DOI: 10.1002/adma.200701474

Fabrication of Oriented Multilayers of Photosystem I Proteins on Solid Surfaces by Auto-Metallization **

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Fabrication of serially-oriented multilayers of photosynthetic reaction center photosystem I (PS I) was mediated by the photo-catalytic specificity that reduced Pt^{4+} ions to metal patches on the reducing side of PSI forming junctions with the oxidizing end of the proteins through Pt-sulfide bond of genetically-engineered cysteine mutants. The dry multilayers can be utilized in hybrid bio-solid-state electronic devices in which an increase in photo-voltage, resulting from the larger absorption cross-section and the serial-arrangement of PS I, is required. PS I is a transmembrane multisubunit protein-chlorophyll complex that mediates vectorial light-induced electron transfer. The nano-size dimension, an absorbed light energy yield of approximately 47% (or ca. 23% of solar radiation) and a photovoltage of 1 V with quantum efficiency of almost 1[1], make the reaction center a promising unit for applications in molecular nano-electronics. The robust PS I used in these experiments, that was isolated from the thylakoid membranes of cyanobacteria, is sufficiently stable to be used in hybrid solid-state electronic device. The dry PS I monolayer was shown earlier[3] to remain stable for more than three months and it stayed active for over one year in the present experiments. The structural stability is due to hydrophobic interactions that integrates 96 chlorophyll and 22 carotenoid pigment molecules and the trans membrane helixes of the core subunits.[2] The light-induced electron transfer at cryogenic temperatures[3] is an indication of little structural motions during function. We have fabricated self-assembled oriented monolayers by the formation of direct sulfide bonds between unique cysteine mutants of PS I from the cyanobacteria and the metal surface which generated, a photovoltage of 0.45 V under a dry environment.[4] In earlier works, only indirect adsorption of single plant PS I molecules[5] and binding of bacterial reaction center monolayers[6] were functioning in such an environment. Although a Schottky junction with PS I monolayer provides electronic coupling with unique photovoltaic properties, oriented multilayers can be advantageous when a larger light absorption cross section and enhanced photovoltage values are desired. As an efficient oriented multilayer, the PS I complexes need to be physically and electronically coupled and organized in a serial fashion. The use of the unique specificity of a photo-catalytic protein with redox potential of –0.53 V enabled the reduction of Pt^{4+} ions and deposition of metallic platinum at the reducing end of PS I (Fig. 1a and b). The met-

