Supplementary Material (ESI) for Chemical Communications

Supporting Information

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

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Details in experiments

Pigment exchange and protein isolation

Native-RC from the photosynthetic bacterium RS601 (one of *Rb. sphaeroides* strain) was separated and purified as described in ESI-Ref. 1.

Pheophytin (Phe)-exchanged RC (Phe-RC) was separated and purified by using the methods developed by Scheer etc. [ESI-Ref. 2] with further modifications. Phe, collected from the spinach (*spanacia oleracea* Mill.) leaves, was suspended in acetone and added into the native-RC suspension ($OD_{800,\ 1\ cm}$=5.0). The final concentration of Phe was 20 times of that of bacteriopheophytin (Bphe) in native-RC. After incubation at 43.5 ^{o}C for 1 h, the system was cooled down and kept at room temperature. Ten folds volume of Tris-LDAO (TL) buffer was then added to dilute the concentration of acetone. The sample was loaded immediately to the DEAE-Cellulose 52 column after incubation to prevent the de-binding of the bound Phe at the $Bphe_A$ site. The column was rinsed in turn with TL buffer containing 0.09 mol/L and 0.12 mol/L NaCl. The eluted Phe-RC was concentrated by ultrafiltration with a 10 KD cut-off membrane to obtain a final concentration with $OD_{800,\ 1\ cm}$=2.0. The samples were then loaded onto the tubes with 10%-40% sucrose gradient and centrifuged at 260,000 *g* for 16 h. The fraction close to 25% gradient was collected and dialyzed against 1 L TL buffer to exclude sucrose.

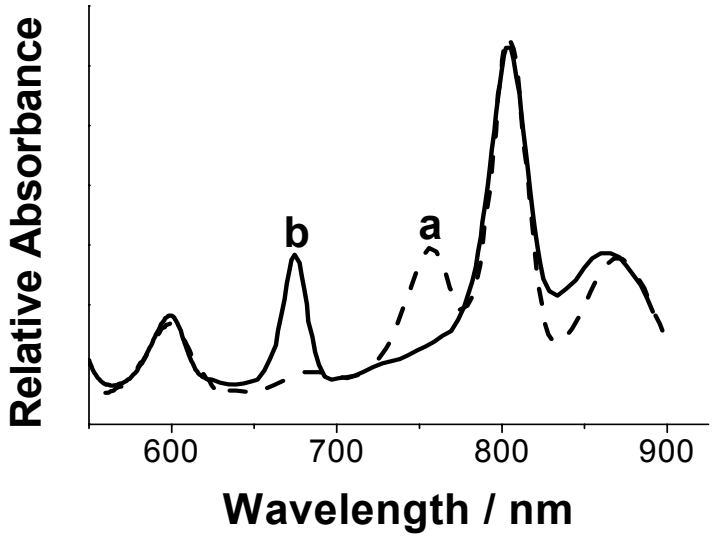
Synthesis of the novel mesoporous WO_3-TiO_2

The tailor-made three dimensional (3D)-wormlike mesoporous WO_3-TiO_2 films (pore size of 7.1 nm) were prepared as follows. Therein, 1 g of triblock copolymer P123 ($EO_{20}PO_{70}EO_{20}$) was dissolved in 10 g of ethanol, then 0.8 g of WCl_6 and 2.4 g of $Ti(OBu)_4$ were added into the solution and the mixture was further stirred for 2 h at room temperature. The target film was achieved by spin-coating of the mother solution on indium tin oxide (ITO) grass with the thickness of *ca.* 150 nm. The solvent was fully evaporated in air (relative humidity: 20 ~ 30%). After gelation at 45 ^{o}C for 1 day, the inorganic framework was obtained via calcination at 350 ^{o}C in air.

