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Supporting Information

Viscosity-sensitive NIR probe for in vivo imaging of Early-Stage Hepatic Fibrosis

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1. General Information

1.1 Materials

All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. HepG 2 cells used in our experiment were purchased from Beyotime Institute of Biotechnology. Mito-Tracker Green was purchased from Beyotime Institute of Biotechnology. Female 6-week-old BALB/c-nu mice were purchased from SPF (Beijing) Biotechnology Co., Ltd. Production Permit No.: SCXK (Beijing) 2019-0010. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05mol/L Na_2HPO_4 water solution and 0.05mol/L KH_2P0_4 water solution with the volume ratio 4:1. All chemicals and solvents used were of analytical grade. All solution samples were made by dissolving their each solid in water or DMSO.

This study was performed in strict accordance with the Chinese guidelines for the care and use of laboratory animals and was approved by the Institutional Animal Care and Use Committee of Scientific Research in Shanxi University (Taiyuan, China).

1.2 Instruments

TLC analysis was performed using precoated silica plates. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanghai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ^{1}H NMR and ^{13}C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI-MS was measured with a Thermo Scientific Q Exactive. The cells imaging experiments were measured by a Zeiss LSM-810 Airyscan confocal laser scanning microscope. The in vivo imaging experiments and ex vivo imaging experiments were performed with a LVIS Lumina LT Series III system.

1.3 Bio-imaging

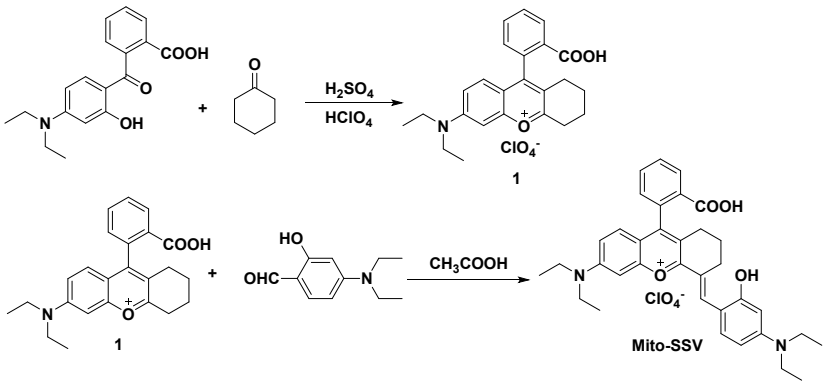
Cell Culture and Imaging. The cells were grown in Dulbecco's Modified Eagle's medium supplemented with 12% Fetal Bovine Serum and 1% antibiotics at 37 °C in humidified environment of 5% CO2. Cells were plated on 6-well plate and allowed to adhere for 24 h. Cells were incubated with Mito-SSV at 37 ℃ for 20 min. Before the experiments, cells were washed with PBS for 3 times.

1.4 In vivo and Ex vivo-imaging

Development of liver fibrosis. Mice were randomly divided into 3 groups (a control group, a CCl_4 group, and a metformin group). The liver fibrosis model was induced by intraperitoneal injection of CCl_4 dissolved in olive oil (V/V=1：4) twice per week for 3 weeks, while the control mice were injected with olive oil alone. Mice in the metformin group were treated with metformin in drinking water at the same time. In vivo imaging was conducted at 1, 2, 3 weeks, respectively. After 3 weeks, mice were sacrificed and the vital organs were harvested immediately and imaged by the IVIS imaging system.

