Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Label-free and "signal-on" homogeneous photoelectrochemical cytosensing strategy for ultrasensitive cancer cell detection

Fangfang Lu,‡ Limin Yang,‡ Ting Hou* and Feng Li*

College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, People's Republic of China

E-mail: thou@qau.edu.cn, lifeng@qau.edu.cn.

‡These authors contributed equally to this work.

Experimental section

Materials and reagents. Indium tin oxide (ITO) glass was obtained from Shenzhen Nanbo Display Technology Co. Ltd. (Shenzhen, China). Methylene blue (MB), sodium chloride (NaCl), magnesium chloride ($MgCl_2 \cdot 6H_2O$), and sodium hydroxide (NaOH), Na_2HPO_4, and NaH_2PO_4 were purchased obtained from Shanghai Titan Scientific Co., Ltd (Shanghai, China). 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diph-enyltetrazolium bromide (MTT) was bought from Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). Fetal bovine serum (FBS), Dulbecco's modified Eagles medium (DMEM), and cell medium RPMI 1640 were purchased from Biological Industries Co., Ltd. (Beit-Haemek, Israel). Ascorbic acid (AA) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). The human breast cancer cell line MCF-7 and human hepatocyte cell line HL-7702 were bought from Procell Life Science Co., Ltd. (Wuhan, China). All oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and their sequences were showed below:

ON1: 5'-GCAGTTGATCCTTTGGATACCCTGGAGAAGAAGGTGTTTAAGTA-3'

ON2: 5'-GGGTTGGGCGGGATGGGT-3'

ON3: 5'-ACCCATCCCGCCCAACCC-3'

Aptamer S2.2: 5'-GCAGTTGATCCTTTGGATACCCTGG-3'

Hairpin (H1): 5'-AGGGCGGGTGGGTGTTTAAGTTGGAGAATTGTACTTAAACACCTTCT TCTTGGGT-3'

Hairpin (H2): 5'-TGGGTCAATTCTCCAACTTAAACTAGAAGAAGGTGTTTAAGTTGGGTA GGGCGGG-3'

The oligonucleotide stock solutions, prepared by diluting with PBS buffer (pH 7.4, 20 mM Na_2HPO_4, 20 mM NaH_2PO_4, 2.5 mM $MgCl_2$, 100 mM NaCl, 25 mM KCl), were heated up the water bath to 95 °C, kept it at this temperature for 5 min and then slowly cooled to room temperature before use.

Apparatus. PEC measurements were performed on a Zahner PEC measurement system (Zahner-Elektrik GmbH & Co. KG, Germany), with a RTR02 light (627 nm) as the accessory light source. Native polyacrylamide gel electrophoresis (PAGE) was performed on a Bio-Rad electrophoresis system (Bio-Rad Laboratories, Inc., U.S.A.), PAGE (8%) experiments were carried out in 1 × TBE buffer (pH 7.9, 9 mM Tris-HCl, 9 mM boric acid, 0.2 mM EDTA) with 110 V for 1.0 h and stained for 20 min in a GelRed solution. and the gel was imaged using a Gel Doc XR+ Imaging System (Bio-Rad Laboratories, Inc., U.S.A.). Absorbance in the MTT assay was performed on microplate reader (Synergy 2, Biotek, USA).

ITO electrode pretreatment and PEC detection. The ITO electrodes were treated with ethanol and ultrapure water for 30 min by ultrasonic treatment, respectively. Subsequently, the obtained cleared ITO electrodes were immersed in 1 mM NaOH solution for 5 h at room temperature, followed by sonication in ultrapure water for 10 min. After dried with nitrogen gas, the resulted ITO electrodes with negatively surface were used in the PEC measurements. A three-electrode system was applied in PEC experiments: an indium tin oxide (ITO) working electrode, an Ag/AgCl reference electrode, and a platinum wire counter electrode. The PEC measurements were carried out at a specific bias potential with 0 V.

Cell culture and treatment. MCF-7 and HL-7702 were cultured in RPMI 1640 and DMEM medium, respectively. All cell lines were supplemented with 10% FBS and 1% antibiotics penicillin/streptomycin and maintained at 37 °C in a 100% humidified atmosphere containing 5% CO_2 at 37 °C. After the cells plated on chamber slides for 24 h, the cells were collected by

centrifugation at 1000 rpm for 4 min and washed twice with buffer solution PBS buffer (pH 7.4, 20 mM Na_2HPO_4, 20 mM NaH_2PO_4, 2.5 mM $MgCl_2$, 100 mM NaCl, 25 mM KCl). Then, the cells were carefully diluted into the buffer solution with various concentrations. The cell number was counted by hemocytometer.

