# Live-Cell Imaging of Nucleolus and Mapping Mitochondrial Viscosity with a Dual Function Fluorescent Probe

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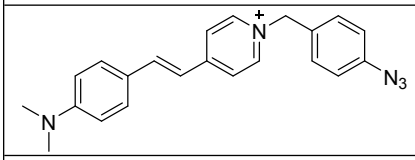
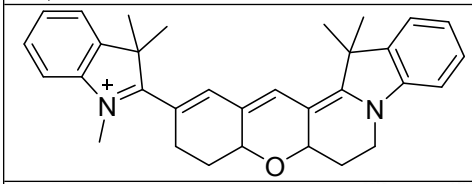
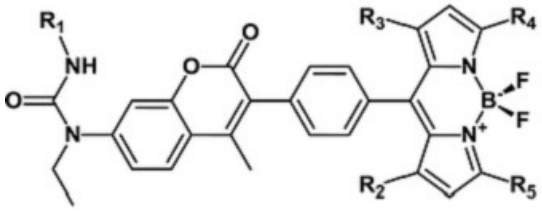
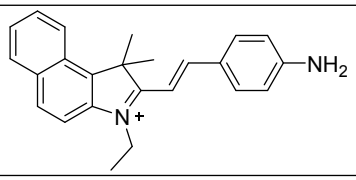
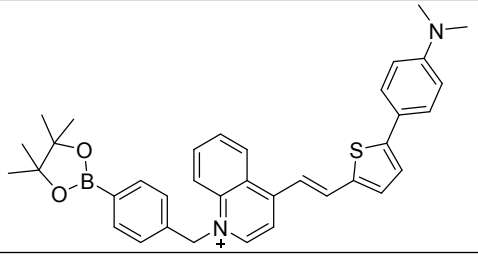
Table S1: Current state of art. (Mitochondria/ dual imaging)

| **Some recently studied Fluorophores** | Synthetic steps | Key Features | General Application |
|---|---|---|---|
|  | Three step synthesis | 10 fold intensity enhancement Viscosity (ethanol-glycerol mixtures)[1] | Mitochondrial viscosity measurement $H_2S$ sensing |
|  | Two step synthesis | 37 times enhancement viscous solution[2] | Mitochondrial viscosity measurement |
|  | Multi-step synthesis | 40 folds intensity in 100% Glycerol[3] | Mitochondrial viscosity measurement |
|  | 3 steps | Viscosity increment 66 times in high viscous solution[4] | Mitochondrial viscosity measurement Role in inflammation |
|  | 3 steps | Viscosity increments, (~7-8 folds)[5] | Mitochondrial viscosity $H_2O_2$ detection |

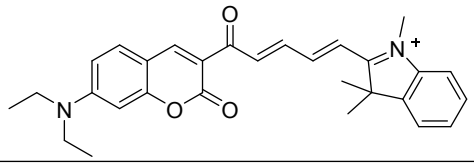
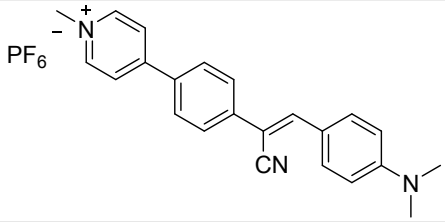
| | | | |
|---|---|---|---|
|  | 3 steps | 22 fold intensity[6] | Mitochondrial viscosity |
|  | 3 steps | No viscosity study[7] | nucleolus and mitochondria imaging |
| (Current Study) | 2 steps | 155 fold viscosity enhancement | Mitochondrial and nucleolus imaging Two-photon compatability |

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Table S1a: Viscosity values of water-glycerol mixtures used in our experiments[8, 9] This can be calculated using the website: http://www.met.reading.ac.uk/~sws04cdw/viscosity_calc.html

| Glycerol percentage | Viscosity value (cP) at 30 °C |
|---|---|
| 0 | 0.79 |
| 20 | 1.52 |
| 40 | 3.492 |
| 60 | 10.304 |
| 80 | 49.694 |
| 100 | 597.92 |



