

## Electronic Supplementary Material

### **An efficient ratiometric fluorescence and colorimetry dual-mode probe for convenient determination of nitrite in real samples and *E. coli***

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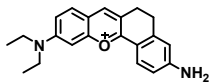
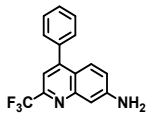
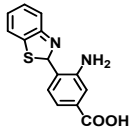
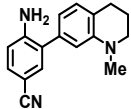
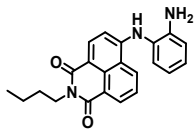
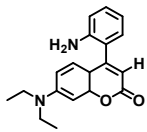

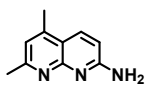
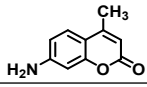
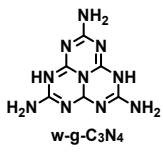
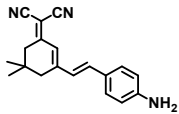
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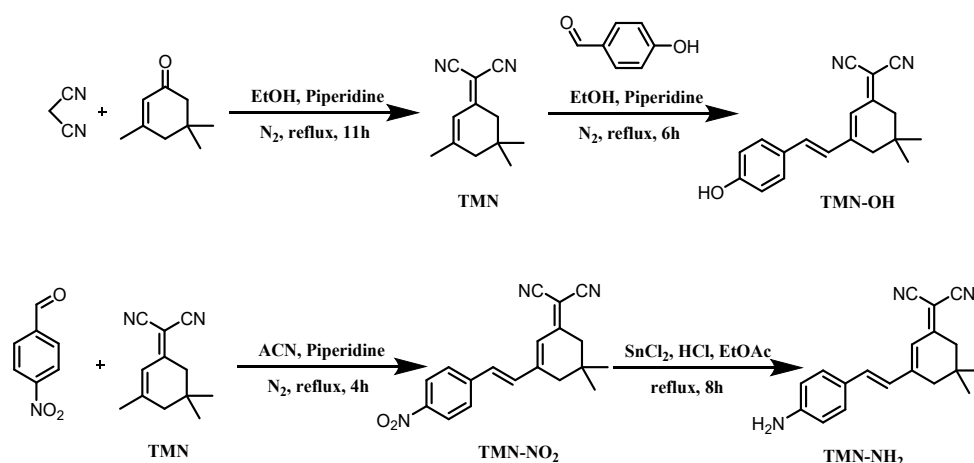
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# 1. Fluorescent probes for the detection of NO<sub>2</sub><sup>-</sup>

**Table S1.** Comparison of fluorescent probes for the detection of NO<sub>2</sub><sup>-</sup>.

reaction mechanism	Probes	Excitation/ Emission wavelength /nm	Stokes shift /nm	reaction temperature / °C	response time / min	Signal transduction mode	References
Diazotization of amines		580/620	40	25	8	“turn-off”	Spectrochim. Acta A., 2022, 282, 121692
		465/580	115	25	40	“turn-off”	Microchem. J., 2021, 169, 106342
Amino diazotization followed by azo coupling with activated aromatic rings		-/463 365/530	- 165	-	< 5S	ratiometric	Adv. Sci., 2020, 7, 2002991.
		285/400 470/555	115 85	room temperature	1	ratiometric	Anal. Chem., 2014, 87, 1274-1280
		355/440	85	room temperature	7	“turn-on”	Anal. Chim. Acta, 2023, 1268, 341403
		567/656	89	room temperature	7	“turn-on”	Food Chem., 2021, 341, 128254
Amino diazotization followed by hydrolysis to hydroxyl		285/350	65	-	10	“turn-off”	Microchem. J., 1999, 62, 371-376
		346/403	57	in boiling water bath	20	“turn-off”	Spectrochim. Acta A., 2007, 66, 586- 589
		325/380	55	in boiling water bath	70	-	Chromatographia 1993, 36, 57-60.
		340/435	95	room temperature	10	“turn-off”	New J. Chem., 2017, 41, 7171- 7176
		400/597 400/663	197 263	room temperature	10	ratiometric	This work

