Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Targetable and Fixable Rotor for Quantifying Mitochondrial Viscosity of Living Cells by Fluorescence Lifetime Imaging

Xinbo Song, Ning Li, Chao Wang, and Yi Xiao

Localization stabilities of Vis-A and Vis-B in mitochondria

As is shown in Figure 1 and Figure 2, Colocalization studies of **Vis-A** and **Vis-B** with Mito Tracker Deep Red (Commercial mitochondrial tracker) have been conducted in 7721 cells, Hela cells, and MCF-7 cells, confirming that the both probes can specially localize in the mitochondria of the living cells.

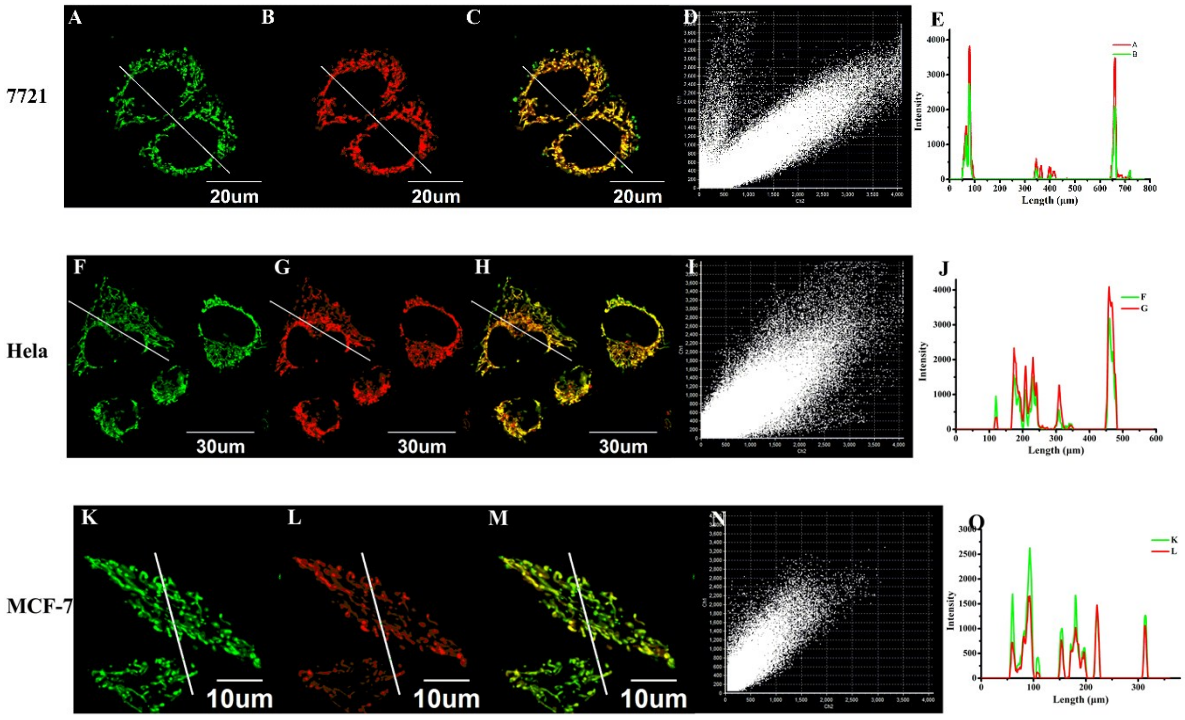


Figure 1. Colocalization imaging studies of **Vis-A** in 7721, Hela, and MCF-7 cells. A, F, and K **Vis-A** Channel 1: λ_{ex}=488nm, λ_{em}=500-550nm. B, G, and L Mito Tracker Deep Red Channel 2: λ_{ex}=635nm, λ_{em}=655-755nm; C overlay of A and B (Pearson coefficient 0.92). H overlay of F and G (Pearson coefficient 0.95). M overlay of K and L (Pearson coefficient 0.95). D, I, and N: Correlation of **Vis-A** and Mito Tracker Deep Red. E, J, and O: Intensity profile of ROIs across the cells.

