

## Supporting information

### **Rational design of a novel mitochondrial-targeted near-infrared fluorescent pH probe for imaging in living cells and in vivo**

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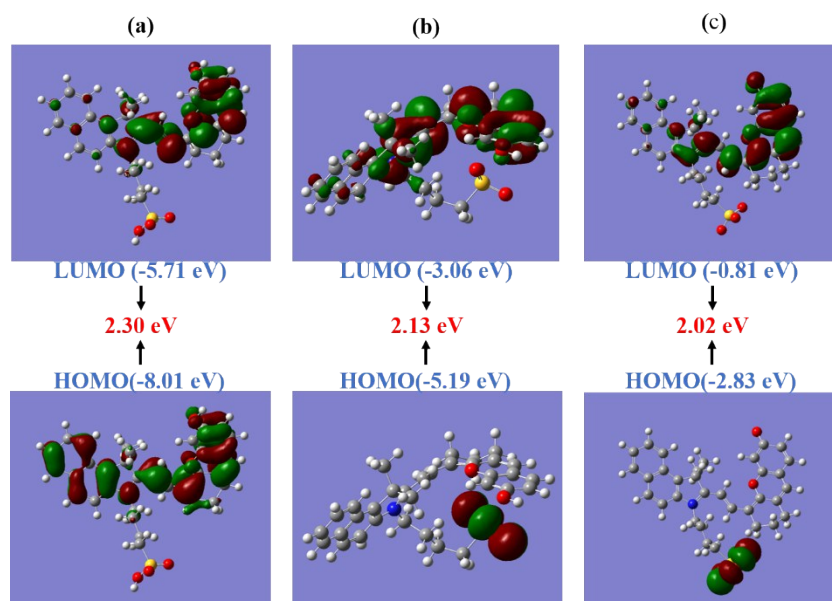
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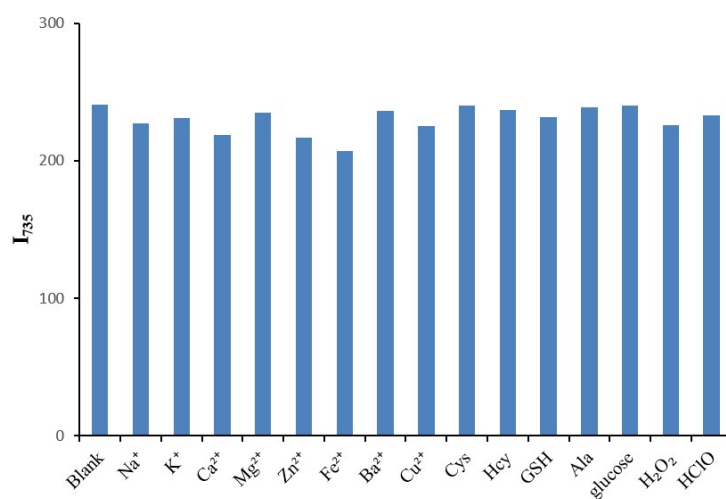
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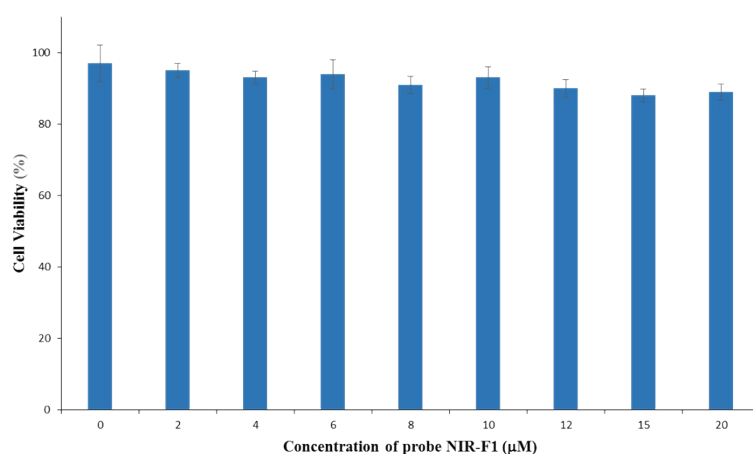
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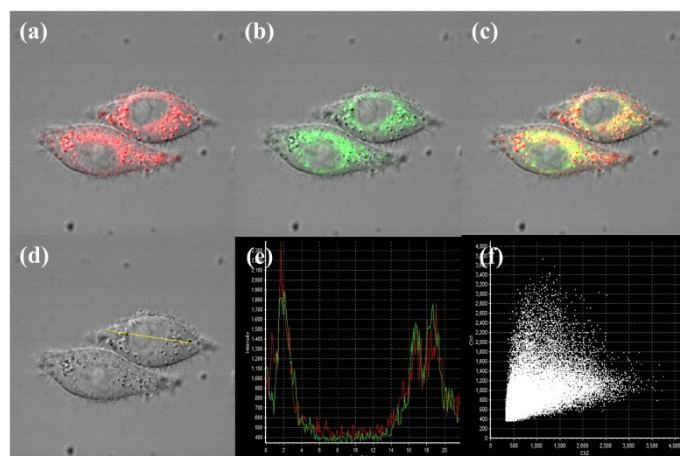
**Figure S1.** Density functional theory (DFT) optimized structures and frontier molecular orbitals (MOs) of (a) form a, (b) form b, and (c) form c. Calculations were based on ground state geometry by DFT at the B3LYP/6-31+G(d) level using Gaussian 09.



**Figure S2.** Fluorescence response ( $I_{735}$ ) of NIR-F1 (10  $\mu$ M) in the presence of diverse ions (10 mM for  $\text{Na}^+$ ,  $\text{K}^+$ , 200  $\mu$ M for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Cu}^{2+}$ ) and bioactive small molecules (5 mM for GSH, Cys, Hcy, Ala, and glucose; 200  $\mu$ M for  $\text{H}_2\text{O}_2$  and HClO) in buffer solution.



**Figure S3.** Cell viability of MCF-7 treated with different concentrations of NIR-F1 for 24 h in fresh medium.



**Figure S4.** NIR-F1 co-localizes to mitochondria in MCF-7 cells. MCF-7 was stained with (a) 10  $\mu\text{M}$  NIR-F1 (Channel 1:  $\lambda_{\text{ex}} = 653 \text{ nm}$ ,  $\lambda_{\text{em}} = 700\text{--}800 \text{ nm}$ ) and (b) 0.1  $\mu\text{M}$  Mito-Tracker Green (Channel 2:  $\lambda_{\text{ex}} = 488 \text{ nm}$ ,  $\lambda_{\text{em}} = 495\text{--}550 \text{ nm}$ ). (c) Overlay of (a) and (b). (d) bright field. (e) Intensity profile of regions of interest (ROI) across MCF-7 cells. (f) Intensity correlation plot of stain NIR-F1 and Mito Tracker Green.