

Supplementary Information

A Two-Photon Fluorescent Probe for Basal Formaldehyde Imaging in Zebrafish and Visualization of Mitochondrial Damage Induced by FA Stress

Fangyun Xin^a, Yong Tian^a, Congcong Gao^a, Bingpeng Guo^a, Yulong Wu^a, Junfang Zhao^b,
Jing Jing^{*a} and Xiaoling Zhang^{*a}

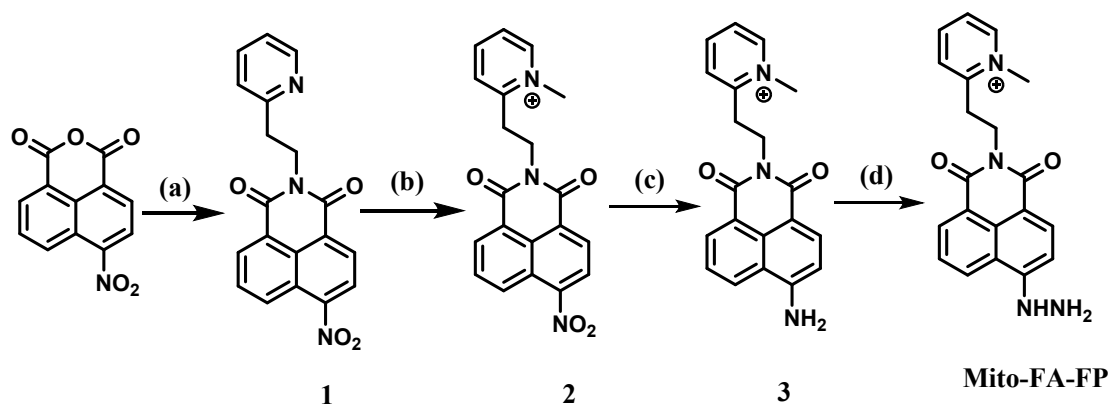
^a Beijing Key Laboratory of Photo-electronic/Electro- photonic Conversion Materials, Key Laboratory of Cluster Science of Ministry of Education, School of Chemistry and Chemical Engineering, Analytical and Testing Centre, Beijing Institute of Technology, Beijing 100081, P. R. China.

^b Technical Institute of Physics and Chemistry, Chinese Academy of Sciences.

E-mail: zhangxl@bit.edu.cn, hellojane@bit.edu.cn

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Scheme S1 The synthesis route of **Mito-FA-FP**. (a) 2-(2-Pyridyl) ethylamine, ethanol, reflux 4 h. (b) CH_3I , toluene, and reflux overnight. (c) tin(II) chloride, concentrated HCl, ethanol, reflux 4 h. (d) (i) 18% HCl aq., NaNO_2 , 0 °C for 1 h. (ii) $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, concentrated HCl, room temperature for 2 h.

Table 1 Photophysical parameters of **Mito-FA-FP** before and after reacting with FA

Dye	Maximal absorption	Molar extinction coefficients/ $\text{M}^{-1} \text{cm}^{-1}$	Fluorescence quantum yield
Mito-FA-FP	0.026	5.2×10^3	0.051
Mito-FA-FP+FA	0.038	7.6×10^3	0.326

