Supporting Information for

Development of a viscosity sensitive fluorescent probe for real-time monitoring of mitochondria viscosity

Kai Zhou, Mingguang Ren, Beibei Deng, Weiying Lin*

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

Email: weiyinglin2013@163.com

*Correspondence to: Weiying Lin, Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, P.R. China. Email: weiyinglin2013@163.com.

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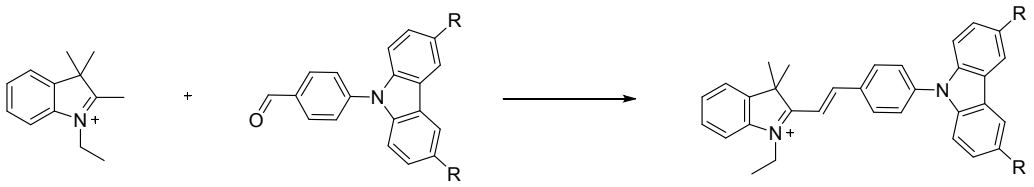
Synthesis



Scheme S1 Synthesis of fluorescent viscosity probes of **MHC-V1** and **MHC-V2**

Synthesis of compound MHC-V1

The crude product **1** (540 mg, 2 mmol,1.1 eq) and Compound **3** (500 mg, 1.84 mmol,1.0 eq) were dissolved in N,N-dimethylformide（DMF）(2 mL), and the mixture was refluxed for 6 h at 90℃ under a nitrogen atmosphere. After completion of the reaction, the mixture was then cooled to room temperature, poured into water, extracted with dichloromethane，washed with water and brine, dried over sodium sulfate, and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (petroleum ether to methanol / dichloromethane = 1: 20, v/v) to afford the compound **MHC-V1** as a red powder (380 mg, yield: 47%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (d, J = 16.3 Hz, 1H), 8.58 (d, J = 8.6 Hz, 2H), 8.31 (d, J = 7.7 Hz, 2H), 8.00 (m, J = 6.1, 3.0 Hz, 1H), 7.95 (t, J = 4.3 Hz, 2H), 7.93 (s, 1H), 7.85 (d, J = 16.4 Hz, 1H), 7.71 – 7.65 (m, 2H), 7.57 (d, J = 8.2 Hz, 2H), 7.54 – 7.47 (dd, 2H), 7.36 (dd, J = 10.9, 3.9 Hz, 2H), 4.81 (q, J = 7.1 Hz, 2H), 1.87 (s, 6H), 1.55 – 1.47 (t, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 181.99 , 153.26 , 144.61 , 141.57 , 140.90 , 139.98 , 133.66 , 133.10 , 130.07 , 129.64 , 127.11 , 127.01 , 123.77 , 123.66 , 121.26 , 121.19 , 115.80 , 113.45 , 110.40 , 55.41 , 52.91 , 26.00 , 14.39. HRMS (ESI) m/z calcd for $C_{32}H_{29}N_2^+$ [M]: 441.2325; Found 441.2328.

Synthesis of compound MHC-V2

The crude product **1** (410 mg, 1.5 mmol, 1.3 eq) and Compound **3** (450 mg, 1.2 mmol, 1.0 eq) were dissolved in DMF (2 mL), and the mixture was refluxed for 6 h at 90 ℃under a nitrogen

atmosphere. After completion of the reaction , the mixture was then cooled to room temperature, poured into water, extracted with dichloromethane，washed with water and brine, dried over sodium sulfate, and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (petroleum ether to methanol / dichloromethane = 1: 20, v/v) to afford the compound **MHC-V2** as a red powder (285 mg, yield: 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (d, J = 16.2 Hz, 1H), 8.55 (d, J = 8.5 Hz, 2H), 8.35 (d, J = 1.5 Hz, 2H), 8.01 – 7.97 (m, 1H), 7.94 (t, J = 4.2 Hz, 2H), 7.92 (s, 1H), 7.83 (d, J = 16.3 Hz, 1H), 7.68 (dd, J = 9.3, 5.2 Hz, 2H), 7.55 (dd, J = 8.7, 1.7 Hz, 2H), 7.50 (d, J = 8.7 Hz, 2H), 4.80 (q, J = 7.1 Hz, 2H), 1.87 (s, 6H), 1.50 (t, J = 7.2 Hz, 3H), 1.44 (s, 18H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 181.95 , 153.45 , 144.58 , 143.88 , 140.91 , 138.26 , 133.18 , 133.10 , 129.99 , 129.63 , 126.45 , 124.45 , 124.01 , 123.65 , 117.41 , 115.75 , 113.11 , 109.92 , 52.86 , 42.84 , 35.04 , 32.26 , 26.04 , 14.40 ; HRMS (ESI) m/z calcd for $C_{40}H_{45}N_2^+$ [M]: 553.3577; Found 553.3572.