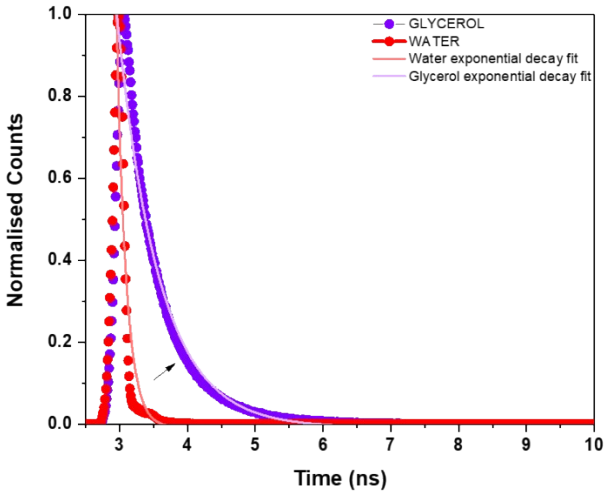



**Fig. S1.** Lifetime decay curve for the probe, Mito-Nucleo-VS in homogenous glycerol and water media (the arrow marked showed the significance difference between the two lifetimes)

**pH dependence**

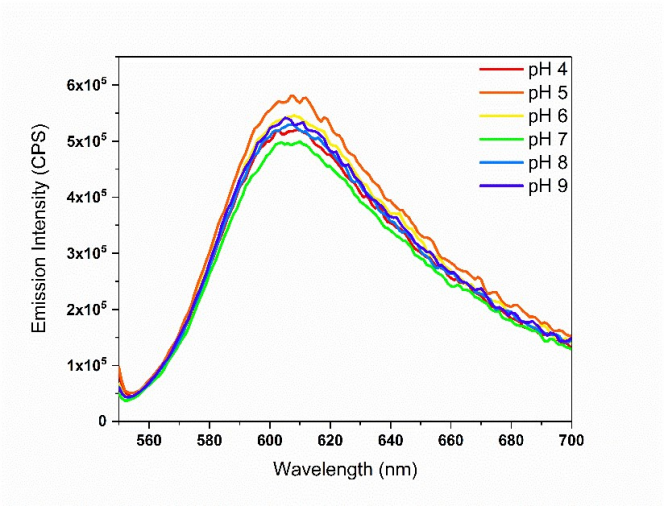



**Fig. S2. Effect of pH on the** emission spectra of the probe, Mito-Nucleo-VS.

**Cell Viability Experiment (MTT Assay)**

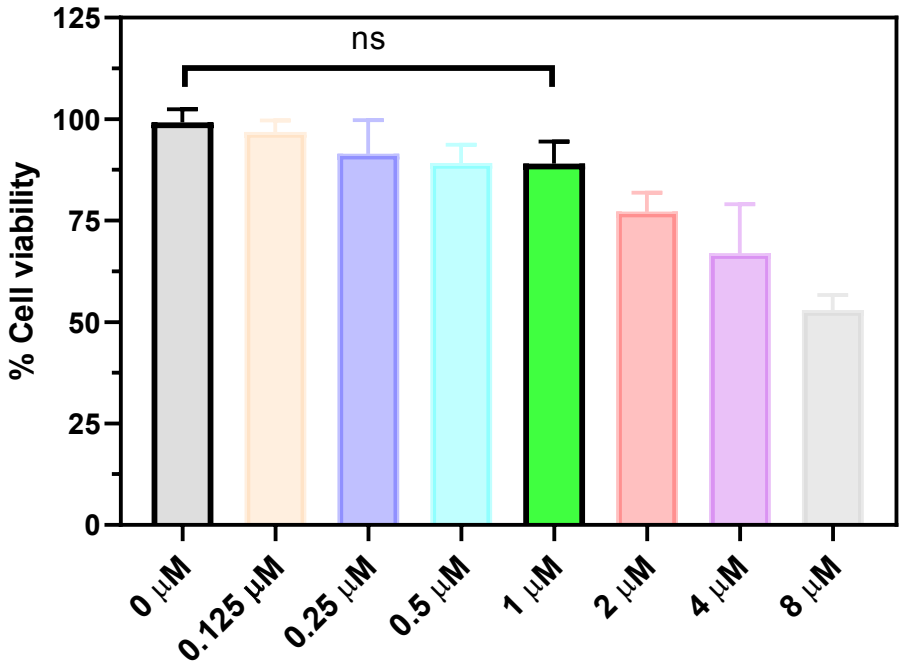



**Fig. S3**. Assessment of Mito-Nucleo-VS's cytotoxicity at various concentrations using the MTT test. Cells without the treatment of probe is considered as negative control set (0 μM). The changes in % cell viability between the sets of negative control and at imaging concentration (1 μM) found to be non-significant (after 24 h of probe incubation).

