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Supporting Information for

A novel mitochondria-targeted fluorescent probe for detecting viscosity in living cells and zebrafishes

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Materials and general information

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. All aqueous solutions were prepared withultrapure waterobtained from a Milli-Q water purification system (18.2 MΩ cm). 1H and 13C NMR spectra were measured on an AVANCE III HD 500 digital NMR spectrometer, using tetramethylsilane (TMS) as the internal standard. High resolution mass spectrometric (HRMS) analyses were measured on Brooke solanX-70FT-MS, Agilent 6540T. The absorbance was recorded by ultraviolet-visible absorption spectrometry (UV-2700, Shimadzu) or microplate reader (TransGen Biotechnology). Fluorescent spectra were recorded with a HITACHI F4700 fluorescence spectrophotometer. The pH measurements were carried out on a PHS-3C pH meter. Viscosity experiments were carried out with a NDJ-8 rotational viscometer, and each viscosity value was recorded. The fluorescence imaging of cells was performed with a Leica TCS-SP8 CARS confocal microscope. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of them being obtained from the Qingdao Ocean Chemicals

Viscosity determination and spectral measurement

The solvents were obtained by mixing methanol-glycerol and ethylene glycol-methanol systems in different proportions. Viscosity value was measured by a NDJ-8 rotary viscometer. The solutions of **CSS-1** of different viscosity were prepared by adding the stock solution (1.0 mM) 30 μL to 3 ml of solvent mixture (methanol-glycerol

solvent systems) to obtain the final concentration of the probe **CSS-1** (10.0 μM). These solutions were sonicated for 30 minutes to eliminate air bubbles. After standing for 1 hour at a constant room temperature, the solutions were measured in a UV spectrophotometer and a fluorescence spectrophotometer.

Measurement of fluorescence quantum yield (Φ)

Fluorescence quantum yield was determined by the relative comparison with Rhodamine B (Φ_s = 0.97 in EtOH) as standard for **CSS-1** in different solvents. And they were calculated by the following formula:

$$\Phi = \Phi_S \frac{IA_s}{I_sA} \cdot \frac{\eta^2}{\eta_s^2}$$

In which, A is the absorbance, I is the integrated fluorescence intensity, and η is the refractive index of the solvent.

The Förster-Hoffmann equation

The relationship between the fluorescence emission intensity of the probe **CSS-1** and the solvent viscosity could be formulated by the Förster-Hoffmann equation:

$$\log I = \mathrm{C} + x \log \eta$$

Where η is the viscosity, I is the emission intensity, C is a constant, and x is the sensitivity of the probe to viscosity.

Cytotoxicity assay

The vitro cytotoxicity of the probe **CSS-1** to Hela cells were studied by standard MTT (Solabio Life Sciences, Beijing, China) assays. cells were seeded in 96-well plates (5×10^3 cells/well) and then incubated with various concentrations of the probe (0, 1, 2,

5, 10, 20, 30 ,40 and 50 μM) for 24 h. After that, 10 μL MTT (5 mg/mL in PBS) 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 (%) = (ODsample-ODblank) / (ODcontrol- ODblank) × 100 %）

Cell culture and fluorescence imaging

HeLa cells were cultured in 90% DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (fetal bovine serum) and 1% antibiotics (100 U/ml penicillin and 100 μg/mL streptomycin, Hyclone) in an atmosphere of 37 °C and 5% CO_2. The HeLa cells were seeded up to appropriate density into a 35 mm glass-bottom culture dishes (Nest). Hela cells was treated with **CSS-1** (10.0 μM) for 30 min at 37 °C. Then the cells were washed with PBS buffer (pH = 7.4) three times, and the cells incubated with lipopolysaccharide, monensin and nystatin (the final concentration is 10 μM) for more 30 min at 37 °C. Finally, the cells were washed three times with PBS buffer. The fluorescence imaging was carried out by a Leica TCS SP8 CARS confocal microscope with a 63*objective lens. The fluorescence images of the slices were acquired using 488 nm excitation and fluorescence emission windows of 550-650 nm.

