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A new strategy to improve the water solubility of organic fluorescent probe using silicon nanodots and fabricate a two-photon SiNDs-ANPA-N_3 for visualizing hydrogen sulfide in living cells and onion tissues

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Reagents and Apparatus

All chemicals were of analytical grade and used without further purification. Phorbol-12-myristate-13-acetate (PMA) was obtained from Sigma. Carboxymethoxylamine hemihydrochloride (AOA) and 4-Bromo-1,8-naphthalic anhydride (98%) were purchased from Annaiji (Shanghai, China). N-hydroxysulfosuccinimide sodium salt (Sulfo-NHS) and (3-Aminopropyl) trimethoxysilane (APTMS) (97%) were purchased from Aladdin. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) was purchased from Shanghai Medpep Co., Ltd. Dulbecco's modified Eagle's medium (DMEM) was purchased from HyClone (Waltham, MA, U.S.A.). Fetal bovine serum (FBS) was taken from Tianhang Biological Technology (Zhejiang, China) and 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Genview (U.S.A). Physiological buffer solution (PBS) consisted of 8.00 g/L NaCl, 0.20 g/L KCl, 0.20 g/L KH_2PO_4, and 2.78 g/L $Na_2HPO_4 \cdot 12H_2O$ (pH 7.4). Sodium phosphate buffer solution was prepared by mixing 0.10 M H_3PO_4 solution with 0.10 M Na_3PO_4 solution and adjusted to the required pH values. Water used throughout the experiments was Milli-Q ultrapure water (Millipore=18.25 MΩ·cm).

UV-vis absorption spectra were acquired with an UV-2600 spectrophotometer (Daojin, Tokyo, Japan) and fluorescence spectra were obtained with a RF-6000 spectrophotometer (Shimadzu, Tokyo, Japan). TEM image was obtained with a JEM-2100 (HR) transmission electron microscope by dropping an aqueous SiNDs-ANPA-N_3 fluorescent probe solution and SiNDs solution onto ultrathin carbon support film

(300 meshes) with the excess solvent evaporated. FTIR spectra over the range 800-4000 cm^{-1} were performed on IS10 Fourier transform infrared spectrometer. ^{1}H NMR spectra were recorded on AVANCE III HD at 400 MHz (Bruker, Switzerland). High-resolution mass spectrometry (HR-MS) was performed on a LTQ-Orbitrap Elite (Thermo Fisher Scientific, USA) mass spectrometer. The confocal microscopy fluorescence images were obtained with a 60 × oil (NA = 0.55) objective lens by UltraVIEW VoX confocal microscope (PerkinElmer, America) and NOL-LSM 710 (Carl Zeiss 710, Germany) and analyzed by EZ-C1 software. Onion tissue images were recorded by Olympus inverted fluorescence microscope IX71 equipped with a cooled CCD camera (Olympus DP72).

