Electronic Supplementary Information

Photoelectrochemical Biosensor for Detection of Adenosine Triphosphate in the Extracts of Cancer Cells

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1. Reagents and Apparatus

Reagents. All the reagents were analytical grade and used without further purification. Doubly distilled water (DDW) was used throughout this work. Carboxyl-modified magnetic beads (MB, diameter 1.0 μm, 100 mg/mL) were ordered from Tianjin BaseLine Chromtech Research Centre. Tri(2-carboxyethyl)phosphine hydrochloride (TCEP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), ATP, CTP, GTP and UTP were purchased from Sigma. Sodium citrate and $AuCl_3HCl{\cdot}4H_2O$ were purchased from Shanghai Chemical Reagent Inc. (Shanghai, China). Fifteen percent tin (IV) oxide, as a colloidal dispersion of 15-nm particles in water, was obtained from Alfa Aesar. APS and glutaraldehyde were purchased from Acros. The oligonucleotides used in this study were purchased from SBS Genetech Co.,Ltd. (China) with the following sequences: ATP aptamer: 5'-NH_2-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3'; Barcode DNA: 5'-SH-TTT TTT CCC CGC GCG AAC CGT ATA-3'; Binding DNA: 5'-SH-TTT TTT CCC CGC GCG AAC CGT ATA TTA CCT TCC TCC GC-3'; Complementary DNA: 5'-TAT ACG GTT CGC GCG GGG AAA AAA-3'.

Apparatus. The photocurrent and electrochemical impedance spectroscopy (EIS) were measured on an electrochemical workstation (Zahner Zennium, Germany). A three-electrode system was employed with Pt wire as an auxiliary electrode, Ag/AgCl as a reference electrode and ITO conductive glass supplied by Weiguang Corp. (Shenzhen, People's Republic of China, ITO coating 180±25 nm, sheet resistance ≤10 Ω/square) as a working electrode. SnO_2 colloidal dispersion was spread on a piece of ITO-coated glass using a Spin Coater (type KW-4A, SIYOUYEN Corp., Beijing, China). Transmission electron microscopy (TEM) image was taken with JSM-6700F instrument (JEOL, Japan).

2. Synthesis of $[Ru(bpy)_2dppz]^{2+}$

$[Ru(bpy)_2dppz]^{2+}$ was synthesized according to references with some modification.[1, 2] Briefly, $RuC1_3 \cdot 3H_2O$ (3.6 g, 9.9 mmol)), bipyridine (3.12 g, 20.0 mmol), and LiCl (2.8 g, 0.7 mmol) were refluxed in 25 mL dimethylformamide for 8 h. The reaction was stirred magnetically throughout this period. After the reaction mixture was cooled to room temperature, 100 mL of acetone was added and the resultant solution cooled at 0°C overnight. Filtering yielded a dark green-black microcrystalline product. The solid was washed with water (3×5 mL) and diethyl ether (3×5 mL), then it was dried by suction to give $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (yield 63%).

$[Ru(bpy)_2dppz]^{2+}$ was assembled by refluxing of $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (0.59 g, 1.06 mmol) with (0.33 g, 1.17 mmol) of dppz (dipyrido[3,2-a:2',3'-c]phenazine) in 150 mL of 50% methanol for 5.5 h, then reaction mixture was concentrated to 10%. After adding 50 mL water and boiling for 10 min, the solution was allowed to cool to room temperature, and then kept at 0 ºC for 12 hours. The complex was then separated from soluble impurities by precipitation with 10% $NaBF_4$ and recrystallization with ethanol (yield 60%).

3. Preparation of SnO_2 modified ITO electrode

SnO_2 electrode was prepared according to literature.[3] Briefly, ITO-coated glass was cleaned in an ultrasonic cleaner sequentially with each of the following solutions: household detergent in water (15 min), deionized water (2 min, twice), acetone (5 min), 2-propanol (5 min), and deionized water (10 min, twice). Fifteen percent tin (IV) oxide was spread on a piece of ITO-coated glass and then dried in air. The film was then sintered at 450 °C for 60 min and the semiconductor thin films were adhered strongly to the glass surface.

