Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Mitochondria-Targeted Polydopamine Nanoprobes for Visualizing Endogenous Sulfur Dioxide Derivatives in Rat Epilepsy Model

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Chemicals and materials. Dopamine hydrochloride (DA) was purchased from BBI Life Sciences. The amino acids glycine (Gly), valine (Val), lysine (Lys), serine (Ser), leucine (Leu), phenylalanine (Phe), glutamine (Gln), isoleucine (Ile), tyrosine (Tyr), tryptophan (Trp), threonine (Thr), methionine (Met), glutamate (Glu), alanine (Ala), aspartic acid (Asp), proline (Pro), glutamic acid (Glu), arginine (Arg), histidine (His), cysteine (Cys), asparagine (Asn), homocysteine (Hcys) and the metal salts NaF, NaCl, NaBr, NaI, $Na_4P_2O_7$, Na_3PO_4, Na_2HPO_4, NaH_2PO_4, Na_2S, Na_2S0_4, Na_2CO_3，$NaHCO_3$, $NaN0_2$, $NaNO_3$, NaSCN, NaAC were purchased from Shanghai Titan Scientific Co. Ltd. (Shanghai, China). Hydrochloric acid (HCl) was ordered from Shanghai Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China). Trihydroxymethyl aminomethane ($C_4H_{11}O_3$) was purchased from Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China). Tris-HCl buffer (50 mM, pH 7.0) was prepared using metal free reagents in distilled water. Human hepatocarcinoma cell (HepG2) cells and fetal bovine serum (FBS) were purchased from ATCC (HB-8065) and Biological Industries (BI, 04-001-01ACS), respectively. Mitochondria (MitoTracker® Red CM-H2XRos) trackers were purchased from Invitrogen. Maleinimide (NEM) and Pentylenetetrazole (PTZ) were purchased from Shanghai Titan Scientific Co. Ltd. (Shanghai, China).

Apparatus. Transmission electron microscope (TEM) images were acquired by using a JEM-2010 (UHR, JEOE, Japan) with a 200 kV accelerating voltage and Atomic Force X-ray photoelectron spectroscopy (XPS) measurements were performed on a PHI Quantum 5000 XPS system (Physical Electronics, USA). Whereas XPS was used to characterize the elemental composition and bonding configuration. Fourier transform infrared (FT-IR) spectra were recorded using a VECTOR 22 spectrophotometer (Bruker, Germany.). Fluorescence spectroscopy was measured in a microplate reader (Cytation5, BIOTEK, USA) using a black 384-well microplate (Thermo, USA). UV-vis absorption spectroscopy of PDA was also measured in a microplate reader (Cytation5, BIOTEK, USA) using a transparent 96-well plate (Thermo, USA). Glass-bottomed culture dish (801001, Nest, China).

EXPERIMENTAL SECTION

1. Synthesis of Probe PDADs. The concentration of DA (1 M) aqueous solution was prepared with dopamine hydrochloride powder and ultrapure water and stored at room temperature in dark. After 6 months of reaction, the reacted solution was transferred to the dialysis bag, which was dialysis for 2 days to remove the unreacted dopamine, and then the solution in the dialysis bag was freeze-dried to obtain PDADs powder. 23.7 mg PDADs powder was dissolved in 5.0 mL ultrapure water to obtain 4.7 $mg \cdot mL^{-1}$ PDADs original solution.

2. Fluorescence Response of PDADs to SO_2 Derivatives. In vitro testing of SO_2 derivatives was performed at room temperature in a 50 mM tris-Hcl buffer (pH 8.5). Typically, The concentration of PDADs (30 $\mu g \cdot mL^{-1}$) solution was added to a 0.5 mL of the buffer solution, followed by a fixed amount of SO_2 derivatives. The solution was mixed in a vortex mixer and incubated for 2 minutes, the fluorescence emission spectra were recorded with a microplate reader (Ex=315 nm).

