

## Full Paper

# Photo-Induced Electron Transfer Between Photosystem 2 via Cross-linked Redox Hydrogels

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## Abstract

Photosystem 2 (PS2) that catalyses light driven water splitting in photosynthesis was 'wired' to electrode surfaces via osmium-containing redox polymers based on poly(vinyl)imidazol. The redox polymer hydrogel worked as both immobilization matrix and electron acceptor for the enzyme. Upon illumination, the enzymatic reaction could be switched on and a catalytic current was observed at the electrode. The catalytic current is directly dependent on the intensity of light used for the excitation of PS2. A typical current density of  $45 \mu\text{A cm}^{-2}$  at a light intensity of  $2.65 \text{ mW cm}^{-2}$  could be demonstrated with a significantly improved operational stability.

**Keywords:** Modified electrodes, Redox polymer, Electron transfer, Photoelectrochemistry, Photocurrent, Photosystem 2

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## 1. Introduction

Photosystem 2 (PS2) is a membrane-integrated redox enzyme complex which is involved in the electron transport chain of photosynthesis. It absorbs visible light under formation of excited electronic states which are efficiently separated in space from the electron vacancy, thus avoiding their recombination. Its remarkable photocatalytic properties make it particularly suitable for the integration in bioelectrochemical devices which are used for conversion of visible light into chemical energy [1]. In order to utilize the photocatalytic properties of PS2 in such devices, an electron transfer (ET) pathway between immobilized PS2 and an electrode surface has to be designed. Direct electron transfer from, e.g., adsorbed PS2 is rather unlikely due to fact that its acceptor site (the  $Q_B$ -binding pocket) is deeply buried within the protein shell. In previous studies, quinone derivatives such as duroquinone or 2,6-dichloro-1,4-benzoquinone were used as free-diffusing redox mediators [2, 3]. In these studies, PS2 with a genetically introduced polyhistidine-tag was immobilized via metal ion affinity on thiolated gold surfaces bearing Ni(II)-nitrilotriacetic acid groups. Poly-mercapto-*p*-benzoquinone as co-immobilized redox mediator was used to overcome the limitations of a free-diffusing mediator but did not lead to devices with sufficiently high current densities [4].

A significant increase of the photocatalytic current is expected if either the amount of PS2 on the surface is

increased or the electron transfer pathway will be optimized. A method that provides both a higher surface concentration of PS2 and a reliable electron transfer pathway may be found in the use of redox polymers. Redox polymers have played an important role in the development of reagentless amperometric biosensors [5–8]. They allow electron transfer via a hopping mechanism between neighboring redox centers that are covalently bound to the polymer backbone. Thus, they are capable of shuttling electrons from a reactive site within an immobilized redox protein towards an electrode surface. The rate of electron transfer is determined by several parameters such as the composition of the polymer backbone (flexibility, swelling behavior, amount of cross-linking), the distance between the polymer-bound redox centers, and the formal potential of the bound mediator [9–11]. Consequently, it is possible to tailor the properties of the redox polymer to find an optimized redox polymer for a certain application [12–15].

Here, we present the immobilization of high amounts of PS2 isolated from the cyanobacterium *Thermosynechococcus elongatus* within the three-dimensional network of a redox polymer allowing concomitantly the efficient electron transfer from immobilized PS2 to the electrode upon illumination with light. The photochemically induced electron transfer of the resulting bioelectrochemical device is elucidated.

## 2. Experimental

### 2.1. Chemicals

Allylamine (99%) and 2,2'-azobis(isobutyronitrile) (98%; AIBN) were obtained from Fluka (Basel, Switzerland). Potassium hexachloroosmat ( $K_2OsCl_6$ ), 2,2'-bipyridine (99%; bipy) were purchased from Acros (Geel, Belgium). 1-Vinylimidazole (99%) was obtained from Aldrich (Taufkirchen, Germany) and ethanol was purchased from Mallinckrodt Baker (Deventer, Netherlands). Acetone was from Riedel-de Haen (Seelze, Germany). Osmium-bis-*N,N*-(2,2'-bipyridil)-dichloride was synthesized according to [5]. Poly(ethylene glycol)diglycidyl ether (PEGDGE) was obtained from Polysciences (Warrington, USA).