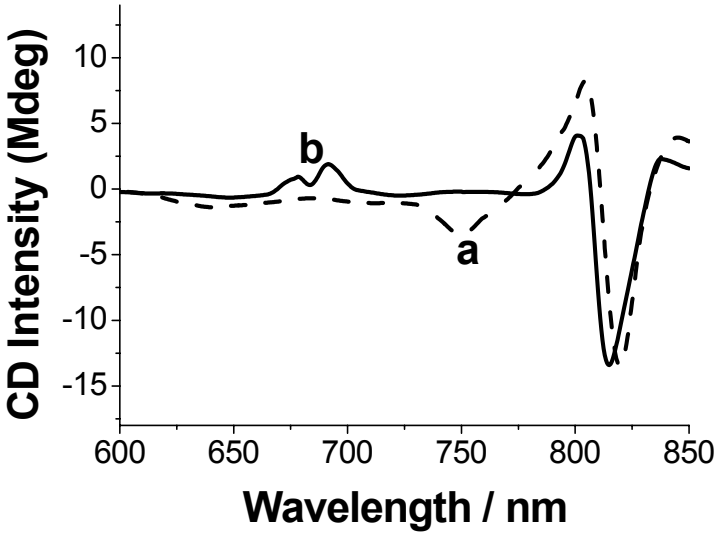
Another 3D-worm-like mesoporous WO_3-TiO_2 films (pore size of 3.4 nm) and the 2D-hexagonal mesoporous WO_3-TiO_2 films (pore size of 9.8 nm) provided for comparison were prepared similarly, except the amphiphiles used were substituted with P85 ($EO_{26}PO_{39}EO_{26}$) and F127 ($EO_{106}PO_{70}EO_{106}$), respectively.

Fabrication of the RC/WO_3-TiO_2 photoelectrodes

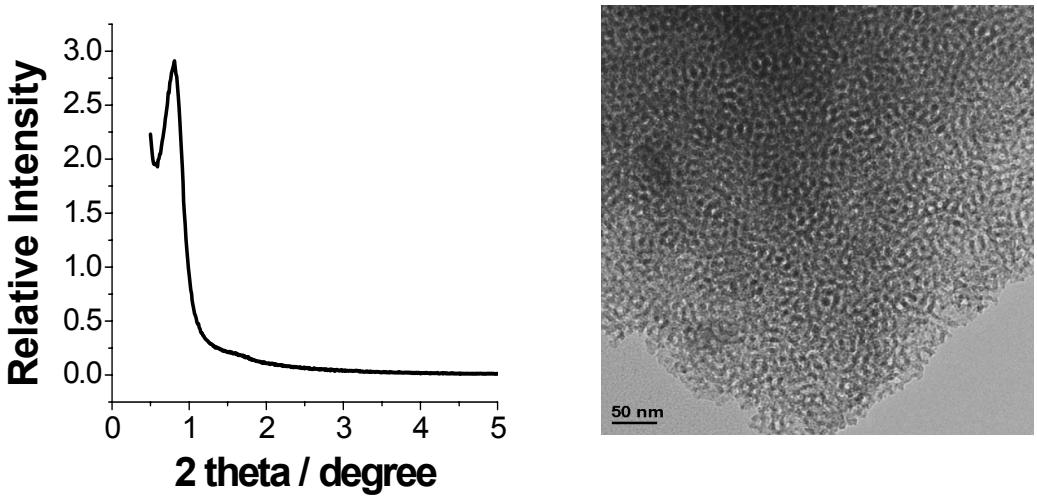
Protein immobilization was achieved by immersing the freshly prepared WO_3-TiO_2 films (~ 1.5 cm^2) in the pH 8.0 Tris-HCl buffer solution of native-RC/Phe-RC (at 4 °C) for 2 ~ 3 days. Prior to all measurements, the films were rinsed and kept in buffer solution. The successful entrapment of RC on the tailored mesoporous WO_3-TiO_2 films was proved by the near infrared (NIR)-visible absorption spectra presented in ESI-Fig. 6. The results from another two kinds of mesoporous WO_3-TiO_2 films mentioned above and data from Al_2O_3 gel films reported in our previous work [ESI-Ref. 3] were also given for comparison, as shown in ESI-Table 1.



