Electronic Supplementary Material (ESI) for Analyst.

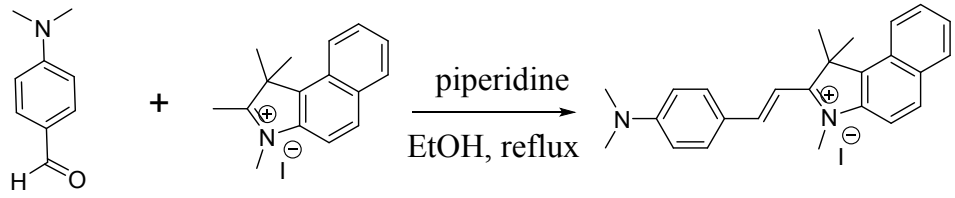
A mitochondria-targeted fluorescence probe for the detection of endogenous SO_2 derivatives in living cells

Ye-Hao Yan[a], Qiu-RongWu[b], Qiao-Ling Che[a], Man-Man Ding[a], Min Xu[a], Jun-Ying Miao[b], Bao-Xiang Zhao[a*], Zhao-Min Lin[c*]

[a]*Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, P.R. China*

[b]*Shandong Provincial Key Laboratory of Animal Cells and Developmental Biology, School of Life Science, Shandong University, Qingdao 266237, P.R. China*

[c]*Institute of Medical Science, the Second Hospital of Shandong University, Jinan 250033, P.R. China*



Scheme S1 Synthesis route of Acceptor

p-Dimethylaminobenzaldehyde and benzoindole derivative were dissolved into EtOH (20 mL) under the catalysis of piperidine and kept refluxing 6 h. Then, purification by column chromatography (DCM: MeOH= 15:1) gave the product in 54% yield. The Acceptor was confirmed by ^{1}H NMR (**Fig. S5**).

1. Energy transfer efficiency was obtained based on the following equation:

$$E = 1- F_{DA}/F_D$$

In the equation, E represents the energy transfer efficiency in probe ZACA FRET system. F_D: Fluorescence intensity of the Donor alone. F_{DA}: Fluorescence intensity of the donor in this FRET system.

2. Detection limit = 3σ/K

Wherein, σ = the standard deviation of I_{490}/I_{620} of blank testing solution without HSO_3^- /SO_3^{2-}, and K = the slope of the linear calibration.

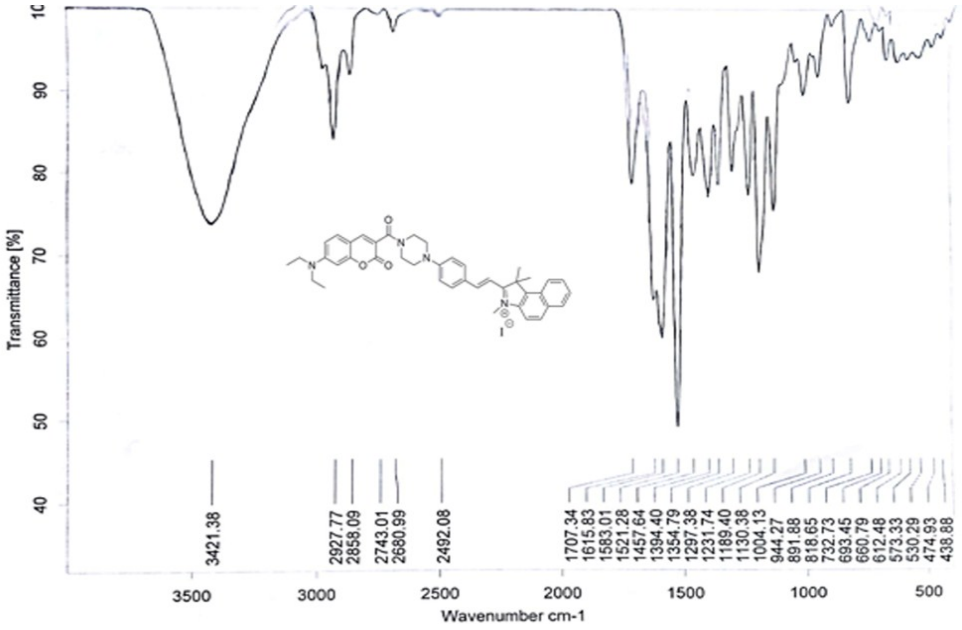


Fig. S1 IR of probe ZACA.

