Supporting information for

Electrochemiluminescence Analysis of Glucose in Single Living Cells using Enzyme-modified Micropipette

Xiaofang He,[a] Qian Zhou,[a] Dechen Jiang[b*] and Danjun Fang[a*]

a. School of Pharmacy, Nanjing Medical University, Nanjing, Jiangsu 211126, China

b. State Key Laboratory of Analytical for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, Jiangsu Province, 210023, China

E-mail: djf@njmu.edu.cn, dechenjiang@nju.edu.cn

Experimental Section

Chemical and Cell Culture.

The compound 8-amino-5-chloro-7-phenylpyrido [3, 4-d] pyridazine-1, 4(2H, 3H)-dione (L012) was obtained from Wako Chemical, Inc. (Richmond, VA). Chitosan (CS, 80−95% deacetylation) was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). 30% of hydrogen peroxide and glucose were obtained from Aladdin (Shanghai, China). All aqueous solutions were prepared with ultrapure water (18.2 MΩ cm).

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

Preparation of enzyme modified micropipettes

A glass capillary (BF100-58-10) was pulled using a micropipette puller (P2000; Sutter Instrument Co.) to produce a tip with ~1 μm opening. A gold layer with a thickness of approx.. 50 nm was deposited on the outer surface of the capillary tip using a vacuum metal evaporator system (JEE-420, Jeol, Japan). Then, the micropipette was immersed in a mixed solution containing 10 μL of glucose oxidase (3 mg/ml) and 10 μL of chitosan (0.5 wt%, pH=5) at 4℃ for 8 hours. After that, the micropipette was air-dried for 0.5 hours. The enzyme modified micropipette was then immersed in 20 μL L-012 solution (2 mM) at 4℃ for 8 hours. After air-dried for 0.5 hour, the enzyme modified micropipette was prepared.

ECL detection

The enzyme modified micropipette was used as the working electrode, and an Ag/AgCl wire was used as the reference electrode. In the solution experiment, the electrode was positioned into a 10 mM PBS to detect the background luminescence signal. Then, hydrogen peroxide or glucose solution with different concentrations was added into the solution for the ECL detection. In single cell experiment, the micropipette was positioned outside the cell to collect the background signal. Then, the micropipette was positioned inside the cell to detect the ECL signal for the quantification of intracellular glucose. Typically, the microelectrode was inserted approximately 4-5 μm into the cytoplasmic matrix to target the cytosolic glucose. Care was taken to avoid contact with the nuclear

membrane in order to minimize mechanical disturbance and potential signal interference. To ensure the positional stability during the measurement, a motorized micromanipulator (MP-225A) in combination with an air-floated optical table was used. This setup allowed for high-precision positioning of the microelectrode and effective suppression of environmental vibrations. Additionally, a CCD camera was used to monitor the electrode position in real time and ensure spatial accuracy during in situ single-cell analysis. The cyclic voltammetry curves were collected using CHI 600E electrochemical workstation (CH Instrumental, USA) with a voltage range of 0 to 1 V. The ECL intensity was collected with a photomultiplier tube (PMT, 7ID101, Beijing Sefan Photoelectric Instrument Co., LTD.) biased at 800 V.

Figure S1. (A) The scanning electron microscopic image of the microcapillary tip; (B) the bright-field image of the enzyme modified micropipette.

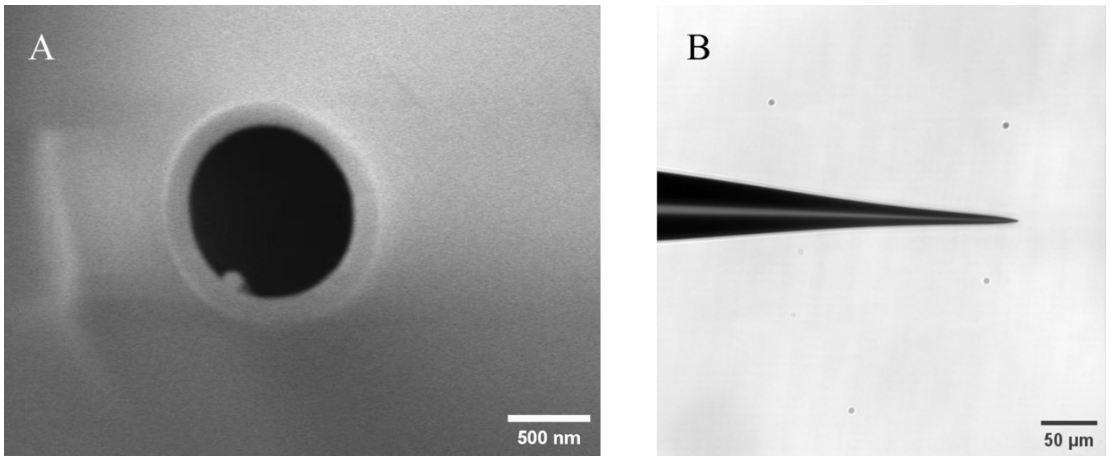

Figure S2. The bright-field image to display the insertion of the micropipette inside one living cell.

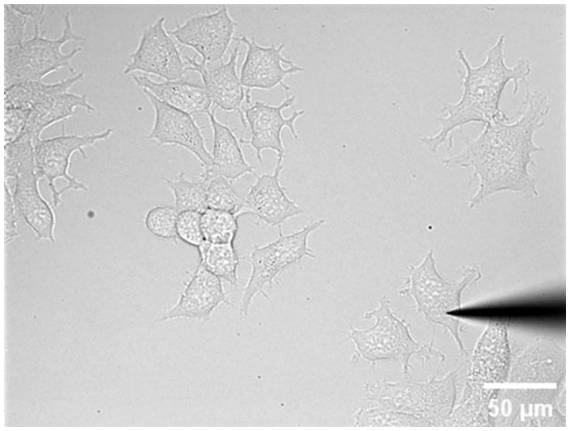

Figure S3. The ECL traces from the enzyme modified micropipette collected from PBS (black line) and single starved MCF7 cell (red line).