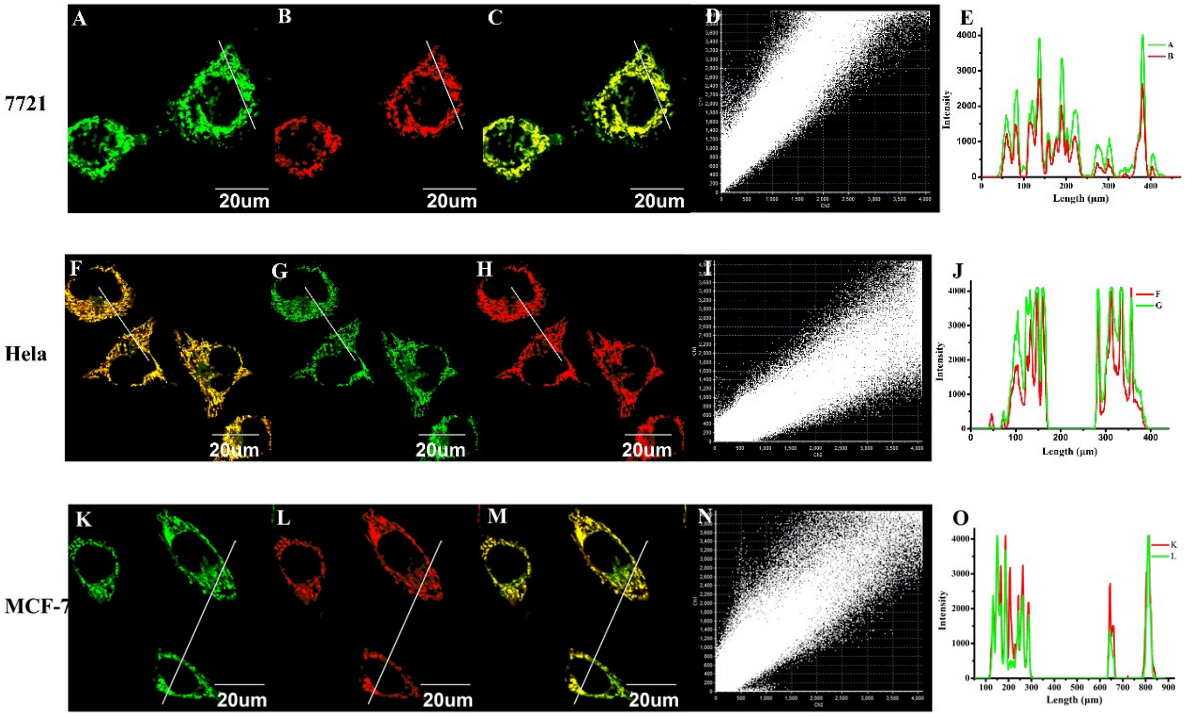


Figure 2. Colocalization imaging studies of **Vis-B** in 7721, Hela, and MCF-7 cells. A, F, and K **Vis-B** Channel 1: λ_{ex}=488nm, λ_{em}=500-550nm. B, G, and L Mito Tracker Deep Red Channel 2: λ_{ex}=635nm, λ_{em}=655-755nm; C overlay of A and B (Pearson coefficient 0.92). H overlay of F and G (Pearson coefficient 0.95). M overlay of K and L (Pearson coefficient 0.95). D, I, and N: Correlation of Vis-A and Mito Tracker Deep Red. E, J, and O: Intensity profile of ROIs across the cells.

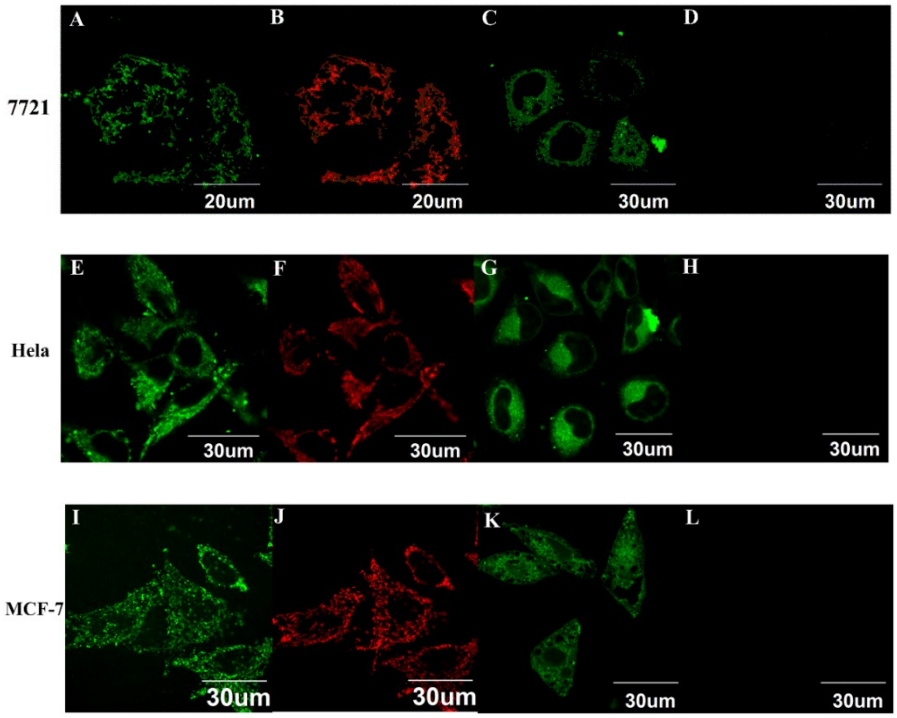


Figure 3. Imaging studies of **Vis-A** stain in 7721, Hela, and MCF-7 cells. A, B, E, F, I, and J: The cells are treated with **Vis-A** (1μM) and Mito Traker Deep Red (0.5μM) for 90 min, washed three times by PBS. A, E, and I Channel 1: λ_{ex}=488nm, λ_{em}=500-550nm. B, F, and J Channel 2: λ_{ex}=635nm, λ_{em}=655-755nm; C, D, G, H, K and L: The cells are treated with **Vis-A** (1μM) and Mito Traker Deep Red (0.5μM) for 90 min, washed three times by PBS, cultured

with four percent formaldehyde solution for 1 hours at 4°C, and then washed by the solution (ethanol : PBS = 1 : 5) every 5min for three times. C, G, and K Channel 1: λ_{ex}=488nm, λ_{em}=500-550nm. D, H, and L Channel 2: λ_{ex}=635nm, λ_{em}=655-755nm .

As is shown in Figure 3 and Figure 4, **Vis-B** and the commercial mitochondrial tracker are washed away from the dead cells. The similar method is used for **Vis-A**. **Vis-A** and the commercial mitochondrial tracker are also successful to label the mitochondria in the living cells. As the cells is killed, the commercial mitochondrial tracker is washed away. And the channel of **Vis-A** is also bright because **Vis-A** has reacted with the proteins and fixed on the proteins.

