Supporting Information for

Catalase assisted Peroxide Quenching for Electrochemical Measurement of Reactive Oxygen Intermediates in Single Living Cell

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Experimental Section/Materials

1.1. Reagents.

DMEM high-glucose medium, fetal bovine serum, and penicillin-streptomycin were purchased from GIBCO (USA). Catalase (CAT), phorbol myristate acetate (PMA), and luciferin were purchased from Sigma (USA). A 30% hydrogen peroxide solution and dimethyl sulfoxide (DMSO) were produced by Aladdin (China). The experiments utilized ultrapure water with a resistivity of 18.2 MΩ·cm (obtained from the Millipore water purification system). All chemical reagents were of analytical grade and required no further purification. Unless otherwise specified, all reagents were sourced from Aladdin.

1.2. Cell Culture.

The MCF-7 cells were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Science of Chinese Academy of Science (Shanghai, China). The MCF-7 cells were seeded in DEME high-glucose medium supplemented with 10% fetal bovine serum (FBS) and 1% antibotics (penicillin/streptomycin). The MCF-7 cells were cultured with 5% CO_2 at 37°C.

1.3. Preparation and Characterization of Platinum-Coated Borosilicate Glass Tubes.

Borosilicate glass capillaries (BF100-58-10) were pulled into glass tubes with a tip opening of 200 nm using a micropipette puller (P-2000, Sutter Instrument, USA). Subsequently, a platinum layer approximately 20 nm thick was sputtered onto the surface of the glass capillary tip using a high-vacuum sputter coater (EM ACE600, Leica, Germany). The characterization of the platinum-coated borosilicate glass tubes with a diameter of 250 nm was performed using an S-4800 field-emission scanning electron microscope (Hitachi, Japan).

1.4. Preparation and Characterization of nanopipettes.

Copper wire was secured to a platinum-coated borosilicate glass tube using epoxy conductive adhesive (CW2400), and the adhesive was dried for 10 minutes at 80°C in an electric thermostatic blast drying oven (DHG-9030A, Shanghai Jinghong Laboratory Equipment Co., Ltd.). Under a stereo microscope (PXS6555, Shanghai Cewei Photoelectric Technology Co., Ltd.), the pre-prepared polydimethylsiloxane (PDMS) adhesive (DW-YL270, Zhongke Meiling Cryogenic Technology Co., Ltd.) was applied to the tip of the platinum-coated borosilicate glass tube and dried for 10 mins at 80°C. Subsequently, phosphate buffer solution (10 mM, pH 7.4) was injected into the glass tube, and the current of the prepared nanopipettes was measured using cyclic voltammetry

(CV) on an electrochemical workstation (CHI 760E, Shanghai Chenhua Instrument Co., Ltd.).

1.5. Electrochemical Detection

Electrochemical measurements were performed using an electrochemical workstation (CHI 760E, CH Instruments, Shanghai, China) with a two-electrode system. The nanopipette served as the working electrode, and an Ag/AgCl wire was used as the reference electrode. In the solution experiments, chronoamperometry (CA) was employed with an applied voltage of 0.6 V and a scan rate of 0.1 V/s. In single-cell experiments, the nanopipette was inserted into the cell, while the reference electrode was placed in the extracellular solution. Channel 1 was set at 0.6 V to record electrochemical signals before and after PMA stimulation of the cell. Meanwhile, channel 2 was set at 1.5 V with a duration of 3 s to electrochemically pump the solution with catalase and fluorescein from inside the glass tube into the MCF-7 cell.

Figure S1. The top view of the capillary tip using scanning electron microcopy.

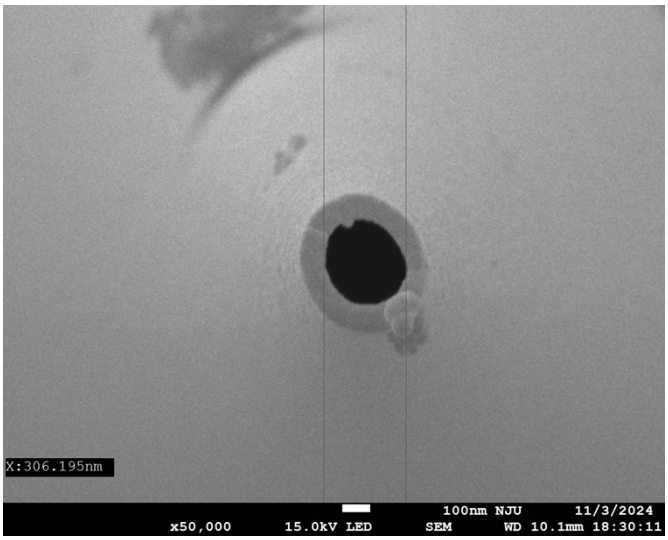

Figure S2. The simulated limiting currents from the nanopipettes with different lengths of Pt region at the tip.

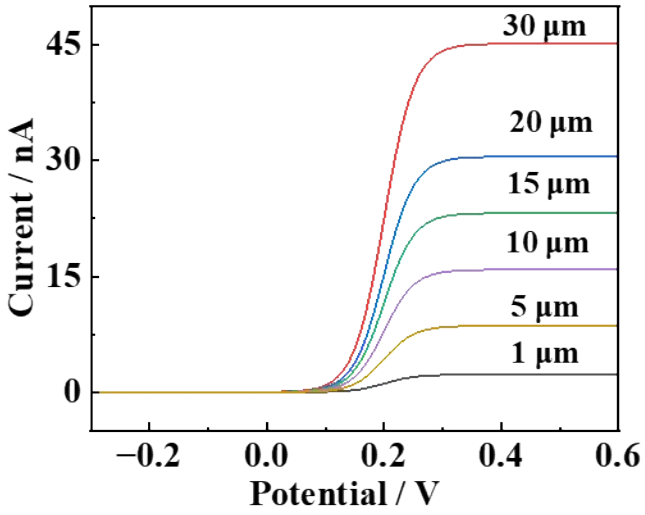

Figure S3. The typical current trace of the nanopipette after the response of hydrogen peroxide and the consequent injection of 10 µL PBS into the buffer. a: stands for injection of 200 µM hydrogen peroxide solution; b: stands for injection of 10 µL PBS into the solution.

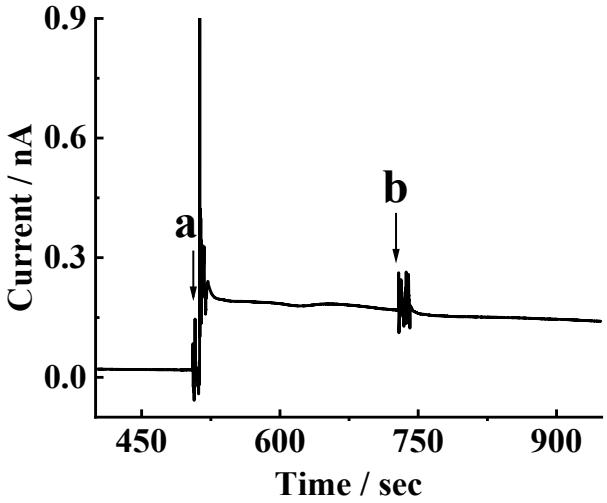

Figure S4. The integrated charges from the nanopipettes collected from eight cells. The bar (deep yellow) presents the data from the cell with the injection of fluorescein; and the bar (green) presents the data from the cell with the injection of catalase and fluorescein.