

Electronic Supplementary Information (ESI)

Localized surface plasmon resonance-enhanced photoelectrochemical biosensing strategy for highly sensitive and scatheless cell assay under red light excitation

Ruyan Li, Rong Yan, Jianchun Bao, Wenwen Tu* and Zhihui Dai*

*Jiangsu Collaborative Innovation Center of Biomedical Functional Materials and
Jiangsu Key Laboratory of Biofunctional Materials, School of Chemistry and
Materials Science, Nanjing Normal University, Nanjing 210023, P. R. China*

*Corresponding author. E-mail: daizhihui@njnu.edu.cn, wwt@njnu.edu.cn; Fax:
+86-25-85891051; Tel: +86-25-85891051

1. Experimental section

1.1 Materials and reagents. Tungsten (IV) sulfide (powder, 2 μm , 99%), gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), tris (2-carboxyethyl) phosphine (TCEP), bovine serum albumin (BSA), cysteamine, 1-Ethyl-3-[3-(dimethylamino)-propyl] carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Polyacrylic acid (PAA, with an average molecular weight of 1800) was obtained from J&L Biological Co., Ltd. (Shanghai, China). Ascorbic acid (AA) and sodium borohydride (NaBH_4) were obtained from Sinopharm Chemical Reagent Co, Ltd (Shanghai, China). All other chemicals were of analytical grade. The ultrapure water used in all of the assays was obtained from a Millipore water purification system ($\geq 18 \text{ M}\Omega \text{ cm}$, Millipore SAS Corporation, France). Phosphate buffer saline (PBS, pH 7.4, 0.1 M) as the supporting electrolyte was made from phosphate

($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}/\text{KH}_2\text{PO}_4$, 81:19 (molar ratio)) and sodium chloride dissolved in the solution with the final concentrations of 0.1 M. The washing solution was PBS (10 mM, pH 7.4, $C_{\text{NaCl}}=0.01$ M). The physiological PBS made of NaCl (136.75 mM), KH_2PO_4 (1.76 mM), $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (10.14 mM) and KCl (2.28 mM) was used to wash the cell culture fluid. The oligonucleotides were bought from Sangon Biological Engineering Technology & Company Ltd. (Shanghai, China) and purified using high-performance-liquid chromatography. The aptamer sequence was 5'-HS-GCAGTTGATCCTTTGGATACCCTGG-3'.

1.2 Apparatus. Scanning electron microscope (SEM) image was recorded on a JSM-7600F scanning electron microscope (JEOL, Japan). Transmission electron microscope (TEM) images were taken using a Hitachi H-7650 type transmission electron microscope (Hitachi, Japan). Atomic force microscope (AFM) image was obtained on a Nanoscope IIIa scanning probe microscope (Agilent, USA) using a tapping mode. Ultraviolet–visible (UV–vis) absorption spectra were obtained on Cary 60 spectrophotometer (Agilent, USA). Fourier transform infrared (FTIR) spectra were detected on Tensor 27 (Bruker, Germany) at room temperature. Electrochemical impedance spectroscopy (EIS) was carried out in 0.1 M KCl solution containing $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ (5 mM, 1:1) mixture as a redox probe with an Autolab potentiostat/galvanostat PGSTAT302N (Metrohm, Netherland) from 0.1 Hz to 100 kHz with a signal amplitude of 10 mV. The cells were cultured with a cell incubator (Thermo, USA). The PEC measurements were performed with a Zahner PEC workstation (Zahner, Germany). The PEC detection was carried out using a conventional three-electrode system at room temperature, where a modified indium tin oxide (ITO) electrode (sheet resistance, 20-25 Ω/sq) with an geometric area of 0.28 cm^2 used as a working electrode, a Ag/AgCl electrode used as a reference

electrode, and a platinum wire used as an auxiliary electrode. MTT assay was carried out on a microplate reader (Bio-Tek synergy, USA).

