--- Electronic Supplementary Information ---

Cell Surface Carbohydrates Evaluation via Photoelectrochemical Approach

Wei-Wei Zhao, Long Zhang, Jing-Juan Xu* and Hong-Yuan Chen

State key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

E-mail address: xujj@nju.edu.cn (J.J. Xu)

Experimental Section

Chemicals and materials

Indium tin oxide (ITO) with type of N-STN-S1-10 was purchased from China Southern Glass Holding Co., Ltd. (China), with coating thickness of 180 ± 20 nm and sheet resistance of 8.1 ± 0.6 $\Omega \cdot cm^{-2}$. TiO_2 (P25), 3-aminophenylboronic acid monohydrate (APBA), 4-dimethylamiopyridine (DMAP), 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), neuraminidase (sialidase) and 4,4',4'',4'''-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)-tetrakis(benzoic acid) (TCPP, 75%) were purchased from Sigma-Aldrich (USA). Acridine orange (AO) was products from Amresco (USA). Ascorbic acid (AA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Dichloromethane, methanol and triethylamine were purchased from Aladdin Reagent Inc.(China). Phosphate buffered saline (PBS) (pH 7.4) contained 137mM NaCl, 2.7 mM KCl, 87.2 mM $Na_2HPO_4 \cdot 12H_2O$, and 14.1 mM KH_2PO_4. All aqueous solutions were prepared using ultrapure water (Milli-Q, Millipore).

Apparatus

Photoelectrochemical (PEC) measurements were performed with a homemade PEC system equipped with a 500W Xe lamp and a monochromator. Photocurrent was measured on a CHI 750a electrochemical workstation (China) with a three-electrode system: a modified ITO electrode with a geometrical area of 0.25 ± 0.01 cm^2 as the working electrode, a Pt wire as the counter electrode

and a saturated Ag/AgCl electrode as the reference electrode. All the photocurrent measurements were performed at a constant potential of 0V (versus Ag/AgCl). A 0.1M PBS containing 0.1M AA was used as the blank solution for photocurrent measurements, which was degassed by highly pure nitrogen for 15 min before PEC experiments and then kept over a N_2 atmosphere for the entire experimental process. Electrochemical impedance spectroscopy (EIS) was carried out with an Autolab potentiostat/galvanostat (PGSTAT 30, Eco Chemie B.V., Utrecht, The Netherlands) and controlled by FRA 4.9 softwares with a three-electrode system as that in the PEC detection in 0.1M KCl containing 5.0mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe. Scanning electron microscopy (SEM) was carried out on a Hitachi S-4800 scanning electron microscope (Hitachi corporation, Japan). The UV-vis absorption spectrum was performed on a Shimadzu UV-3600 UV/vis spectrophotometer (Shimadzu Co., Japan). A DMIRE2 inverted fluorescence microscope (Leica, Germany) equipped with a DP71 CCD (Olympus, Japan) was used for fluorescence imaging.

Cell line, culture and treatment

The HL-60 cell line was kindly provided by the Gulou Hospital (Nanjing, China). HL-60 cells were cultured in RPMI 1640 medium (GIBCO) supplemented with fetal bovine serum (10%) (FBS, GIBCO), penicillin (60 $\mu g \cdot mL^{-1}$), and streptomycin (100 $\mu g \cdot mL^{-1}$)at 37℃ in a humid atmosphere containing CO_2 (5%). After 48 h, the cells were collected and separated from the medium by centrifugation at 1000 rpm for 5 min and then washed twice with sterile PBS (pH 7.4). The sediment was resuspended in PBS to obtain a homogeneous cell suspension at a certain concentration. The cell number was determined using a Petroff-Hausser cell counter (USA). Sialidase treatment was performed by incubating the cells in a culture medium containing 10μg/mL sialidase for varying times.

Construction of PEC sensor

The ITO slices were made as the working electrode. ITO slices were cleaned by immersion in 2 M boiling KOH solution solved in 2-propanol for 15min, followed by washing copiously with water and dried at 120℃ for 2 h. 0.25 g TiO_2 powder was dispersed ultrasonically in 50 mL water (the concentration of the suspension is 5.0 mg/mL), and then 30 μL of the suspension was applied

onto a piece of ITO slice with fixed area of ~0.2 cm^2. After drying in air, the film was sintered in a Muffle furnace at 450 ℃ for 30min and finally cooled down to room temperature. An ITO/TiO_2 electrode was submerged into a stock solution of porphyrin (1 mM, 0.8 mg/mL, in methanol) for 30 min before it was washed by water and dried under air naturally. The functionalized electrode was then submerged into another dichloromethane solution which contained APBA (1.6 mg/mL), EDC (2.4 mg/mL), DMAP (0.6mg/mL) and triethylamine (1.0 mg/mL) at room temperature, followed by washing with water and drying under air. The above-functionalized ITO/TiO_2/porphyrin/APBA electrode was put into the cell solution of certain concentration in PBS (pH 7.4) and was incubated for one hour, followed by washing with PBS (pH 7.4) to remove the cells that were not captured.