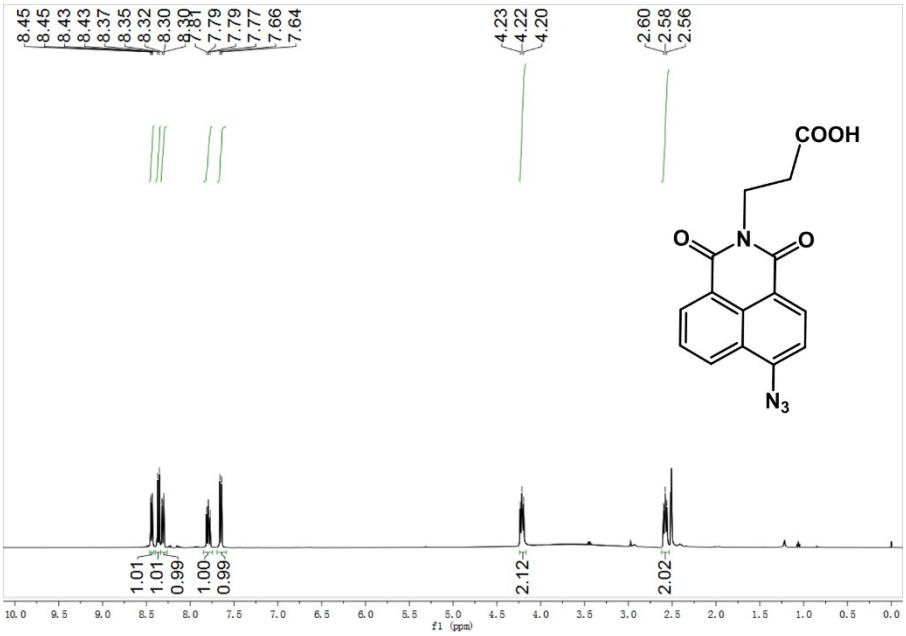

Fig. S1. The ^{1}H NMR (400 MHz, DMSO-d6) spectrum of ANPA-N_3. δ 8.44 (d, J = 7.3 Hz, 1H), 8.36 (d, J = 8.0 Hz, 1H), 8.31 (d, J = 8.4 Hz, 1H), 7.79 (t, J = 7.4 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 4.22 (t, J = 7.8 Hz, 2H), 2.58 (t, J = 7.8, 2H).

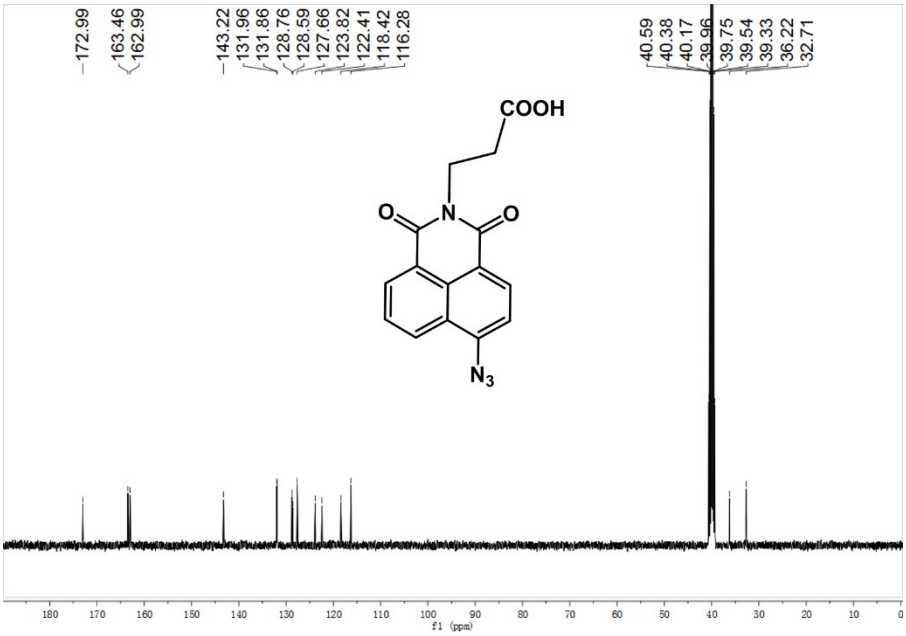

Fig. S2. The ^{13}C NMR (400 MHz, DMSO-d6) spectrum of ANPA-N_3. δ 173.0, 163.5, 163.0, 143.2, 132.0, 131.9, 128.8, 128.6, 127.7, 123.8, 122.4, 118.4, 116.2, 36.2, 32.7.

