Electronic Supplementary Information for

A novel mitochondria-targeted rhodamine analogue for detection of viscosity changes in living cells, zebra fishes and living mice

Rui Guo,[a] Junling Yin[b], Yanyan Ma,[b] Qiuan Wang[a,*], and Weiying Lin[a,b,*]

[a] *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan 410082, P.R. China*

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

* Corresponding authors: weiyinglin2013@163.com (W. Lin) ； WangQA@hnu.edu.cn (Q. Wang)

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Materials and general information

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Twice distilled water was used throughout all experiments. High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP spectrometer; NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a UV/Vis absorption spectra were recorded with Shimadzu RF-5301PC; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer; The fluorescence imaging of cells was performed with OLYMPUS FV1000 (TY1318) confocal microscopy; 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.

Viscosity determination and fluorescence spectral measurement detection

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 **RV-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 dye (10.0 μM). These solutions were sonicated for 5 minutes to eliminate air bubbles. After standing for 1 hour at a constant temperature, the solutions were measured in a UV spectrophotometer and a fluorescence spectrophotometer.

Cytotoxicity assay

The toxicity of the probes was tested by an MTT (Solabio Life Sciences, Beijing, China) assay. The day before the experiments, 4×103 of Hela cells in 100 μL culture were seed into 96 well-plates. Next day, 100 μL culture contained various concentrations of probes (0, 1, 2, 5, 10, 15, 20 and 40μM) were added into the cells instead of the old culture. After incubation for 24 h, 10 μL MTT (5 mg/mL in PBS) was added into the each well and incubated for another 4 h. Then, the culture was removed and replaced with 100 μL DMSO, and the plates were put onto a shaker for 20 min at 110 rpm to dissolve the formazan crystals. The absorbance was measured at 570 nm with Microplate Reader (Thermo Fisher Scientific, USA). The cell viability (%) = (OD570 (Experiments) − OD570 (Blank)) / (OD570 (Control) − OD570 (Blank)). OD570 (Experiments) denotes cells treated with the probes of various concentrations; OD570 (Control) denotes cells without the probes; OD570 (Blank) denotes plates without the cells treated with the same OD570 (Control). Each concentration was conducted with five parallel samples, and the results were expressed as mean ± standard deviation (SD).

Cell culture and fluorescence imaging

The HeLa cells were seeded up to appropriate density into a 35 mm glass-bottom culture dishes (Nest). Then the cells further incubated with the probe **RV-1** (the final concentration is 10 μM) for another 30 min at 37 °C. Then the cells were washed with PBS buffer (pH = 7.4) three times, and the cells incubated with Monensin or 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 imaging experiments were recorded through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera. The fluorescence emission was collected at TRITC channel (570-620nm) upon excitation at 561 nm.

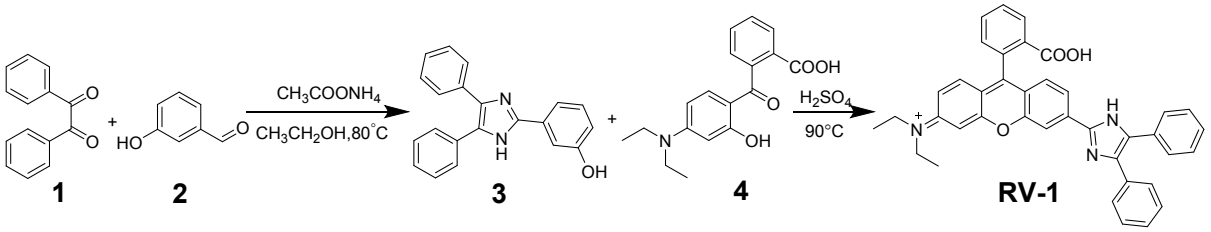
Fluorescence imaging in living zebrafishes

Wild type zebra fishes were purchased from the Nanjing EzeRinka Biotechnology Co., Ltd. All procedures for this study were approved by the Animal Ethical Experimentation Committee of Shandong University according to the requirements of the National Act on the use of experimental animals (China). The zebra fishes 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. 10 μM probe **RV-1** was added for incubated for 30 min, followed by washing away gently. Then 10 μM Monensin or nystatin 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. The imaging experiments were recorded through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera. The fluorescence emission was collected at TRITC channel (570-620nm) upon excitation at 561 nm.

