Supporting Information

Proteorhodopsin-based Biohybrid Light-powering pH Sensor †

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Supporting 1.The preparation of reconstituted-pR

The TCM PR-expression plasmid the same as the reported[1] was provided by Dr. Deliang Chen, and used to transform into *Escherichia coli* UT5600 cells (the *E.coli* outer-membrane-deficient strain UT5600 (ompT$^-$)). The LB growth medium was supplemented by *Ampicillin* and *Streptomycin* (final concentration 100 μg/mL and 10 μg/mL) and cultivated at 30 ℃ until an OD600 of 0.4-0.6 was reached. Induction was started by adding 0.2% L(+)-Arabinose and 50-100 mM all-trans retinal (Toronto research chemicals inc.), and cultivation was continued for another 3-5 h. The cells were collected by centrifuging (7000g, 15min, 4 ℃) and the pellets were suspended in Buffer I (100 mM NaCl, 100 μg/mL lysozyme, 100 μg/mL DNAse I) to remove cell walls by enzymolysis for 3-5 h. The protoplasts were collected by centrifuging (7000g, 30min, 4 ℃) and sonicated in Buffer II (50 mM Tris-HCl, 5 mM $MgCl_2$, 0.2 mM PMSF, pH 7.0). The membrane fraction was collected by centrifugation (10000g, 1.5h, 4 ℃) and then sonicated in Buffer III (5 mM imidazole, 50 mM Tris-HCl, 300 mM NaCl, 1% Triton X-100, pH 7.0). The unsolubilizd portions were removed by centrifugation (13000g, 1.5h, 4℃) and the supernatant was applied to a Ni-NTA agarose (QIAGEN, Shanghai, China) column after a 0.22 μm filter.

After the adsorption of proteins, nonspecific materials were removed from the column by washing with Buffer W (50 mM imidazole, 50 mM Tris-HCl, 300 mM NaCl, 0.1% Triton X-100, pH 7.0). The elution of pR was carried out by applying Buffer E (300 mM imidazole, 50 mM Tris-HCl, 300 mM NaCl, 0.1% Triton X-100, pH

7.0). The concentration of pR solution was enhanced by ultra-centrifuging in a Mw 30000 ultra-centrifuging filter. The amount of pR was estimated using a molar extinction coefficient of 50000 at 525 nm, and Phosphatidylcholine (PC) from soybean (Beijing Biodee biotechnology Co. Ltd) in 25% v/v DMF solution was added at a pR:PC molar ratio of 1:50. The detergent was removed by applying into a SM-2 Adsorbent Bio-Beads (Bio-Rad laboratories, Inc.) column to form reconstituted-pR. All chemicals used were of the highest grade commercially available.

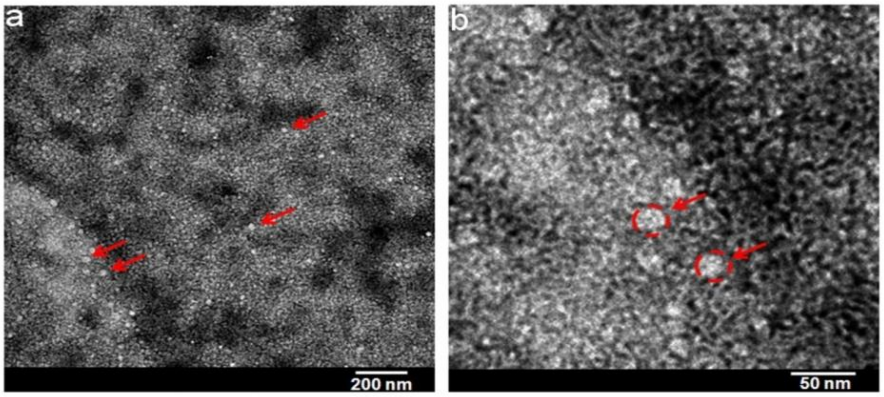

Fig. S1 Transmission electron microscope (TEM) examination: To investigate the homogeneity of the prepared protein, transmission electron microscopy (TEM, Philips TECNAI-20) was conducted before deposition. As shown, nanoscale protein particles are well distributed in the scope.

