

Supplementary information

Silicon nanowires-based fluorescent sensor for in situ detection of hydrogen sulfide in extracellular environment

Huimin Wang,^{a,b} Lixuan Mu^{a,*}, Guangwei She,^a and Wensheng Shi^{a,*}

^a Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of

Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, China

^b University of Chinese Academy of Sciences, Beijing 100190, China

*Corresponding author: Dr. Lixuan Mu and Prof. Wensheng Shi,

Tel: +86-10-82543513 Fax: +86-10-82543513

Email: mulixuan@mail.ipc.ac.cn; shiws@mail.ipc.ac.cn

1. Reagents and Materials

3-aminopropyl-triethoxysilane (APTES), Trifluoroacetic acid and Isopropyl nitrite were purchased from Alfa Aesar. 4-Amino-1,8-naphthalic anhydride was purchased from Sigma-Aldrich. Other reagents were purchased from Beijing Chemical Regent Co. All reagents and chemicals were AR grade and used without further purifications unless otherwise noted. Water used for measurement was purified by Millipore filtration system.

2. Measurements

Fluorescence spectra were recorded on a Hitachi F-4600 spectrophotometer. Fluorescence images were taken with laser scanning confocal microscope (LSCM, Nikon- Ti + Ultra-VIEWVoX) with an excitation of 488 nm (Coherent). TEM images were recorded by a JEOL-2100 at an acceleration

voltage of 200kV. SEM images were recorded by a Hitachi S-4800FEG. The X-ray photoelectron spectroscopy (XPS) was recorded by a PHIQUANTERA-II SXM at a voltage of 15kV.

3. Preparation of SiNWs

SiNWs were prepared by a typical chemical vapor deposition (CVD) method.¹ An alumina boat containing SiO powder (Aldrich, 99%) was placed at the center of a horizontal alumina tube mounted inside a high temperature tube furnace. The system was evacuated to 10^{-3} Pa. Then Ar (95%) and H₂ (5%) (carrier gas) was introduced at the flow rate of 49 sccm, and the system pressure rised to 800 Pa. The furnace was heated to 300 °C at a speed of 10 °C/min and then to 800 °C at a speed of 15 °C/min. After ten minutes' maintenance, the carrier gas and the pump were cut off. Then the furnace was heated to 1350 °C, while the system pressure was maintained at 10^4 Pa. The system was maintained for 6 h and then cooled to room temperature naturally. The slightly yellow cottonlike product was collected at both sides of the alumina boat. The structures and morphologies of the as-prepared SiNWs were characterized using transmission electron microscopy (TEM). (Figure S1)

4. Modification of SiNWs

The SiNWs by CVD method were immersed in a mixture containing 7.5 mL concentrated H₂SO₄ (98%) and 2.5 mL 30% H₂O₂ (V/V=3:1) at 90°C for 1.5 h, then cooled down to room temperature. After repeatedly washed with deionized water, the clean SiNWs were immersed in a mixture of 5 mL H₂O, 1 mL 30% H₂O₂ and 1 mL NH₄OH (V/V/V=5:1:1) at room temperature for 2 h. Then the SiNWs were repeatedly rinsed with deionized water and dried under vacuum at 50 °C to obtain the hydroxyl groups terminated SiNWs for the following modification.

The procedure of the SiNWs modification was shown in Figure S2.

20 mg of dried SiNWs were dispersed in 10 mL anhydrous toluene (distilled) under nitrogen in a two-neck round bottomed flask, then 0.80 mmol of 3-aminopropyl-triethoxysilane (APTES) was added by syringe. The suspension was heated to 90 °C and stirred for 20 h. After cooling down to room temperature, the product (defined as APTES-SiNWs) was collected by a micro filter and repeatedly

washed by ethanol.

The as-prepared APTES-SiNWs were suspended in a round flask contain 6 mL absolutely anhydrous ethanol under nitrogen, then 0.1mmol (21.3mg) of 4-Amino-1,8-naphthalic anhydride dissolved in anhydrous ethanol was added. The mixture was stirred for 18 h at 80 °C, protected from light. The modified SiNWs were collected by filtration and repeatedly washed with ethanol. Unreacted organic materials were removed completely by monitoring the fluorescence of the washing liquid. The product was defined as 4-A-SiNWs.

8mg of 4-A-SiNWs were suspended in 10mL 2:1 CH₃CN:CH₂Cl₂ and cooled to 0 °C, Trifluoroacetic acid (10μL, 0.135mmol) was added to the mixture, then followed by addition of *iso*-amyl nitrite (12μL, 0.09mmol). After stirring for 2h at 0 °C, sodium azide (12mg, 0.185mmol) was added and the solution was stirred for additional 1h. The mixture was poured into 15mL saturated aq NaHCO₃, after stirring for 5min, the final product was filtered and washed with deionized water, EtOH and CH₂Cl₂. The obtained final product was named as MSiNWs.

