Photoactive Electrodes Incorporating Electrosprayed Bacterial Reaction Centers

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Highly efficient light absorption and charge separation within the photosystem and reaction center (RC) complexes of photosynthetic plants and bacteria are of great interest for solar cell and photo detector applications, since they offer almost unity quantum yield and expected ultimate power conversion efficiencies of more than 18% and 12%, respectively. In addition, the charge separated states created by these protein complexes are very long lived compared to conventional semiconductor solar cells. In this work, a novel technique is presented for the deposition of photosynthetic protein complexes, by electrospraying RCs of *Rhodobacter sphaeroides* onto highly ordered pyrolytic graphite (HOPG) electrodes. Remarkably, it is shown that the RCs not only survive exposure to the high electric fields but also yield peak photocurrent densities of up to 7 μA cm^{-2}, which is equal to the highest value reported to date.

1. Introduction

Through millions of years of evolution, natural selection has yielded very efficient biological solar harvesters, notably as photosynthetic bacteria and plants, which rely on finely tuned electron transfer pathways residing in photosynthetic protein complexes (termed reaction centers (RC) or photosystems). These protein complexes harvest light to form a charge-separated state at a quantum conversion efficiency of around unity. The RC of the bacterium *Rhodobacter sphaeroides* is one of the simplest and most robust examples of these protein complexes, with a theoretical power conversion efficiency exceeding ≈12% (see Supporting Information) and a long recombination time of up to 1 s (compared to ≈5 ms in silicon-based devices).[1,2] The RC consists of three protein subunits, H, M, and L, and ten cofactors: the primary electron donor P, which is a bacteriochlorophyll dimer known as the special pair (P_A and P_B); two accessory bacteriochlorophylls (B_A and B_B); two bacteriopheophytins (H_A and H_B); two quinones (Q_A and Q_B); a carotenoid; and one iron atom (Fe^{2+}) (Supporting information, Figure S2A,B).[3] Upon photo-excitation of P, electron transfer occurs from P* through the A-branch cofactors to the primary quinone Q_A, then to the secondary quinone Q_B (Supporting information, Figure S2A,B). At neutral pH, the RC is a net negative complex which has a complicated spatial distribution of charged functional groups (shown for pH 8 in supporting information Figure S2C), with a high concentration of negative charges at the P side.

The high expected power conversion efficiency (12%) combined with the long recombination time of the *R. sphaeroides* RC makes it of great interest for use in solar cell applications, bio-sensors, and photo-sensors.[4–7] One method for turning these promising biological materials into solar cells is through deposition onto solid electrode surfaces. Different methods of depositing RCs involve using linker molecules,[8–11] or other chemical modifications of either the RC or the electrode;[12–16] however in this work, we use a new technique to deposit RCs on HOPG electrodes by electrospraying the proteins directly onto the surface. Peak photocurrent densities of up to 7 μA cm^{-2} is measured which is equal to the best current densities achieved[12] and 1000 times higher than what is often seen in the literature for RC-coated electrodes and floating RC cells.[4,8,9,17,18] The electrospray technique works by connecting a high voltage power supply to a conductive needle attached to a syringe containing the protein solution. The electrode substrate is connected to the opposite polarity of the power supply and the protein solution is forced through the needle. As the solution exits the needle it disperses into small droplets in air, due to a high coulombic repulsion of the droplets in the electric field between the needle and the substrate. As a result, the charged droplets hit the substrate at a high velocity and are attached to the electrode. Light-harvesting pigment-protein complexes have previously been deposited on a substrate using electrostatic discharge and found to interact energetically with synthetic dye.[19]

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We have chosen to test the efficacy of electrospray deposition (ESD) for the deposition of RCs from the bacterium Rhodobacter Sphaeroides, which were prepared using the procedure detailed in the supporting information and reference.[20] In principle, if the catalytic activity of the RC were retained after deposition, it could function to both harvest light energy as well as initiate electron transfer leading to current in an electrical circuit. Evidence will be presented to suggest that the RCs remain intact and functional after the electrospray procedure. The direct electrospray method was investigated as a method to form an uninhibited electron transfer pathway between the RC and the HOPG surface, due to the affinity of the charged RC droplets for the oppositely charged HOPG surface, and the lack of any insulating molecular linker functionality. After electrospraying with RC, the HOPG electrode was studied by measuring the photocurrent at dark open circuit potential, the external quantum efficiency over the wavelength range of the RC absorption and atomic force microscopy (AFM). We hypothesized that applying positive potential to the HOPG during electrospray would lead to a stronger interaction with the negatively charged RC than the converse, and tested this by comparing HOPG electrosprayed under both these conditions.