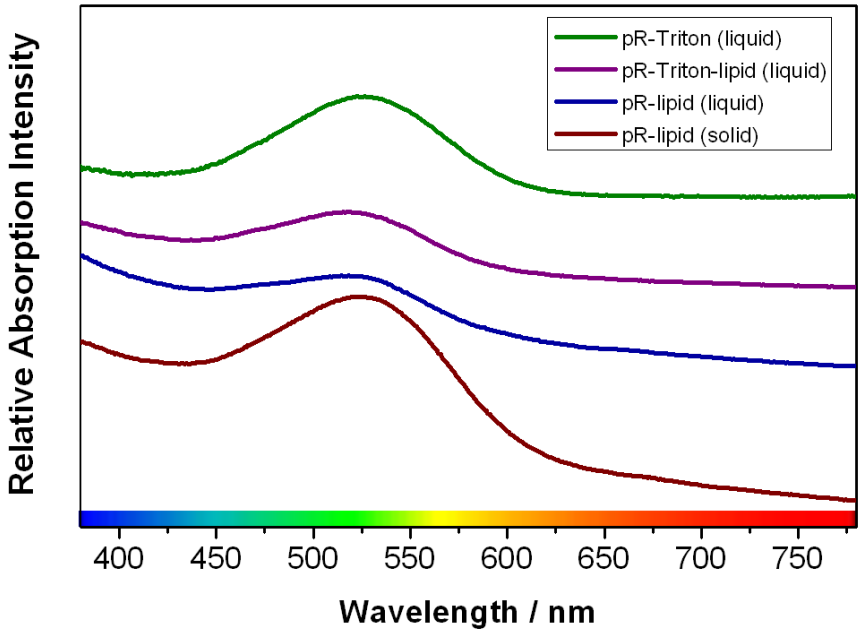

Fig. S2 UV-*vis* spectrum examination: UV-*vis* spectra tests were conducted with pR in Buffer E solution, pR reconstitution process and reconstituted-pR deposited on electrode. Comparing the characteristic absorption peaks, the Uv-*vis* spectrum displays the little-changed absorption peaks around 525 nm, demonstrating appreciable preservation of protein conformation of Green-pR.

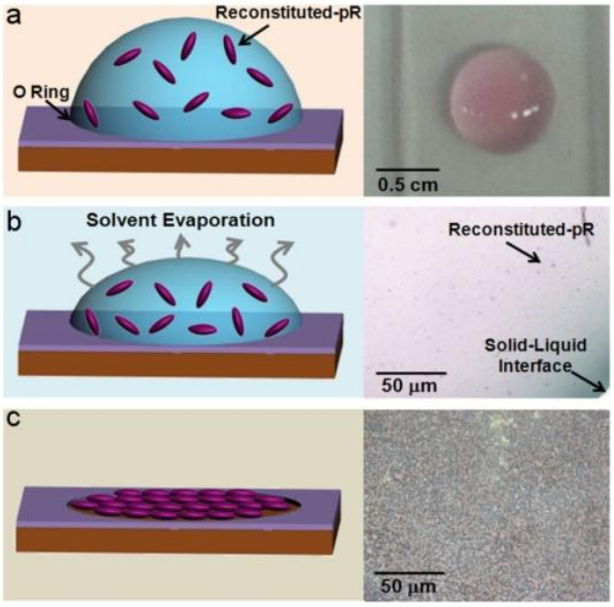

Fig. S3 Reconstituted-pR deposition by evaporation-induced assembly: As widely used in nanoparticles assembling[2, 3], evaporation-induced assembly is chosen to depose reconstituted-pR onto the electrode. The optical micrographs of reconstituted-pR deposition process were investigated through an optical microscope (Vision Engineering Co., UK), which was coupled to a CCD camera and connected to a desktop computer. (a) A drop of reconstituted-pR suspension (in 10mM PBS, pH 7.4) of volume 50 μL was deposited in an o-ring (radius, r=7mm) on the cleaned ITO glass. Due to the suspension state, the membrane patches are distributed inside the solution with random direction. (b) Along with the process of solvent evaporation, the membrane patches are induced by several different forces to assemble[4]. Unlike critical sphere particles, the membrane patches are well-distributed deposited on the electrode surface with suppression of the coffee-ring effect[5], as shown on the right. (c) After the reconstituted-pR deposition, a reconstituted-pR layer was formed densely and homogeneously on the surface of the ITO glass. A successive distilled water washing was applied on the deposited membrane to remove the weakly-bound proteins.