3. Monitoring Total SO_2 Derivatives in Biological Fluid Samples by PDADs. Human serum and urine samples were supplied by the Hospital of Nanjing in accordance with approved institutional protocols and then instantly stored frozen until used. The use of human serum and urine samples was approved by the donors. The serum sample (4 mL) was added to acetonitrile (2 mL) to precipitate proteins, and then the solution was mixed in a vortex mixer and incubated for 2 min. The fully mixed solution was placed in a centrifuge and centrifuged at 8000 rpm for 10 minutes to obtain the supernatant. Finally, the obtained supernatant was filtered with a 0.45 mm microporous filter to obtain the further serum sample. For the determination of SO_2 derivatives, PDADs (30 $\mu g \cdot mL^{-1}$) was injected into 0.5 mL of diluted human serum or urine samples in tris-HCl buffer (50 mM, pH 8.5) directly spiked with different concentrations of SO_2 derivatives, the fluorescence spectra of the solutions were collected after reaction for 40 min at room temperature.

4. Intracellular Fluorescence Imaging. HepG2 cells were cultured in Dulbecco's modified eagle's medium (DMEM), containing streptomycin (100 μg/mL), 10% fetal bovine serum, and penicillin (100 units/mL). These cells were maintained in a humidified incubator at 37°C under 5% CO_2 for 2 days on the culture dish (801001, Nest, China). For intracellular fluorescence imaging, the HepG2 cells were incubated with DMEM (1 mL) containing 10% fetal bovine serum and 20 μg/mL probe PDADs at 37 °C for 2 h, the probes that did not enter the cell were washed away by PBS of three times. Cell images were obtained by a Zeiss LSM880 NLO (2+1 with BIG) confocal microscope system. MitoTracker Red CM-H2XRos (100 nM, Ex=579 nm, PMT range=600-650 nm) were used as mitochondria trackers. To discover the sensing property of probe for SO_3^{2-} in HepG2 cells, the cell culture medium was added to Na_2SO_3 at 37°C for 2 hours, and then cell imaging were performed.

5. Fluorescence Imaging in Zebrafish. The animal procedures were in accordance with the guidelines of the Institutional Animal Care and Use Committee, Nanjing Tech University. This committee approved the experiments in zebrafish. Zebrafishes samples were supplied by TiYing biological corporation (Shanghai, China). The same batch of Zebrafishes egg and larvae (three-days) were incubated in nutrient solution supplemented with 40 μg/mL PDADs probe at 28°C for 2 h, the probes that did not enter the sample were washed away by the nutrient solution. Zebrafish egg and larvae were placed into a dish filled with a nutrient solution for imaging the SO_3^{2-}. The sample to be fluorescence imaged will be anesthetized by0.01%-0.02% tricaine. Fluorescence images were captured by a confocal microscope system (Zeiss LSM880 NLO), and the collection windows were set at 420-500 nm (Ex=405 nm). All animal experiments were performed in full compliance with international ethical guidelines.

6. Imaging of Hippocampus Tissue of Rat Epilepsy Model. The animal procedures were in accordance with the guidelines of the Institutional Animal Care and Use Committee, Nanjing Tech University. This committee approved the experiments in rat. By following a

reported protocol, we established a PTZ-treated rat epilepsy model[1]. Rats in good health from the same batch were randomly divided into a control group and a PTZ -treated disease group. Simply, rats in the PTZ -treated disease group were induced by injecting 50 mg/kg of PTZ into the abdominal cavity. Rats in the control group were intraperitoneally injected with normal saline of the same volume. After 120 h of observation, all the rats were euthanized and samples of hippocampal tissue were collected. This was repeated three times, and the detection effect of the probe was good. This experiment was done three times in total, and 12 rats were used.