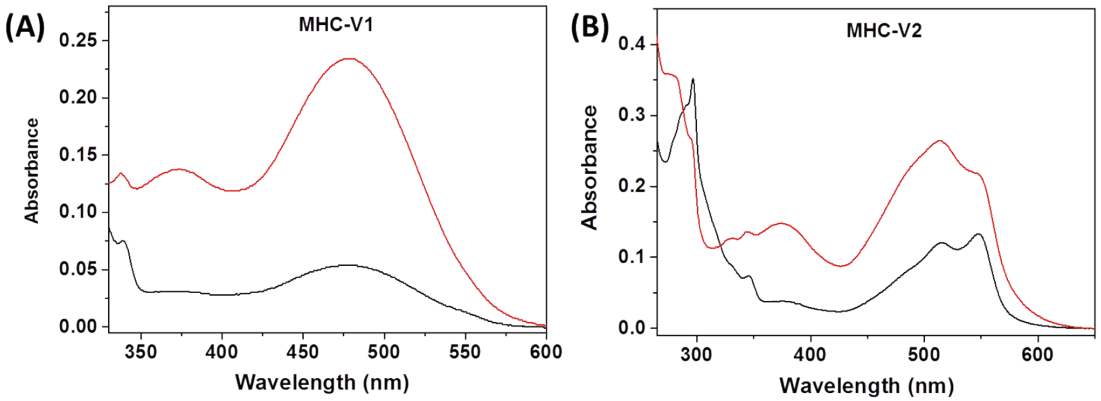


Figure S1 The absorption spectra of probes (A) **MHC-V1** (10 μM) and (B) **MHC-V2** (10 μM) in the solution of ethanol-glycerol systems, pure ethanol (black line) and 90 % glycerol (red line).

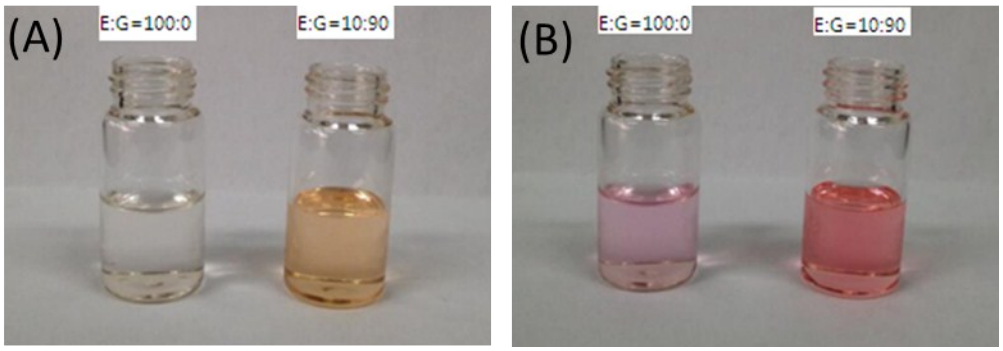


Figure S2 The color changes of the probes **MHC-V1** (A) and **MHC-V2** (B) solutions (0.5 mM) in different viscosity solutions.

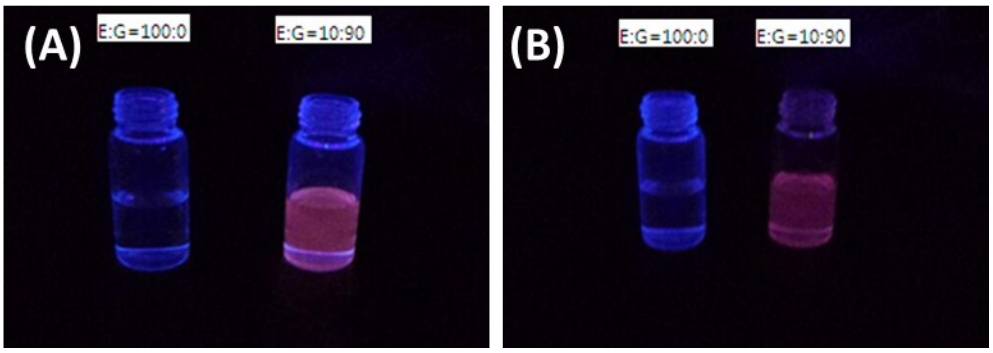


Figure S3 The fluorescence changes of the probes **MHC-V1** (A) and **MHC-V2** (B) solutions (0.5 mM) in different viscosity solutions with 365 nm ultraviolet light.

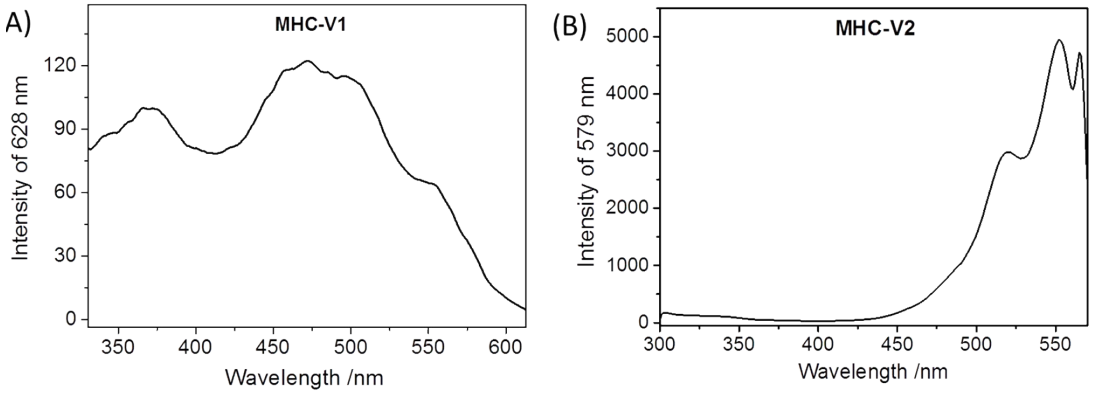


Figure S4 The excitation spectra of the probes (A) **MHC-V1** (10 μM) and (B) **MHC-V2** (10 μM) in the solution of ethanol-glycerol systems.