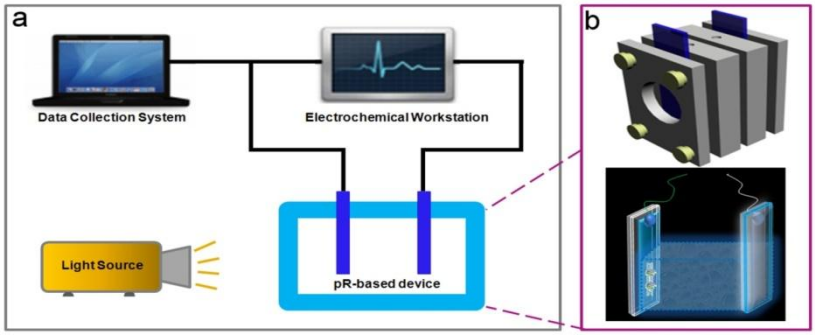

Fig. S4 Photoelectric measuring system: Photoelectric measurements were carried out in a custom-designed cell. The electrolyte solution of 100 mM PBS was filled in a Teflon O-ring (20 mm inside diameter, 2 mm cross section) with two electrodes made with transparent indium tin oxide (ITO) glass (14 mm × 35 mm × 1mm) fixing on each side. The ITO glass electrode exhibits surface resistance of 4–8 Ω (CG-40IN-1115, Delta Technologies). A continuous wave (cw) broadband light was used as the excitation sources. The photocurrent was measured under the bias voltage of 0.1 V, which was added between positive and negative electrodes. The light was introduced from a Xenon Lamp (Beijing Perfectlight Technology Co. Lt, China) through an optical filter (500-800 nm) and modulated with a photochopper (Model SR540 Stanford Research System, Inc.). The photocurrent signal generated by the device was collected by an electrochemical station (CHI 660D, Shanghai Chenhua Apparatus Co. China). Each test was repeated ten times to obtain the average current value at different external stimulus.

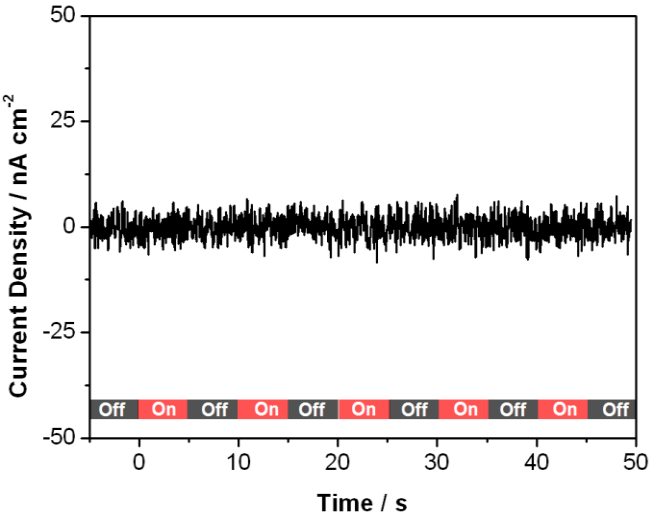

Fig. S5 ITO with phosphatidylcholine (PC) photoelectric detection as a control: PC patches without pR protein were prepared and deposited on the ITO electrode as the same method as the reconstituted-pR. Under the same electrochemical measurement, the PC-based device exhibits no apparent photoelectric responses while illuminating with flickering light.