Ammoniumsulfate (AMS), 2-(*N*-morpholino)ethanesulfonic acid (MES),  $CaCl_2$ ,  $MgCl_2$  and  $H_2SO_4$  were purchased from Mallinckrodt Baker (Deventer, Netherlands). *D*-mannitol was obtained from Riedel-de Haen (Seelze, Germany). *N*-Dodecyl- $\beta$ -*D*-maltoside ( $\beta$ -DM) was supplied by Biomol (Hamburg, Germany). Aluminium oxide pastes with 3  $\mu m$ , 1  $\mu m$ , and 0.3  $\mu m$  grain sizes were purchased from Leco (Mönchengladbach, Germany).

### 2.2. Biochemical Preparations

PS2 was prepared from mass cultures of *Thermosynechococcus elongatus* in a 20 L photobiofermenter as reported in [16]. Solubilized membrane proteins were purified by hydrophobic interaction chromatography (Waters AP-2, filled with Poros 20 OH, Waters, Eschborn, Germany) as a first chromatographic step. The concentration of AMS in the crude extract was adjusted to 1.65 M. The elution of PS2 was induced by a linear gradient from 1.65 M AMS down to 0.6 M. After dialysis in 20 mM MES pH 6.5, 10 mM  $MgCl_2$ , 10 mM  $CaCl_2$  and 0.03% (w/v)  $\beta$ -DM the isolated PS2 complexes were further purified by ion exchange chromatography (Uno-Q6 column, Bio-Rad, München, Germany) yielding an active dimeric subpopulation of PS2 complexes with a typical oxygen evolution activity of 4000–6000  $\mu mol O_2$  (mg Chlorophyll) $^{-1} h^{-1}$ . Samples were stored in buffer containing 0.5 M *D*-mannitol at 1–2 mg Chl mL $^{-1}$  at  $-70^\circ C$  prior to use.

### 2.3. Synthesis of the Redox Polymer

#### 2.3.1. Synthesis of Copolymer of Vinylimidazole and Allylamine

Synthesis was done according to a protocol described previously [14]. In brief, a mixture of 0.2 mL (4.6 mmol) allylamine and 3 mL (30.6 mmol) vinylimidazole was copolymerized under argon atmosphere in a specifically designed reaction tube with reflux condenser. The reaction was initiated by 240 mg (1.5 mmol) of 2,2'-azobis(isobutyronitrile). After stirring for 2 hours at  $70^\circ C$ , the reaction

mixture was allowed to cool to room temperature. The product was dissolved in 6 mL of ethanol and added dropwise to 60 mL of acetone under heavy stirring. The filtered product was washed 2 times with 5 mL acetone and dried in vacuum at  $50^\circ C$  for 2 hours. Copolymer composition and molecular weight were found to be 10.2% of allylamine and 89.8% of vinylimidazole;  $M_n \approx 6000$ . Yield: 2.06 g (64 %).

$^1H$  NMR: 7.6–6.4 (46%, br.d, Im); 4.9 (s, water); 3.8–3.4 (4%, br.q,  $H_2NCH_2$ , EtOH); 3.3 (s, MeOH); 3.2–1.5 (48%, br.m, backbone CH–CH $_2$ ); 1.19 (1%, t, EtOH); 1.1–0.9 (2.5%, br.s, CH $_3$ ).

#### 2.3.2. Coordinative Binding of $[Os(bipy)_2Cl_2]$ to the Polymer Backbone

168 mg of the polymer was dissolved in 2 mL ethanol and  $[Os(bipy)_2Cl_2]$  (102 mg, 0.18 mmol) was added. The reaction mixture was heated to  $90^\circ C$  and stirred at this temperature for 5 days. After cooling to room temperature, the reaction mixture was used for electrode modifications.