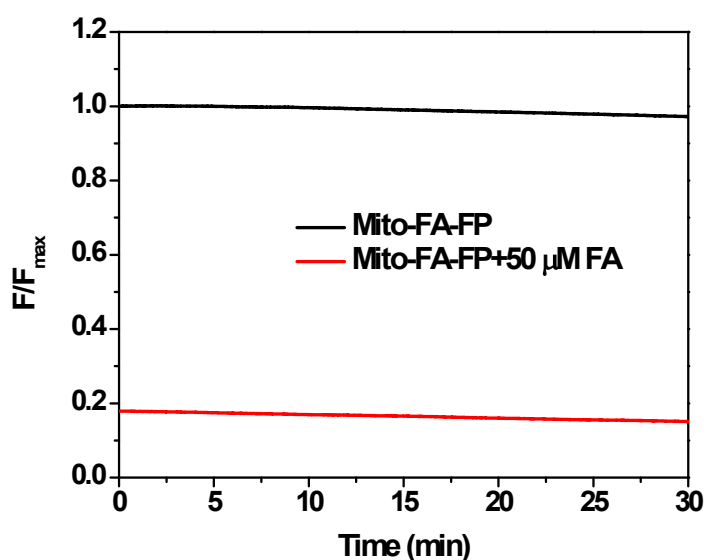


Fig. S1 The photostability of 5 μM **Mito-FA-FP** with and without 50 μM FA at 550 nm wavelength.

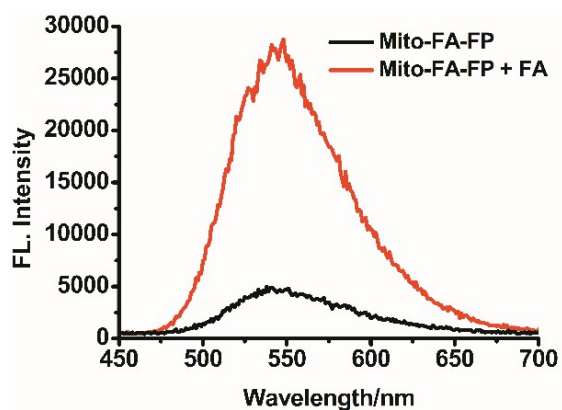


Fig. S2 Two-photon fluorescence emission of the probe **Mito-FA-FP** before and after treatment with FA. Excited at 880nm.

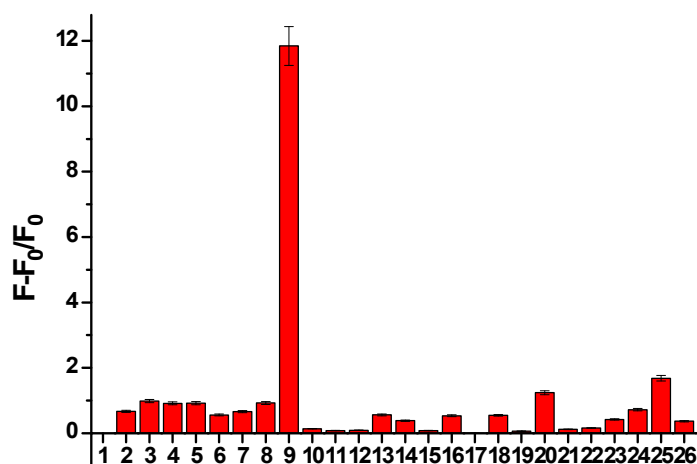


Fig. S3 Fluorescence intensity of the probe **Mito-FA-FP** (5 μ M) in the presence of various analytes in 10 mM PBS (pH 7.4) at 550 nm. Legend: (1)PBS, (2) glyoxal, (3) methylglyoxal, (4) sodium pyruvate, (5) trichloroacetaldehyde, (6) acetaldehyde, (7) 4-nitro-benzaldehyde, (8) acetone, (9) FA, (10) NaClO, (11) H₂O₂, (12) tert-butyl hydroperoxide, (13) NO, (14) CaCl₂, (15) MgCl₂, (16) Na₂SO₃, (17) NaNO₂, (18) NaHSO₃, (19) NaHS, (20) L-Arg, (21) L-Cys, (22) DL-Hcy, (23) D-phe, (24) N-Acetyl-glycine, (25) N-Acetyl-L-cysteine, (26) GSH. The data was obtained after treatment **Mito-FA-FP** with relevant analytes for 30 min. The concentrations of the representative analytes are amino acids, cations and anions, 5mM; reactive oxygen species and reactive nitrogen species, 100 μ M; ketones and aldehyde, 50 μ M.