2. Experimental Section

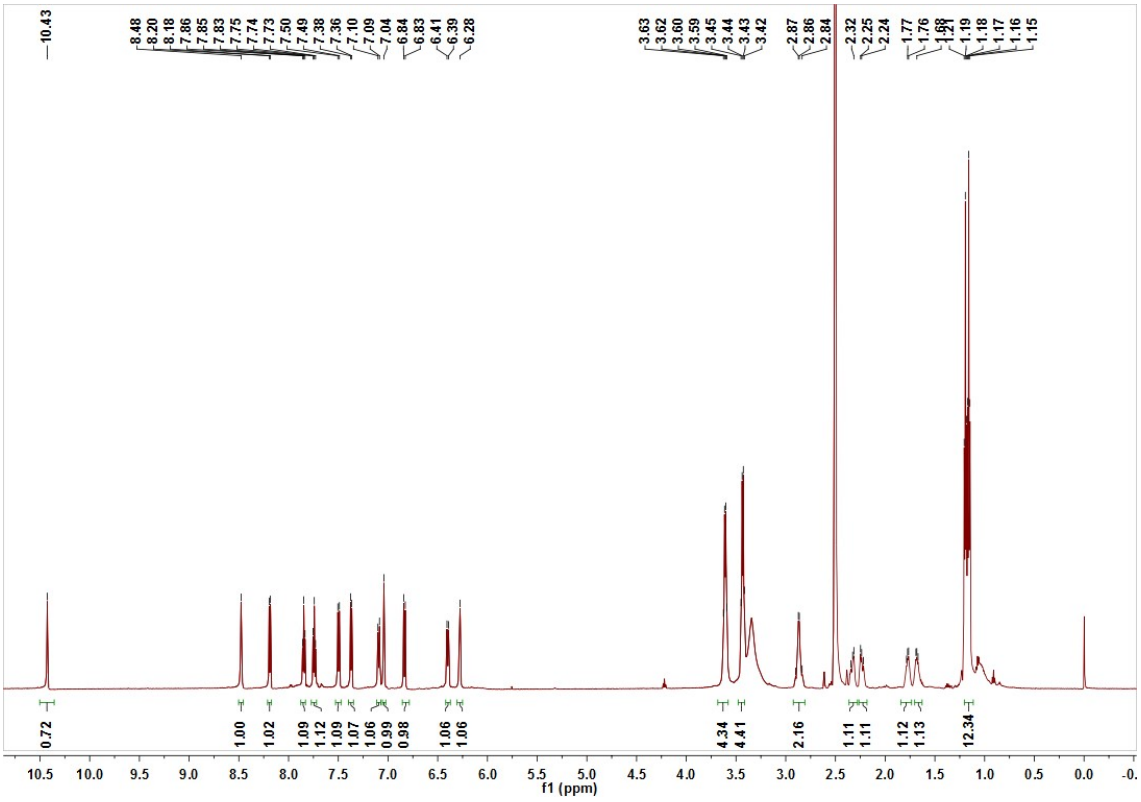
2.1 **Scheme S1**. Synthesis route of probe **LV**.



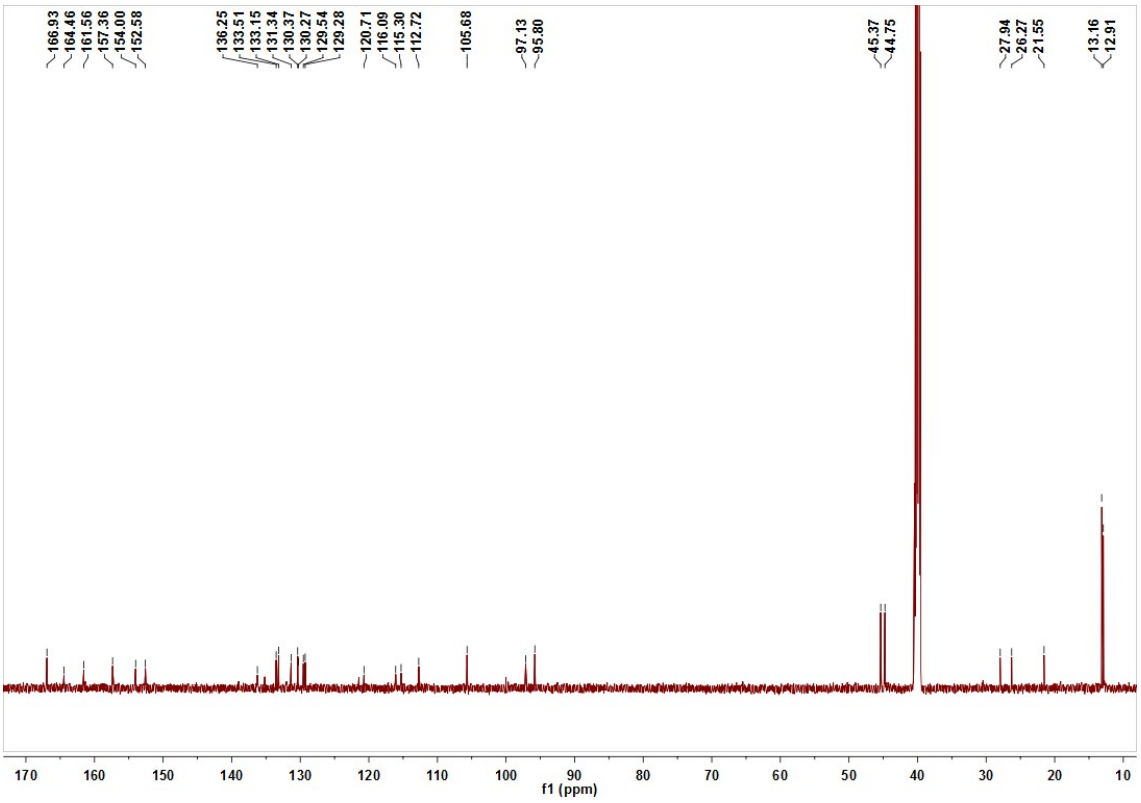
2.2 Synthesis and characterization of **LV.**