4. Synthesis of AuNPs

AuNPs were synthesized according to the procedure of literature.[4] Briefly, 100 mL of 1 mM

$HAuCl_4$ was brought to a reflux while stirring and then 10 mL of 38.8 mM trisodium citrate solution was added quickly, which resulted in a change in solution color from pale yellow to deep red. After refluxed for an additional 15 min, the solution was allowed to cool to room temperature. The final AuNPs prepared by this method have an average diameter of approximately 20 nm as measured by TEM.

5. Preparation of MB-bio-barcode conjugate

The oligomer coated MBs were prepared according to the modification of the literature.[5] Briefly, suspension of carboxyl-modified MBs (20 μL, 100 mg/mL) were washed three times with 0.1 M imidazole buffer (pH 7.0), and then activated in the same buffer solution containing 0.2 M EDC with gentle shaking for 60 min. 150 μL Tris-HCl containing 5'-amino group capped aptamer (50 μL, 2.0×10^{-6} M) was added into the activated MBs and incubated overnight at 37 °C. The resulting probes were washed three times with 200 μL Tris-HCl to remove unreacted DNA, and resuspended in 100 μL Tris-HCl before use.

The bio-barcode was prepared according to the reference with a little modification.[6] Briefly, binding DNA (20 μL, 1×10^{-6}M) and barcode DNA (180 μL, 1×10^{-5}M) in 1 mL Tris-HCl were activated by TCEP (5 μL, 10 mM) for 1 h. Then 1 mL of freshly prepared AuNPs was added. After standing for 16 h, the DNA-AuNPs conjugates were “aged” in salts (300 mM NaCl, 10 mM Tris-HCl, pH 7.0) for 24 h. Free oligonucleotides were removed by centrifuging at 13000 rpm for 30 min. The red precipitate was washed, re-centrifuged and dispersed in 1 mL of 10 mM Tris-HCl containing 100 mM NaCl (pH 7.0).

The prepared bio-barcode was then assembled on MBs through the hybridization of aptamer with binding DNA. Bio-barcode (250 μL) was added into aptamer-coated MBs (100 μL) and

incubated for 1 h. After magnetic separation, unreacted bio-barcode could be removed easily by washing three times with Tris-HCl. Then the produced MB-bio-barcode conjugates were resuspended in 180 μL Tris-HCl (pH 7.4) and stored at 4℃ before use.

6. ATP recognition

Titration experiments were carried out by adding ATP (20 μL, both in the standard form and that released from cells) to the MB-bio-barcode conjugate solution and kept for 90 min to make the aptamer change its structure to bind ATP. After magnetic separation, the supernatant was kept for later use.

7. Assembly processes on the electrode and photoelectrochemical detection

The SnO_2 modified ITO electrode obtained above was then silanized in 200 μL ethanol solvent containing 1 mM APS through sonication for 2 h at room temperature. After this time, the electrode was rinsed with double distilled water, dried under N_2 atmosphere and then cured at 110 °C for 15 min in an oven.

Aptamer DNAs were assembled on electrode using cross-linking reagent glutaraldehyde. Firstly, silanized ITO electrode was immersed into tube containing 5% glutaraldehyde solution (200 μL) for 2 h in a water bath at 37 °C. Secondly, the modified electrode was immersed in Tris-HCl (200 μL, 10 mM) containing 5'-amino group aptamer (50 μL, 2.0×10^{-6} M) for 12 h in a water bath (37°C). The assembled ITO electrode was then incubated with the released bio-barcode obtained above and allowed to react for 60 min at 37 °C. Thirdly, complementary DNA (200 μL, 1×10^{-5} M) was added for further hybridization. Finally, the electrode was immersed in Tris-HCl (200 μL, 10 mM) containing 5×10^{-5}M $Ru(bpy)_2(dppz)^{2+}$ solution for 30 min allowing intercalation to take place. The electrode surface was rinsed with 10 mM Tris-HCl (pH 7.0) containing 1‰ SDS after

each step of fabrication process in order to remove non-specific adsorption.

Photoelectrochemical detection was carried out in 10 mM Tris-HCl (pH=7.0) containing 30 mM sodium oxalate, which was served as a sacrificial electron donor during the photocurrent measurement. Light excitation of 470 nm was switched every 20 s. The ITO electrode area in contact with the electrolyte was 0.25 cm^2.