### 2.4. Electrode Modification

Prior to the modification, gold electrodes were cleaned by polishing with aluminum oxide paste (down to 0.3  $\mu m$  grain size). After rinsing with water, electrodes were electropolished in 0.5 M  $H_2SO_4$  by cycling between  $-100$  mV and  $+1700$  mV at a scan rate of 100 mV s $^{-1}$  for 10 cycles. Electrodes were modified using 2 mg mL $^{-1}$  PS2 in buffer (20 mM MES pH 6.5, 10 mM  $MgCl_2$ , 10 mM  $CaCl_2$  and 0.03% [w/v]  $\beta$ -DM), 5 mg mL $^{-1}$  redox polymer and 5 mg PEGDGE dissolved in distilled water. The stock solution was prepared by manual mixing of 30  $\mu L$  PS2 solution, 10  $\mu L$  polymer solution and 4  $\mu L$  PEGDGE. A droplet of 7  $\mu L$  was deposited on the surface of a 2 mm gold disk electrode using a pipette. The electrodes were kept in the dark at  $4^\circ C$  for 24 h to enabling cross-linking by means of PEGDGE. Prior to use, the electrodes were washed with buffer solution to remove loosely bound compounds.

### 2.5. Electrochemical Measurements

For electrochemical measurements, an AUTOLAB PGSTAT 12 potentiostat (Eco Chemie, Utrecht, Netherlands) was used. Gold disk electrodes (active electrode diameter 2 mm) were from CH Instruments (Austin, USA), a Ag/AgCl reference electrode was from WPI (Sarasota, USA). 3 M KCl was used as internal electrolyte. Platinum electrodes with 1 mm diameter were made in-house.

Differential pulse voltammetry of the Os-modified polymer was performed using a three-electrode set-up with a 1 mm platinum disk electrode as working electrode, a Ag/AgCl reference electrode and a Pt wire as counter electrode. Prior to measurements, the Pt disk electrodes were polished on a polishing cloth using decreasing size of alumina paste

(3  $\mu\text{m}$ , 1  $\mu\text{m}$ , 0.3  $\mu\text{m}$ ) and ultrasonicated in water for 5 minutes.

Electrochemical experiments with immobilized PS2 complexes were carried out at 25 °C in buffered electrolyte (MES) pH 6.5, 10 mM  $\text{MgCl}_2$ , 10 mM  $\text{CaCl}_2$  and 0.03% [w/v]  $\beta$ -DM. Current response of immobilized PS2 was registered by applying a potential of 0.3 V and recording the current over time. Immobilized PS2 was illuminated with a halogen cold light reflection lamp using a 675 nm interference filter (Schott, Mainz Germany) and a cold light filter Calflex 3000 (Balzers, Liechtenstein) to protect the sample from excessive heat. Cyclic voltammetry under continuous illumination with different light intensities was performed using additional neutral gray filters with different transmission values (Schott, Mainz, Germany).

### 3. Results and Discussion

The basic concept of PS2 'wired' to an electrode surface via an osmium-modified redox polymer is depicted in Figure 1. The polymer immobilizes the PS2 firmly on the electrode surface while its hydrogel character still allows small molecules to diffuse almost freely through the immobilization matrix. Electrons from the PS2-driven splitting of water will be accepted by the redox centers of the polymer and transferred to the electrode surface via a hopping mechanism. To proof the principle suitability of osmium-modified redox polymers to act as both immobilization matrix and electron acceptor for PS2, an osmium modified poly(vinyl)imidazol was used as shown in Figure 2b. Amino functions at the polymer and lysine residues at the protein complex can be cross-linked by means of a bifunctional molecule such as poly(ethylene glycol)diglycidyl ether (PEGDGE) to yield a polymer network with securely integrated PS2. The formal potential of the redox polymer was determined by differential pulse voltammetry (DPV, Fig. 2a). The peak at +190 mV vs. Ag/AgCl (3 M KCl) can be attributed to the  $\text{Os}^{2+}/\text{Os}^{3+}$  redox couple. The absence of other peaks indicates that the coordination sphere is uniform for all osmium complexes bound to the polymer backbone. Since the formal potential of the  $Q_A$ -cofactor of PS2 was determined to be around  $-300$  mV vs. Ag/AgCl [17], the driving force for the reduction of the redox polymer by PS2 is high and assures an efficient ET. However, future work will be directed to the investigation of redox polymers bearing Os-centers with lower formal potentials in order to assure a lower potential loss during the electron transfer reaction.

