Supporting information for

A viscosity-sensitive fluorescent probe with a large Stokes shift for monitoring lipid droplets and its application in cell, tobacco leaf and food detection

Yuan He, *[a] Yun-Hao Yang, ‡[b] Guo-Yu Chen, ‡[a] Lin Li, [c] Lin-Qing Wang, [c] Long-Ke Li, [a] and Jian-Yong Wang *[b]

[a] Collaborative Innovation Center of Food Production and Safety, College of Food and Bioengineering, Zhengzhou University of Light Industry, Zhengzhou 450002, P. R., China. E-mail: heyuan818@163.com

[b] Faculty of Light Industry, State Key Laboratory of Biobased Material and Green Papermaking, Key Laboratory of Paper Science and Technology of Ministry of Education, Qi Lu University of Technology (Shandong Academy of Sciences), Jinan, 250353, P. R., China. E-mail: wjy@qlu.edu.cn.

[c] College of Life Sciences, Zhengzhou Normal University, Zhengzhou, 450044, P. R., China.

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1. Materials and instrument

High-resolution electrospray mass spectra (HRMS) were gained from Bruker APEX IV-FTMS 7.0T mass spectrometer; NMR spectra were examined from AVANCE II 400 MHz Digital NMR Spectrometer with TMS as an internal standard; Electronic absorption spectra were recorded on a LabTech UV Power spectrometer; Fluorescence spectra were obtained with a HITACHI F4600 fluorescence spectrophotometer; The fluorescent images of the cells were obtained with Leica SP8 inverted fluorescence confocal microscope. The pH measurements were implemented on a Mettler-Toledo Delta 320 pH meter; analysis was exhibited on silica gel plates and column chromatography was carried out over silica gel (mesh 200-300). Both TLC and silica gel were purchased from Qingdao Ocean Chemicals.

2. General information for spectroscopic studies

Malononitrile-based dyes with different substituents were dissolved separately in dimethyl sulfoxide (DMSO) to obtain a stock solution (1 mM). 10 μM fluorescence was used for all spectroscopic experiments and 20 μL of the dye reserve solution was added to the cuvette after dilution to 2 mL with different solvents. Solutions of various interfering substances (10 mM) were prepared in ultrapure water. PBS buffer solutions of different pH values ranging from 1-12 were measured and prepared with a pH meter. Probe **LDCN** excitation wavelength was 510 nm; volume 600 v; excitation slit width and emission slit width were both 5 nm.

3. Cell culture and cytotoxicity assays

HeLa cells were provided by Jiangsu Kaiji Biotechnology Co., Ltd. The living HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10% FBS) under the atmosphere containing 5% CO_2 and 95% air at 37 °C. The cytotoxic effects of the probe **LDCN** were tested by the MTT assay. The living cells line was treated in DMEM (Dulbecco's Modified Eagle Medium) supplied with fetal bovine serum (10%, FBS), penicillin (100 U/mL), and streptomycin (100 μg/mL) under the atmosphere of CO_2 (5%)

and air (95%) at 37 °C. The HeLa cells were then seeded into 96-well plates, and 0, 2, 5, 10, 20, and 30 μM (final concentration) of the probe **LDCN** (99.9% DMEM and 0.1% DMSO) were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 hours. Then the HeLa cells were washed with PBS buffer, and DMEM medium (500 μL) was added. Next, MTT (50 μL, 5 mg/mL) was injected into every well and incubated for 4 h. Violet formazan was treated with sodium dodecyl sulfate solution (500 μL) in the H_2O-DMF mixture. The absorbance of the solution was measured at 570 nm by way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **LDCN**.

4. General procedures for probe LDCN biological experiments

The concentration of the probe in the cell imaging experiments was 10 μM, and the HeLa cells were incubated at a temperature of 37°C with 5% CO_2 for 30 min. To remove the residual probe, the cells were rinsed three times with PBS buffer solution before imaging. HeLa cells co-localization experiment: Probe 10 μM and commercial lipid droplet dye BODIPY 5 μM were co-incubated with HeLa cells for 30min. Finally, the cells were imaged with a Leica SP8 inverted fluorescence confocal microscope with an excitation wavelength of 493 nm and emission wavelengths of 480-530 nm (green channel) excitation wavelength of 405 nm, and emission wavelengths of 600-670 nm (red channel), respectively.

