined the resulting photocur-

rents. The highest photocurrent enhancement of 33% was obtained with cell extracts obtained after a freeze-thaw cycle. This is a promising step for the future use of living photosynthetic cells, in contrast to the dry cells used here, which could assure constant regeneration of the light-harvesting complexes by biomass renewal. The next step is then to investigate biofilms of algal strains on photoelectrodes.

Ultimately, for PEC reactors it is the H_2 production that accounts for its function. When we consider entire cyanobacteria as the bio-organic component on hybrid PEC electrodes, it may be worthwhile to consider their H_2 evolving complex. One interesting avenue of H_2 production by cyanobacteria is via nitrogenase, the protein responsible for converting N_2 into NH_3. It is yet unclear why and how the reduction of N_2 to NH_3 involves the production of H_2 as this makes an energetically expensive metabolic process even more costly.

Therefore, selection of the appropriate N_2 fixing cyanobacteria is crucial as microorganisms that fix N_2 during periods of photosynthetic activity have an unlimited supply of H_2O-derived electrons, rendering the high-energy requirement to break the triple bond of a N_2 molecule redundant; provided they can avoid the toxicity of O_2 molecules to nitrogenase. Consequently a fraction of photosynthetic and respiration derived electrons flow towards nitrogenase and are then redistributed between N_2 reduction leading to N_2 fixation, and proton reduction leading to H_2 production.

The value of considering the cyanobacteria cell as a complete 'metabolic unit' becomes evident when quantifying H_2 yields under increasing light levels. It has been observed that saturation in the rate of N_2 fixation, as measured by ethylene production, at light levels of ~200 μmol photons m^{-2} s^{-1} does not cause corresponding saturation in the rate of H_2 production.[55] The continued increase in net H_2 production under conditions of levelled nitrogenase activity has been attributed to a decrease in the reassimilation of H_2 by uptake hydrogenase, coinciding with an increase in plastoquinone (PQ) reduction (Figure 6). This indicates that net H_2 production is affected by the redox state of the PQ pool, whereby the ability of the PQ pool to accept H_2 derived electrons from uptake hydrogenase decreases as it becomes progressively reduced. The ability to experimentally map this electron flow will lead to a better determination on the potential for nitrogenase and photosynthetic cyanobacteria as a source of H_2 produced by biological systems.

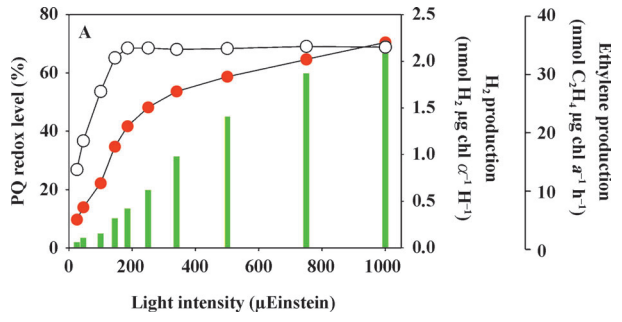

Figure 6. Effect of increasing light intensity on H_2 production (filled symbols), ethylene production (open symbols), and the PQ redox level (bars) in cultures of *Trichodesmium erythraeum* IMS101 (redrawn from ref. [55]).

Electrical Resistance of Hematite is Lowered by Cyanobacteria

As discussed above, pigments and proteins adsorbed or linked to metal oxide surfaces can degrade depending on their specific half-life under the local physicochemical conditions and would have to be replaced to maintain a constant charge transport. Likewise, photosynthetic organisms constantly replace pigments and proteins in their PS that have been inactivated by reactive species such as ROS.