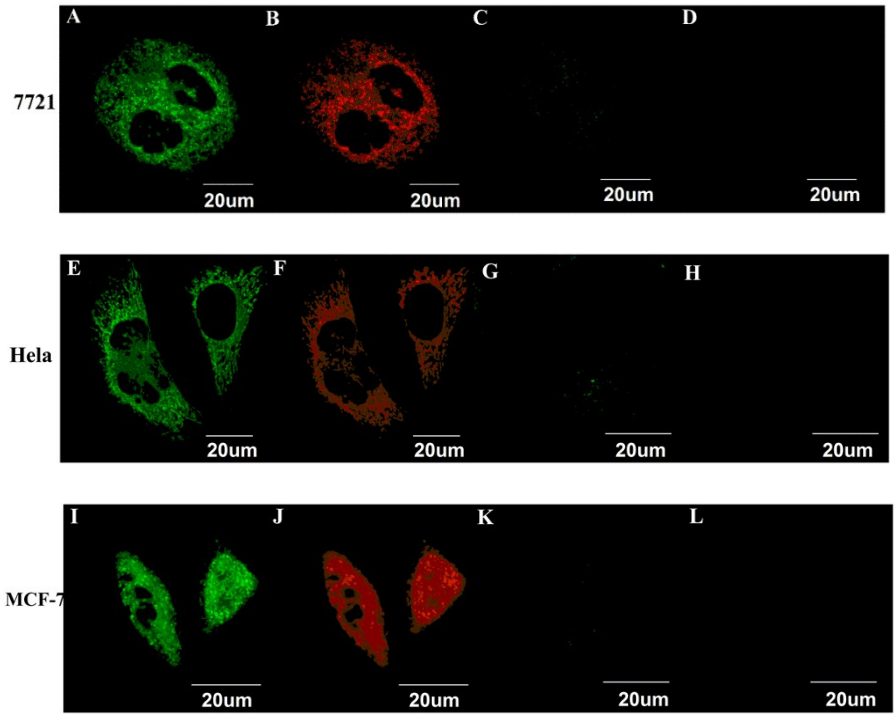


Figure 4. Imaging studies of **Vis-B** stain in 7721, Hela, and MCF-7 cells. A, B, E, F, I, and J: The cells are treated with **Vis-B** (1μM) and Mito Traker Deep Red (0.5μM) for 90 min, washed three times by PBS. A, E, and I Channel 1: λ_{ex}=488nm, λ_{em}=500-550nm. B, F, and J Channel 2: λ_{ex}=635nm, λ_{em}=655-755nm; C, D, G, H, K and L: The cells are treated with **Vis-B** (1μM) and Mito Traker Deep Red (0.5μM) for 90 min, washed three times by PBS, cultured with four percent formaldehyde solution for 1 hours at 4°C, and then washed by the solution (ethanol : PBS = 1 : 5) every 5min for three times. C, G, and K Channel 1: λ_{ex}=488nm, λ_{em}=500-550nm. D, H, and L Channel 2: λ_{ex}=635nm, λ_{em}=655-755nm.

Monitoring normal and abnormal mitochondrial viscosity

Figure 5 shows **FLIM** images of 7721 cells, Hela cells, and MCF-7 cells, in which fluorescence lifetime in the mitochondria have been mapped with considerable spatial resolution.

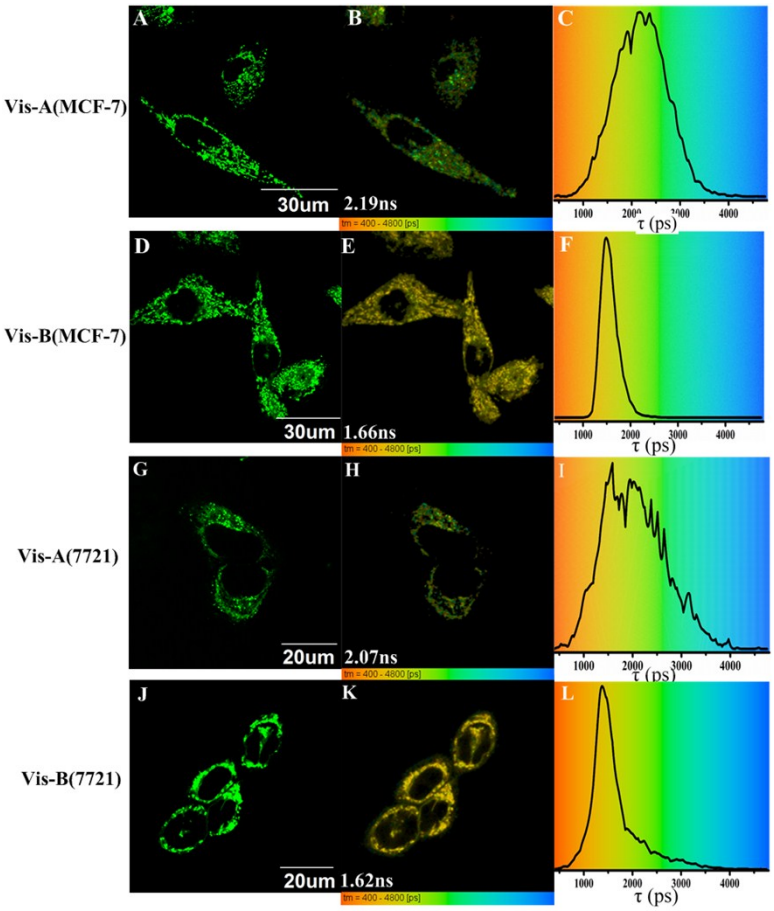


Figure 5. Imaging studies of **Vis-A** and **Vis-B** stain in MCF-7 and 7721 cells. A: Fluorescence imaging of **Vis-A** in MCF-7 cells. B: Fluorescence life time imaging of **Vis-A** in MCF-7 cells. C: Fluorescence lifetime distribution histogram for B. D: Fluorescence imaging of **Vis-B** in MCF-7 cells. E: Fluorescence lifetime imaging of **Vis-B** in MCF-7 cells. F: Fluorescence lifetime distribution histogram for E. G: Fluorescence imaging of **Vis-A** in 7721 cells. H: Fluorescence life time imaging of **Vis-A** in 7721 cells. I: Fluorescence lifetime distribution histogram for H. J: Fluorescence imaging of **Vis-B** in 7721 cells. K: Fluorescence lifetime imaging of **Vis-B** in 7721 cells. L: Fluorescence lifetime distribution histogram for K. MCF-7 cells and 7721 cells are treated with **Vis-A** (1μM) for 90 min, washed three times by PBS. MCF-7 cells and 7721 cells are treated with **Vis-B** (1μM) for 90 min, washed three times by PBS.

Fluorescence lifetime imaging microscopy after the cells stimulated by rotenone.

