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Photoelectron Generation by Photosystem II Core Complexes Tethered to Gold Surfaces

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By using a nondestructive, ultrasensitive, fluorescence kinetic technique, we measure in situ the photochemical energy conversion efficiency and electron transfer kinetics on the acceptor side of histidine-tagged photosystem II core complexes tethered to gold surfaces. Atomic force microscopy images coupled with Rutherford backscattering spectroscopy measurements further allow us to assess the quality, number of layers, and surface density of the reaction center films. Based on these measurements, we calculate that the theoretical photoelectronic current density available for an ideal monolayer of core complexes is $43 \mu\text{Acm}^{-2}$ at a photon flux density of

$2000 \mu\text{molquanta m}^{-2}\text{s}^{-1}$ between 365 and 750 nm. While this current density is approximately two orders of magnitude lower than the best organic photovoltaic cells (for an equivalent area), it provides an indication for future improvement strategies. The efficiency could be improved by increasing the optical cross section, by tuning the electron transfer physics between the core complexes and the metal surface, and by developing a multilayer structure, thereby making biomimetic photoelectron devices for hydrogen generation and chemical sensing more viable.

Introduction

In 1912, Giacomo Ciamician was dreaming of “industrial colonies without smoke and without smokestacks” and “forests of glass tubes...and glass buildings...everywhere” inside of which “the photochemical processes that hitherto” had “been the guarded secret of plants” were taking place.^[1] This powerful vision is still a distant future despite a great deal of progress in the understanding of photosynthesis. The possibility to reconstruct molecular components of natural photosynthesis in a photoelectrochemical system is one of the most promising approaches for direct manipulation of basic photochemical nanomachines outside of plants.

In all oxygenic photosynthetic organisms, the reaction centers of photosystems I and II (PSI and PSII RCs) convert photon energy into electrical potentials with extraordinary efficiency ($45 \pm 10\%$ and $80 \pm 15\%$, respectively^[2]) under a wide range of light and temperature conditions. PSII RCs oxidize water to generate oxygen and protons on the luminal side of the thylakoid membrane, while translocating electrons toward the stromal side.^[3] In principle, this reaction can be re-engineered on solid interfaces to convert light into chemical energy in the form of hydrogen or into a direct electron flow^[4] for transmission, storage, or direct usage. Although progress in the fields of genomics, molecular genetics, biochemistry, biophysics, materials science, and engineering makes such devices tantalizingly close, a functional device has remained elusive. Membranes with oriented photosynthetic RCs have been assembled,^[5–7] and attempts have been made to integrate photosynthetic protein complexes into solid-state devices, such as photodetectors and photovoltaic cells.^[8] PSII core complexes have been immobilized onto electrodes for assembling biosen-

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sors^[9–12] and as components of a future biohydrogen production device.^[13] Hydrogen generation through solid-state integration of PSII and PSI RCs with hydrogenases has been suggested, although a full device has yet to be assembled. One of the main bottlenecks in the assembly of such optoelectronic nanodevices is a method to rapidly and nondestructively assess the photochemical properties in situ.

The architecture of PSII from the thermophilic cyanobacterium *Thermosynechococcus elongatus* has been elucidated by high-resolution X-ray crystallography.^[14] In vitro the PSIIs are dimeric units with a physical dimension of 10.5 nm depth, 20.5 nm length, and 11.0 nm width. In each PSII monomer the two proteins D₁ and D₂, which harbor all the cofactors necessary for photochemical charge separation, are flanked by the CP₄₃ and CP₄₇ subunits binding Chl a of the core antenna. Upon illumination, the excited primary electron donor, P₆₈₀, ejects an electron into the final electron acceptor, plastoquinone Q_B, through chlorophyll D₁ (Chl_{D1}), pheophytin D₁ (Pheo_{D1}) and plastoquinone Q_A. While Q_A is fixed within the structure, Q_B in vivo is released into the membrane matrix after accepting two electrons and undergoing protonation. The cationic radical P₆₈₀⁺ is reduced by a neutral tyrosine forming Tyr_z[•], which in turn oxidizes Mn and ultimately H₂O.

