Supporting Information

Two-photon fluorescent probe for detecting lipid droplets viscosity in living cells and zebra fishes

Min Peng, Junling Yin, and Weiying Lin*

Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, P.R. China.

Email: weiyinglin2013@163.com

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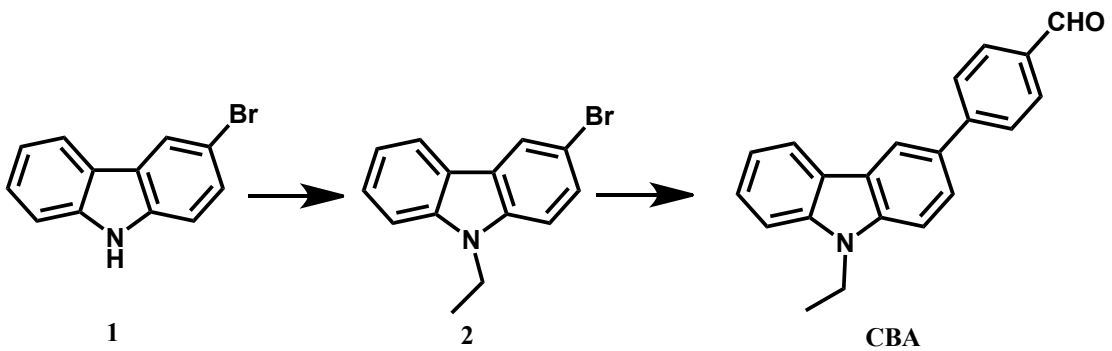
Apparatus and reagents

Unless otherwise stated, all solvents and reagents were commercially available and used without further purification. Doubly distilled water was used throughout all experiments. The absorption spectra were recorded in 1 cm cells with a Shimazu UV-2700 spectrophotometer (Suzhou, China). The emission spectra were measured on Hitachi F4600 fluorescent spectrophotometer. High resolution electrospray mass (HRMS) was recorded on Apex-Ultra Bruker instrument. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. The fluorescence imaging of cells was performed using a Nikon A1MP confocal microscope. The viscosity measurement was carried out on a NDJ-8 rotational viscometer. All reagents were analytical in experiment and used deionized water.

Synthesis of the probe CBA



1 2 CBA

Scheme. S1 Synthetic pathway for the probe **CBA**.

Synthesis of compound 2

Firstly, 3-Bromo-9H-carbazole(compound **1**; 2.46g, 10 mmol) and NaOH(0.8 g, 10 mmol) were dissolved in 15 mL mixtures (V_{THF}:V_{H2O}=1:3) and stired at room temperature for 1.5 h; then bromoethane (6.54 g, 60 mmol) were added and then the reaction mixture was heated at 55°C for 12 hours. The resulting was purified by silica gel column chromatography (petroleum ether/ethyl acetate,30:1)to afford compound 2 (white product ;70%).

Synthesis of CBA

A mixture of compound 2 (274.1 mg, 1 mmol), 4-Formylphenylboronic acid (180 mg, 1.2 mmol), K_2CO_3 (447 mg,0.03 mmol) and Tetrakis(tripheylphosphine)platinum in THF (10 ml) was heated to 55℃ for 12 h and the reaction was flushed with nitrogen. After the reaction, the solvent was removed under reduced vacuum. The resulting was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 30:1) to afford yellow product (74%). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.05 (s, 1H), 8.65 (d, J = 1.6 Hz, 1H), 8.28 (d, J = 7.6 Hz, 1H), 8.03 (q, J = 8.8 Hz, 4H), 7.90 (dd, J_1=8.4 Hz, J_2= 2, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.49 (m, 1H), 7.24 (t, J = 7.4 Hz, 1H), 4.48 (q, J = 7.0 Hz, 2H), 1.33 (t, J = 7.0 Hz, 3H)； ^{13}C NMR (101 MHz, DMSO-d6) δ 192.34, 146.71, 139.85, 139.52, 134.00 , 129.96, 129.18, 126.79, 125.91, 124.73, 122.72, 122.14, 120.50, 118.97,