Co-localization Experiment

Hela cells were incubated with 10 μM the probe and 1μM positioning dye in culture medium for 30 min. Then the co-location experiment was carried out. （Mito

Tracker Green: λ_{ex} = 488 nm, λ_{ex} = 500–550 nm.Hoechst 33342: λ_{ex} = 405 nm, λ_{ex} = 425–475 nm.Lyso Tracker Blue: λ_{ex} = 405 nm, λ_{ex} = 425–475 nm.Bodipy 500/510 (green): λ_{ex} =500 nm, λ_{ex} = 510–620 nm.）

Fluorescence imaging in living zebrafishes

Wild type zebrafishes were purchased from the Nanjing EzeRinka Company Limited. All procedures for this study were approved by the Animal Ethical Experimentation Committee of Guangxi University according to the requirements of the National Act on the use of experimental animals (China). For the fluorescence imaging experiments, 3-day-old zebrafishes were transferred into a 30 mm glass culture dishes using a disposable sterilized dropper. The probe **CSS-1** (10 μM) was added for incubated for 30 min, followed by washing away gently. Then 10 μM Monensin were put into dishes respectively for another 30 min. After that, the zebrafishes were transferred into new glass bottom dishes for imaging. Prior to the imaging, we adopted 1% agarose gel for immobilization of zebrafishes, and put zebrafishes onto agarose with a little media to ready imaging. The imaging experiments were recorded through a Leica TCS SP8 CARS confocal microscope with a 4*objective lens. The fluorescence emission was collected at TRICT channel (550-650 nm) upon excitation at 496 nm.

Table S1. The photo-physical data of probe CSS-1 in different solvent systems.

solvents	η[a] (cP)	Dielectric constant	λ_{ab} (nm)	λ_{em} (nm)	Stokes shift (nm)	Stokes shift (cm^{-1})	Fluorescence Quantum Φ (%)
H_2O	1.01	78.4	446	592	146	6849	0.1
CH_2Cl_2	0.43	9.1	509	589	80	1250	0.05
CH_3CN	0.37	37.5	475	596	121	8264	0.15
Acetone	0.32	20.7	467	592	125	8000	0.08
$CHCl_3$	0.54	5.1	505	589	84	1190	0.02
DMSO	2.24	48.9	465	596	131	7633	0.16
Ethylene glycol	2.35	37	458	595	137	7299	0.32
EtOH	1.07	24.5	462	585	123	8130	0.11
DMF	0.8	36.7	475	596	121	8264	0.14
MeOH	0.65	32.6	455	590	135	7407	0.022
Glycerol	953	45.5	475	592	117	8547	0.652
Methanol: Glycerol=1:1	7.8	-	460	590	130	7692	0.068

Table S2. Test viscosity in the varied of the Methanol /Glycerol and Methanol / Ethylene glycol (v/v) mixtures of **CSS-1.**

Methanol: Glycerol	Viscosity / CP	Methanol: Ethylene glycol	Viscosity / CP
10:0	0.65	**10:0**	0.65
9:1	0.97	**9:1**	0.66
8:2	1.56	**8:2**	0.67
7:3	2.3	**7:3**	0.71
6:4	4.5	**6:4**	0.72
5:5	7.8	**5:5**	0.84
4:6	37	**4:6**	0.96
3:7	72	**3:7**	1.21
2:8	155	**2:8**	1.38
1:9	395	**1:9**	1.62
0:10	953	**0:10**	2.35

Table S3. The Pearson's colocalization coefficient of the positioning dye and probe **CSS-1** obtained from the internal software of the Leica Confocal Microscopy Imaging Instrument.

Organelles	Positioning dye	Pearson's colocalization coefficient
Mitochondria	Mito Tracker Green	0.85
Nuclei	Hoechst 33342	0.32
Lysosomal	Lyso Tracker Blue	0.31
Lipid droplet	Bodipy 500/510 (green)	0.50
Endoplasmic reticulum	ER-Tracker Blue	0.21