8. Preparation of ATP Extracts from K562 Cells[7]

The K562 leukemia cells were cultured in cell flasks according to the instructions from the American Type Culture Collection. The cell line was grown to 90% confluence in RPMI-1640 Medium supplemented with 10% fetal bovine serum and 100 IU/mL penicillin-streptomycin at 37 °C, and the cells were harvested by trypsinization. The cell density was determined by a hemocytometer prior to each experiment. Then, a suspension of 2.5×10^6 K562 cells in 1.0 mL was dispersed in RPMI cell media buffer, centrifuged at 3000 rpm for 5 min, washed with phosphate-buffered saline (18.6 mM phosphate, 4.2 mM KCl and 154.0 mM NaCl, pH 7.4) three times and resuspended in 1.0 mL of deionized water. Finally, the cells were disrupted by sonication for 20 min at 0 °C and the lysate was centrifuged at 10 000 rpm for 10 min at 4 °C in order to remove the homogenate of cell debris.

9. Detection condition of photoelectrochemistry.

Fig. S1 shows the photocurrent response of ITO/SnO_2 electrode in 9.0×10^{-8} M $Ru(bpy)_2dppz^{2+}$ solution as the excitation light was turned on and off. The result showed that the produced anodic current showed quick response to 470 nm light and good stability for six cycles. Thus, it can be used as detection signal.

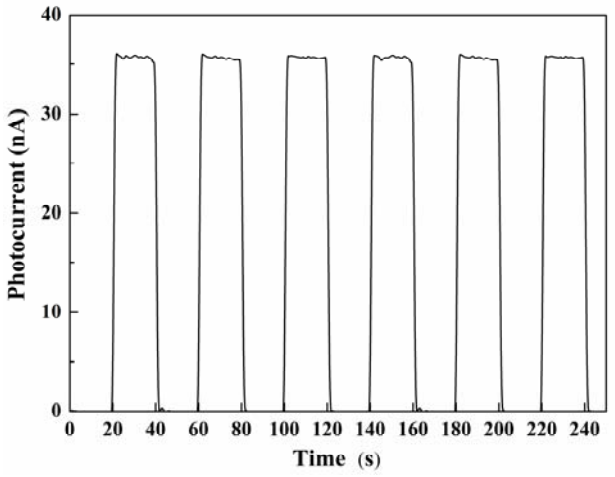

Fig. S1. Time-based photocurrent response of ITO/SnO_2 electrode in 10 mM Tris-HCl (pH=7.0) containing 30 mM sudium oxalate and 9.0×10^{-8} M $Ru(bpy)_2dppz^{2+}$. The excitation light was 470 nm and the light was turned on and off every 20 s. The applied potential was 0.1 V.

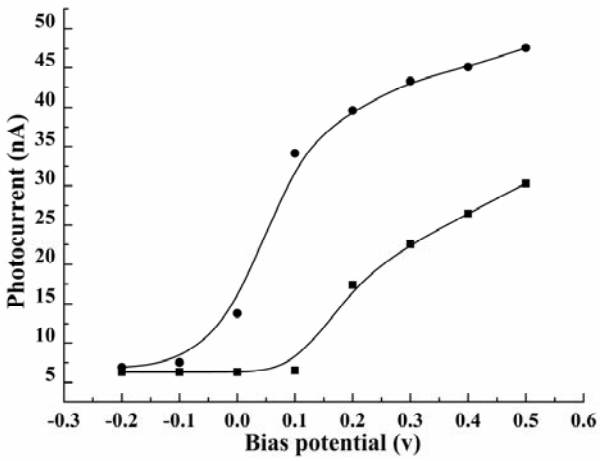

Fig. S2. Effect of potential bias on the measured photocurrents in (●) 10 mM Tris-HCl (pH=7.0) containing 30 mM sudium oxalate and 9.0×10^{-8}M $Ru(bpy)_2dppz^{2+}$; (■) 10 mM Tris-HCl (pH=7.0). Phototcurrents were recorded at λ=470 nm.