118.88, 109.47, 109.14, 36.88, 13.49. HRMS (ESI): calcd. for $C_{21}H_{17}NO$, $[M+H]^+$, m/z, 299.1305, found: 300.1325

Calculation of fluorescence quantum yield of CBA

The fluorescence quantum yields (Φ_f) were determined by using Quinine sulfate as the reference according to the literature method. Quantum yields were corrected as follows:

$$\phi_f = \phi_r \left(\frac{A_r \eta_s^{\,2} D_s}{A_s \eta_s^{\,2} D_r} \right)$$

Where the s and r indices designate the sample and reference samples respectively. A is the absorbance at λ_{ex}, η is the average refractive index of the appropriate solution, and D is the integrated area under the corrected emission spectrum.

Preparation of solutions of probe CBA

The stock solution of **CBA** was dissolved in DMSO in 1 mM, 10 μM of the probes were used in photophysical experiments by addition of 30 μL of the stock solution to a 3.0 mL test solution

Spectral test

The solvents were obtained by mixing a methanol-glycerol system in different proportions. Measurements were carried out with a NDJ-8 rotational viscometer, and each viscosity value was recorded. The solutions of **CBA** of different viscosity were prepared by adding the stock solution (1.0 mM) 30 μL to 3 ml of solvent mixture (methanol-glycerol

systems) to obtain the final concentration of the dye (10.0 μM). These solutions were sonicated for 5 minutes to eliminate air bubbles, the solutions were measured in a UV spectrophotometer and a fluorescence spectrophotometer.

Cytotoxicity assay

The cytotoxicity of the probe **CBA** to Hela cells were studied by standard MTT assays. 2×10^4 cells/mL cells were seeded in 96-well plates and then incubated with various concentrations of the probe (0, 1, 2, 5, 10, 15, 20and 50μM) for 24 h. After that, 10 μL MTT (5 mg/mL) was added to each well and incubated for another 4 h. Finally, the media was discharged, and 100 μL of DMSO was loaded to dissolve the formazan crystals. The plate was shaken for about 10 min, and each well was analyzed by the microplate reader and detected at the absorbance of 490 nm. The cell viability (%) = $(OD_{sample}-OD_{blank}) / (OD_{control}- OD_{blank}) \times 100\ \%$）.

Cell culture and fluorescence imaging

HeLa cells were cultured in 90% Dulbecco's Modified Eagle Medium (DMEM, Gibico) supplemented with 10%FBS (Hyclone) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) in an atmosphere of 37 °C and 5% CO_2 The Monensin or Nystatin was first added into the Hela cells in a glass bottom culture dishes (Nest)for 30 min, then they were washed by PBS for three times, after that, the

probe(1mM,10μL) was added into the Hela cells treated with Monensin or nystatin for another 30 min. After washed with PBS for three times, the fluorescence imaging were carried out by a Nikon A1MP inverted fluorescence confocal microscope. The fluorescence emission of the probe was collected at FITC channel (500nm-550nm), the excitation wavelength was488 nm. Monensin and nystatin could induce structural changes or swelling, leading to viscosity changes in the lipid droplets.

Fluorescence imaging in living zebra fishes

Wild type zebra fish were kindly gifted from Shandong Academy of Sciences, and were kept at 28 °C and optimal breeding conditions. For the fluorescence imaging experiments, 3-day-old zebra fishes were transferred into a 30 mm glass culture dishes using a disposable sterilized dropper. The 10 μM Monensin or nystatin was added for incubated for 30 min, followed by washing away gently. Then probe **CBA** (1mM,10μL)were put into dishes respectively for another 30 min. After that, the zebra fishes were transferred into new glass bottom dishes for imaging. Prior to the imaging, we adopted 1% agarose gel for immobilization of zebra fishes, and put zebra fishes onto agarose with a little media to ready imaging. Fluorescence images were acquired with Nikon A1Rconfocal microscope with a 4 × objective lens. The OP fluorescence emission was collected at FITC channel (500 nm-550 nm) upon excitation at 488 nm, the TP fluorescence at 730 nm.