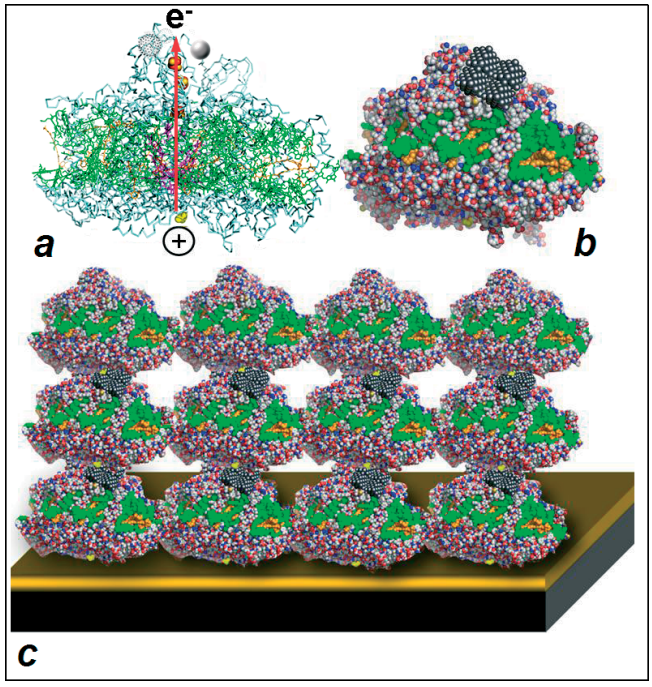

Figure 1. Simulation of the molecular structure of platinized PS I and their multilayer coverage of a gold surface. (a) Light-induced charge separation (arrow) across the electron transport chain (rods, purple and space fill) showing chlorophyll and carotenoid molecules (rods, green and orange) in PS I modelled as polypeptide back-boned structure (cyano) with cysteine mutants Y635C shown in space fill, yellow. Pt ion (dots) bound to PS I is reduced to Pt (space fill, dark gray) by electrons from the terminal iron sulphur cluster (space fill). The electron transport chain in PS I contains a special pair of chlorophyll a (P700) that transfers electrons following photo excitation in 1 picoseconds (ps) to a monomeric chlorophyll a (Chl), through two intermediate phylloquinones (PQ) to the final acceptors: three [4Fe-4S] iron sulfur centers (FeS) that are reduced in 0.2 μs.[7] The redox potential of the primary donor P700 is +0.49 V and that of the final acceptor FeS is –0.53 V. (b) A schematic presentation of Pt crystal deposited on a PS I molecule (space fill model). (c) A schematic presentation of a PS I multilayers (space fill model) on gold surface. The real multilayer structure might have some symmetrical distortion. Atom color codes are: C gray, O red, N blue and S yellow and Pt dark gray. The images were modelled by PyMole software from the coordinates in PDB 1JB0 file.

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[**] Thanks are due to L. Frolov and O. Wilner for equal contribution to this work and to the Institute of Nano Science and Nanotechnology of Tel Aviv University for use of facilities. Supporting Information is available online from Wiley InterScience or from the author.

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al junction facilitated sequential binding of the PS I oxidizing end at the preceding platinized reducing end, yielding an electronically coupled multilayer (Fig.1c).

Initially, an oriented monolayer was fabricated using cysteine mutant Y635C in subunit PsaB of PS I from the cyanobacteria *Synechosystis* sp. PCC 6803.[4,8] The mutated amino acid is located near P700 in the external membrane loops and does not have stereo hindrance when placed on a solid surface, assuring the formation of sulfide bonds and close electronic junction (Fig. 1a). The photochemical properties of the isolated unique PS I mutant Y635C[4] were similar to that of the native complex.[9] The fabrication of oriented monolayers was carried out by directly reacting the cysteine in the mutant PS I with a 150 nm thick gold surface on a silicon slide to form an Au-sulfide bond. Excess protein was washed and the monolayer was dried under nitrogen. AFM images clearly show a dense monolayer of 15–21 nm particles (Fig. 2a) as expected from the size of PS I as obtained by crystallography.

Platinum was deposited on the protein by photo-reducing Pt^{4+} ions in solution by the vectorially oriented PS I layers, according to the reaction: $[PtCl_6]^{2-} + 4e + h\nu = Pt\downarrow + 6Cl^-$. The source of electrons for reduction of $[PtCl_6]^{2-}$ were the electrons from the light-activated PS I, that was continuously re-reduced by ascorbate dichlorophynol indophenol in the solution. Ascorbate did not reduce or photo-reduce Pt^{4+} ions in solution in the absence of PS I monolayer. AFM images of the monolayer show that the size of the PS I slightly increased because of platinization. The phase image, however, clearly demonstrated the presence of metal deposited on top of each PS I. The phase angle of PS I (Fig. 2c) increased on top of the particles following deposition of platinum (Fig. 2d); while being lower at the bottom of each platinized PS I as a result of the lower stiffness of the protein. It is possible that the flat tops of the images of the platinized PS I were due to the formation of crystalline-like platinum patches on the top of PS I (Fig. 2d, zoom). A simulation of deposited platinum crystals of about 2 nm at the reducing end of PS I and of the assembled multilayer is shown (Fig. 1b and c).