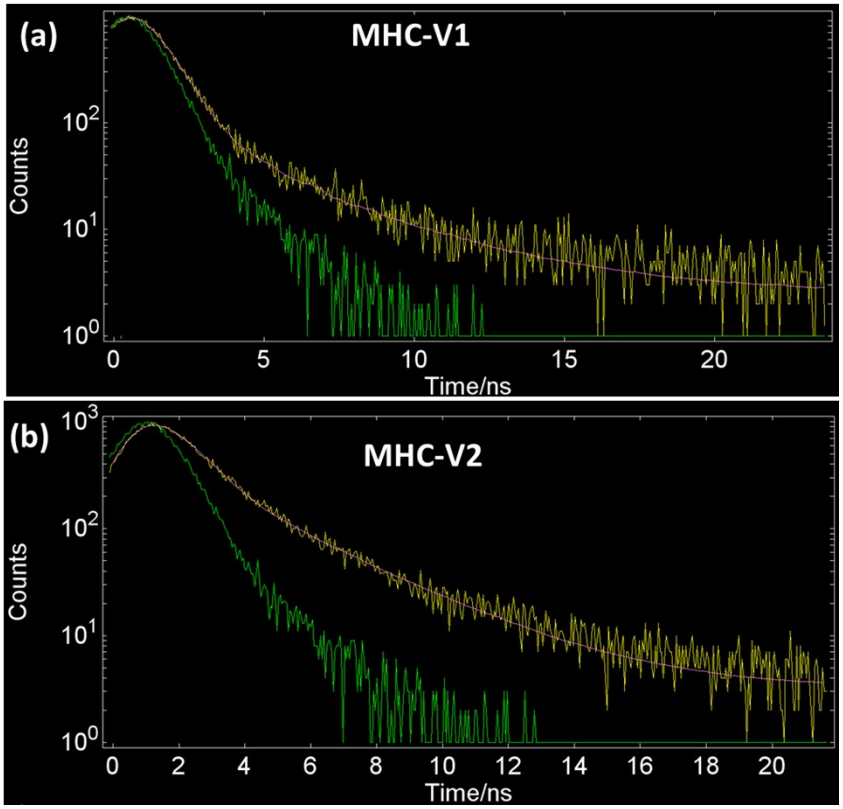


Figure S5 The fluorescence decay of the probes (A) **MHC-V1** (10 μM) and (B) **MHC-V2** (10 μM) with the variation of solution viscosity (ethanol/glycerol system), pure ethanol (green line) and 90 % glycerol (yellow line).

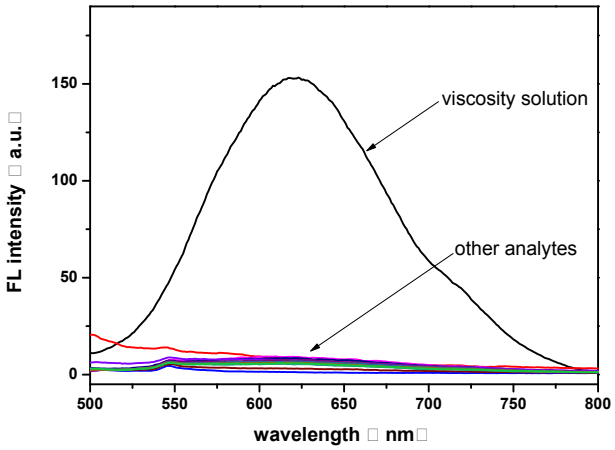


Figure S6 The fluorescence spectra changes of probe **MHC-V1** (10 μM) in the presence of various analytes (50 μM).

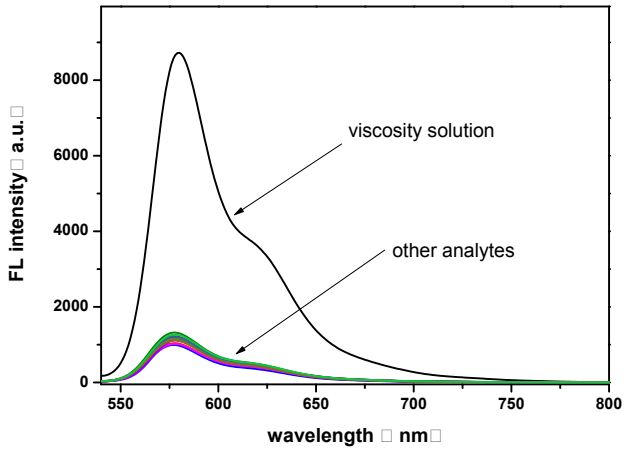


Figure S7 The fluorescence spectra changes of probe **MHC-V2** (10 μM) in the presence of various analytes (50 μM).