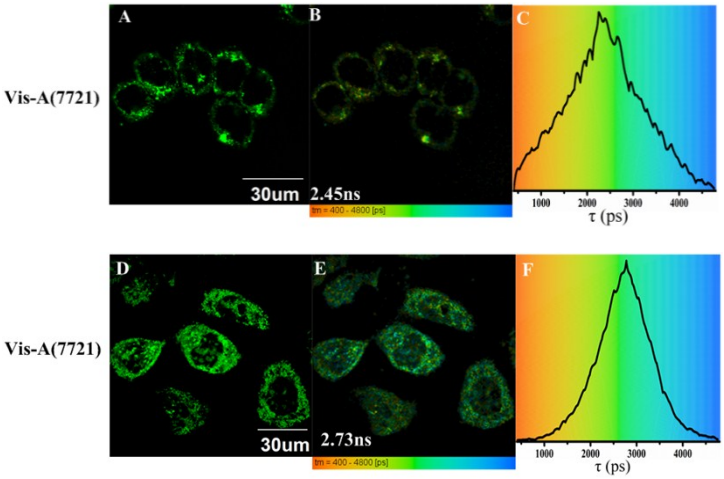


Figure 6.7721 cells are treated with **Vis-A** (2μM) for 90 min, washed three times by PBS. A, B, C are stimulated by rotenone (10μM) for 8.5h and D, E, F are stimulated by rotenone (10μM) for 18h. A: Imaging studies of **Vis-A** in 7721 cells. B: Fluorescence life time imaging of **Vis-A** in 7721 cells. C: Fluorescence lifetime distribution histogram for B. D: Fluorescence imaging of **Vis-A** in 7721 cells. E: Fluorescence lifetime imaging of **Vis-A** in 7721 cells. F: Fluorescence lifetime distribution histogram for E.

Fluorescence lifetime imaging microscopy after the cells stimulated by CCCP

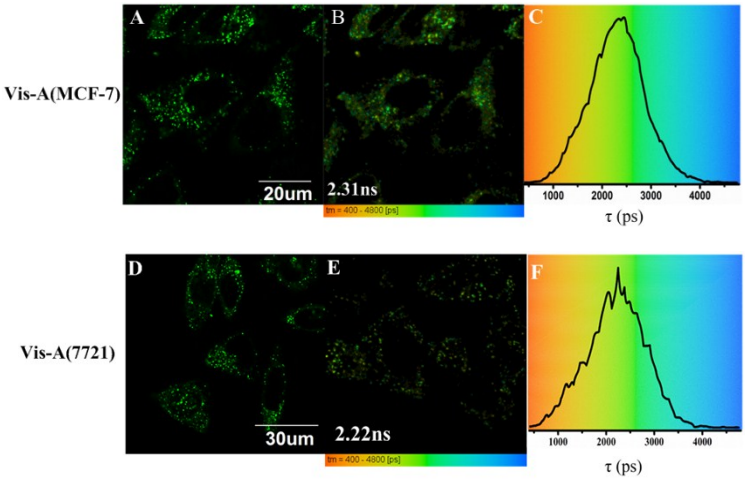
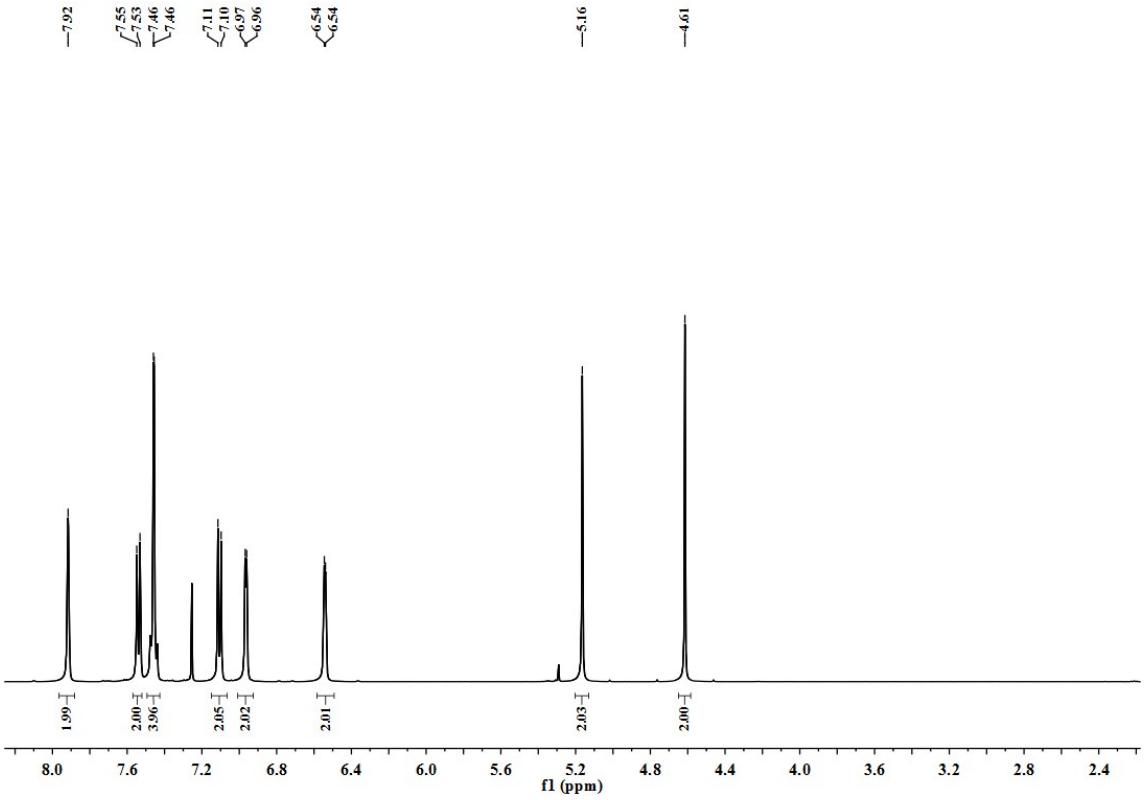
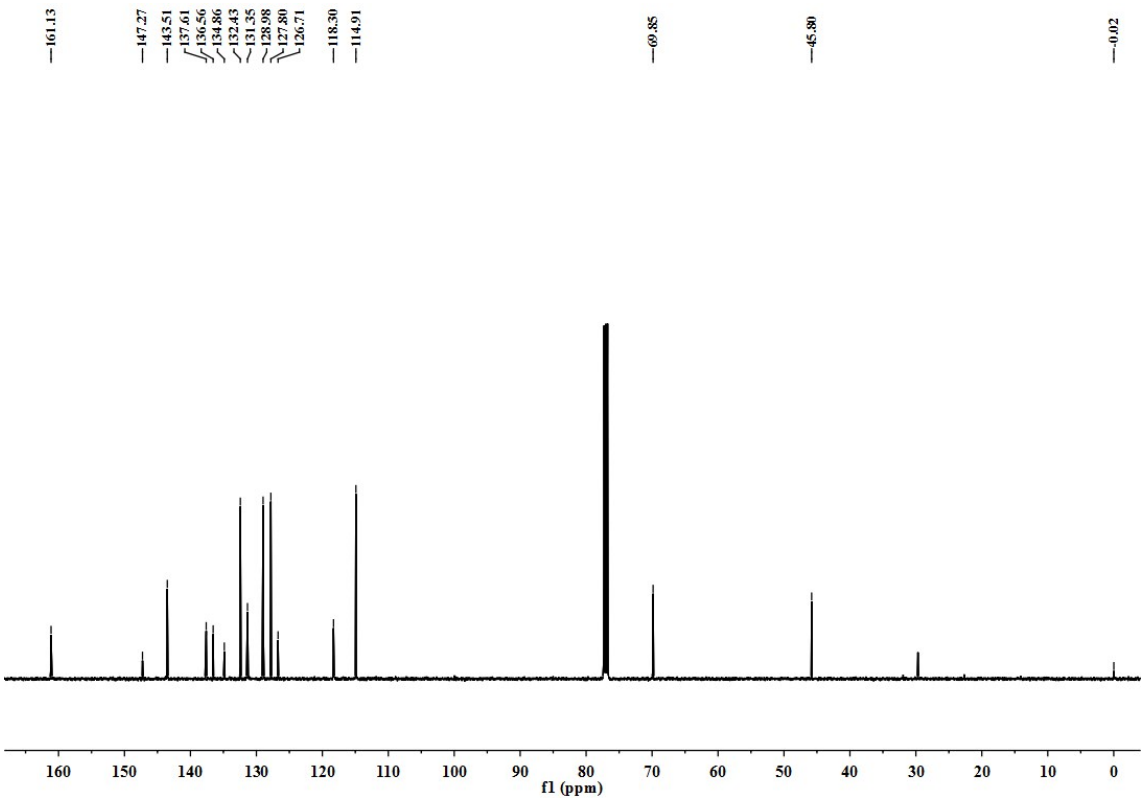