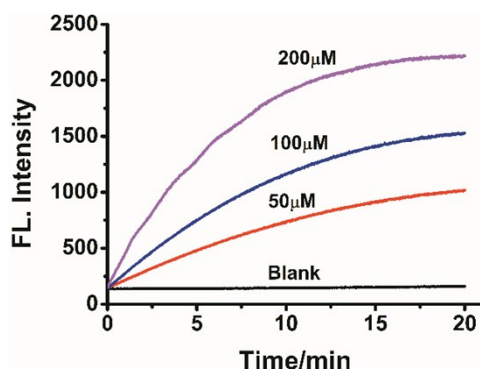


Fig. S4 Time response profiles of **Mito-FA-FP** (5 μM) to FA.

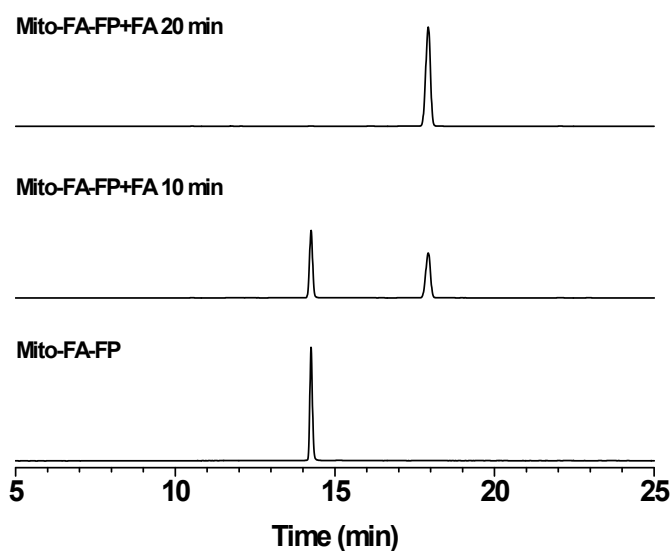


Fig. S5 HPLC traces of 5 μM **Mito-FA-FP** before and after treatment with 200 μM FA for different time. Eluent solvent: acetonitrile/ H_2O (v/v = 8/2), flow rate = 1 mL min^{-1} , detection wavelength: 440 nm.

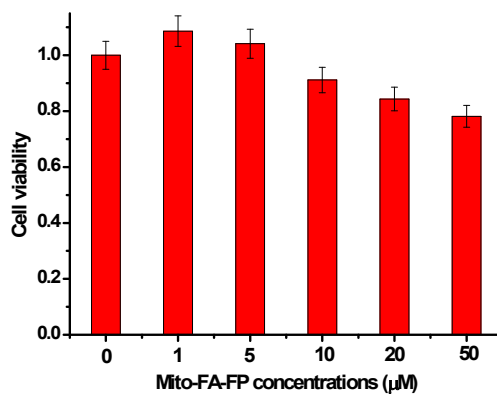


Fig. S6 Effects of the probe **Mito-FA-FP** with different concentrations on the viability of the

HeLa Cells. The probe with varied concentrations was incubated with the HeLa cells for 24 h. The viability of the cells in the absence of the probe is defined as 1, and the data are the mean standard deviation of five separate measurements.

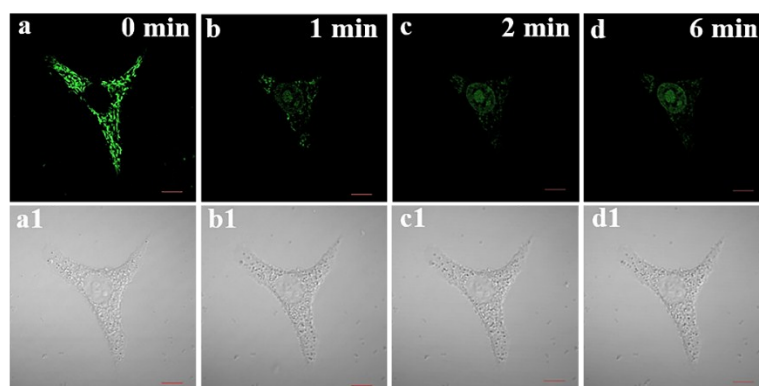


Fig. S7 Time dependent effect of 10 μM CCCP on the fluorescence intensity of **Mito-FA-FP** (5 μM) in HeLa cells. First, the HeLa cells were treated with **Mito-FA-FP** (5 μM) for 30 min, then incubated with 10 μM CCCP for different time period. The fluorescence imaging of cells at different time point after addition of CCCP are as follows: (a) 0 min; (b) 1 min; (c) 2 min; (d) 6 min; (a1), (b1), (c1), (d1) are the bright field imaging at 0 min, 1 min, 2 min, 6 min respectively. Scale bar: 20 μm .