Tobacco seedling co-localization experiment：The leaves of tobacco seedlings were sliced and incubated with LDCN (10μM) for 30 minutes, then placed on an imaging plate containing trace water, and imaged under a confocal microscope with excitation wavelengths of 405nm and emission wavelengths of 480-530nm (green channel) and 600-670nm (red channel). The images of leaf slices of tobacco seedlings were observed by laser confocal microscope.

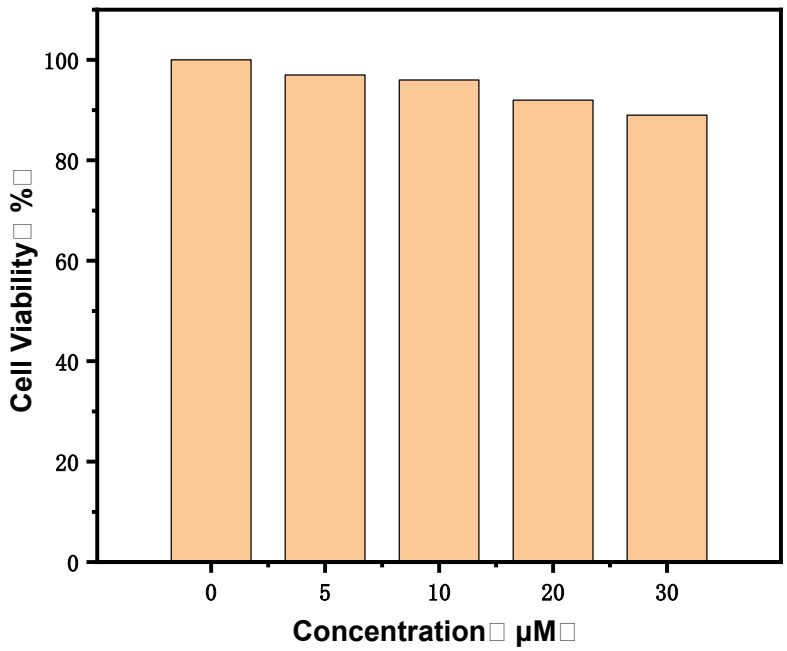

Fig. S1 Cytotoxicity assays of probe **LDCN** at different concentrations (0 μM; 2μM; 5 μM; 10 μM; 20μM; 30 μM) for HeLa cells.