5. Preparation of SiNW arrays

The high-quality SiNW arrays were fabricated according to previous work.² A clean n-type silicon wafer with [100] orientation was cut into 1 cm×8 cm pieces. After cleaning ultrasonically in acetone, ethanol and water for 5min respectively, the clean pieces were immersed into 5% hydrofluoric acid (HF) solution for 15min to bring Si-H bonds on the surface of the wafer. Afterwards, the wafer pieces were soaked in a 5mM silver nitrate solution to deposit the Ag particles on its surface, and the particles would act as catalyst in the following etching process. Finally, the wafer attached with Ag particles was soaked in the etching solution consisting of 4.8M HF and 0.2M H₂O₂ at 50 °C for 30 min. After etching, the samples were immersed into a solution of condensed hydrochloric acid (HCl) and condensed nitric acid (HNO₃) (V:V=3:1) for 2 h to remove the Ag particles. Finally, the SiNWs arrays were obtained after copiously rinsing with water and ethanol. The SEM images of SiNW arrays were shown in Figure S3.

6. Preparation of SiNW arrays-based sensor (MSiNW arrays)

The modifying procedures applied to SiNW arrays were the same as to SiNWs obtained by CVD methods mentioned above. After modification of SiNW arrays, the modified SiNW arrays (MSiNW arrays) were obtained.

7. Preparation of cell-captured MSiNW arrays

MSiNW arrays were cut into pieces of 1.0 cm×0.5 cm. The pieces were placed into a 24-well plate and then 1 mL of a cell suspension (10^6 cells/mL) was loaded. After incubating for 16 h at 37 °C and 5% CO₂, the pieces were rinsed with PBS (pH 7.4) for six times to remove the uncaptured cells, and the cell captured MSiNW arrays was prepared. In order to observe the captured cells, the MSiNW arrays were incubated with 2 μL DAPI solution (2 μg/mL in deionized H₂O) for 20 min (at 37 °C and 5% CO₂) and then followed by six washes with sterile PBS (pH 7.4). Afterwards, the piece of MSiNW arrays was placed upside down in a specific dish contains 2mL sterile PBS. The images of the piece of MSiNW arrays before and after treated with H₂S were recorded with LSCM.

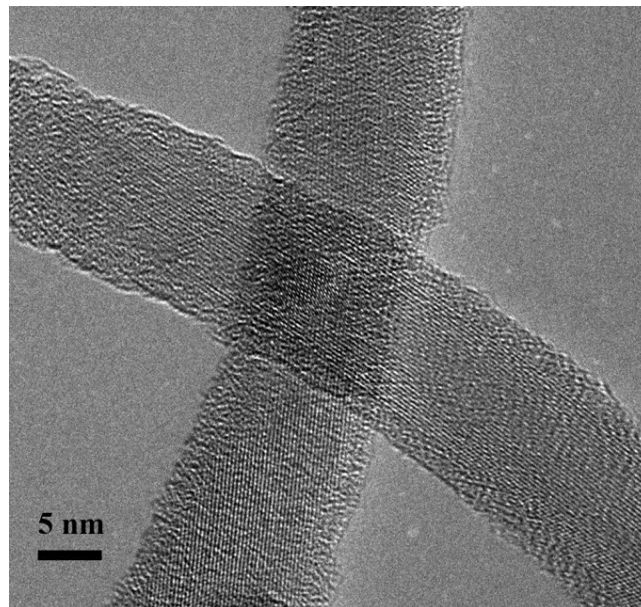


Figure S1 The TEM image of SiNWs by CVD method.

