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# A reversible fluorescent probe based on C=N isomerization for the selective detection of formaldehyde in living cells and in vivo

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# 1. General Methods

**Materials and apparatus:**

The solution of the probe BOD-$NH_2$ (1 mM) could be dissolved in dimethyl sulfoxide (DMSO) and maintained in refrigerator at 4 °C. The purity of probe was tested on a Shimadzu LC-20AT HPLC system equipped with fluorescence and UV-vis absorption detectors. When it was used for imaging, the purity of our probe was greater than 99.89%. Stock solutions of FA, acetaldehyde, methylglyoxal, glyoxal; benzaldehyde, pyridoxal, 4-nitro-benzaldehyde, sodium pyruvate, alanine, glycine, serine, arginine, cysteine, glutathione, glucose, hydrogen peroxide, hydrogen sulfide, methane acid, dehydroascorbate were diluted to desired concentrations when needed. All the reagents were obtained from Aladdin (USA). All other chemicals were from commercial sources and of analytical reagent grade, unless indicated otherwise. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich. The following rabbit antibodies were purchased from Cell Signaling Technology (β-actin: #4970, 1:1000; LSD1: #2139, 1:1000). Ultrapure water was used throughout.

Absorption spectra were obtained on Lambda 35 UV-visible spectrophotometer (PerkinElmer). Fluorescence spectra were measured by FluoroMax-4 Spectrofluorometer with a Xenon lamp and 1.0-cm quartz cells. $^{1}H$ and $^{13}C$ NMR spectra were taken on a Bruker spectrometer. High-resolution mass spectra were carried on LCQ Fleet LC-MS System (Thermo Fisher Scientific). The fluorescence images of cells were acquired using a LTE confocal laser scanning microscope (Olympus FV1000 confocal laser-scanning microscope) with an objective lens (× 40). Flow cytometry data were collected by BD Biosciences FACSAria. Hippocampus slices were obtained from LeicaVT1200S. BALB/c mice and x-ray images were collected by Bruker In-vivo Imaging System.

**Absorption and fluorescence analysis**

Absorption spectra were obtained with 1.0-cm glass cells. Fluorescence emission spectra were obtained with a Xenon lamp and 1.0-cm quartz cells. The fluorescence intensities were measured at $\lambda_{ex/em}$ = 495/515 nm. BOD-$NH_2$ (0.10 mL, 1.0 mM) was added to a 10.0-mL color comparison tube. After dilution to 10 μM with 10 mM HEPES buffers, analytes were added. The mixtures were equilibrated 2 h before measurement.

**Cell culture**

Human hepatocellular carcinoma cell line (SMMC-7721), human embryonic kidney cell line (HEK), and human lung carcinoma cell line (PC9), human epitheloid cervix carcinoma cell line (HeLa), human hepatocellular carcinoma cell line (HepG2), human neuroblastoma cell line (SH-SY5Y) and human lung carcinoma cell line (A549) were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). All the cells were incubated in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10 % fetal bovine serum (FBS) or RPMI (Roswell Park Memorial Institute) 1640 Medium supplemented with 10 % FBS at 37 °C under a humidified atmosphere containing 5 % $CO_2$.

**2. Reversibly spectral profiles of BOD-$NH_2$ towards FA**

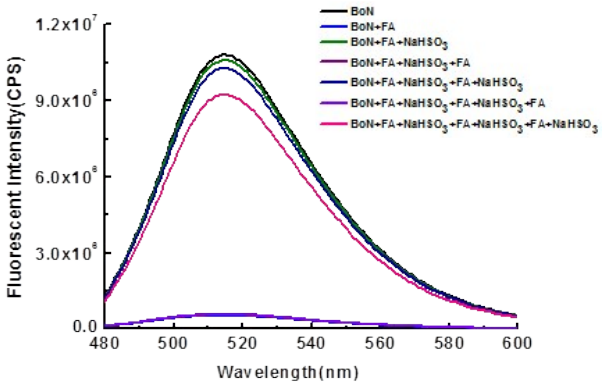


Fig. S1 Reversibly fluorescent response of BOD-$NH_2$ (5 μM) after the sequential treatment of FA (500 μM, 2 h), $NaHSO_3$ (500 μM, 10 min). Spectra were recorded in PBS (10 mM, pH 7.4) at 37 °C. $\lambda_{ex}$ = 495 nm, $\lambda_{em}$ = 515 nm..