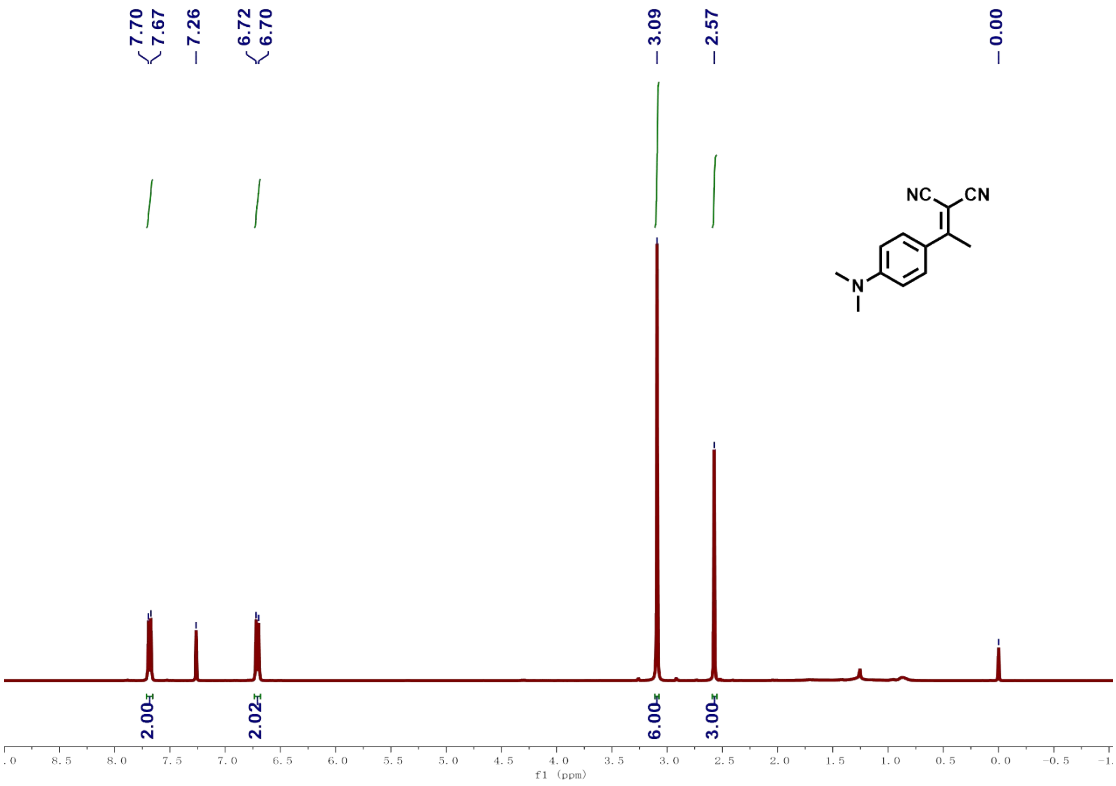

Fig. S2 ^{1}H NMR spectrum of compound 2 in Chloroform-*d*.

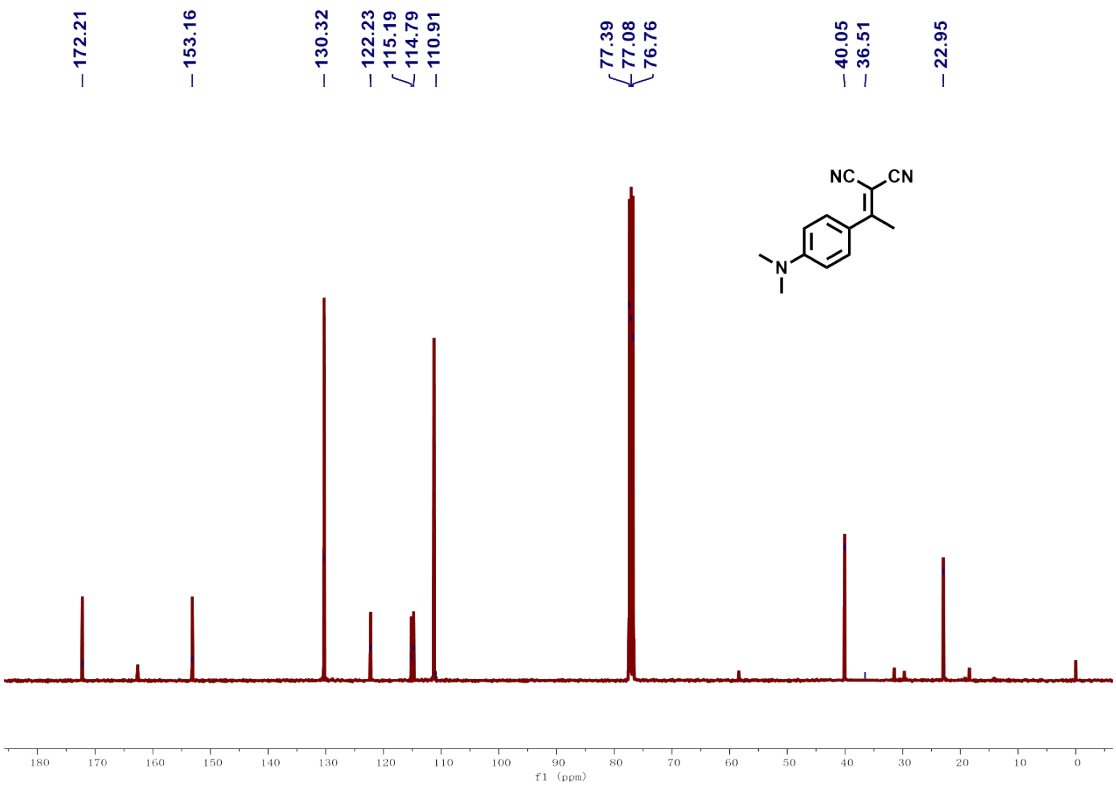

Fig. S3 ^{13}C NMR spectrum of compound 2 in Chloroform-*d*.

