Supplementary data

A ratiometric fluorescent probe for detection of the endogenous mitochondrial SO₂ based on FRET mechanism

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Fig. S1 Ultraviolet absorption spectra of IPIN-SO₂ (10 μ M) with the addition of sulfite (0-30 equiv.) in 0.1M PBS (pH = 7.4) buffer solution.



Fig. S2 Linearity between the fluorescence intensity ratio (F_{475}/F_{580}) and the concentrations of SO₃²⁻ in PBS (pH = 7.4) solution.



Fig. S3 Photostability for fluorescent images of Glioma cells incubated with 0.1μ M IPIN-SO₂ at 37°C. First row: fluorescence images from the <u>blue</u> channel (460/50 nm); second row:fluorescence images at the red channel (605/55 nm); third row: overlay images of the first and second rows; fourth row: bright field images; fifth row: overlay images of the first, second third and fourth rows.



Fig. S4 Cytotoxicity assays of IPIN-SO₂ at different concentrations (1-16 μ M) for Glioma cells.



Fig. S5 Fluorescence images of Glioma cells incubated with IPIN-SO₂ (0.1 μ M) for 60 min at 37 °C and further incubated with Na₂SO₃ (0, 0.05, 0.5 mM) for 30 min at 37 °C. first row: fluorescence images from the <u>blue</u> channel (460/50 nm); second row: fluorescence images at the red channel (605/55 nm); third row: overlay images of the first and second rows; fourth row: bright field images; fifth row: overlay images of the first, second third and fourth rows.



Fig. S6 ¹H NMR spectrum of probe IPIN-SO₂.



Fig. S7 ¹³C NMR spectrum of probe IPIN-SO₂.



(A)



(B)

Fig. S8 HRMS spectra of **IPIN-SO**₂ (A) and **IPIN-SO**₂ in the presence of Na₂SO₃ (B). Table S1 Comparison of ratiometric fluorescent probes for HSO_3^-

Structures	λ_{ex}/nm	λ_{em}/nm	Detection	Response	Ref.
			limit	time	
	380	475/580	130 nM	180 s	This Work
	380	477/576	70 nM	1200 s	Dyes Pigments, 2019, 165, 212-216
	380	465/640	62 nM	120 s	RSC. Adv., 2019, 9, 1147-1150
Ph N N N N N N N N N N N N N	380	470/578	26.7 nM	120 s	Anal. Chim. Acta, 2019, 1053, 148- 154
Ph O N N N N N N N N N N N N N	380	470/578	40 nM	120 s	Anal. Chim. Acta, 2019, 1055, 133- 139
HO O O	466/580	523/663	0.27 nM	90s	Chem. Commun., 2014, 50, 183
Et ₂ N O O CN	446	480/578	5800 nM	30s	Analyst, 2013, 138, 3018
	445	478/633	380 nM	300 s	Chem. Commun., 2013, 49, 2637
	405	480/650 8	90 nM	180s	Biomateri als, 2015, 56, 1

	450	518/610	89 nM	900s	Org. Biomol. Chem., 2014, 12, 4637
N N SO ^O SO ^O NEt ₂	450/550	485/667	27 nM		Sens. Actuators, B, 2015, 211, 377
HO COOEt HO	415/500	460/600	100 nM		Chem. – Asian J., 2014, 9, 1817
HO O NEt2	430/605	485/640	34 nM	300s	Sens. Actuators, B, 2015, 206, 268
	415	485/605	53 nM	3600s	Anal. Chim. Acta, 2015, 888, 138
Et ₂ N O O OR	410	465/592	200 nM	$t_{1/2} \approx$ 300s	Anal. Chim. Acta, 2013, 788, 165
	322/470	460/595	3.0 nM	180s	Chem. Commun., 2015, 51, 1154
	410	530/580	100 nM	120s	Chem. Commun., 2016, 52, 2760