Electronic Supplementary Material (ESI) for ChemComm.

Supporting information for:

Step-wise Functionalization of Polysiloxane Towards Versatile Dual-response Fluorescent Probe and Elastomer for the Detection of H_2S in Two-photon and NO in Near-infrared Modes

Yujing Zuo, Xiaoni Wang, Zhiming Gou, and Weiying Lin*

Institute of Fluorescent Probes for Biological Imaging, School of Materials Science and Engineering, School of Chemistry and Chemical Engineering, University of Jinan, Shandong 250022, P.R. China

Email：weiyinglin2013@163.com

1. Materials

2-Bromo-1,8-naphthalic anhydride and Sodium thiomethoxide solution were purchased from Aladdin Co. (China) and used as received. (aminopropyl)methyldimethoxylsilane, dimethyldimethoxylsilane, and were obtained as commercial products and used directly. All procedures for this study were approved by the Animal Ethical Experimentation Committee of Shandong Academy of Sciences according to the requirements of the National Act on the use of experimental animals (China). The zebrafish were a kind gift by Shandong Academy of Sciences (Jinan, China). HeLa cells were obtained from the College of Life Science, Nankai University (Tianjin, China).

2. Characterization and measurements

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AVANCE 400 spectrometer at 25 °C using $CDCl_3$ as solvent and without tetramethylsilane as an interior label. Ultraviolet absorption (UV) spectra in THF solution were detected using a Beijing TU–1901 double beam UV–Vis spectrophotometer. The luminescence (excitation and emission) spectra of the samples were determined with a Hitachi F–4500 fluorescence spectrophotometer. Excitation and emission slits measured were 5 mm and 5 mm, respectively.

3. Synthesis of compound 1

4-Bromonaphthalene-1,8-dicarboxylic anhydride (135 mg, 0.5 mmol), NaN_3 (65 mg, 1 mmol) were mixed in DMF (5.0 mL) and stirred at 50 °C for 5 h. Then 10 mL of water was added into the mixture and extracted with CH_2Cl_2, washed three times with water, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified using a silica column chromatography (CH_2Cl_2 :EtOH= 4 : 1) to afford compound 1 (120 mg, yield: 49 %) as an black powder. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.57–8.60 (m, 1H), 8.40–8.45 (m,1H), 8.24–8.36 (m, 1H), 7.29-7.37 (m, 1H).

4. Synthesis of Cy-7-Cl

Cy-7-Cl was synthesized according to the classical procedure. ^{1}H NMR (DMSO-d_6, 400 MHz):δ 8.25 (d, 4H), 7.62 (d, 2H), 7.54 (d, 4H), 7.42 (t, 2H), 6.32 (t, 2H), 4.31

(t, 4H), 2.72 (t, 4H), 1.86 (m, 2H), 1.67 (s, 12H), 1.31 (m, 6H).

5. Synthesis of aminopropyl–functional polysiloxanes (P0)

Aminopropyl–functional polysiloxanes (**P0**) was synthesized according to the classical procedure. A mixture of (aminopropyl)methyldimethoxylsilane (3.98 g, 0.02 mol) and dimethyldimethoxysilane (7.31 g, 0.6mol) was added dropwise to the distilled water (200 mL), then KOH (3.00 g) was added to the solution. The mixture was then stirred at ambient temperature for 2 h, and then heated to 70 °C for 3 h. Then cooled to room temperature, water layer was removed. The solution was washed by distilled water (200 mL) for three times to remove the residual KOH. Then, the product was dried over vacuum drying for 24 h and P0 was obtained as a colorless viscous liquid. Yield: 90 %.^{1}H NMR (400 MHz, $CDCl_3$):2.68 (2H), 1.52 (2H), 1.41 (2H), 0.55 (2H), 0.10 (40 H). ^{13}C NMR (100 MHz, $CDCl_3$): 44.96, 27.10, 14.06, 0.56, -0.83.

6. Synthesis of naphthalimides–functional polysiloxanes (P-N)

The synthetic route and the structure were shown in **Scheme 2**. **P0** (2 g) and compound 1 (0.15 g) were mixed in ethanol (100 mL). The reaction mixture was refluxed for 10 h. After cooled to room temperature, the solvent was filtered and then evaporated, **P-N** was obtained after precipitation using water as yellow viscous liquid. Yield: 71 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (m, 1H), 8.35 (m, 1H), 7.27 (m, 1H), 2.67-2.60 (m, 15H), 2.04 (m, 15H), 1.64-1.37 (m, 15H), 0.55 (m, 15H), 0.08 (m, 85 H). PDI=1.24.

7. Synthesis of Cy-7-functional polysiloxanes (P-CY)

P0 (2.00 g) and Cy-7-Cl (0.22 g) were mixed in ethanol (100 mL). The reaction mixture was refluxed for 9 h. After cooled to room temperature, the solvent was evaporated, **P-CYN** was obtained after precipitation using water as dark green viscous liquid. Yield: 50 %.

^{1}H NMR ($CDCl_3$, 400 MHz):δ 8.23 (d, 4H), 7.60 (d, 2H), 7.55 (d, 4H), 7.42 (t, 2H), 6.32 (t, 2H), 4.32 (t, 4H), 2.72 (t, 4H), 1.86 (m, 2H), 1.67 (s, 12H), 1.31 (m, 6H), 2.65-2.60 (m, 19H), 2.04 (m, 20H), 1.64-1.37 (m, 20H), 0.55 (m, 20H), 0.08 (m, 103 H).

8. Synthesis of P-CYN

The synthetic route and the structure were outlined in **Scheme 2**. **P-N** (2.10 g) and

Cy-7-Cl (0.20 g) were mixed in ethanol (80 mL). The reaction mixture was refluxed for 9 h. After cooled to room temperature, the solvent was evaporated, **P-CYN** was obtained after precipitation using water as dark green viscous liquid. Yield: 50 %.
^{1}H NMR (DMSO-d_6, 400 MHz):δ 8.60 (m, 1H), 8.35 (m, 1H), 8.23 (d, 4H), 7.60 (d, 2H), 7.55 (m, 4H), 7.42 (t, 2H), 7.27 (m, 1H), 6.32 (t, 2H), 4.32 (t, 4H), 2.72 (t, 4H), 1.86 (m, 2H), 1.67 (s, 12H), 1.31 (m, 6H), 2.65-2.60 (m, 19H), 2.04 (m, 20H), 1.64-1.37 (m, 20H), 0.55 (m, 20H), 0.08 (m, 103 H). ^{13}C NMR (101 MHz, DMSO) δ 172.27, 160.66, 148.47, 143.56, 142.10, 141.66, 134.14, 133.71, 133.01, 132.24, 132.16, 131.02, 130.73, 130.46, 130.04, 129.62, 129.14, 128.43, 126.55, 125.65, 123.03, 120.35, 119.54, 111.76, 101.72, 56.51, 49.48, 39.13, 27.80, 26.33, 18.99, 12.68, -0.32.