Compound **1** was prepared according to our previous reported works.[1] Then, compound **1** (0.92 g, 2 mmol) and 4-Dimethylaminobenzaldehyde (0.596 g, 4 mmol) was dissolved in 50 mL CH_3COOH. The mixture was heated at 110 °C for 4 h. After the reaction was completed, the solvent was removed to give the crude product. Then, dried and subjected to purification by flash chromatography (CH_2Cl_2 : CH_3OH = 20:1) to give probe **LV** as a black solid (1.96 g, 73.2%). ^{1}H NMR (600 MHz, DMSO) δ 10.43 (s, 1H), 8.48 (s, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 7.85 (t, *J* = 7.5 Hz, 1H), 7.74 (t, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 9.2 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.09 (d, *J* = 9.4 Hz, 1H), 7.04 (s, 1H), 6.83 (d, *J* = 9.4 Hz, 1H), 6.40 (d, *J* = 9.2 Hz, 1H), 6.28 (s, 1H), 3.61 (dd, *J* = 14.0, 6.9 Hz, 4H), 3.43 (dd, *J* = 13.8, 6.8 Hz, 4H), 2.92 – 2.81 (m, 2H), 2.36 – 2.28 (m, 1H), 2.26 – 2.18 (m, 1H), 1.84 – 1.73 (m, 1H), 1.70 – 1.63 (m, 1H), 1.20 – 1.11 (m, 12H). ^{13}C NMR (151 MHz, DMSO) δ 166.93 (s), 164.46 (s), 161.56 (s), 157.36 (s), 154.00 (s), 152.58 (s), 136.25 (s), 133.51 (s), 133.15 (s), 131.34 (s), 130.32 (d, *J* = 15.7 Hz), 129.54 (s), 129.28 (s), 120.71 (s), 116.09 (s), 115.30 (s), 112.72 (s), 105.68 (s), 97.13 (s), 95.80 (s), 45.37 (s), 44.75 (s), 27.94 (s), 26.27 (s), 21.55 (s), 13.16 (s), 12.91 (s).

Figure S1: Structure characterization of **LV**.



^{1}H-NMR spectrum of **LV** in DMSO-d_6



^{13}C-NMR spectrum of **LV** in DMSO-d_6

Figure S2: Fluorescence intensity change **LV** at 740 nm of in PBS and glycerol when excited at 650 and 700 nm.