In vivo inflammation experiments and fluorescence imaging in living mice

4 weeks old Kunming mice were purchased from Shandong University Laboratory Animal Centre (Shandong, China). Unless otherwise noted, all procedures for this study were approved by the Animal Ethical Experimentation Committee of Shandong University according to the requirements of the National Act on the use of experimental animals (China). The mice were given an i.p. injection of LPS (1 mg in 400 μL saline). After 4 h, the mice were anesthetized by i.p. injection of Nembutal (40~50 mg/kg), and their abdominal fur was removed with an electric shaver. Then, the mice were injected i.p. with **RV-1**(200 μM in DMSO). As a control, untreated (neither treated with LPS nor RV-1) or unstimulated mice i.p. injected only with **RV-1** (200 μM in DMSO) were also prepared. The mice were then imaged (30 min after the injection of **RV-1**) by using a FMT 2500 LX quantitative tomography in vivo imaging system, with an excitation filter of 580 nm and an emission filter of 660 nm.

Synthesis



Scheme. S1 Synthetic pathway for the probe **RV-1**.

The compound **3** was prepared according to the previously reported methods with minor modifications.[1]

Table S1 Fluorescence lifetime measurement in various solvent systems.

Methanol/glycerol (v: v)	$\tau^{[a]}$(ns)
5:5	0.5746
2:8	0.6315
1:9	0.7509
0:10	0.9757

[a] the fluorescence lifetime of the probe **RV-1**.

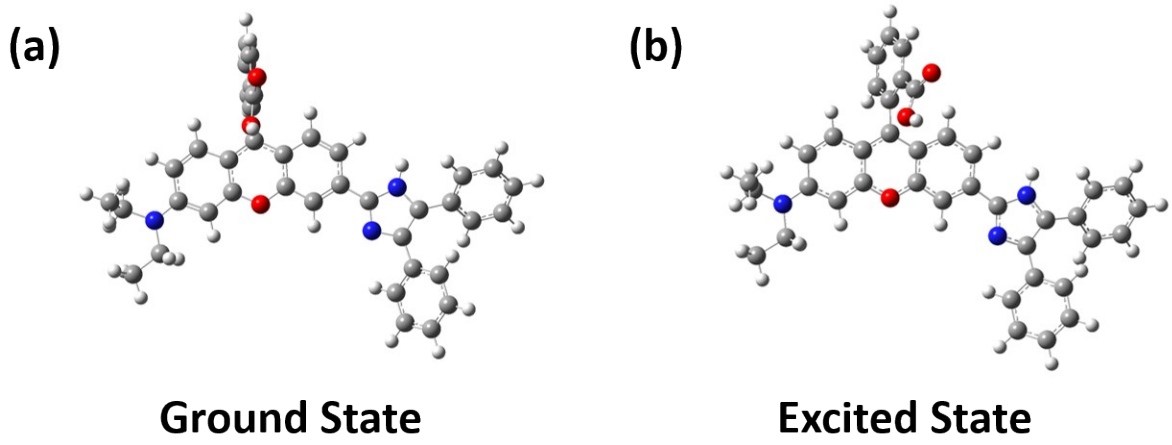


Fig.S1 Optimized conformations of the probe **RV-1** in (a) ground state using Gaussian 09 program by DFT B3LYP/6-31G (d) methods; (b) excited state using Gaussian 09 program by TD-DFT B3LYP/6-31G (d) methods.