9. Fluorescence imaging in HeLa cells

HeLa cells were incubated on a confocal plate for 24 hours, and washed three times with PBS buffer. HeLa cells were then incubated with P-CYN (concentration 10 μM) in an incubator for 30 min prior to cell imaging. The fluorescence cell images were achieved by a Nikon A1MP confocal microscopy. The fluorescence emission was obtained under excitation of 405 nm and 647 nm laser respectively.

10. Zebrafish pretreatment and fluorescence imaging

2 days old zebrafish was incubated with 10 μM P-CYN for 0.5 h. At the same time, the second group was incubated with 10 μM P-CYN for 0.5 h, and then treated with 10 and 20 μM of H_2S, and finally the fluorescence images were acquired. The fluorescence emission was obtained at 500 to 550 nm under the excitation of 405 nm laser excitation. The third group was incubated with 10 μM P-CYN for 0.5 h, and then treated with 100 μM of NO donor solution, and finally the fluorescence images were acquired. The fluorescence emission was obtained at 675 to 725 nm under the excitation of 647 nm laser excitation.

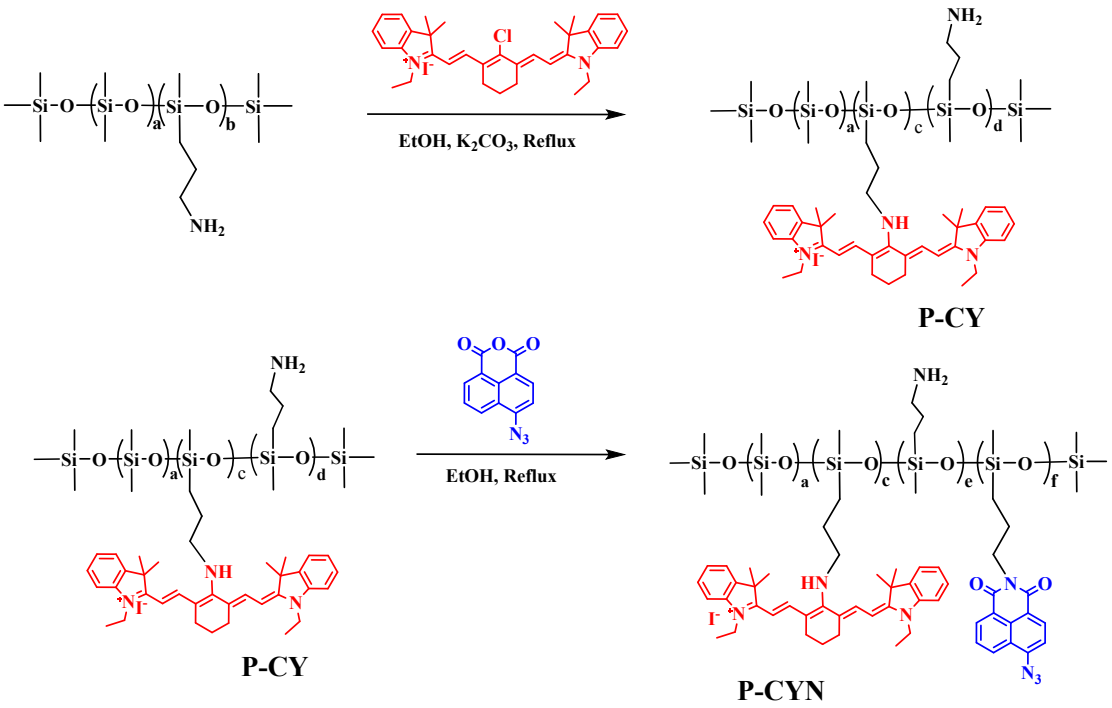
11. Preparation of silicon elastomers (E-P-N and E-P-CY)

The silicon elastomer was crosslinked by facile aldimine condensation reaction. terephthalaldehyde was used as chemical crosslinking agent. Crosslinking was achieved by using a solution of the crosslinkable polymer (P-N, and P-CY) and terephthalaldehyde. 2.0 g of P-N was dissolved in 5 mL of THF, then 0.10 g of

terephthalaldehyde was added to the solution. Majority of THF was then removed by evaporation before the crosslinking step. Then the mixed solution was poured onto a Teflon mode. The crosslinking was performed at room temperature for another 20 min in air to form an insoluble crosslinked network (**Scheme 3**). All of the elastomers were swollen within the THF to interchange the substrates and remove the uncrosslinked molecules. They were then dried at room temperature for 1d, and then dried at vacuum to ensure that all uncrosslinked materials were removed from the network. Transparent crosslinked networks were finally obtained and named as E-P-N and E-P-CY.

Table S1. Molecular weights of **P0**, **P-CY**, **P-N**, and **P-CYN**.

	M_n (g/mol)	M_w (g/mol)	PDI (M_w/M_n)
P0	2300	3600	1.56
P-N	3900	5200	1.33
P-CY	4200	5700	1.35
P-CYN	6100	7800	1.28



b=c+d **where a=0.6, b=0.4,**
d=e+f **c=0.05, f=0.05, and e=0.3**

Scheme S1. The synthesis route for probe **P-CYN**.

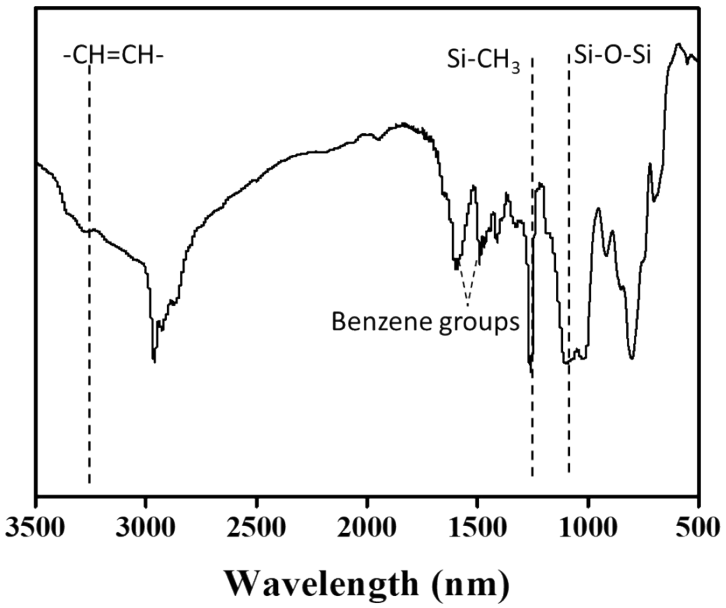


Figure S1. FT-IR spectra of **P-CY**.