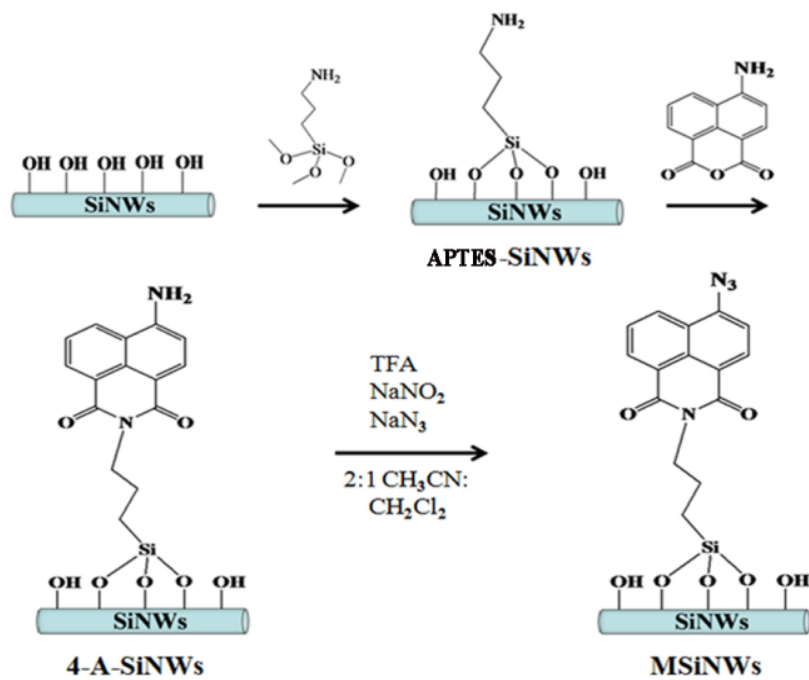


Figure S2 The procedure for SiNWs modification.

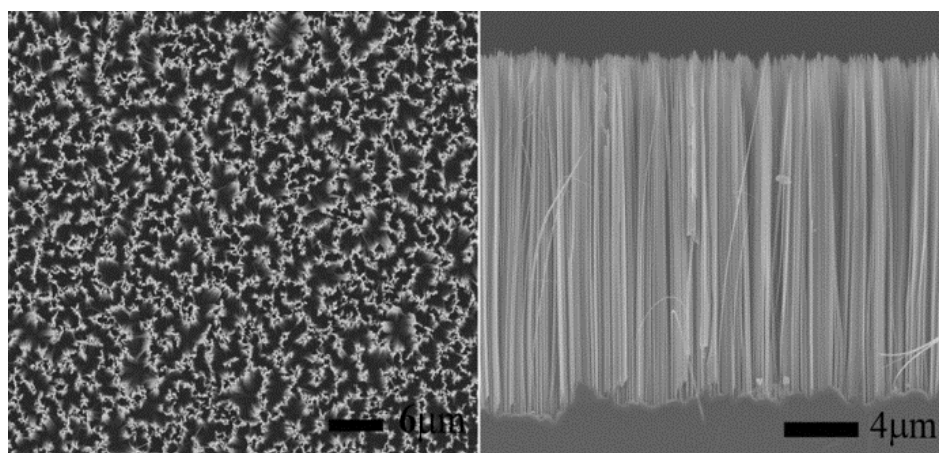


Figure S3 SEM images of the top view (left) and the side view (right) of the SiNW arrays.

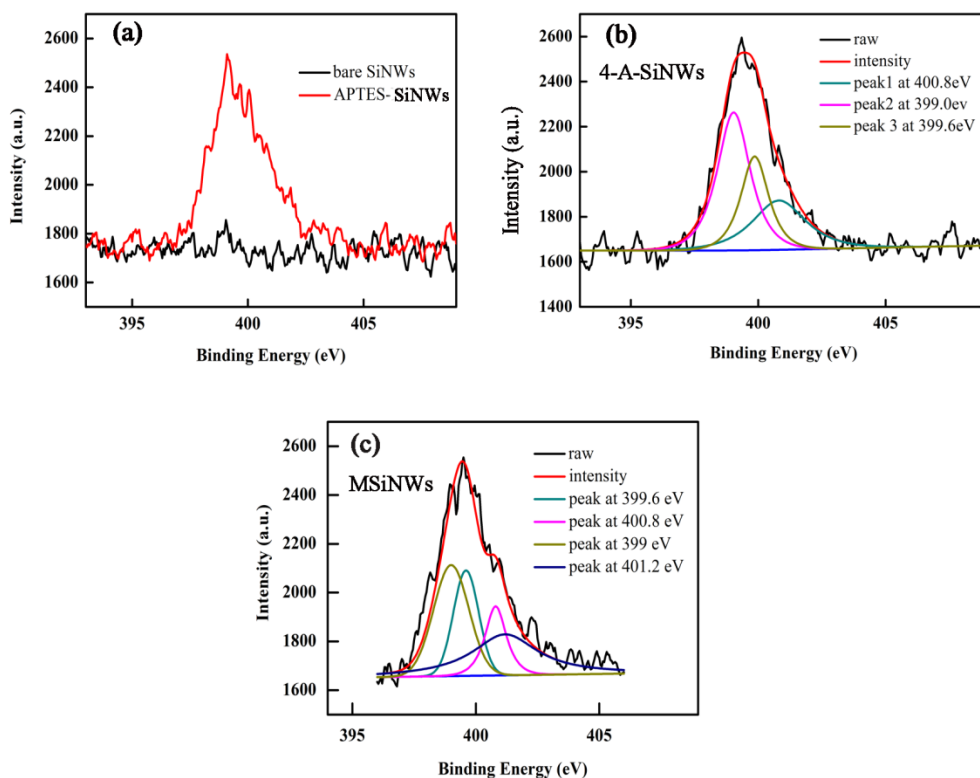


Figure S4 XPS spectra of N(1s) region. (a) Bare SiNWs and APTES-SiNWs (b) 4-A-SiNWs (c) MSiNWs.