**Probe binding to mitochondria (Mitochondrial membrane potential impairment experiment)**

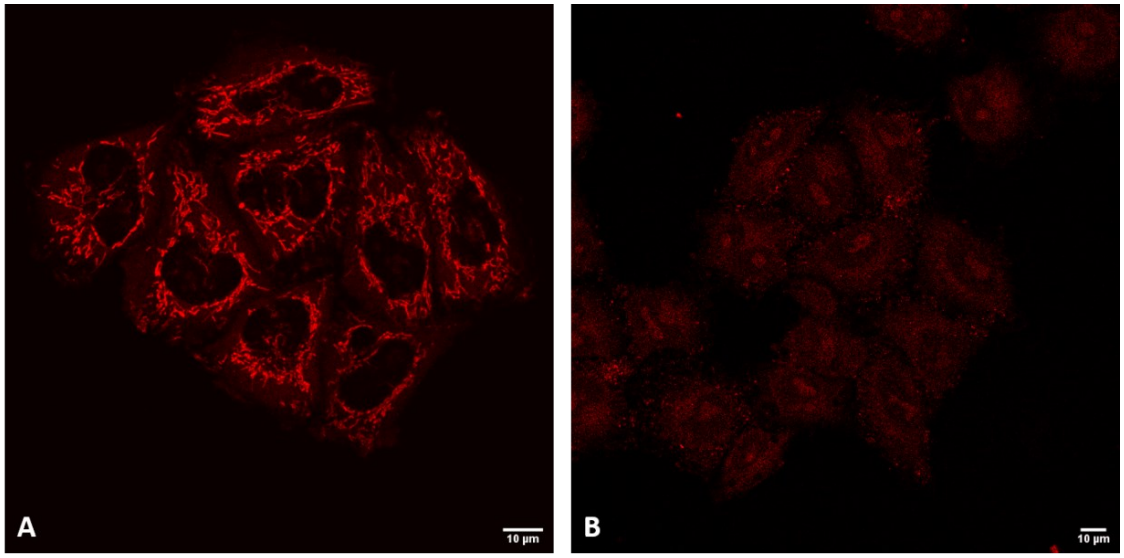



**Fig.** S**4** (A) Cell incubated with Mito-Nucleo-VS probe without CCCP, (B) Cells treated with CCCP prior to the probe incubation

**Probe's sensitivity towards mitochondrial viscosity during apoptosis**

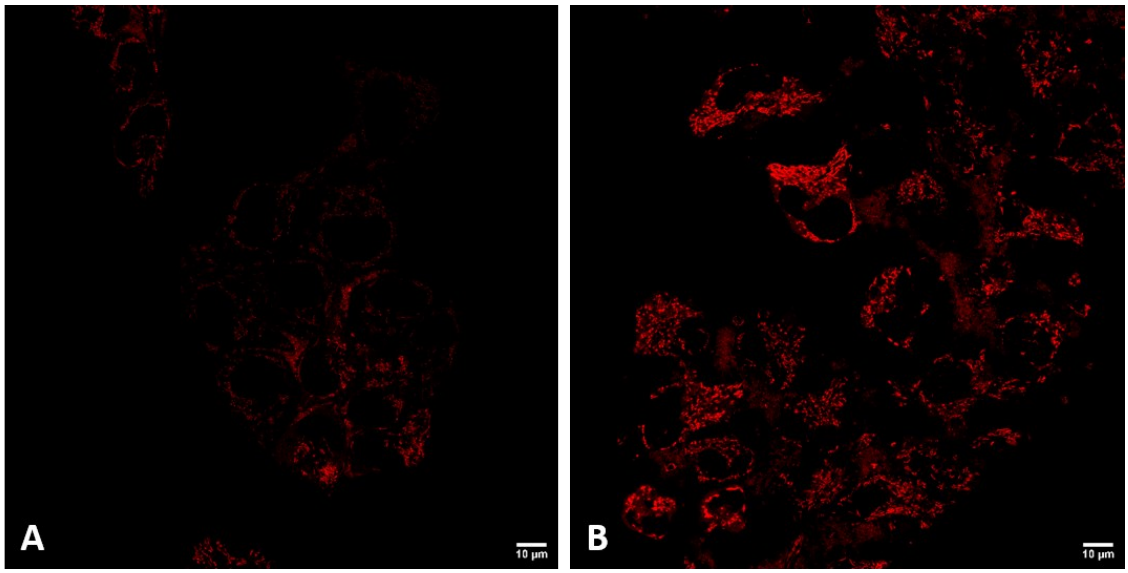



**Fig. S5** (A) Cell incubated with 5 µM staurosporine for 1.5 h prior to Mito-Nucleo-VS probe, (B) Cells treated with Mito-Nucleo-VS probe alone