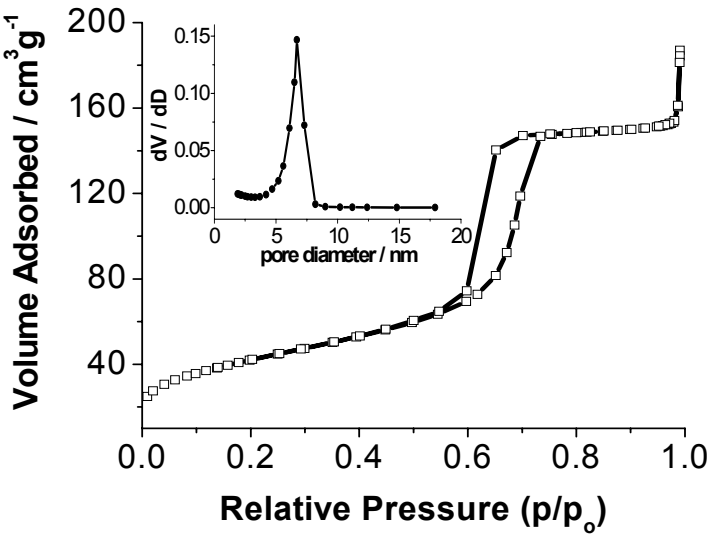
ESI-Fig. 1 Normalized NIR-Vis absorption spectra of native-RC (**a**, dash line) and Phe-RC (**b**, solid line) in pH 8.0 Tris-HCl buffer at 293 K.



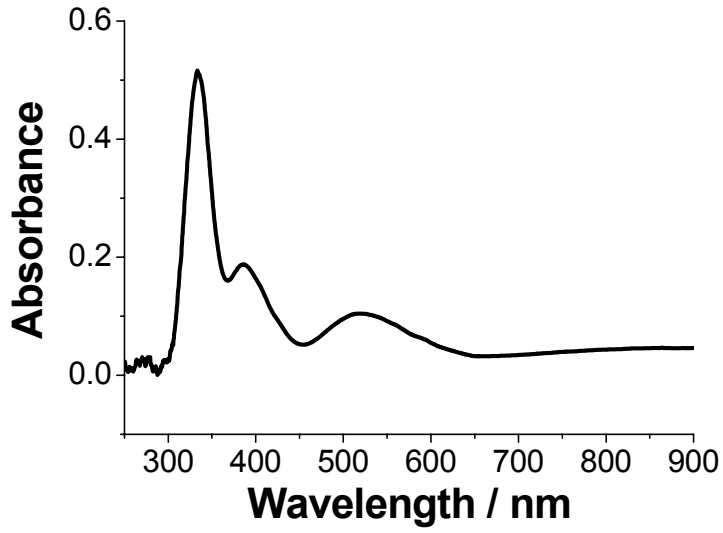
ESI-Fig. 2 Normalized CD spectra of native-RC (**a**, dash line) and Phe-RC (**b**, solid line) in pH 8.0 Tris-HCl buffer at 293 K.



ESI-Fig. 3 XRD pattern (left) and TEM image (right) of the tailor-made 3D-wormlike mesoporous WO_3-TiO_2.



ESI-Fig. 4 Nitrogen sorption isotherms and pore-size distribution plots (inset) for the calcined tailor-made 3D-wormlike mesoporous WO_3-TiO_2 film.



ESI-Fig. 5 UV-Vis-NIR absorption spectrum of the tailor-made 3D-wormlike mesoporous WO_3-TiO_2 film recorded using blank ITO as background.

ESI-Table 1 Structural Characterization of different matrix prepared for entrapping RC