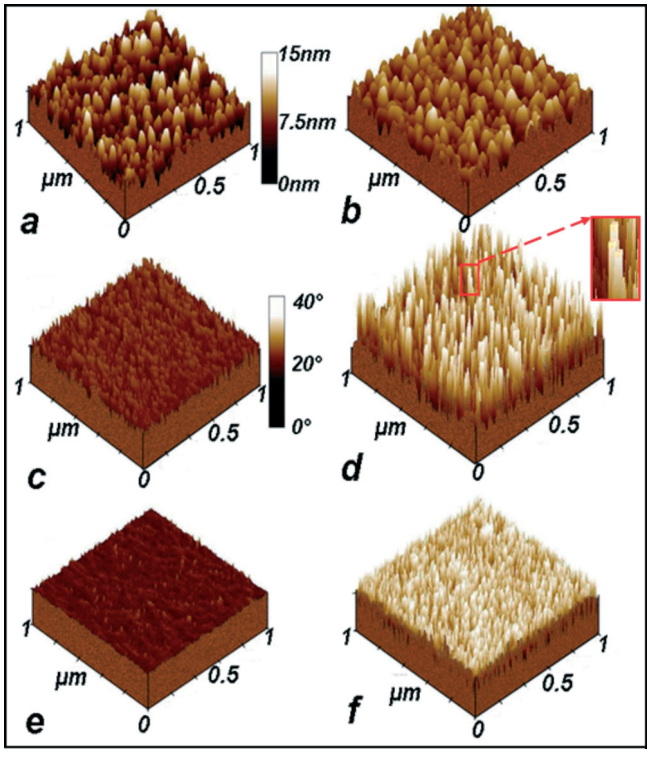

Figure. 2. Scanning probe microscopy images of platinized PS I monolayer. Topographic 3D images of PS I (a) and of the platinized PS I (b) monolayers obtained by AFM. Phase contrast 3D images of PS I (c) and platinized PS I (d), proteinase K digested PS I (e) and platinized PS I (f) monolayers. In the phase contrast measurements, features of the protein can be seen under the metal surfaces of the platinized PS I.

The first row of oriented monolayer of PS I is shown to be attached to the solid gold surface by formation of a sulfide bond between the unique cysteine at the oxidizing end of PS I. The photo-reduction of Pt^{4+} ions which resulted in the deposition of Pt patches at the reducing end of each PS I molecule, is used to attach the next monolayer of PS I through the formation of sulfide bonds. Digestion of the protein in the monolayer with proteinase K in solution after Pt deposition resulted in a decrease in the size of the particles in the monolayers, as would be expected. However, particles with high phase angle remained attached to the gold surface following the digestion of the protein and intensive washing with water of the platinized PS I monolayer (Fig. 2f). This procedure can be utilized for modification of metal electrode surfaces by a monolayer of platinum nano particles. The present demonstration is in harmony with earlier work that suggested the deposition of Pt on PS I during photo-reduction of Pt^{4+} ions. This suggestion was based on the appearance of metal aggregates on thylakoide membranes and the production of H_2 following the photo-reduction of Pt^{4+} ions by thylakoids and by isolated PS I in solution,[10] and the deposition of ca. 50 nm particles on a solid surface following acetone extraction of layered thylakoids that were pre-illuminated in a solution containing a mixture of Pt and Ru ions.[11] X-ray photoelectron spectroscopy (XPS) analysis of monolayers indicated, in the present work, the deposition of 427 Pt atoms per PS I in the platinized monolayer. The calculation is based on the finding of a ratio 0.9/5.87 Pt/N assuming 2786 N atoms per PS I. In order to estimate the size of the patch, we calculated that a crystal of ca. 2 nm can be formed this a number of Pt atoms (Fig. 1b).[12] No Pt atoms were detected in unplatinized PS I monolayers. The results of the analysis concur with the imaging of Pt patches on the PS I in the platinized monolayers.

The platinized monolayer was washed and incubated again in a solution of cysteine mutants of PS I for binding of a second layer by a formation of sulfide bond between the oxidizing end of the proteins and platinum patches on top of the PS I complexes (the reducing side). This process was repeated several times. The multilayers were successfully fabricated many times. The formation of new layers of PS I and their platinization were monitored by observation of changes in the phase angles. The electric properties of the surface of PS I

monolayer were expected to be modified following deposition of metal on the surface. We therefore measured the contact potential difference (CPD) of the metalized PS I monolayer by Kelvin probe force microscopy (KPFM). Current can not be measured by this method, because the AFM probe is raised ca. 20 nm above the sample surface for CPD determination. The CPD and the photovoltage were determined by a novel KPFM system that uses a 1300 nm wavelength feedback laser not absorbed by PS I.[14] Self-assembly of PS I monolayer caused a decrease of ca. 0.7 V in the CPD of the gold surface. Such a change is a result of formation of a Schottky junction between the gold and PS I on binding of the photosystem to the metal surface. Oxidation of P700 by the metal, which has a 0.52 eV higher work function, resulted in the positively-charged PS I expressed as a decrease in the CPD (Table 1). However, deposition of Pt caused a large increase of 0.231 *V* in the CPD. The increase in CPD of the platinized surface is due to a Pt work function of –5.6 eV[15], which affected surface potential.

Table 1. Contact potential difference (CPD) of PS I layers on gold slide.