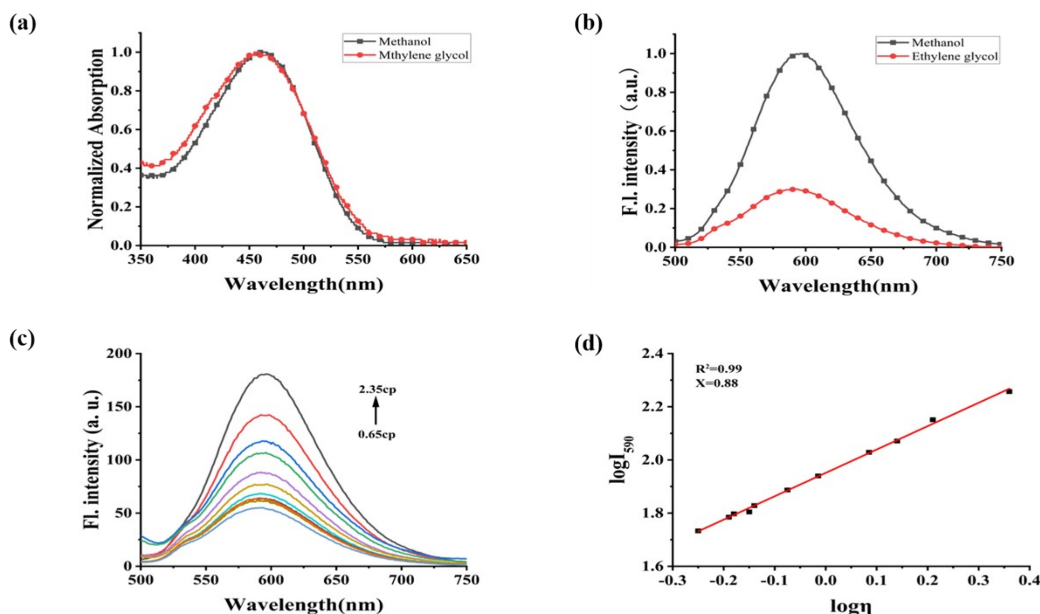

Fig.S1. (a) Normalized absorption spectra of the probe **CSS-1** in methanol and ethylene glycol. (b) Fluorescence emission spectra of the probe **CSS-1** in methanol and ethylene glycol; (c) Fluorescence spectra of probe **CSS-1** (10 μM) in ethylene glycol-methanol mixtures with different ratios, $\lambda_{ex} = 460$ nm, slit width: $d_{ex} = d_{em} = 10$ nm; (d) the linear response between log I_{590} and log η in the ethylene glycol-glycerol system, $R^2 = 0.99$, X = 0.88.

Calculation of detection limit [1-3]

The detection limit was determined from the fluorescence titration data based on a reported method. [1-3] According to the result of titrating experiment, the fluorescence intensity data at 590 nm were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to these normalized fluorescence intensity data, and the point atwhich this line crossed the ordinate axis was considered as the detection limit (0.9142 cp).

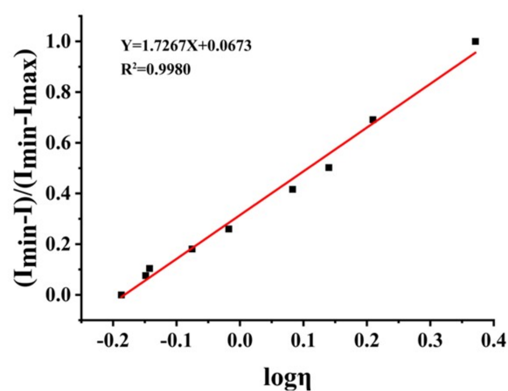

Fig.S2. Normalized response of the fluorescence signal to changing η.

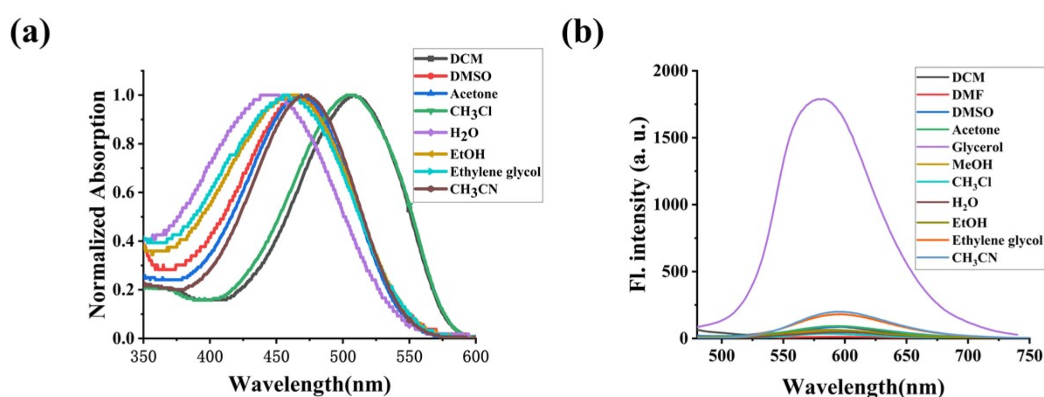

Fig.S3. Fluorescence emission and normalized absorption spectra of the probe **CSS-1** in several solvents with various polarities, λ_{ex} = 460 nm, slit width: d_{ex} = d_{em} = 10 nm.