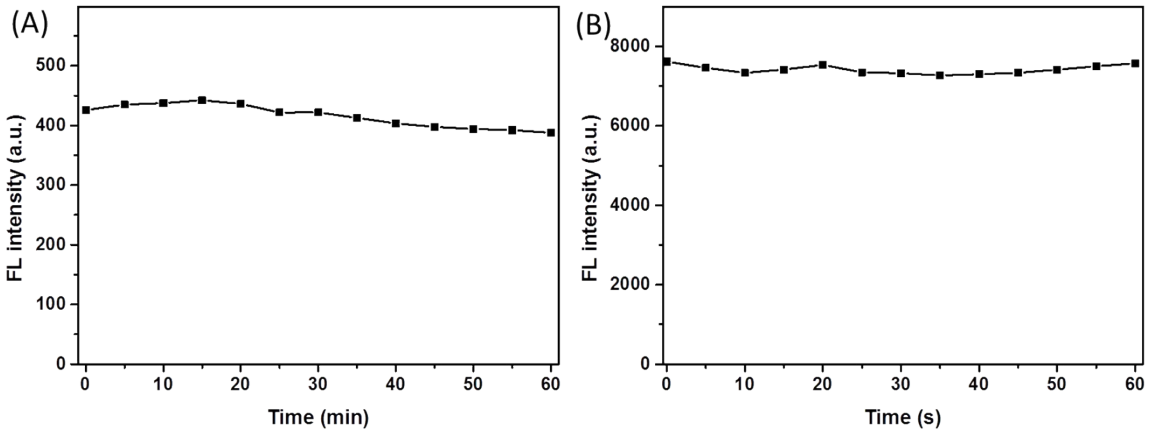


Figure S8 Photostability profiles of the free probes **MHC-V1** and **MHC-V2** (10 μM) in the presence of UV-irradiated (365 nm). The fluorescence intensities at 628 nm for **MHC-V1** and 579 nm for **MHC-V2** were continuously monitored at time intervals in 90 % glycerol. Time points represent 0 - 60 min.

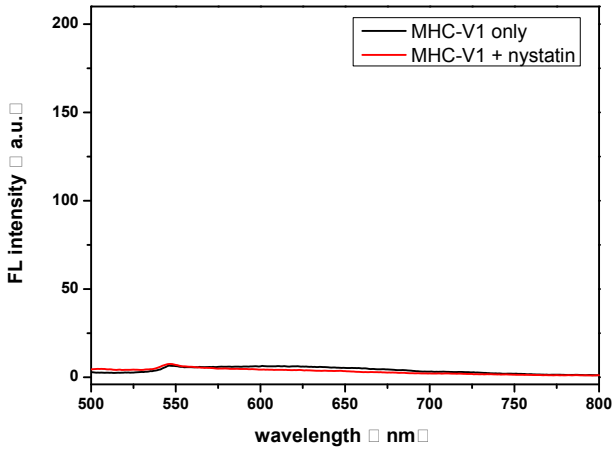


Figure S9 Fluorescence spectra of probe **MHC-V1** (10 μM) in the absence and presence of nystatin (60 μM) in PBS (30 % DMF) at 37℃ for 30 min.

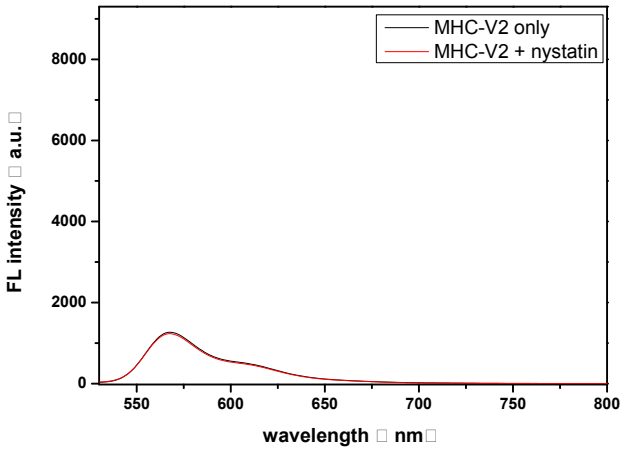


Figure S10 Fluorescence spectra of probe **MHC-V2** (10 μM) in the absence and presence of nystatin (60 μM) in PBS (30 % DMF) at 37℃ for 30 min.

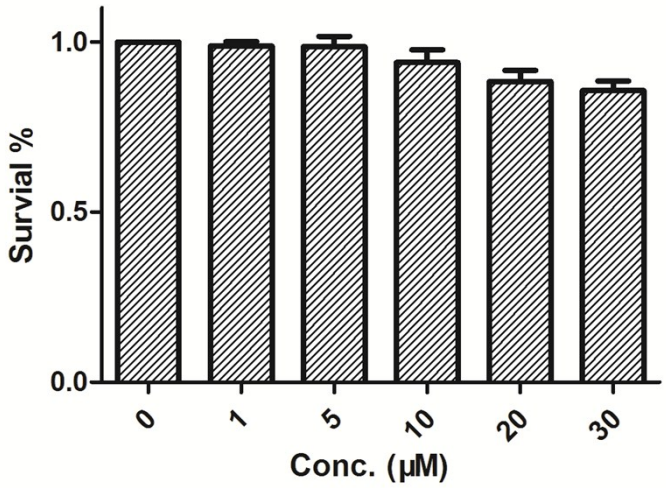


Figure S11 Cytotoxicity assays of **MHC-V1** at different concentrations for HeLa cells

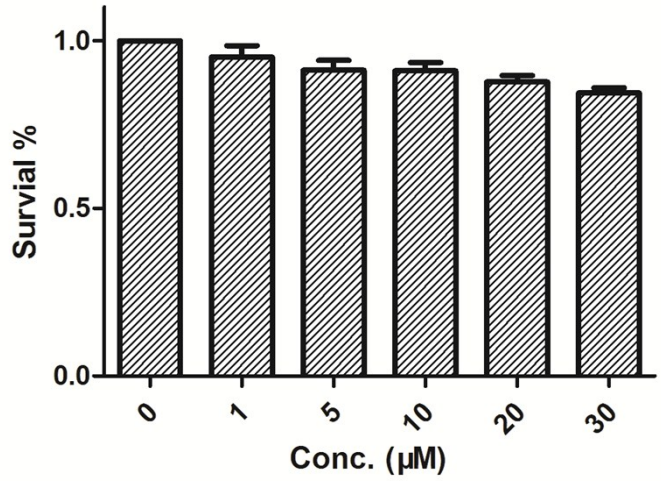


Figure S12 Cytotoxicity assays of **MHC-V2** at different concentrations for HeLa cells