## 2. Synthesis procedures



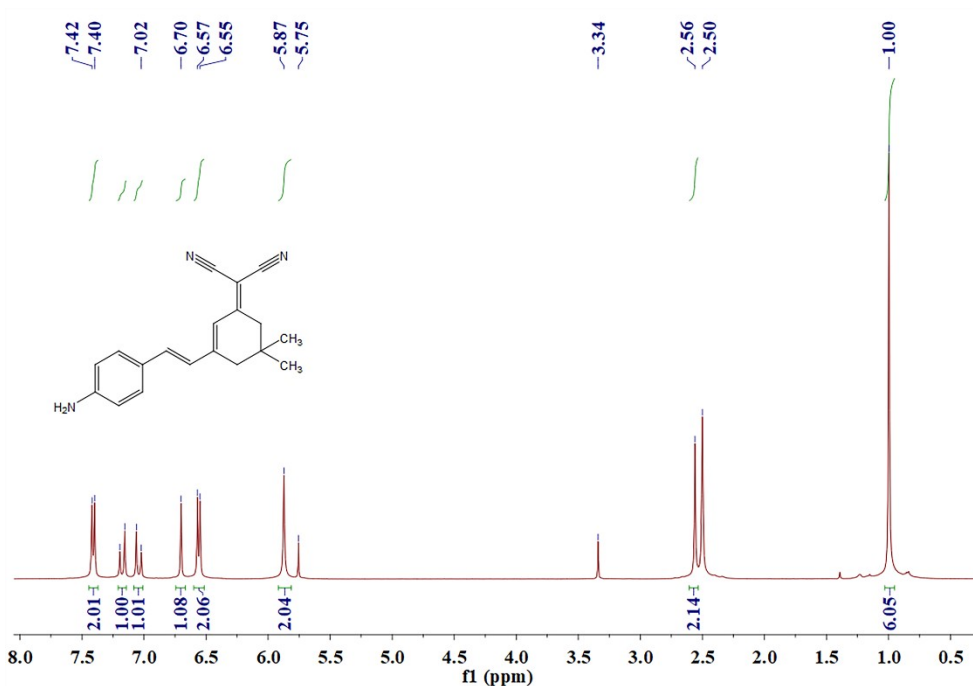
**Scheme S1.** Synthetic scheme of fluorophore **TMN-OH** and probe **TMN-NH<sub>2</sub>**

TMN (2-(3,5,5-trimethylcyclohex-2-en-1-ylidene)malononitrile) and TMN-NO<sub>2</sub> ((*E*)-2-(3-(4-nitrostyryl)-5,5-dimethylcyclohex-2-en-1-ylidene)malononitrile) were prepared as described in the literature [1]

**Synthesis of TMN-NH<sub>2</sub>** ((*E*)-2-(3-(4-aminostyryl)-5,5-dimethylcyclohex-2-en-1-ylidene)malononitrile): TMN-NO<sub>2</sub> (802 mg, 2.5 mmol) and SnCl<sub>2</sub>·2H<sub>2</sub>O (565 mg, 2.5 mmol) were mixed in 30 mL ethyl acetate, then hydrochloric acid (0.5 mL) was slowly added to the above solution. The mixture was refluxed at 80 °C for 8 h until the reaction was completed. The mixture was adjusted pH to neutral with 15% sodium hydroxide, and then extracted with dichloromethane. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, concentrated in vacuo and the crude product was purified by silica column chromatography using petroleum ether/dichloromethane (5:1, v/v) as eluent to afford **TMN-NH<sub>2</sub>** as black-purple powder (586 mg, yield: 81.1%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.41 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 15.9 Hz, 1H), 7.04 (d, *J* = 15.9 Hz, 1H), 6.70 (s, 1H), 6.56 (d, *J* = 8.5 Hz, 2H), 5.87 (s, 2H), 2.56 (s, 2H), 1.00 (s, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 169.90 (s), 157.61 (d, *J* = 5.8 Hz, 1H), 151.38 (s), 139.85 (s), 130.18 (s, 2C), 123.42 (d, *J* = 8.4 Hz), 119.95 (s), 114.58 (s), 113.78 (s, 2C), 72.66 (s), 42.31 (s), 38.21 (s), 31.64 (s), 27.46 (s, 2C). ESI-Mass: calcd for C<sub>19</sub>H<sub>19</sub>N<sub>3</sub>: 289.1579, found [M-H]<sup>-</sup> at m/z 288.1523. (Fig. S1 - S3).

**Synthesis of TMN-OH** (*(E)*-2-(3-(4-hydroxystyryl)-5,5-dimethylcyclohex-2-en-1-ylidene)malononitrile): TMN (93 mg, 0.5 mmol), *p*-hydroxybenzaldehyde (122 mg, 1.0 mmol) and some piperidine were added in 20 mL EtOH. The mixture was refluxed at 80 °C for 6 h with stirring under a nitrogen atmosphere. After being cooled to room temperature, the solvent was removed under reduced pressure and the residue was purified by column chromatography (PE/EA, 3/1, v/v, as eluent) to afford the desire product as a yellow solid (135 mg, yield 93.1%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.97 (s, 1H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 5.0 Hz, 2H), 6.81 – 6.77 (m, 3H), 2.59 (s, 2H), 2.52 (s, 2H), 1.01 (s, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 170.23 (s), 159.29 (s), 156.67 (s), 138.25 (s), 129.82 (s, 2C), 127.09 (s), 126.22 (s), 121.32 (s), 115.83 (s, 2C), 114.07 (s), 113.26 (s), 74.76 (s), 42.29 (s), 38.18 (s), 31.61 (s), 27.39 (s, 2C). ESI-Mass: calcd for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O: 290.1419, found [M-H]<sup>-</sup> at *m/z* 289.1342. (Fig. S4 - S6)