Figure.7 7721 cells and MCF-7 cells are treated with **Vis-A** (2μM) for 90 min, washed three times by PBS. A, B, C, D, E, F are stimulated by CCCP (100μM) for 3h. A: Imaging studies of **Vis-A** stain in MCF-7 cells. B: Fluorescence life time imaging of **Vis-A** in MCF-7 cells. C: Fluorescence lifetime distribution histogram for B. D: Fluorescence imaging of **Vis-A** in 7721 cells. E: Fluorescence lifetime imaging of **Vis-A** in 7721 cells. F: Fluorescence lifetime distribution histogram for E.

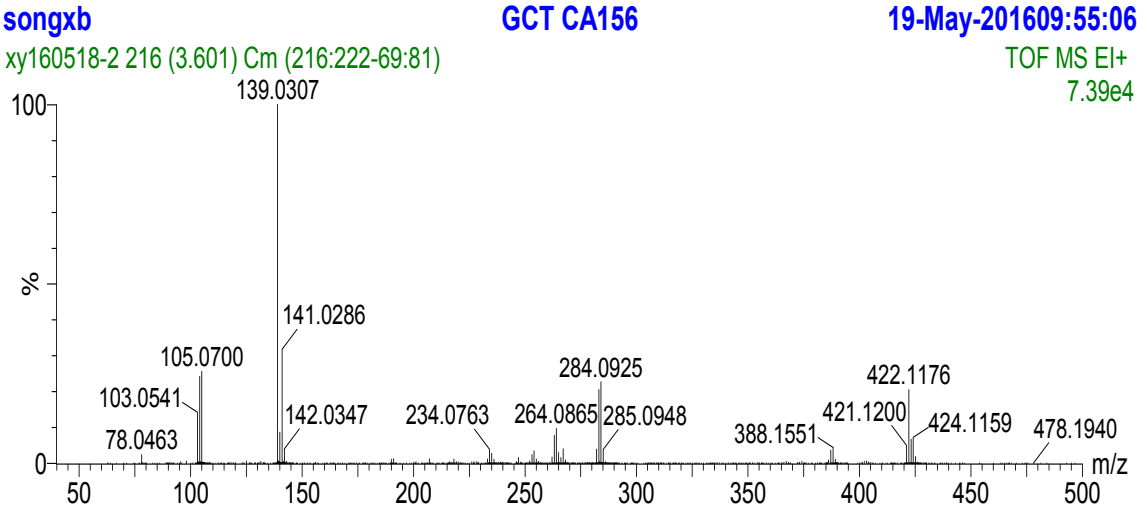
Compound d ^{1}HNMR (500 MHz, $CDCl_3$)