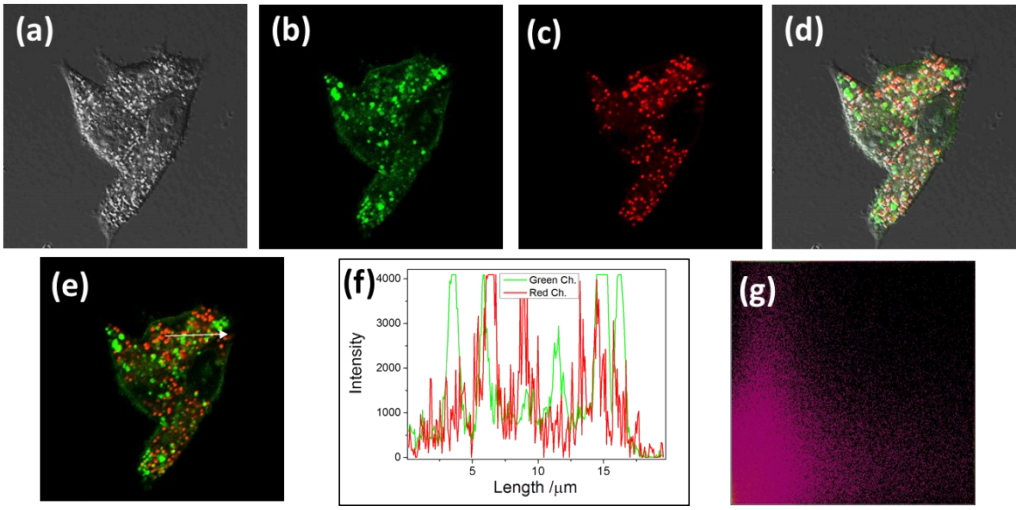


Figure S13 Brightfield and fluorescence images of HeLa cells stained with the probe **MHC-V2** (10 μM) and lysosome dye. a) brightfield image; b) from green channel (lysosome staining); c) from the red channel (fluorescence image viscosity with **MHC-V2**); d) overlay of brighfield, green and red channels; e) overlay of green and red channels; f) Intensity profile of linear region of interest across the HeLa cell costained with green channel of Lyso Tracker green and red channel of **MHC-V2** imaging of viscosity ; g) Intensity scatter plot of green and red channels.

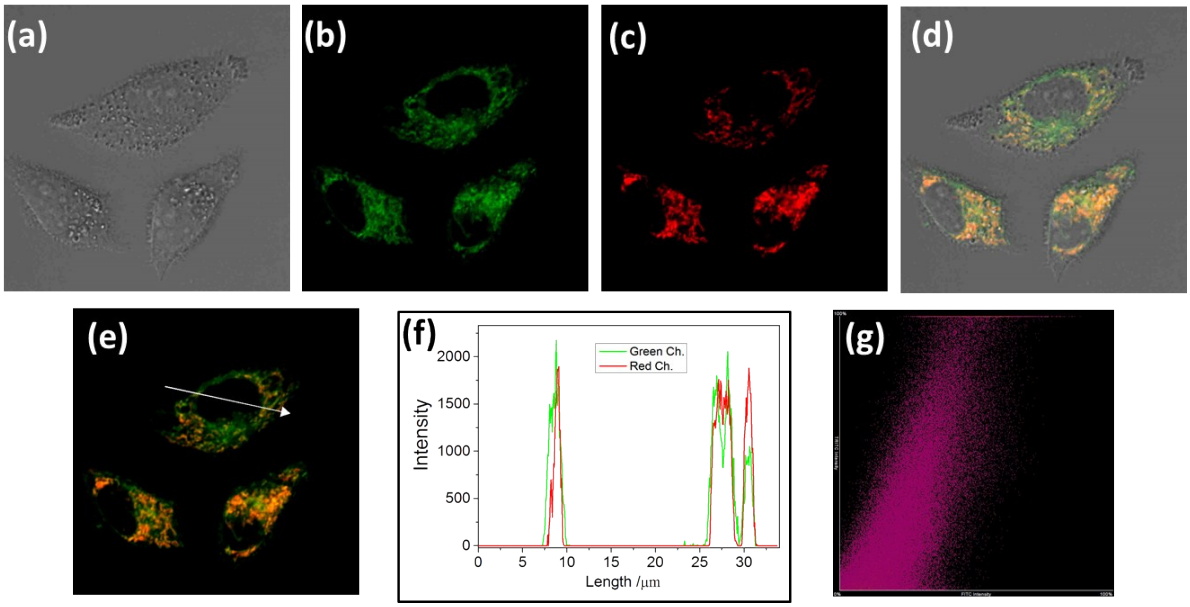


Figure S14 Brightfield and fluorescence images of HeLa cells stained with the probe **MHC-V1** (10 μM) and mitochondrial dye. a) brightfield image; b) from the green channel

(mitochondria staining); c) from red channel (fluorescence image viscosity with **MHC-V1**); d) overlay of brighfield, green and red channels; e) overlay of green and red channels; f) Intensity profile of linear region of interest across the HeLa cell costained with red channel of **MHC-V1** imaging of viscosity and green channel of Mito Tracker Green ; g) Intensity scatter plot of green and red channels.