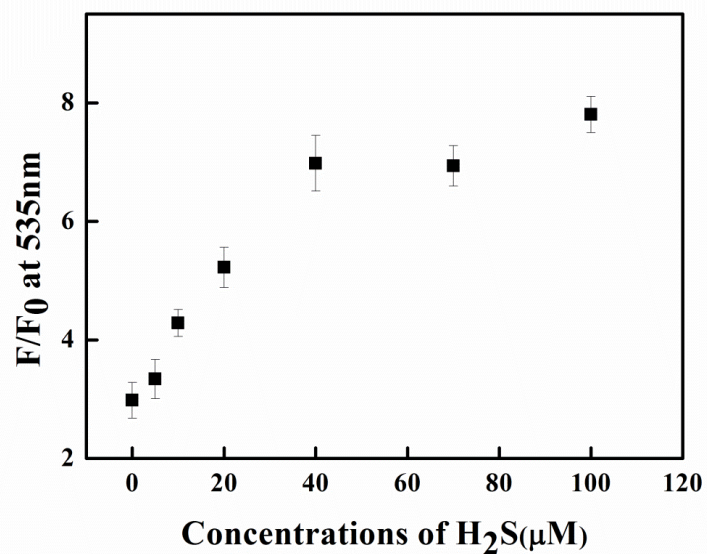


Figure S5 Relative fluorescence intensity of MSiNWs at 535nm as a function of H₂S concentration.

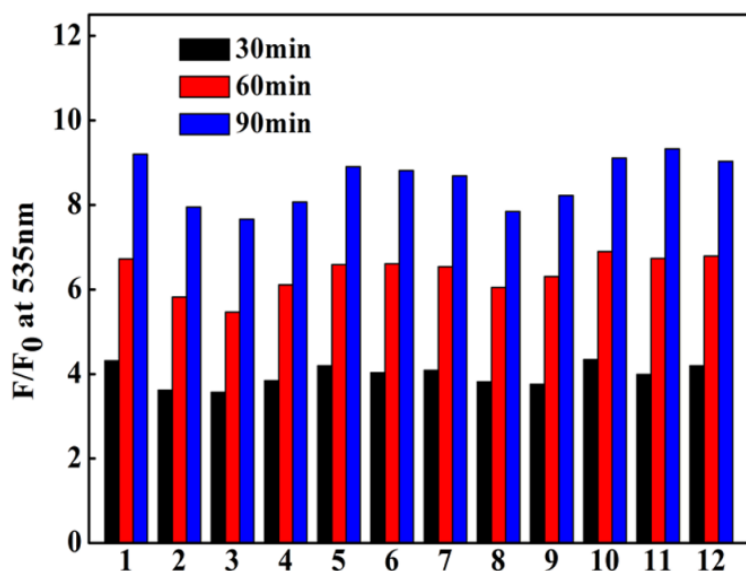


Figure S6 Fluorescence response of MSiNWs (150µg/mL) in the presence of 300µM H₂S with biologically relevant RSNOS. Bars represent relative responses at 535 nm at, 30, 60, and 90 min after addition of H₂S and the interfering RSNOS. Data shown are for 5 mM GSH, 5 mM Cys, and 300µM for other RSONS. (20 mM HEPES, pH 7.4, $\lambda_{\text{ex}} = 430 \text{ nm}$.) 1-12 is successively for H₂S with (1) Na₂SO₃; (2) HOCl (3) Na₂S₂O₃; (4) H₂O₂; (5) KSCN; (6) NaNO₂; (7) KO₂; (8) ALA; (9) tBuOOH; (10) Cys; (11)GSH; (12) H₂S;

References

- [1] Shi, W. S., Peng, H. Y., Zheng, Y. F., Wang, N., Shang, N. G., Pan, Z. W., Lee, C. S., and Lee, S. T. Synthesis of Large Areas of Highly Oriented, Very Long Silicon Nanowires, *Adv.Mater.* 2000, *12*, 1343-1345.
- [2] Peng, K. Q, Lu, A. J, Zhang, R., and Lee, S.-T. Motility of Metal Nanoparticles in Silicon and Induced Anisotropic Silicon Etching, *Adv. Funct. Mater.* 2008, *18*, 3026-3035.