# Supporting information for

## A Golgi-located fluorescent probe for the detection of hydrazine in biological and environmental systems

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*S1 Experimental section*

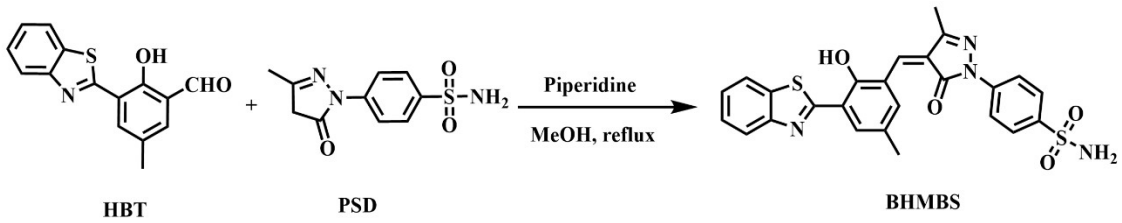
*S1.1. Materials and instrumentation*

Solvents and starting materials for syntheses were purchased commercially and used as received. Elemental analyses were carried out on an Elemental V ario EL analyzer. $^1$H NMR spectra are recorded on a Bruker AV400 NMR spectrometer in DMF-$d_6$ solution. The UV spectra were recorded on a Purkinje General TU-1800 spectrophotometer. Fluorescence spectra were determined on the Varian CARY eclipse spectrophotometer. ESI-MS spectra were obtained on a Bruker Daltonics Esquire 6000 mass spectrometer. The X-ray diffraction measurement for **BHMBS** was performed on a Bruker SMART APEX II CCD diffractometer equipped with a graphite monochromatized MoKα radiation (λ = 0.71073 Å) by using φ-ω scan mode. Semi-empirical absorption correction was applied to the intensity data using the SADABS program. The structures were solved by direct methods and refined by full matrix least-

square on $F^2$ using the SHELXTL-97 program. The cytotoxic effect exerted by **BHMBS** on cultured HepG2 cells was ascertained by a standard MTT assay according to the literature method. Fluorescent images were taken on Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope.

*S1.2. Synthesis of probe **BHMBS***

Compound **HBT** (0.2693 g, 1 mmol) and Compound **PSD** (0.2530 g, 1mmol) were mixed in methanol (15 mL) with five drops of piperidine as a catalyst. After the reaction, the red solid was precipitated and filtered to obtain probe **BHMBS**, which was further purified by recrystallization in DMF. Yield, 69 %. m.p.＞300 °C. $^1$H NMR (400 MHz, DMF-$d_6$ ) δ 13.67 (s, 1H), 9.18 (s, 1H), 8.31 (d, $J$ = 7.9 Hz,1H), 8.27 (d, $J$ = 3.7 Hz, 1H), 8.24 (s, 1H), 8.22 (s, 1H), 8.09 (s, 2H), 8.03 (s, 2H), 7.70 (t, $J$ = 7.3 Hz,1H), 7.61 (t, $J$ = 7.6 Hz,1H), 7.42 (s, 2H), 2.49 (s, 3H), 1.29 (s, 3H). $^{13}$C NMR was not detected due to the poor solubility of the probe. ESI-MS: m/z = 505.1098 for $[M+H]^+$,Calc: 505.0999. Crystal data for **BHMBS** ($C_{25}H_{20}N_4O_4S_2$): crystal size: 0.15 × 0.04 × 0.03 mm, Triclinic, space group P -1. a = 4.697 (8) Å, b = 11.160 (2) Å, c = 11.554 (2) Å, α =67.350 (5) °, β= 82.509 (4) °, γ= 87.050 (5) °, Z = 1, T = 296 K, θ =2.2-25.0°, 12080 reflections measured, 3606 unique (Rint = 0.044). Final residual for 325 parameters and 2953 reflections with I>2σ(I): $R_1$ = 0.043, $wR_2$ =0.111, and GOF = 1.062. (CCDC: 2426031).




**Scheme S1**. Synthetic route of the probe **BHMBS**.

*S1.3. Fluorescence spectra measurements*

A 10 μM stock solution was prepared by dissolving probe **BHMBS** in N, N-Dimethylformamide

(DMF). 50% hydrazine hydrate solution is used for the preparation of stock solution. The analytes were prepared in water. All the spectral analyses were performed in an almost aqueous solution (PBS buffer pH = 7.4, 10 mM, including 1% DMF) at room temperature. The test solutions for UV–vis and fluorescence measurements were prepared by putting 20 μL of probe **BHMBS** (1 mM) and appropriate aliquots of each analyte into test tube and then diluting to the scale mark (2 mL) with a solution of almost aqueous solution at room temperature.