Table S1 Test viscosity in the varied of the methyl alcohol/glycerol (v/v) mixtures and fluorescence quantum yield (Φ_f) of **CBA**

Methanol/glycerol (v: v)	η/cp	Φ_f/%
10:0	1.7	Almost zero
9:1	2.0	0.6
8:2	2.4	1.6
7:3	3.2	2.4
6:4	9.8	3.8
5:5	16.1	5.0
4:6	35	8.6
3:7	81	9.6
2:8	242	14.3
1:9	351	17.6
0:10	656	21.3

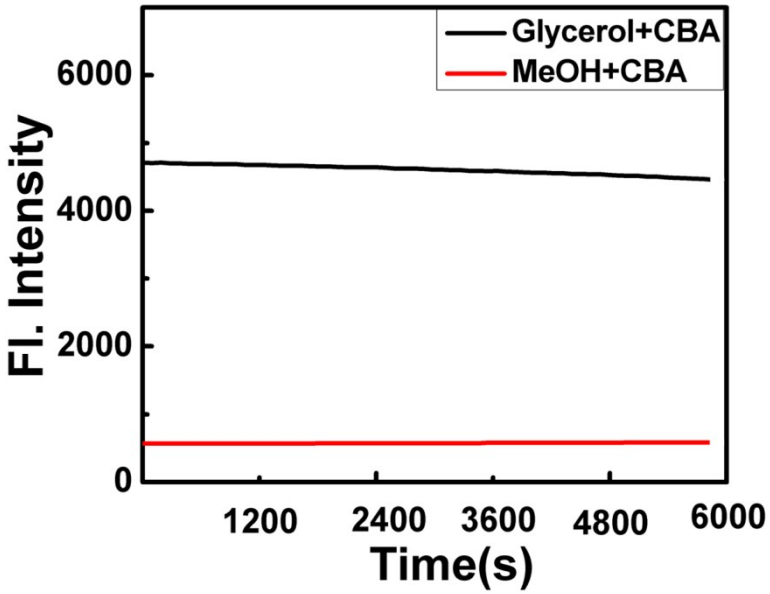


Fig. S1 The photostability of the probe **CBA** in glycerol and methanol with the continuous irradiation by laser light for 90 min.