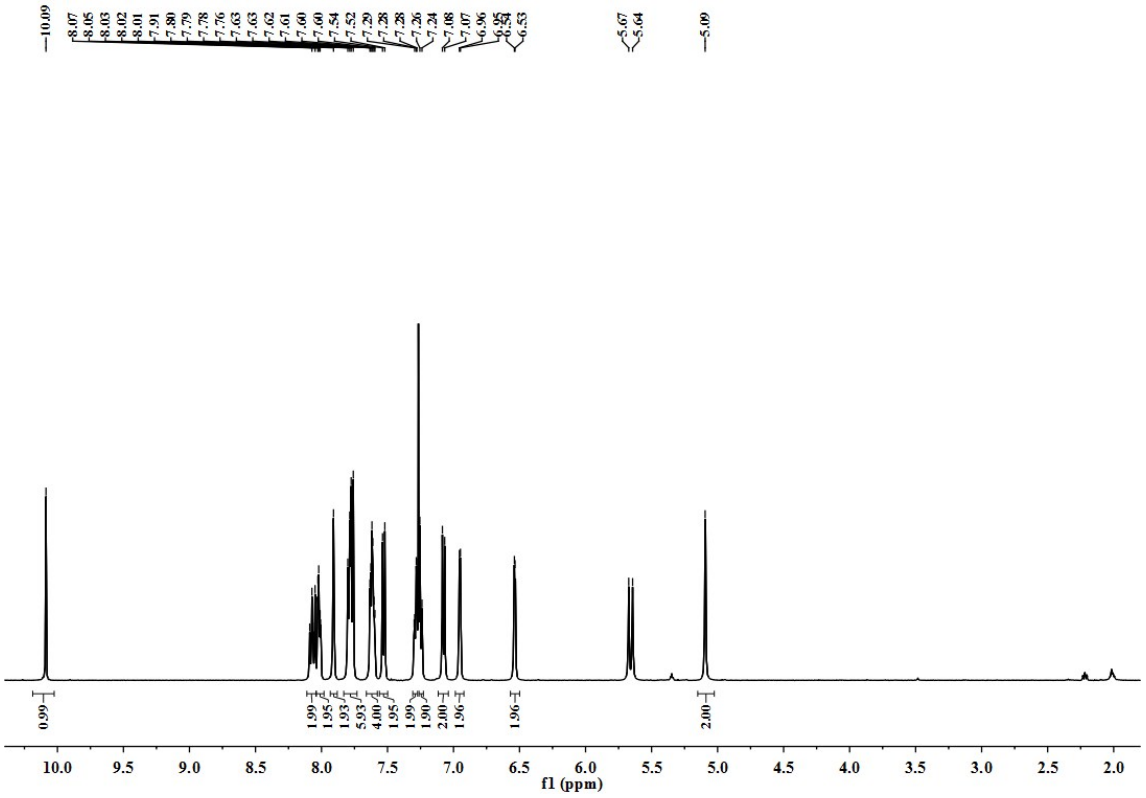
Compound d ^{13}CNMR (126 MHz, $CDCl_3$)