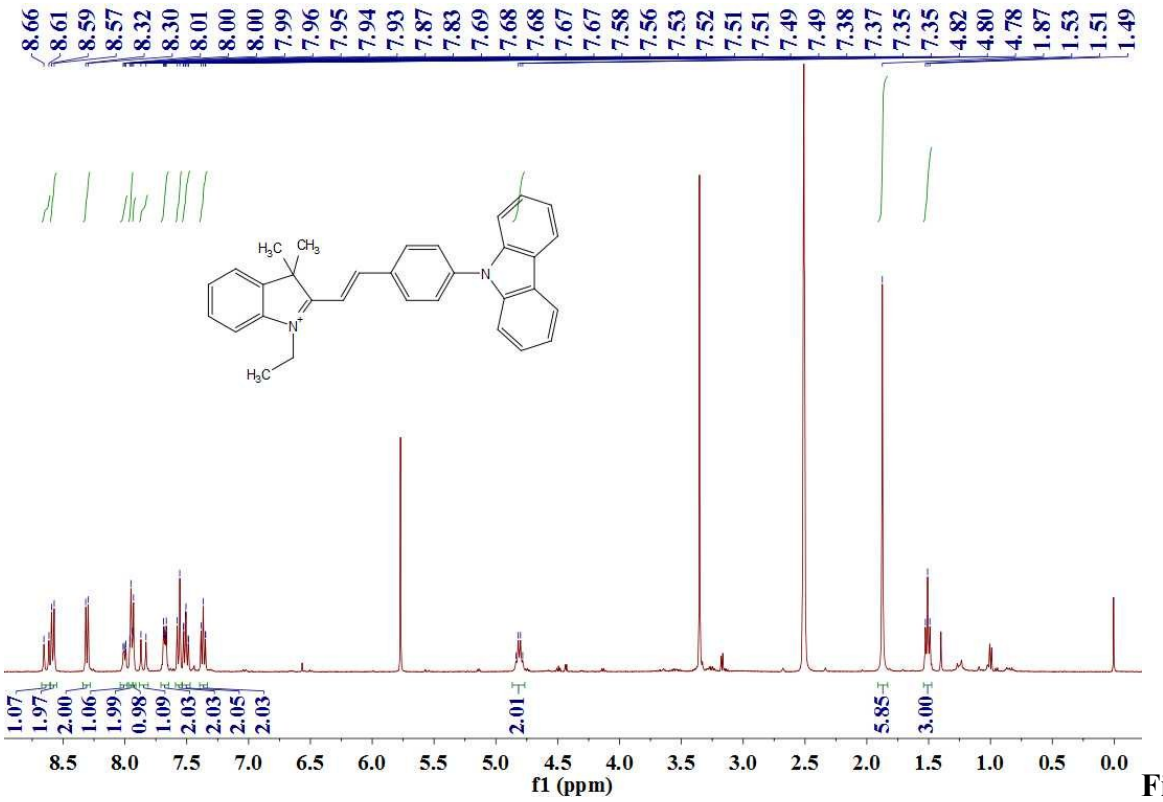


Figure S15 ^{1}H-NMR (DMSO-d_6) spectrum of **MHC-V1.**

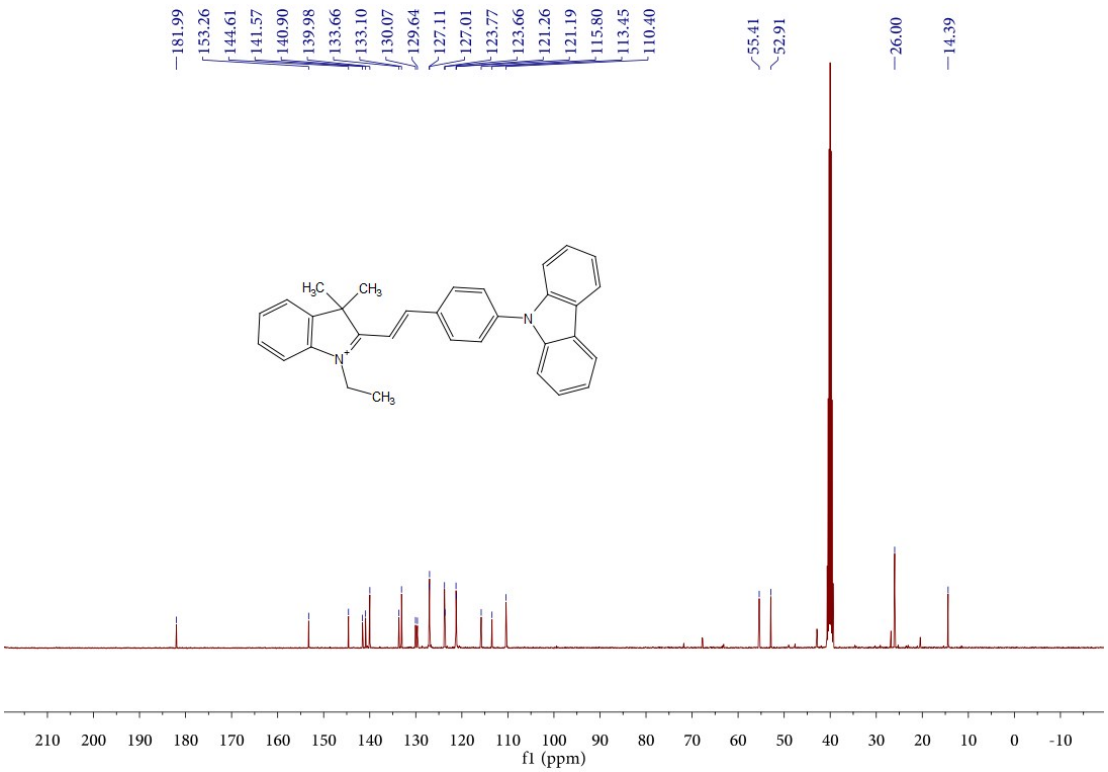