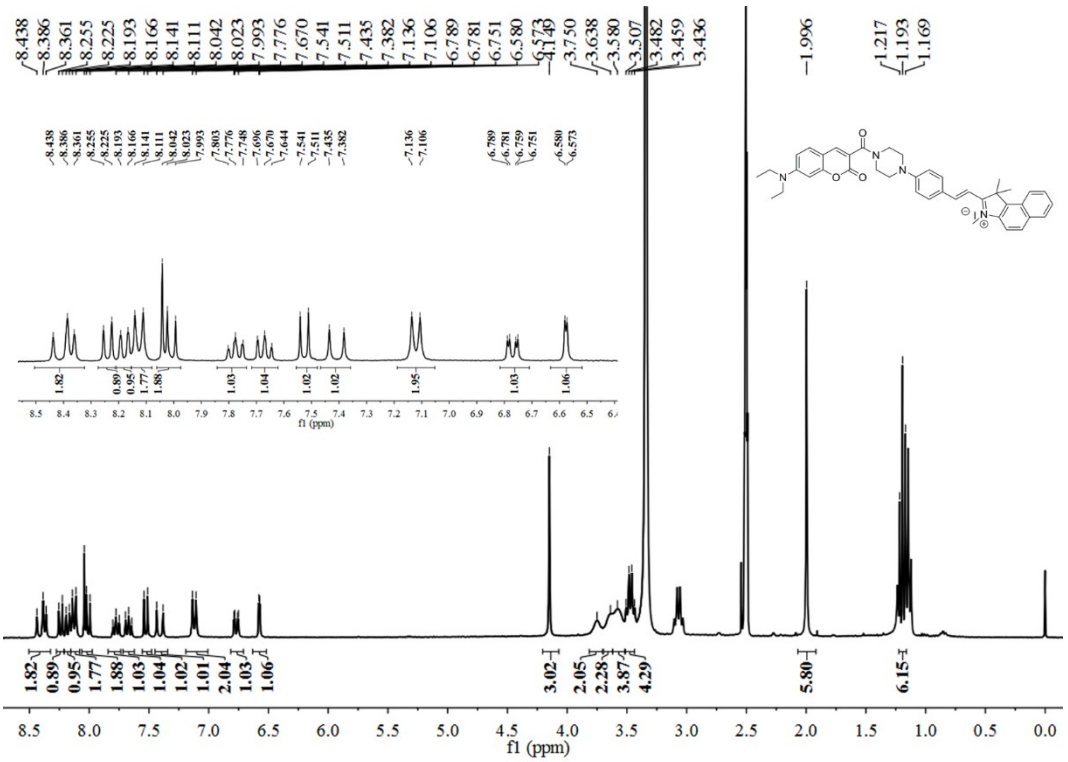


Fig. S2 ^{1}H NMR of probe ZACA (300 MHz, DMSO-d_6).