## 3. pH Effects on probe

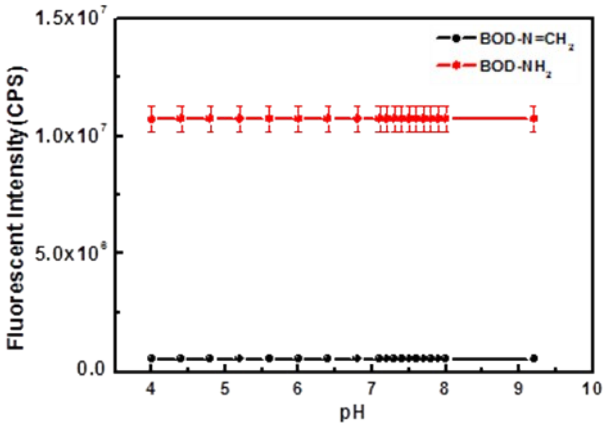


Fig. S2 Fluorescence emission changes at 515 nm with the pH titration curve of BOD-$NH_2$ (5 μM) and BOD-N=$CH_2$ (5 μM). pH: 4.0, 4.4, 4.8, 5.2, 5.6, 6, 6.4, 6.8, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 9.2 (10 mM HEPES buffer solution). The data were shown as mean (± s.d.) ($n$ = 7).

## 4. MTT Assay for BOD-$NH_2$

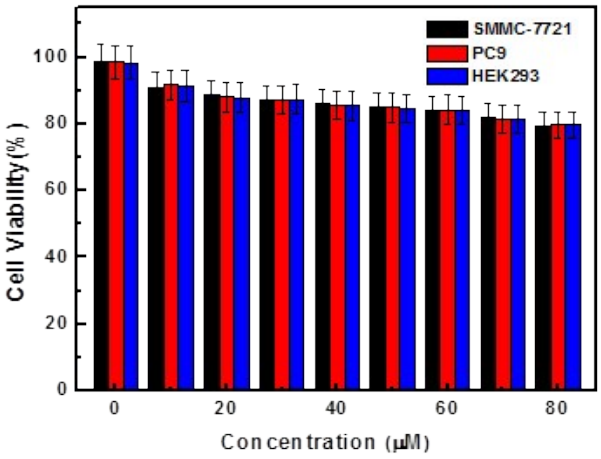


Fig. S3 Cell viabilities of BOD-$NH_2$ by using SMMC-7721 cells, HEK293 cells and PC9 cells. The concentrations of probe were 0 - 80 μM. The data were shown as mean (± s.d.) ($n$ = 7).

## 5. Bright-field image of Fig. 5

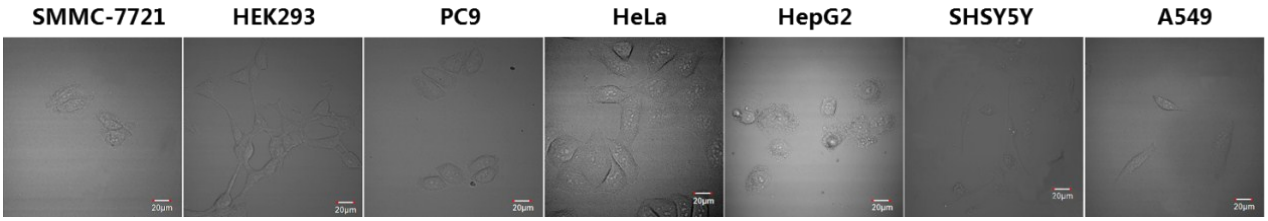


Fig. S4 Bright-field image of Fig. 5.

## 6. Bright-field image of Fig. 6

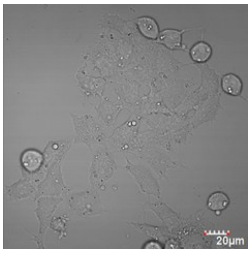


Fig. S5 Bright-field image of Fig. 6.

## 7. Selectivity towards various anions and cations

We further examed the selectivity of BOD-$NH_2$ towards various anions and cations. As shown in Fig.S6 the fluorescence intensity of BOD-$NH_2$ was also hardly affected by various ions and anions, such as, $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Fe^{3+}$, $Cu^{2+}$, $Cl^-$, $Br^-$, $SO_4^{2-}$, $CO_3^{2-}$, $H_2PO_4^-$ and $NO_3^-$. Therefore, the probe was highly selective for FA over other anions and cations.

