Electronic Supplementary Information

Electroactive Polymer Tag Modified Nanosensors for Enhanced Intracellular ATP Detection

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Table of Contents

1. Experimental Sections

1.1 Materials and Instruments

Fc-ATP aptamer DNA oligonucleotides and ATP aptamer DNA oligonucleotides were purified with HPLC and supplied by Sangon Biotech Co. (Shanghai,China). The specific sequence is shown in the Table S1.

Table S1. DNA sequences.

No.	Sequence (5'-3')
$\mathbf{K_5}$	5'-S-S-C_6-ACCTGGGGGAGTATTGCGGAGGAAGGT-NH_2-C_7-3'
$\mathbf{K_{single}}$	5'-S-S-C_6-ACCTGGGGGAGTATTGCGGAGGAAGGT-Ferrocene-3'

SiC nanowires (SiC NWs) were purchased from Nanjing/Jiangsu XFNANO Materials Tech Co., Ltd. (Nanjing, China). $HAuCl_4 \cdot 3H_2O$, $Ru(NH_3)_6Cl_3$, Tris (2-carboxyethyl) phosphine (TCEP), Adenosine 5'-triphosphate disodium salt hydrate (ATP), Calcein acetoxymethyl ester (Calcein-AM), and propidium iodide (PI) were bought from Sigma-Aldrich (China). 4-Cyano-4-(phenylcarbonothioylthio) pentanoic Acid (CPAD), 6-Mercapto-1-hexanol (MCH), Adenosine 5'-monophosphate monohydrate (AMP), Adenosine 5'-diphosphate monopotassium salt dihydrate (ADP), Cytidine 5'-triphosphate disodium salt (CTP), Uridine 5'-triphosphate trisodium salt hydrate (UTP), Guanosine 5'-triphosphate trisodium salt hydrate (GTP) were purchased from Aladdin Industrial Co., Ltd. (China). 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044), FcMMA were purchased from Macklin Biochemical Co. (Shanghai, China). Oligomycin was purchased from MedChemExpress. Human breast cancer cells (MCF-7 cells) were supplied by Central South University Xiangya School of Medicine. DMEM basic (1×) culture medium, fetal bovine serum (FBS), penicillin and streptomycin for cell culture were obtained from GIBCO (USA). 80# Microcrystalline wax was purchased from Shuangfeng Wax Co., Ltd (Cangzhou, China). Borosilicate capillary (1B100-4) was purchased from World Precision Instruments (USA) and was used to fabricate micropipette holders. All other chemicals and solvents of analytical grade were obtained from Sinopharm Chemical Reagent Co., Ltd. (China) and used as received unless stated otherwise. Ultrapure water (Millipore, 18.2 MΩ·cm) was used for

preparing all aqueous solutions during the whole experiments.

Scanning electron microscopy (SEM) images were performed on a scanning electron microscope (TESCAN CLARA) equipped with an energy-dispersive X-ray spectroscopy spectrometer (Ultim Extreme). X-ray photoelectron spectroscopy (XPS) measurements were implemented by a photoelectron spectrometer (ESCALAB250Xi, Thermo Fisher Scientific). The C 1s peak (284.6 eV) was used as a reference to calibrate binding energies and Al Kα X-ray radiation was used as the X-ray source. A microforge (World Precision Instruments, 40× objective) was used to fabricate NWEs. Electrochemical measurements were carried out with a CHI 660e electrochemical workstation (CHI Instruments) in a two-electrode system with an Ag/AgCl electrode as the reference/counter electrode. An inverted fluorescent microscope (AxioObserver Z1 and Axiovert 200M, Zeiss) was used for bright-field (BF) and fluorescence (FL) imaging. A micromanipulator (TransferMan 4r, Eppendorf) was used for single-cell detection at the nanoelectrodes.

