Supporting Information for

A ratiometric fluorescent formaldehyde probe for bioimaging application

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Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the fluorescence quantum yield. Fluorescence quantum yield ($\Phi_f$) was determined by using quinine sulfate ($\Phi_f = 0.58$, in 0.1 M H$_2$SO$_4$ aqueous solution) as the fluorescence standard. The quantum yield was calculated using the following equation.

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X/A_X F_S) (n_X/n_S)^2$$

Where $\Phi_F$ is the fluorescence quantum yield, $A$ is the absorbance at the excitation wavelength, $F$ is the area under the corrected emission curve, and $n$ is the refractive index of the solvent used. Subscripts $S$ and $X$ refer to the standard and to the unknown, respectively.

Determination of the detection limit. The linear relationship between the fluorescence intensity ratio ($I_{451}/I_{359}$) and the concentration of FA was fitted based on the fluorescence titration. The detection limit was calculated using the following equation based on the fluorescence titration.

$$\text{Detection limit} = 3\sigma/k$$

Where $\sigma$ is the standard deviation of the blank sample and $k$ is the slope of the linear regression equation.
**HeLa cells culture.** HeLa cells were cultured in DMEM (Dulbecco’s modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO₂ and 95% air at 37 °C.

**Imaging of FA in living cells.** HeLa cells were incubated with 10.0 μM RFFP for 30 minutes in an atmosphere of 5% CO₂ and 95% air, and then treated with 1 mM or 3 mM FA for 2 hours. Subsequently, the cells were imaged using Nikon A1MP confocal microscope with an excitation of mercury lamp and emission collection of blue channel and green channel.

**Synthesis of compound RFFP.**

![Chemical structure of RFFP](image)

The mixture of the starting compound 6-hydroxy-2-naphthaldehyde (86 mg, 0.5 mmol) and 25% ammonia (378 μL, 5 mmol) in 5 mL of methanol was stirred at 0 °C for 30 min. After warming to room temperature, allylboronic acid pinacol ester (101 mg, 0.6 mmol) was added and continued to stir overnight. The solvent was removed under reduced pressure and the solid residue was purified by flash chromatography column using ethanol/dichloromethane (v/v 1:10) to afford a white solid as compound RFFP (70 mg, yield 66 %). ¹H NMR (400 MHz, CDCl₃), δ (ppm): 2.47-2.63 (m, 2H), 4.10-4.13 (t, J = 6.8 Hz, 1H), 5.09-5.18 (m, 2H), 5.69-5.79 (m, 1H), 6.94-6.95 (1H), 6.98-7.01 (dd, J = 8.8, 2.4 Hz, 1H), 7.31-7.34 (dd, J = 8.8, 1.6 Hz, 1H), 7.43-7.45 (d, J = 6.8 Hz, 1H), 7.54-7.56 (d, J = 8.8 Hz, 1H), 7.60 (s, 1H); ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 43.34, 55.31, 109.46, 118.27, 118.75, 124.96, 126.78, 128.32, 129.39, 134.06, 13.96, 138.78, 15.39. HRMS (EI) m/z calcd for C₁₄H₁₄N,O₁ (M⁺): 213.1017. Found 213.1064.
Fig. S1 Absorption spectra of 10 μM RFFP with 300 eq. of FA in 25 mM PBS buffer (pH 7.4, containing 1 % acetone) within 240 min.

Fig. S2 ESI-MS spectrum of compound RFFP in the presence of excessive FA in pH 7.4 PBS buffer.
**Fig. S3** DFT optimized structures of (a) RFFP and (b) naphthaldehyde. The numbers represent corresponding atom charge and the carbon atom conjugated at 2-position was highlighted in bright blue.

**Fig. S4** Time-dependent (0 to 240 min) fluorescence intensity ratio ($I_{451}/I_{359}$) responses of sensor RFFP (10 μM) to FA (3 mM) in 25 mM PBS buffer (pH 7.4, containing 1 % acetone).
Fig. S5 The pH influence on the fluorescence intensity ratio ($I_{451}/I_{359}$) of RFFP (10 μM) in the absence (■) or presence (★) of FA (3 mM).
Fig. S6 $^1$H NMR spectrum of compound RFFP.

![Fig. S6 $^1$H NMR spectrum of compound RFFP.](image)

Fig. S7 $^{13}$C NMR spectrum of compound RFFP.

Reference
