Manipulated photocurrent generation from pigment-exchanged photosynthetic proteins adsorbed to nanostructured WO_3–TiO_2 electrodes†

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A novel photoelectrode (PE) consisting of the pigment-exchanged photosynthetic reaction center (RC) trapped on the mesoporous WO_3–TiO_2 film was fabricated to facilitate bio-photoelectric conversion by manipulating the excitation relaxation of the proteins.

In natural bacterial photosynthetic transmembranes, a delicate arrangement of cofactors and peptides contributes to the highly efficient near-infrared (NIR) light-harvesting and charge separation that cannot be simulated.[1] The core element involved in the bacterial photosynthetic process is a pigment–protein complex called the reaction center (RC). The stable and robust character of the RC makes it possible for it to be integrated as a molecular electronic device.[2] Unfortunately, two factors may greatly restrict the utility of the RC. Firstly, the long-lived final charge separation state of the RC is achieved by sacrificing a great deal of energy.[3] Secondly, inevitable charge recombination for isolated RC partly quenches the separated electron-hole pairs.[4] Such disadvantages have been partly overcome in our recent work, according to which a novel RC photoelectrode (PE) fabricated by entrapping the proteins on a tailored mesoporous WO_3–TiO_2 matrix exhibited good NIR photoelectric responses, thanks to the matched energy levels of the WO_3–TiO_2 and the RC.[2h] With the aim of further promoting the photoelectric performance of the RC, we describe here a RC mutant (containing plant pheophytin (Phe) instead of bacteriopheophytin (Bphe), termed Phe-RC) as a replacement for the native-RC entrapped in the above-mentioned WO_3–TiO_2 matrix (Scheme 1). Remarkably enhanced NIR incident photon-to-current conversion efficiency (IPCE value of ~23% at around 800 nm), compared with that of any other RC PE reported to the best of our knowledge, (with the IPCE value normally less than 1% at around 800 nm), strongly indicated that the electron injection (EI) (from Phe-RC to the WO_3–TiO_2 matrix) was manipulated as a feasible excitation relaxation pathway of the Phe-RC for improving the design of biomimic photoelectric devices.

In this work, specific pigment replacement was introduced for altering both the energetics and kinetics of the light-initiated electron transfer (ET) in the RC. Herein, Bphe molecules, the primary electron acceptors involved in the ET process of RC, were replaced with Phe ones from spinach.[5]† The normalized steady-state absorption spectrum of Phe-RC indicated that Bphe (Qy absorption band at ~760 nm) located at both the $Bphe_A$ and $Bphe_B$ binding sites was replaced by Phe (Qy absorption band at ~675 nm) with a high yield of more than 95%.[5]† The NIR light-harvesting capability of the bacteriochlorophyll (Bchl) dimer (abbreviated as P, Qy absorption band at ~870 nm for native–RC and a slight blue-shift of 5 nm for Phe-RC) and Bchl monomer (Qy absorption band at ~802 nm) remained almost unaltered after pigment substitution. The NIR circular dichroism (CD) spectrum of Phe-RC further exhibited evidently the substitution of Bphe with Phe, as the negative band at ~750 nm disappeared completely while a new positive band at ~678 nm emerged.[5]† Similar CD signals observed at the 780 to 840 nm band of the two proteins displayed that the excitonic coupling of Bchl was not affected obviously after pigment exchange.

Then, the as-made Phe-RC was entrapped on the three dimensional (3D)-wormlike mesostructured WO_3–TiO_2, with uniform pore size centered at 7.1 nm, for fabrication of the Phe-RC PE.[2h,6]† The band gap energy (E_g) of ~3.3 eV was derived from a plot of $(\alpha h\nu)^{0.5}$ against $h\nu$ (α, absorption coefficient) based on the optical data for the tailored mesoporous WO_3–TiO_2 matrix.[6]† The higher E_g compared with that of the bulk WO_3 (2.8 eV) and TiO_2 (3.2 eV)[7] demonstrated the quantum size effect of these WO_3–TiO_2 films. The matched conduction band (Cb) values of the two components in the WO_3–TiO_2 products resulted in higher capability of separating photogenerated carriers than single TiO_2 or WO_3.[8] Accordingly, these structural features might promote the photo-induced EI of RC specifically entrapped on the tailor-made WO_3–TiO_2 films.