*S1.4. Detection for water and soil samples*

The water samples were collected from the tap water, Xinyue Lake and in Henan Polytechnic University. These samples were firstly filtered. Then, $N_2H_4$ was added into 2 mL of pretreated water samples. Finally, 20 μL of probe stock solution (1 mM) was added into each sample, and the photograph were recorded by smartphone.

Soil samples were collected from different places (field soil and sandy soil,) in Henan Polytechnic University. In experimental groups, field and sandy soil samples (1 g) were pretreated with $N_2H_4$ (10 mM, 1 mL) for 20 min initially. Next, each sample was soaked in PBS buffer and left to stand for 30 min at indoor temperature, followed by filtering to obtained the soil extract. Lastly, probe **BHMBS** solution was spiked in samples and the fluorescent photographs and spectra were recorded.

*S1.5 paper-based test strips*

The filter paper was cut into 5 cm × 1 cm rectangles, which were firstly immersed in the probe's stock solution (1 mM) and naturally dried in air. The trace amount of hydrazine hydrate (10 μL ethanol containing different quantities of $N_2H_4$) was placed in a bottle (10 mL), then the probe-loaded strips were deposited on top of the bottles immediately.

*S1.6 Fluorescence imaging in bio-systems*

The HepG2 cells were incubated in the Dulbecco 's modified Eagle's medium supplemented with 10 % fetal bovine serum at 37 ℃ in a humidified incubator with 5% $CO_2$ for 24 h. In the imaging experiment, the cells were pre-incubated with **BHMBS** (10 μM) at 37 ℃ for 30 min, then treated with $N_2H_4$ for 30 min to image exogenous $N_2H_4$. To image endogenous $N_2H_4$, the cells were treated with INH (1 mM), subsequently incubated with the **BHMBS** (10 μM) for 30 min. For zebrafish, all the steps were conducted in agreement with the above procedures.

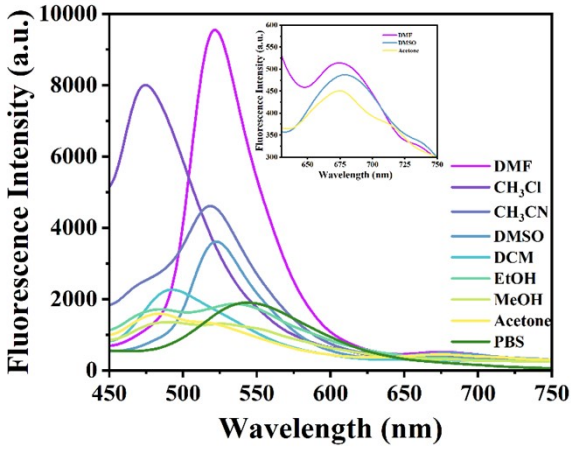



**Fig. S1** Fluorescence emission of probe **BHMBS** (10 μM) in various solvents with different polarity.

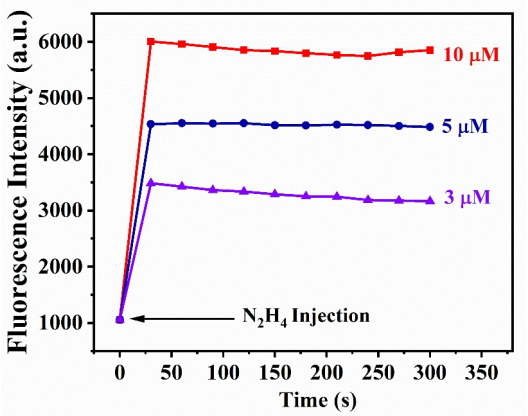



**Fig. S2** Time course for the fluorescence response of 10 μM **BHMBS** with the addition of various concentrations $N_2H_4$. $\lambda_{ex}$ = 430 nm.