2. Results and Discussion

The photo-response (photocurrent generated at the dark open circuit voltage) was measured for HOPG electrodes treated with RCs by either electrospray (with positive potential at HOPG) or dip-coating (see methods), in a three-electrode electrochemical cell with ferrocene (Fc) solution as the electrolyte (Supporting Information, Figure S4C). The photocurrent was found to be negative in both cases, but the dip coated electrode showed a much weaker photo response compared to the electrosprayed electrode (**Figure 1**A). The photo-response of the clean, untreated HOPG electrode was also measured, and found to be negligible (see supporting information).

Having shown that the HOPG becomes photoactive only after treatment with RCs (Figure 1A), we wished to determine whether the photocurrent originated from the light absorption of RCs that remained intact after the electrospray procedure. The light wavelength absorption spectrum of the RC is sensitive to the degree of protein denaturation or remaining in the native state.[21] Furthermore the absorption spectrum of the RC determines the light wavelength action spectrum. Therefore, the external quantum efficiency (EQE) of photocurrent generation (the percentage of charge carriers collected per incident photon) was measured as a function of incident wavelength in the range 950 to 340 nm. Comparing the EQE of photocurrent with the absorption spectrum of RCs (Figure 1B), reveals a similar pattern of peaks, strongly suggesting that the photocurrent originated from light absorbed by structurally intact RCs on the HOPG surface.

Atomic force microscopy (AFM) was performed on freshly cleaved HOPG, and on electrosprayed HOPG which had