**Probe's sensitivity towards mitochondrial viscosity during inflammation**

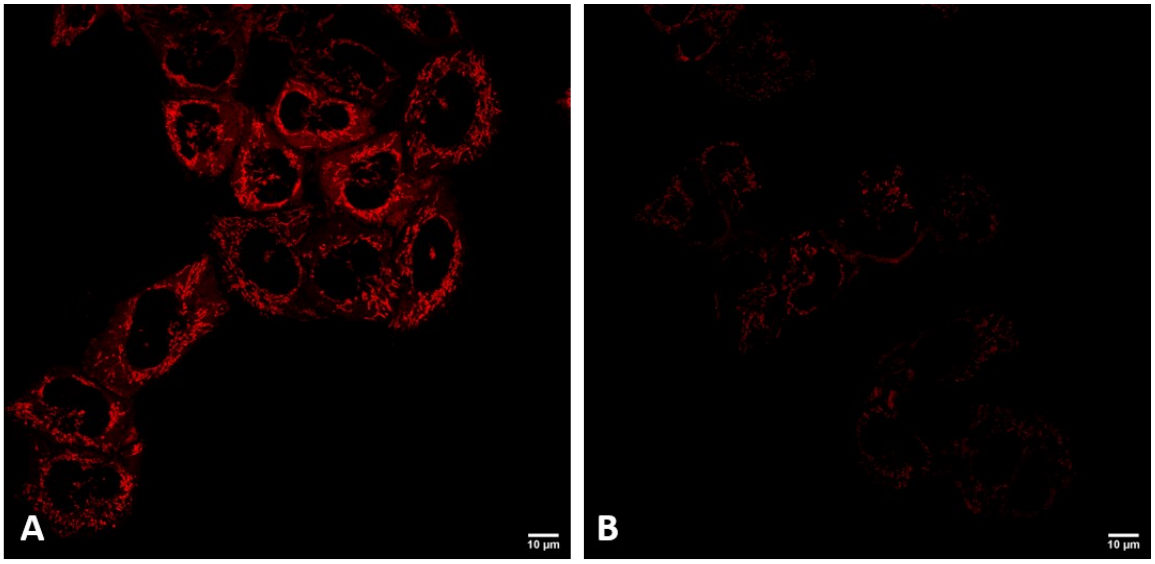



**Fig. S6** (A) Cell incubated with 10 µg/mL LPS for 10 hours prior to Mito-Nucleo-VS probe, (B) Cells treated with Mito-Nucleo-VS probe alone

**Comparison between neutral and cationic derivative for nucleolus binding**

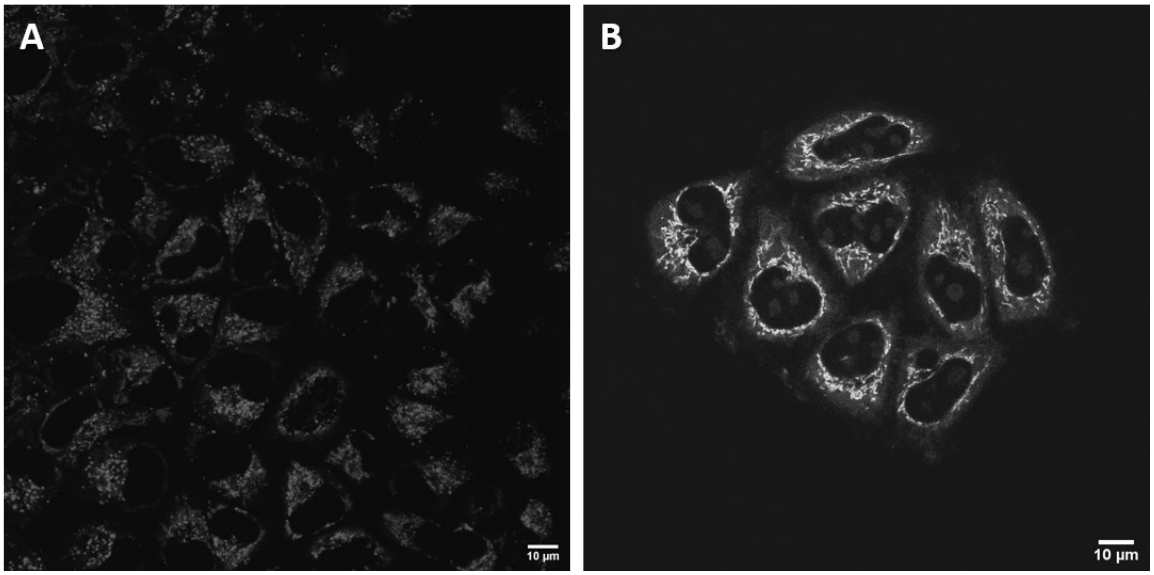



**Fig. S7** Grayscale single plane image of (A) Cell incubated with **1** (neutral derivative of Mito-Nucleo-VS), (B) Cells treated with Mito-Nucleo-VS probe