### 3. HRMS and NMR spectra



**Fig. S1** <sup>1</sup>H NMR of TMN-NH<sub>2</sub> in DMSO-*d*<sub>6</sub>

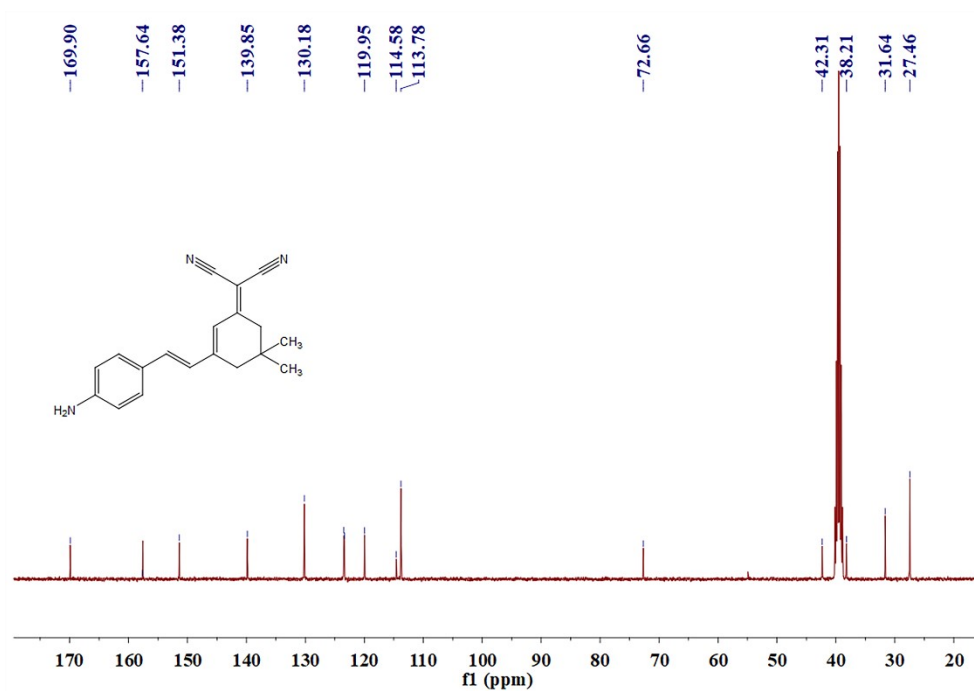


Fig. S2  $^{13}\text{C}$  NMR of TMN-NH<sub>2</sub> in DMSO-*d*<sub>6</sub>