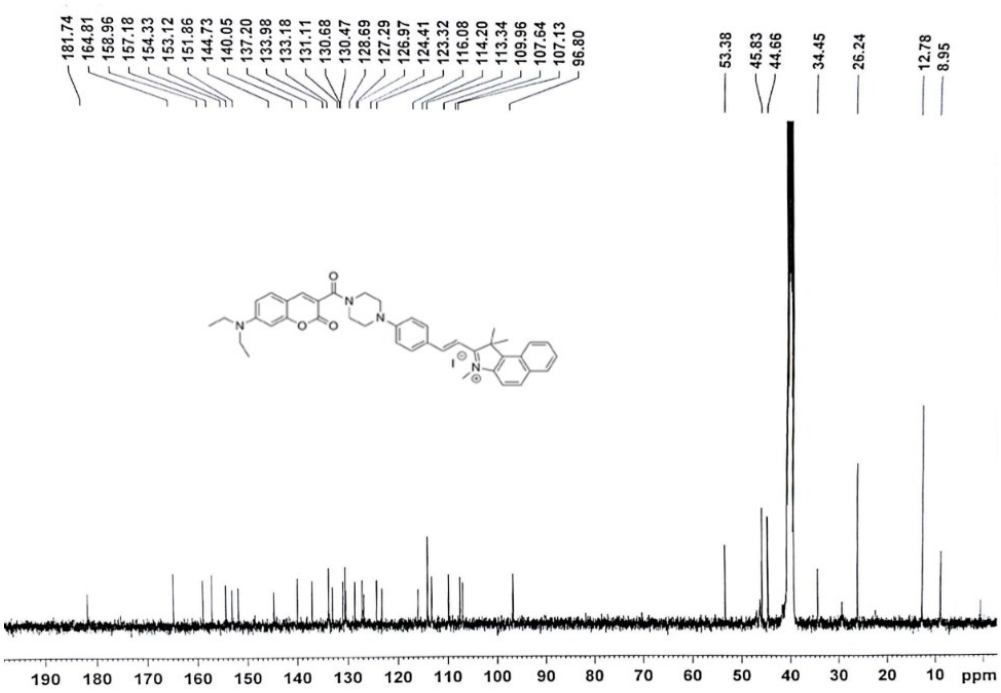


Fig. S3 ^{13}C NMR of probe ZACA (100 MHz, DMSO-d_6).

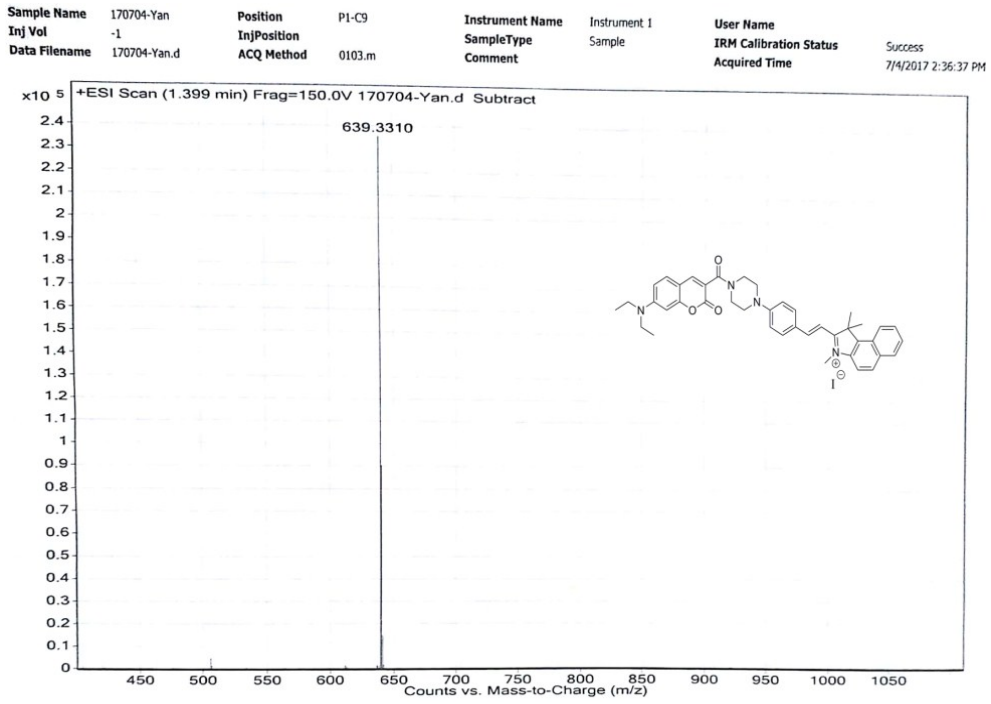


Fig. S4 HR-MS of probe ZACA.