To investigate the excitation relaxation of the entrapped Phe-RC presented in Scheme 1, femtosecond pump–probe dynamics, fluorescence (FL) emission and NIR photoelectric responses were studied in detail.

First of all, femtosecond pump–probe measurements exhibited in Fig. 1 mapped the deferred evolution of ultrafast excited-state dynamics induced by pigment exchange, which thus enhanced the tendency of photo-induced EI from the photo-excited state of P (P*) or P^+Bchl^- in RC directly to the WO_3–TiO_2 matrix. The excited-state dynamical trace for native-RC demonstrated a biexponential decay at both 800 nm (τ_1 = 220 fs and τ_2 = 2.0 ps) and 850 nm (τ_1 = 130 fs and τ_2 = 2.6 ps) with pulse-width limited timescale, revealing a crude three-step mechanism (*via* the excited state of Bchl (B*) → the upper excitonic state of P (P_+) → the lower excitonic state of P (P_-) → P^+Bphe^-) and a two-step one (*via* P_+ → P_- → P^+Bphe^-) of the excitation relaxation, respectively.[9] The time constant measured correspondingly for

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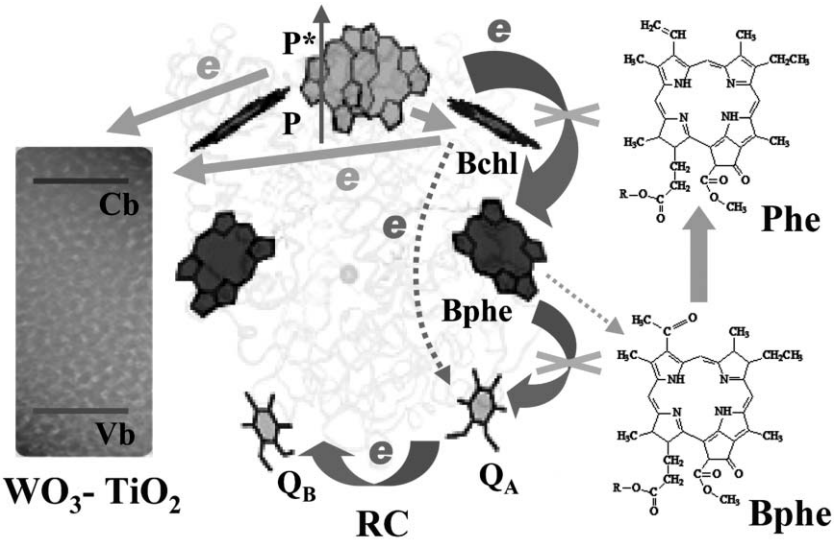

Scheme 1 Illustration of the Phe-RC/WO_3–TiO_2 PE and photo-induced ET. Cb, conduction band; Vb, valence band; P, bacteriochlorophyll (Bchl) dimer; Bphe, bacteriopheophytin; Phe, pheophytin; Q_A, the primary quinone; Q_B, the secondary quinone.

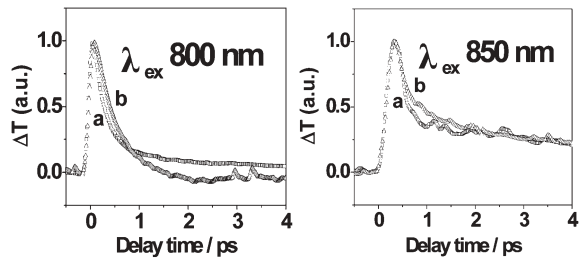

Fig. 1 Time-resolved one-color dynamics of native-RC (a, square) and Phe-RC (b, triangle) excited at 800 nm (left) and 850 nm (right) with magic angle (54.7°) polarization.

