Supporting Information (SI)

In Situ Monitoring of ROS Species Secretion from Single Cell with a Dual-nanopore Biosensor

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Experiment section

Chemicals and Materials

The G-rich DNA oligomers (HS-5′TTTTTTTTTTTTTTTGGGTAGGGCGGGTTGGG-3′) to preparation G-quadruplex DNAzyme were synthesized and purified by Shanghai Sangon Biological Engineering Technology (China), trisodium citrate dihydrate, hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), SH-PEG 2000, tris (2-carboxyethyl) phosphine hydrochloride (TCEP), phorbol 12-myristate-13-acetate (PMA), dimethyl sulfoxide (DMSO), dopamine (DA) and ascorbic acid (AA), catechol (CT), tyrosine (TR), citric acid (CA), glucose (Glu), Uric acid (UA), hemin, mineral oil (CAS：8042-47-5), chloride of magnesium, calcium, zinc, copper were purchased from Sigma-Aldrich (St. Louis, Mo, USA), mPEG-Silane was purchased from Laysan Bio, Inc (Alabama, USA). The commercial hydrogen peroxide detection kit was purchased from Shanghai Sangon Biological Engineering Technology (Shanghai, China). All reagents were of analytical grade. All the solutions were prepared in double-distilled water which was purified by a Milli-Q system (Millipore, Bedford, MA) and had an electric resistance >18.25 MΩ.

Fabrication and Characterization of glass nanopore

All quartz capillaries used in the experiments were thoroughly cleaned by immersing and sonicating in acetone and water for 10 min in each time of washing and then dried prior to use. Dual-nanopores fabricated from dual theat quartz capillaries with outer diameter 1.2 mm, inner diameter 0.9 mm (Sutter Instrument, USA) using a two-line protocol: (1) HEAT, 850; FIL, 4; VEL, 30; DEL, 160; PUL, 80, followed by (2) HEAT, 860; FIL, 3; VEL, 20; DEL, 140; PUL, 160. The scanning electron microscopic (SEM) characterization of nanopore was performed on a JSM-7800F instrument (JEOL, Japan).

Preparation of Au-Coated glass nanopore

For the preparation of Au-coated nanopore, firstly, the nanopore channel was treated with piranha acid (98% H_2SO_4/30% H_2O_2, V:V = 3:1, 80 °C, 30 min) to remove organic impurities, followed by washing with ultrapure water and absolute ethanol to obtain cleaned inner surface, then a gold layer was coated onto the inner surface of a glass nanopore by the photochemical reduction of chloroauric acid. Briefly, one barrel of glass nanopore was filled with a solution containing of C_2H_5OH/$HAuCl_4$ solution (8 mM) =2:3 (v/v), the other nanopore was filled with distilled water, and placed under the UV light (254 nm, 18 W, Model ZF-2, Anting Electronic Instrument, Shanghai, China) for 2−3 h at room temperature until a clear gold layer formed on the inner surface. After washing with ethanol and water 1−2 min for each, the Au-coated nanopore dried in 80 °C for 60 min.

Preparation of the G-quadruplex DNAzyme.

A solution of 100 μM G-rich DNA oligomers solution was heated at 95 °C for 10 min in 10 mM Tris buffer, pH 7.4 and gradually cooled to room temperature. Then, an equal volume of TBS (25 mmol/L Tris-HCl, 140 mmol/L NaCl, 40 mmol/L KCl pH 7.4) with 0.05% Triton X-100 was added to this solution. The mixture was allowed to appropriate folding at room temperature for 30 min, followed by the addition of an equal volume of hemin solution (50 μM) and allowed it to form the DNAzyme with G-quardruplex structure for 3 h.

Chemical modification of Au-Coated glass nanopore

Firstly the Au-Coated glass nanopore was filled with fresh reduced (50 times excess TCEP reduce for 1h) G-quadruplex DNAzyme in 1×PBS (pH=7.4) by an Eppendorf Microloader and incubated at room temperature for 8 h. Then rinsed with 1×PBS (pH=7.4) and subsequently filled with 500 μM SH-PEG at room temperature for 30 min to avoid non-specific adsorptions at the inner surface of the glass nanopore, and then rinsed with 1×PBS (pH=7.4). The other interior pore was blocked with 500 μM silane-mPEG and then rinsed with 1× PBS (pH=7.4). The resulting modified nanopore was stored in refrigerator at 4 °C for further use. During the process of backfill aqueous solution, if found air bubbles, can remove them by centrifugation at 4000 rpm for 1 min.