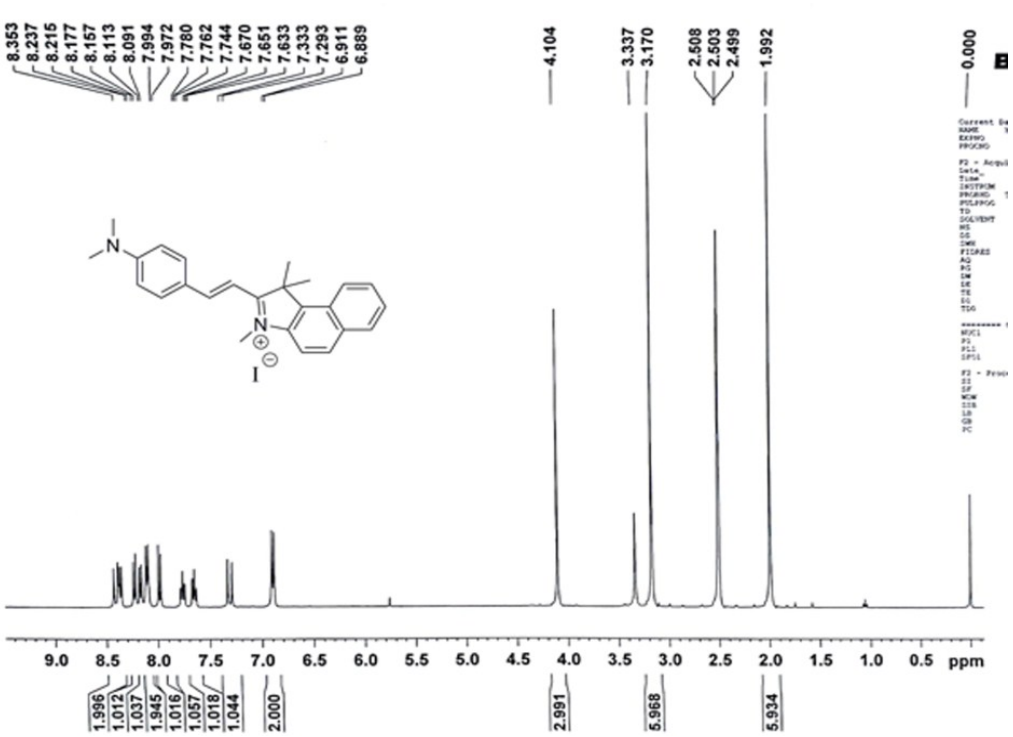


Fig. S5 ^{1}H NMR of Acceptor (400 MHz, DMSO-d_6).

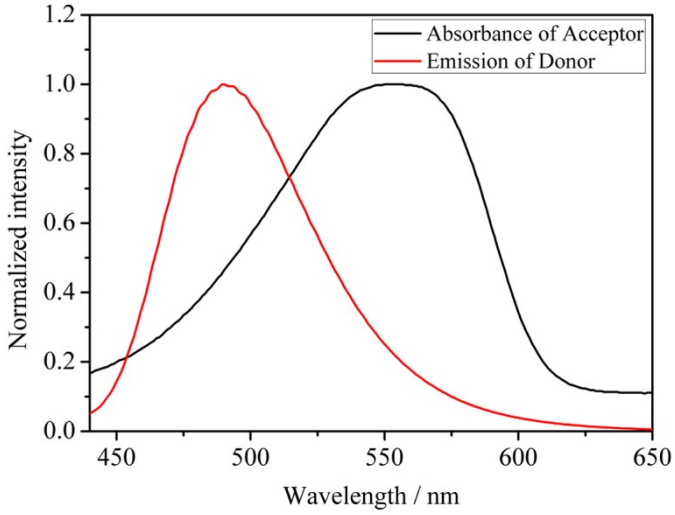


Fig. S6 The overlap between the emission band of Donor and the absorbance band of Acceptor.