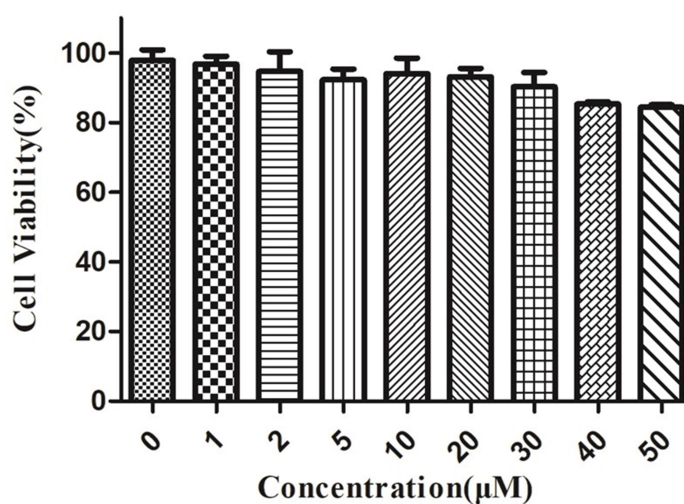

Fig.S4. Cytotoxicity assays of **CSS-1** at different concentrations for HeLa cells

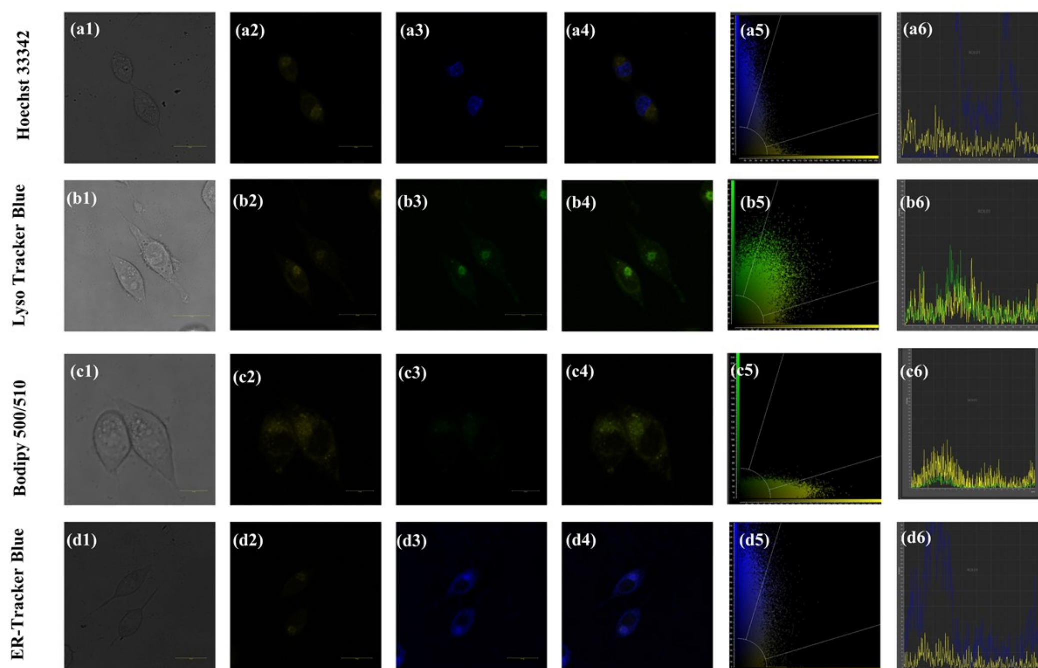

Fig.S5. Co-localization image obtained by using four positioning dyes to act on HeLa cells separately, and then adding 10 μM probe **CSS-1**. (The figure a corresponds to the nuclear localization dye Hoechst 33342, the figure b corresponds to the Lyso Tracker Blue, the figure c corresponds to the Bodipy 500/510 (green), and the figure d corresponds to the endoplasmic reticulum localization dye ER-Tracker Blue; picture 1 of a, b, c, d, e, d are bright field pictures, picture 2 is the yellow channel picture of the probe, picture 3 is positioning dye Fluorescence picture, picture 4 is the overlay picture, picture 5 is the intensity scatter plot of fluorescence intensity, picture 5 is the intensity trends of **CSS-1** and positioning dye in the cell ROI region.)