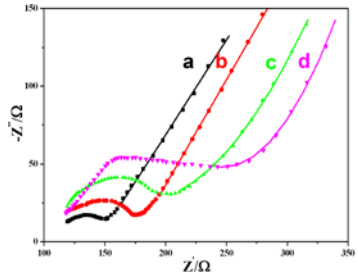

Figure S1. Electrochemical impedance Nyquist plot of electrodes (a) ITO/titania, (b) ITO/titania/porphyrin, (c) ITO/titania/porphyrin/APBA and (d) ITO/titania/ porphyrin/APBA/cell. The frequency range is between 0.01 and 100 000 Hz with applied voltage of 5 mV.

EIS was employed to monitor the assembly process of the sensor. ESI Fig. 1 shows the EIS changes of different electrodes in $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution. After functionalizing the electrodes with porphyrin and APBA sequentially, increased electrochemical impedance was observed, suggesting the effective bridging of titania and APBA by employed porphyrin. The passivation layers consisted of porphyrin and APBA hindered the electron transfer between the redox probe and electrodes. After cell attachment, due to the excellent insulating properties of cells, further barrier effect would be produced, resulting in an increase in the electron-transfer resistance.[1]

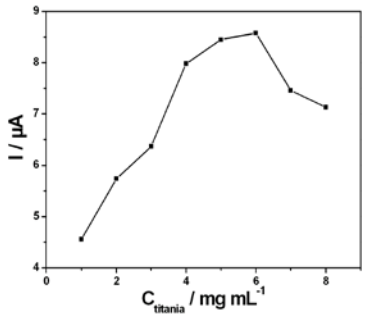

Figure S2. The change of photocurrent on ITO/titania/porphyrin/APBA as a result of titania concentration change.

We further did the experiments to reveal the influence of titania membrane thickness on the photoelectric effect of the sensor. Increasing the concentration of titania suspension could increase the thickness of the titania film,[2] so in this work, the membrane thickness was tuned by the concentration of titania suspensions. Experimentally, titania suspensions from 1.0-8.0 mg/mL was prepared and used to assemble the corresponding sensors, and the photocurrents were recorded with the results shown in ESI Figure 2. As can be seen, with the concentrations of the titania suspension increased, the photocurrent of the modified electrode enhanced. As the thickness of titania membrane increased, the current strength raised significantly, and the electrode made by 5.0 mg/mL titania suspension showed the maximum photocurrent intensity. The phenomenon was due to that the titania layer possessed the mesoporous structure (as demonstrated by Figure 1A) and that increasing the thickness of the titania film would result in the increase of the surface area.[3] In our work, the visible light responsibility of the semiconductor hybrid originated from the sensitization of porphyrin on the titania surface. Obviously, the increment of surface area would lead to more porphyrin adsorption and hence higher interfacial electron communication between electron holes and AA molecules. Thus, the photocurrent intensity increased with the increase of titania thickness due to the enhanced porphyrin adsorption. However, the diffusion resistance for electron motion also increased in thicker titania film due to the increase of the surface recombination centers (for higher possibility of hole-electron re-association).[2] As a result, thicker titania film caused lower photocurrent strength. Thus, the optimal concentration of 5.0 mg/mL titania suspension was selected in the experiments.

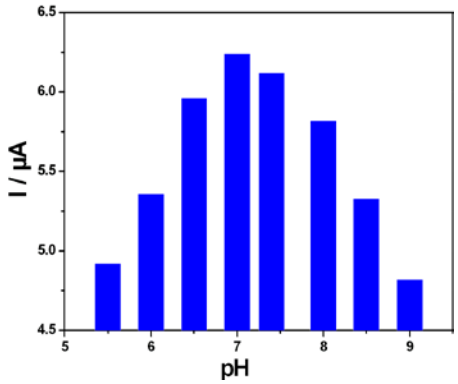

Figure S3. Illustration of the pH effect on the photocurrent intensity produced by ITO/titania/porphyrin/APBA/cell after 0.5 h incubation.

Since the function of APBA in principle associated exquisitely with its pKa (*ca.* 8-9), the influence of ambient pH for the cell incubation were further studied with results shown in Figure 2B. Incubation time of 0.5 hour and pH range of 5.5-9.0 were selected because the cells tend to unstable in both more acidic and basic solutions. Experimental results demonstrated the signal reached its maximum in 7.0-7.4 pH range, indicating the predominant APBA-SA binding which rendered least amount of captured cells and hence minimal steric effect. The lowered photocurrent at base condition could be satisfactorily attributed to the ordinary nonspecific APBA bonding with various *cis*-diols, while the operation under weak acidic condition could also lead to intensified photocurrent decrease that perhaps caused by the thermodynamically favorable APBA-SA binding therein. Although more cells could be adsorbed in non-neutral conditions, physiological pH was still chosen as the optimized condition to retain the biological activity of living cells for the operation of detection.

REFERENCES

(1) X. Zhong, H. J. Bai, J. J. Xu, H. Y. Chen, Y. H. Zhu, Adv. Funct. Mater., 2010, 20, 922-999.

(2) D. Kuang, S. Ito, B.Wenger, C. Klein, E. Jacques, J. Am. Chem. Soc. 2006, 128, 4146-4154.

(3) Z. S. Wang, H. Kawauchi, T. Kashima, H. Arakawa, Coord. Chem. Rev. 2004, 248, 1381-1389.