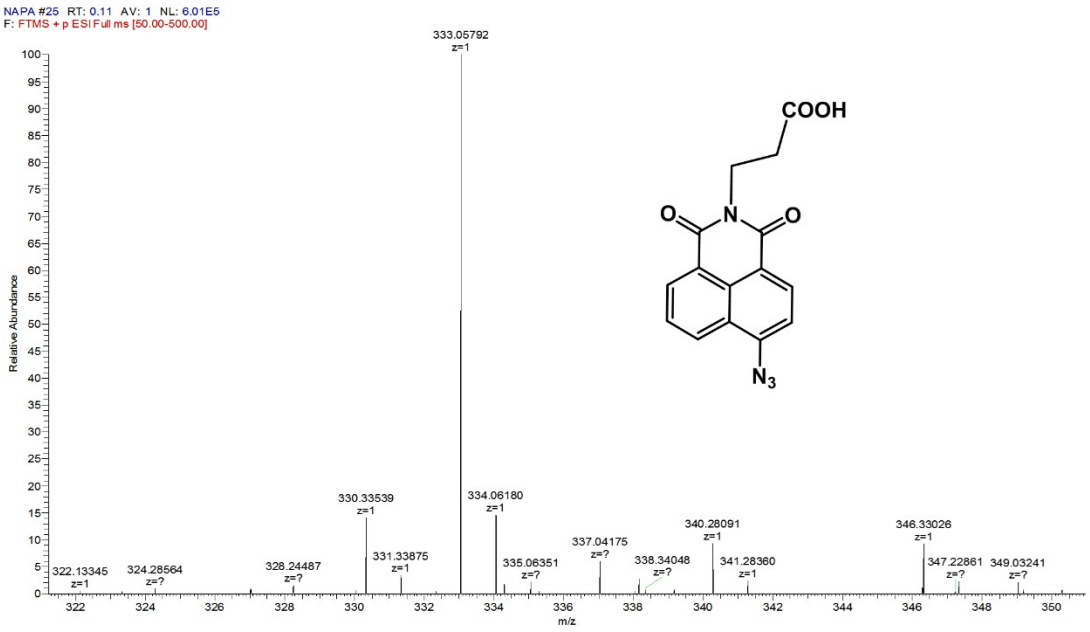

Fig. S3. The HRMS of ANPA-N_3: Calcd. for $C_{15}H_{10}N_4O_4Na$ $(M+Na)^+$ 333.0594, found 333.0579.

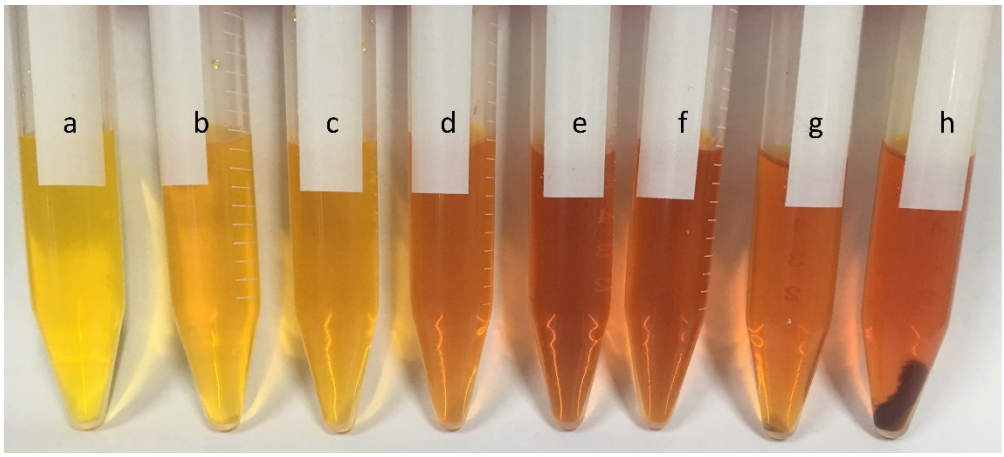

Fig. S4. Different amounts of ANPA-N_3 (a, 0.025 mmol; b, 0.05 mmol; c, 0.075 mmol; d, 0.1 mmol; e, 0.2 mmol; f, 0.3 mmol; g, 0.4 mmol; h, 0.5mmol) coupled onto the SiNDs.