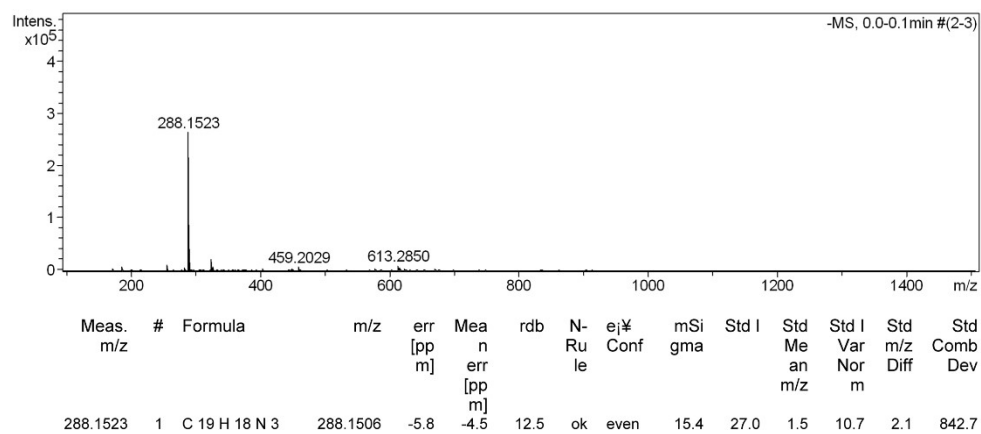


Fig. S3  $^1\text{H}$  NMR of HBTMS in DMSO-*d*<sub>6</sub>

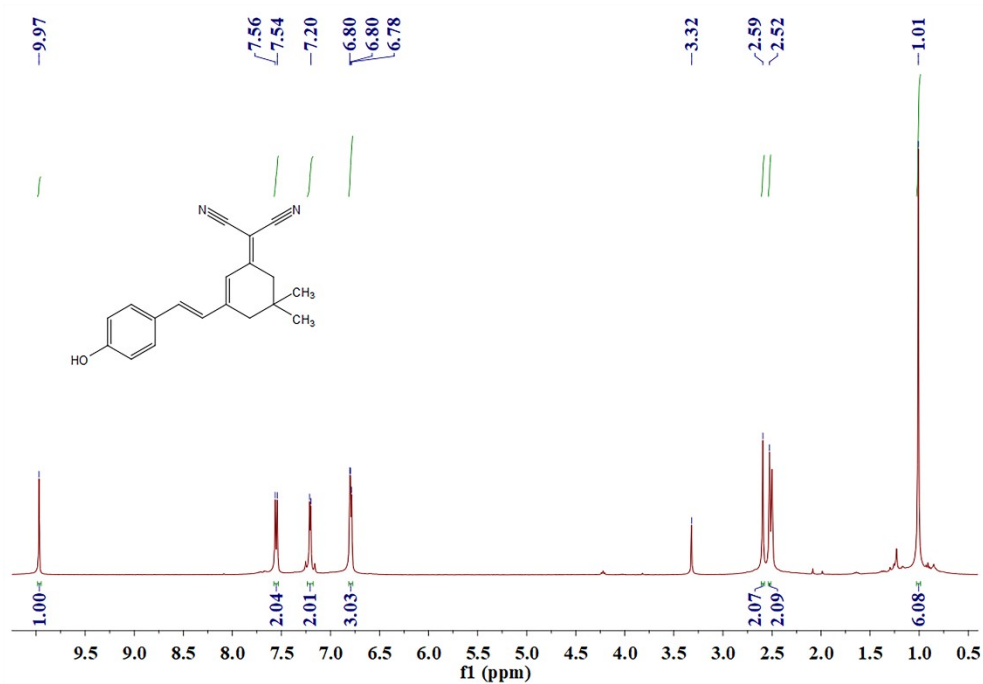


Fig. S4 <sup>1</sup>H NMR of TMN-OH in DMSO-*d*<sub>6</sub>

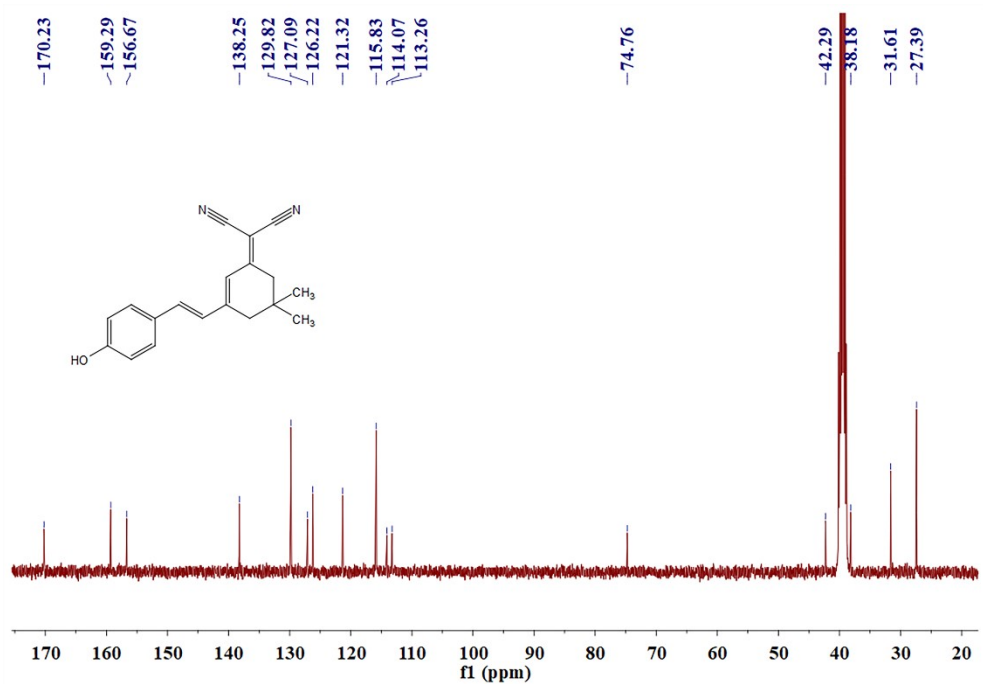