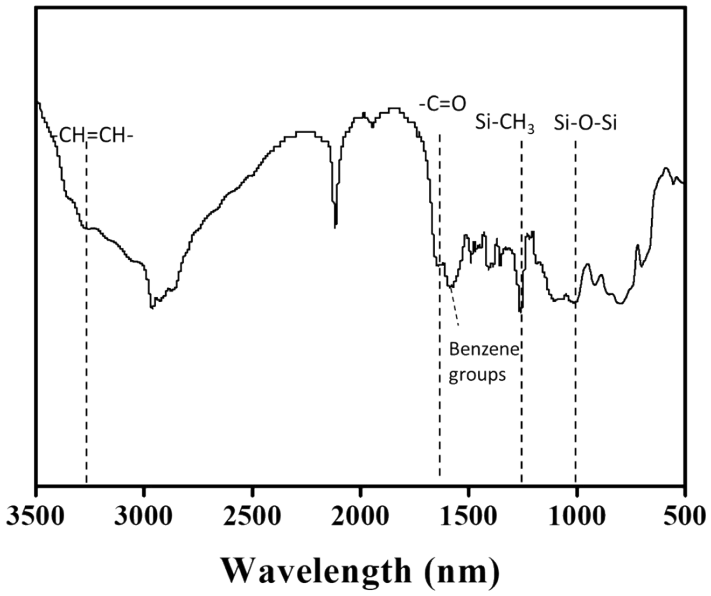


Figure S2. FT-IR spectra of **P-CYN**.

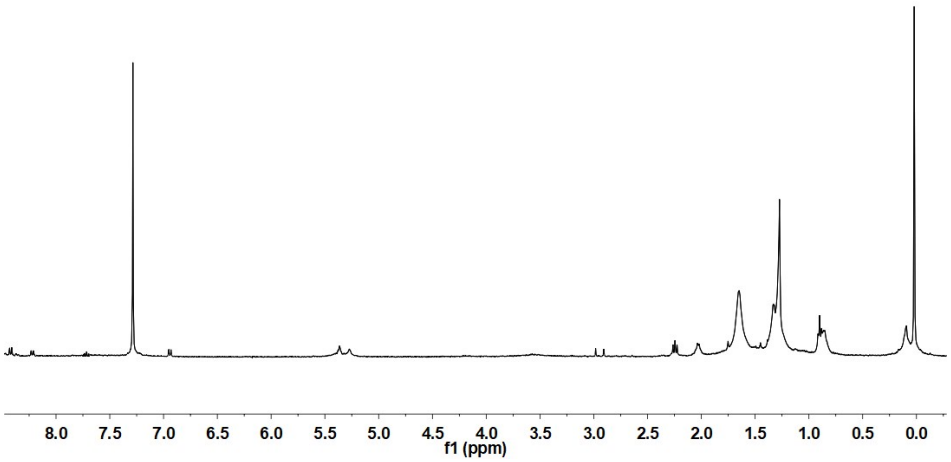


Figure S3. ^{1}H-NMR spectra of **P-CY** ($CDCl_3$).

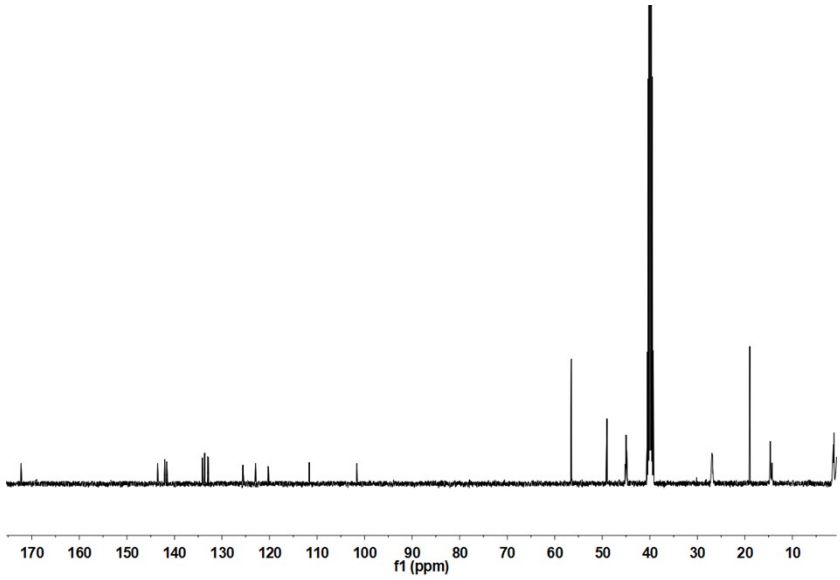


Figure S4. ^{13}C-NMR spectra of **P-CY** (DMSO-d6).

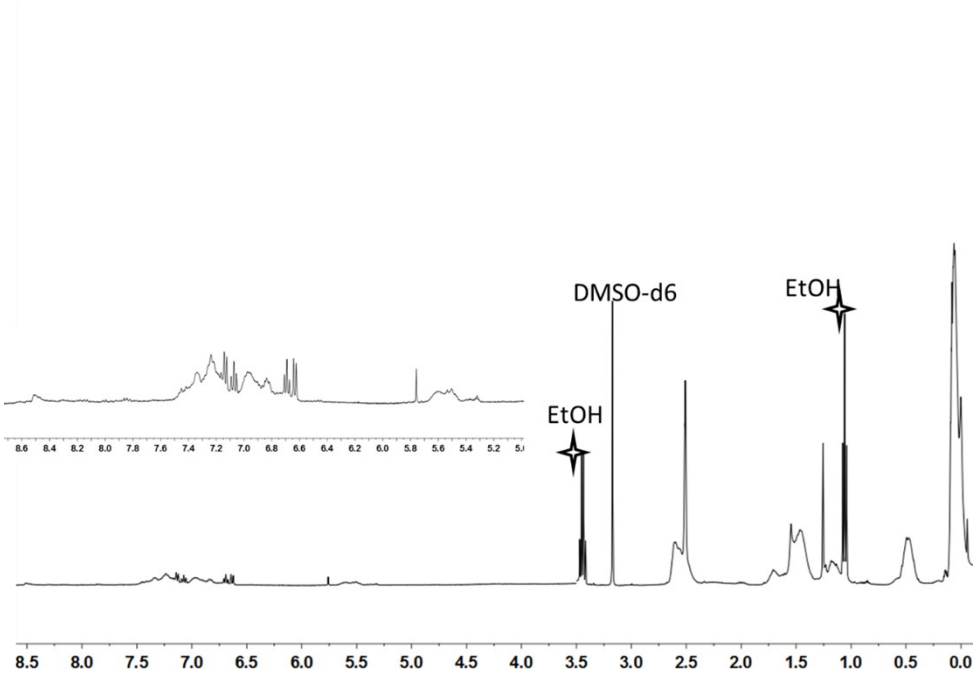


Figure S5. ^{1}H-NMR spectra of **P-CYN** (DMSO-d6).