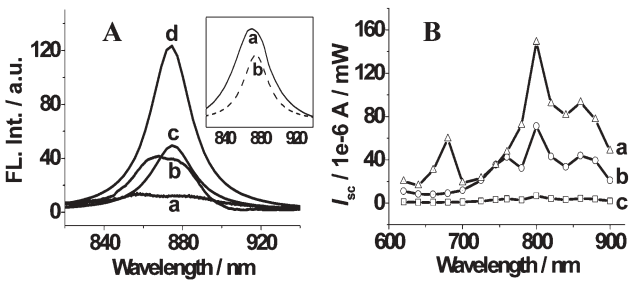

Fig. 2 (A) Steady FL emission spectra of the bare WO_3–TiO_2 film (a), Phe-RC/WO_3–TiO_2 film (b), native-RC/WO_3–TiO_2 film (c), and native-RC/Al_2O_3 film (d) excited at 800 nm. Inset displays steady FL emission spectra of 2 μM Phe-RC (a, solid line) and native-RC (b, dash line) in pH 8.0 Tris-HCl buffer. (B) NIR photocurrent action spectra of the Phe-RC/WO_3–TiO_2 film (a, triangle), native-RC/WO_3–TiO_2 film (b, circle), and native-RC/Al_2O_3 film (c, square) excited using a Ti-sapphire laser with tunable output wavelength from 600–900 nm and detected in pH 8.0 Tris-HCl buffer containing 8 mM sodium dithionite.

Phe-RC (τ_1 = 450 fs and τ_2 = 3.0 ps at 800 nm, τ_1 = 310 fs and τ_2 = 4.2 ps at 850 nm) was substantially slower, indicating the distinct delay of excitation transfer in the mutant species (for more details, see ESI†). The relative slow-down formation of the final charge-separated state in Phe-RC was presumably attributed to the higher free energy level of P^+Phe^- than that of P^+Bchl^-.[10]

Meanwhile, FL emission spectra (shown in Fig. 2A) suggested the increased photo-induced EI (*via* P* or P^+Bchl^- to WO_3–TiO_2) to be an alternative for excitation relaxation of the Phe-RC/WO_3–TiO_2 PE (**1**). Herein, data plots of a native-RC/WO_3–TiO_2 PE (**2**) [2h] and native-RC/Al_2O_3 PE (**3**) [2e] are provided for comparison. FL intensity of the three RC photoelectrodes (PEs) was normalized to the protein-loading amount. Results of the proteins in buffer are also presented in the inset. Apparently, no distinct FL intensity differences were found for **1** and **2** while obviously higher intensity was observed for Phe-RC in buffer compared with its native counterpart. On the other hand, FL intensity of **1** and **2** was less than half of that derived for **3**. Since the steady FL peaks centered at ~875 nm (native-RC) and ~871 nm (Phe-RC) were attributed definitely to the emission of energy transferring from Bchl to P,[11] the degressive intensity tendency of the photo-induced EI within the three RC PEs could be reflected accordingly as **1** > **2** > **3**.

Furthermore, NIR photoelectric measurements also proved the favored, manipulated photo-induced EI within **1**. Fig. 2B displayed comparatively the NIR photocurrent action spectra of the above-mentioned three RC PEs. Compared with **2** and **3**, **1** exhibited remarkably enhanced photocurrent responses (maximal IPCE value of **1** reached ~23% at around 800 nm), however, only minor differences on protein loading were displayed (details in protein loading and photoelectric tests are displayed in the ESI†). Therefore, the intensity sequence of the photo-induced EI within these RC PEs was further confirmed as follows: **1** > **2** > **3**.

The likely operation for the manipulated excitation relaxation of **1** is briefly discussed here. For isolated RC in buffer, charge separation and FL emission serve as two primary ways to quench the photo-generated P*. With specific immobilization of the proteins, a third ET pathway, that is, the photo-induced EI, may occur thanks to the mutual sites of energy levels for P^*/P^+Bchl^- and WO_3–TiO_2.[2h] Moreover, the specific pigment exchange further accelerates the EI by somewhat hindering the spontaneous charge separation inside RC (Scheme 1). Consequently, the facilitated EI

contributes significantly to the excitation relaxation of the adsorbed Phe-RC.

In summary, a novel Phe-RC-trapped WO_3–TiO_2 PE was fabricated with a feasible pathway manipulated for excitation relaxation of the proteins. The facilitated photo-induced EI from Phe-RC to the WO_3–TiO_2 matrix remarkably improved the bio-photoelectric performances of the photosynthetic proteins and might arouse further concerns for developing versatile bio-photoelectric devices.

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5 Details in protein isolation, pigment exchange, and NIR-Vis absorption as well as CD spectra of the two kinds of RC are shown in ESI. Relevant references are also presented.

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