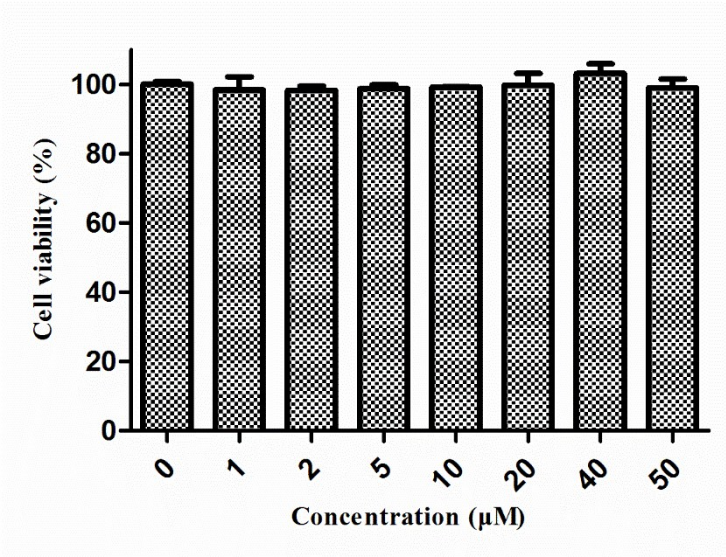


Fig.S2 Cytotoxicity data of **RV-1** (HeLa cells incubated for 24 h).

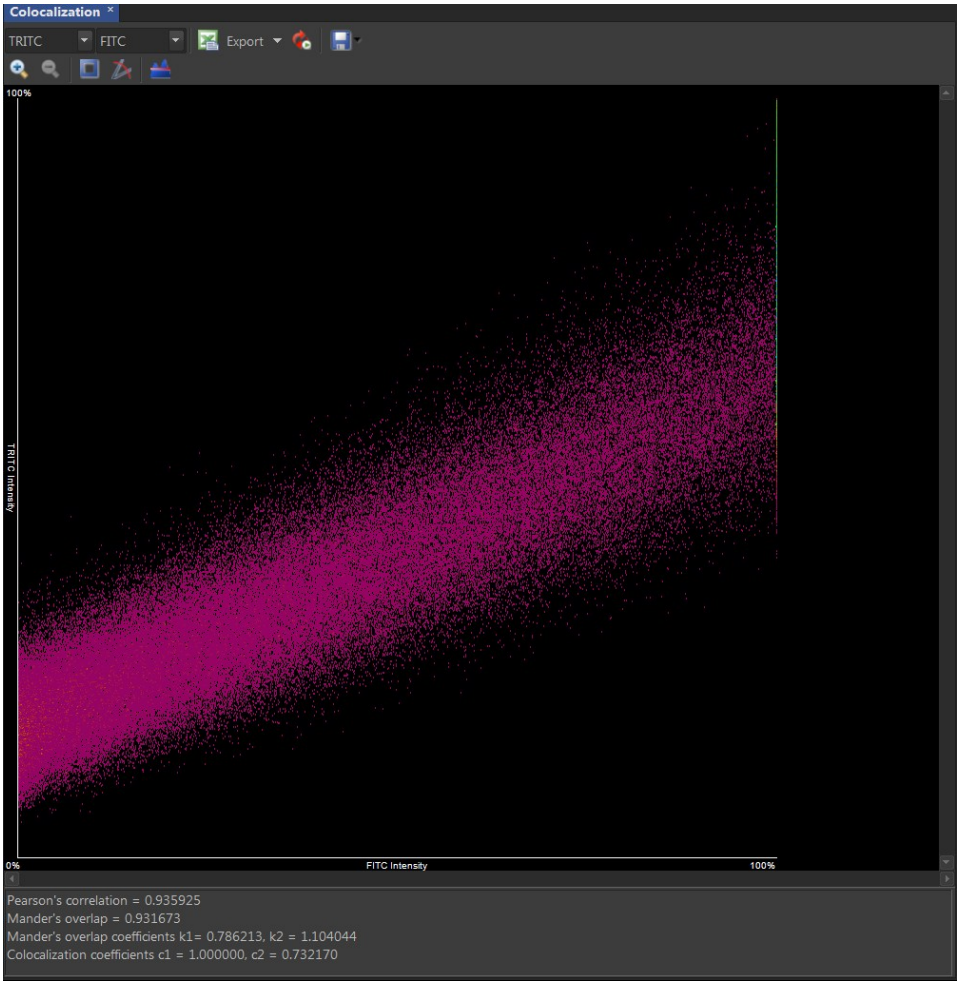


Fig.S3 Intensity correlation plots of **RV-1**(Y-axis) and Mito-Tracker Green (X-axis) in HeLa cells.