10. Sensitivity of ATP analysis without the amplification of bio-barcode.

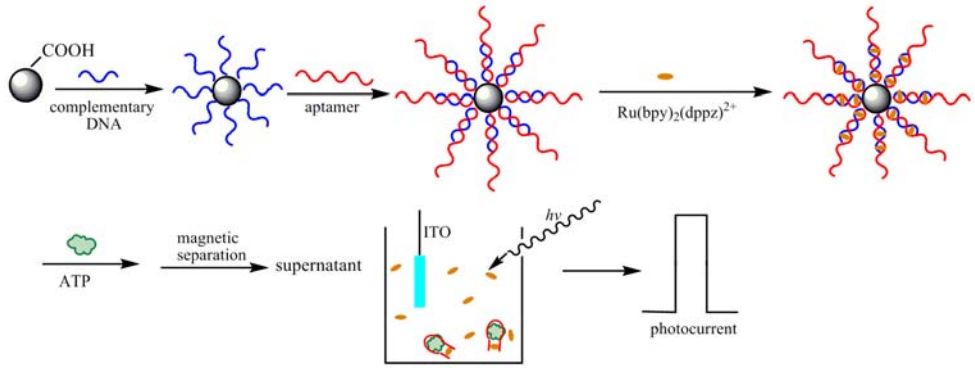

Fig. S3. Schematic diagram for the fabrication of ATP biosensor and photoelectrochemical

detection without the amplification of bio-barcode.

Analysis of ATP without the amplification of bio-barcode was conducted aiming at know the amplification effect of bio-barcode. The fabrication of the sensing design is shown in Fig. S3. Firstly, NH_2-functionalized complementary DNA was coupled to carboxyl-modified MBs. After hybridization with aptamer, photoelectrochemically active species $Ru(bpy)_2dppz^{2+}$ can intercalate into the double helix of double-stranded DNA. When target molecule ATP was added, the double helixes were opened and photoelectrochemically active species $Ru(bpy)_2dppz^{2+}$ was entered into solution. The supernatant was then subjected to photoelectrochemical measurement. The sensitivity of the developed biosensor was investigated in Fig. S4. A linear range from 6.0×10^{-7} to 1.0×10^{-5} M was achieved with an equation of ΔI=0.28041+0.80332C_{ATP} (10^{-7}M) (n = 9, R = 0.9990) and a detection limit of 1.3×10^{-7} M.

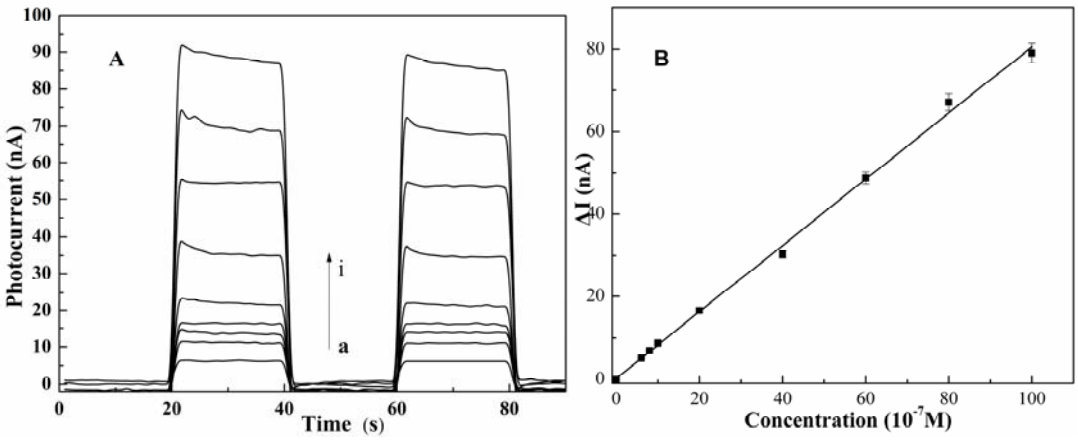

Fig. S4. (A) Photocurrent responses for increasing levels (a–i) of ATP (a) 0 (b) 6×10^{-7} (c) 8×10^{-7} (d) 1×10^{-6} (e)2×10^{-6} (f) 4×10^{-6} (g) 6×10^{-6} (h) 8×10^{-6} (i) 1×10^{-5} M. (B) The calibration plot of peak current *versus* the concentration of ATP from 6×10^{-7}M to 1×10^{-5} M. The excitation light was 470 nm and the light was turned on and off every 20 s. The applied potential was 0.1 V.