Fig. S5 <sup>13</sup>C NMR of TMN-OH in DMSO-*d*<sub>6</sub>

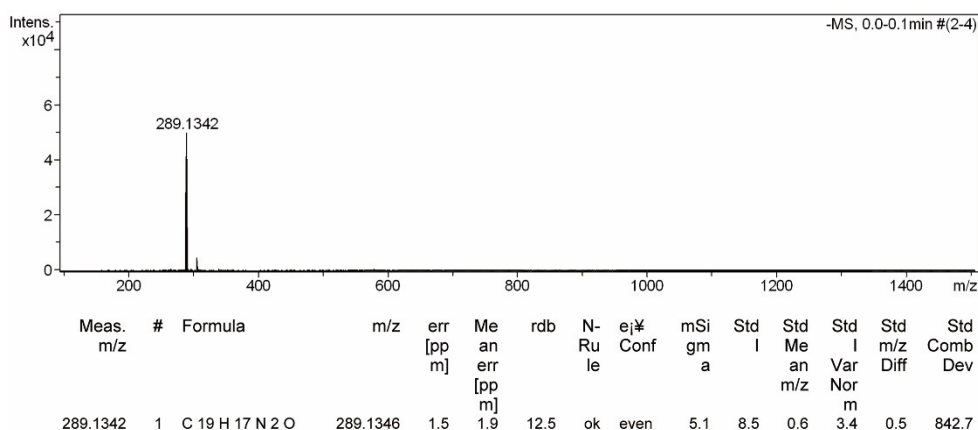


Fig. S6 ESI-HRMS [M-H] of TMN-OH

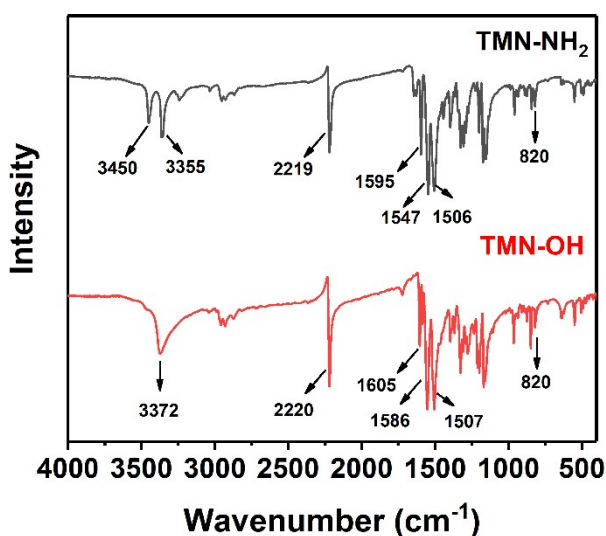
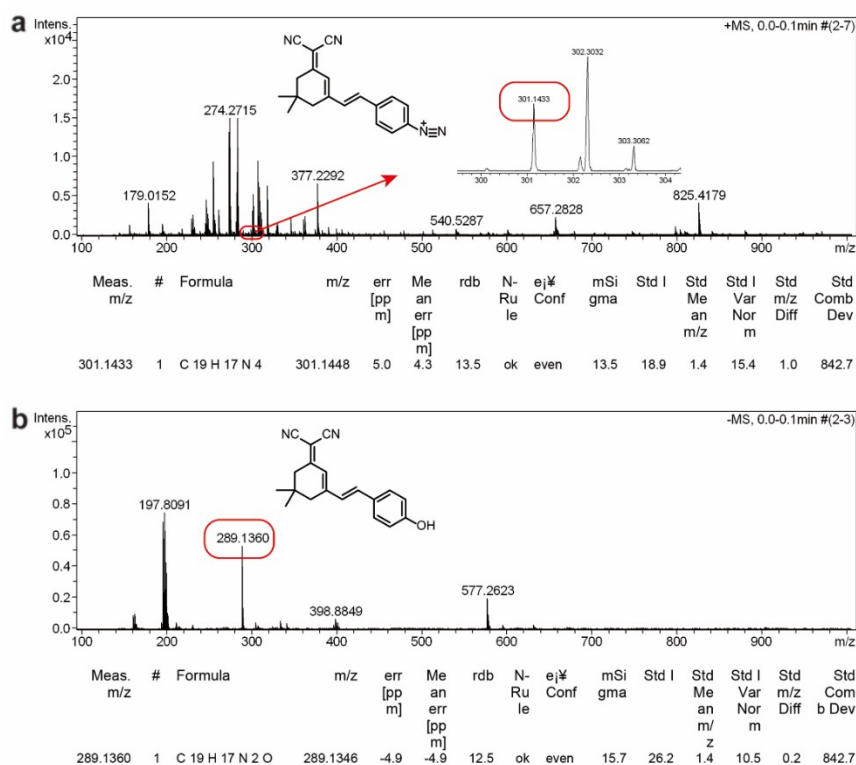