The Morphology and Performance Analysis of PDADs:

Transmission electron microscopy (TEM) was applied for the observation of the synthesized PDADs; the results revealed the fabrication of monodispersed 3–8 nm PDADs with narrow size distribution (Fig. S1a, ESI†). Highr esolution TEM (HR-TEM) images showed a distinct latticefringe image with a spacing of 0.334 nm, indicating the typical distance between graphite layers (Fig. S1b, ESI†).20 These results of atomic force microscopy (AFM) images and typical section analysis of PDADs demonstrated that the PDADs exhibited a height distribution from 3 to 6 nm with spherical shapes (Fig. S1c–e, ESI†). The as-obtained PDADs showed the typical absorption band of around 310–400 nm and emission band centered at 425 nm with a low blue fluorescence in water (Fig. S2, ESI†). Meanwhile, they exhibited excitation and pH dependent fluorescence behaviors (Fig. S3, ESI†), and the zeta potential of the PDADs was measured for positive charges (+15.53 mV) (Fig. S4, ESI†), which was consistent with our previous reports.19 X-ray photoelectron spectroscopy (XPS) displayed four obvious peaks of C 1s, N 1s, O 1s, and Cl 1s. As Q7 shown in Fig. S5 (ESI†), the high-resolution C 1s scan spectrum result showed the presence of C–C/CQC, C–N, and CQO/CQN functional groups. The N 1s scan spectrum can be

divided into three peaks corresponding to pyridinic N, amino N, and pyrrolic N, respectively. Furthermore, the O 1s scan spectrum is fitted with two peaks assigned to CQO and C–OH/C–O–C groups. The contents and ratios of surface groups of the PDADs are summarized in Table S1 (ESI†) according to the XPS results.

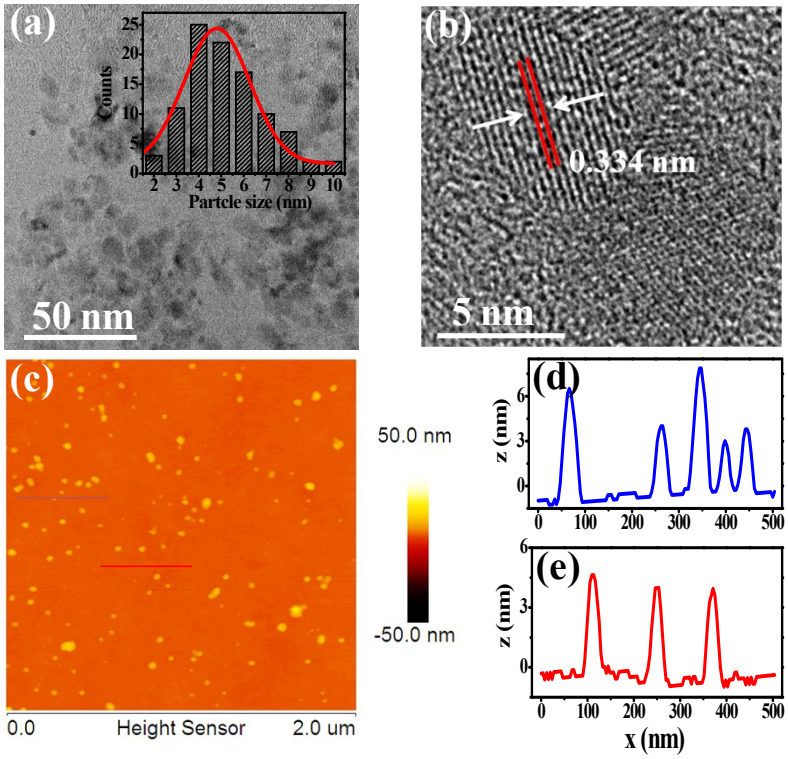

Fig. S1. Characterization of PDADs: TEM images of PDADs (a) and the HR-TEM of PDADs (b). AFM images (d), and profile analyses of PDADs (d and e).