1.3 Synthesis of PAA modified WS₂ nanosheets and cysteamine-stabilized Au NPs.

The PAA modified WS₂ nanosheets were synthesized according to a previous report with a little modification.^{S1} It was a simple method to prepare water-soluble WS₂ nanosheets with a one-step sonication-assisted exfoliation of bulk WS₂. The detailed synthetic process was described as follows: 0.75 g bulk WS₂ powder and 0.25 g PAA were added into a 250 mL serum bottle, and then 100 mL ultrapure water was added into the reaction vessel as the solvent. The mixture was sonicated continuously for 8 h by an ordinary ultrasonic cleaner with 100 W power and 40 kHz frequency. After ultrasonic treatment, the upper suspension would gradually turn dark green, followed by removal of the large-size masses of WS₂ with centrifugation at 3000 rpm for 5 min. The supernatant was washed with ultrapure water several times to remove the excess PAA and collected by centrifugation at 10000 rpm for 5 min. Finally, the as-obtained black product was redispersed in water with a certain concentration and stored at 4 °C in the dark for further experiments.

The cysteamine-stabilized Au NPs were synthesized according to the previous protocol.^{S2} All of the glassware used in the synthetic process was cleaned in the newly prepared aqua regia (HNO₃:HCl=1:3), rinsed thoroughly with deionized water and dried under 70 °C before use. Briefly, 100 μL cysteamine solution (213 mM) was added into 10 mL HAuCl₄ solution (1.42 mM). After stirring continuously for 20 min, 2.5 μL NaBH₄ solution (10 mM) was added in the mixture for reducing HAuCl₄. With vigorously stirring for 15 min in dark, the brownish solution changed into clear wine red. The concentration of the resulting Au NPs solution was 10.5 nM, which was

evaluated by the original concentration of gold ion solution. The as-prepared Au NPs solution was stored in the refrigerator (4 °C) for further use.

1.4 Cell culture and treatment. The MCF-7 cells were cultured in a cell culture flask in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 µg/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO₂. The HepG2 cells and L02 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 µg/mL penicillin, and 100 µg/mL streptomycin at the same condition. After the cells grew to the logarithmic growth phase, they were collected and separated from the medium by centrifugation at 1000 rpm for 5 min, and then washed with sterile PBS (0.1 M, pH 7.4) twice. The cell sediment was resuspended in the PBS to obtain a homogeneous suspension. The concentration of cell suspension was determined using a blood counting chamber.

1.5 Fabrication of the PEC biosensor. The ITO electrode was cut into 5 cm×1.2 cm pieces cleaned with acetone, NaOH (1 M, V_{ethanol} : V_{water} = 1:1) and pure water, respectively, followed by drying in the dryer at 70 °C. Firstly, 25 µL of the WS₂ nanosheets solution (0.2 mg/mL) was dropped on the ITO electrode and dried at 35 °C in the vacuum oven. Then, the modified electrode was activated in 25 µL ultrapure water containing 10 mg/mL EDC and 5 mg/mL NHS for 60 min at room temperature. After rinsing carefully with 60 µL washing solution, 25 µL Au NPs solution was cast onto the modified electrode surface. The nanocomposites of WS₂ nanosheets/Au NPs were formed on the electrode surface by the classical EDC coupling reactions between the -COOH groups on the PAA-modified WS₂ nanosheets and -NH₂ groups of the cysteamine-stabilized Au NPs. After drying, conjugation of SH-aptamer on the nanocomposites modified electrode was achieved through Au-S bond. In Detail, TECP-activated SH-DNA (1 µM, 25 µL) was applied to the above-mentioned

electrode surface and incubated at 4 °C overnight for generating coordination bond between gold and sulfur. Subsequently, the electrode was washed with the washing solution twice carefully to remove the free DNA. Following this step, 25 μL (w/v, 2%) BSA was used to block nonspecific binding sites, and then it was washed with washing solution thoroughly to obtain the PEC biosensor.