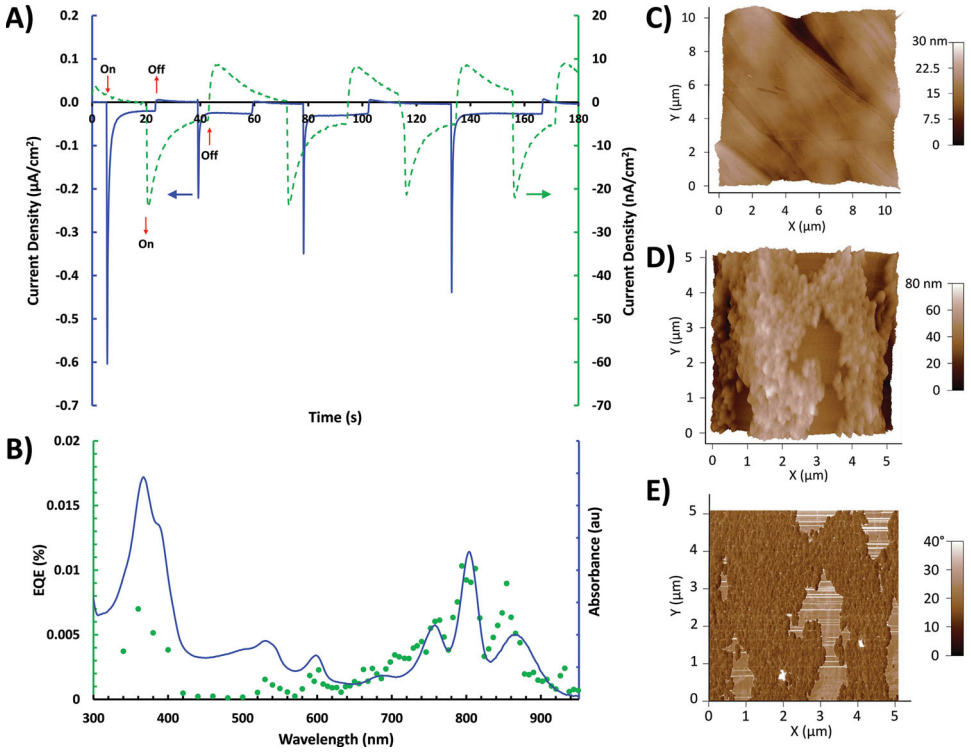

Figure 1. A) Photo-response of the RC treated electrodes under 146 mW cm^{-2} illumination: electrosprayed HOPG (blue solid line) and dip-coated HOPG (green dashed-line). The dark open circuit potential was 45 mV. B) External quantum efficiency (EQE) of the photocurrent, per incident photon, generated on the RC-electrosprayed HOPG electrode (green dots), compared to the absorption spectrum of the RC (blue line). Atomic force micrographs: C) Topography of clean, freshly cleaved HOPG; D) The corresponding topography, and E) the phase images of the HOPG surface after RC electrospray and rinsing. One lightly deposited area is displayed to show the contrast between the coated and clean HOPG.

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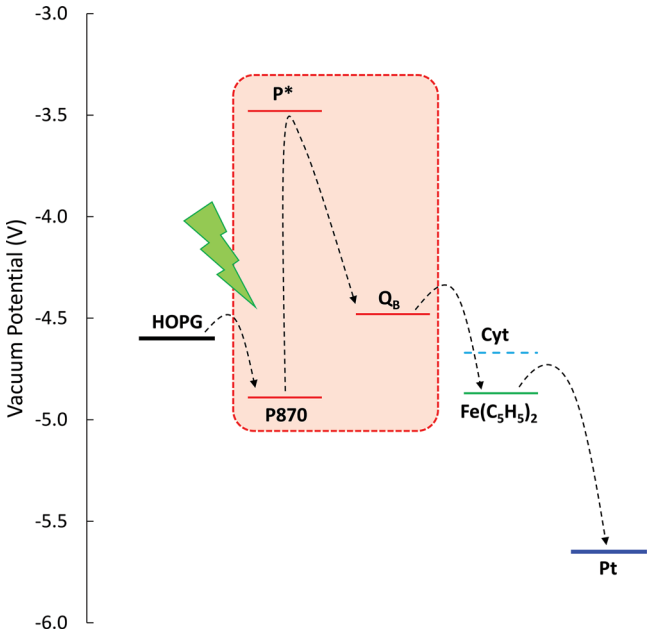

Figure 2. The potential levels of the cell components shown relative to vacuum.

been rinsed with DI water to remove extraneous material (Figure 1C–E). An incomplete layer of a grainy-textured surface coating was observed, which could be distinguished as a distinct material from the HOPG (due to differences in surface energy shown by the contrast in the phase image). Prompted by the EQE measurements above, we conclude that this surface coating consists of electrospray deposited RC material. The thickness of the layer is approximately 30 nm, consistent with three to four layers of RCs.

In light of these findings, the photo-response in Figure 1A can be explained via Fc and Fc^+ concentrations before and during illumination. **Figure 2** depicts an energy diagram of the cell components.

The work functions values used for HOPG and Pt suggest that electron flow is unidirectional from HOPG to the P^+ state in the RC and from ferrocene to Pt. However, in solution the Fermi levels of HOPG and Pt equilibrate with the solution potential governed by the concentration of ferrocene ([Fc]) and ferrocenium ([Fc^+]) ions. At the onset, [Fc] and the much smaller [Fc^+] are constant across the cell (**Figure 3**A). At the dark open circuit potential (OCP), the HOPG, bulk electrolyte and Pt are all at the same potential (Figure 3C), thus yielding zero cell current. The HOPG is maintained at this potential throughout the experiment by applying a potential equal to the dark OCP. Upon illumination, the initial spike can be explained by the fact that the RCs form a charge-separated state and reduction of P^+ occurs via direct electron transfer from HOPG and/or by local mediators at the RC/HOPG boundary. Concurrently, oxidation of Q_B^- by Fc^+ starts occurring at the Q_B^- site of the RC, along with oxidation of Fc to Fc^+ at Pt with transfer of electrons from Pt to the HOPG. These processes perturb the concentrations of Fc and Fc^+ near the electrodes (Figure 3B). Diffusion of ions between these localized populations is unlikely on the timescales of these experiments as the electrodes are too widely separated (≈1 cm) for ions to diffuse rapidly across the cell. Once Fc^+ at the HOPG is depleted, the electron transfer process is likely limited by the diffusion of Fc^+ from the bulk to the HOPG which causes the photocurrent to drop from the initial spike and plateau at a stable value. Such DC currents are also observed in similar photoelectrochemical cells made with various photosynthetic proteins.[4,9,12,17,18,22,23] At steady-state, the changes in Fc and Fc^+ concentrations are expected to cause the solution potential to be lowest near the HOPG, highest near Pt and at the dark OCP value in the bulk (Figure 3D). Since the HOPG potential is fixed at dark OCP, the Fermi level of HOPG is at a lower energy compared to the solution potential in its neighborhood. This may lead to some parasitic oxidation of Fc at HOPG but the measurement of a cathodic current suggests that the current from the HOPG to the RC is dominant.