Figure S16 ^{13}C-NMR (DMSO-d_6) spectrum of **MHC-V1.**

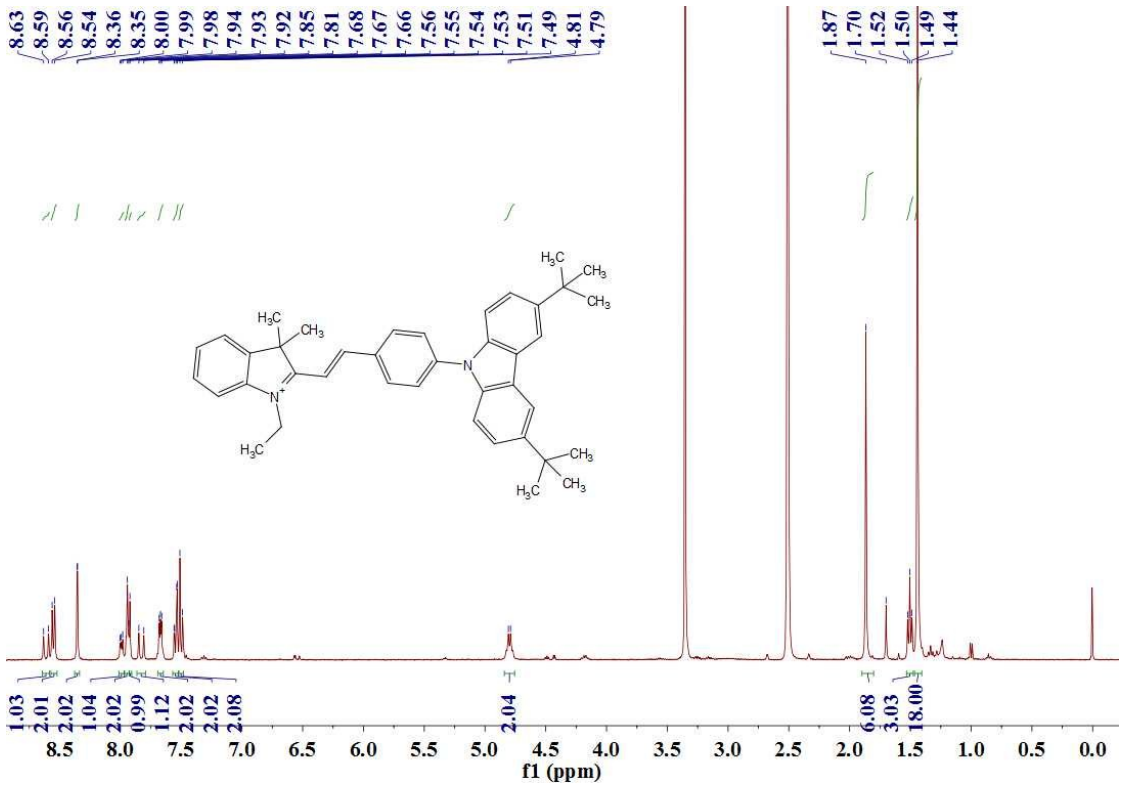


Figure S17 ^{1}H-NMR (DMSO-d_6) spectrum of **MHC-V2**.