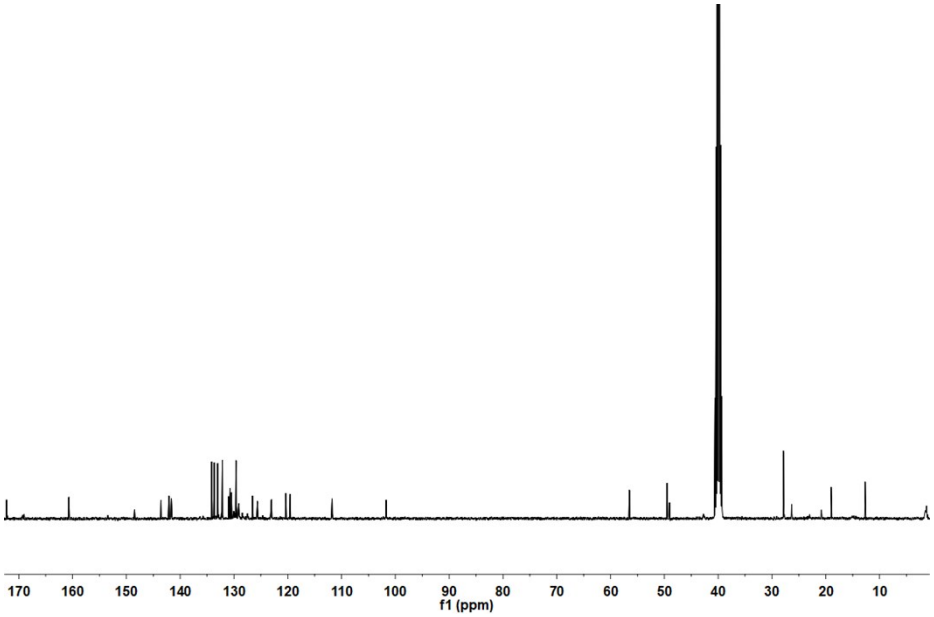


Figure S6. ^{13}C-NMR spectra of **P-CYN** (DMSO-d6).

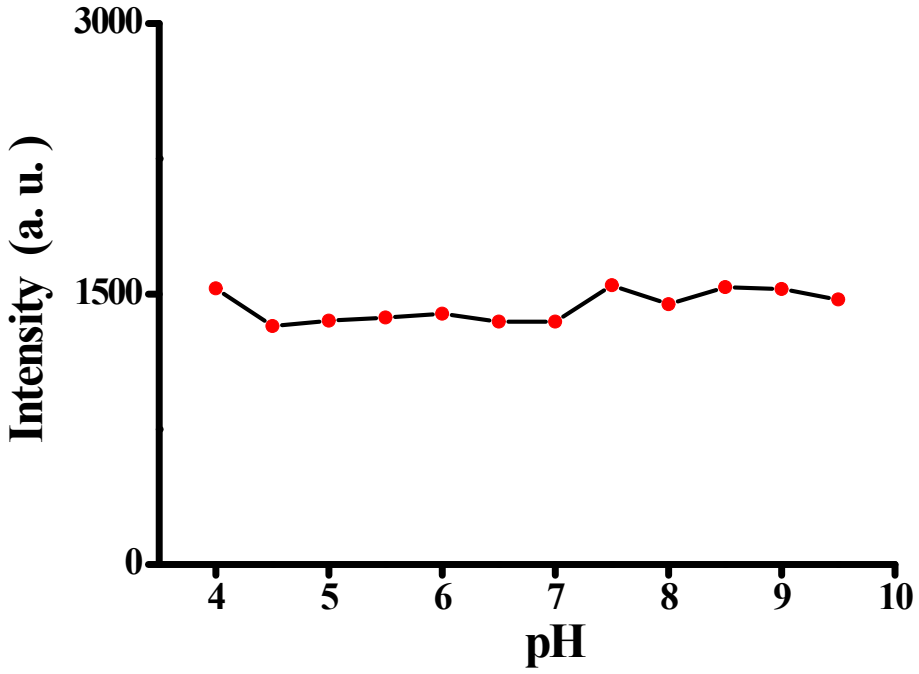


Figure S7. Effect of pH on the fluorescent intensity of **P-CYN** (10 uM). Fluorescence was measured at $\lambda_{ex}/_{em}$= 640/750 nm.

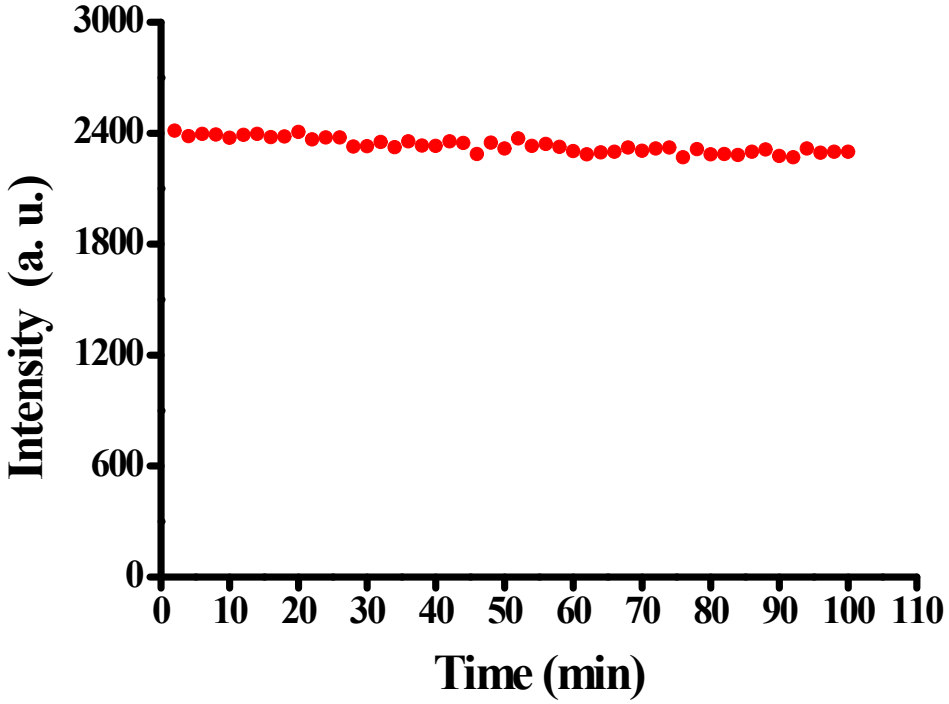


Figure S8. Photostability testing result for **P-CYN** in PBS solution. Fluorescence was measured at $\lambda_{ex}/_{em}$= 640/750 nm.