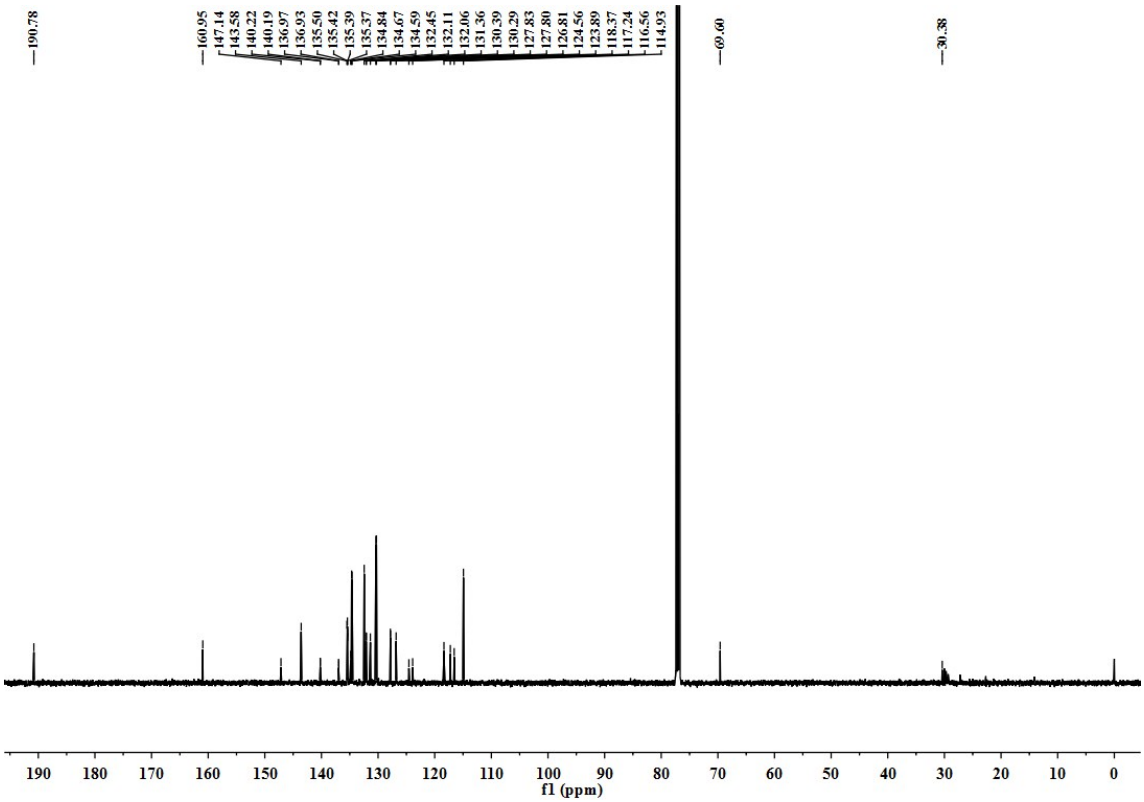
Compound d MS



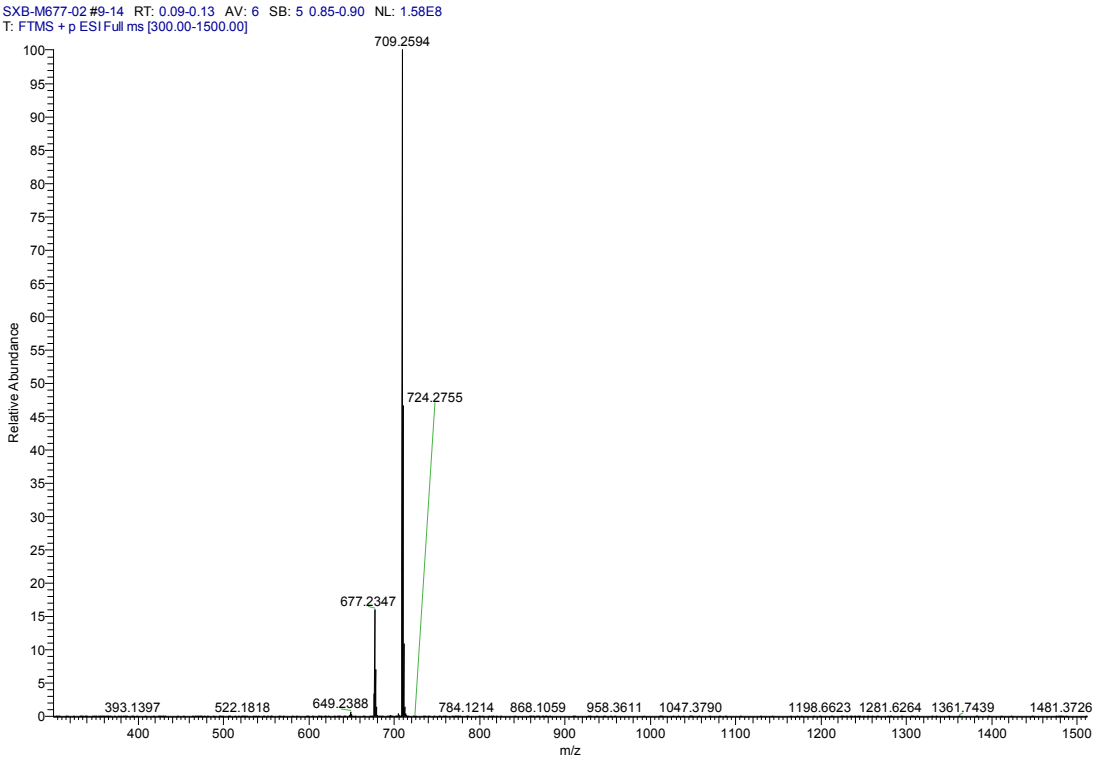
Vis-A ^{1}HNMR (500 MHz, $CDCl_3$)



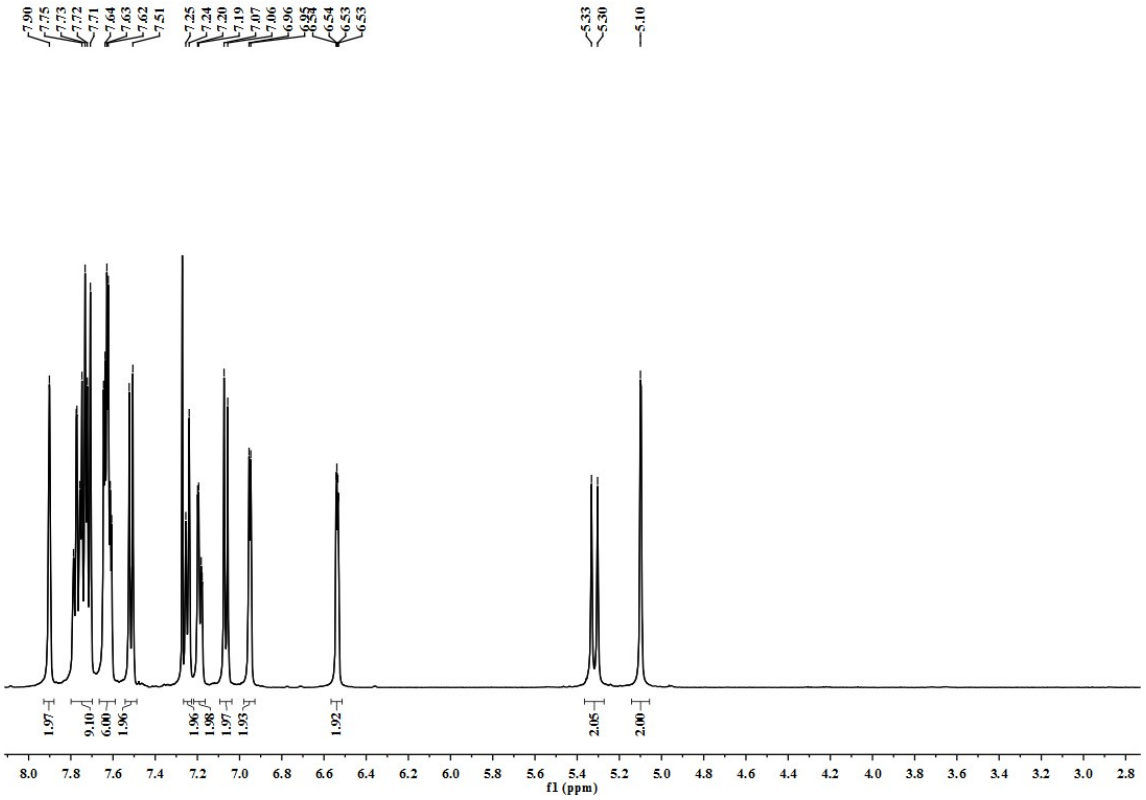
Vis-A ^{1}HNMR (126 MHz, $CDCl_3$)