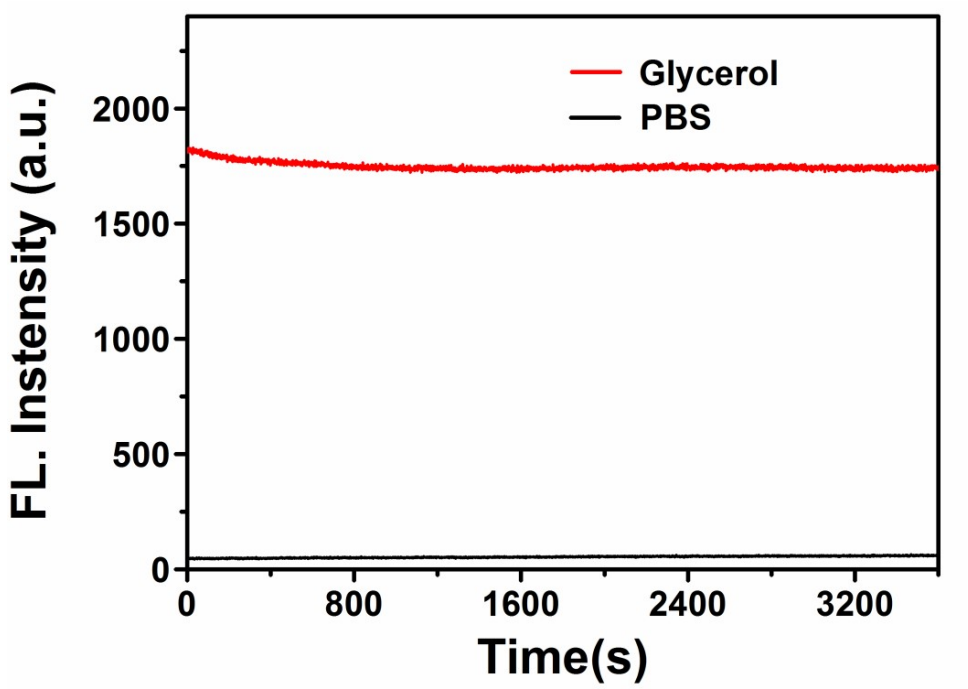


Figure S3. Absorption spectra of **LV** (10 μM) in the solvent of low viscosity (PBS) and high viscosity (glycerol).

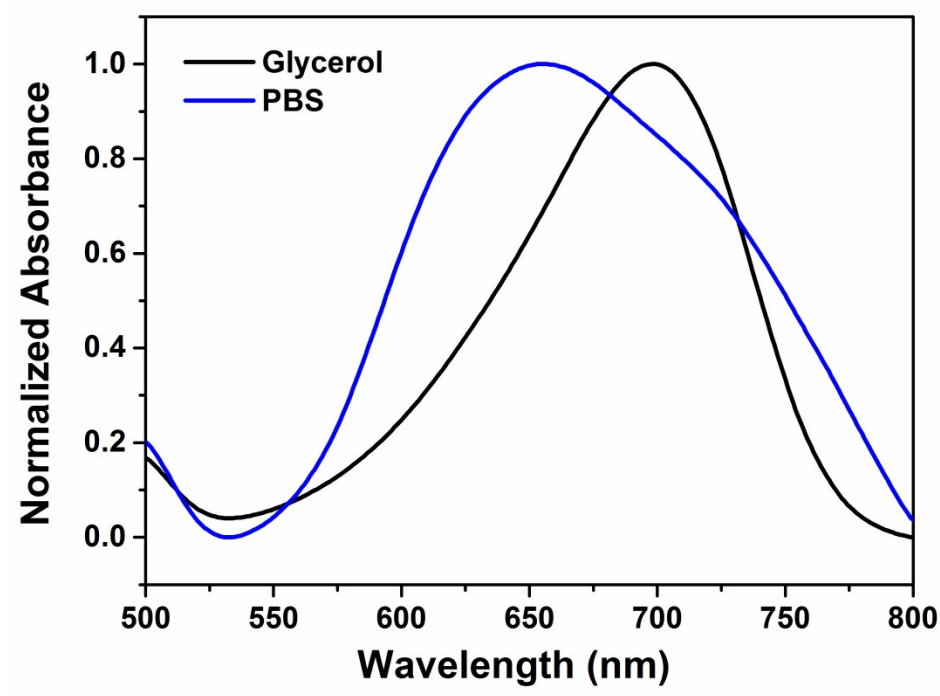


Figure S4: The fluorescence intensity changes of LV with different pH values and temperature.

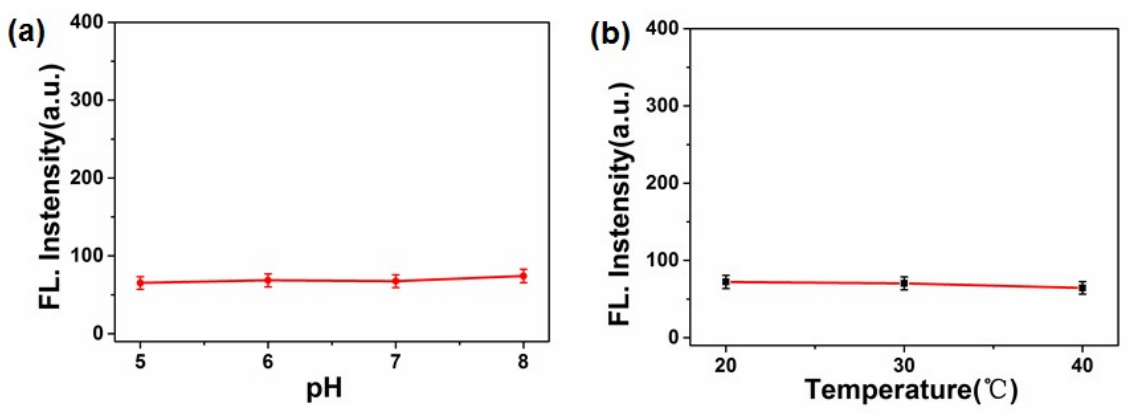


Figure S5: The cytotoxicity test of **LV**.