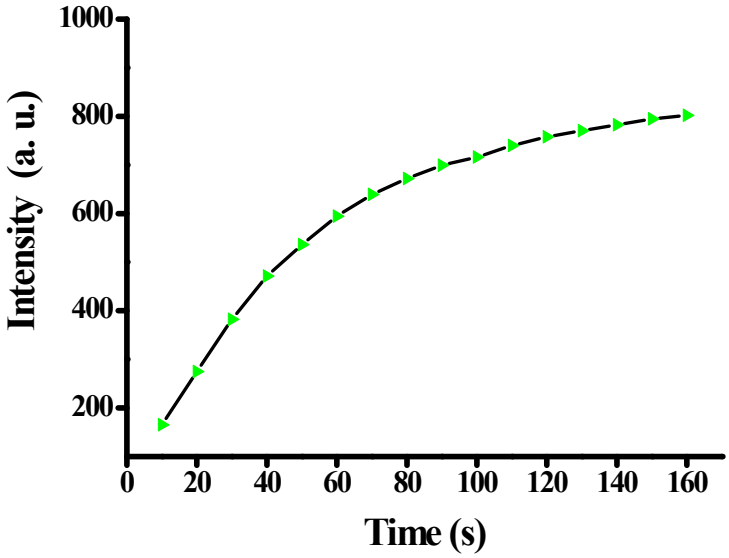


Figure S9. Time course study for the reaction between **P-CYN** with H_2S.

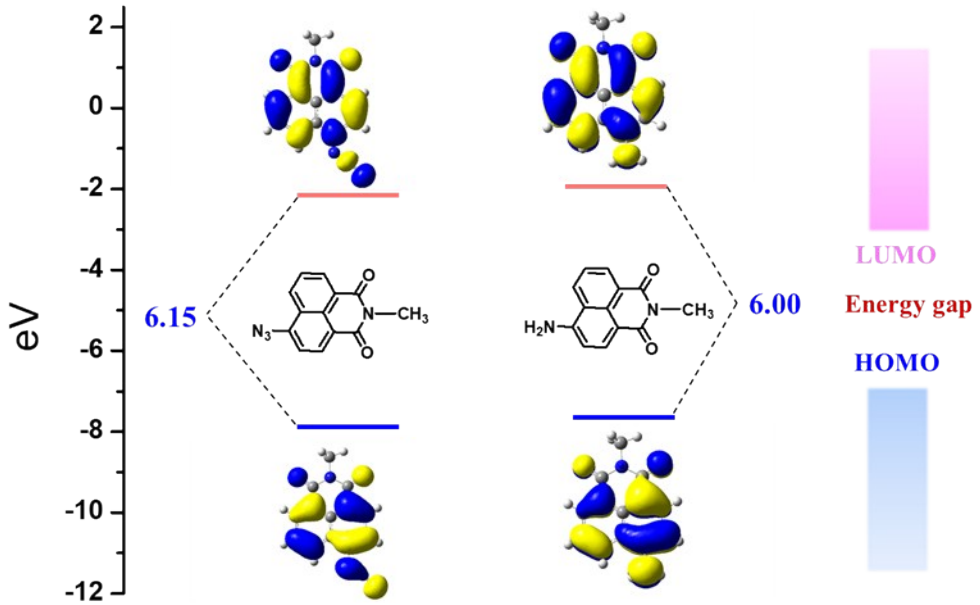


Figure S10. Frontier molecular energy (LUMO (red line) and HOMO (blue line)), the corresponded energy gap, and simplified structure of fluorescent group for detecting H_2S.

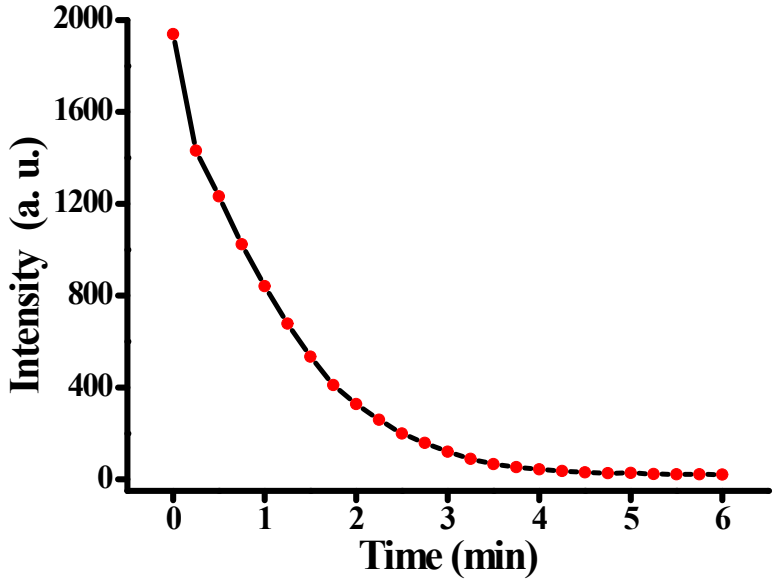


Figure S11. Time course study for the reaction between **P-CYN** with NO.