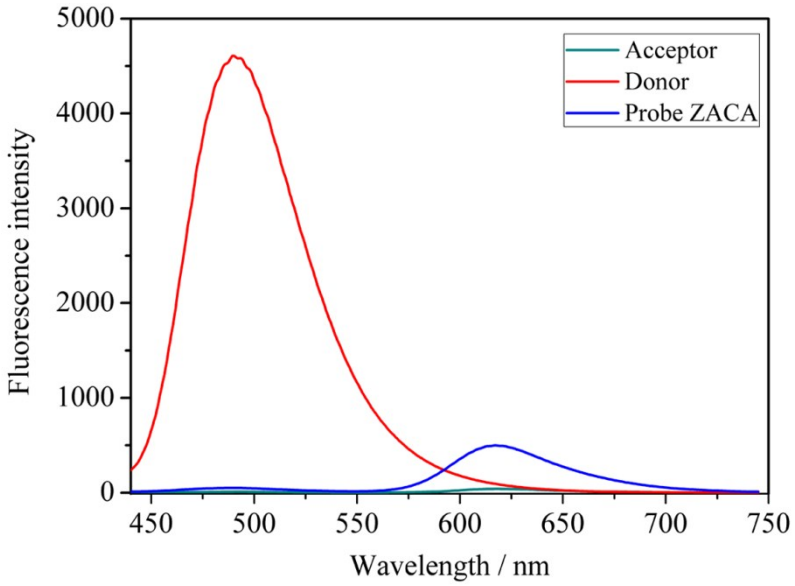


Fig. S7 The fluorescence spectra of Acceptor, Donor and probe ZACA. (5 μM, DMSO: PBS = 3:7, pH = 7.4, Excitation wavelength: 420 nm, slit: 5/5, speed: 1200 nm/s).

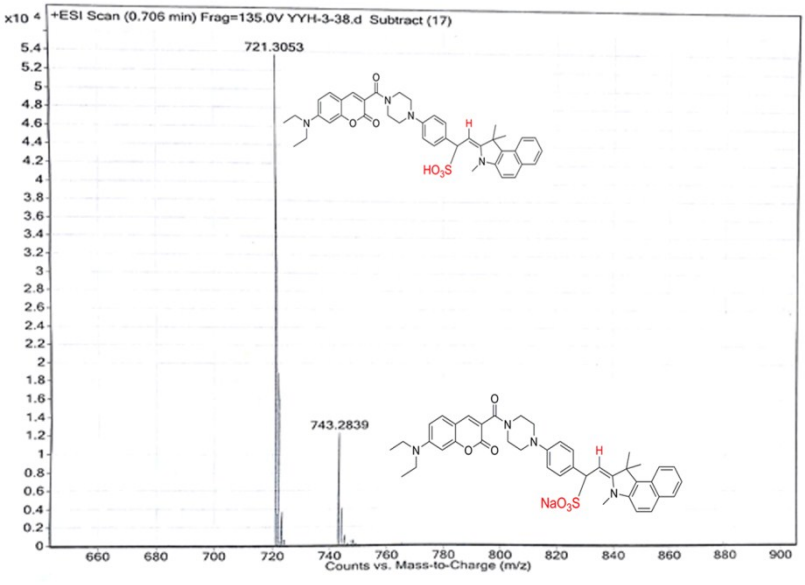


Fig. S8 HR-MS of the mixture of probe ZACA and HSO_3^-/SO^{2-}_3.