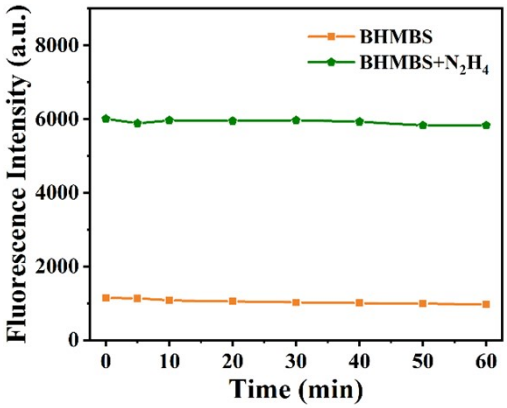



**Fig. S3** The fluorescence intensity of **BHMBS** (10 μM) with the addition of $N_2H_4$ (50 μM). $\lambda_{ex}$ = 430 nm.

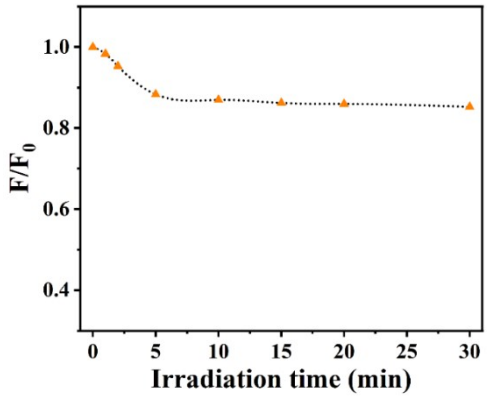



**Fig. S4** Effect of irradiation time under 365 nm UV light on the intensity ratio ($F/F_0$) of **BHMBS** solution with $N_2H_4$ (50 μM). $F_0$ was the original fluorescence intensity tested at 0 min and F was the fluorescence intensity tested at the exact irradiation time. $\lambda_{ex}$ = 430 nm.

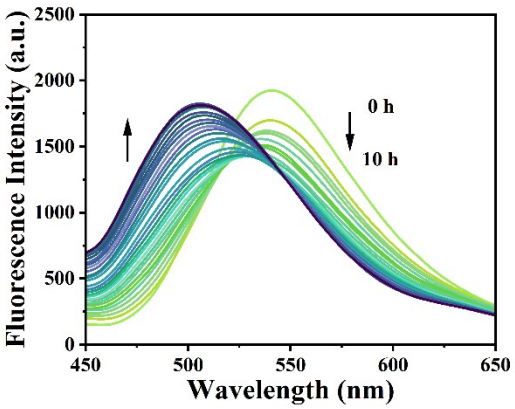



**Fig. S5** Kinetics behaviors of **BHMBS** (10 μM) hydroxylamine (50 μM) at different time. $\lambda_{ex}$ = 430 nm.

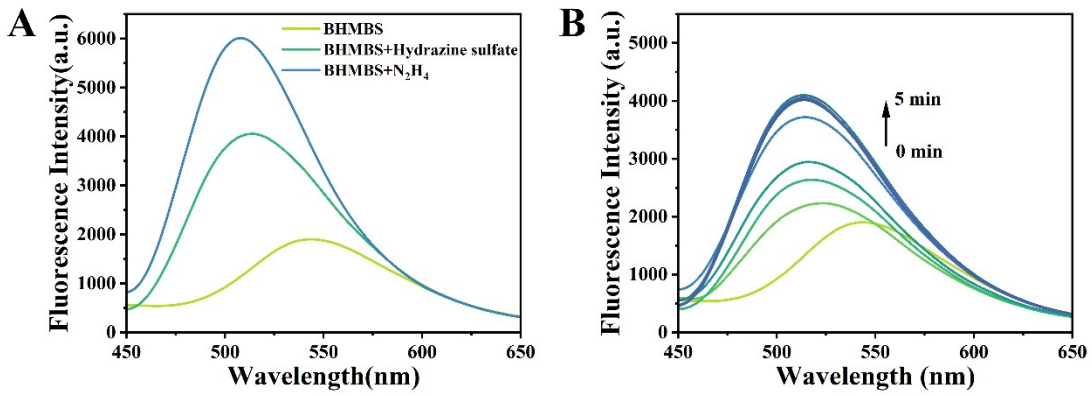



**Fig. S6** (A) Fluorescence emission of probe **BHMBS** (10 μM) with $N_2H_4$ (50 μM) and hydrazine sulfate (250 μM), repectively. (B) Kinetics behaviors of **BHMBS** (10 μM) with hydrazine sulfate (250 μM) at different time. $\lambda_{ex}$ = 430 nm.