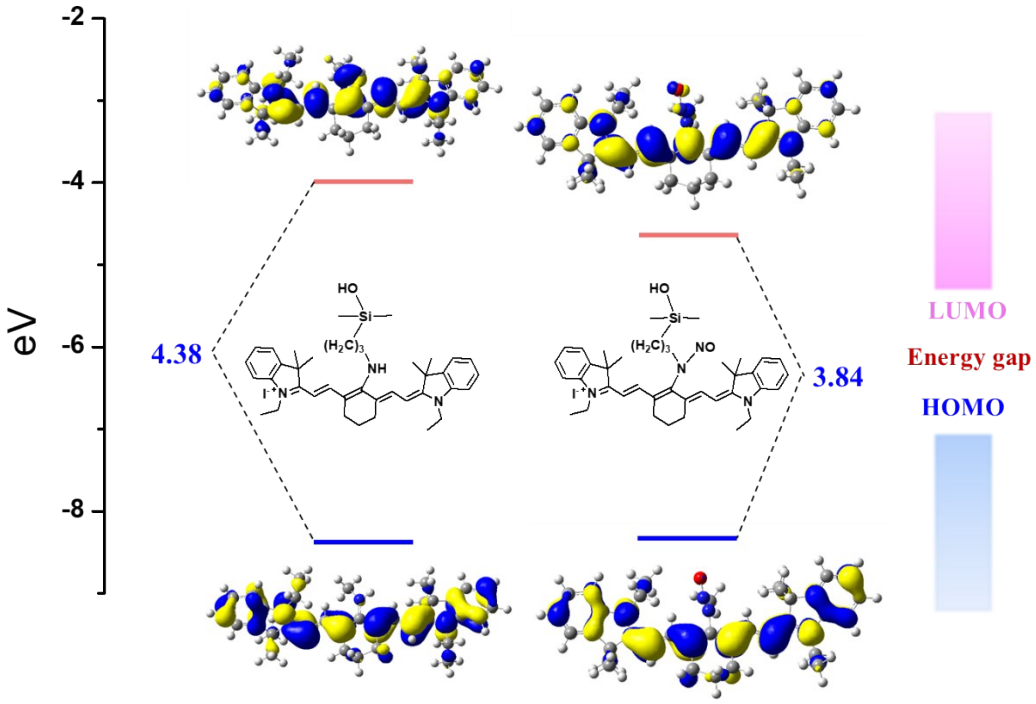


Figure S12. Frontier molecular energy (LUMO (red line) and HOMO (blue line)), the corresponded energy gap, and simplified structure of fluorescent group for detecting NO.

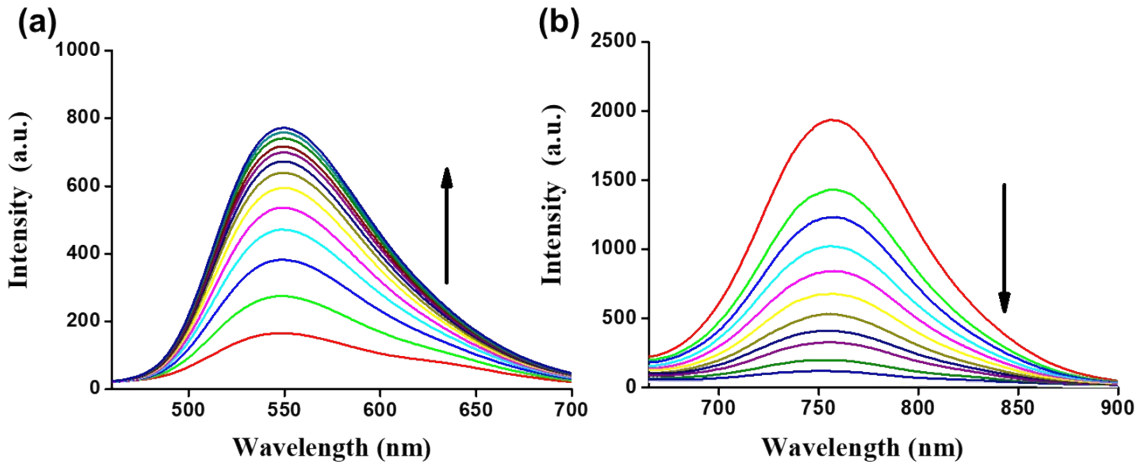


Figure S13. (a) Fluorescence spectra of **P-CYN** (10 μM) in aqueous solution (PBS bu□er, pH = 7.4, PBS : EtOH =9 :1) upon adding NO from 0 to 20 μM, (b) fluorescence spectra of **P-CYN** (10 μM) in aqueous solution (PBS bu□er, pH = 7.4, PBS : EtOH =9 :1) upon adding NO from 0 to 20 μM. (each time 1 μM for H_2S, while 2 μM for NO).

Note:

The detection of H_2S and NO has been performed in the same experimental system. (PBS bu□er, pH = 7.4, PBS : EtOH =9 :1) upon adding different amounts of H_2S from 0 to 15 μM and NO from 0 to 20 μM. H_2S and NO have been added to the testing solution at the same time, (each time 1 μM for H_2S, while 2 μM for NO). Then, the fluorescent spectra have been tested under different excitation wavelength. As shown in Figure S13, the fluorescent intensity of P-CYN at 550 nm enhanced with the concentration enhancement of H_2S, meanwhile, the the fluorescent intensity at 760 nm decreased gradually with the concentration enhancement of NO. The results indicated that the detection of H_2S and NO could be performed simultaneously in the same experimental system in vitro.

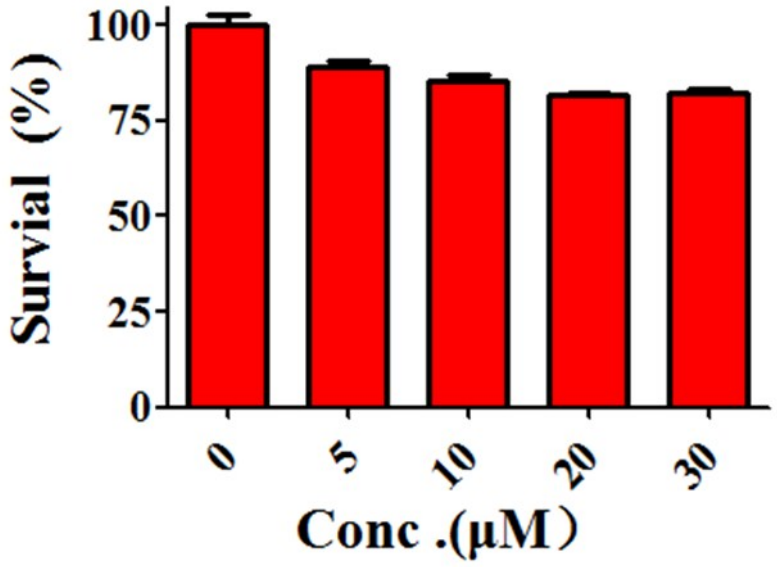


Figure S14. Cytotoxicity of **P-CYN** on HeLa cells determined by MTT.