Results and Discussion

We tethered PSII core complexes (PSII CCs) from *T. elongatus* to gold surfaces by using Ni²⁺-nitrilotriacetic acid (NiNTA) coordination sites (described in the Supporting Information). The photochemical activity of the PSII CCs was monitored with a highly sensitive fluorescence technique.^[15] In this approach, the rate of rise of fluorescence driven by a short (ca. 100 μs) saturating pulse of actinic light provides information about the effective absorption cross section (σ_{PSII}) of the photochemical reaction, the average quantum efficiency of the process (F_v/F_m), and the kinetics of electron transfer on the acceptor side (τ).^[16] F_m and $F_v = F_m - F_0$ (where F_0 is the minimum fluorescence yield parameter) are the maximum and the variable fluorescence yield parameters, respectively. We applied this technique to investigate heterogeneous samples comprising photosynthetic CCs interfaced with electrodic surfaces.

Based on the kinetic profiles from single-turnover flashes followed by relaxation (Figure 1 and Supporting Information), we calculated a σ_{PSII} from 22 to 34 Å² at 470 nm (Table 1). To calculate the spectrally averaged cross section, we integrated the measured cross section at 470 nm between 365 and 750 nm (Figure 2). The spectrally integrated average cross section is 25 Å².

In PSII CCs immobilized on Au foils, the basic fluorescence kinetics features are preserved. The photochemical energy conversion efficiency of freshly isolated PSII core complexes is 0.70, which lies at the upper limit of values measured in vivo. The value for F_v/F_m of PSII CCs immobilized on Au was ca. 0.53. These measurements clearly confirm that PSII CCs remain photochemically competent after immobilization.^[13] Atomic force microscopy (AFM) and Rutherford backscattering spectroscopy (RBS) measurements indicated that these samples comprise a

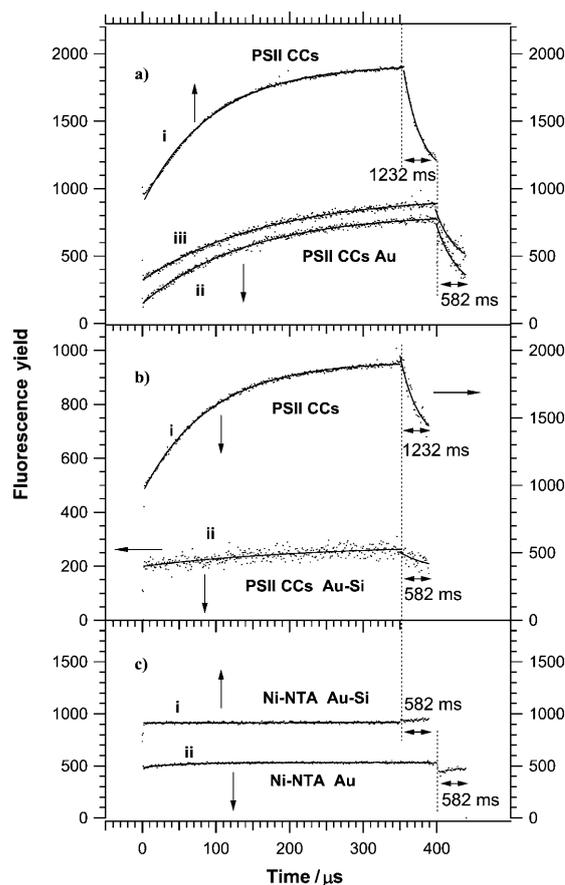


Figure 1. a–c) Results of single-turnover flash (STF) experiments on isolated and tethered PSII core complexes, as well as NiNTA-modified gold surfaces. Fluorescence kinetics versus time of PSII core complexes from *T. elongatus*: a) [trace i] In vitro, STF = 350 μs, τ = 1232 ms; [trace ii] tethered to Au (prepared from original suspension of 0.92 mg Chl mL⁻¹, 50 min incubation time) and washed 5 times with buffer, STF = 400 μs, τ = 582 ms; [trace iii] tethered to Au and washed 5 more times with buffer, STF = 400 μs, τ = 582 ms. b) [trace i] In vitro, STF = 400 μs, τ = 1232 ms; [trace ii] tethered to Au–Si (prepared from original suspension of 0.20 mg Chl mL⁻¹, 50 min incubation time) and washed 5 times with buffer, STF = 400 μs, τ = 582 ms. c) [trace i] Ni²⁺-NTA surface-modified Au–Si, without PSII proteins, STF = 400 μs, τ = 582 ms; [trace ii] Ni²⁺-NTA surface-modified Au, without PSII proteins, STF = 400 μs, τ = 582 ms. All measurements are the result of 64 iterations with the exception of (b), trace ii, which is the result of only 1 iteration.

few layers of PSII complexes (Figure 3a). Assuming that the molecular mass of the PSII core complexes is between 680 to 500 kDa and that one layer is defined as an ideal monolayer of PSII complexes (20.5 × 11.0 nm²),^[14] we measured 1.6–2.5 (±0.4) layers and a numerical surface density of 1.19–1.84 pmol cm⁻² based on RBS in a glancing angle configuration.