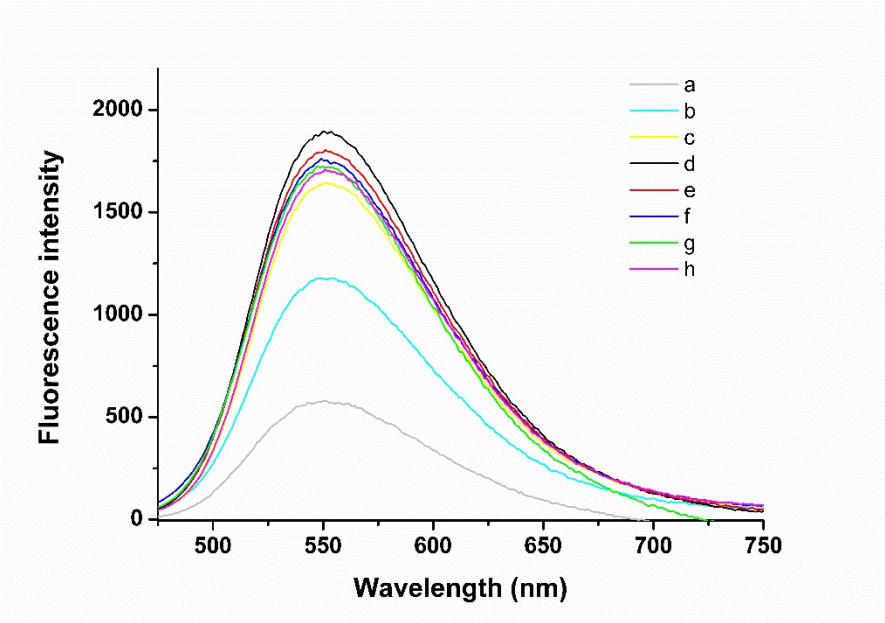

Fig. S5. Fluorescence spectra of SiNDS-ANPA-N_3 (0.075 mg/mL) response to 200 μM NaHS in PBS buffer (pH 7.4). The SiNDS-ANPA-N_3 were prepared by 200 mg SiNDs with different amounts of ANPA-N_3 (a, 0.025 mmol; b, 0.05 mmol; c, 0.075 mmol; d, 0.1 mmol; e, 0.2 mmol; f, 0.3 mmol; g, 0.4 mmol; h, 0.5 mmol). Excitation was at 430 nm; slit widths of excitation and emission, 3 nm/3 nm.

Calculation of the amount of the ANPA-N_3 in the stock solution

SiNDs and ANPA-N_3 produce the characteristic absorption band at 337 nm (Fig. S6B) and 360 nm (Fig. S6A), respectively. Since the SiNDs-ANPA-N_3 exhibits characteristic absorption band at 360 nm which overlaps with the absorption of SiNDs; while upon reacting with NaHS, SiNDs-ANPA-N_3 turns into SiNDs-ANPA-NH_2 and displays characteristic absorption at 430 nm which will not overlap with the absorption of SiNDs (Fig. S6B); hence to more accurately determine the amount of the amount of the ANPA-N_3 in the stock solution, the SiNDs-ANPA-N_3 solution and ANPA-N_3 solution were added excessive amount of NaHS, so as to transform the SiNDs-ANPA-N_3 into SiNDs-ANPA-N_3 and ANPA-N_3 into ANPA-NH_2.[1]