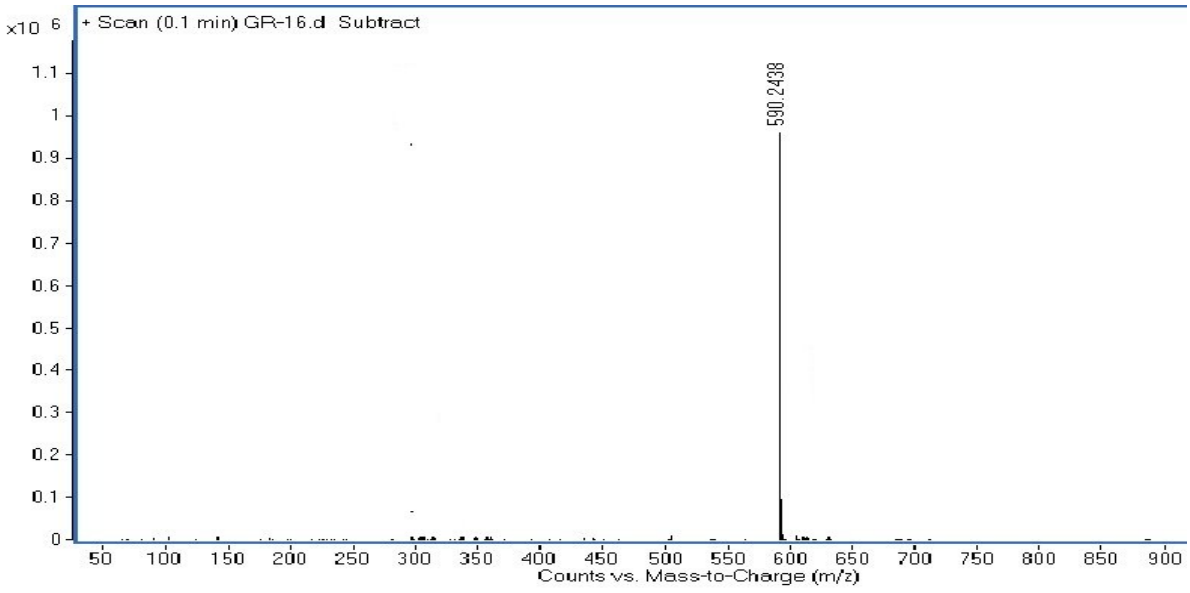


Fig. S4 HRMS ESI-MS spectrum of **RV-1**.