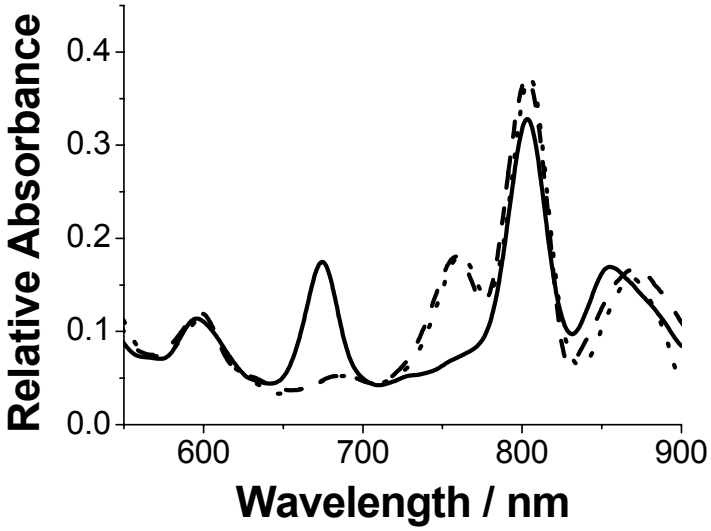
Matrix	Pore structure	Pore size (nm)	Thickness (nm) [b]	Contact angle [c]	M_{RC} immobilized (μmol/g) [d]
Al_2O_3 gel [a]	Disordered voids	Widely distributed	800 ~ 1200	31 ~ 33°	0.15 ~ 0.35
WO_3-TiO_2	2D-hexagonal	9.8 ± 0.8	~ 150	23.4°	0.29/0.26
WO_3-TiO_2	3D-wormlike	3.4 ± 0.3	~ 150	24.6°	0.31/0.32
WO_3-TiO_2	3D-wormlike	7.1 ± 0.6	~ 150	24.2°	0.63/0.59

[a] Preparation of both the bare and RC-embedded Al_2O_3 gel films was according to our work reported previously. ESI-Ref.. 3 [b] Thickness of the matrix was determined with a SEA 5120 element monitor MX instrument with an average of five measurements. [c] All data for contact angle were measured with a Phoenix-300 analyzer at 298 K in air with an average of four times. [d] Molar amount (M) listed here were calculated from the differential absorption spectra of RC solution before and after immobilization (molar extinction coefficient of RC at 802 nm is ca. 2.88 x 10^5 M^{-1} cm^{-1}) with an average of three measurements. The data presented in front of and behind the diagonal are the M for native-RC and Phe-RC, respectively.

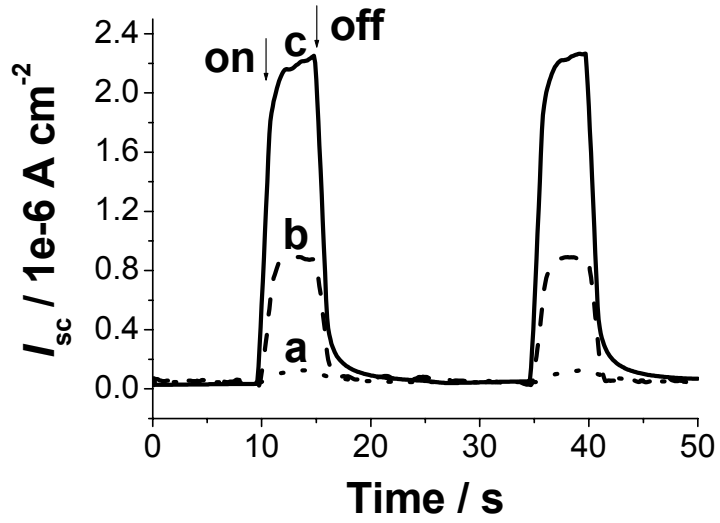
ESI-Table 2 The ultrafast pump-probe dynamics of native-RC and Phe-RC in buffer [a]

Sample	Excitation wavelength (nm)	τ_1 (fs)	τ_2 (ps)	τ_3
Native-RC	800	220	2.0	N/A
Phe-RC	800	450	3.0	N/A
Native-RC	850	130	2.6	N/A
Phe-RC	850	310	4.2	N/A