Cell cytotoxicity assay. MCF-7 cells were plated on chamber slides for 24 h under 5% CO_2 at 37 °C. Next, the cells were incubated with culture medium and ON1 probe with various concentrations for 24 h. After washed with PBS buffer twice, MTT (0.5 mg mL^{-1}) was added into each well. After 4 h, the MTT solution was discarded and 150 μL dimethyl sulfoxide was put into each well. Finally, the absorbance was recorded at 490 nm with a microplate reader.

Photoelectrochemical detection of MCF-7 cells. For cancer cell detection, 5 μL of ON1 probe (10 μM) and 5 μL of MCF-7 cells with different concentrations were added to 75 μL PBS buffer (20 mM, pH 7.4, 2.5 mM $MgCl_2$, 100 mM NaCl, 25 mM KCl). The resulting mixture was incubated at 37 °C for 40 min. After centrifugated at 1500 rpm for 5 min, the cells and ON1 probe bound to the cell surface was removed, and the supernatant solution containing unbound ON1 probe was obtained. Subsequently, 5 μL of H1 (10 μM), 5 μL of H2 (10 μM) and 5 μL MB (50 μM) were put into the obtained supernatant solution. After incubated with 2 h at 37 °C, 100 μL of PB buffer (100 mM, pH 7.4) containing 0.2 M AA was added to the above reaction solution, and then the PEC measurement was carried out. In the experiments for selectivity investigation, HL-7702 cells were tested.

Figures

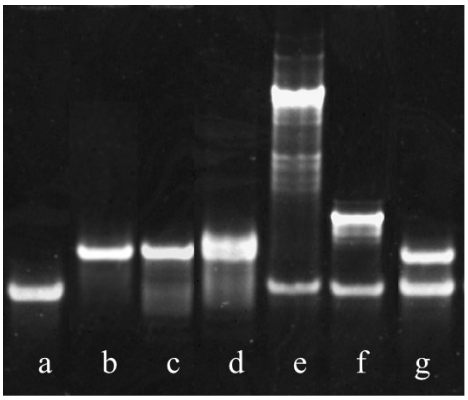

Fig. S1 PAGE characterization of the HCR products. Lane a: ON1, Lane b: H1, Lane c: H2, Lane d: H1 + H2, Lane e: ON1 + H1 + H2, Lane f: ON1 + H1, Lane g: ON1 + H2.

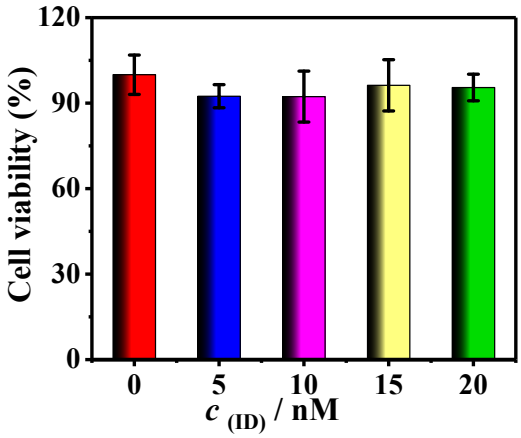

Fig. S2 Cell viability of the cells incubated with different concentrations of ON1 probe for 24 h respectively. Red bar stands for the control, blue bar, pink bar, yellow and green bars stand for 5 nM, 10 nM, 15 nM and 20 nM, respectively.