Samplelayers	[a]CPD (dark)	CPD (light) [V]	Photovoltage
Au	$0.510 \pm 1.4^{e-3}$	$0.527 \pm 1.5^{e-3}$	$0.017 \pm 2.0^{e-4}$
Au-PSI	$-0.191 \pm 5.2^{e-4}$	$0.061 \pm 3.1^{e-4}$	$0.252 \pm 9.0^{e-4}$
Au-PSI-Pt	$0.040 \pm 2.5^{e-4}$	$0.105 \pm 4.5^{e-4}$	$0.065 \pm 3.0^{e-4}$
Au-PSI-Pt-PSI	$-0.177 \pm 5.1^{e-4}$	$0.153 \pm 5.0^{e-4}$	$0.330 \pm 1.3^{e-3}$
Au-PSI-Pt-PSI-Pt	$0.043 \pm 2.5^{e-4}$	$0.123 \pm 4.7^{e-4}$	$0.079 \pm 3.1^{e-4}$
Au-PSI-Pt-PSI-Pt-PSI	$-0.155 \pm 5.1^{e-4}$	$0.231 \pm 8.5^{e-4}$	$0.386 \pm 1.4^{e-3}$

[a] The CPD of PS I and platinized PS I reaction center mono- and multi-layers surfaces were measured by KPFM[13]. The structure and the composition of the mono- and multi-layers and the surfaces of gold, PS I and platinized PS I are indicated as Au, -PS I and -Pt, respectively. The measurements were carried out either in dark or in light. Photovoltage was determined from the difference between the CPD in the dark and the light. The illumination was provided by a diode laser with output power of 40 mW at 670 nm. Each value is an average of 6 samples of 512 × 512 line scans of the various surfaces. All the CPD measurements were calibrated against highly-oriented, freshly-cleaved pyrolytic graphite that gave a CPD of 0.04 V.

The observed increase in CPD is a clear indication of the deposition of Pt on top of the PS I and is in agreement with the observed increase in the phase angle of platinized PS I. This result is also in harmony with earlier measurements done by scanning tunneling microscopy,[16] that indicated a change in the voltage current pattern of platinizated single PS I molecules. Sequential assembly of PS I monolayer and platinization decreased and increased the CPD at approximately similar magnitudes, as was observed in the first monolayer (Table 1). These sequential changes of about 0.225 V in the CPD are independent indicative of the formation of the multilayers Illumination of PS I monolayer caused an increase of 0.252 V in the CPD due to a light-induced charge separation that drives electron transfer across the reaction center, and resulted in the appearance of a negative charge at the reducing end of the protein away from the gold surface. This value is smaller by ca. 0.7 V than the expected 1.0 V difference in the energies of the primary donor P700 and the final acceptor FeS. The difference can be partially explained by a loss caused by a Schottky barrier of 0.5 eV formed between the gold and P700. The energy levels were calculated by conversion of the redox potentials at pH 7[1] to NHE values by addition of 0.41 V. The solid state energy levels were related to the NHE redox levels.[17] The solid state energy levels were –5.1 and –5.6 eV[15] for gold and platinum Fermi-level, respectively. The energy levels in PS I were: –4.58, –2.78, –3.06, –3.52 eV for the primary electron donor (P700), excited P700*, the primary and the final electron acceptors (FeS cluster), respectively. The photovoltage of the platinized monolayer was only 0.065 V, due to charge screening[18] by the coating platinum layer. A Schottky barrier of 1 eV between Pt and P700 connecting the second and the third layer caused an increase in the photopotential that was smaller than the expected additive photopotential in a serial arrangement. The decay of the photopotential was faster than the shutter-off time (0.7ms) in all the layers (Fig. 3b), and within the decay time of light induced charge separation in PS I in solution.

Limited access of the incident light to the surface of the sample in the KPFM instrument prevented the generation of maximal photopotential. Indeed, an increase in the photovoltage as a function of light intensity was linear but did not reach saturation in the mono- and multi-layers (Fig. 3c). However, there was an increase of up to 2.4 fold in photopotential of the multilayers, due to both an increase in the absorption