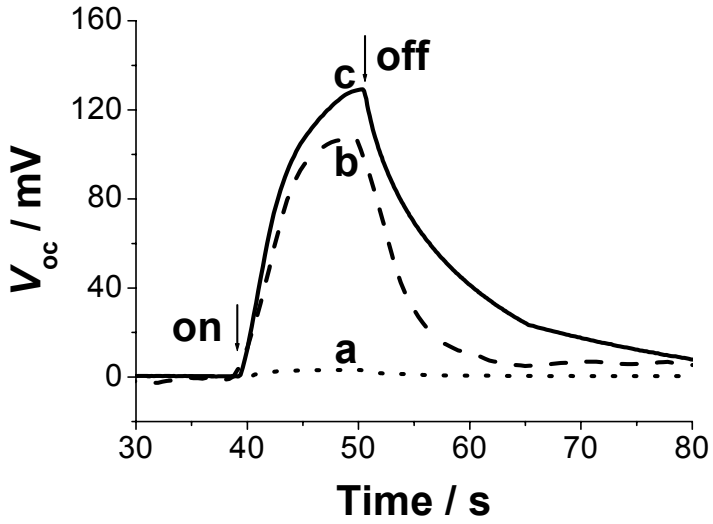
[a] τ_1(excited at 800 nm): B* → P_+ → P_-, τ_1(excited at 850 nm): P_+ → P_-; τ_2(excited at 800 nm, 850 nm): P_- → P^+Bphe^- (P_- → P^+Bchl^-); τ_3(excited at 800nm, 850 nm): P^+Bphe^- (P^+Bchl^-) → $P^+Q_A^-$



ESI-Fig. 6 NIR-Vis absorption spectra of the native-RC/Al_2O_3 film (dot line), native-RC/WO_3-TiO_2 film (dash line), and Phe-RC/WO_3-TiO_2 film (solid line) at 293 K. Absorption of blank Al_2O_3 and WO_3-TiO_2 films was subtracted as background.



ESI-Fig. 7 Short-circuit photocurrent (I_{sc}) responses of the native-RC/Al_2O_3 film (**a**, dot line), native-RC/WO_3-TiO_2 film (**b**, dash line), and Phe-RC/WO_3-TiO_2 film (**c**, solid line) in pH 8.0 Tris-HCl buffer containing 8 mM sodium dithionite illuminated with a 20 W incandescent lamp coupled with a filter ($\lambda > 600$ nm, $I_{inc} = 0.1$ mW cm^{-2}). The bias was set at the open-circuit voltage.



ESI-Fig. 8 Open-circuit photovoltage (V_{oc}) responses of the native-RC/Al_2O_3 film (**a**, dot line), native-RC/WO_3-TiO_2 film (**b**, dash line), and Phe-RC/WO_3-TiO_2 film (**c**, solid line) in pH 8.0

Tris-HCl buffer containing 8 mM sodium dithionite illuminated with a 20 W incandescent lamp coupled with a filter (K> 600 nm, I_{inc} = 0.1 mW cm^{-2})

ESI-Table 3 Photoelectric performance of different RC-modified electrodes [a]

Sample	M_{RC} (μmol/g)	I_{sc} (μA cm^{-2})	V_{oc} (mV)	IPCE% (at 800 nm)
Phe-RC/WO_3-TiO_2 (**1**)	0.59	2.2	130	23
Native-RC/WO_3-TiO_2 (**2**)	0.63	0.9	108	11
Native-RC/Al_2O_3 (**3**)	0.35	0.08	3	1

[a] Short-circuit photocurrent (I_{sc}) (detected at λ > 600 nm, I_{inc} = 0.1 mW cm^{-2}), open-circuit photovoltage (V_{oc}) (detected at λ > 600 nm, I_{inc} = 0.1 mW cm^{-2}), and incident photon-to-current conversion efficiency (IPCE) listed here were all measured in pH 8.0 Tris-HCl buffer containing 8 mM sodium dithionite with an average of five measurements.

ESI-References:

1. X.H. Zeng, H. Yu, Y.Q. Wu, M.J. Wu, J.M. Wei, H.X. Song and C.H. Xu, *Acta. Biochim. Biophys. Sinica.* 1997, **29**, 46.

2. M. Meyer and H. Scheer, *Photosynth. Res.* 1995, **44**, 55.

3. J.Q. Zhao, N. Ma, B.H. Liu, Y.L. Zhou, C.H. Xu and J.L. Kong, *J. Photochem. Photobiol. A: Chem.* 2002, **152**, 53.