1.6 Measurement procedure. The fabricated PEC biosensor was immersed in cell suspension at a certain concentration and incubated at 37 °C for 2 h, followed by washing with 0.01 M pH 7.4 PBS twice to remove the nonspecifically adsorbed cells. Different electrodes were used for continuous incubation of MCF-7 cells from low concentration to high value. Then the PEC biosensor was inserted into the supporting electrolyte containing 0.1 M AA as electron donor to record the photocurrent response at an applied potential of 0.1 V under 630 nm irradiation.

1.7 MTT assay. MTT assay of cell viability was used for evaluating the cytotoxicity of WS_2/Au NPs nanocomposites. Briefly, MCF-7 cells were seeded in 96-well plates ($\sim 1 \times 10^4$ cells per well) and cultured overnight under 5% CO_2 at 37 °C. Various concentrations of the WS_2/Au nanocomposites were added into the experimental groups, and the control groups were incubated under the same condition without the addition of WS_2/Au NPs nanocomposites. After 24h post-treatment, the medium was removed. The cells were washed with PBS twice before the addition of 10 μL MTT (5 mg/mL in PBS) and 90 μL serum-free medium, and then they were further incubated for 4 h. Next, the culture medium containing MTT was discarded and 100 μL dimethyl sulfoxide was added into each well to solubilize the formazan crystals precipitate with gentle shaking. At last, the absorbance was measured at 490 nm with a microplate reader.

2. Characterization

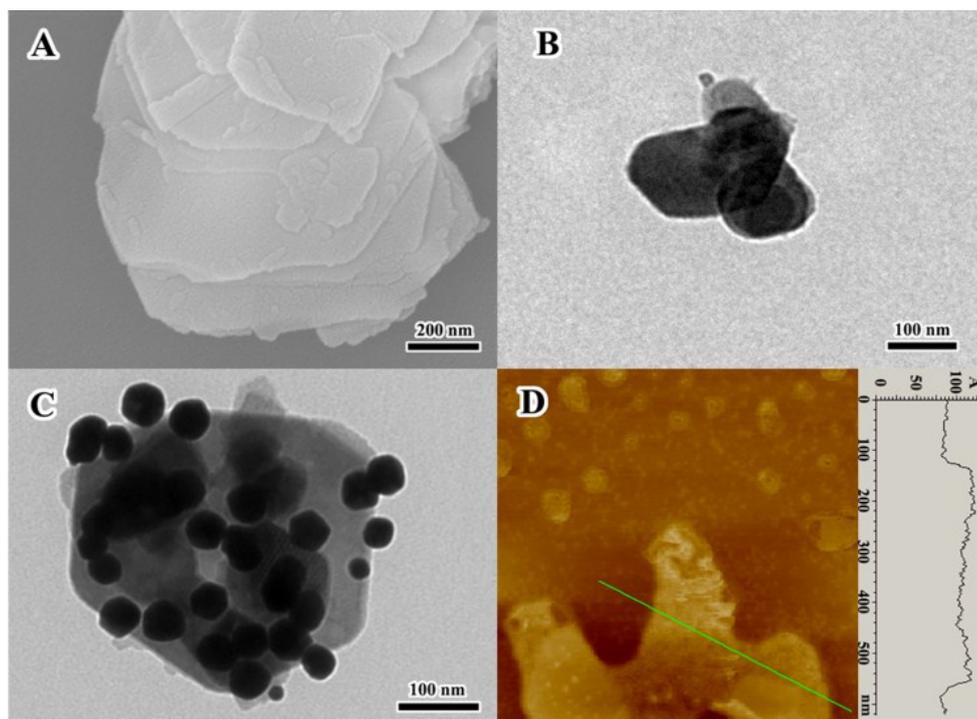


Fig. S1 (A) SEM image of the bulk WS₂. TEM images of (B) the as-synthesized layered WS₂ nanosheets and (C) the as-prepared WS₂/Au NPs nanocomposites. (D) AFM image of the layered WS₂ nanosheets and the corresponding height image.