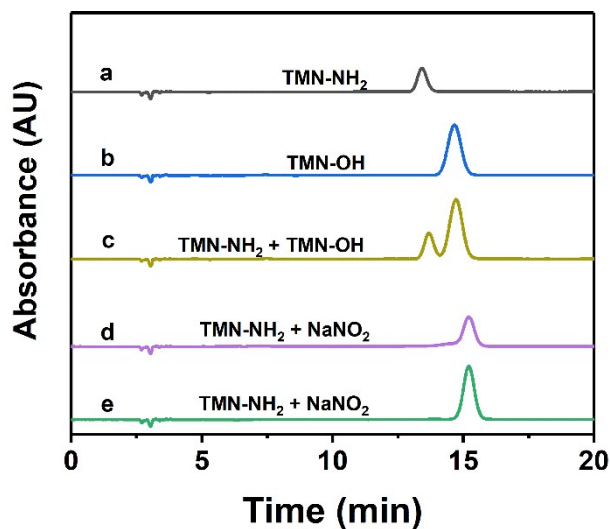
Fig. S7 FTIR spectra of TMN-NH<sub>2</sub> and TMN-OH

The infrared spectra were evaluated to verify the structures of TMN-NH<sub>2</sub> and TMN-OH in Fig. S7. In the infrared spectra of TMN-NH<sub>2</sub>, the peaks at 1506, 1547 and 1595 cm<sup>-1</sup> demonstrated a benzene structure. The band at 820 cm<sup>-1</sup> was caused by the out-of-plane bending vibrational mode of C-H, indicating that there was 1,4-disubstituted benzene in TMN-NH<sub>2</sub>. The absorption band at 2219 cm<sup>-1</sup> in TMN-NH<sub>2</sub> correspond to the stretching vibration of C≡N bands. The peaks at 3355 and 3450 cm<sup>-1</sup> were assigned to the characteristic peak of N-H bond of primary amines. The infrared spectrum of TMN-OH has similar characteristic peaks to that of TMN-NH<sub>2</sub> in the wavenumber range of 3000-400 cm<sup>-1</sup>, but there was a characteristic peak of the O-H stretching vibration with a broad absorption at 3372 cm<sup>-1</sup>. The above infrared data further validated the structure of TMN-NH<sub>2</sub> and TMN-OH.

## 4. Validation of the reaction mechanism



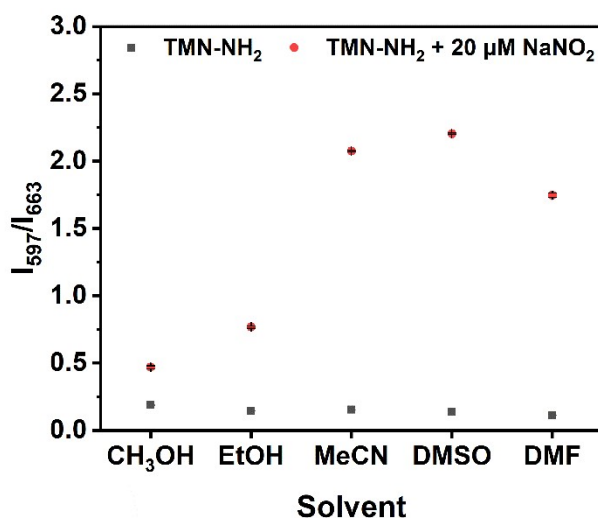
**Fig. S8** ESI-HRMS analysis of the reaction solution of probe **TMN-NH<sub>2</sub>** (10  $\mu$ M) with **NO<sub>2</sub><sup>-</sup>** (20  $\mu$ M) in positive ion mode (a) and in negative ion mode (b).