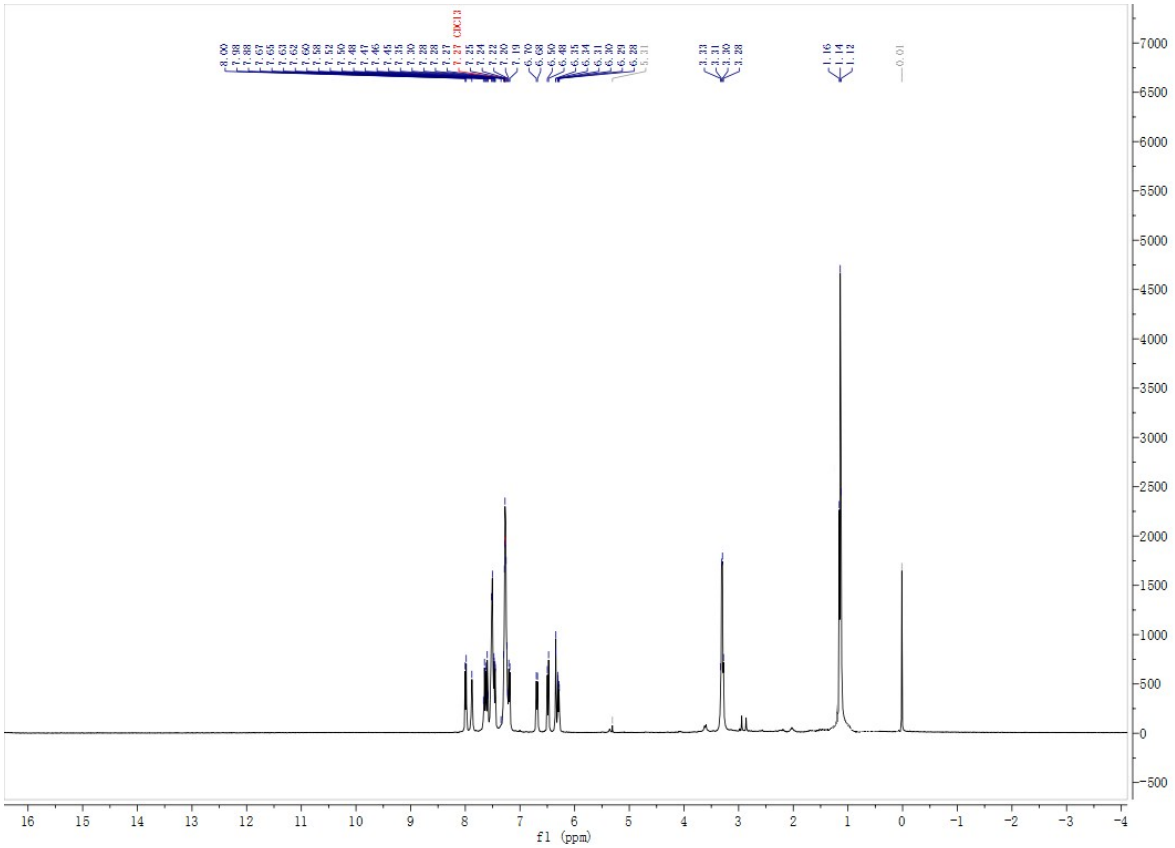


Fig. S5 ^{1}H NMR spectrum of **RV-1** in $CDCl_3$.

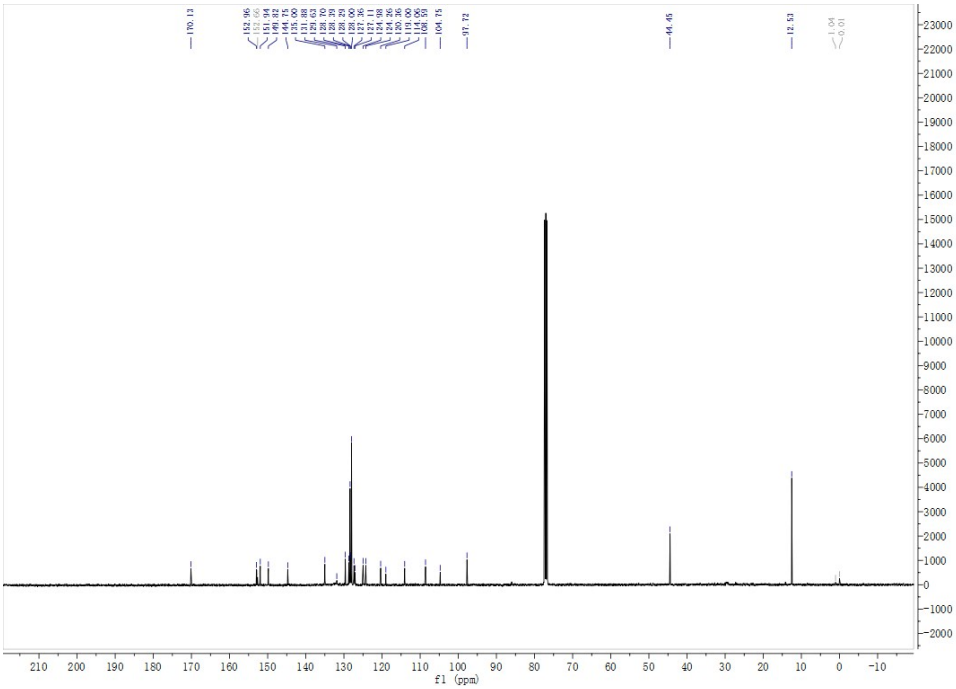


Fig. S6 ^{13}C NMR spectrum of **RV-1** in $CDCl_3$.

References

1. N. Azizi, N. Dado and A. K. Amiri, *Can. J. Chem.,* 2011, **90**, 195-198.