It was clearly observed that several sheets of bulk WS₂ overlapped each other (Fig. S1-A). After ultrasonic processing of bulk WS₂ suspension in the presence of PAA molecules, bulk WS₂ was exfoliated into layered WS₂ nanosheets with the size ranging from tens to a few hundreds nanometers which were much smaller than the bulk WS₂ material (Fig. S1-B), owing to the introduction of water-soluble PAA molecules on WS₂ nanosheets. The strong coordination effect of the carboxyl group in PAA molecules with the unsaturated W atom of WS₂ might facilitate the exfoliation and functionalization of WS₂ nanosheets. Au NPs uniformly distributed throughout the thin layer of WS₂ nanosheets as shown in the TEM image of WS₂/Au NPs nanocomposites (Fig. S1-C). The average diameter of the Au NPs was estimated to be

40 nm. The two-dimension thin layer nanosheets with higher specific surface area greatly increased the load capacity of Au NPs, which was beneficial for developing LSPR-enhanced PEC biosensing platform. The average thickness of layered WS₂ nanosheets was measured to be 5.0 nm (Fig. S1-D). Considering the thickness of 0.8–1.2 nm for a monolayer WS₂ nanosheet prepared via chemical methods,^{S3} it was about 5 layers of WS₂ nanosheets.

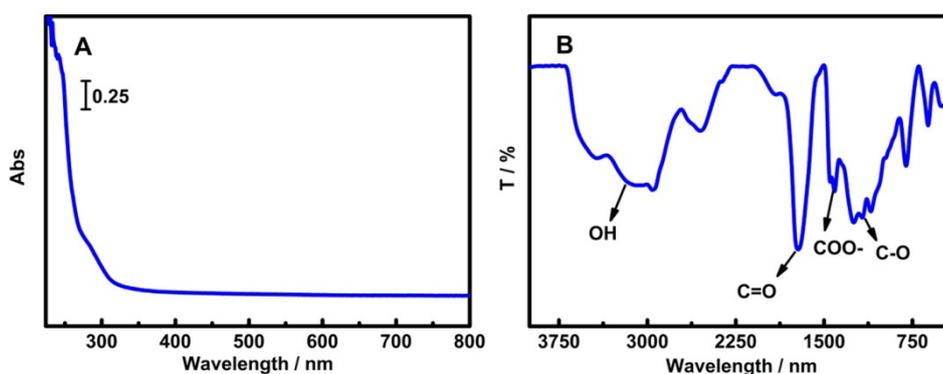


Fig. S2 (A) UV-vis spectrum of the PAA molecules and (B) FT-IR spectrum of PAA molecules.

As shown in Fig. S2-A, the PAA molecules showed no obvious absorption peak from ~300 nm to 800 nm. And the obvious characteristic peaks of carboxyl group were observed on the FT-IR spectrum of PAA molecules (Fig. S2-B).

3. PEC responses

As shown in Fig. S3, No obvious photocurrent response was observed at Au NPs /ITO electrode (curve a), WS₂ nanosheets modified electrode exhibited a slight photocurrent of ~80 nA (curve b). After the assembly of Au NPs on WS₂/ITO electrode, the photocurrent enlarged to ~2510 nA (curve c), which was ~31 times than that of WS₂ nanosheets.