Electrochemical measurements

The modified nanopore was mounted in a home-made electrochemical cell**,** the modified cavity was backfilled with 1×PBS (pH 7.4) containing 2 mM ABTS, two Ag/AgCl electrodes were inserted into modified cavity as working electrode and unmodified cavity as reference electrode respectively. Electrochemical measurements were recorded using a PGSTAT12 Autolab (metrohm, Switzerland), using a linear sweep voltammetry setting between -0.5 V to 0.5 V at scan rate of 50 mV/s. For situ monitoring H_2O_2 secretion, took a continuous record of the current at -0.4V.

Detection of H_2O_2 in vitro

All detections were performed at room temperature. For detection of H_2O_2, the nanopore sensor modified cavity was backfilled with 1×PBS containing 2 mM ABTS (pH=7.4) and immersed into the solution containing different concentration of H_2O_2. Incubation for a given time at room temperature in dark, then carry out the electrochemical measurements to record I–V curves. The regeneration of the nanopore sensor was performed by immersed in fresh 1×PBS (pH 7.4). For situ monitoring H_2O_2, We took a continuous record of the current at -0.4V.

Cell culture

MCF-7 cells were obtained from the cell bank of Central Laboratory at Xiangya Hospital (Changsha, China) and MCF-10A cells were purchased from Cell Bank of the Committee on Type Culture Collection of Chinese Academy of Sciences (Beijing, China). MCF-7 Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin at 37℃ in humidified atmosphere containing 5% CO_2. MCF-10A Cells were cultured under the same condition except for using DMEM medium.

H_2O_2 secretion measurement by the dual-nanopore sensor and the commercial hydrogen peroxide detection kit

For H_2O_2 secretion measurements, MCF-7 cells were cultured in a home-made microwell chip array to investigate the H_2O_2 secretion. The individual microwells of the chip may randomly entrapped single cell or multiple cells. For measurement, different buffer solutions could be exchanged to give different extracellular environments. To achieve this purpose, solution was added above the chip and a cover glass was utilized to press the chip to ensure the microwell was

filled with solution. A filter paper was placed above the microchip to draw out the solution above the chip and in the microwells. The process can be repeated several times to obtain required extracellular environment in the microwell. Then an elastic PDMS chip was utilized to press the microwell array chip to carefully remove solution above the chip and the solution in individual microwells was retained. Then the water-immiscible oil was added above the chip to isolate individual microwells. We put the chip under the microscope to choose the well only captured a single MCF-7 cell, so we can monitor the H_2O_2 secretion of a single MCF-7 cell. Measurements of H_2O_2 in cell lysate and secreted H_2O_2 of large number cells were also detected using a commercial hydrogen peroxide detection kit according to the instructions.

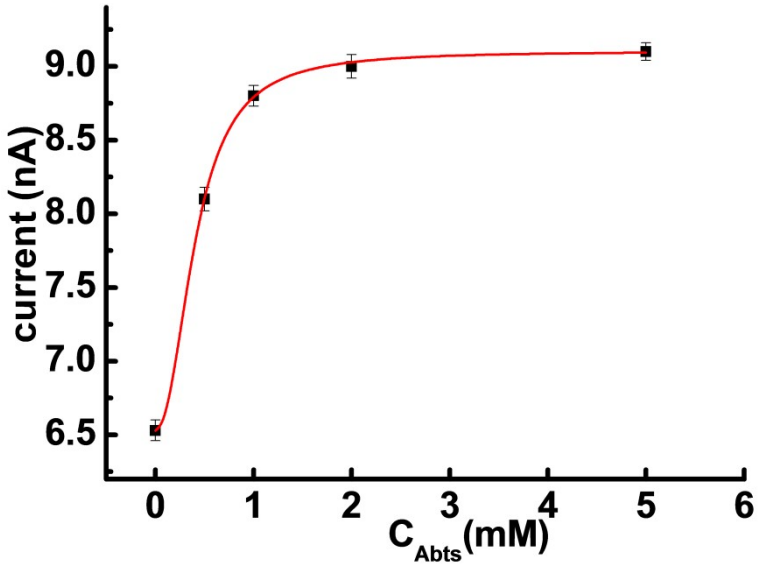

Figure S-1. Optimized the ABTS concentration of the biosensor with incubation time of 10 min in 1×PBS (pH=7.4). Error bars are the SD of three repetitive experiments.