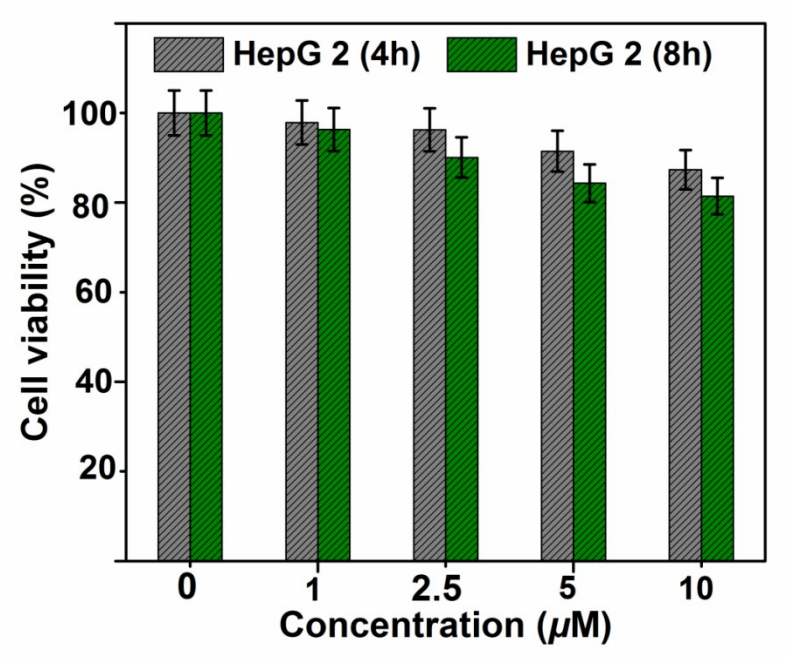


Figure S6: The fluorescence imaging of probe LV (10 μM) without H_2O_2 and Nystatin.

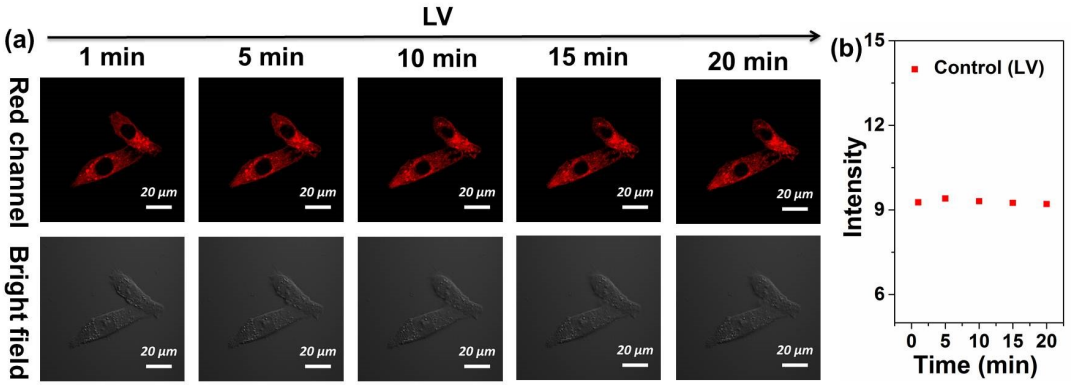
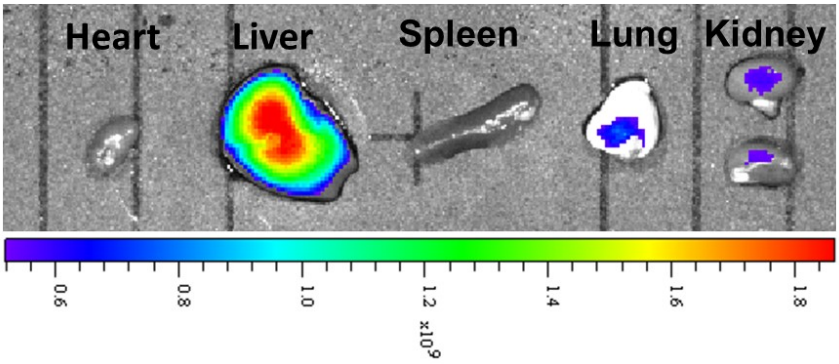


Figure S7: Fluorescence images of excised organ after tail intravenous injection of LV.



REFERENCES

1. Zhang, W. J.; Huo, F. J.; Zhang, Y. B.; Yin, C. X. *J. Mater. Chem. B,* **2019***, 7, 1945-1950*