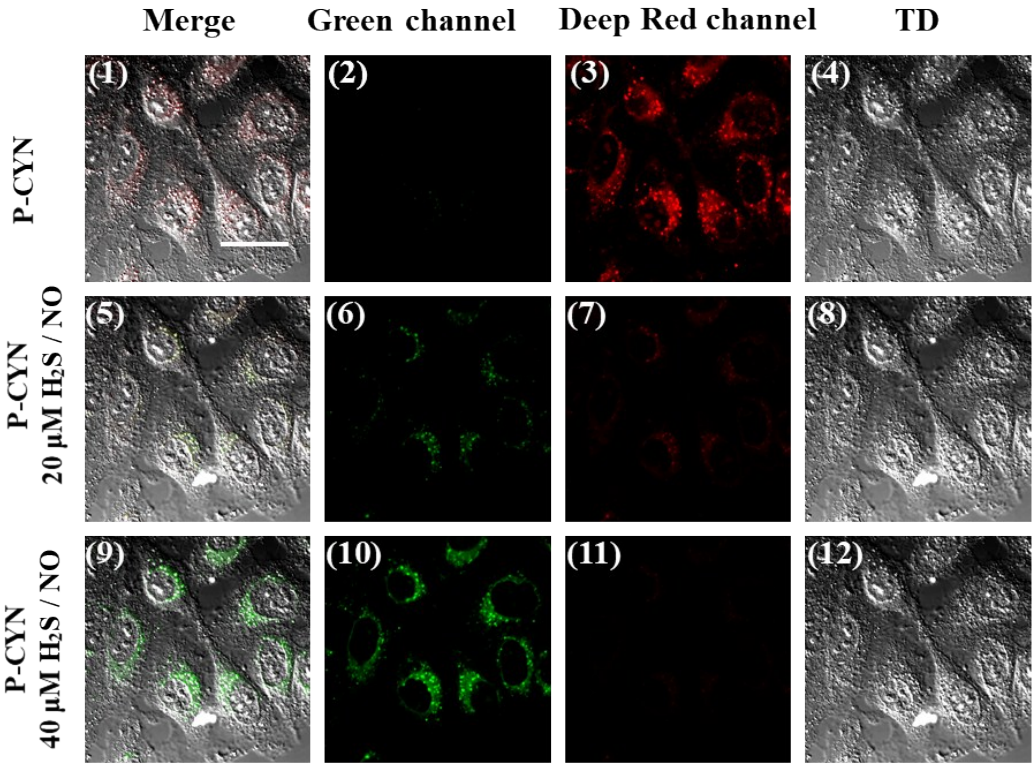


Figure 15. (a) Fluorescent imaging of HeLa cells pre-treated by **P-CYN** (10 μM) and H_2S and NO with concentrations varied (0, 20 and 40 μM, respectively), green channel: λ_{ex} = 405 nm, λ_{em} = 500 to 550 nm, deep red channel: λ_{ex} = 647 nm, λ_{em} = 663 to 738 nm. Scale bar=20 μm.

Note:

The dual-response fluorescent probe did need different excitation light for each target. Therefore, to detect H_2S and NO simultaneously, we performed the experiment under a confocal laser scanning microscope to obtain different excitation light. As shown in Figure S15, H_2S and NO have been added into the cell culture simultaneously. As a result, the fluorescence in the green channel emerged, meanwhile, the fluorescence in the deep red channel diminished with the enhancement of the concentration of H_2S and NO. Therefore, monitoring the H_2S and NO simultaneously has been achieved under different excitation light using a confocal laser scanning microscope. The results indicated that the detection of H_2S and NO could be performed simultaneously in the same live system.

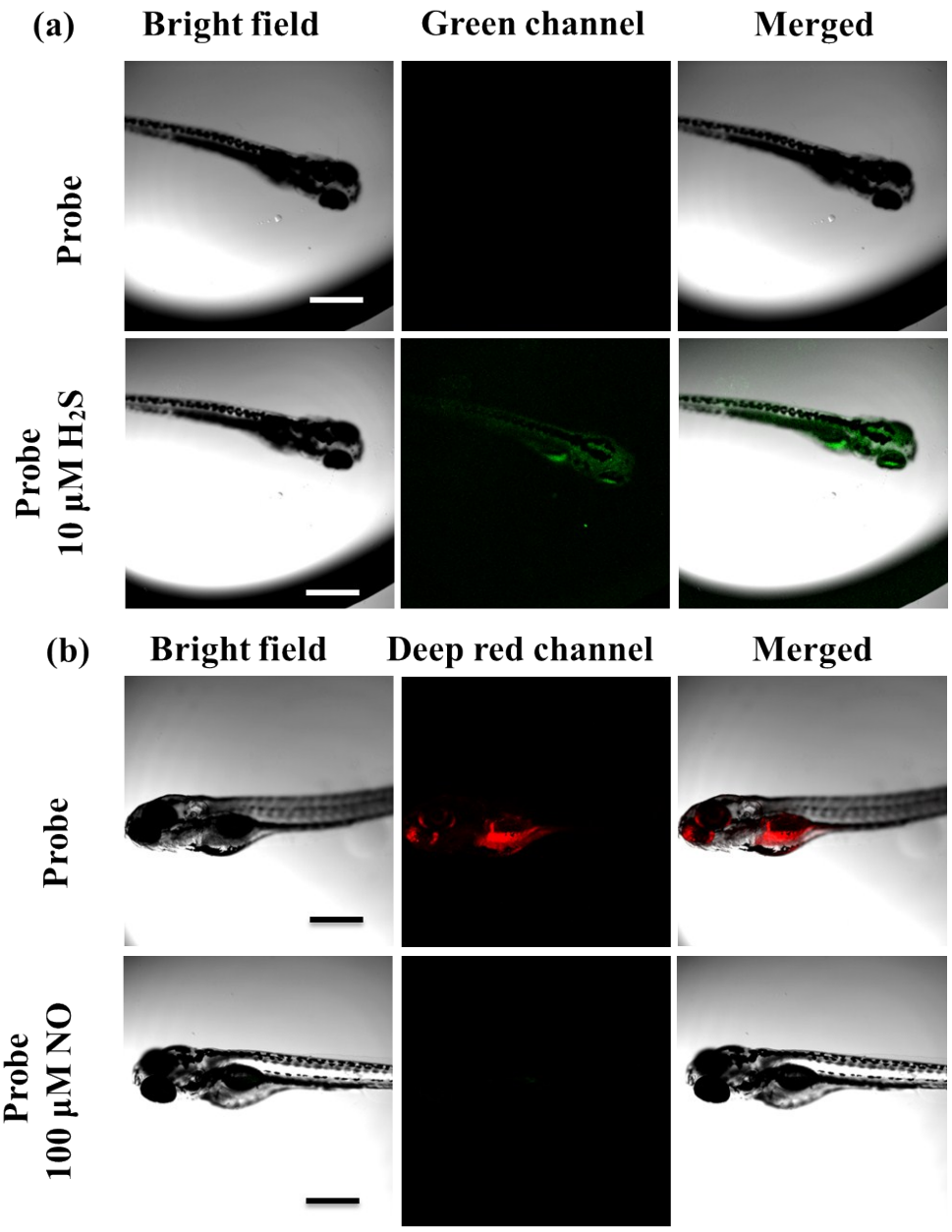


Figure S16. (a) Confocal images of zebrafishes treated by **P-CYN** (10 μM) and **P-CYN + H_2S** (10 μM), (b) confocal images of zebrafishes treated by **P-CYN** (10 μM) and **P-CYN + NO** (100 μM), λ_{ex} = 647 nm, λ_{em} = 665 to 735 nm. Scale bar = 20 μm.