To optimize the possibility of direct electron transfer from the PSII to the electrode, we produced a structure closer to a protein monolayer by adjusting the concentration of the PSII suspension^[7,11] using gold-coated silicon as a substrate (Figure 1c). For comparison, a 0.10 mg Chl mL⁻² suspension led to a monolayer dotted by second-layer features (Figure 2b). The number of layers of this latter sample obtained by RBS was 1.3–2.0 (±0.4) and the numerical surface density was 0.93–1.45 pmol cm⁻², depending on the assumed molecular mass.

Table 1. Fluorescence parameters of PSII from *T. elongatus* cells, in vivo, in vitro, and on gold surfaces.

Fluorescence profile (see Figure 1)	Description	F_0	F_m	F_v/F_m	σ_{PSII} [\AA^2]	$I^{[a]}$ [$\text{q \AA}^{-2} \text{s}^{-1}$]	$\tau_i^{[b]}$ [ms]	α_i
Spectrum not shown	<i>T. elongatus</i> (exponential growth, light-acclimated)	867.2	1498	0.42	22.7	344 ^[c]	$\tau_{\text{avg}} = 4.10$ or $\tau_1 = 0.958$, $\tau_2 = 9.05$, $\tau_3 = 109$	$\alpha = 1$ or $\alpha_1 = 0.562$, $\alpha_2 = 0.331$, $\alpha_3 = 0.106$
Spectrum not shown	<i>T. elongatus</i> (stationary, light-acclimated)	851.4	1427	0.40	33.8	344 ^[c]	$\tau_{\text{avg}} = 5.88$ or $\tau_1 = 0.878$, $\tau_2 = 13.8$, $\tau_3 = 498$	$\alpha = 1$ or $\alpha_1 = 0.486$, $\alpha_2 = 0.292$, $\alpha_3 = 0.221$
(a), [i]	Isolated PSII RCs	438.4	1469.5	0.70	30.9	344 ^[c]	$\tau_{\text{avg}} = 34.8$ or $\tau_1 = 1.67$, $\tau_2 = 18.9$, $\tau_3 = 286$	$\alpha = 1$ or $\alpha_1 = 0.200$, $\alpha_2 = 0.481$, $\alpha_3 = 0.319$
(a), [ii]	PSII RCs on Au	598.0	1265	0.53	30.9 ^[d]	230 ^[e]	$\tau_{\text{avg}} = 56.5$	$\alpha = 1$
(a), [iii]	PSII RCs s on Au	670.9	1301	0.48	30.9 ^[d]	191 ^[e]	$\tau_{\text{avg}} = 24.6$	$\alpha = 1$
(b), [i]	Isolated PSII RCs	945.3	1919	0.51	31.9	344 ^[c]	$\tau_{\text{avg}} = 26.1$ or $\tau_1 = 2.00$, $\tau_2 = 25.3$, $\tau_3 = 878$	$\alpha = 1$ or $\alpha_1 = 0.215$, $\alpha_2 = 0.390$, $\alpha_3 = 0.395$
(b), [ii]	PSII RCs on Au–Si	199.6	281.6	0.29	31.9 ^[d]	132 ^[f]	$\tau_{\text{avg}} = 90.0$	$\alpha = 1$
(c), [i]	NiNTA Au–Si	411.9	418.9	0.017	N/A	132 ^[f]	N/A	N/A
(c), [ii]	NiNTA Au	483.5	531.3	0.090	N/A	132 ^[f]	N/A	N/A

[a] Irradiance considering quanta (q) at 470 nm. [b] Time constant τ_i ($i = 1, 2, 3$) or average time constant of the fluorescence relaxation after the STF fluorescence induction. [c] Measured in a quartz cuvette. [d] It is assumed that σ_{PSII} is conserved in going from isolated PSII CCs to immobilized PSII CCs. [e] Measured in a Teflon cavity; the irradiance values are slightly different because of slightly different positions of the samples in the cavity. [f] Measured in a Delrin cavity.