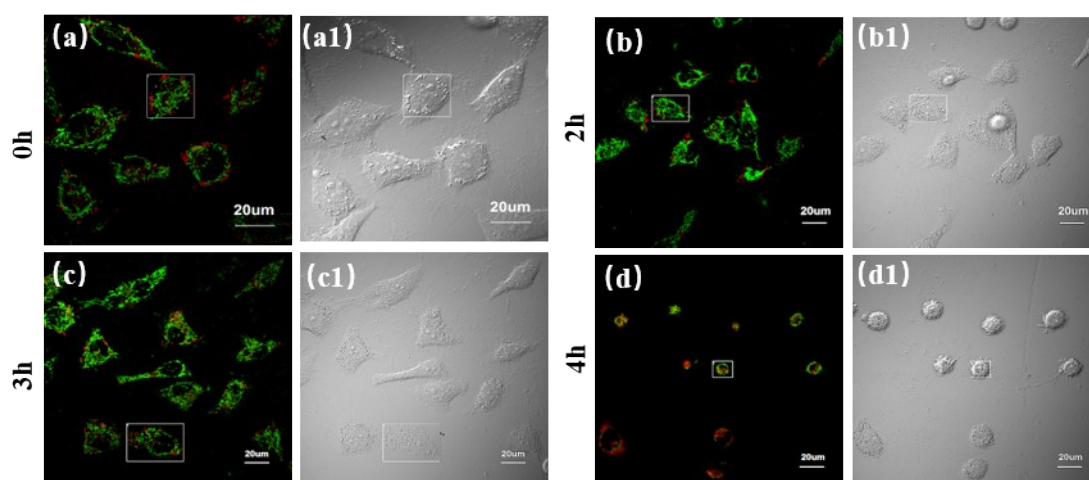


Fig. S8 Fluorescence confocal microscopy analysis of HeLa cells after treatment with 150 μM FA for different time (0, 2, 3, and 4 h). The white region is randomly chosen for mitochondrial morphology observation. Mitochondria and lysosomes were stained with **Mito-FA-FP** (5 μM) and Lyso-Tracker Blue (1 μM) respectively for 30 min. The excitation wavelength of blue channel and green channel are at 405 nm and 488 nm, and the emission collection are 420-470 nm and 500-600 nm respectively.