11. Detection of ATP in Cell Extracts with HPLC Method

HPLC analyses were carried out using a Hitachi HPLC system consisting of an A-2130 pump, a L-2400 detector and Chromeleon software. Separation was carried out using a Waters Allsphere

ODS-C18 column (4.6 mm × 250.0 mm, 5 μm). The calibration curve for the determination of ATP standard by HPLC method was shown in Fig. S5. The peak area was linear with the concentration of ATP ranging from 1.0 to 80 μM. The correlation coefficient was 0.9996 and the regression equation was y = 9388.64273+7473.49214x (x was the concentration of ATP, μM; y was the peak area, AU). After diluting, ATP extracts from K562 cells were then measured by HPLC and the ATP level in cells was quantified in the regression equation of ATP standards.

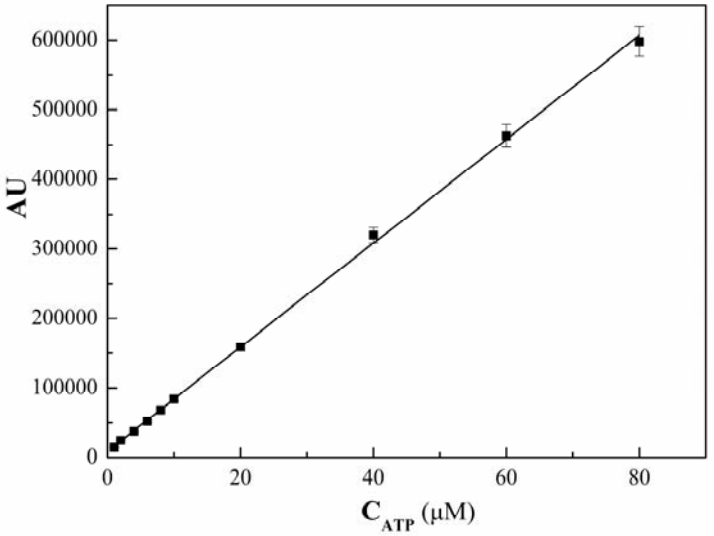

Fig. S5. Plot of peak area *vs.* the concentration of ATP. The gradient mobile phase is acetonitrile and water in the proportion of 60 / 40 (v/v). Samples were injected in 10 μL volume and eluted at a flow rate of 1.0 mL/min. The detection wavelength was set at 258 nm. The mobile phase consisted of 20 mM NaH_2PO_4 and Na_2HPO_4 phosphate buffer (pH 5.95), degassed by vacuum extraction for 10 min.

12. Data processing

The measured photocurrent shows a little decline with time between each light on and light off cycle. In order to provide a definite data for calibration plot, average data of three independent measurements (see Fig.4 A-C in manuscript) were calculated, and the simulated photocurrent response was shown in Fig. S6 for calibration plot.

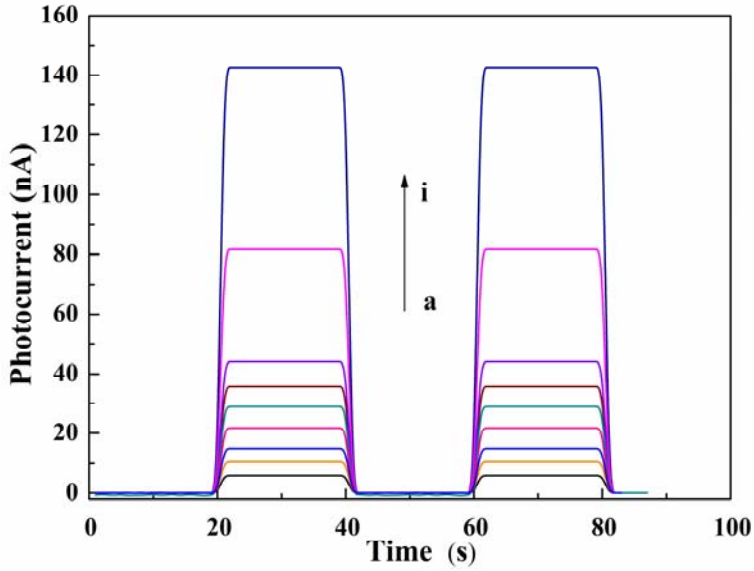

Fig. S6. Simulated photocurrent responses from the average of three independent measurements in Fig. 4 (A-C) with increasing levels (a–i) of ATP (a) 0 (b) 1×10^{-8} M (c) 2×10^{-8} M (d) 4×10^{-8} M (e) 6×10^{-8} M (f) 8×10^{-8} M (g) 1×10^{-7} M (h) 2×10^{-7} M (i) 4×10^{-7} M.

References

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