The entrapment of PS2 within the polymer matrix was realized by manual mixing of PS2, redox polymer, and PEGDGE as cross-linking agent. Drop-coating of the mixture onto gold disc electrodes with active electrode diameters of 2 mm resulted in PS2/Os-polymer modified electrodes. The cyclic voltammogram of the resulting polymer film on the electrode surface in the absence of light (Fig. 3a, solid line) shows a quasireversible redox wave with a peak separation of 45 mV. Since a cyclic voltam-

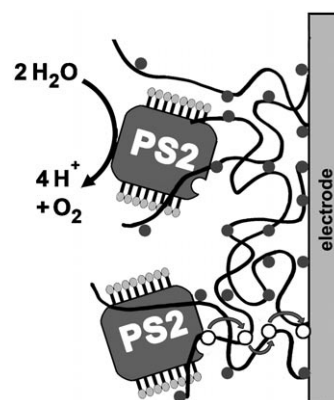


Fig. 1. PS2 entrapped within a mediator modified redox polymer. Arrows depict the electron transfer pathway by a hopping mechanism. (— polymer chains, ● osmium complexes).

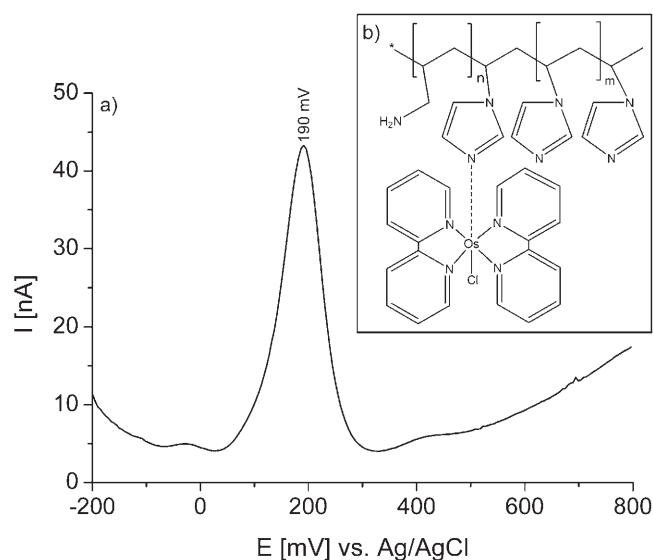


Fig. 2. a) Differential pulse voltammograms for  $\text{Os}(\text{bipy})_2\text{-P}$  (1 M KCl, step potential 49.5 mV, interval time 0.2 s, modulation amplitude 150 mV, modulation time 0.06 s). b) Structure of poly(vinyl)imidazol  $\text{Os}(\text{bipy})_2\text{Cl}$ -polymer.

gram of a surface confined redox species should not exhibit any peak separation it can be assumed that the electron transfer kinetics is limited by counter ion mobility or limitations in the self-exchange process between neighboring redox centers in the polymer. Such behavior was observed previously for similar polymers [18, 19]. The surface concentration of the Os complexes was calculated from the peak area in the CV to be in the range of 25  $\text{pmol cm}^{-2}$ .