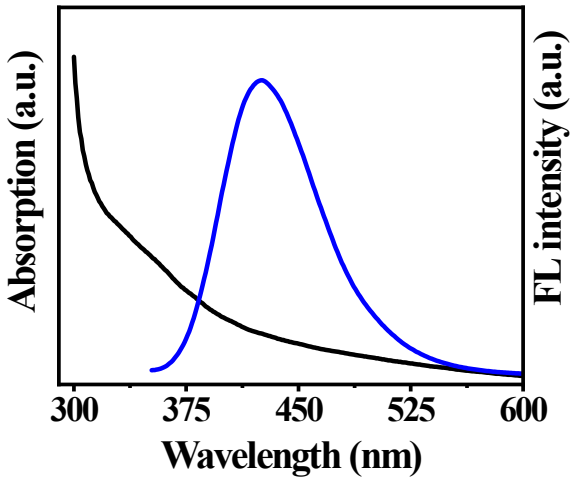

Fig. S2. UV-vis absorption spectra and fluorescence emission spectrum of PDADs.

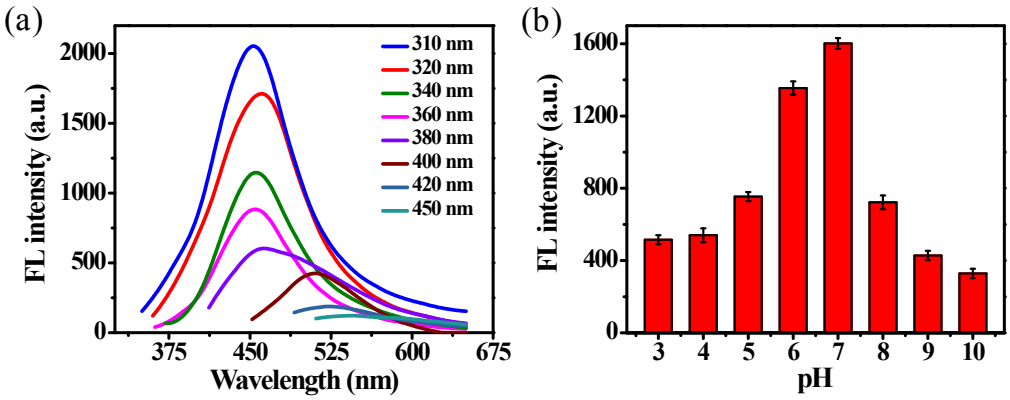

Fig. S3. (a) Fluorescence spectra of PDADs under different excitation wavelengths. (b) Fluorescence intensity change of PDADs in tris-HCl buffer (50 mM) at different pH.

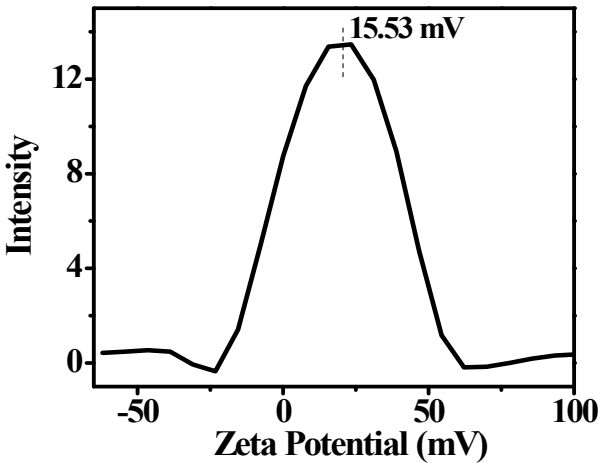

Fig. S4. Zeta potential of PDADs.