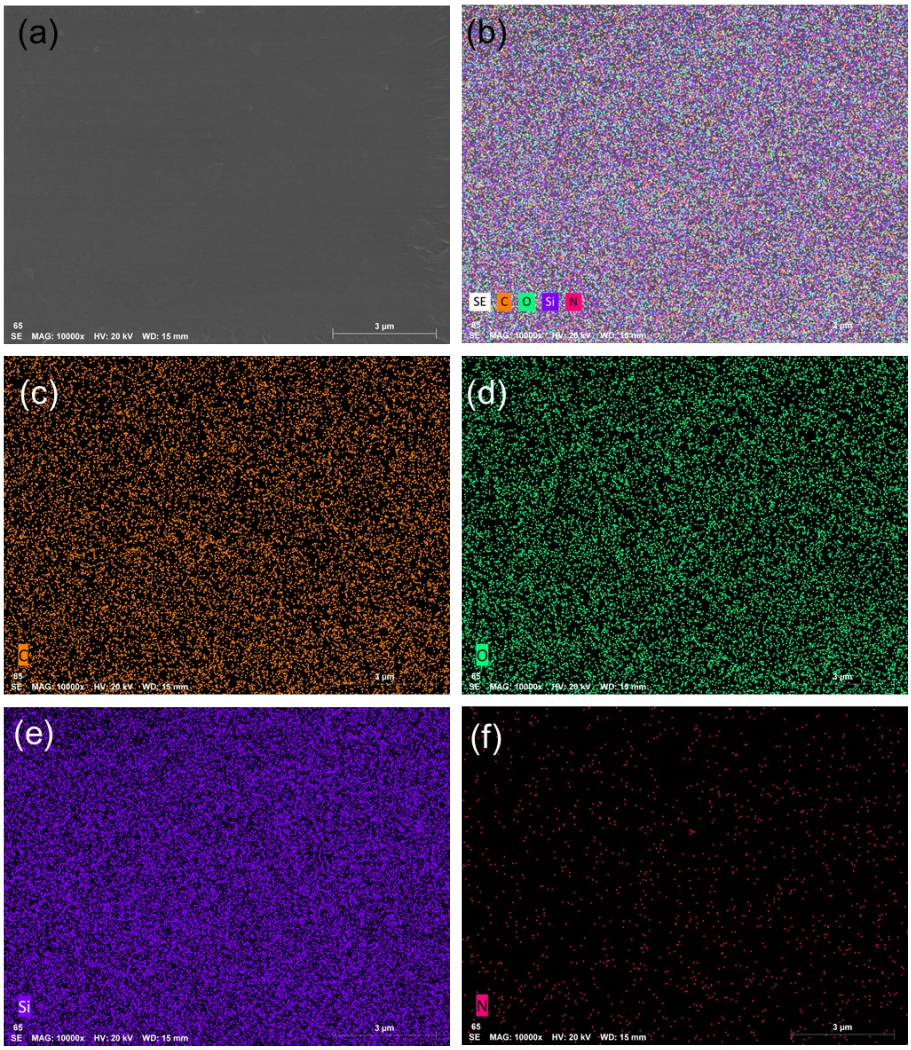


Figure S17. Energy dispersive spectroscopy images of **E-P-N** tested by a field emission scanning electron microscopy (FESEM), bar= 3 μm.

Contact angles of E-P-N and E-P-CY:

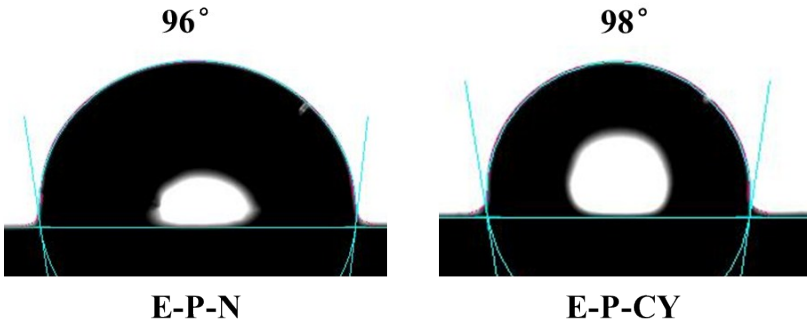


Figure S18. Contact angles of **E-P-N** and **E-P-CY.**

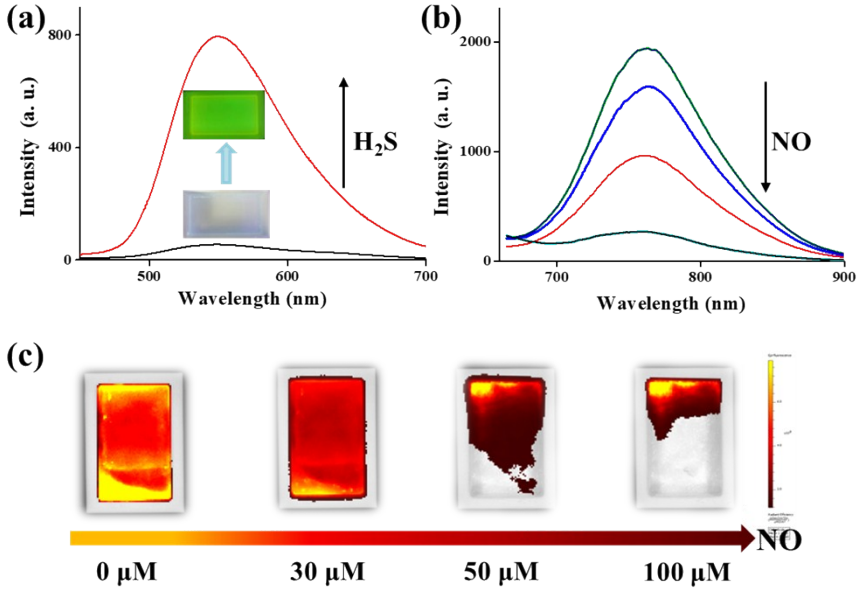


Figure S19. (a) Solid state photoluminescence of **E-P-N** after treated with H_2S donor solution with different concentrations from 0 to 20 μM, the inserted is the digital image of **E-P-N** before and after treated with 100 μM of H_2S under a 365 nm UV light, (b) solid state photoluminescence of **E-P-CY** obtained from (a) after treated with NO donor solution with different concentrations from 0 to 100 μM, (c) representative fluorescence images of **E-P-CY** after treated with NO donor solution with different concentrations from 0 to 100 μM. E_x = 425 nm, E_m = 750 nm.