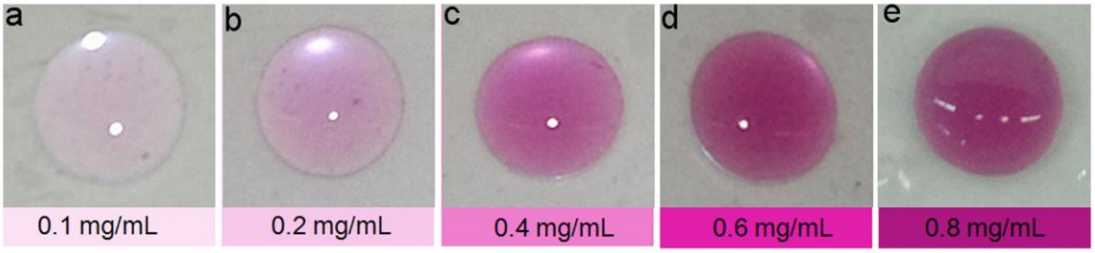

Fig. S6 Concentration dependent deposition: In this process, reconstituted-pR suspensions with different concentrations were deposited on the transparent electrode. Along the increasing of pR concentration, the characteristic colour of magenta becomes more significant.

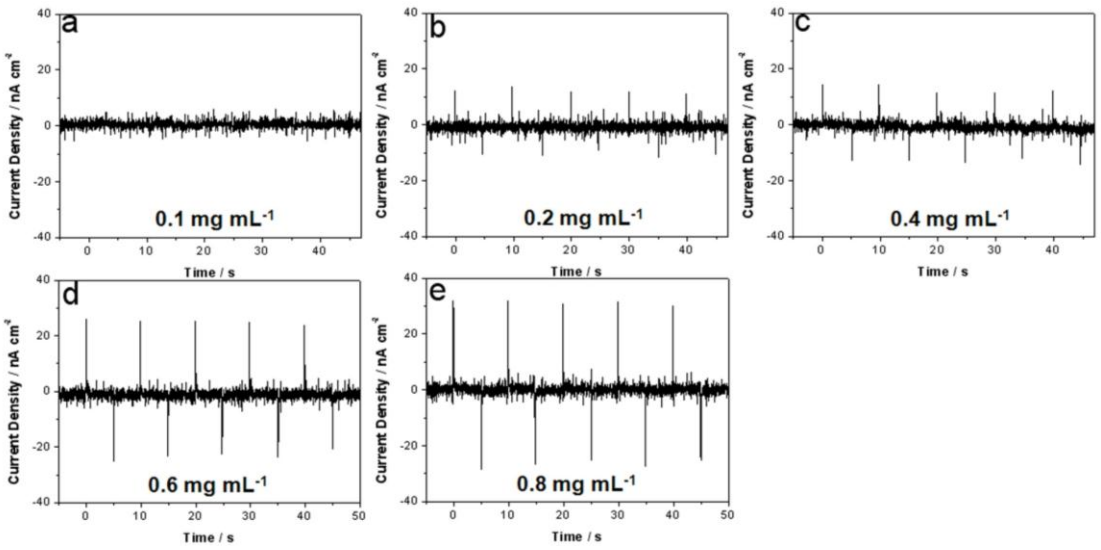

Fig. S7 Photocurrent regulation with pR concentration: pR-based photoelectric devices with a series of pR concentration,

0.1mg mL^{-1}, 0.2 mg mL^{-1}, 0.4 mg mL^{-1}, 0.6 mg mL^{-1} and 0.8 mg mL^{-1} were investigated respectively in our experiment. In this range, the detected photocurrent enhances with the protein concentration increase. All the samples were tested under chronoamperometric measurement with 0.1 V bias voltages, neutral electrolyte environment (100mM PBS, pH 7.0) and same light intensity of 318 mW cm^{-2}.

