Supporting Information for

Development of a Novel Near-Infrared Molecule Rotator for Early Diagnosis and Visualization of Viscosity Changes in Acute Liver Injury Models

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Experimental section

Quantum yield calculation

The fluorescence quantum yield $\Phi_s$ was estimated from the absorption and fluorescence spectra of probe according to equation, where the subscript $s$ and $r$ stand for the sample and reference (fluorescein as standard $\Phi_F = 0.85$), respectively. $\Phi$ is the quantum yields, $A$ represents the absorbance at the excitation wavelength, $S$ refers to the integrated emission band areas and $n_0$ is the solvent refractive index. The fluorescence quantum yields ($\Phi_F$) were estimated with equation as follows:

$$\Phi_s = \frac{\Phi_F S_A n_0^2}{S_R A_r n_{r0}^2}$$

Cytotoxicity of probe DJM

The cytotoxicity of probe DJM was tested by MTT method. Miha cells were seeded at a density at $1 \times 10^4$ cells per well into 96-well plate, and incubated in 37 °C cell incubator (containing 5% CO$_2$) for 12 hours. The culture medium was high glucose DMEM with fetal bovine serum and appropriate antibodies (penicillin and streptomycin). Then the probe DJM (0, 1, 2, 5, 10, 20 μM) was added and incubated for 24 h. 50 μL MTT was added to each pore and the cells were incubated at 37 °C and 5% CO$_2$ for 4 h. Then, removed the medium and replaced it with DMSO (150μL), and detected the absorption values at 490 nm.

Cell culture and fluorescence imaging

DMEM containing 10% fetal bovine serum and 1% penicillin was used for Miha cell culture in an incubator supplemented with 95% air and 5% CO$_2$ at 37 °C. Then, nutrient solution was removed and cells were washed three times with PBS buffer (pH=7.0, 10 mM) before imaging. To compare the difference in viscosity level of Miha cells, they were treated with 10 μM DJM for 15 min and then washed three times with PBS buffer.

Imaging in zebrafish

The 3-day-old zebrafish was incubated with DJM (10 μM) for 30 min, and then washed with PBS buffer and imaged as control group. The 3-day-old zebrafish firstly
incubated with APAP (200 µM) for 4 h, then incubated with **DJM** (10 µM) for 30 min, washed with PBS buffer and imaged as APAP-treated group. The third group is firstly treated with APAP (200 µM) for 4 h, and then treated with NAC (100 µM) for 1h. After that, these zebrafish was then incubated with **DJM** (10 µM) for 30 min, washed with PBS buffer and imaged as APAP+NAC group. Thereafter, the treated zebrafish was washed with PBS buffer three times and imaged using a confocal microscope. Fluorescence images were acquired with Nikon A1R confocal microscope.
Scheme S1. Synthesis route of DJM.

Fig. S1 Absorption spectra of DJM (10 μM) in different ratios of PBS/glycerol mixtures.
**Fig. S2** MTT results of Miha cells viabilities after incubation with **DJM** for 24 h. Data are expressed as mean ± SD (*p < 0.05, experiment times n = 3).

**Fig. S3** Quantitative analysis of Miha cell labeled with **DJM** (10 μM) for 2 h (a) control; (b) after treatment with APAP (200 μM) for 4 h; (c) after treatment with APAP (200 μM) for 4 h, and then treated with NAC (100 μM) for 1h.
**Fig. S4** Quantitative analysis of living 3-day-old zebrafish. (a) only labeled with **DJM** (10 μM) for 2 h. (b) after treatment with APAP (200 μM) for 4 h. (c) after treatment with APAP (200 μM) for 1 h, and then treated with NAC (100 μM) for 1 h.
**Fig. S5** Quantitative analysis of normal and APAP-induced liver tissue in mice.

**Fig. S6** Mass spectra of DJM.
Figure S7 $^1$H NMR spectrum of DJM in CHCl$_3$-$d_6$.

Figure S8 $^1$C NMR spectrum of DJM in CHCl$_3$-$d_6$. 