

Supporting Information

Enhanced electrochemiluminescence imaging of single cell membrane proteins based on Co₃O₄ nanozyme catalysis

Jingjing Zhang^a, Lin Hao^{b*}, Jie Chao^a, Lianhui Wang^c, Shao Su^{c*}

^aSchool of Geographic and Biologic Information, Nanjing University of Posts and Telecommunications, Nanjing 210023, China

^bDepartment of Urology, Xuzhou Central Hospital, Xuzhou 221009, Jiangsu, China

^cKey Laboratory for Organic Electronics and Information Displays, Jiangsu Key Laboratory for Biosensors, Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing University of Posts and Telecommunications, Nanjing 210023, China

*Corresponding authors:

E-mail addresses: haolinxuzhou@163.com (Lin Hao); iamssu@njupt.edu.cn (Shao Su)

EXPERIMENTAL SECTION

Materials and Reagents. Dulbeccos modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin were obtained from Life Technologies. 8-Amino-5-chloro-7-phenylpyrido[3,4-d]pyridazine-1,4 (2H,3H)-dione (L012) was purchased from Wako Corporation. Hydrogen peroxide was purchased from Sangon Biotech Ltd (Shanghai, China). CEA antibody was obtained from Abcam Ltd (Shanghai). MCF-7 and Hela cells were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences of Chinese Academy of Science (Shanghai, China). Cells were cultured in DMEM medium supplemented with 10% FBS and 1% antibiotic (penicillin/streptomycin) in an incubator containing 5% CO₂ at 37 °C.

Apparatus. Indium tin oxide conductive glass (ITO, square resistance < 17 ohm/sq, 10 × 10 cm², ITO thickness 100 ± 20 nm) was purchased from Zhuhai Kaiwei Optoelectronic Technology Co., Ltd. Electrochemical luminescence analyzer (MPI-A) was purchased from Xi'an Remei Corporation. The ECL imaging device consists of a water-immersion objective (40X, Olympus, Japan), a microscope (Olympus, Japan), an electron multiplying CCD (EM-CCD) (Evolve, Photometrics, Tucson, AZ), and voltage emitter (DG1021, Beijing Puyuan Precision Technology Co., Ltd).

Synthesis and functionalization of Co₃O₄ nanoparticles. 40 mL 0.025g/mL Co(NO₃)₂ aqueous solution was evenly mixed with 0.8 mL 30% hydrogen peroxide, and ammonia was then added to adjusted the pH of solution to 9.0. Subsequently, the mixture solution was transferred to a reaction vessel and reacted at 180 °C for 12 h. After the reaction, Co₃O₄ nanoparticles were obtained through centrifugation, washing and drying. The product was ultrasonically dispersed in a mixture of ethanol and water, then 3-aminopropyltriethoxysilane was added and stirred overnight at 50°C. Finally, the aminated Co₃O₄ was obtained through centrifugation, washing, and drying.

Synthesis of Co₃O₄-tagged CEA antibody. 1 mg/mL Co₃O₄ solution mixed with 2.5wt% GA solution and stirred for 6 h at room temperature. 10 mg/mL CEA antibody was added into the mixture and continued for 30 min. 20 μL of 1% BSA was then added

into the above mixture to block excess binding sites. The obtained Co_3O_4 -CEA antibody were purified by centrifugation, and then resuspended in PBS. The Co_3O_4 -CEA probes were stored at 4°C for future use.

ECL imaging. An “O” ring was used to fix a certain size of conductive area on the ITO electrode as the working electrode. The Ag/AgCl electrode and the platinum wire performed as the reference electrode and auxiliary electrode, respectively. The surface aminated Co_3O_4 prepared was dispersed on the ITO electrode and dried at 80°C to spread the particles evenly and firmly on the electrode surface. For ECL imaging, a system was assembled with a water-immersion objective (40X, Olympus, Japan), a tube, and an electron multiplying CCD (EMCCD). The solution to generate ECL from ITO and Co_3O_4 surface was 10 mM PBS containing $200\ \mu\text{M}$ L012 and $20\ \mu\text{M}$ H_2O_2 by applying a conversion mode voltage among 1.0 V (2 s) and -1.0 V (0.5 s) using voltage transmitter. After that, the pictures were acquired continuously using EMCCD.