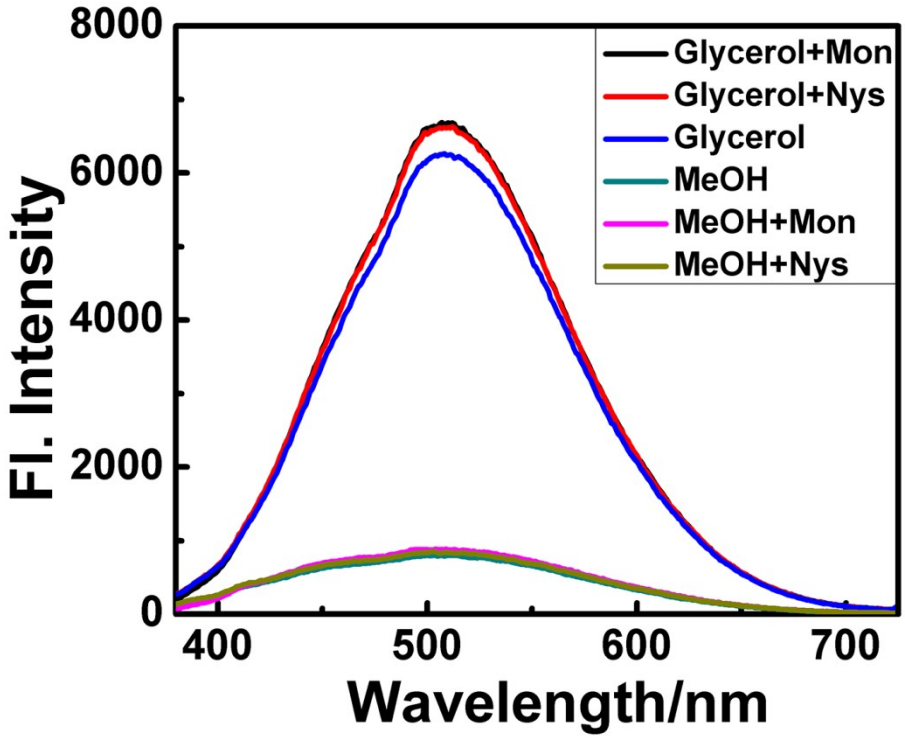


Fig. S2 The fluorescence emission of the probe **CBA** in glycerol; glycerol+Mon; glycerol+Nys; methanol; methanol+Mon; methanol+Nys.

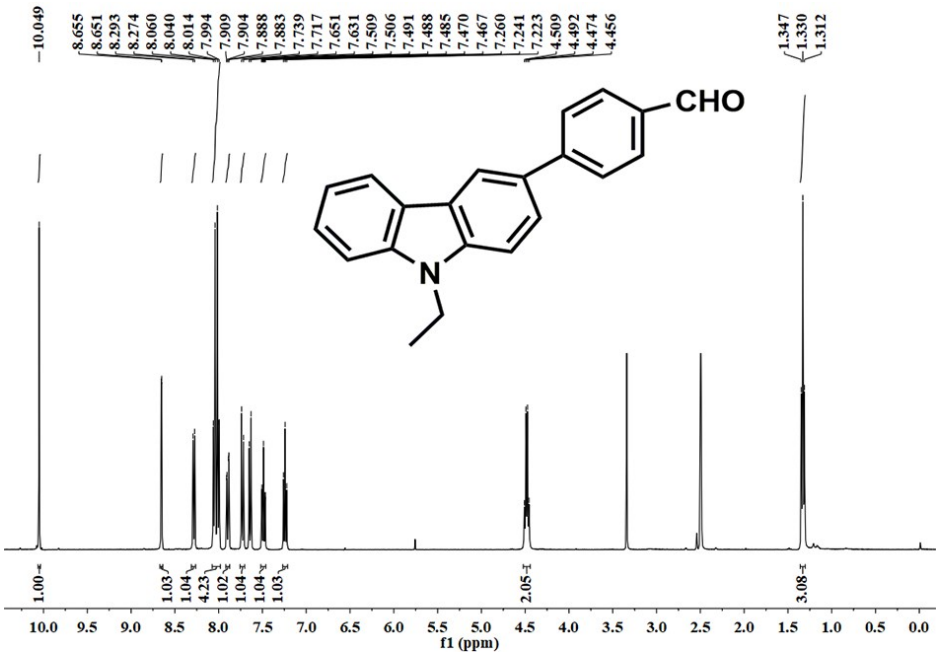


Fig. S3 ^{1}H NMR spectrum of **CBA** in DMSO-d6.