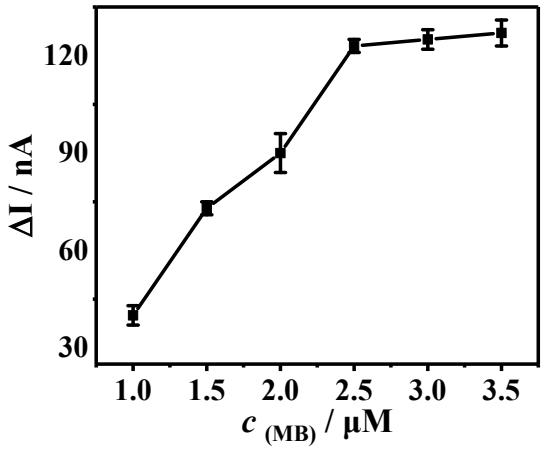

Fig. S3 The photocurrent changes ΔI (ΔI=I –I_0, in which I and I_0 are the photocurrents in the presence and absence of MCF-7 cells, respectively) versus the MB concentration ranging from 1.0 μM to 3.5 μM. The concentration of MCF-7 cells, ON1 probe, HP1, and HP2 were 5×10^3 cells mL^{-1}, 5 nM, 0.5 μM, and 0.5 μM.

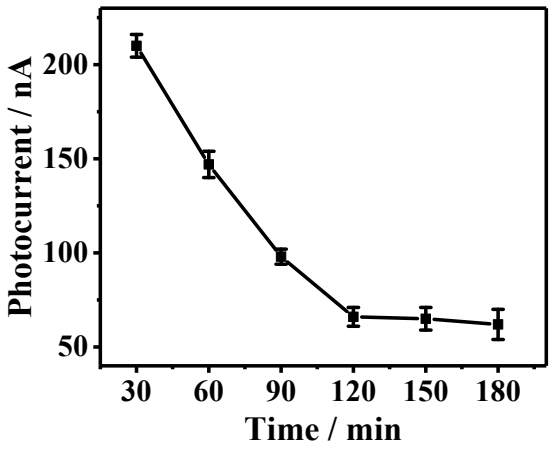

Fig. S4 The relationship between the photocurrent and the reaction time (ranging from 30 min to 180 min) in the presence of 2.5 μM MB, 5 nM ON1 probe, 0.5 μM HP1, and 0.5 μM HP2.

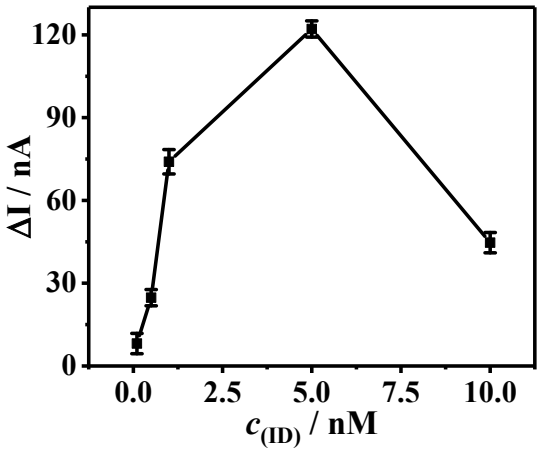

Fig. S5 The photocurrent changes ΔI (ΔI=I $-I_0$, in which I and I_0 are the photocurrents in the presence and absence of MCF-7 cells, respectively) versus the ON1 concentration (ranging from 0.1 nM to 10 nM). The concentration of MCF-7 cells, ON1 probe, HP1, and HP2 were 5×10^3 cells mL^{-1}, 5 nM, 0.5 μM, and 0.5 μM.

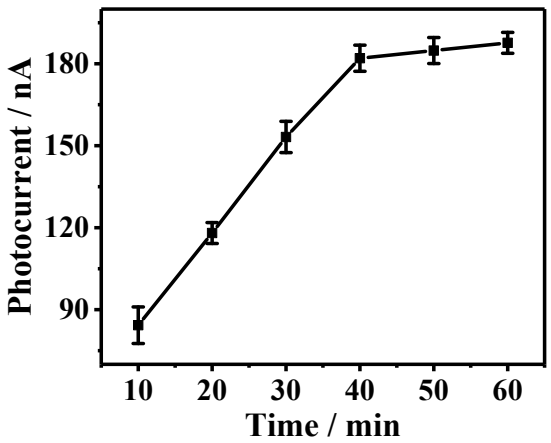

Fig. S6 The photocurrent of MB versus the MCF-7 cells incubated with ON1 probe for different times (ranging from 10 min to 60 min). The concentration of MCF-7 cells, ON1, HP1, and HP2 were 5×10^3 cells mL^{-1}, 5 nM, 0.5 μM, and 0.5 μM.