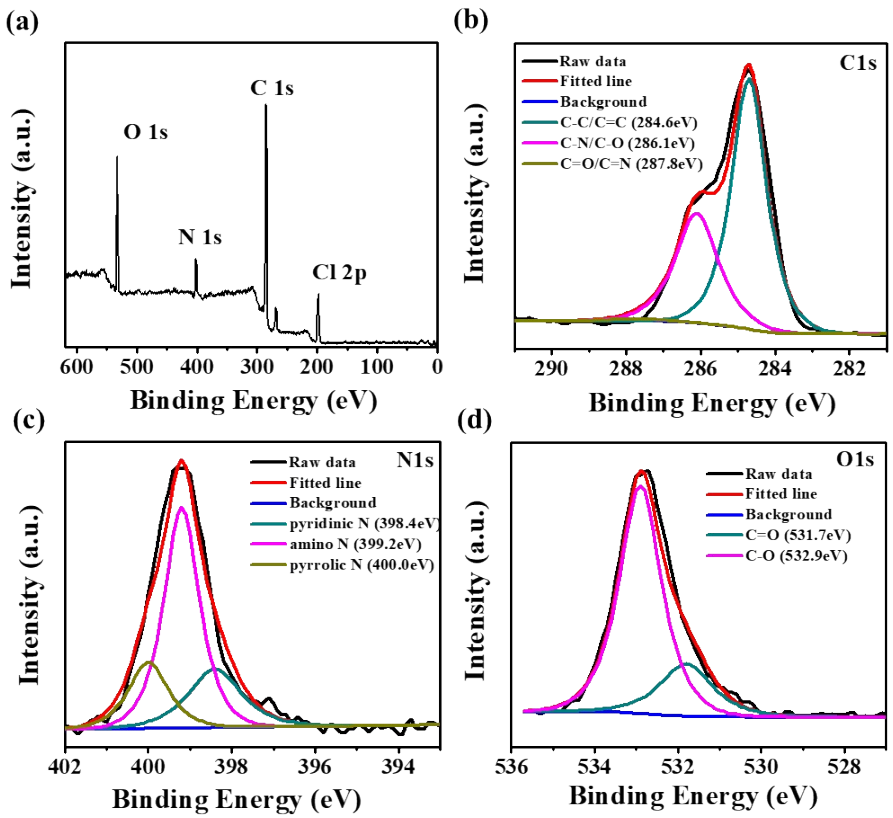

Fig. S5. Full-survey X-ray photoelectron spectroscopy (XPS) of PDADs (a), C1s spectrum (b), N1s spectrum (c), and O1s spectrum of PDADs (d).

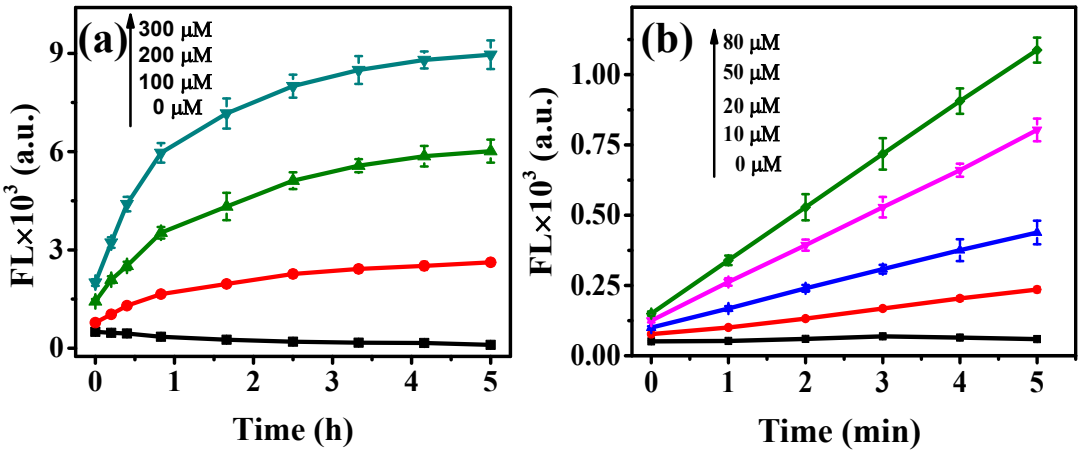

Fig. S6. (a) Time-dependent fluorescence responses of the PDADs before and after the addition of SO_3^{2-} with different concentrations (0, 100, 200, 300 μM) in tris-HCl buffer (50 mM, pH 8.5). (b) Time-dependent fluorescence responses of the PDADs before and after the addition of SO_3^{2-} with different concentrations (0, 10, 20, 50,80 μM) in tris-HCl buffer (50 mM, pH 8.5).