When the light is switched off, it is expected that the current will drop to zero, as in the case of dye, silicon, and polymer-based solar cells.[24] However, for all of the samples, some current "overshoot" in positive direction was observed. This current can be explained by the steady state solution potentials that develop in the cell (Figure 3D), as have previously been observed in the work of others.[12] When the light is switched off, the RC current no longer exists. The energy differences between the electrode and the solution will dissipate with oxidation occurring at HOPG and reduction occurring at Pt until the entire cell is back in equilibrium. This current is recorded as a positive spike and subsequent diffusive current decay after light is switched off.

The photo-response of the dip-coated electrode (green line in Figure 1A) shows a symmetrical rise and decay in current which can be explained by the same rationale, but in this case since there is no spike in current, the rise in current can be primarily due to the mediator oxidization by RCs and reduction by the HOPG than direct electron transfer. For dip coated samples, it is expected that some RCs are detached from the HOPG when dipped in electrolyte (now floating RCs), therefore, the negative current can be explained by the surface energy of the HOPG. HOPG was characterized to be hydrophilic (see supporting information). It is previously shown[12,25] that RCs are more likely to interact with hydrophilic electrodes at the P side, which justifies the direction of the current during light illumination.

The effect of electrode polarity was investigated by changing the polarity of the HOPG and needle's tip between separate electrospray depositions. The depositions were performed in the absence of illumination (see methods). No significant difference was seen in the response when polarity was changed (**Figure 4**,5).

The steady-state response of the photo-current produced by the electrospray deposited RCs are between 0.1 to 0.25 μA cm^{-2} which is in around the same range as the values reported: 0.13 to 0.2 μA cm^{-2} (floating reaction centers with superhydrophobic carbon nanotube electrodes),[12] 0.08 to 0.1 μA cm^{-2} (Photosystem I attached to a self-assembled monolayer on gold electrodes),[22] 0.04 to 0.06 μA cm^{-2} (photosynthetic reaction centers bound using a linker to carbon electrodes).[23]

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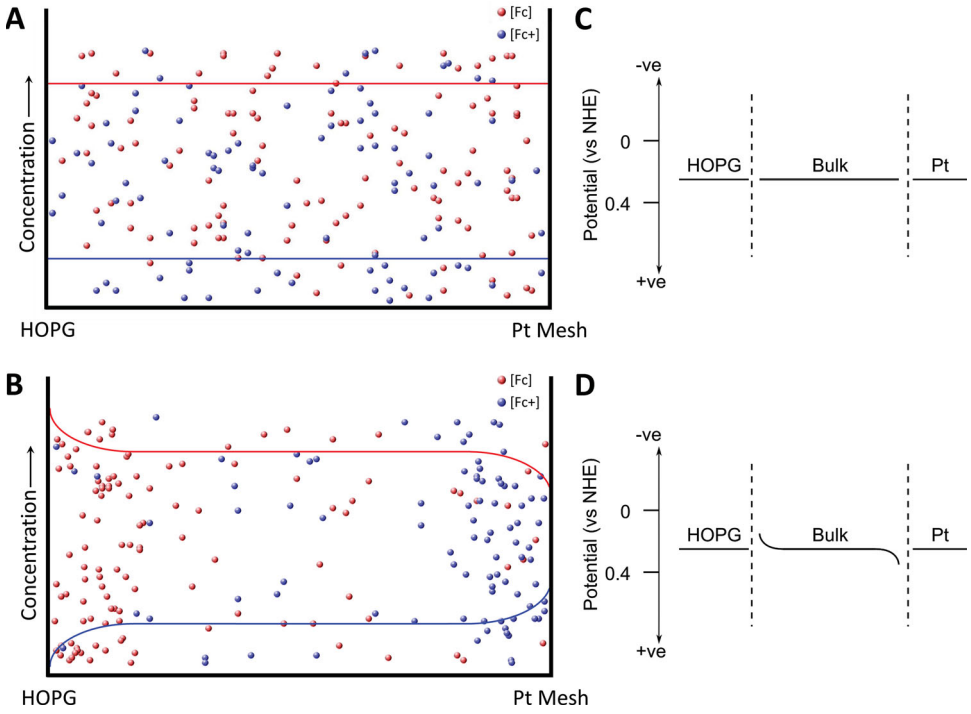

