Electronic Supplementary Information

An anti-poisoning nanosensor for in-situ monitoring of intracellular endogenous hydrogen sulfide

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1. Experimental Sections

1.1 Materials and Instruments

SiC nanowires (SiC NWs) were purchased from Nanjing/Jiangsu XFNANO Materials Tech Co., Ltd. (Nanjing, China). Molybdenum (IV) sulfide (MoS_2), $HAuCl_4 \cdot 3H_2O$, $Ru(NH_3)_6Cl_3$, glutathione (GSH), reduced nicotinamide adenine dinucleotide (NADH), cysteine (Cys), homocysteine (Hcy), dopamine (DA), ascorbic acid (AA), o-(Carboxymethyl)hydroxylamine hemihydrochloride (AOAA, an inhibitor of hydrogen sulfide synthetase), tetraacetoxymethylester (Calcein-AM), and propidium iodide (PI) were bought from Sigma-Aldrich (China). Quercetin (Qu) and 3,4-ethylenedioxythiophene (EDOT) were purchased from Aladdin Industrial Co., Ltd. (China). 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, 18.0 MΩ·cm) was used for preparing all aqueous solutions during the whole experiment.

Scanning electron microscopy (SEM) images were obtained with field-emission scanning electron microscopes (Zeiss Sigma). Energy-dispersive X-ray spectroscopy (EDX) images were obtained by an EDX spectrometer (X-MaxN). 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. The UV absorption spectra were obtained by UV-2600 UV-vis spectrophotometer (Shimadzu, Japan). Zeta potential was acquired on Zetasizer (Nano-ZS90, Malvern). 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 electrode. An inverted fluorescent microscope (AxioObserver Z1 and Axiovert 200M, Zeiss) was used for bright-field and fluorescence imaging. Amperometric recordings of cell experiments were obtained by a patch clamp amplifier (EPC-10, HEKA Electronics) coupled with a micromanipulator (TransferMan 4r, Eppendorf).

1.2 One-Pot Synthesis of MoS_2 and Qu Composite Functionalized Nanowires

Based on our group's previous work of synthesizing functionalized nanowires in one pot,[1] after modification, the functionalized nanowires were prepared as follows:

To synthesize SiC@Au-PEDOT nanowires: firstly, 2 mg SiC nanowires were uniformly dispersed in 30 mL acetonitrile by ultrasonic treatment. Secondly, 500 μL EDOT was added to the SiC nanowire dispersion under stirring. Thirdly, 2 mL $HAuCl_4$ aqueous solution (25 mM) was added to the second step mixture to initiate the chemical polymerization reaction. Finally, the mixture was stirred for 48 h at room temperature. With the above steps, the SiC@Au-PEDOT nanowire dispersion was obtained.

To synthesize Qu and MoS_2 functionalized nanowires (Qu@MoS_2-NWs): 0.1 g Qu was dissolved in 20 ml mixed organic solvent (V_{DMSO}:$V_{acetonitrile}$ = 1:3) with ultrasonic treatment; subsequently, 10 mL freshly prepared SiC@Au-PEDOT nanowire dispersion was added to 20 mL Qu solution under stirring. Then 500 μL EDOT and 2 mL $HAuCl_4$ aqueous solution (25 mM) were added to the mixture. After reacting for 24 h, Qu functionalized SiC@Au-PEDOT nanowires (Qu-NWs) dispersion was obtained. Finally, 5 mL MoS_2 dispersion (1 mg/ml in DMF) which was ultrasonicated for 4 h, was added to Qu-NWs dispersion. After stirring for 24 h, Qu@MoS_2-NWs dispersion was obtained. The Qu@MoS_2-NWs were then collected through centrifuging and washing with ethanol for 3 times.

MoS_2 functionalized SiC@Au-PEDOT nanowires (MoS_2-NWs) were also synthesized with the same method simply by omitting the steps of Qu addition.

1.3 Fabrication of MoS_2 and Qu Composite Functionalized NWEs

Procedures of functionalized NWEs fabrication were according to the method in our previous work with some modifications.[1,2] Firstly, the prepared Qu@MoS_2-NWs dispersed in ethanol were spread on a glass slide and heated to evaporate ethanol, and then the glass slide was cut into two parts for the purpose of allowing nanowires to partially protrude over the glass slide edge. Secondly, to obtain a micropipette holder, a borosilicate capillary was first pulled to obtain a glass micropipette with the tip diameter about 2 μm, and then liquid metal was injected into the glass micropipette and centrifuged to a distance of 8-16 μm from the tip of micropipette, after which the micropipette tip was insulated with wax. Finally, after the wax was heated to melt, a nanowire was inserted into the prepared micropipette holder to fabricate a functionalized NWE by using the microforges. For comparison, SiC@Au-PEDOT NWEs, Qu-NWEs and MoS_2-NWEs were also fabricated by a similar protocol with changing the corresponding nanowires.