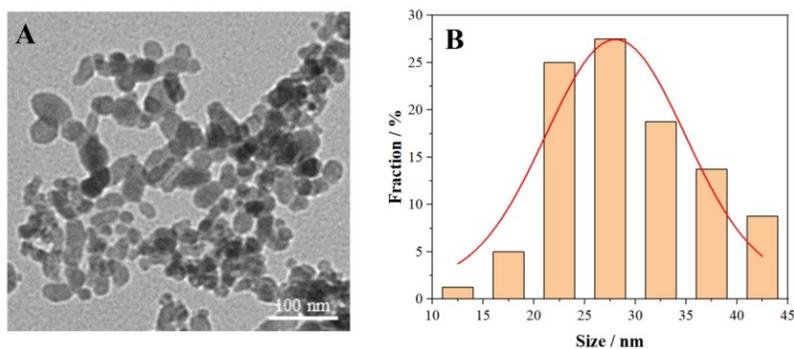


Figure S1. TEM image (A) and the statistical map of particle size distribution of Co_3O_4 nanoparticles.

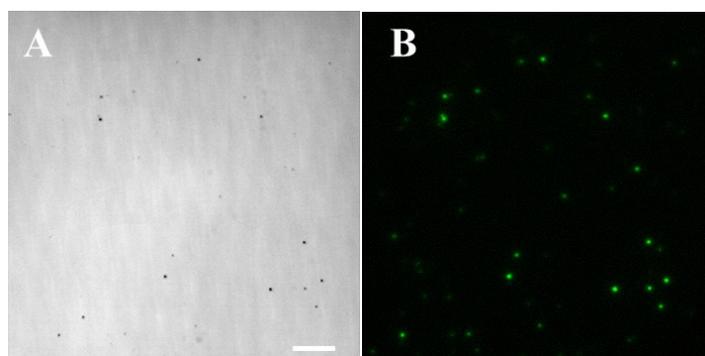


Figure S2. (A) Bright-field image and (B) ECL image of Co_3O_4 on the ITO slide. The exposure time was 1 s, the applied potential was 1 V. Scale bar: 5 μm.

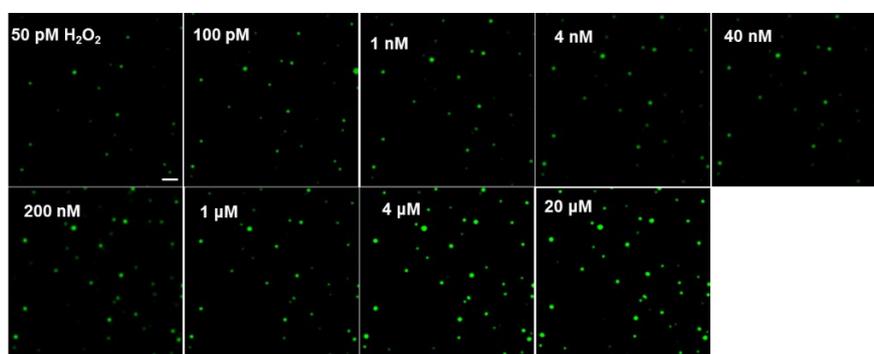


Figure S3. The ECL images of Co₃O₄ at ITO slides with 50 pM, 100 pM, 1 nM, 4 nM, 40 nM, 200 nM, 1 μM, 4 μM and 20 μM H₂O₂. The exposure time was 500 ms, the applied potential was 1 V. Scale bar: 5 μm.

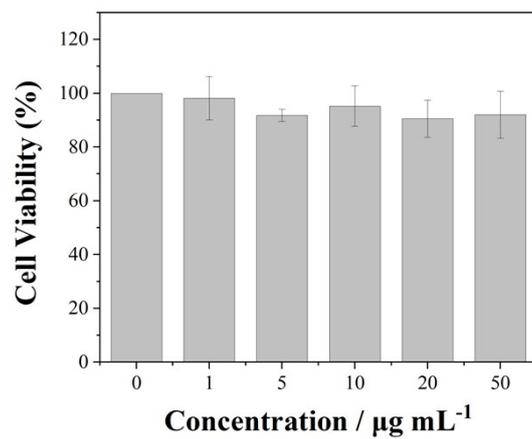


Figure S4. Cell viability of MCF-7 cells in 10 mM PBS with the different concentration of Co₃O₄ nanozymes.