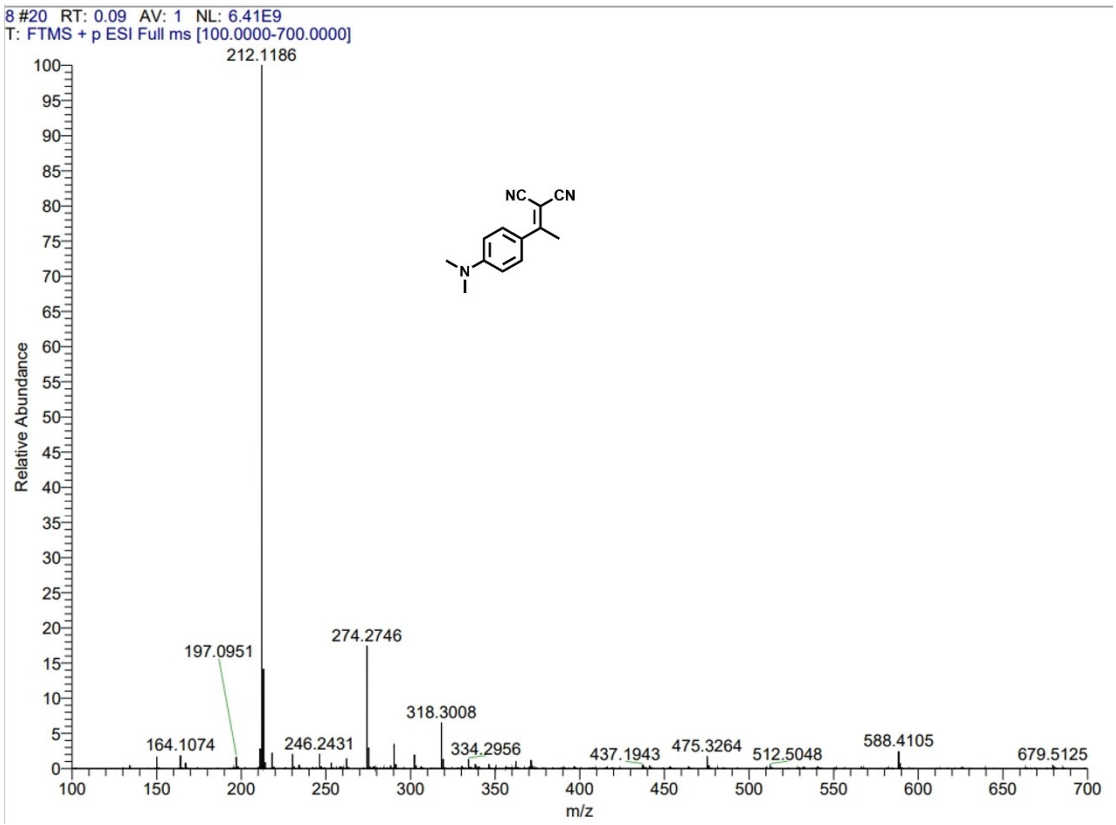

Fig. S4 HR-MS spectrum of compound 2 in CH_3OH.