The growth and architectural evolution of such complex biohybrid structures is being investigated in fields that are not related with photoelectrochemistry and AP, but their investigation may turn out valuable for what we propose here, in particular, as the understanding of the covalent attachment and charge-transfer between inorganic framework and living matter is concerned.[56]

Making use of the automatic renewal process in employing living organisms as coating materials instead of isolated proteins or whole-cell extracts would potentially reduce maintenance costs and increase the lifetime of a PEC. Owing to their fast life-cycle and robustness, model planktonic algae are used in applications, for example, for the production of biofuel. Ideally, the algae of choice for use in a PEC would adhere easily to the photoelectrode, have a broad ecological niche regarding water chemistry and incident light intensity, and significantly reduce the electrical resistance to the electrode. The cyanobacterium *Anabaena sp.* has been shown to be capable of several energy-generating growth modes, that is, mixotrophic, photo-organoheterotrophic, and chemo-organoheterotrophic growth.[57] It forms filaments that produce a large surface in contact with the surrounding medium and attaches to surfaces in surface waters where it is found in naturally occurring biofilms. *Anabaena* also fixes nitrogen similar to symbiotic bacteria in legumes used for organic fertilization which may open other additional applications when employed in a PEC-system.

It appears that *Anabaena* and similar organisms could be suitable enhancers for photocurrents in PEC. As a first proof of concept, we established *Anabaena sp.* at two different cell densities on hematite films on FTO and subjected it to EIS under light and dark conditions at bias potentials from 0 to 1400 mV vs. Ag/AgCl. Figure 7 shows how the impedance measured under dark conditions ranges in the 100 kΩ range for potentials up to 800 mV, but experiences a decrease to the 1 kΩ range in the 600 to 800 mV range and above upon illumination.

Upon comparison with the impedance spectra of the pristine (this is noncoated) and CPC-coated film (Figure 8) we find that for 800 and 1000 mV the biofilm PEC assembly has a lower impedance upon illumination than the pristine hematite electrode. This impedance is still larger than that for the CPC-coated electrode.

We have subjected this biofilm-electrode to so-called "ambient pressure XPS" (AP-XPS) spectroscopy (Ambient Pressure

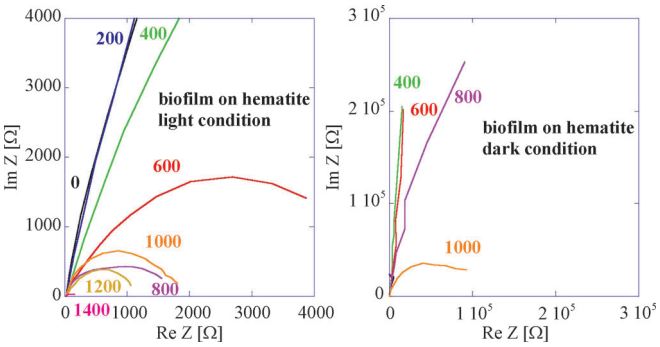

Figure 7. Comparison of impedance spectra of *Anabaena sp.* biofilm grown on hematite under light (left panel) and dark (right panel) conditions in 0.05 molar PBS. Spectra were recorded from 100 kHz to 10 mHz in the dark and 1.5 AM light from 0 to 1400 mV DC bias vs. Ag^{+}/AgCl reference in a so-called cappuccino cell. The bold numerals in the graphs (0, 200, 400, 600, 800, 1000, 1200, and 1400) denote the bias potentials in mV. Note the impedance window is 4000 Ω for the light condition and 3×10^{5} for the dark condition.

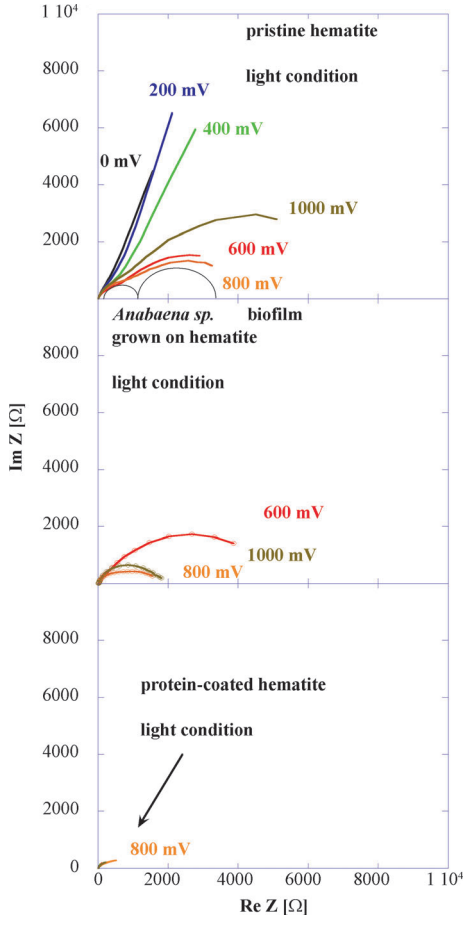

Figure 8. Comparison of impedance spectra recorded in light conditions from pristine hematite (top) and *Anabaena sp*. Biofilm grown on hematite (middle) and protein (CPC) coated hematite (bottom) films. The films were measured in PBS electrolyte. Bold numerals in the panels (0 mV–1000 mV) denote the bias potential. The length of the real axis and imaginary axis is 1×10^{4} Ω for all panels.