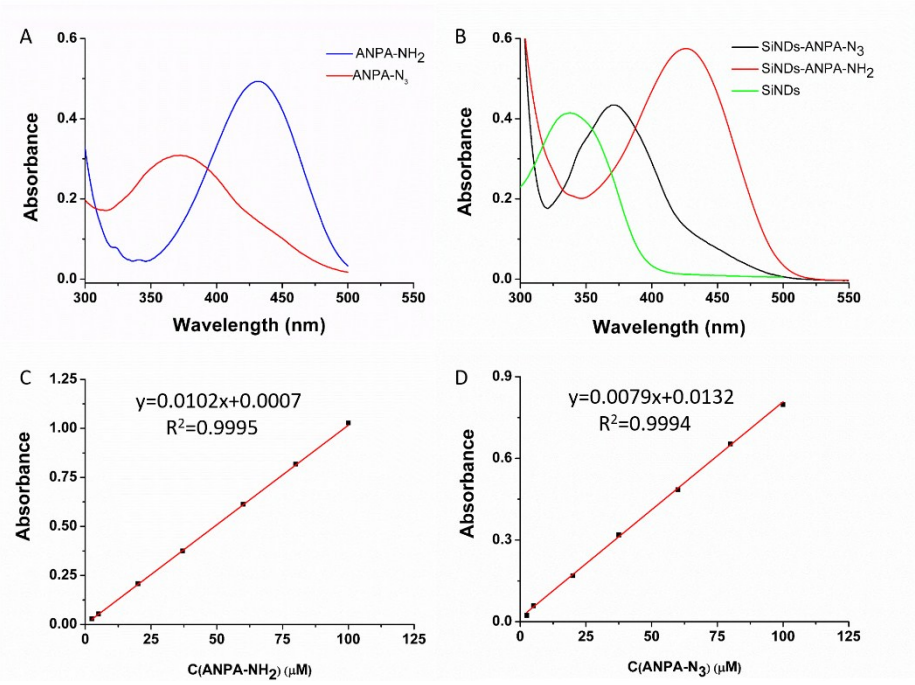

Fig. S6. (A) Absorption spectra of ANPA-N_3 (red) and ANPA-N_3 solution was added excessive amount of NaHS then transformed to ANPA-NH_2 (blue) in PBS buffer (pH 7.4). (B) Absorption spectra of SiNDs (green), SiNDs-ANPA-N_3 (black) and SiNDs-ANPA-N_3 solution was added excessive amount of NaHS then transformed to SiNDs-

ANPA-NH_2 (Red) in PBS buffer (pH 7.4). (C) Standard calibration curve of known concentrations of ANPA-NH_2 [y = 0.0102x + 0.0007, R^2 = 0.9995]. (D) Standard calibration curve of known concentrations of ANPA-N_3 [y = 0.0079x + 0.0132, R^2 = 0.9994].

References

1 C. Yu, X. Li, F. Zeng, F. Zheng and S. Wu, *Chem. Commun.*, 2013, **49,** 403-405.

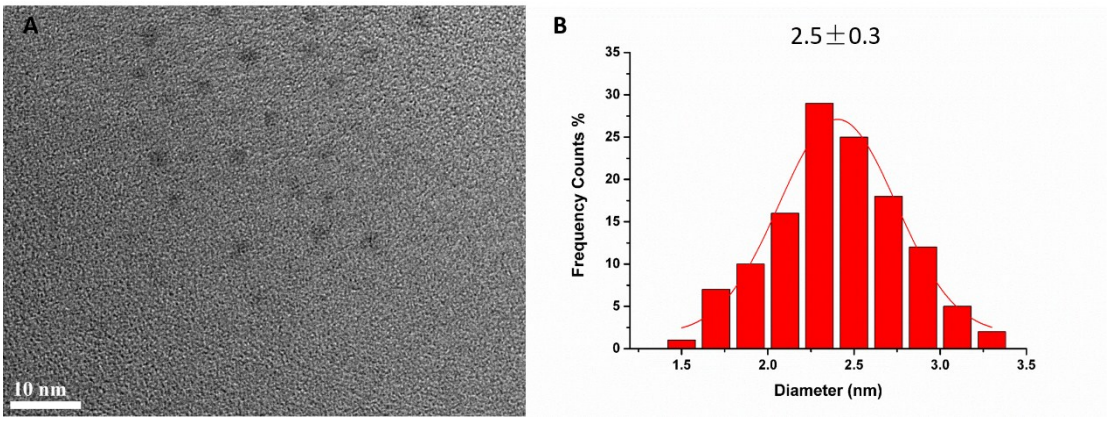

Fig. S7. (A) TEM images of label-free SiNDs. (B) The TEM diameter distribution.