Figure 3. Suggested concentration of Fc and Fc^+ A) before and B) during illumination. The horizontal axis denotes the distance across the cell. Energy diagram of the HOPG, bulk, and Pt C) before and D) during illumination.

3. Conclusions

In conclusion, in this work we have demonstrated that an electrospray process can be used to deposit RCs on HOPG electrodes without any need for binders and chemical modifications of the RCs or the electrode surface. It is also shown that the RCs survive the harsh deposition process without losing their protein structure-dependent light wavelength action spectrum. The functionality of HOPG electrosprayed with RC was also compared to HOPG treated with RC by dip coating in the absence of an electric field, to determine the effectiveness of the electrospray technique for integrating RCs into a photovoltaic device, allowing the functionality of the RC to be harnessed. By depositing RCs using this technique, peak current densities of up to 7 μA cm^{-2} were achieved, which is almost

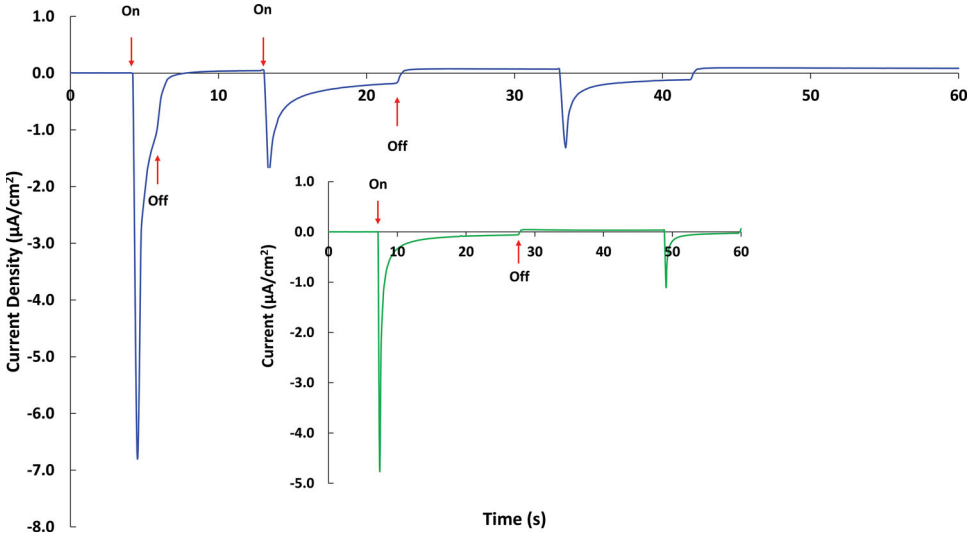

Figure 4. Photoresponse of a RC-electrosprayed HOPG electrode under 146 mW cm^{-2} illumination. The blue and green (inset) plots represent the photoresponse of the RC-electrosprayed HOPG electrode for the case of connecting the needle to ground and the HOPG to ground respectively during deposition.