1.2 Cell Experiments

1.2.1. Cell culture

The MCF-7 cells were cultured in DMEM added with 10% FBS and 1% penicillin-streptomycin at 37 °C under 5% CO_2 atmosphere. Before further experiments, the MCF-7 cells were seeded on small round slides (diameter of 7 mm) and cultured for 12 h. The conditions of cells incubation with different reagents were as follows: $CaCl_2$ (10 mM) for 2 h; oligomycin (3 μg/mL) for 1 h.

1.2.2. Cell vitality experiments

After a $Fc_{(n)}$-ATP aptamer NWE was inserted into several MCF-7 cells and then withdrawn, the vitality of penetrated cells was investigated by the fluorescence staining. MCF-7 cells were incubated with 1 mL fluorescence dye solution (Calcein-AM + PI) (3 μg/mL) for 30 min and then washed with PBS 3 times to remove the residual dyes. BF and FL microphotographs were taken with an inverted fluorescent microscope.

1.2.3. Data acquisition and analysis of single-cell intracellular ATP assays

The $Fc_{(n)}$-ATP aptamer NWE was connected to a patch-clamp amplifier probe. Subsequently, the NWE was moved to the vicinity of the cell membrane under a micromanipulator (TransferMan® NK2, Eppendorf) with a 40-fold objective lens, and then the NWE was slowly moved forward to insert it into the cell. After incubation for 7 min, the signal of ferrocene polymers on the electrode surface was monitored by square-wave voltammetry (SWV) with an Ag/AgCl electrode as the reference/counter electrode, applying a voltage from -0.2 V to 0.4 V to the $Fc_{(n)}$-ATP aptamer NWE NWE. To minimize noise interference, the above operations were carried out on a Faraday cage shield as well as an optical anti-shaking platform, and the relevant instruments were well grounded. The data were analyzed and processed by "Origin 2019" software.

2. Supporting Figures

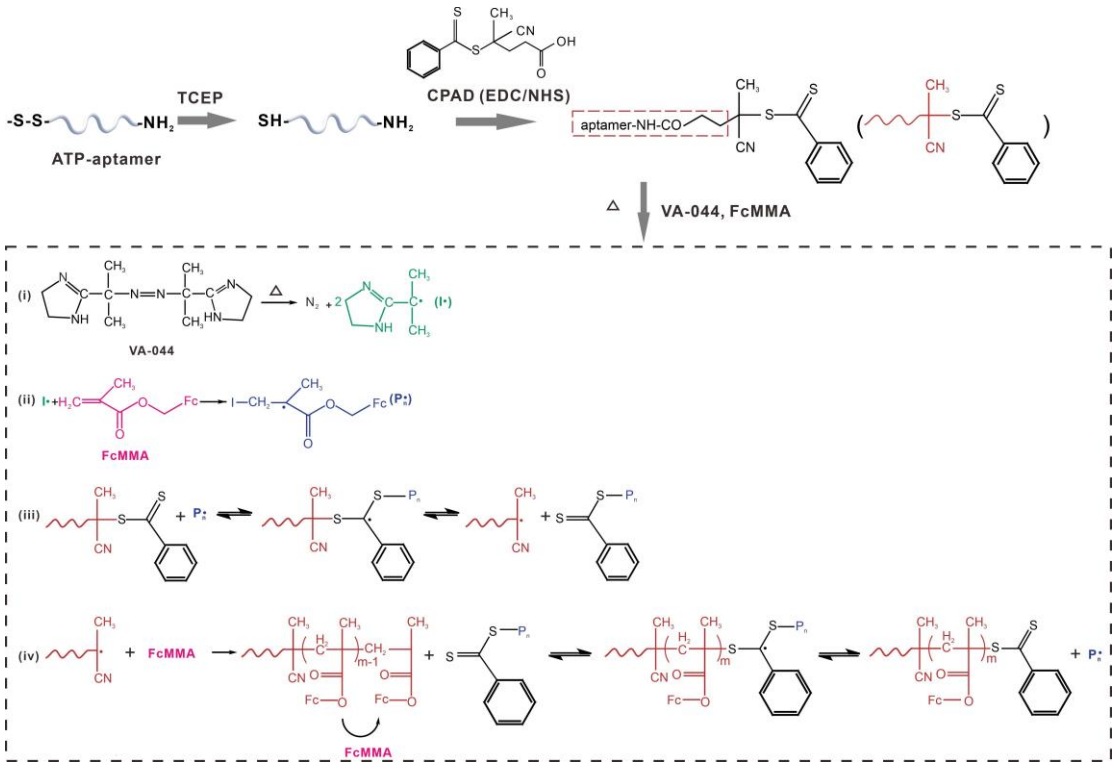

Fig S1. The preparation of $Fc_{(n)}$-ATP aptamer via RAFT polymerization.