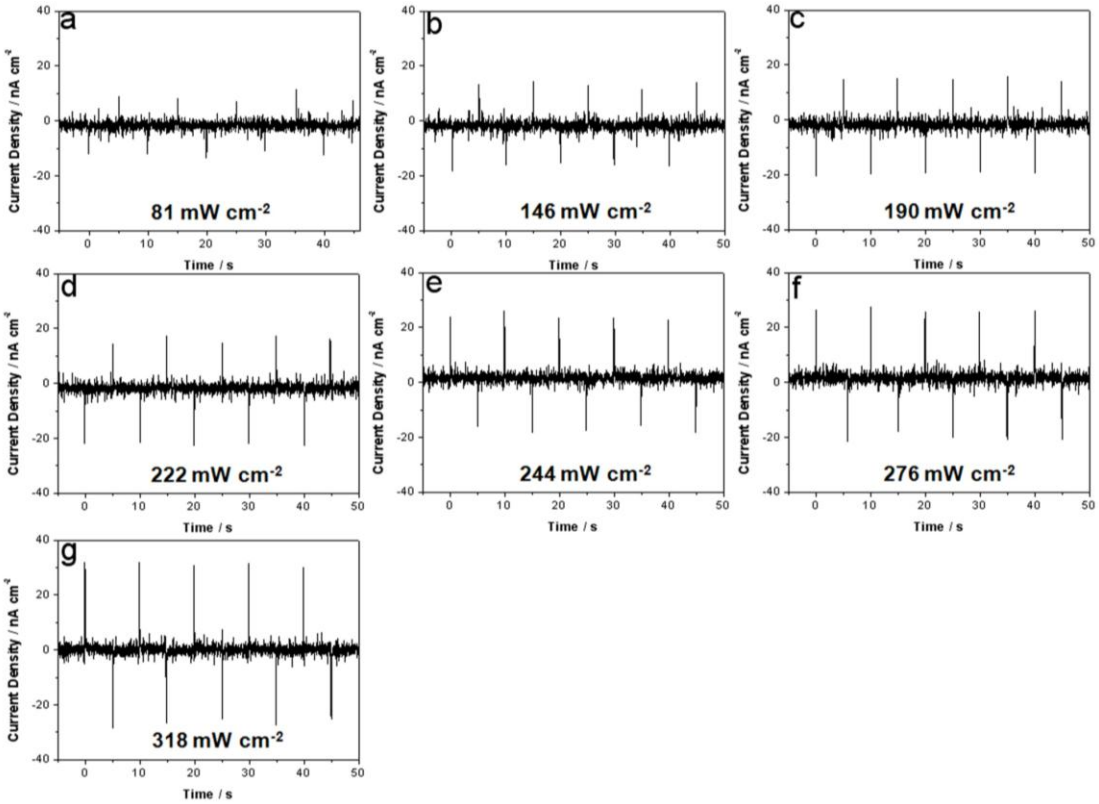

Fig. S8 Photocurrent regulation with stimulating light intensity: Light intensity of 81 mW cm^{-2}, 146 mW cm^{-2}, 190 mW cm^{-2}, 222 mW cm^{-2}, 244 mW cm^{-2}, 276 mW cm^{-2} and 318 mW cm^{-2} was applied in this regulation respectively. The photocurrent increases steadily with the reinforcement of light intensity in this range. The pR concentration of all the samples was fixed with 0.8 mg mL^{-1}. All the photocurrent was tested under chronoamperometric measurement with 0.1 V bias voltages and neutral electrolyte environment (100mM PBS, pH 7.0).

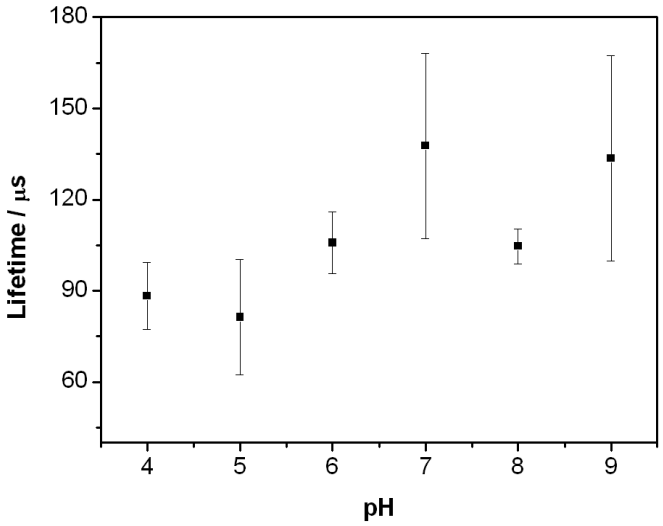

Fig. S9 The lifetime of K or O intermediate exhibits no obvious relationship with environmental pH. The detection light wavelength was set on 580 nm to investigate K or O intermediate photokinetic property. The lifetime of K or O intermediate displays unregulated behaviour upon pH variation.