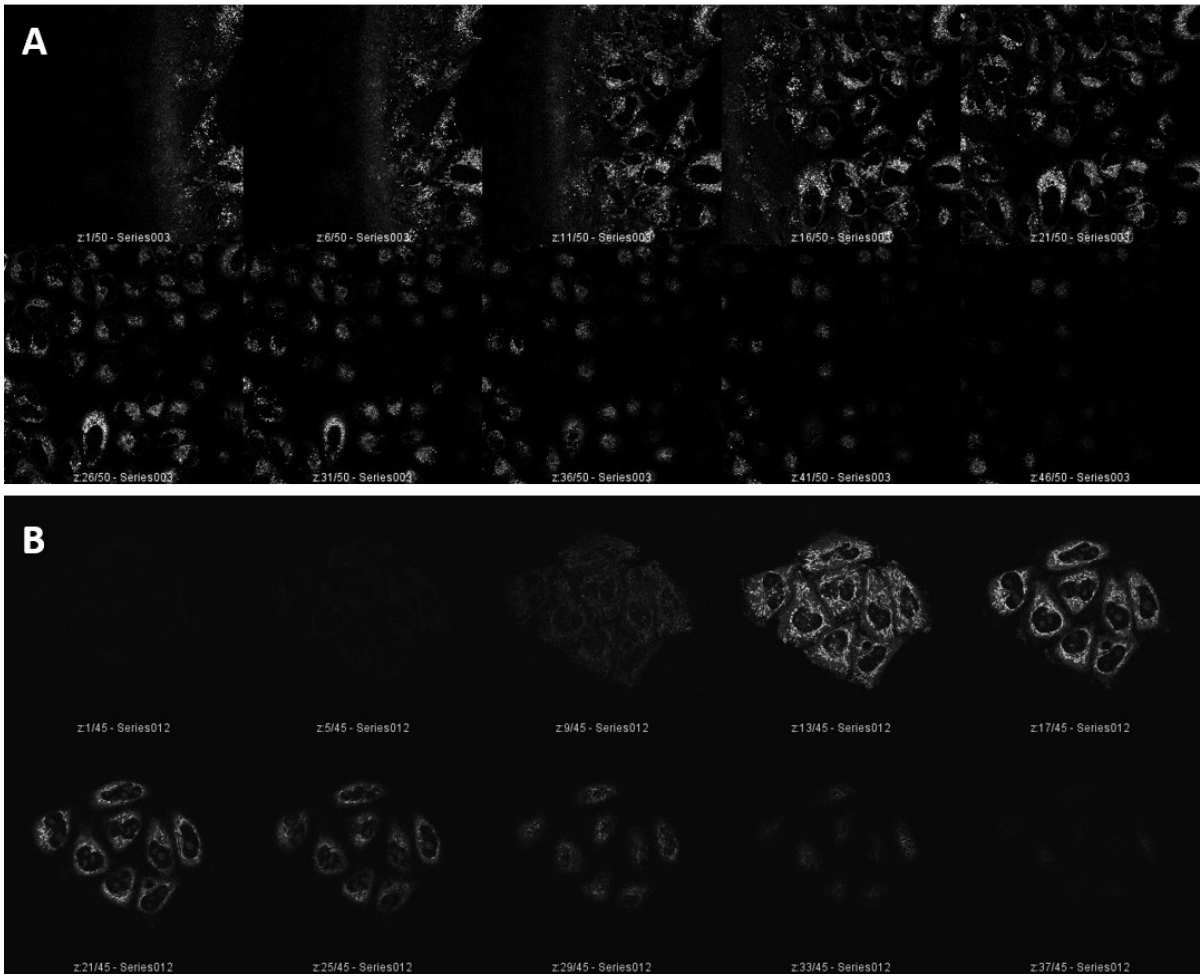



**Fig. S8** Grayscale montage of different z-axis planes of (A) Cell incubated with 1 (neutral derivative of Mito-Nucleo-VS) and (B) Cells treated with Mito-Nucleo-VS probe