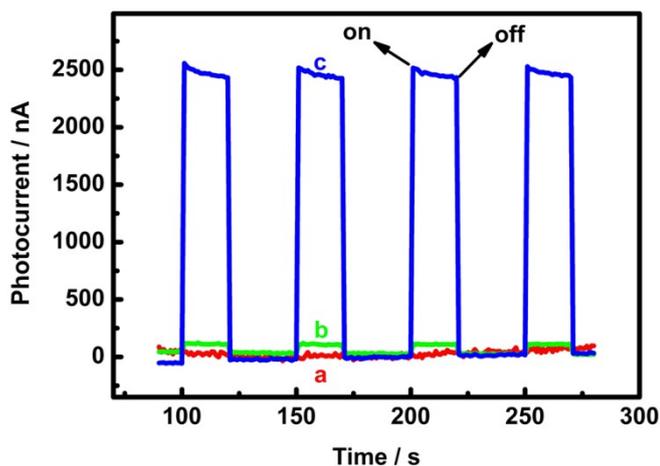


Fig. S3 Photocurrent responses of (a) Au NPs/ITO, (b) WS₂/ITO and (c) Au NPs/WS₂/ITO electrodes in 0.1 M PBS containing 0.1 M AA.

4. Optimization of the detection conditions

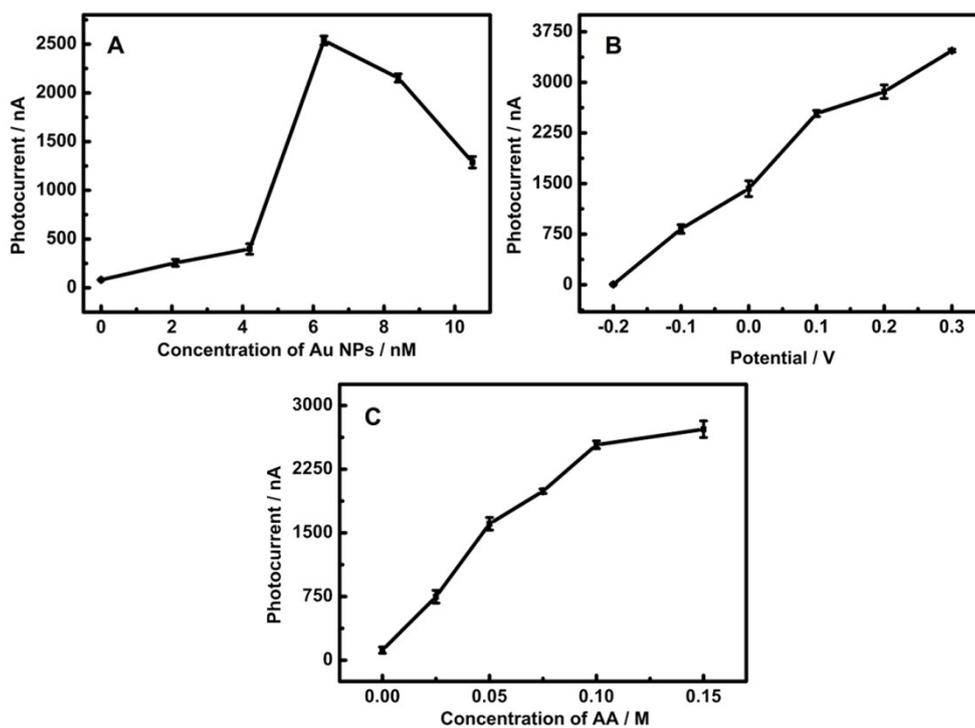


Fig. S4 Influence of Au NPs concentration (A), bias potential (B) and AA concentration (C) on the PEC response of Au NPs/WS₂/ITO electrode. The PEC measurements were recorded in 0.1 M PBS. The error bars were derived from the standard deviation of three measurements.

The conjugation of the WS₂ nanosheets with the Au NPs facilitated to a vast enhancement of photocurrent. Thus, the concentration of Au NPs was studied to obtain the optimal PEC response. The photocurrent was recorded in the electrolyte containing 0.1 M AA under 630 nm illumination by applying the bias potential at 0.1 V. The photocurrent intensity rose slowly with increasing the Au NPs concentration from 0 nM to 4.2 nM (Figure S4-A). A sharp promotion of the photocurrent intensity was noticed and reached a maximum value at the Au NPs concentration of 6.3 nM. Therefore, 6.3 nM was selected as the optimal concentration of Au NPs for the PEC determination.