Upon illumination of the electrode with light ( $\lambda = 675$  nm) a catalytic current is observed (Fig. 3a, dashed lines). The anodic current increases with increasing light intensity while the cathodic peak decreases concomitantly. With the on-set of the light-induced charge separation in PS2 upon illumination, the polymer accepts electrons from the enzymatic oxidation of water resulting in a rereduction

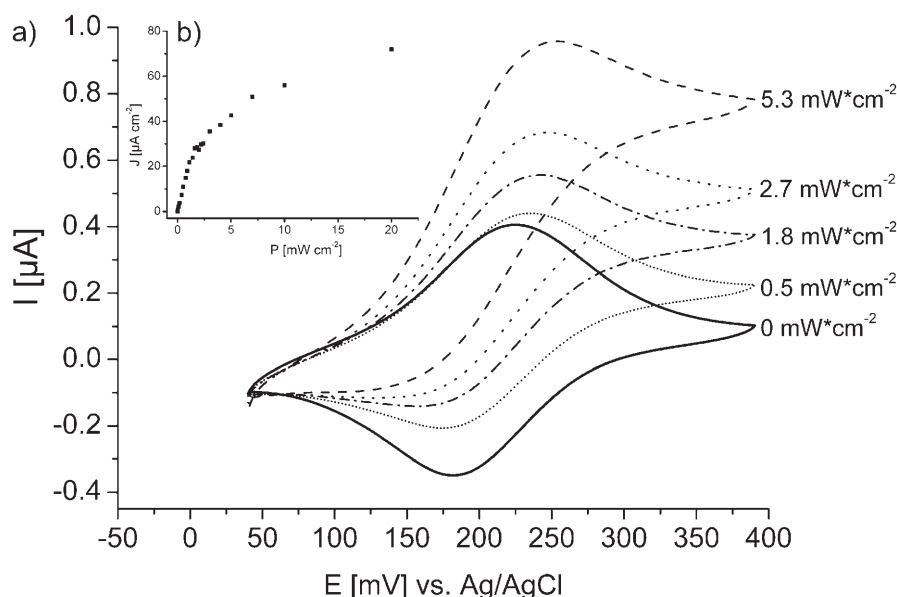


Fig. 3. a) Cyclic voltammogram of entrapped PS2 complexes within a matrix of Os(bipy)<sub>2</sub>Cl-polymer and PEGDGE (full line). Dashed lines are representative CVs upon illumination with different light intensities (0.5–5.3 mW cm<sup>-2</sup>) of the electrode at 675 nm. b) Plot of obtained current from chronoamperometry vs. excitation intensity of a gold electrode modified with Os(bipy)<sub>2</sub>Cl-polymer, PEGDGE and PS2.

of the polymer by PS2. Hence, no significant reduction current is observed in the cyclic voltammogram. The anodic current increases linearly with increasing light intensity over a wide range as depicted in Figure 3b. Saturation occurs at a light intensity of 23 mW cm<sup>-2</sup>. The data strongly support that PS2 is securely immobilized within the redox polymer while maintaining most of its enzymatic activity. Electron transfer to the electrode surface via the redox centers within the polymer seems to be highly efficient.

To monitor the power and long-term stability of the system, chronoamperometry was carried out at a constant potential of +300 mV vs. Ag/AgCl (Fig. 4). In the dark, only a small background current is observed. Upon illumination with a light intensity of 2.65 mW cm<sup>-2</sup>, the current is dramatically increased as expected. Without PS2 or redox polymer such signal was not obtained (data not shown). The current density of up to 45 μA cm<sup>-2</sup> represents an improvement of nearly one order of magnitude over a previously described system based on an electrodeposited layer of poly-mercapto-*p*-benzoquinone [4]. Obviously, either the amount of immobilized PS2 or the efficiency of the electron transfer or both were increased drastically.

Additionally, the long-term stability of the PS2/redox polymer system is superior to another previously described system making use of freely diffusing mediators [2]. As seen in Figure 5, the half life of the conventional system is in the range of 18 minutes. The here described system shows a significant improved operational stability. This improvement of stability can be attributed to a fast ET process between PS2 and the redox polymer and, thus, further reducing the light-induced damage of PS2, which is either caused by reactive oxygen species formed during recombination processes if ET is inefficient [20] or by damage of the