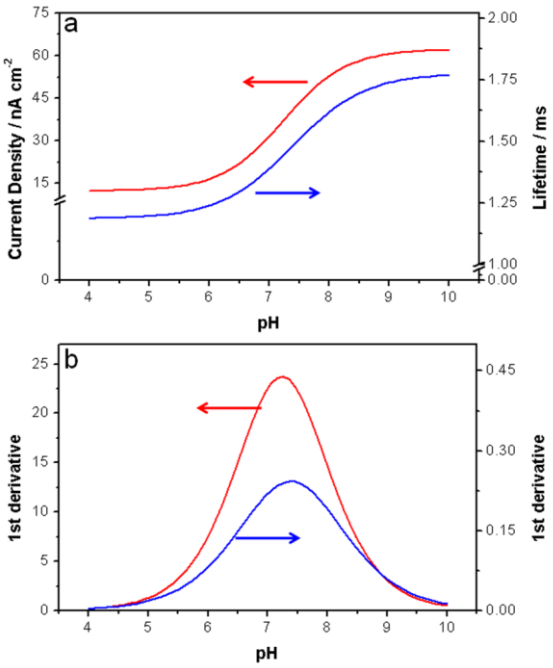

Fig. S10 The behaviour of photocurrent and M intermediate lifetime under a series of environmental pH: (a) the fitting curves of photocurrent (red) and M intermediate lifetime (blue) from pH 4 to 10. (b) The 1st derivative curves of photocurrent (red) and M intermediate lifetime (blue) from pH 4 to 10. Each curve shows a peak around pH 7.3, which implies the most sensitive rate of change upon environmental pH.

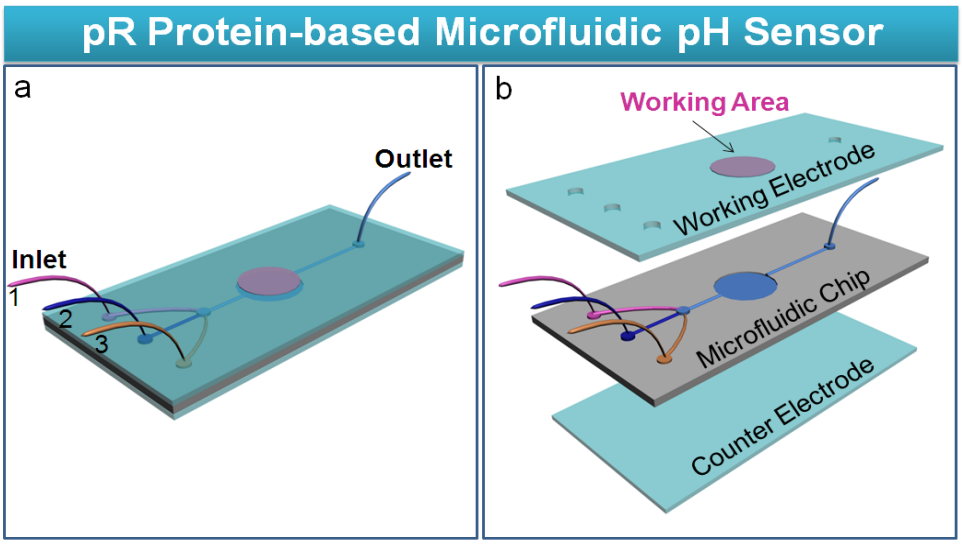

Fig. S11 Design of pR-based microfluidic pH sensor: A microfluidic device is designed with 3 channels as separated inlets to conduct influent into the pR-based working chamber, which has adjustable volume (< 20 μL). The two electrodes are made by transparent ITO glasses while the working electrode is deposited by reconstituted-pRs. The influent injecting rate is controlled by a micro flow pump within 5-200 μL/min.

Reference:

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