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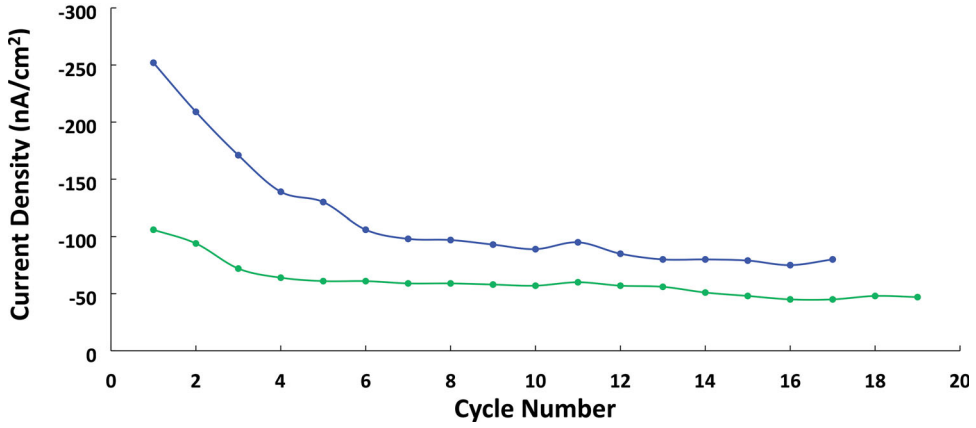

Figure 5. Steady state component of the light-induced current of an electrosprayed HOPG sample with HOPG connected to +V (top line) and HOPG connected to -V (bottom line) during the electrospray process.

1000 times greater than in our previous work,[8] and greater than when RCs are deposited by dip coating, in the absence of an electric field. Atomic force microscopy imaging provided evidence for the presence of RCs on the surface of the HOPG, and along with EQE measurements demonstrated that the photo-current is generated by intact deposited RCs. The proposed deposition technique is fast, low cost, and chemical-free, and can be used to deposit other proteins that have net electric charges on their surface.

4. Experimental Section

Electrode Preparation: The HOPG electrodes were prepared by attaching a 1 cm by 1 cm HOPG block to a flattened stainless steel rod (10 cm), using copper tape adhesive. To ensure high conductivity of the contact between copper and HOPG, one small drop of silver paint was applied to the edges of the copper tape. The electrodes were encapsulated in epoxy, leaving only the front 1 cm^2 HOPG unprotected. Each HOPG surface was cleaned by removing a few layers of HOPG using scotch tape. Clean HOPG electrodes were electrosprayed with 4.8 μM solution of wild type RC or dipped in an RC solution of the same concentration and incubated at −40 °C for three days. The samples were rinsed with deionized (DI) water three times before performing further tests.

Electrospray Setup: The electrospray apparatus was made of a 1 in. Luer-lok 26G stainless needle connected to a BD 10 mL Syringe. At the top, the HOPG electrode was attached to the setup glass cover. One HV power supply was used to apply 15 kV between the needle and the HOPG that was apart from the needle by 75 mm (Supporting Information, Figure S4A,B). The apparatus was housed in glass, under a positive pressure of N_2 to reduce arcing. To study effect of HOPG polarity, 0.3 mL solution of 3.6 μM RC was used for the electrospray process. For both experiments, one HOPG electrode was used to minimize any possible process variation in fabrication of the HOPG electrodes. Before performing the second test, the HOPG electrode was rinsed with DI water and a few layers of HOPG were removed by adhesion to adhesive tape.

Photo-Response Measurements: A three-electrode electrochemical cell was made (Supporting Information Figure S4C) using a Pt mesh as the counter electrode, HOPG electrode as the working electrode and an Ag/AgCl reference electrode, in an electrolyte consisting of 1 mM ferrocene solution (1.4 mg ferrocene (Sigma Aldrich) dissolved in 7.35 mL deionized water) and 1 mL of 1 M pH 8 Tris buffer solution. The current density was measured before and after illuminating light on the HOPG and at the dark open circuit potential using a potentiostat device. The platinum mesh counter electrode was washed with DI water and flamed each time before use.

External Quantum Efficiency (EQE) Measurements: EQE measurements were taken in a two-electrode cell (working electrode: electrosprayed HOPG; counter electrode: Pt mesh), in a solution of 0.9 mM ferrocene in Tris buffer; the voltage was set to the dark open circuit voltage (−0.05 V). Illumination at each chosen wavelength was for 1 min duration (provided by a 150 W Xenon arc lamp, passed through a Newport 77250 grating monochromator), followed by 1 minute of darkness. The wavelength was stepped by 6 nm (in the 950–590 nm range), 10 nm steps (580–520 nm range) and 20 nm steps (500–349 nm range).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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