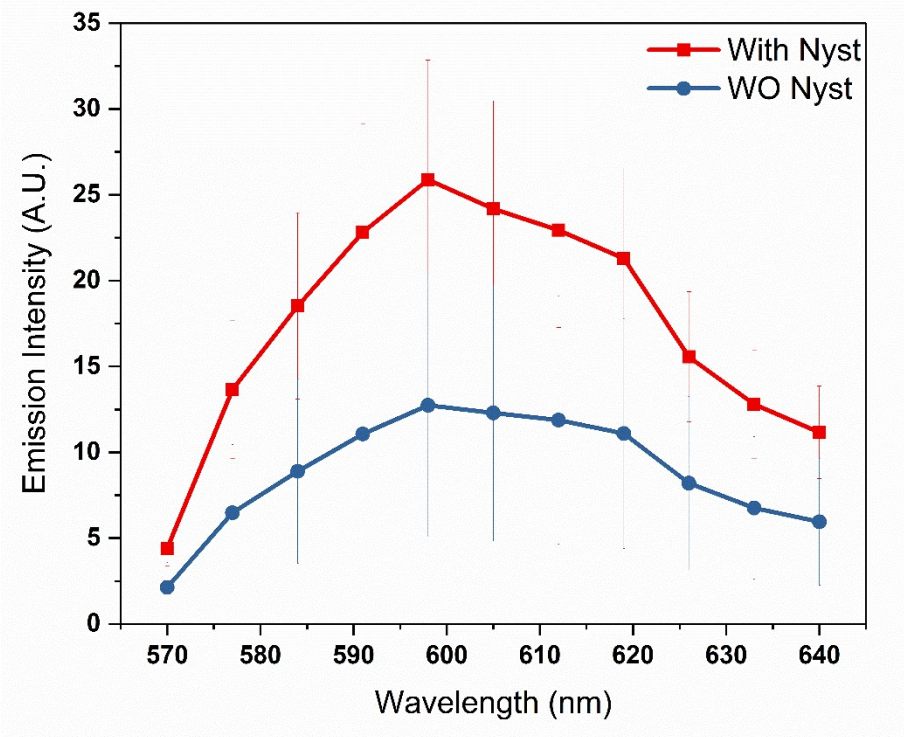



**Fig. S9** Lambda scanning to showcase the impact of nystatin on mitochondrial viscosity dependent intensity changes (excitation wavelength: 561 nm, Emission scan: 570-640 nm on the xyλz scan, no of cells (n) = 25]