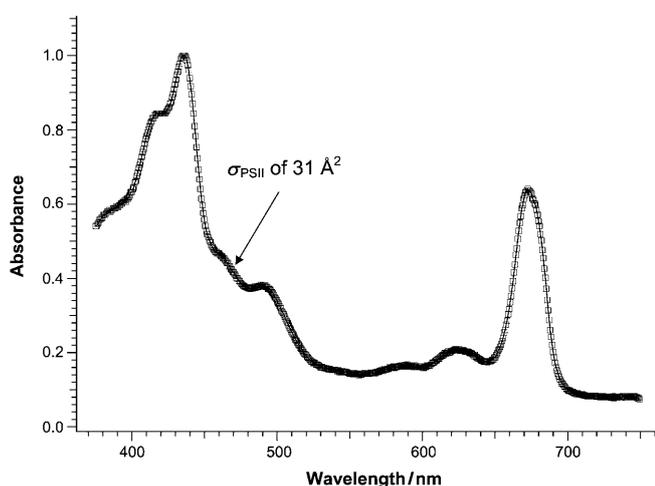


Figure 2. Background-corrected and normalized optical absorption spectrum of PSII core complexes in vitro measured in washing buffer (see Supporting Information).

The results clearly reveal that the immobilized PSII CCs are photochemically active, albeit with a lower photochemical energy conversion efficiency of 0.29.

The kinetics of electron transfer on the acceptor side of PSII in vivo, in vitro, and on gold-coated surfaces were derived from analyses of fluorescence relaxation kinetics.^[17] A comparison of the values reported in Table 1 indicates that the fastest average decay (4–6 ms) occurs in vivo. A significantly slower decay (25–35 ms) was measured for isolated PSII core complexes and for thicker PSII dimer layers on Au (25–57 ms), reflecting over-reduction of the quinone pool on the acceptor side. Spectral resolution allowed us to obtain reliable values for all three time constants τ_i only in the case of PSII in vivo and in vitro. The fluorescence kinetics clearly reveals that the electron transfer mechanism on the acceptor side of PSII is the same before and after isolation. However, a very slow average

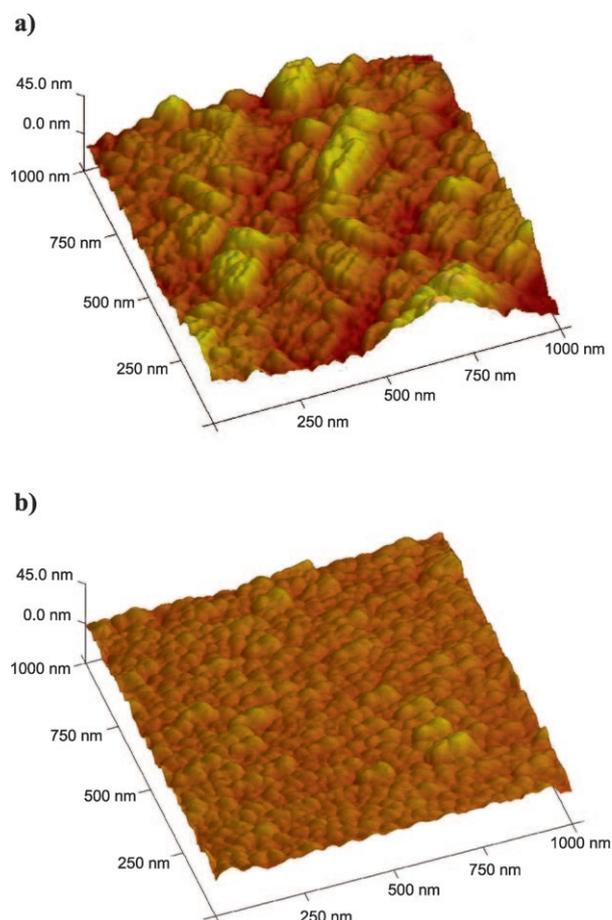


Figure 3. Three-dimensional perspectives of AFM height images of a) PSII dimers on Au–mica obtained from a PSII suspension having a concentration of 0.92 mg Chl mL⁻¹ (50 min of incubation time), and b) PSII dimers on Au–Si substrate obtained from a PSII suspension having a concentration of 0.10 mg Chl mL⁻¹ (50 min of incubation time). Especially in Figure 3a, granular features can be recognized, which correspond to the PSII dimers (with physical dimensions of 10.5 nm depth, 20.5 nm length, and 11.0 nm width) oriented on the surface. A few layers of PSII dimers can be seen in Figure 3a and a monolayer dotted by second-layer features in Figure 3b.

decay (90 ms) was measured for PSII dimers on Au–Si. These results clearly suggest that the oxidation of secondary quinones is the rate-limiting step in electron generation from the core complexes tethered to gold.