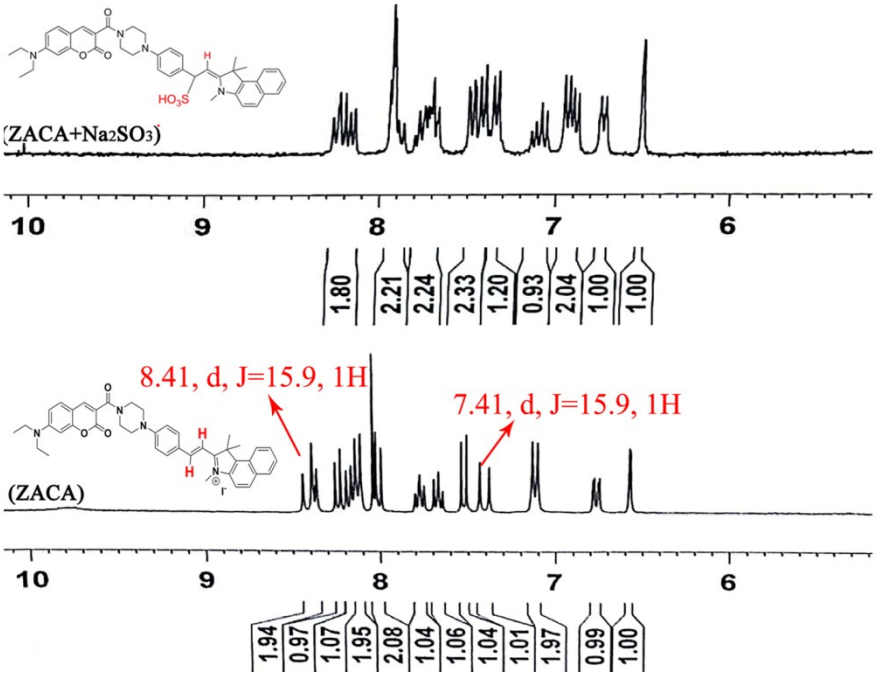


Fig. S9 ^{1}H NMR of the mixture of probe ZACA and HSO_3^-/SO_3^{2-}.

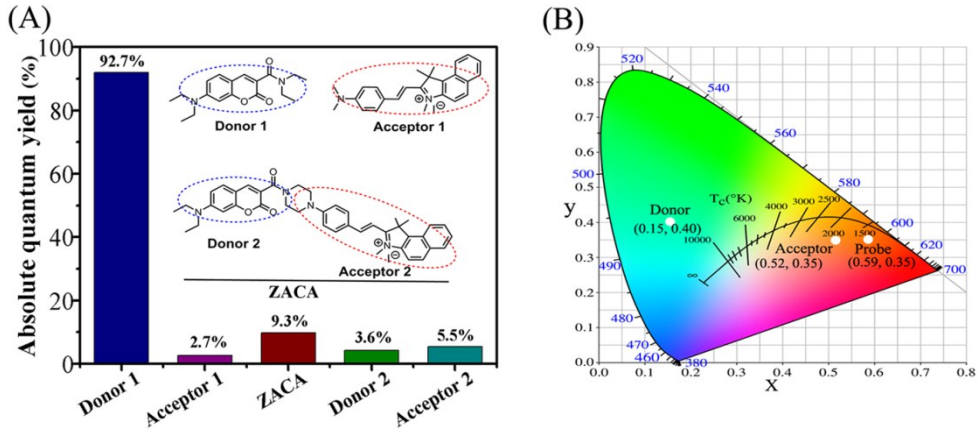


Fig. S10 The quantum yield and CIE chromaticity diagram of Donor, Acceptor and the probe.

(A) The absolute quantum yield of Donor, Acceptor and the probe in DCM solution, wherein Donor 2 is the donor moiety in probe ZACA molecule, Acceptor 2 is the acceptor moiety in probe ZACA molecule. (Excitation range: 405-435 nm) **(B)** The CIE chromaticity diagram of Donor, Acceptor and probe ZACA.