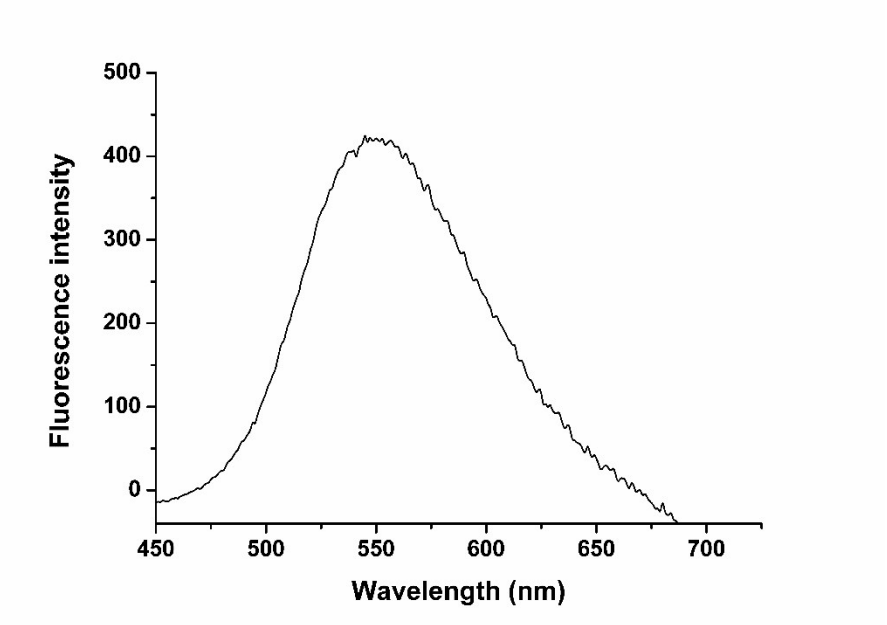

Fig. S8. Two-photon excited fluorescence spectrum of SiNDs-ANPA-N_3 (0.075 mg/mL) towards 200 μM NaHS in PBS buffer (pH 7.4). Excitation was at 853 nm; slit widths of excitation and emission, 3 nm/3 nm.

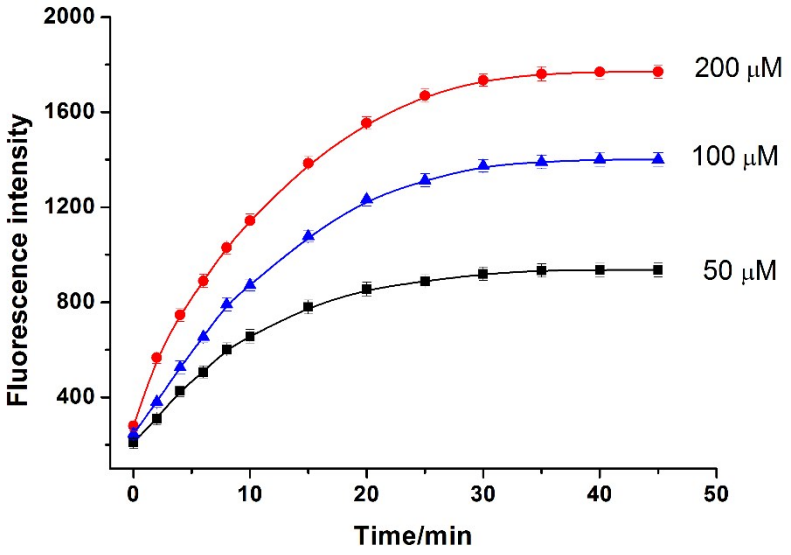

Fig. S9. Time-dependent fluorescence intensity changes of SiNDs-ANPA-N_3 (0.075 mg/mL) in PBS buffer (pH 7.4) in the presence of 50 μM, 100 μM, 200 μM NaHS. Excitation was at 430 nm; slit widths of excitation and emission, 3 nm/3 nm.