Principle: (i): By cleavage of two C-N bonds, the azo initiators (i.e., VA-044) thermally decompose to produce N_2 and alkyl radicals (I·); **(ii):** These alkyl radicals attack the methacrylic monomers (i.e., FcMMA) to form oligomeric radicals (Pn·); **(iii):** The Pn· radicals react with the CPAD connected to the aptamer to produce the radical intermediates, which can fragment either back into the CPAD and the Pn· radicals or into the reinitiated free radicals containing aptamer chains and the thiocarbonylthio-group-capped dormant chains; **(iv):** By repeatedly reacting with the FcMMA monomers, each aptamer will carry a large number of electroactive Fc to improve electrochemical signal.[1]

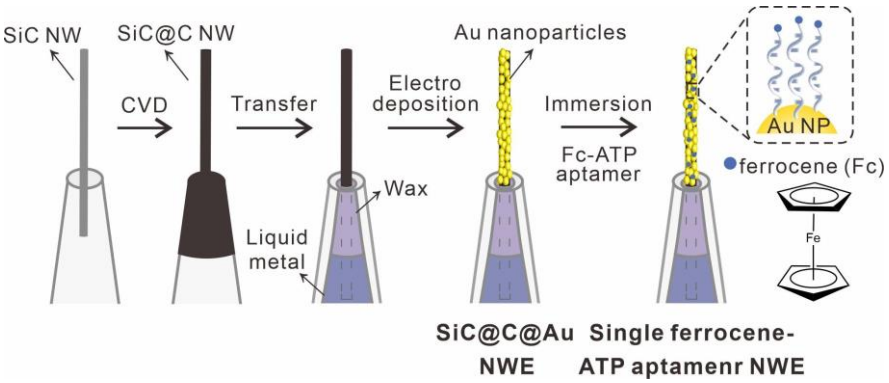

Fig S2. Preparation process of the single ferrocene-ATP aptamer NWEs.

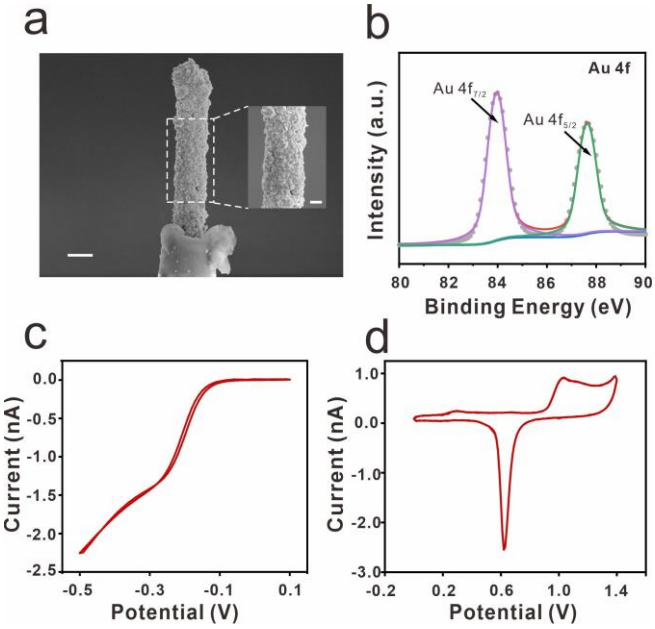

Fig S3. Characterization of a SiC@C@Au NWE. (a) SEM image of a SiC@C@Au NWE (scale bar: 1 μm). Inset: a magnified SEM image of its detail (scale bar: 400 nm). (b) XPS data of Au 4f states from SiC@C@Au NWs. (c) Cyclic voltammogram of $Ru(NH_3)_6^{3+}$ (1 mM) at a SiC@C@Au NWE. (d) Cyclic voltammogram of an Au NWE in 0.05 M H_2SO_4.