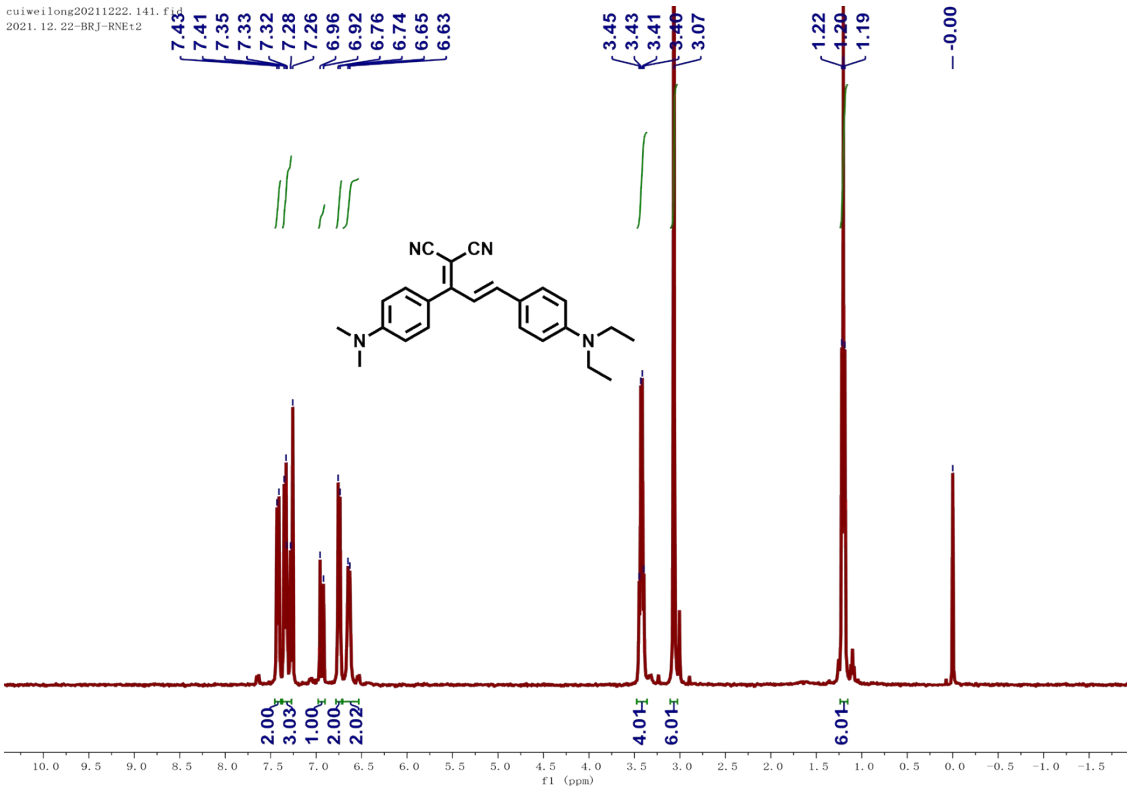

Fig. S5 [1]H NMR spectrum of compound **LDCN** in Chloroform-*d*.