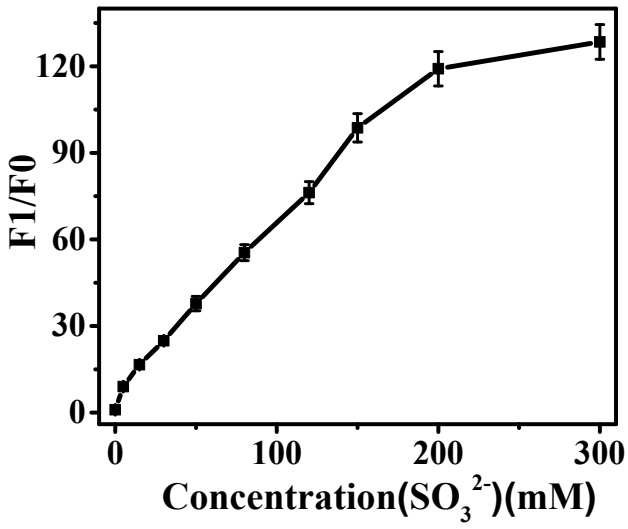

Fig. S7. Relationship between the F1/F0 versus the concentration of SO_3^{2-} from 0 to 300 μM in tris-HCl buffer (50 mM, pH 8.5).

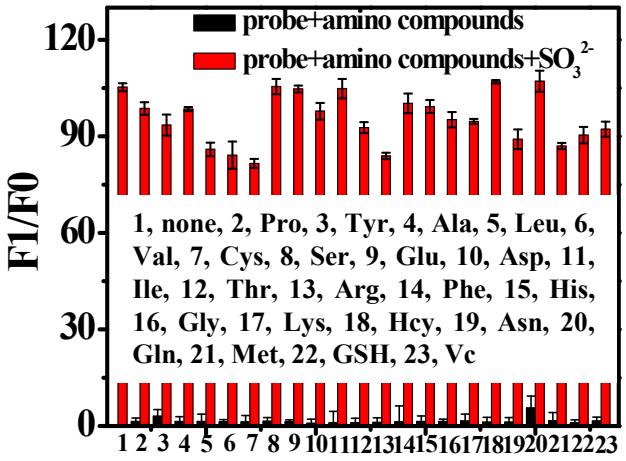

Fig. S8. Competing responses of PDADs (1 M, 0.5 μL) towards various amino acids and biothiols (200 μM) in tris-HCl buffer (50 mM, pH 8.5).