XPS Beamline 9.3.2 at the Advanced Lightsource in Berkeley, California[58]), see Figure 9. This means the biofilm sample subject to XPS spectroscopy was under a high water-vapor pressure of 150 mTorr and under electrochemical polarization from 0 to 1500 mV under dark conditions and under illumination.

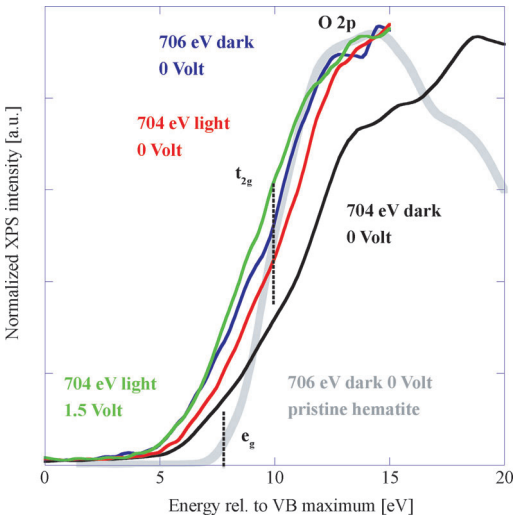

Figure 9. Valence band XPS spectra of the *Anabaena sp.* biofilm grown on hematite. Spectra were recorded with synchrotron photon energies of 704, 706, and 707 eV so as to have Fe2p resonant spectroscopic enhancement by the iron in the hematite. Note that the biofilm was under 150 mTorr water vapor pressure and electrified at 0 and 1500 mV in dark and light conditions. The dashed vertical lines indicate the Fe2p e_g and t_{2g} transitions at 7.6 and 10 eV relative to the valence band (VB) maximum.

Under illumination by visible light, the VB spectrum shifts by 1.8 eV towards the Fermi energy E_F. The difference spectrum at 0 V between light on/off yields intensity ranging from 5 to 15 eV with a maximum at the binding energy of 11 eV. To gain access to direct information under photoelectrochemical and close-to-physiological conditions, ambient pressure XPS (AP-XPS) is necessary. With differentially pumped electron optics system, the information of surface chemical and electronic structures can be retrieved while the sample is exposed to realistic conditions, for example, elevated water vapor pressure and temperature.[58] To the best of our knowledge, this is the first XPS or photoemission study on a biofilm under photoelectrochemical and near-physiological conditions.

We have recorded these spectra in the Fe2p resonant range at 704, 706, and 707 eV at 0 and 1500 mV bias in dark and in light. The widest distance from the Fermi level is under dark conditions at 704 and 707 eV, whereas the spectrum recorded under a resonant energy of 706 eV shows a substantial shift towards E_F. Illumination at 704 eV without bias yields a noticeable shift towards E_F. Illumination at the photon energies 706 and 707 eV yields a larger shift. Applying the 1500 mV bias for example at 704 eV increases the shift towards E_F substantially.

Here it is important to note that XPS studies are generally extremely surface sensitive. Hence, the above spectra show predominantly the biofilm and only little spectral intensity comes from the hematite underneath. This is why we tune the

synchrotron X-ray photon energy to an absorption threshold of the iron, that is, Fe2p resonant around 706 eV. We explained already that this enhances the chemical contrast. Upon comparison with spectra from pristine hematite recorded under identical conditions (p_{H_2O}, T, U, E_{photon}, light on/off), we can discriminate the contributions to some extent.

A problematic case arises when the biofilm thickness increases to an extent that all light is being absorbed by the biofilm, as is shown in Figure 10. There we detected no photocurrent and also no photoresponse upon chopping the light. This shows that biofilm growth for PEC applications needs to be actively controlled and limited.