1.4 Cell Experiments

1.4.1. Cell culture

MCF-7 cells were cultured in DMEM basic (1×) culture medium added with 10% FBS and 1% penicillin-streptomycin at 37 °C under 5% CO_2 atmosphere. Before further experiments, 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: Cys (10 mM) for 1 h; AOAA pre-treated (1 mM) for 1 h and subsequently treated with Cys (10 mM) for 1 h.

1.4.2. Cell vitality experiment

Qu@MoS_2-NWEs were inserted into MCF-7 cells and then withdrawn, and 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 for 3 times to remove the residual dyes. Bright field (BF) and fluorescence (FL) microphotographs were taken with an inverted fluorescent microscope.

1.4.3. Amperometric data acquisition and analysis

All amperometric experiments for cells monitoring were performed by an inverted microscope equipped with a patch clamp amplifier. All apparatuses were placed in a well-grounded Faraday cage. We first applied a constant potential of 300 mV (Ag/AgCl as the reference/counter electrode) to the Qu@MoS_2-NWE and then moved it to contact with the cell membrane with the aid of a micromanipulator. The Qu@MoS_2-NWE was gently moved forward to ensure complete insertion inside the cell for intracellular H_2S detection. The current response of the entire process was recorded by a patch-clamp amplifier. Signals were sampled at 1 kHz and Bessel-filtered at 2.9 kHz. The amperometric data were collected with “Pulse” software and analyzed by “Origin 2018 Graphing &Analysis” software.

2. Supporting Figures

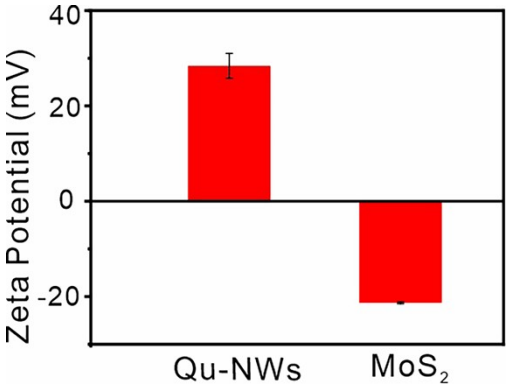

Fig. S1: Zeta potentials of Qu-NWs and MoS_2.

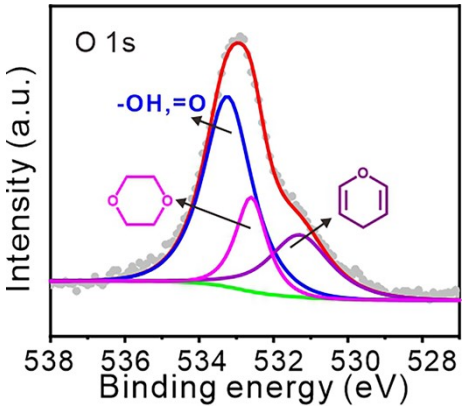

Fig. S2: XPS data of O 1s states from Qu@MoS_2-NWs.

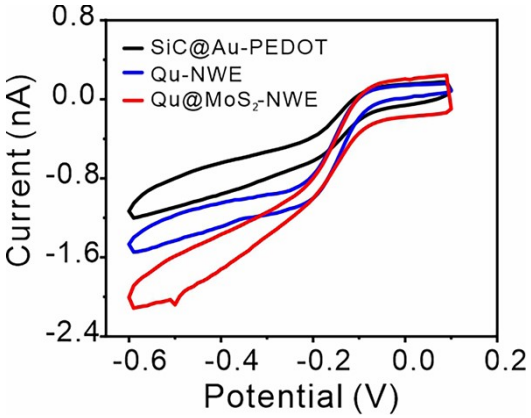

Fig. S3: Cyclic voltammograms of 1 mM $Ru(NH_3)_6^{3+}$ at SiC@Au-PEDOT NWE (black), Qu-NWE (blue) and Qu@MoS_2-NWE (red), 1 M KCl as the supporting electrolyte, scan rate was 0.1 V/s.