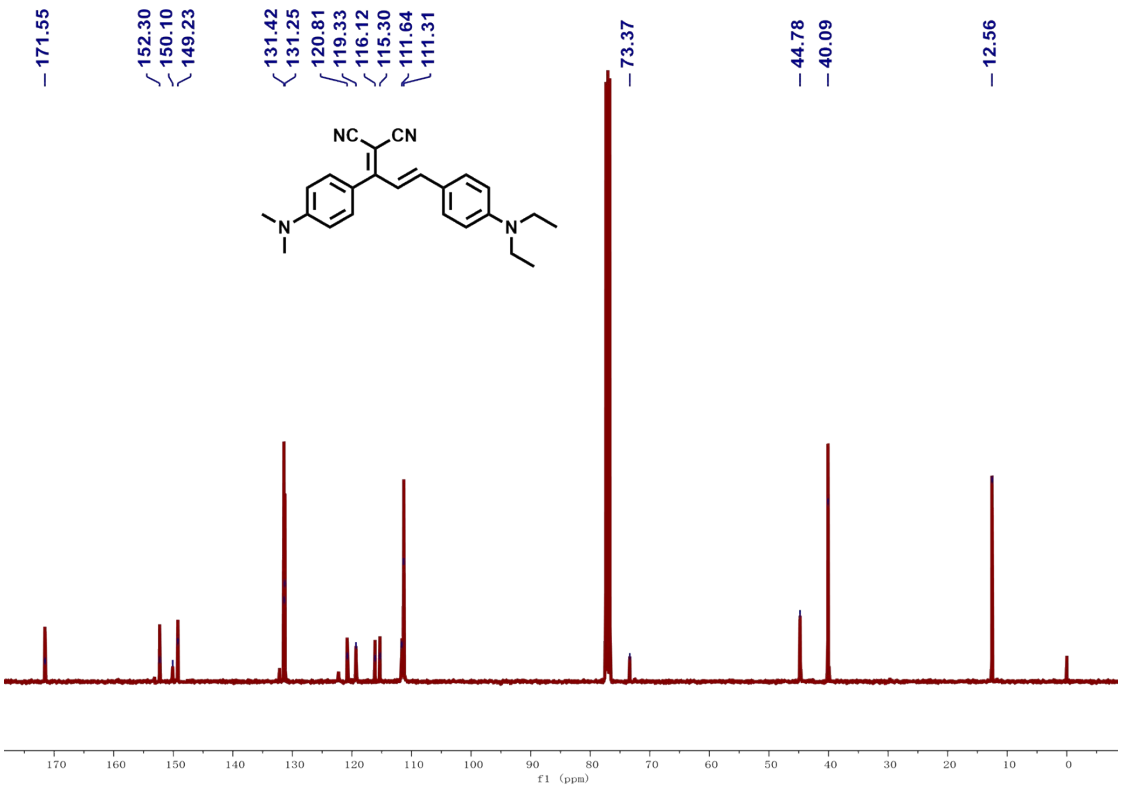

Fig. S6 [13]C NMR spectrum of **LDCN** in Chloroform-*d*.

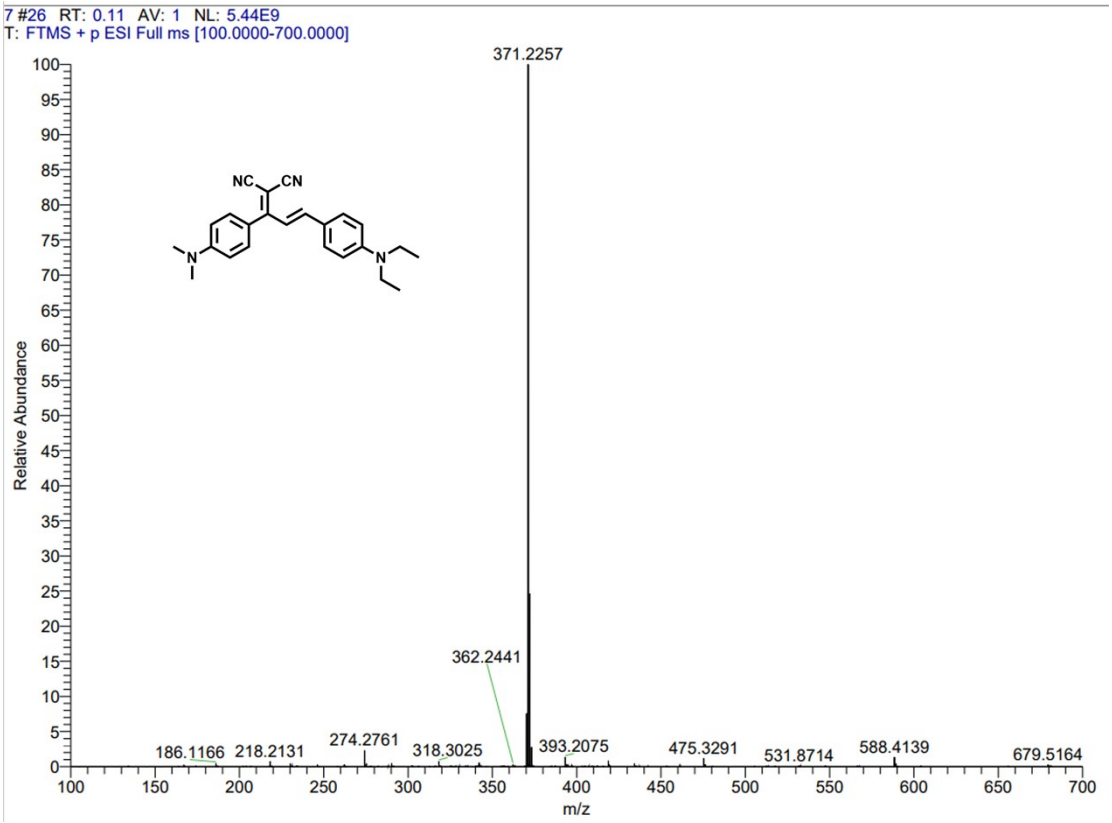

Fig. S7 HR-MS spectrum of **LDCN** in CH_3OH.