Figure 10. Photos of two hematite film photoelectrodes coated with a film of *Spirulina* cell extracts of several 100 μm thickness.

Charge Transfer through Algal Cell Walls

Shawanella bacteria have an iron oxide wire that can be used for charge transport to the metal oxide, so this can be considered a macroscopic hem-tag.

The exploitation of whole microbial photosynthetic cells in PEC offers the advantage of a whole array of evolutionary optimized photosynthetic proteins forming complex apparatus, in comparison to studies focusing on the enlargement of the radiation spectrum absorbed by photoelectrochemical devices by using molecules with a narrow absorbance band such as dyes and single photosynthetic proteins.

The observation that bacteria release electrons to the environment and thus produce electricity is a phenomenon known since the early 20th century.[59] Early studies involved *Anabaena* and *Synechocysts* strains in the presence of redox mediators (indirect electron transfer) and showed pronounced power generation especially during the dark phase as electrons are released by oxidation of the carbon accumulated as glycogen during the light phase.[60] Of interest for PEC applications, electrogenic activity with direct electron transfer of pelagic and biofilm-forming cyanobacteria has been proven in the presence of light on carbon painted anodes coated with conductive polypyrrole for one entire month.[61]

Direct transfer of electrons from the bacterial cell to metal surfaces can proceed by direct contact of either the cell membrane or ad hoc produced conductive extensions called nanowires that protrude through the outer bacterial membrane (Figure 11). In this respect, well-studied organisms belong to

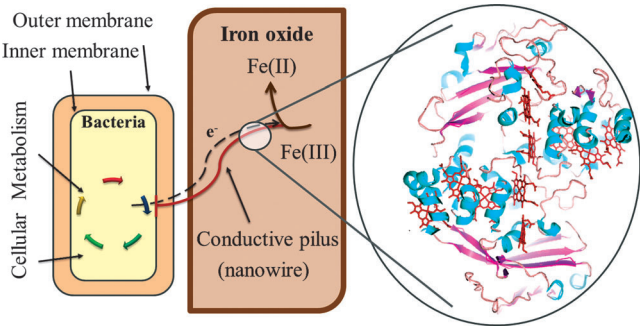

Figure 11. Schematic representation of the dissimilatory reduction of iron oxide by bacteria such as some *Geobacter* and *Shewanella* species that produce conductive nanowires. Electrons produced by the cellular metabolism are vehicled through the outer bacterial membrane along the conductive nanowire that contains multiheme cytochrome-type proteins. As an example, the extracellular 11-heme cytochrome UndA from *Shewanella* sp. HRCR-6 (PDB ID:3UCP) is reported in the inset that shows heme molecules in red, α-helices in blue, and β-sheets in purple.

the genera *Shewanella* and *Geobacter*[62] and electrode substrates can be iron and manganese oxides. In both cases proteins carrying a high number of heme cofactor molecules, for example, even more than 20, are involved. Recent studies suggest that the protein-based composition of the pilus is itself conductive and that the multiheme cytochromes present mediate the electron transfer to the electrode surface.[63]

Some photosynthetic organisms, such as *Synechocystis* PCC6803,[64] have been shown to perform extracellular electron transport through these specialized appendages and these including dissimilatory metal reduction. This way, the specialized bacteria makes metal hydroxides available, as these are otherwise insoluble in water solutions and difficult to absorb by the cell.

Integration of Algal PEC Converters into the Built Environment

The photographs in Figure 12 show details of the BIQ house in Hamburg, Germany, built for the *Internationale Bau Ausstellung* IBA 2013. Engineers and architects have built window-like façades that are filled with algal liquids for biomass. Exposed to the sun, the algae in the window shades will grow and eventually constitute sufficient biomass for a solar-based biomass fuel.[65,66] Compared with the bio-photo-electrochemical approach demonstrated in this concept paper so far, the approach of the BIQ house is quite simple. Yet, it constitutes an important pioneering step forward for applied renewable

Figure 12. BIQ house in Hamburg, Germany, equipped with hollow facades which are filled with algal water (green color) for biomass production. Image source: (top) Strategic Science Consulting GmbH, (bottom) Otto Wulff Bauunternehmung GmbH, Hamburg. For references see [65], [66].

energy supply for solar fuels. We feel that our approach could eventually be integrated in the BIQ house philosophy.