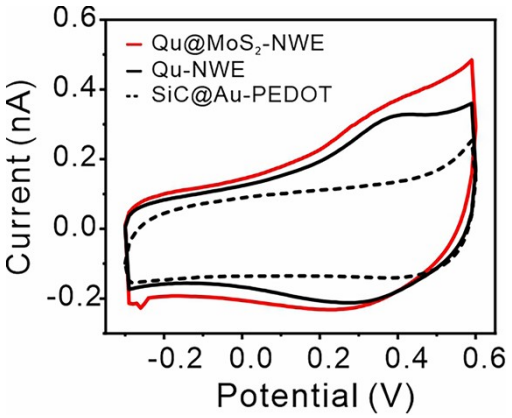

Fig. S4: Cyclic voltammograms of SiC@Au-PEDOT NWE (black dotted curve), Qu-NWE (black solid curve) and Qu@MoS_2-NWE (red solid curve) in 0.01 M PBS solution, scan rate was 0.1 V/s.

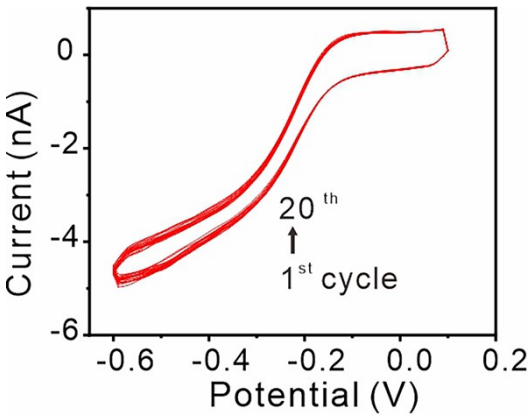

Fig. S5: Repetitive cyclic voltammograms (20 cycles) of 1 mM $Ru(NH_3)_6^{3+}$ at Qu@MoS_2-NWE, 1 M KCl as the supporting electrolyte, scan rate was 0.1 V/s.

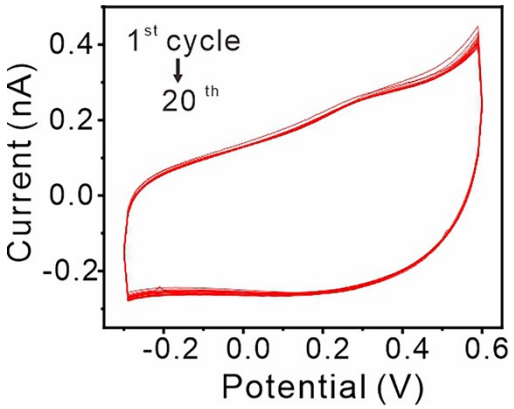

Fig. S6: Repetitive cyclic voltammograms (20 cycles) of Qu@MoS_2-NWE in 0.01 M PBS solution, scan rate is 0.1 V/s.

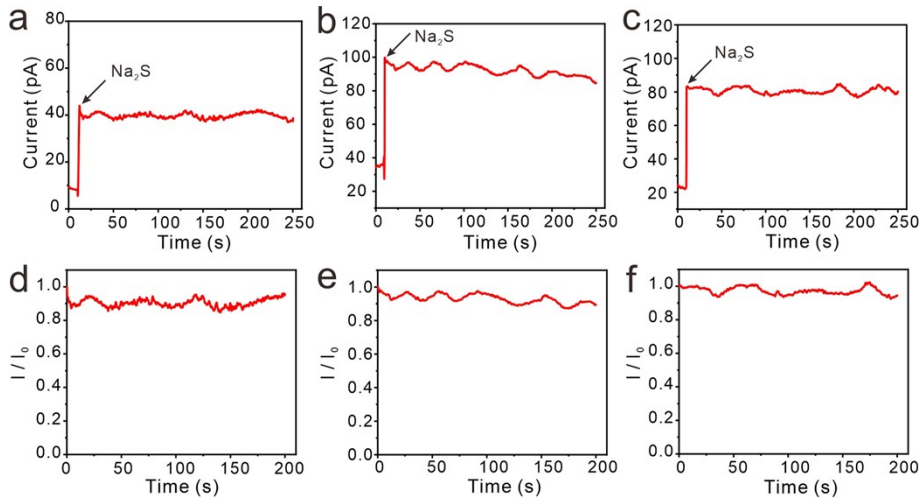

Fig. S7: (a-c) Amperometric current response respectively recorded with three Qu@MoS_2-NWEs in PBS upon the addition of 50 μM Na_2S at +300 mV (vs. Ag/AgCl) and (d-f) the corresponding normalized current (I/I_0), I_0 and I were the current values at starting time and given time after the addition of 50 μM Na_2S, respectively.