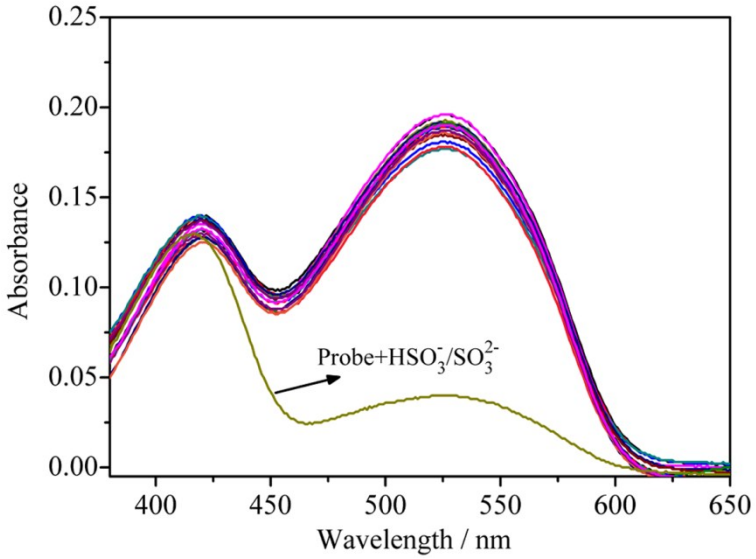


Fig. S11 The Uv-vis spectra of ZACA (10 μM) in presence of various species (blank, $^1O_2$, Fe^{2+}, H_2O_2, HClO, HO·, OH^-, *t*-BuOOH, NO_3^-, CN^-, GSH, Fe^{3+}, Cys, H_2S, Ca^{2+}, Hg^{2+}, Pb^{2+}, and 1.2 equiv. HSO_3^-/SO_3^{2-}). (DMSO: PBS = 3:7, pH = 7.4).

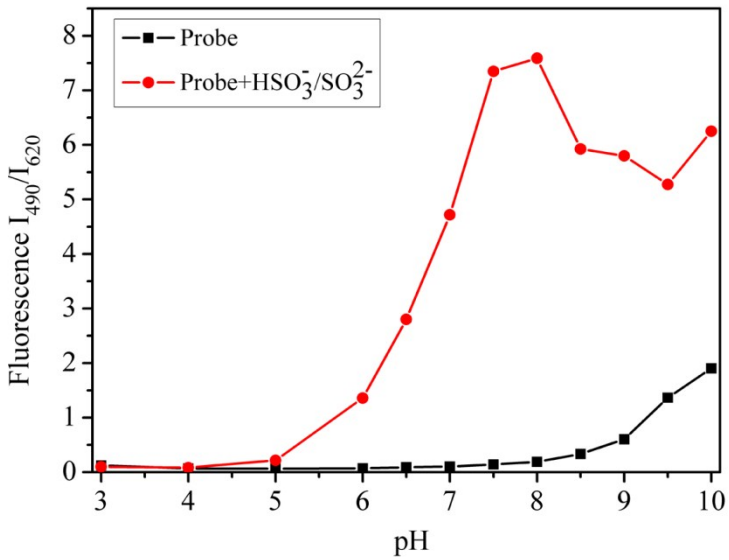


Fig. S12 The pH dependence of the ZACA (5 μM) toward HSO_3^-/SO_3^{2-} (1.2 equiv.). (DMSO: PBS = 3:7, excitation wavelength: 420 nm, slit: 5/5, speed: 1200 nm/min).

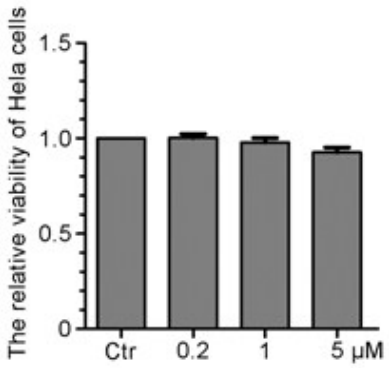


Fig. S13 The toxicity analysis of ZACA for Hela cells.