Table S1. Comparison of the as-proposed strategy with other reported methods

Method	Liner range (cells mL^{-1})	Detection limit	Refs.
Robust photoelectrochemical cytosensor in biological media using antifouling property of zwitterionic peptide	1.0×10^2 - 1.0×10^6	34 cells mL^{-1}	1
Construction of self-powered cytosensing device based on ZnO nanodisks@g-C_3N_4 quantum dots and application in the detection of CCRF-CEM cells	20- 2.0×10^4	20 cells mL^{-1}	2
A label-free aptamer-based cytosensor for specific cervical cancer HeLa cell recognition through a g-C_3N_4–AgI/ITO photoelectrode	10 - 1.0×10^6	5 cells mL^{-1}	3
A localized surface plasmon resonance-enhanced photoelectrochemical biosensing strategy for highly sensitive and scatheless cell assay under red light excitation	1×10^2-5×10^6	21 cells mL^{-1}	4
Synergistic bio-recognition/spatial-confinement for effective capture and sensitive photoelectrochemical detection of MCF-7 cells	8-6×10^3	2 cells mL^{-1}	5
Stackable Lab-on-Paper Device with All-in-One Au Electrode for High-Efficiency Photoelectrochemical Cyto-Sensing	5.0×10^2 - 5.0×10^6	198 cells mL^{-1}	6
Photoelectrochemical cell enhanced by ternary heterostructured photoanode: Toward high-performance self-powered cathodic cytosensing	1.0×10^2 -1.0×10^5	28 cells mL^{-1}	7
Red light-driven photoelectrochemical biosensing for ultrasensitive and scatheless assay of tumor cells based on hypotoxic $AgInS_2$ nanoparticles	1.5×10^2-3.0×10^5	16 cells mL^{-1}	8
Low-toxic Ag_2S quantum dots for photoelectrochemical detection glucose and cancer cells	3×10^2-2.0×10^3	98 cells mL^{-1}	9
A Conjugated Polymer-Based Photoelectrochemical Cytosensor with Turn-On Enable Signal for Sensitive Cell Detection	1.0×10^2 -5.0×10^5	24 cells mL^{-1}	10
Near-Infrared Light-Driven Photoelectrochemical Aptasensor Based on the Upconversion Nanoparticles and TiO_2/CdTe Heterostructure for Detection of Cancer Cells	1×10^3 - 1×10^5	400 cells mL^{-1}	11
Label-free and "signal-on" homogeneous photoelectrochemical cytosensing strategy for ultrasensitive cancer cell detection based on G-quadruplex-inserted long dsDNA structure	10 - 5×10^4	2 cells mL^{-1}	This work

References

1. G. C. Fan, Z. Li, Y. Lu, L. Ma, H. Zhao and X. Luo, *Sens. Actuators, B*, 2019, **299**, 126996.
2. X. Pang; C. Cui, M. Su, Y. Wang, Q. Wei and W. Tan, *Nano Energy*, 2018, **46**, 101-109.
3. R. M. Mazhabi, L. Ge, H. Jiang and X. Wang, *J. Mater. Chem. B*, 2018, **6**, 5039-5049.
4. R. Li, R. Yan, J. Bao, W. Tu and Z. Dai, *Chem. Commun.*, 2016, **52**, 11799-11802.
5. D. Wu and Z. Zhang, *Chem. Commun.*, 2019, **55**, 14514-14517.
6. H. Yang, Y. Zhang, L. Zhang, K. Cui, S. Ge, J. Huang and J. Yu, *Anal. Chem.*, 2018, **90**, 7212-7220.
7. G. C. Fan, Y. Lu, H. Zhao, Q. Liu, Z. Li and X. Luo, *Biosens. Bioelectron.*, 2019,**137**, 52-57.
8. J. Li, X. Lin, Z. Zhang, W. Tu and Z. Dai, *Biosens. Bioelectron.*, 2019, **126**, 332-338.
9. X. Zhang, M. Liu, H. Liu and S. Zhang, *Biosens. Bioelectron.*, 2014, **56**, 307-312.
10. S. Liu, P. He, S. Hussain, H. Lu, X. Zhou, F. Lv, L. Liu, Z. Dai and S. Wang, *ACS Appl. Mater. Interfaces*, 2018, **10**, 6618-6623.
11. K. Wang, R. Zhang, N. Sun, X. Li, J. Wang, Y. Cao and R. Pei, *ACS Appl. Mater. Interfaces*, 2016, **8**, 25834-25839.