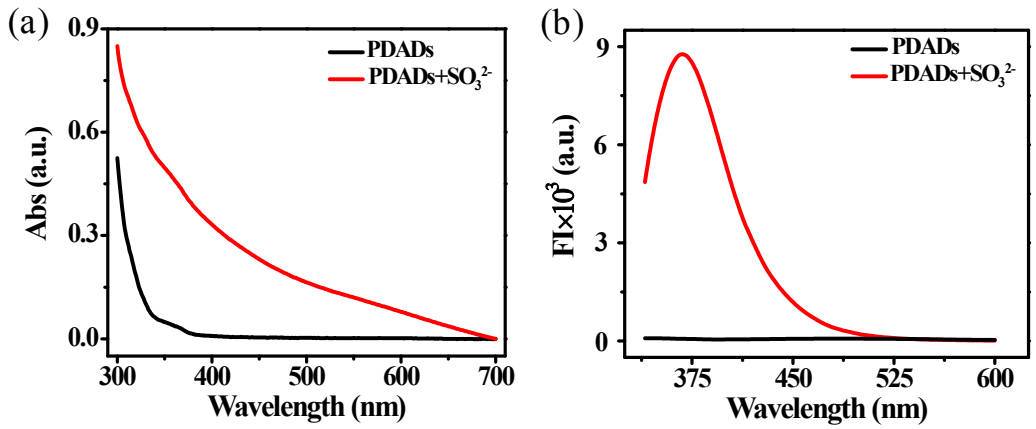

Fig. S9. (a) UV-vis absorption spectra (abs) of PDADs (microwave irradiation, 100 ^{o}C, 30 min) before and after the addition of SO_3^{2-} (200 μM). (b) Fluorescence spectra of PDADs before and after the addition of SO_3^{2-} (200 μM) in tris-HCl buffer (50 mM, pH 9.0). The inset presents a colorimetric photograph of PDADs (microwave irradiation, 100 ^{o}C, 30 min).

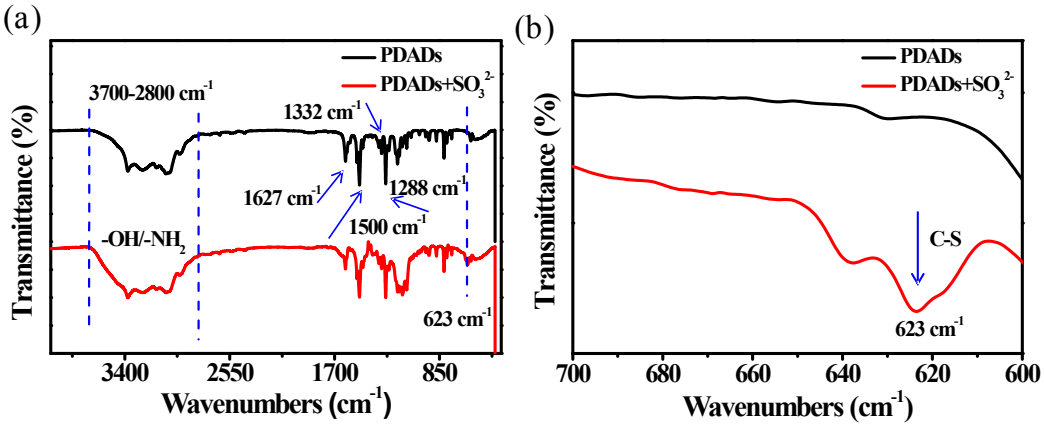

Fig. S10. (a) FT-IR of PDADs before and after the addition of SO_3^{2-} (Wavenumbers: 4000-350 cm^{-1}). (b) FT-IR of PDADs before and after the addition of SO_3^{2-} (Wavenumbers: 600-700 cm^{-1}).

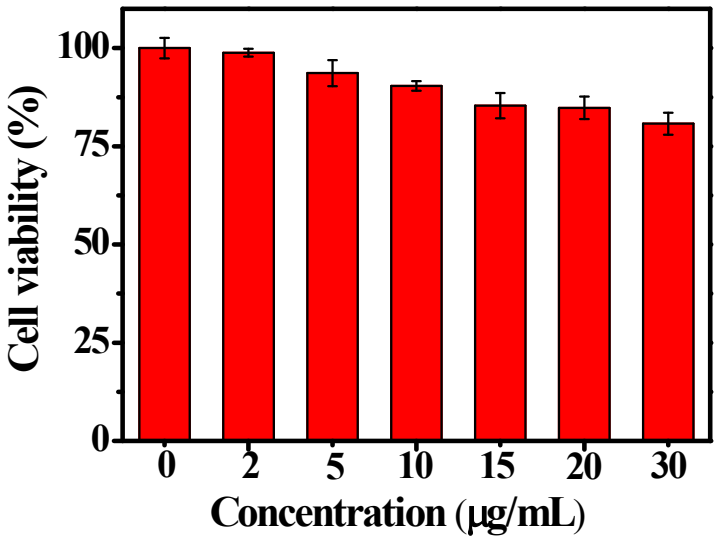

Fig. S11. Percentage of viable HepG2 cells after treatment with indicated concentrations of PDADs after 12 h.