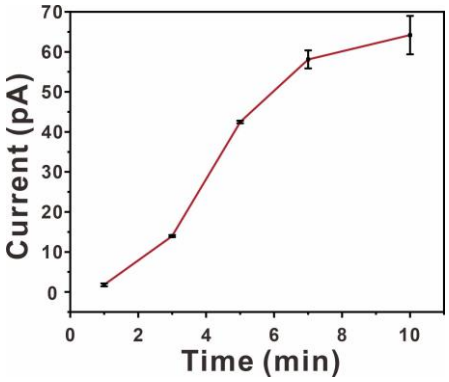

Fig S4. Effect of incubation time on the SWV peak current for 1 mM ATP.

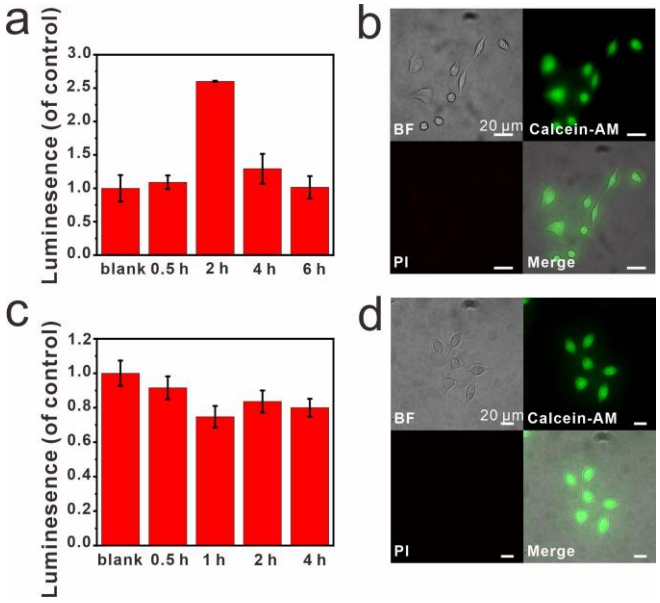

Fig S5. Estimation of intracellular ATP level in responses to drug incubation at different times by ATP assay kit. (a) Statistical results of the luminescence intensity from cells at different Ca^{2+} incubation times. (b) BF and FL imaging of MCF-7 cells stained with Calcein-AM (green) and PI (red) after incubation with $CaCl_2$ (10 mM) for 2 h. (c) Statistical results of the luminescence intensity from cells at different oligomycin incubation times. (d) BF and FL imaging of MCF-7 cells stained with Calcein-AM (green) and PI (red) after incubation with oligomycin (3 μg/mL) for 1 h.

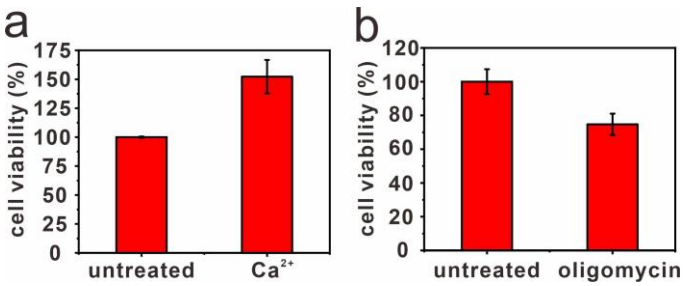

Fig S6. The effect of (a) Ca^{2+} and (b) oligomycin on cell viability was measured by MTT experiment (n=4).

3. References

1. Q. Hu, D. Han, S. Gan, Y. Bao and L. Niu, *Anal. Chem.*, 2018, **90**, 12207-12213.