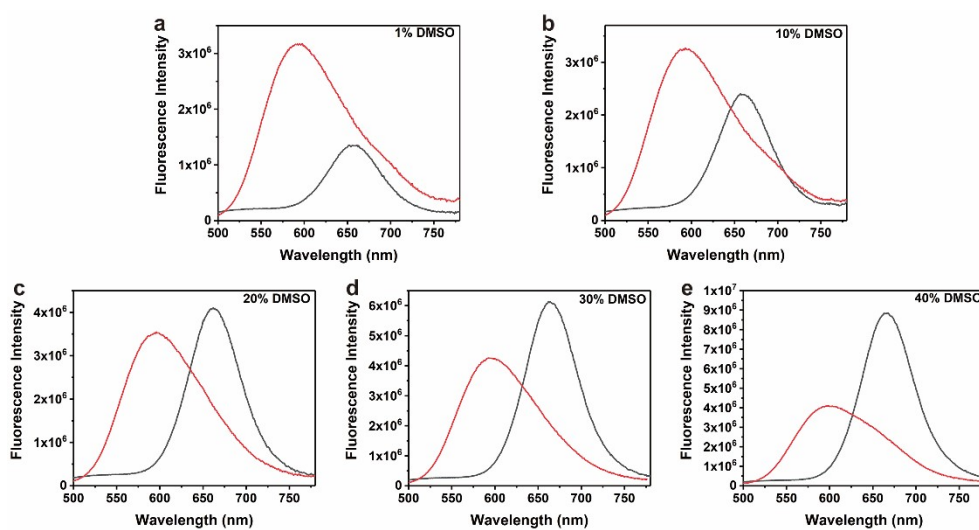
**Fig. S9** HPLC analysis chromatograms. (a) **TMN-NH<sub>2</sub>**; (b) **TMN-OH** (c) the mixture of **TMN-NH<sub>2</sub>** and **TMN-OH**; (d) and (e) were the reaction system of 40  $\mu$ M probe **TMN-NH<sub>2</sub>** with 80  $\mu$ M **NO<sub>2</sub><sup>-</sup>** for 5 min and 10 min, respectively; Chromatographic column: ECHWAY JADE-PAK C18 II (4.6 $\times$ 250 mm, 5  $\mu$ m); Mobile phase: CH<sub>3</sub>OH-H<sub>2</sub>O, 70:30 (v/v); Detection: UV-vis detector (400 nm); Flow rate: 1 mL/min; 20  $^{\circ}$ C; Injection volume: 20  $\mu$ L. Chromatographic retention time: **TMN-NH<sub>2</sub>** 13.6 min, **TMN-OH** 15.2 min.



## 5. Optimization of response conditions

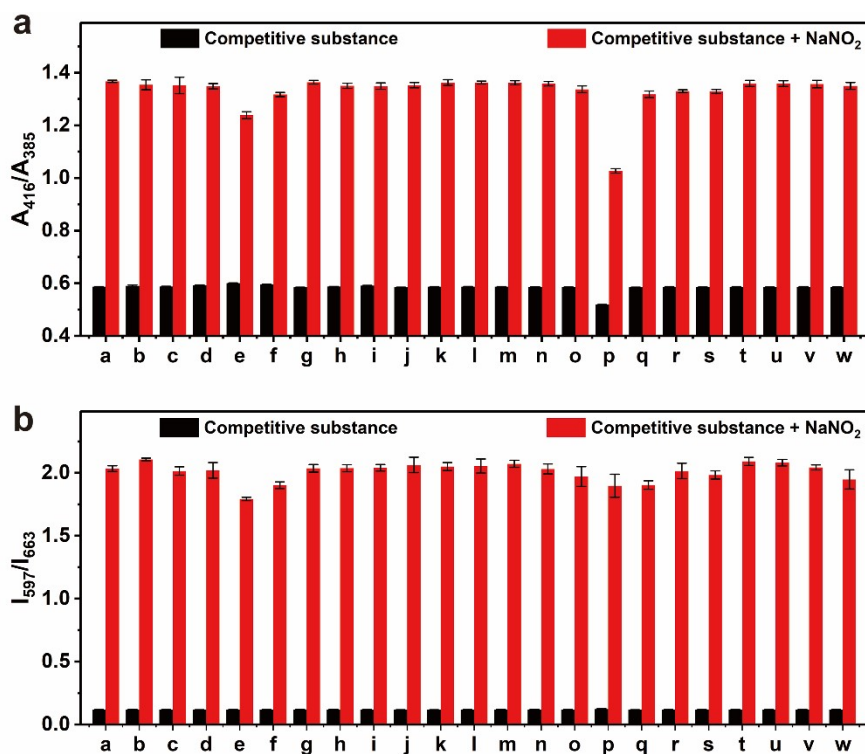


**Fig. S10** The effect of various organic solvent on the  $I_{597}/I_{663}$  of  $\text{TMN-NH}_2$  in the absence and presence of  $\text{NO}_2^-$  (20  $\mu\text{M}$ ). In organic solvent / $\text{H}_2\text{O}$  solution ( $v/v$ , 3:7, pH 1.0),  $\lambda_{\text{ex}} = 400$  nm.