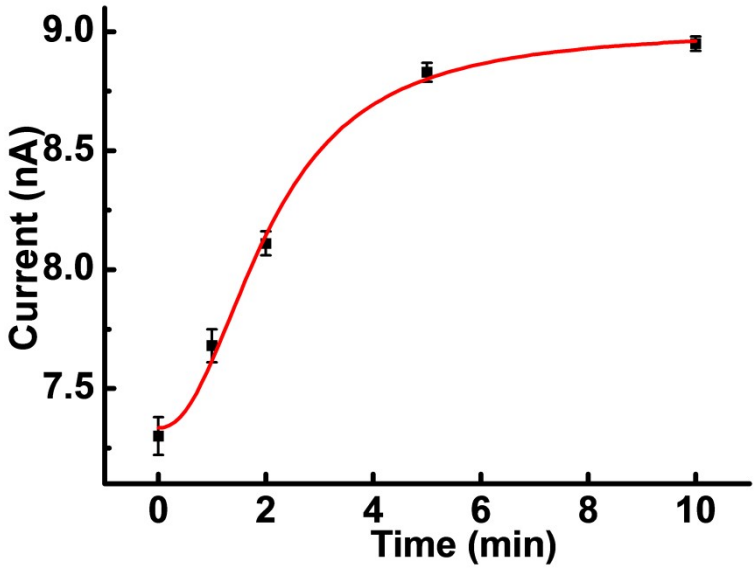

Figure S-2. Current responses to 1 mM H_2O_2 with reaction time of the biosensor in 1×PBS (pH=7.4) with 2 mM ABTS. Error bars are the SD of three repetitive experiments.

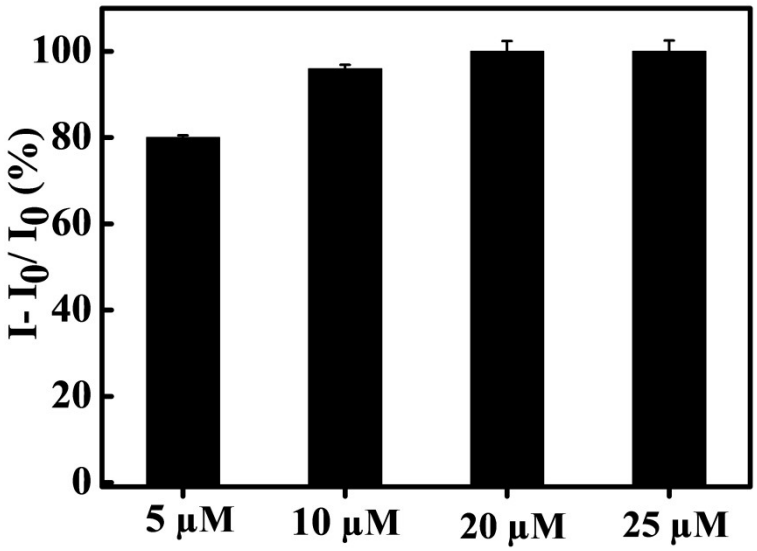

Figure S-3. Optimized the incubation concentration of DNAzyme during the sensor assembly under optimized conditions. Error bars are the SD of at least three repetitive experiments.

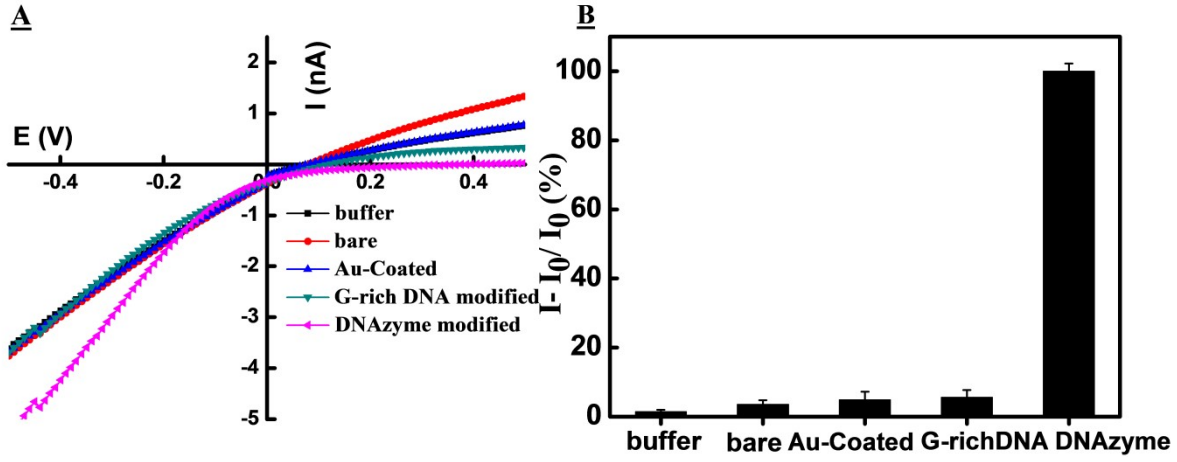

Figure S-4. The bare, Au-coated, modified with G-rich DNA oligomers and G-quadruplex DNAzyme glass nanopores responded to 1 mM H_2O_2. Error bars are the SD of at least three repetitive experiments.