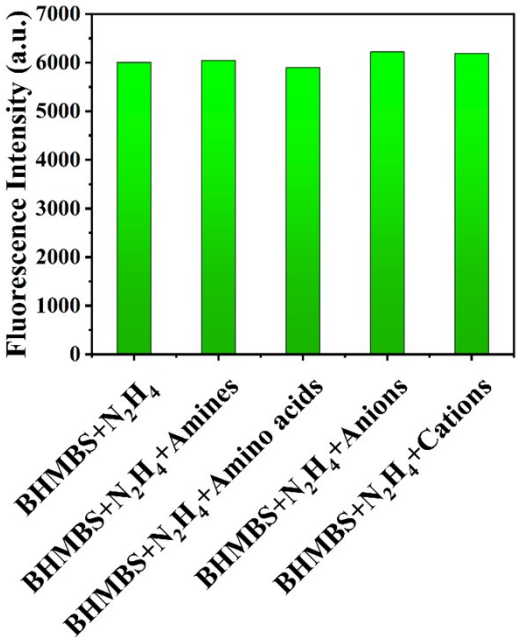



**Fig S7** Interference study results obtained as a result of $N_2H_4$ (50 μM) addition in the presence of a mix of amines (50 μM of methylamine, butylamine, dimethylamine, triethylamine, ethylenediamine, piperazine, pyridine, phenylhydrazine, hydroxylamine, hydrazine sulfate), amino acids (50 μM of INH, Hcy, GSH, Cys), anions(50 μM of $F^-$, $Cl^-$, $I^-$, $ClO^-$, $ClO_4^-$, $PO_4^{2-}$, $SO_4^{2-}$) and cations (50 μM of $Al^{3+}$, $Ca^{2+}$, $Fe^{3+}$, $Mg^{2+}$, $Na^+$, $Ni^+$, $K^+$) in BHMBS. $\lambda_{ex}$ = 430 nm.

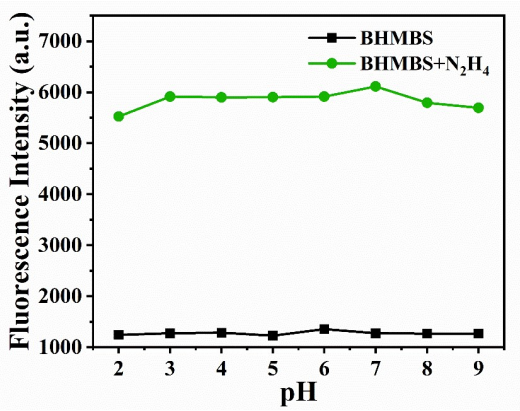



**Fig. S8** Effect of pH value on the fluorescence intensity of **BHMBS** (10 μM) solution in the absence and presence of 10 μM $H_2H_4$. $\lambda_{ex}$ = 430 nm.

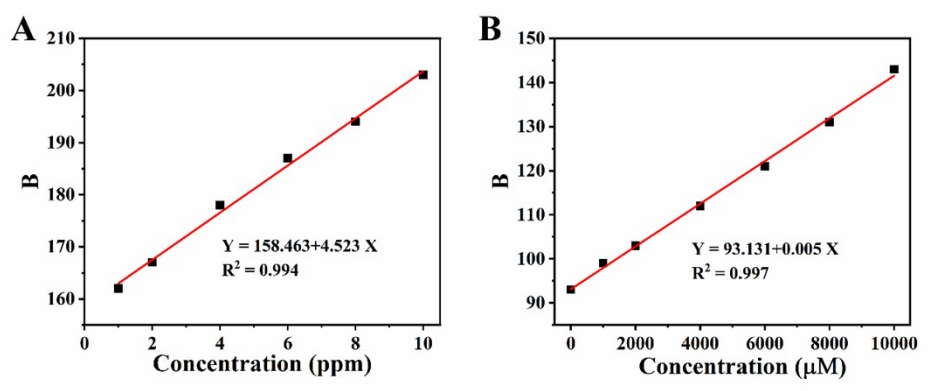



**Fig. S9** Linear plot of B value with $N_2H_4$ vapor (A) and solution (B).

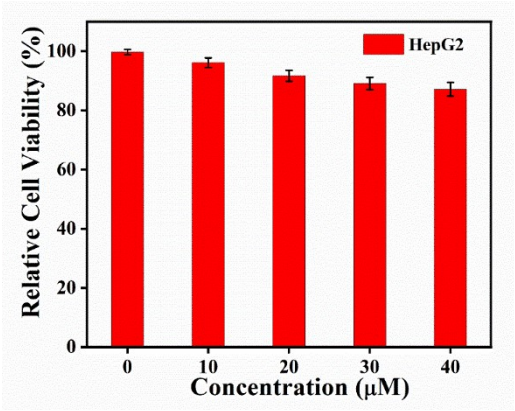



**Fig. S10** The viability assay of cell with different concentrations of probe **BHMBS** (0–40 μM) on HepG2 cells using the MTT assay for 24 h. All samples were done in triplicate.