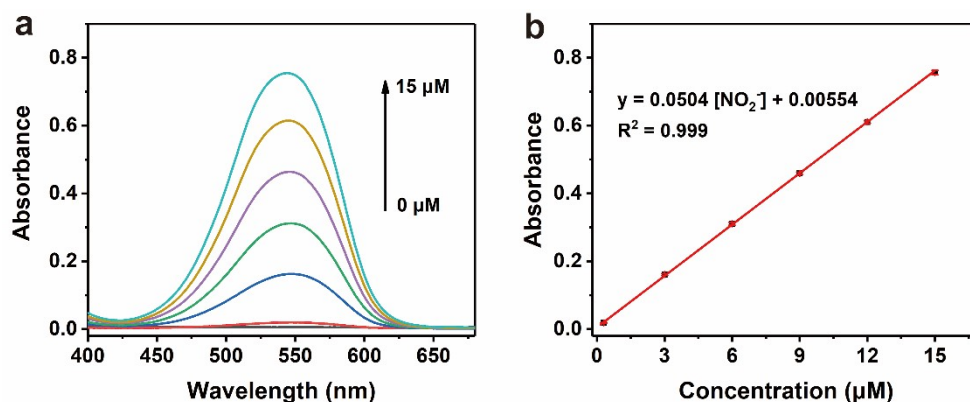
**Fig. S11** The influence of the proportion of DMSO on emission intensity. (a) 1% DMSO, (b) 10% DMSO, (c) 20% DMSO, (d) 30% DMSO, (e) 40% DMSO. Black line:  $\text{TMN-NH}_2$  (10  $\mu\text{M}$ ); red line:  $\text{TMN-NH}_2$  (10  $\mu\text{M}$ ) +  $\text{NO}_2^-$  (20  $\mu\text{M}$ ).  $\lambda_{\text{ex}} = 400$  nm.

## 6. Specificity of TMN-NH<sub>2</sub>



**Fig. S12** (a) Absorbance ratio ( $A_{416}/A_{385}$ ) and (b) fluorescence intensity ratio ( $I_{597}/I_{663}$ ) of TMN-NH<sub>2</sub> (10  $\mu$ M) with various interferents (100  $\mu$ M) in the absence and presence of NO<sub>2</sub><sup>-</sup> (10  $\mu$ M). Tested interferents: (a) blank, (b) KF, (c) CaCl<sub>2</sub>, (d) KBr, (e) KI, (f) MgSO<sub>4</sub>, (g) Na<sub>2</sub>SO<sub>3</sub>, (h) Na<sub>2</sub>S, (i) Na<sub>2</sub>HPO<sub>4</sub>, (j) KH<sub>2</sub>PO<sub>4</sub>, (k) K<sub>2</sub>CO<sub>3</sub>, (l) NaHCO<sub>3</sub>, (m) Zn(NO<sub>3</sub>)<sub>2</sub>, (n) CH<sub>3</sub>COONa, (o) Al(NO<sub>3</sub>)<sub>3</sub>, (p) FeCl<sub>3</sub>, (q) Cu(NO<sub>3</sub>)<sub>2</sub>, (r) Leu, (s) Phe, (t) Glu, (u) Lys, (v) Thr, (w) Val. In DMSO/H<sub>2</sub>O solution ( $v/v$ , 3:7, pH 1.0),  $\lambda_{ex}$  = 400 nm.

## 7. Detection of $\text{NO}_2^-$ in real samples



**Fig. S13** Absorption spectra (a) and the calibration plot (b) of the absorbance intensity versus the concentration of  $\text{NO}_2^-$  by the Griess assay.

Different volumes (0, 1.5, 15, 30, 45, 60 and 75  $\mu\text{L}$ ) of  $\text{NO}_2^-$  standard solution (1 mM) and 2 mL of water mixed with 2 mL p-aminobenzene sulfonic acid solution (4 mg  $\text{mL}^{-1}$ ) in 10 mL centrifuge tube respectively. After standing for 5 min, 1 mL naphthalene ethylenediamine hydrochloride (2 mg  $\text{mL}^{-1}$ ) solution were added and leave for 15 min. The absorption spectra were obtained in the range from 400 nm to 680 nm. The procedure for  $\text{NO}_2^-$  detection of real sample extracts (0.5 mL) is the same as above.

**Table S2** Determination of  $\text{NO}_2^-$  in water samples using Griess Assay and **TMN-NH<sub>2</sub>** by ultraviolet spectrophotometry

Sample	Griess Assay ( $\mu\text{M}$ )	This method ( $\mu\text{M}$ )	Add ( $\mu\text{M}$ )	Funded ( $\mu\text{M}$ )	Recovery (%)	RSD (n = 9, %)
natural drinking water			0.50	0.52	103.3	2.16
	Not detected	Not detected	0.75	0.74	98.1	2.37
			1.00	1.06	106.2	1.87
Tap water			0.50	0.49	97.6	2.86
	Not detected	Not detected	0.75	0.70	93.9	3.06
			1.00	0.96	96.3	2.32

### Reference:

[1] D. Shen, W. Jin, Y. Bai, Y. Huang, H. Lyu, L. Zeng, M. Wang, Y. Tang, W. Wan, X. Dong, Z.

Gao, H.L. Piao, X. Liu, Y. Liu, Rational Design of Crystallization-Induced-Emission Probes To Detect Amorphous Protein Aggregation in Live Cells, *Angew. Chem. Int. Ed.* 60 (2021) 16067-16076. <https://doi.org/10.1002/anie.202103674>.