The possibility of successful integration of PSII core complexes in solid-state devices requires an assessment of their long-term stability at ambient temperature. A stability study (Supporting Information) indicated that the quantum yield of photochemistry, F_v/F_m , of isolated PSII core complexes rapidly decreases to ca. 0.1 in less than 1 h with zero-order kinetics. Based on the results we calculate that ca. 1300 photochemical turnovers were required for half deactivation of PSII core complexes. The estimated number of turnovers is based on the assumption that within one single turnover flash only one exciton is captured per RC, thus producing one electron. This value contrasts with ca. 10^4 turnovers required to damage the D₁ protein in intact cells.^[18] Furthermore, the cross section was substantially unaltered throughout the stability study, suggesting that the coarse structural integrity of the PSII core complexes is not compromised in spite of their functional deactivation. We propose that the rapid decay of photosynthetic activity of PSII core complexes is caused by inefficient electron transfer from Q_A to the residual Q_B owing to the slow oxidation of the mobile quinone. This rate-limiting step leads to the generation of reactive oxygen species (ROS).^[19]

Conclusions

Based on our biophysical measurements, we calculate a theoretical current density generated by a monolayer of PSII dimers to the gold junction of $43 \mu\text{A cm}^{-2}$ at an hypothetical photon flux density of $2000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ equally distributed between 365 and 750 nm. Knowledge of this crucial parameter will determine future directions in this field. This value is approximately two orders of magnitude smaller than the best experimental short-circuit current densities of organic photovoltaic cells under standard white-light irradiance (9.39 – 16.2 mA cm^{-2}).^[20,21] This comparison reveals that successful strategies for improving the current density will be to achieve a higher optical cross section, much higher rates of electron transfer, or to mediate electron transfer to gold from multiple layered samples.^[22] Indeed, our results strongly suggest that when PSII core complexes are tethered to gold surfaces, the rate limiting reaction for the photochemical production of electrons is the oxidation on the acceptor side of the reaction centers.

Experimental Section

Purification of PSII core complexes: His-tagged photosystem II core complexes (PSII CCs) were isolated from the thermophilic cyanobacterium *T. elongatus* mutant strain WT* (His-tagged on the C-terminus at CP43 with *psbA*₁ and *psbA*₂ genes deleted^[23]). Because only the *psbA*₃ gene was expressed in this mutant, the core complex of the D1 reaction centre protein is homogeneous. The optical absorption spectrum of PSII core complexes in vitro (ca.

$10^{-2} \text{ mg Chl mL}^{-1}$) was measured with a SIM AMINCO DW-2000 UV/Vis spectrophotometer.

Directional immobilization of PSII CCs: The immobilization of PSII core complexes on the stromal side onto gold substrates was carried out by following the immobilization procedure proposed by Badura et al.^[13]

Fluorescence induction and relaxation (FIRe) measurements: A custom-built FIRe system^[15] was used in two configurations: (1) samples were placed in a quartz cuvette for measuring *T. elongatus* cells and isolated PSII CCs in buffer solution, and (2) monolayers of PSII CCs on gold and gold-coated substrates were measured by using a dual-fiber optic probe carrying the excitation to and the induced signals from the sample.

T. elongatus cells used in FIRe measurement: FIRe measurements on whole cells of *T. elongatus* were conducted on a novel His-tagged CP43 mutant strain,^[24] similar to WT* and equivalent to that described in Refs. [25] and [26].

AFM and RBS measurements: AFM images were obtained using a Digital Instruments Multimode AFM with a Nanoscope IV controller operating in “tapping mode.” RBS studies were performed using He²⁺ ions from a standard 1.7 MV tandem accelerator. The sample was placed on a two-axis goniometer oriented near-normal to the incident beam and the RBS detectors were positioned at 154° (back-scattering angle, BA) and 98° (glancing angle, GA) scattering geometries. The RBS spectra were analyzed using the SIMNRA simulation program^[27] with typical Rutherford cross-sections.

Further experimental details are available online as Supporting Information.

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