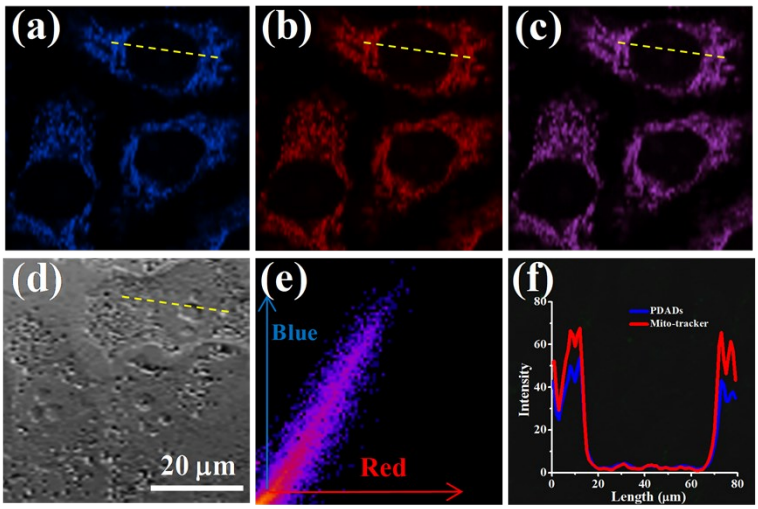

Fig. S12. Confocal fluorescence images of HepG2 cells stained with (a) 20 μg/mL PDADs incubated with SO_2 donor (GSH 500 μM/$S_2O_3^{2-}$ 250 μM) (blue channel: λex=405 nm, λem=420-500 nm) and (b) 200 nM MitoTracker Red CM (red channel: λex=579 nm, λem=600-630 nm) at 37° C for 2 hours (c) Merged image of (a) and (b). (d) Bright field image. (e) Correlation plot of MitoTracker Red CM and PDADs intensities. (f) Intensity profile of regions of interest (ROI) across HepG2 cells. Scale bar: 20 μm.

Table S1 XPS functional group percentages of obtained PDADs

C 1s			N 1s			O 1s	
C-C/C=C 284.6 eV (%)	C-N/C-O 286.1 eV (%)	C=O/C=N 287.8eV (%)	C=N-R 398.4 eV (%)	R_1-NH-RH_2 399.2 eV (%)	R-NH_2 400 eV (%)	C=O 531.7 eV (%)	C-O 532.9 eV (%)
64.42	34.21	1.37	22.90	57.68	19.42	25.31	74.69

Table S2. Determination of SO_2 derivatives in human serum and urine samples.

Samples	original	added (μM)	measured (μM)	recovery (%)	RSD (%) (n=3)
1 (urine)	5	15	19.83	99.13	2.21
2 (urine)	5	25	23.51	94.04	1.86
3 (urine)	5	55	59.65	99.41	4.32
4 (serum)	5	15	19.55	97.75	2.93
5 (serum)	5	25	30.63	102.1	4.49
6 (serum)	5	55	61.44	102.4	3.06

Table S3 Comparison of some sensor platforms for SO_2 derivatives detection

Methods	Detection limit	Selective	Toxicity (Cell viability < 90%)	Reproducibility	References
PDADs	8 nM	Good	1.28 mM	Good	[this method]
CZBT	28 nM	Good	5 μM	Good	[2]
Probe 1	1.22 μM	Good	5 μM	Good	[3]
CSP	1.76 μM	Good	10 μM	Good	[4]
Rh-TPA	3.2 μM	Good	20 μM	Good	[5]
Cou-PCL	5.91 μM	Good	5 μM	Good	[6]

Ref:

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