The bias potential played an important role in the generation of PEC signals since it influenced the recombination of the photoinduced electrons and holes. The photocurrent responses of the Au NPs/WS₂/ITO electrodes were recorded in electrolyte containing 0.1 M AA under 630 nm illumination by applying the bias potential at the range of -0.2 V to 0.3V. As shown in Figure S4-B, the photocurrent intensity went up dramatically from -0.2 to 0.1 V, and the growth trends become slow from 0.1 to 0.3 V. The photocurrent at 0.1 V and 0 V was 73.13% and 41.03% of that at 0.3 V, respectively. Although less interference was from other species coexisting in the samples without applied potential, the photocurrent at 0 V was not sensitive enough for PEC measurement. Considering the above aspect, 0.1 V was chosen as the optimal applied potential for the PEC detection.

To achieve stable and enhanced photocurrent, AA was used as electron donors to prevent the recombination of electrons and holes. The effect of AA concentration on the photocurrent was tested under 630 nm illumination by applying the bias potential at 0.1 V. The photocurrent response was found to enhance with the change of AA concentration from 0 to 0.1 M (Figure S4-C). While the AA concentration further

increased to 0.15 M, the photocurrent response leveled off, implying the equilibrium of PEC reaction. Consequently, 0.1 M was fixed as the optimized concentration for the PEC biosensing.

Table S1. Comparison of the analytical performances of the previous detection methods with the proposed PEC biosensing strategy.

Detection technique	Cell line	Linear range (cell·mL ⁻¹)	Detection limit(cell·mL ⁻¹)	Reference
Amperometric	Leukemia	$1 \times 10^3 \sim 1 \times 10^6$	40	S4
Colorimetric	MCF-7	—	1000	S5
ECL	K562	$1 \times 10^3 \sim 1 \times 10^7$	600	S6
EIS	HMy2	$1 \times 10^4 \sim 4 \times 10^5$	4000	S7
PEC	SK-BR-3	$1 \times 10^2 \sim 1 \times 10^6$	58	S8
PEC	Ramos	160~1600	84	S9
PEC	MCF-7	$1 \times 10^2 \sim 5 \times 10^6$	21	This work

5. Cell viability investigation

The cytotoxicity of WS₂/Au nanocomposites was tested by MTT assay, and the result was displayed in Fig. S5. The cell viability was investigated at the different concentrations of WS₂/Au nanocomposites. After incubating with the nanocomposites for 24 h, the absorbance declined slightly and more than 84% of MCF-7 cells were still alive until the concentration was as high as 210 ppm (the concentration of WS₂/Au nanocomposites adopted in this work was 200 ppm), indicating the hypotoxicity of the PEC biosensing substrate.

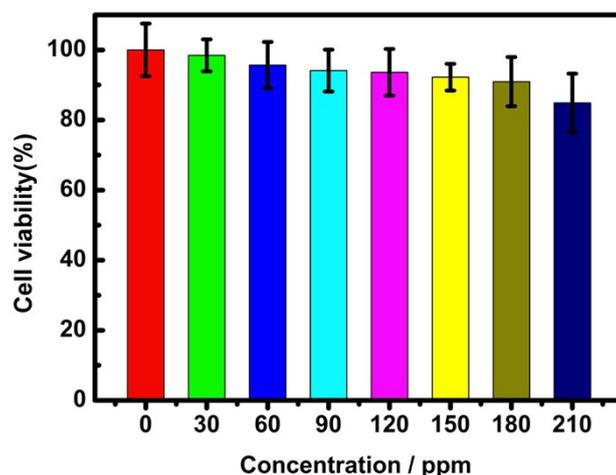


Fig. S5 Cytotoxicity effect of Au/WS₂ nanocomposites on MCF-7 cells.