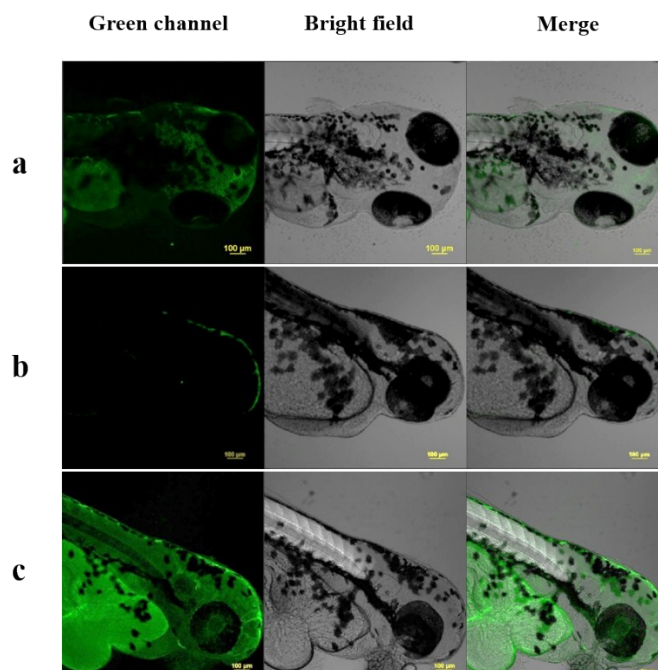


Fig. S9 One-photon confocal imaging of basal FA in zebrafish. (a) Zebrafish was treated with 10 μ M **Mito-FA-FP** for 1h in E3 medium; (b) Zebrafish was pre-treated with 500 μ M of NaHSO₃ for 1h, then treated with **Mito-FA-FP** (10 μ M) for 1 h; (c) Zebrafish with the same conditions of group b was treated with 1mM FA for 1h. Excited at 440 nm.