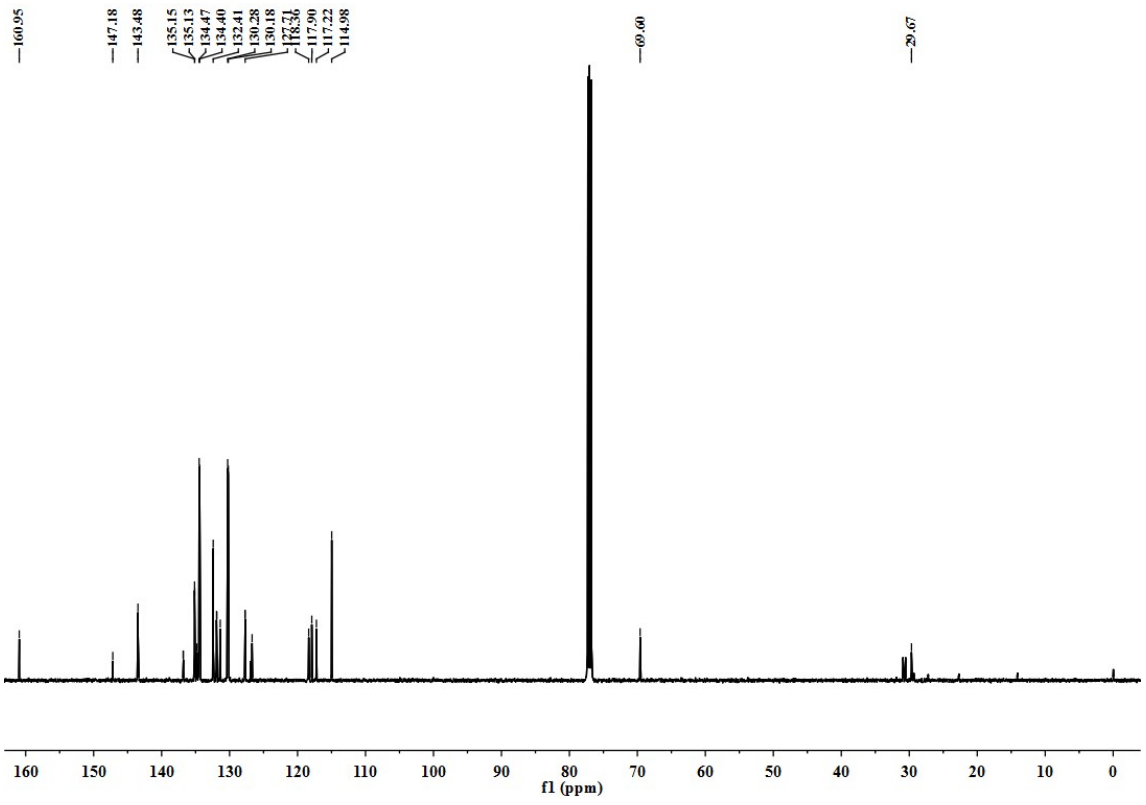
Vis-A MS



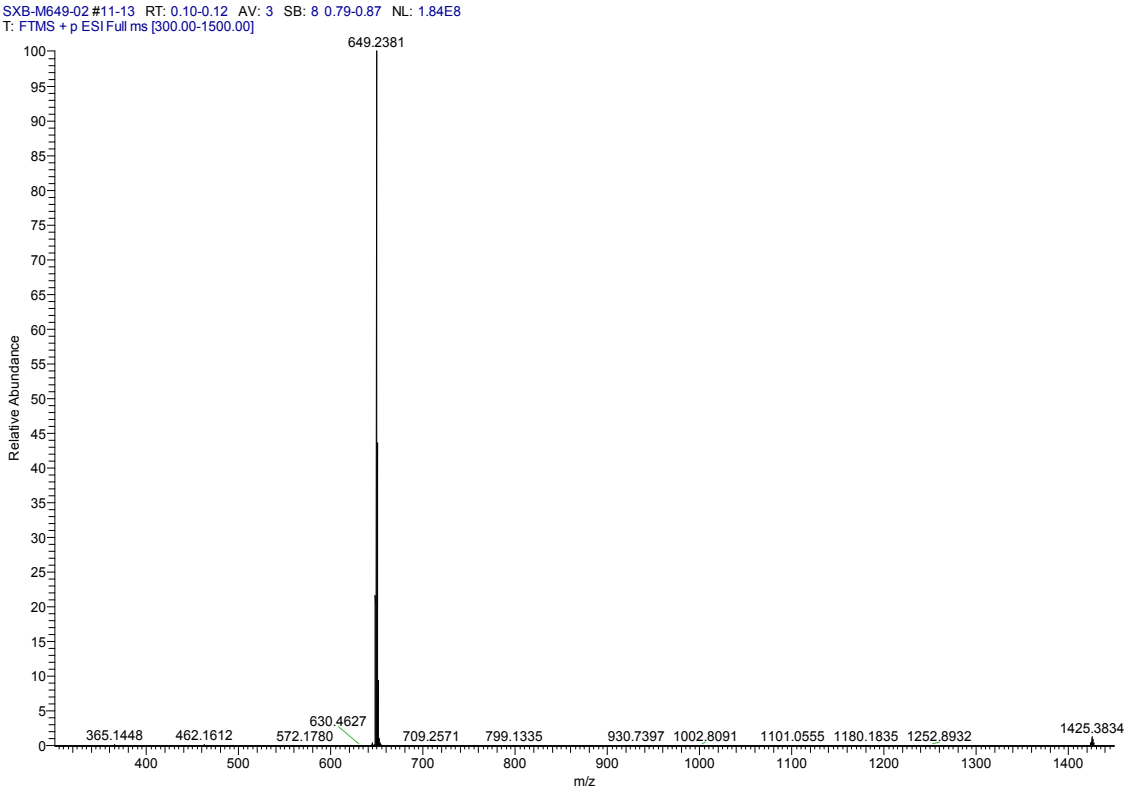
Vis-B ^{1}HNMR (500 MHz, $CDCl_3$)



Vis-B ^{13}CNMR (126 MHz, $CDCl_3$)



Vis-B MS


SXB-M649-02 #11-13 RT: 0.10-0.12 AV: 3 SB: 8 0.79-0.87 NL: 1.84E8
T: FTMS + p ESI Full ms [300.00-1500.00]
649.2381
630.4627
365.1448
462.1612
572.1780
709.2571
799.1335
930.7397
1002.8091
1101.0555
1180.1835
1252.8932
1425.3834
Relative Abundance
m/z