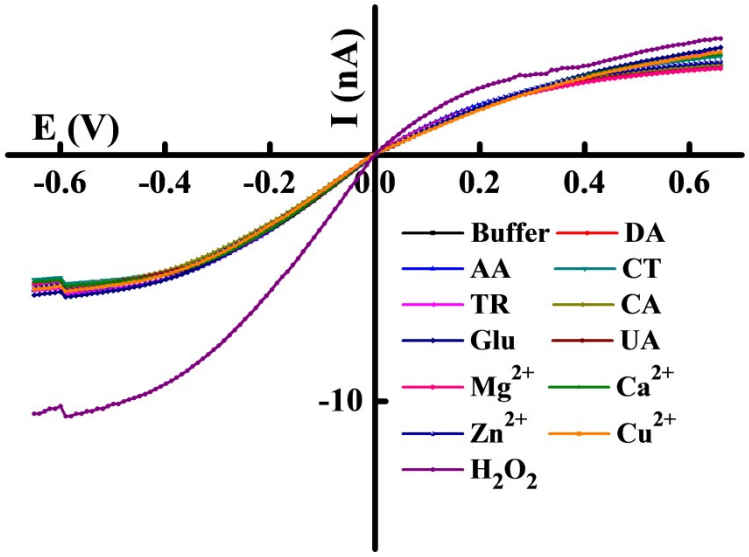

Figure S-5. The typical I-V curves of the dual-nanopore biosensor for 1 mM DA, AA, CT, TR, CA, Glu, UA, H_2O_2 and 100 μM Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}.

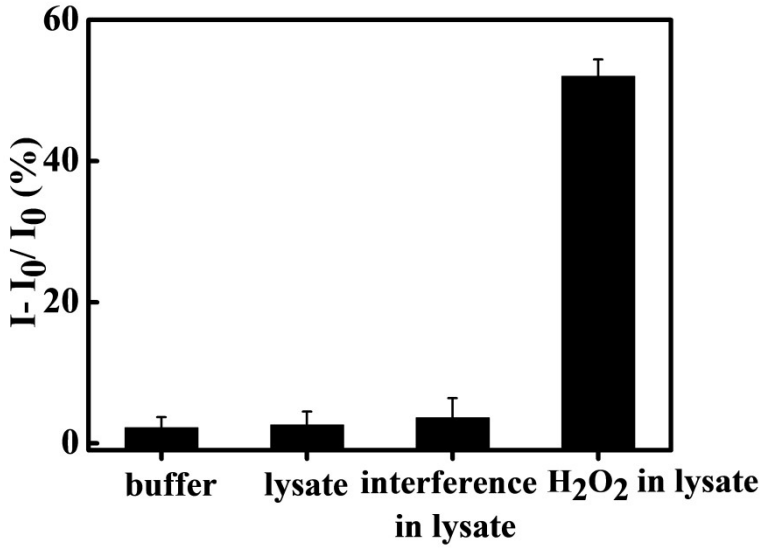

Figure S-6. The dual-nanopore biosensor challenged with buffer; cell lysate of MCF-7; interference spiked in lysate including DA, AA, CT, TR, CA, Glu, UA all at a concentration of 1 mM and ions of Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+} all at a concentration of 100 μM; and 1 mM H_2O_2 spiked in cell lysate. Error bars are the SD of three repetitive experiments.