6. The selectivity of the proposed PEC assay method

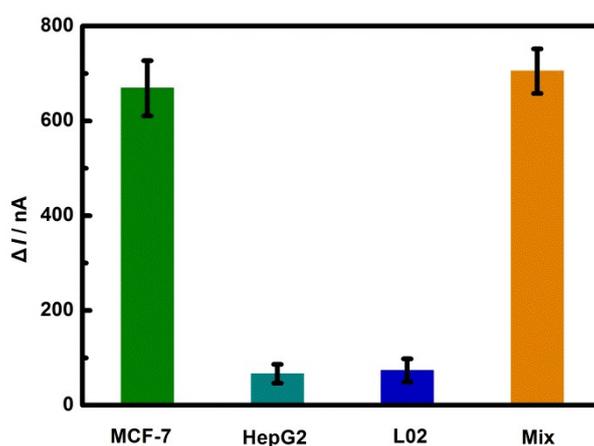


Fig. S6 PEC response of the biosensor towards MCF-7 cells, HepG2 cells, L02 cells, and the mixture of MCF-7 cells, HepG2 cells and L02 cells.

To evaluate the selectivity of the proposed PEC biosensing strategy, HepG2 cells (MUC1-negative),^{S10} L02 cells (MUC1-negative)^{S11} and the mixture of the three type of cells were selected to investigate the analytes versus the photocurrent change ($\Delta I = I_0 - I$, where I_0 is the photocurrent of $C_{\text{cell}} = 0$ and I is the photocurrent of $C_{\text{cell}} = 5000 \text{ cell} \cdot \text{mL}^{-1}$) under the same conditions (Fig. S6). The PEC response of HepG2 cells and L02 cells were 9.9% and 10.8% of that of MCF-7 cells, respectively. While the mixture of the three type of cells also could induce the obvious change of

photocurrent, indicating the interference of HepG2 cells and L02 cells might be negligible. These results illustrated only MCF-7 cells excluding the other test cells could induce the obvious change of photocurrent, confirming a good selectivity of the designed PEC assay method.

References

- S1 Y. X. Yuan, R. Q. Li, Z. H. Liu, *Anal. Chem.*, 2014, **86**, 3610.
- S2 R. Cao, B. X. Li, *Chem. Commun.*, 2011, **47**, 2865.
- S3 M. Zirak, M. Zhao, O. Moradlou, M. Samadi, N. Sarikhani, Q. Wang, H. L. Zhang, A. Z. Moshfegh, *Sol. Energ. Mat. Sol. C.*, 2015, **141**, 260.
- S4 T. T. Zheng, J. J. Fu, L. H. Hu, F. Qiu, M. J. Hu, J. J. Zhu, Z. C. Hua, H. Wang, *Anal. Chem.*, 2013, **85**, 5609.
- S5 Y. Tao, Y. H. Lin, Z. Z. Huang, J. S. Ren, X. G. Qu, *Adv. Mater.*, 2013, **25**, 2594.
- S6 Z. H. Chen, Y. Liu, Y. Z. Wang, X. Zhao, J. H. Li, *Anal. Chem.*, 2013, **85**, 4431.
- S7 A. E. Muniz, C. S. Espinel, B. D. Freitas, A. G. Fernandez, M. M. Costa, A. Merkoci, *Anal. Chem.*, 2009, **81**, 10268.
- S8 F. Liu, Y. Zhang, J. H. Yu, S. W. Wang, S. G. Ge, X. R. Song, *Biosens. Bioelectron.*, 2014, **51**, 413.
- S9 X. R. Zhang, S. G. Li, X. Jin, X. M. Li, *Biosens. Bioelectron.*, 2011, **26**, 3674.
- S10 H. Yoo, H. Jung, S. A. Kim, H. Mok, *Chem. Commun.*, 2014, **50**, 6765.
- S11 Y. Hu, J. Duan, Q. Zhan, F. Wang, X. Lu, X. D. Yang, *PLoS One*, 2012, **7**, e31970.