## Lowering LPS concentration for the inflammation related probe's response

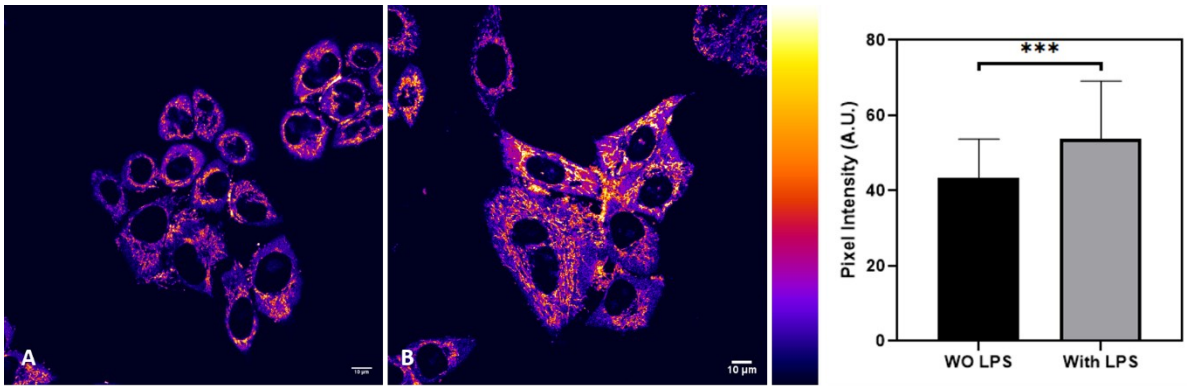
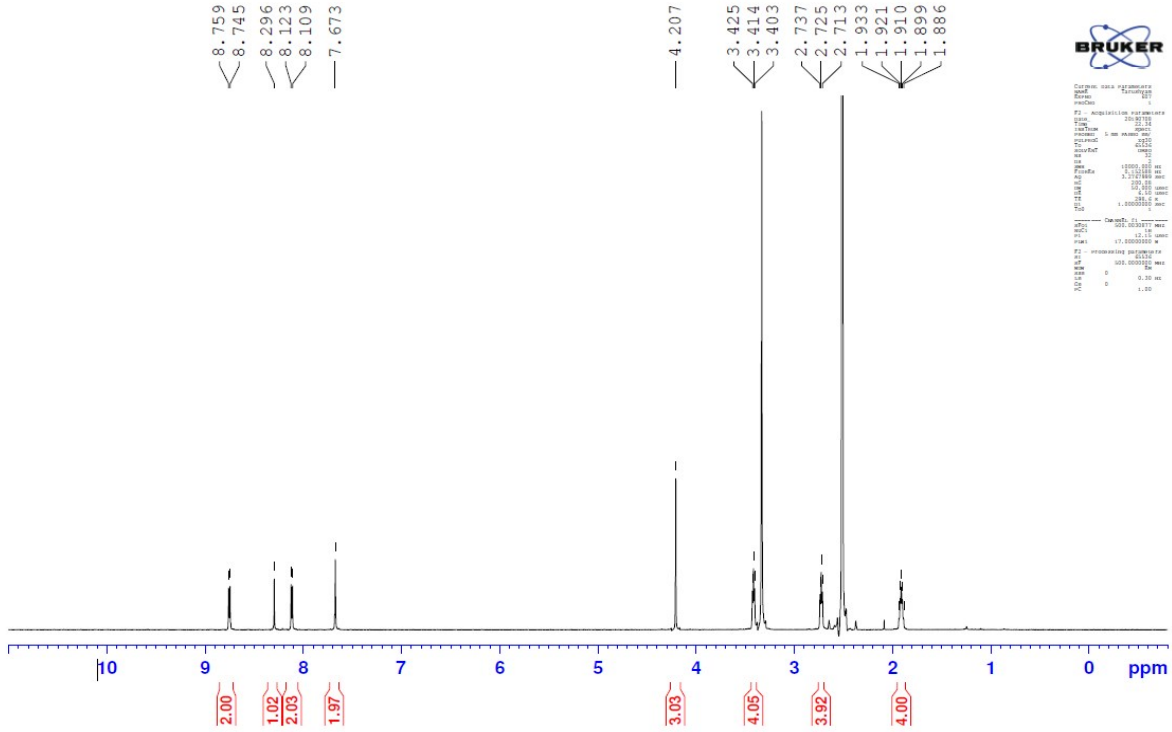