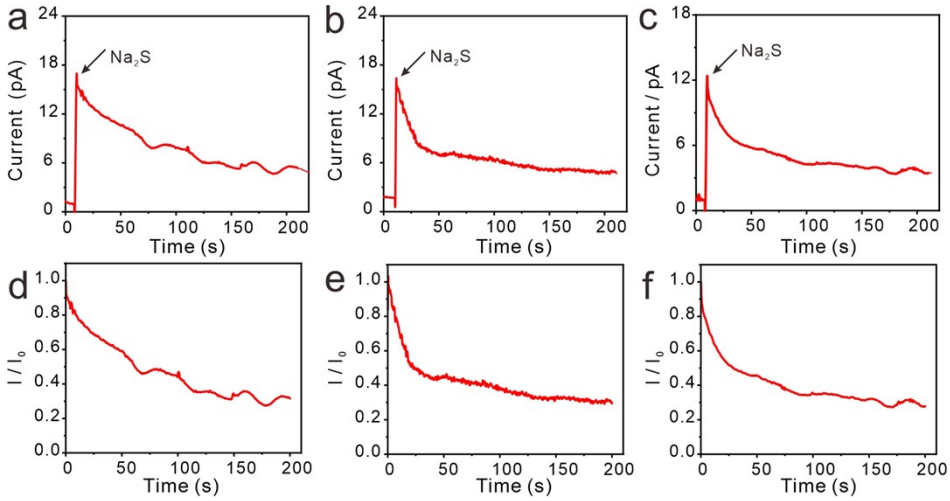

Fig. S8: (a-c) Amperometric current response respectively recorded with three Qu-NWEs in PBS upon the addition of 50 μM Na_2S at +0.3 V (vs. Ag/AgCl) and (d-f) the corresponding normalized current (I/I_0), I_0 and I were the current values at starting time and given time after the addition of 50 μM Na_2S, respectively.

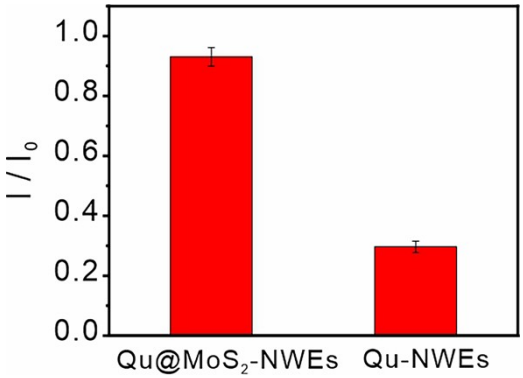

Fig. S9: Statistics data of normalized current (I/I_0) of Qu@MoS_2-NWEs and Qu-NWEs at 200 s (n=3).

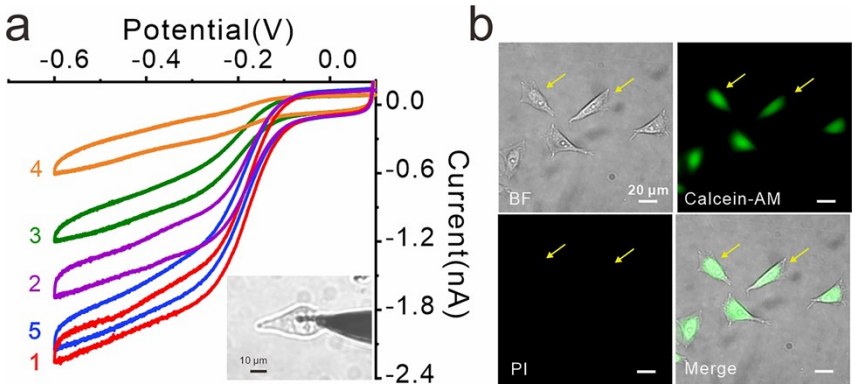

Fig. S10: (a) cyclic voltammograms at a Qu@MoS_2-NWE inserted into an MCF-7 cell for different depths when adding $Ru(NH_3)_6^{3+}$ (1 mM) to a PBS cell bath. Curve 1: before insertion, curve 2: circa 25 % insertion, curve 3: circa 50 % insertion, curve 4: circa 75 % insertion, curve 5: following withdrawal from the cell. Inset: a NWE being completely inserted into a MCF-7 cell. (b) Bright-field and fluorescence imaging of MCF-7 cells stained with Calcein-AM (green) and PI (red) after penetration by Qu@MoS_2-NWEs (indicated by arrows).

3. References

1 W.-T. Wu, H. Jiang, Y.-T. Qi, W.-T. Fan, J. Yan, Y.-L. Liu and W.-H. Huang, *Angewandte Chemie International Edition*, 2021, **60**, 19337-19343.

2 X.-W. Zhang, Q.-F. Qiu, H. Jiang, F.-L. Zhang, Y.-L. Liu, C. Amatore, W. Huang, *Angewandte Chemie International Edition*, 2017, ***56***, 12997-13000.