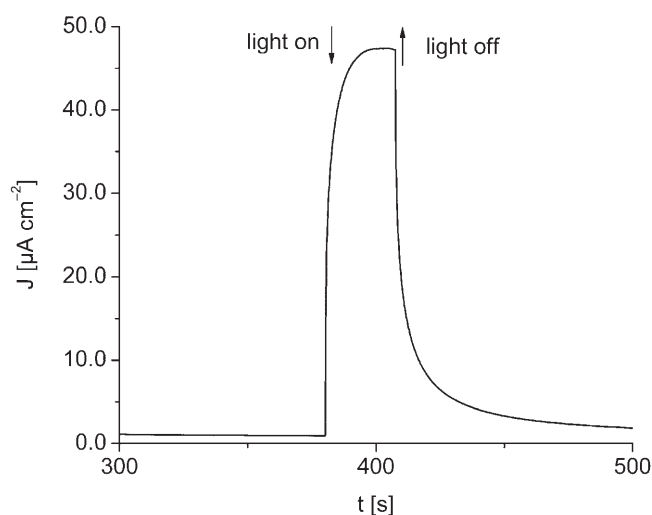


Fig. 4. Current response upon illumination at 675 nm with 2.65 mW cm<sup>-2</sup> of a gold electrode modified with Os(bipy)<sub>2</sub>Cl-polymer, PEGDGE and PS2. The applied potential was +300 mV vs. Ag/AgCl.

oxygen-evolving manganese complex itself as discussed previously [21].

#### 4. Conclusions

In our study we could demonstrate the suitability of Os(bpy)<sub>2</sub>Cl modified poly(vinyl)imidazol redox polymers as both immobilization matrix and electron mediator for isolated PS2 complexes. Gold electrodes modified with cross-linked redox polymer and PS2 showed higher current

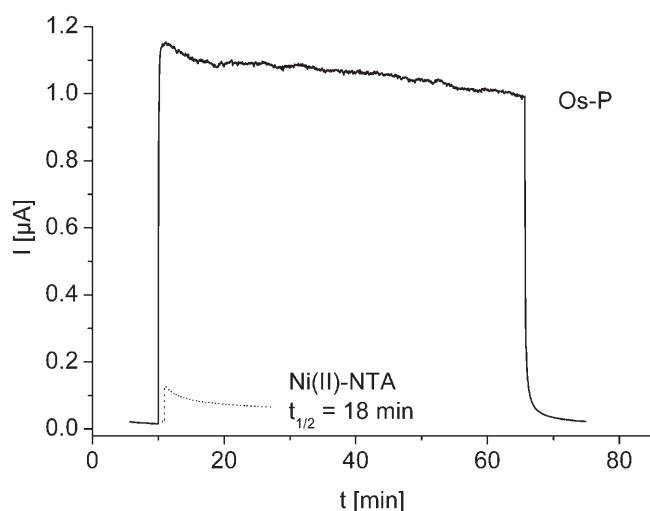


Fig. 5. Current response under continuous illumination (675 nm,  $2.65 \text{ mW cm}^{-2}$ ) of a gold electrode modified with Os(bipy<sub>2</sub>)Cl-polymer, PEGDGE and PS2. The applied potential was +300 mV vs. Ag/AgCl (full line). The dashed line represents the current response of PS2 particles immobilized via his-tag on thiolated gold electrode bearing Ni(II)-NTA groups with 2,6-dichloro-1,4-benzoquinone as soluble redox mediator. For further details see text.

densities and better long-term stability than comparable systems that were studied previously [2–4]. A typical current density of  $45 \mu\text{A cm}^{-2}$  for the redox polymer/PS2 system represents a 10-fold increase while a significant improvement of the operational life time was achieved.

Future studies will mainly aim at the optimization of the redox polymer. Tailoring of the polymer backbone is expected to further improve the electron transfer characteristics of the polymer. Influences of polymer backbone composition on the ET of PS2 will be studied systematically. Additionally, an optimized polymer will allow a higher PS2 loading with improved stability. Thus, redox polymer/PS2 systems might be the basis for a novel type of photo-bioelectrochemical devices to be integrated in photochemical cells or biosensors for the detection of herbicides.

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