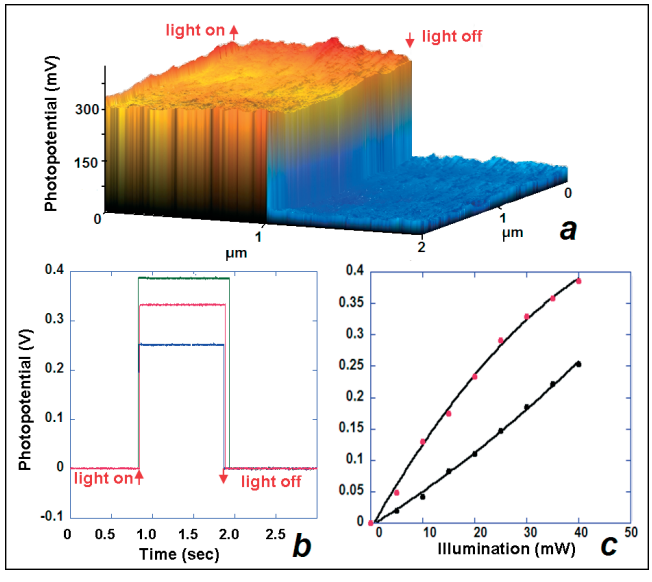

Figure. 3. Kelvin probe microscopy images of PS I mono- and bi- and tri-layers. (a) Light-induced surface potential differences of 3D images PS I bi-layer. (b) Kinetic recording of reversible light induced (shutter off time 0.7 ms) photo-potential of mono- (blue), bi- (red) and tri-layers (green) of PS I are shown. (c) Light intensity dependence of the photo-potential of PS I mono- (black) and tri-layers (red). Surface potential measurements were done by KPFM. Illumination was provided by a diode laser with maximum power output of 40 mW at 670 nm.

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cross-section and to the electronic coupling between the serially-arranged PS I complexes. Electronic coupling between the gold electrode and the multilayers is also indicated by the light-induced photocurrent of 0.12 mA cm^{-2} as measured by cyclic voltammetry. The almost molecular recognition used for fabrication of multilayers in this work, seems to be more efficient than the approach used for the generation of enhanced photovoltage produced by the stacking of hundreds of layers of loosely-oriented bacteriorhodopsin membrane patches.[19,20] Each of the bacteriorhodopsin proteins contains only a single chromophore, and a monolayer generates ca. 40 mV when excited by photons. In PS I, 120 pigment molecules harvest photon energy that is transferred in femtoseconds to a common reaction center, where a photovoltage of 1 V is generated with quantum efficiency of 1. The superior photo-electronic properties can yield an almost total absorption of visible light by the multilayers and a generation of photovoltage to be utilized in the fabrication of hybrid devices.

Photosynthetic reaction center proteins can potentially be utilized in hybrid bio-solid-state electronic devices as single molecules and as oriented monolayers. Here we reported on the fabrication of serially-oriented multilayers mediated by the deposition of platinum on top of PS I layers, and sequential binding of layers through the formation of sulfide bonds between genetically-engineered unique cysteine mutants of the protein and the metal. The photo-catalytic specificity of PS I mediates the reduction of Pt^{4+} ions, which are deposited as metallic platinum patches of ca. 2 nm at the reducing end of the protein to form junctions with the oxidizing side of the next layer. The photovoltaic function and electronic coupling of the dry multilayers were monitored by Kelvin probe force microscopy and by cyclic voltammetry. The larger absorption cross-section and the serial arrangement of PS I resulted in an increase in the photovoltage generated by the multilayer.

Experimental

For site-directed mutagenesis in the *psaB* gene from Synechocystis sp. PCC 6803 was induced by homologous recombination using plasmids pZBL for induction of cysteine Y634C mutations and pBLΔB for *psaB* interruption in recipient cells, as previously described.[4,8,9] PS I was isolated from thylakoid membranes by solubiliztion with *n*-dodecyl *β*-D-maltoside and purification on DEAE-cellulose columns and on a sucrose gradient. The isolation of PS I, the analysis chlorophyll content and photochemical activity determined by flash-induced transient oxidation of P700 at ΔA820 and at ΔA700 nm were as described.[9] In both the cysteine mutant and the native PS I, a half-time of 25 ms for the reduction of P700 was recorded. Surface-exposed cysteines on PS I were probed by biotin-maleimide, as previously described.[21] Pt was deposited on monolayers of PS I by photoreduction of Pt^{4+} ions in solution. Slides of PS I monolayer on gold were incubated in a reaction medium containing: 0.2 mM $PtCl_6^{2-}$, 50mM KH_2PO_4, pH 8, 20 mM Na-ascorbate as an electron donor and 0.05 mM 2,6-Dichloroindophenol (DCIP) as an electron carrier. The reaction was illuminated by a tungsten lamp, with intensity of 35 Watt per cm^2, for 10 min at 20 °C. Slides were then washed with distilled water and dried with ultrapure nitrogen. For multi-layer formation, slides were sequentially incubated in solutions containing PS I, washed and then platinized. CPD was determined by KPFM in a 'lift mode' in an AFM model NTMDT, equipped with a custom-made 1300-nm wavelength feedback laser. The CPD is extracted in the conventional way by nullifying the output signal of a lock-in amplifier, which measures the electrostatic force at the first resonance frequency.[14] AFM topography and KPFM were recorded in sequential scans at a scan rate of 1 Hz; 512 lines.

Received: June 20, 2007
Revised: September 11, 2007
Published online: December 20, 2007

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