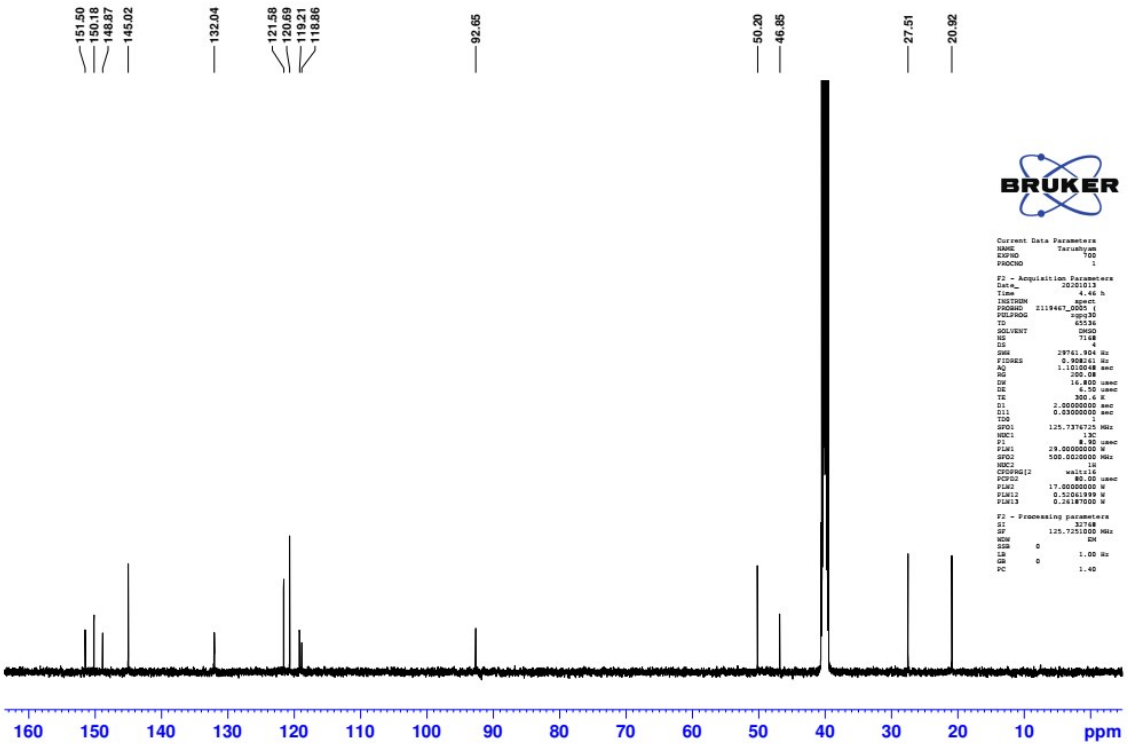

**Fig. S10** Effect on inflammation on mitochondrial viscosity (A. HeLa cell without treatment of LPS, B. HeLa cells were treated with 1.5 µg/mL LPS for 1.5 hour);[no of cells (n) = 40]

## Spectral Characterization

julolidine cation




Julo-WCN-N+-C13-12102020



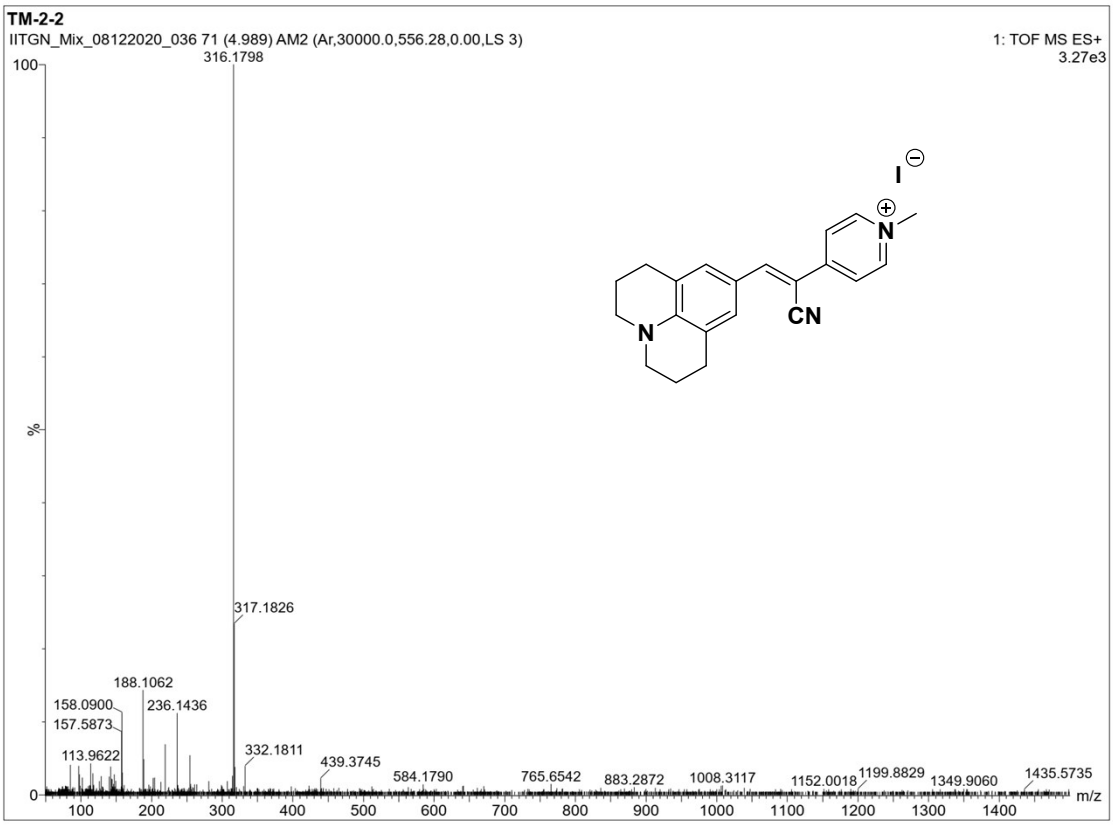



**Fig. S10.** $^{1}H$ and $^{13}C$ NMR and HRMS spectra of Mito-Nucleo-VS. [Theoretical mass (m/z): 316.1808, Obtained mass (m/z): 316.1798]

References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