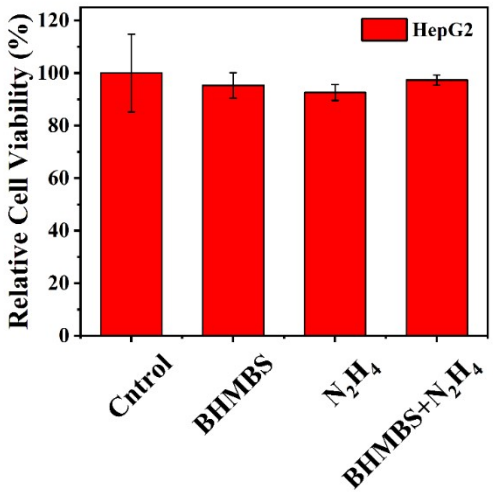



**Fig. S11** The viability assay of cell with **BHMBS** (10 μM), $N_2H_4$ (50 μM) and **BHMBS** (10 μM) + $N_2H_4$ (50 μM) respectively on HepG2 cells using the MTT assay for 24 h. All samples were done in triplicate.

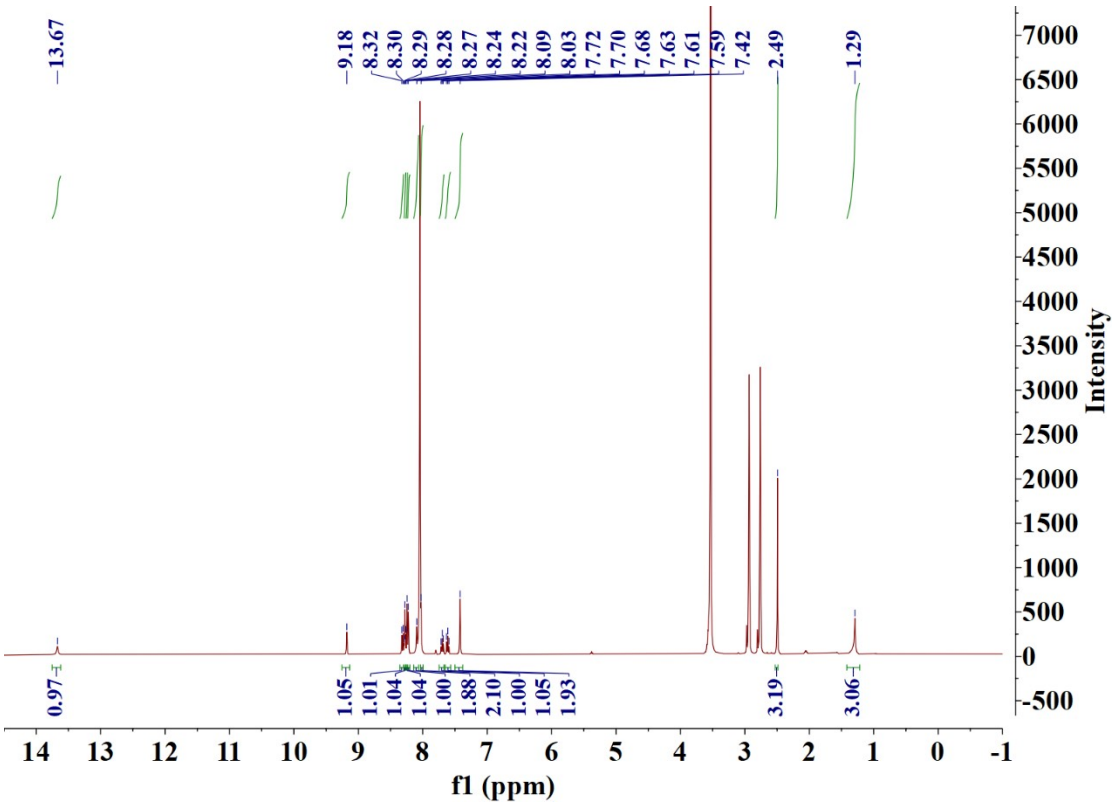



**Fig. S12** $^{1}$H NMR spectrum of **BHMBS** in DMF-$d_6$.