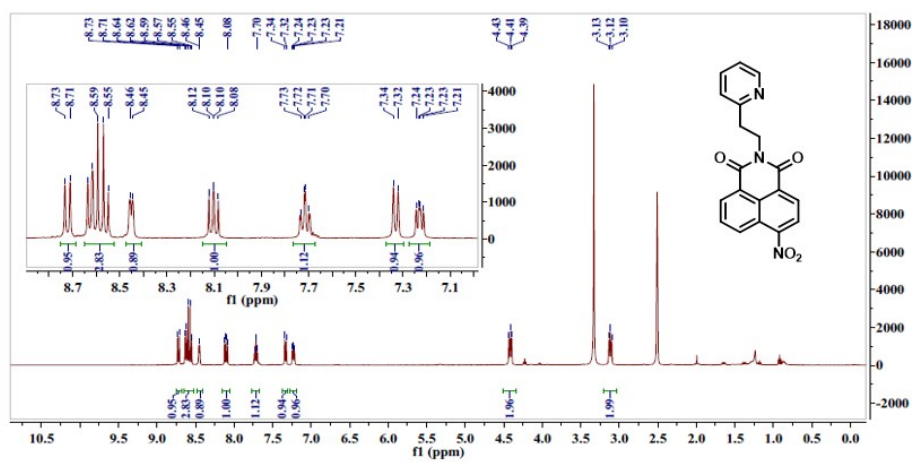


Fig. S10 ¹H NMR spectrum of compound 1

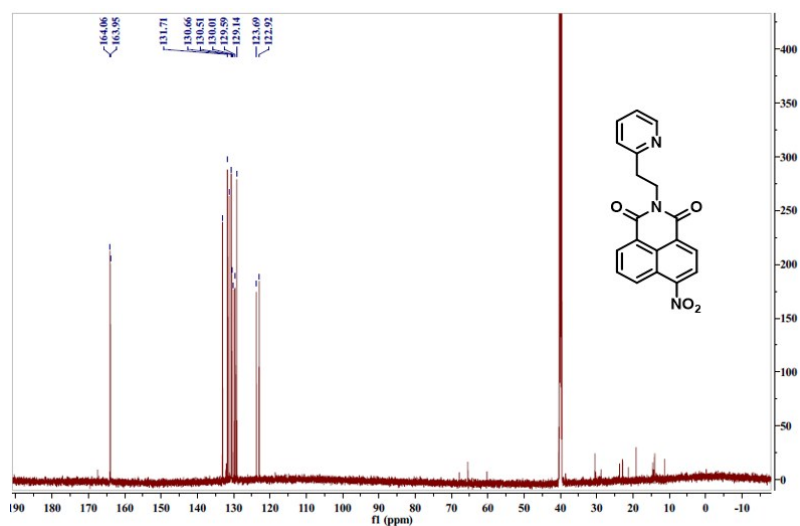


Fig. S11 ^{13}C NMR spectrum of compound 1

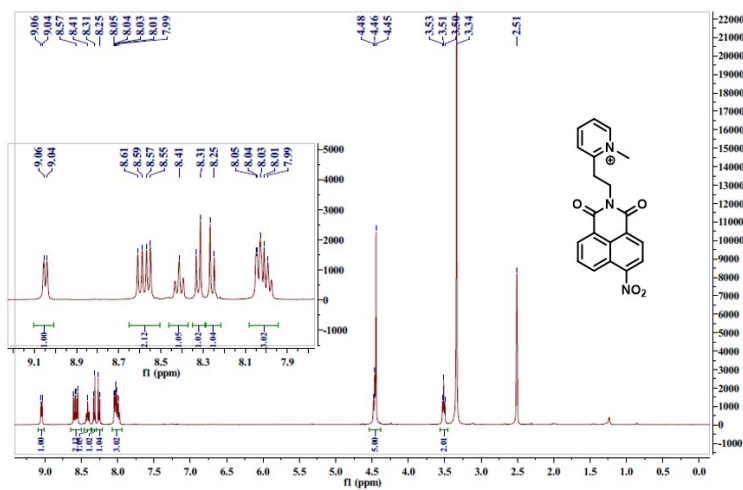


Fig. S12 ^1H NMR spectrum of compound 2

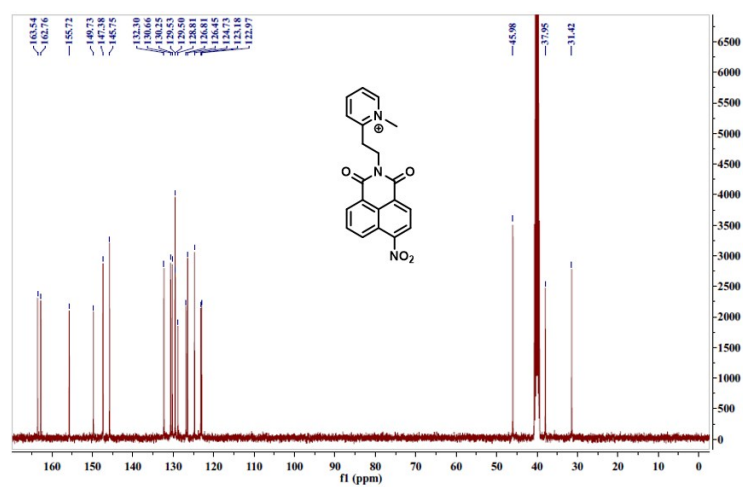


Fig. S13 ^{13}C NMR spectrum of compound 2