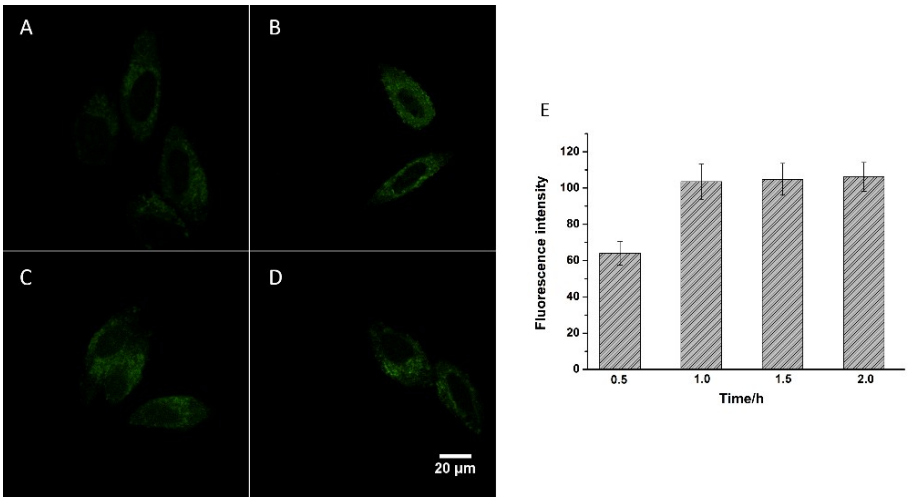

Fig. S10. One-photon confocal microscopy images of HeLa cells incubated with SiNDs-ANPA-N_3 (3 mg/mL) upon 405 nm excitation for different times (A, 0.5 h; B, 1.0 h; C, 1.5 h; D, 2.0 h). Then treatment amount of 100 μM NaHS. E, The average fluorescence intensity of the above images. Scale bar: 20 μm.

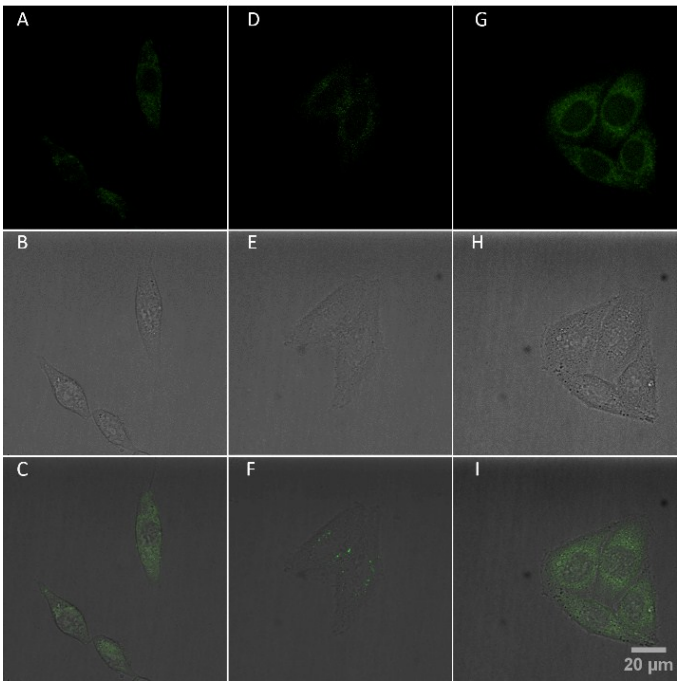

Fig. S11. One-photon confocal microscopy images of HeLa cells incubated with SiNDs-ANPA-N_3 (3 mg/mL) upon 405 nm excitation in the absence or presence of stimulant or scavenger. A-C, the cells incubated with only SiNDs-ANPA-N_3 for 1 h; D-F, the cells incubated with PMA (2 μL, 1 μg/mL) for 1 h then treated with SiNDs-ANPA-N_3 for 1 h; G-I, cells incubated with Cys (400 μM) for 1 h and then treated with SiNDs-ANPA-N_3 for 1 h. Scale bar: 20 μm.