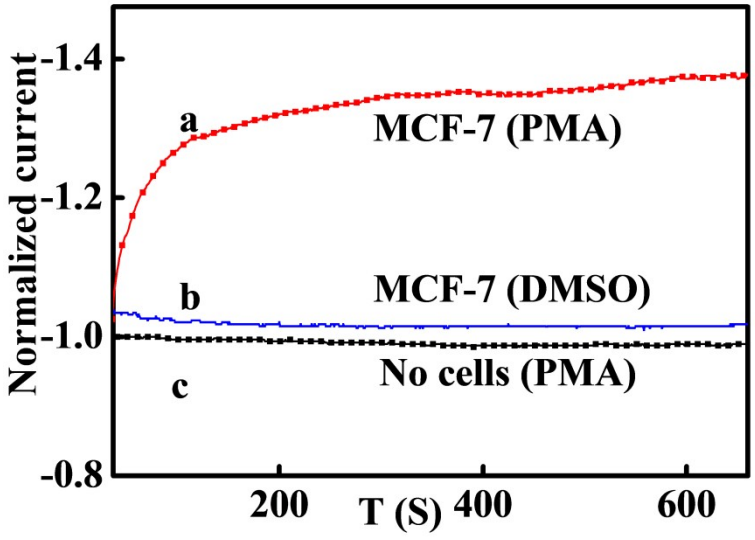

Figure S-7. Real-time current responses of the biosensor to the microwell array chip (curve a) addition of PMA with the cultured MCF-7 cells, (curve b) DMSO with the cultured MCF-7 cells, (curve c) PMA without cultured MCF-7 cells.

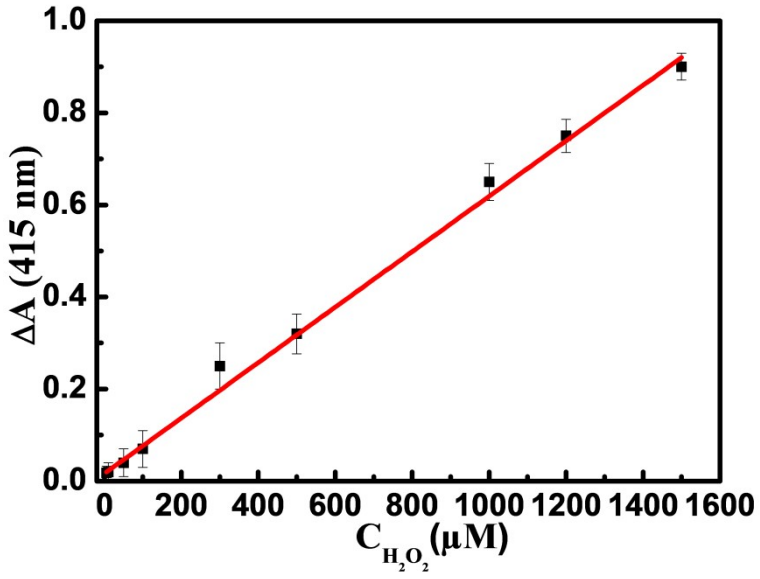

Figure S8. Calibration curve of the commercial H_2O_2 kit for different concentrations of H_2O_2 solution. Error bars are the SD of three repetitive experiments.

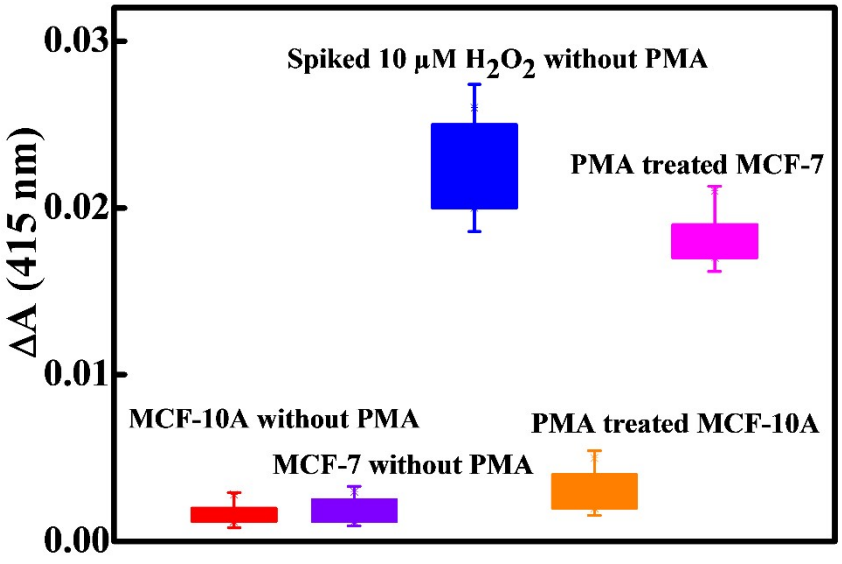

Figure S9. The detection of H_2O_2 utilizing the commercial detection kit for components from cell secretion without stimulation (24 h of culture) or stimulated with 1 μg/mL PMA from ~2×10^6 cells in 1 mL (cell cultured for 24 h and PMA stimulation for 2 h) of two different cell lines of MCF-7 and MCF-10A. Error bars are the SD of three repetitive experiments.