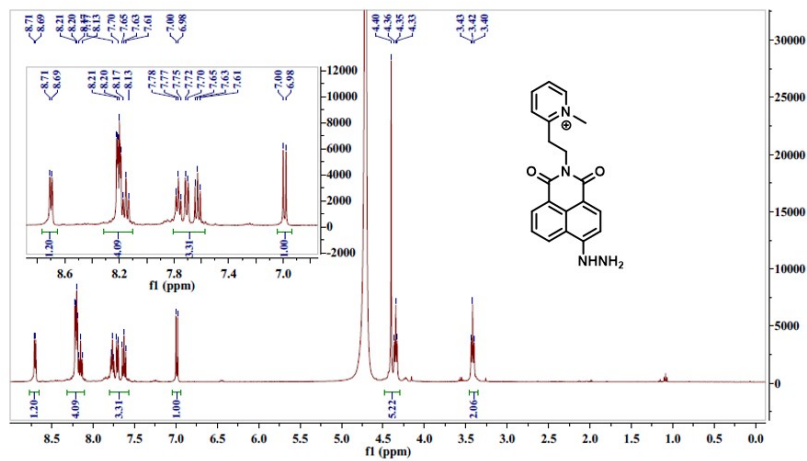


Fig. S14 ^1H NMR spectrum of Mito-FA-FP

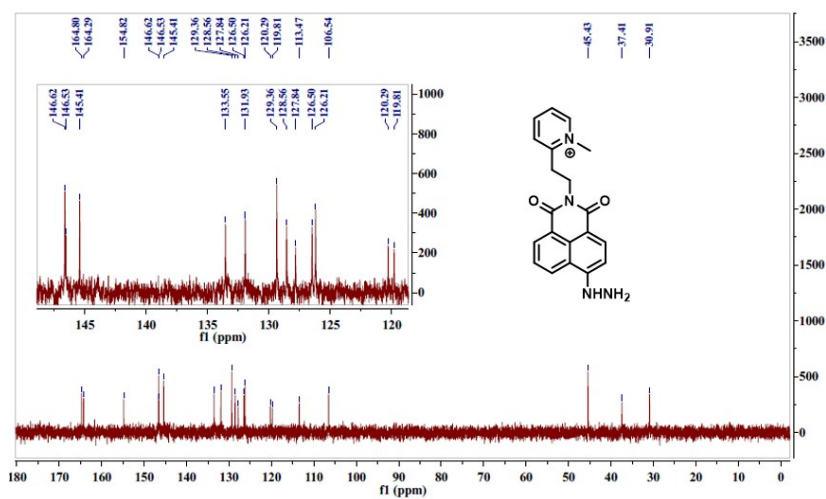


Fig. S15 ^{13}C NMR spectrum of Mito-FA-FP

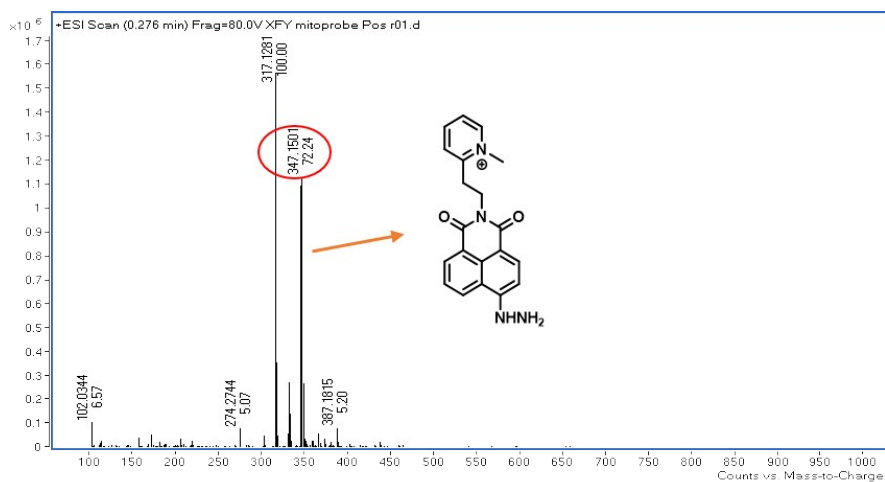


Fig. S16 HR-MS spectrum of Mito-FA-FP

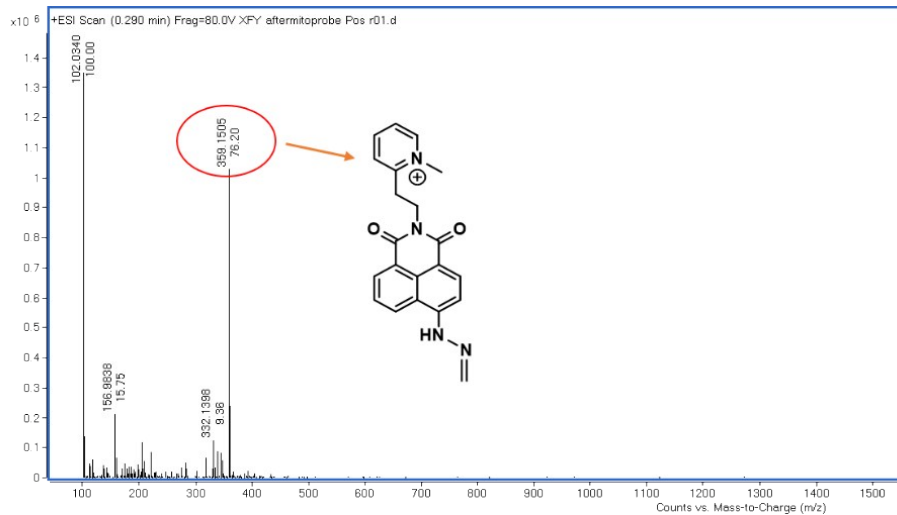


Fig. S17 HR-MS spectrum of Mito-FA-FP+FA