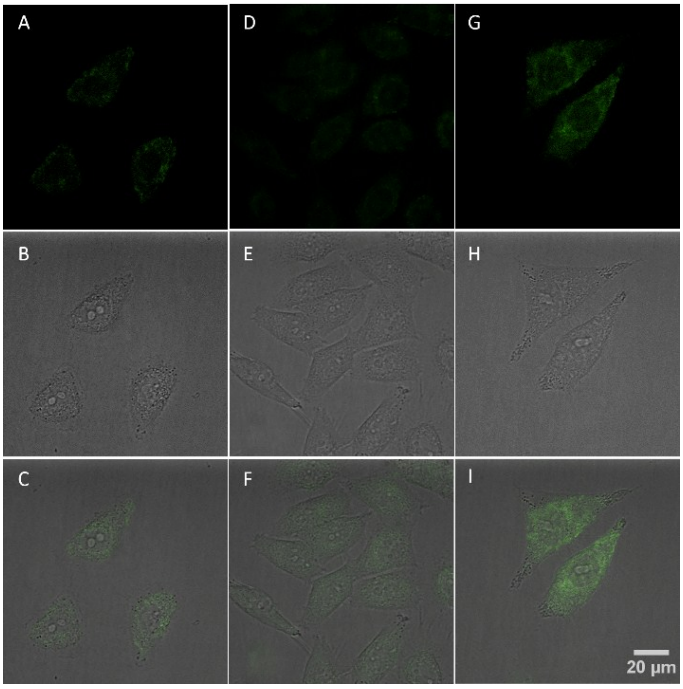

Fig. S12. One-photon confocal microscopy images of MCF-7 cells incubated with SiNDs-ANPA-N_3 (3 mg/mL) upon 405 nm excitation in the absence or presence of stimulants and scavengers. A-C, the cells incubated with only SiNDs-ANPA-N_3 for 1 h; D-F, the cells incubated with PMA (2 μL, 1 μg/mL) for 1 h then treated with SiNDs-ANPA-N_3 for 1 h; G-I, the cells incubated with Cys (400 μM) for 1 h and then treated with SiNDs-ANPA-N_3 for 1 h. Scale bar: 20 μm.

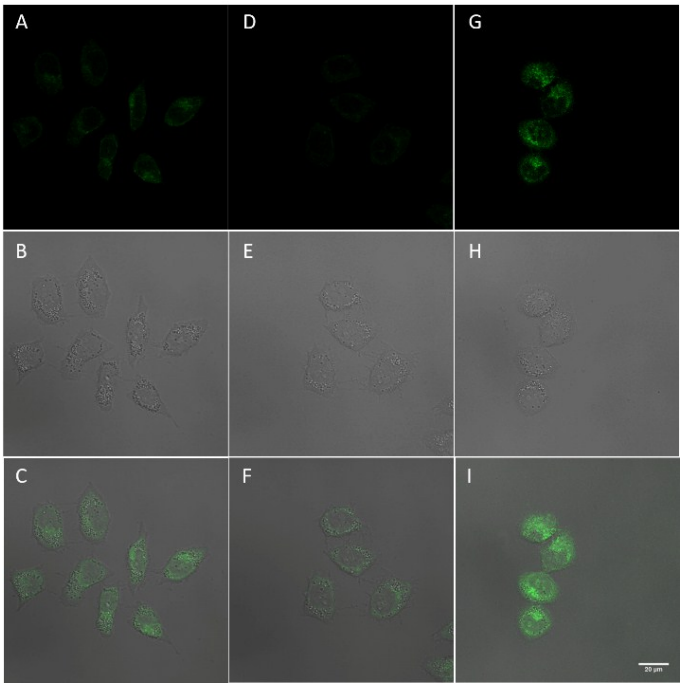

Fig. S13. Two-photon confocal microscopy images of HL-7702 cells incubated with SiNDs-ANPA-N_3 (3 mg/mL) upon 853 nm excitation in the absence or presence of stimulants and scavengers. A-C, the cells incubated with only SiNDs-ANPA-N_3 for 1h; D-F, the cells incubated with PMA (2 μL, 1 μg/mL) for 1 h then treated with SiNDs-ANPA-N_3 for 1 h; G-I, the cells incubated with Cys (400 μM) for 1 h and then treated with SiNDs-ANPA-N_3 for 1 h. Scale bar: 20 μm