Hela cells were cultivated with Dulbecco's modified Eagle's medium (DMEM) which contains supplement of 10% FBS (Fetal Bovine Serum) in the carbon dioxide incubator with an atmosphere of 5% CO_2 and 95% air at 37℃. HeLa cells were placed to a 96-well plate in the concentration of 40000 per mL for 24 h, and then incubated with probe ZACA (0, 0.2, 1, 5 μM) for 6 h, respectively. After that SRB assay was conducted to measure the viability of cells.

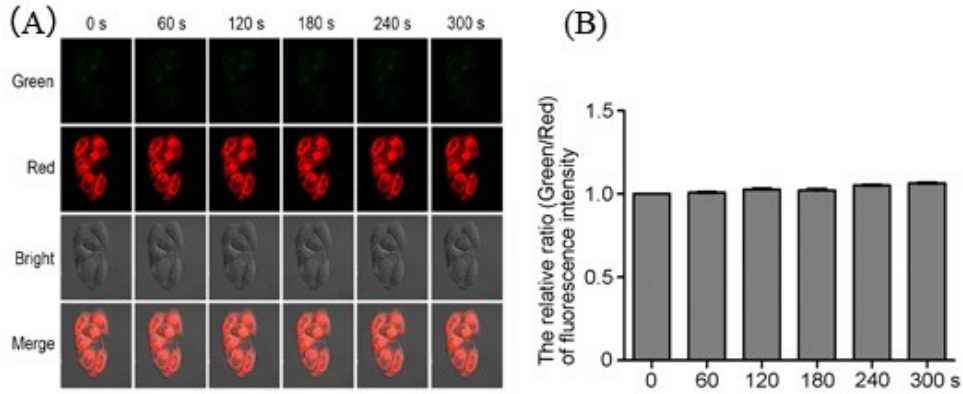
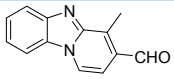
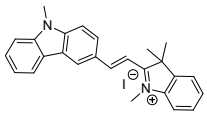
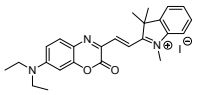
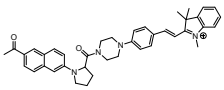
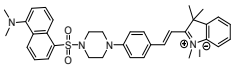
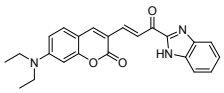
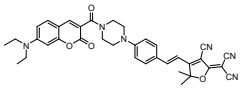
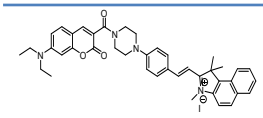


Fig. S14 The photo-stability analysis of ZACA in HeLa cells.HeLa cells were pre-treated with ZACA (1 μM) for 1 h. **(A)** First line: fluorescence images at the green channel (405-555 nm). Second line: fluorescence images at the red channel (560-700 nm). Third line: images at bright field. Forth line: merged images of the first and second lines. **(B)** The relative ratio of fluorescence intensity.

Table S1 Comparison of probe ZACA to other probes:

Probe	Response ions	ratiometric	Detection limit	Targeted subcellar	Reference
	HSO_3^-	No	76 nM	No	S1
	HSO_3^-	Yes	0.15 μM	Mitochondria	S2
	HSO_3^-	Yes	87 nM	No	S3
	HSO_3^- /SO_3^{2-}	Yes	0.24 μM	No	S4
	HSO_3^- /SO_3^{2-}	Yes	0.1 μM	Mitochondria	S5
	HSO_3^-	Yes	53 nM	No	S6
	HSO_3^-	Yes	45 nM	No	S7
	HSO_3^- /SO_3^{2-}	Yes	15.6 nM	Mitochondria	This work

Reference

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[3] M.X. Li, W.Y. Feng, H.Y. Zhang and G.Q. Feng, Sens. Actuators B 2017, **243**, 51-58.

[4] X.Y. Zhu, L.M. Zhu, H.W. Liu, X.X. Hu, R.Z. Peng, J. Zhang, X.B. Zhang and W.H. Tan, Anal. Chim. Acta 2016, **937**, 136-142.

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