In analogy to the algal facades in the BIQ house, the window panels of the facades could be replaced by photoelectrodes that are functionalized with an algal biofilm, waste water being the electrolyte. The produced hydrogen and oxygen would be guided away safely for storage.

Outlook

The benefit of using living cells in PEC systems is that they principally can regenerate, thus counting against terminal degradation of the system. While we have succeeded so far to covalently bond light-harvesting proteins to the metal oxide substrate electrodes by employing histidine tags, where necessary even with genetic engineering, it remains to be shown that one can do the same with living cells or with bacteria without losing functionality. This should generally be possible because there is existing at least one bacteria or microbe class that has a tag extending from its interior to its exterior, that is, *Shawanella* bacteria that can grow electrically conducting nanowires through their cell membrane.[64]

This functionality should be transferred and combined with light-harvesting systems. In the end, we would have a biofilm growing in a controlled manner on a semiconducting photoanode and/or photocathode substrate and work as a light harvesting and maybe photocatalytic active component, which regenerates self-controlled in the conventional understanding, or controlled by external but invasive factors. Since this film is "wired", we should be able to control this behavior electrically. For the spatial arrangement of the components that constitute this biofilm, self-assembly principles could apply that we may have to steer or influence as well from the outside.[67] This biofilm could be sustained with nutrient-rich waste water, for example. This implies that the pH, for example, should be environmentally benign. It has been shown that some enzymes and proteins survive surprisingly well in the standard 1 M KOH electrolyte, which is, however, strongly alkaline aggressive and not compatible with the just mentioned approach. However, we have recently shown that also a buffer system adjusted to pH 7–8 (phosphate buffer saline, PBS) is a suitable and well-performing electrolyte for protein-based PEC systems, once the phycocyanin is co-polymerized with the semiconducting melanin.

It is the cell bilayer membrane by which living organisms communicate with each other and with their environment. These membranes must be immobilized to substrates and also electrically wired for signal transduction. It remains to be addressed in future studies, which power and energy density can be transferred through their interfaces.

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Keywords: artificial photosynthesis · bioelectric interface · charge transfer · energy transfer · solar hydrogen

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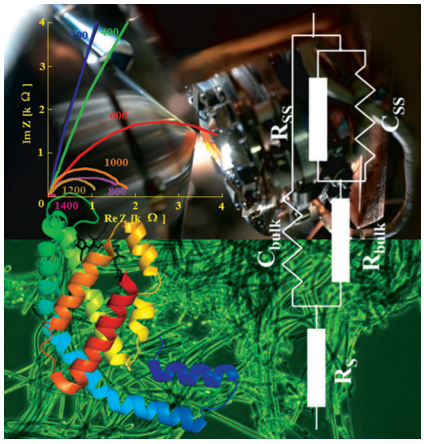
CONCEPT

Artificial photosynthesis is inspired by photosynthesis in nature. Solar hydrogen can be produced by water splitting in photoelectrochemical cells (PEC). The necessary photoelectrodes are inorganic semiconductors. Light-harvesting proteins and biocatalysts can be coupled with these photoelectrodes. We expand this concept toward PEC devices with vital bioorganic components and interfaces, and their integration into the built environment.



Green Chemistry

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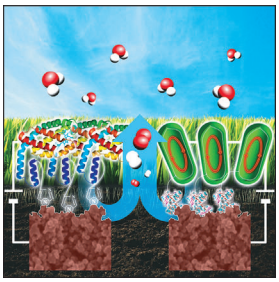
Biological Components and Bioelectronic Interfaces of Water Splitting Photoelectrodes for Solar Hydrogen Production



Let Nature be Your Guide

The energy supply of the developed world depends on fossil fuels, a finite resource, and faces frequently recurring energy crises. Artificial photosynthesis, inspired by nature, is one approach to sustainable energy production. In their Concept article on page ■■ ff., A. Braun et al. expand the concept of water splitting in photoelectrochemical cells with vital bioorganic components and interfaces, and their integration into the built environment.