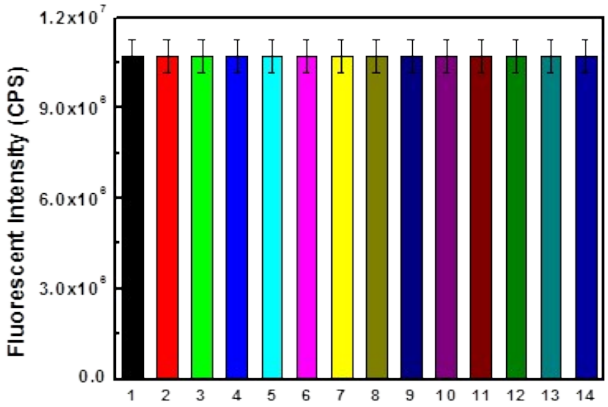


Fig.S6 Fluorescence responses of BOD-$NH_2$ (5 μM) to anions and cations. (1) blank; (2) 1 mM $K^+$; (3) 1 mM $Na^+$; (4) 1 mM $Ca^{2+}$; (5) 1 mM $Mg^{2+}$; (6) 1 mM $Zn^{2+}$; (7) 1 mM $Fe^{3+}$; (8) 1 mM $Cu^{2+}$; (9) 1 mM $Cl^-$; (10) 1 mM $Br^-$; (11) 1 mM $SO_4^{2-}$; (12) 1 mM $CO_3^{2-}$; (13) 1 mM $H_2PO_4^-$; (14) 1 mM $NO_3^-$. All the above data were recorded in 10 mM HEPES buffer (10 mM, pH 7.4) at 37 °C for 2 h. $\lambda_{ex}$ = 495 nm, $\lambda_{em}$ = 515 nm. The data were shown as mean (± s.d.) ($n$ = 7).

## 8. The response of BOD-$NH_2$ toward FA in different pHs

The response of BOD-$NH_2$ toward FA in different pH surroundings was investigated. As shown in Fig.S7, the fluorescent intensity in 515 nm didn't change with the pH variation. The results indicated that the response of BOD-$NH_2$ toward FA could not be affected by pH in 4.0 - 9.0.

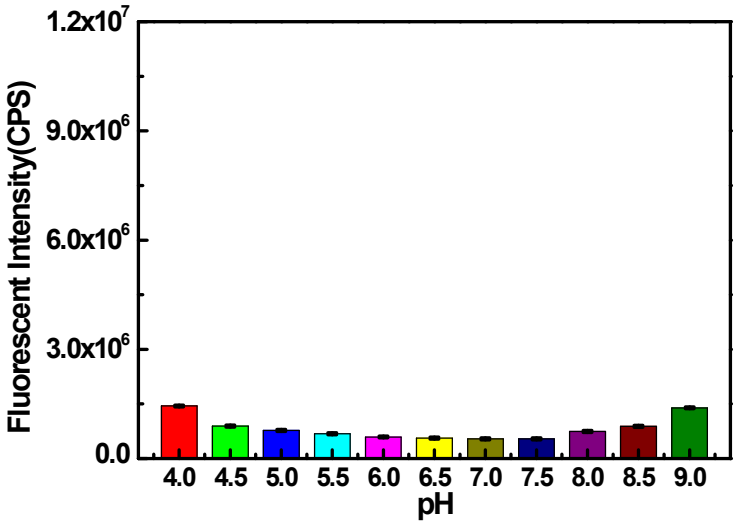


Fig.S7 The response of BOD-$NH_2$ toward FA in different pH (4.0 - 9.0). Fluorescence emission changes at 515 nm with the pH titration curve of BOD-$NH_2$ (5 μM) towards FA (500 μM). pH: 4.0, 4.5, 5.0, 5.6, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 (10 mM HEPES buffer solution). The data were shown as mean (± s.d.) ($n$ = 7).

## 9. Viscosity-dependent fluorescence response

The fluorescence response of BOD-N=$CH_2$ towards viscosity in ethylene glycol and glycerol mixtures with different glycerol fraction ($f_{Gly}$) was tested.

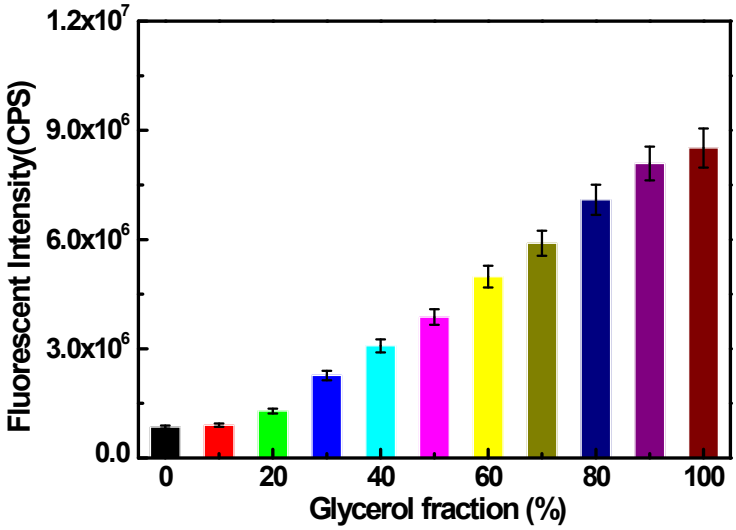


Fig.S8 Viscosity-dependent fluorescence response of BOD-N=$CH_2$ in the mixtures of ethylene glycol and glycerol with different glycerol fraction ($f_{Gly}$). The data were recorded in 10 mM HEPES buffer (10 mM, pH 7.4) at 37 $^{o}$C for 2 h. $\lambda_{ex}$ = 495 nm, $\lambda_{em}$ = 515 nm. The data were shown as mean (± s.d.) ($n$ = 7).

**10. H NMR and C NMR for the probe**