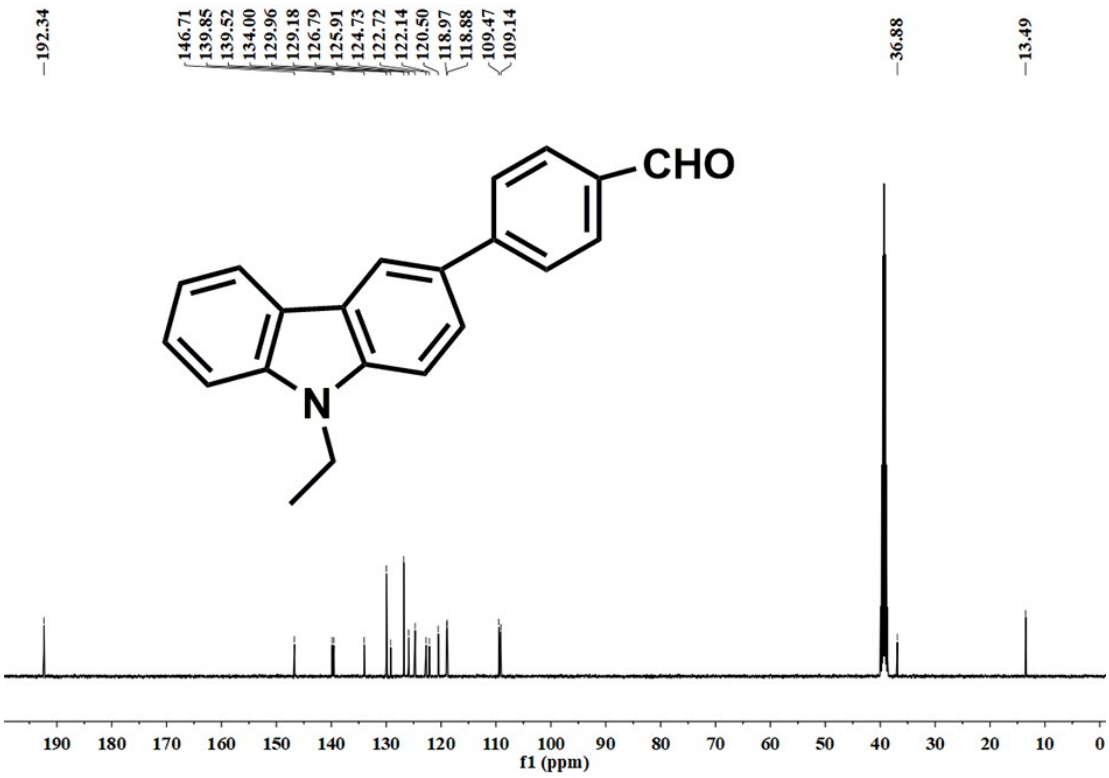


Fig. S4 ^{13}C NMR spectrum of **CBA** in DMSO-d6.

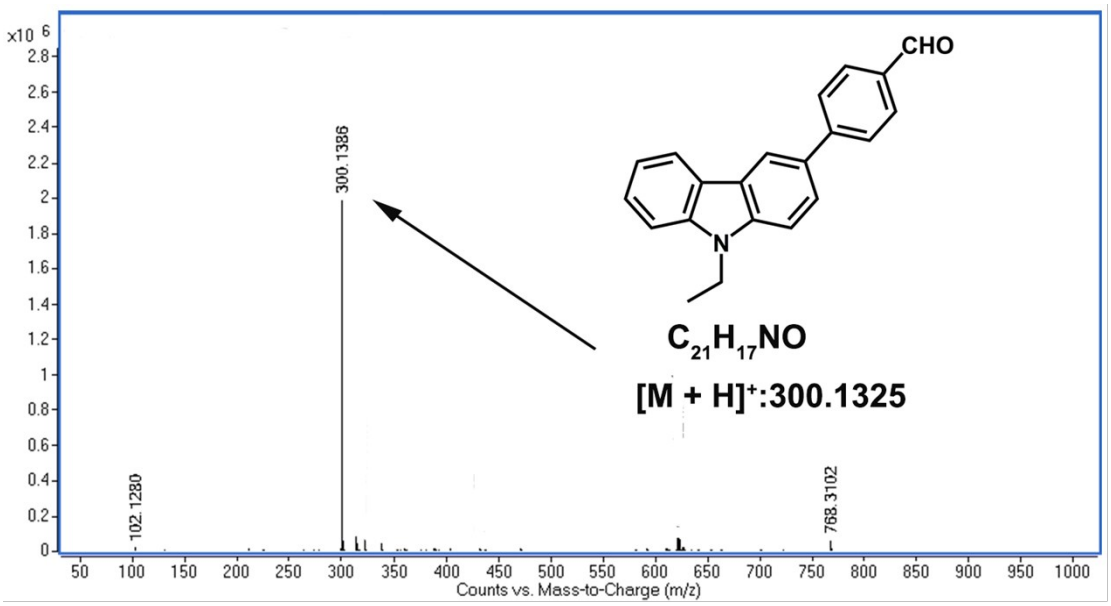


Fig. S5 HRMS spectrum of **CBA**.