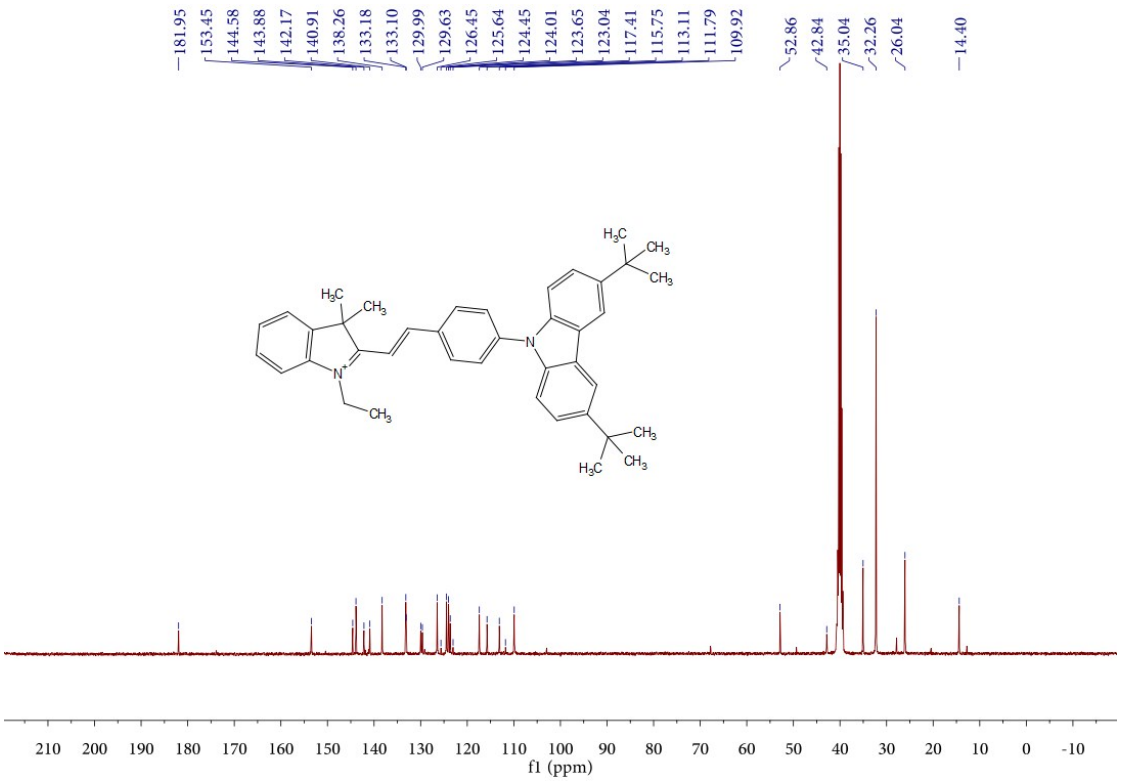


Figure S18 ^{13}C-NMR (DMSO-d_6) spectrum of **MHC-V2.**

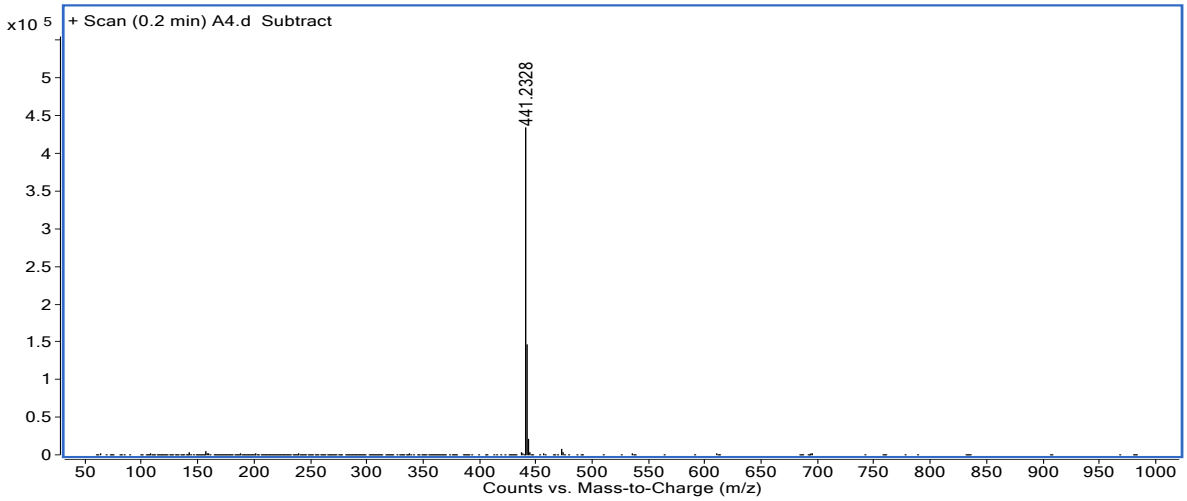


Figure S19 HRMS (ESI) spectrum of **MHC-V1.**

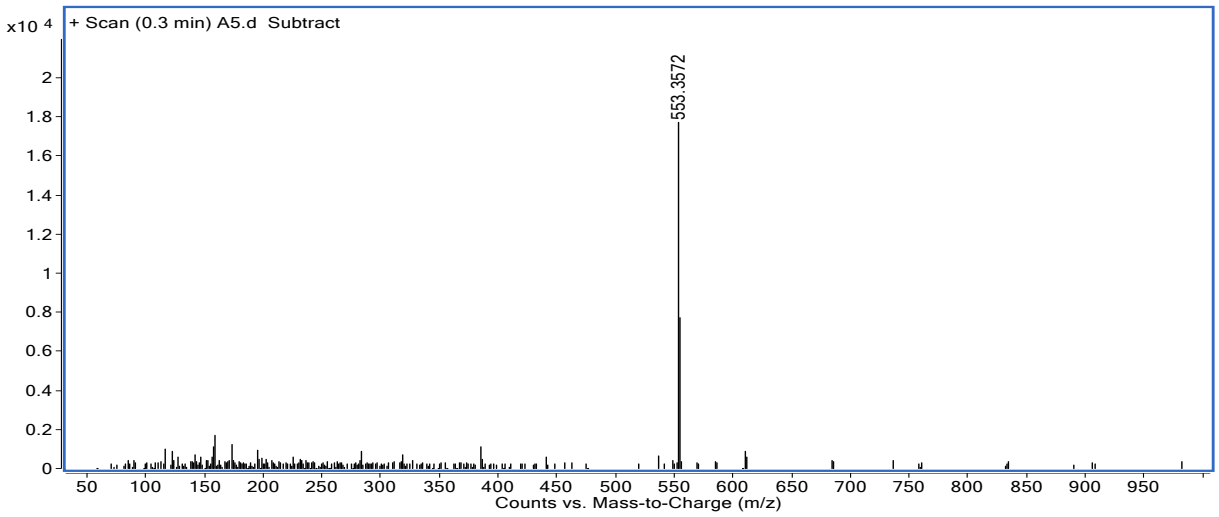


Figure S20 HRMS (ESI) spectrum of **MHC-V2.**