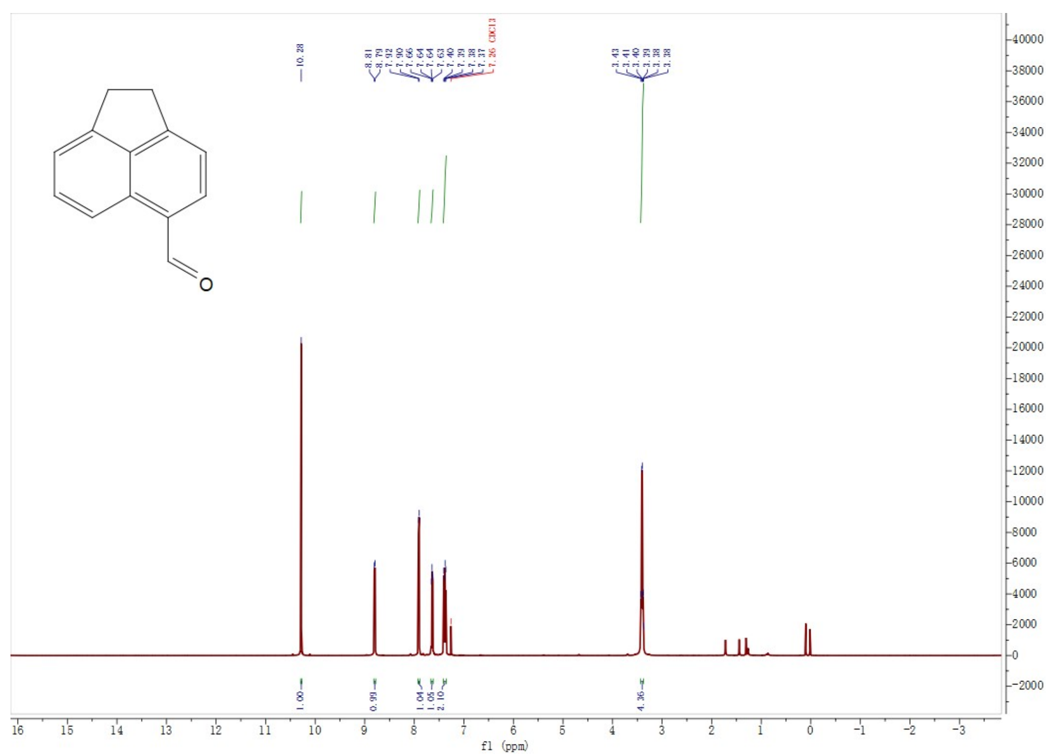

Fig. S6. ^{1}H NMR spectra of Compound 2 in Chlorofoem-d

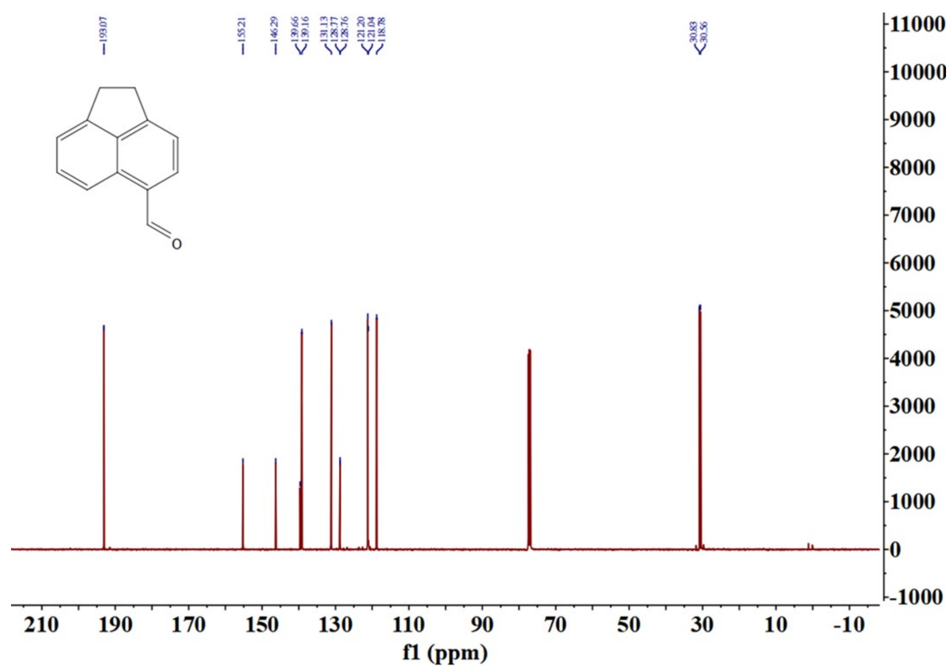

Fig. S7. ^{13}C NMR spectrum of Compound 2 in Chlorofoem-d

Fig. S8. HRMS ESI-MS spectrum of Compound 2

Fig. S9. ^1H NMR spectrum of **CSS-1** in $CDCl_3$

Fig. S10. ^{13}C NMR spectrum of **CSS-1** in $CDCl_3$

Fig. S11. ESI-MS spectrum of **CSS-1**

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