Supporting Material

A diketopyrrolopyrrole-based fluorescent probe for investigating mitochondrial zinc ions

Chenchen Du [a,†] Shibo Fu [b,†] Xiaolei Ren, [a] Xiaohua Wang, [a] Zhong Wang, [b,*] Juan Zhou, [b,*] Hongyu Wang [a,c,d**]

[a] *Department of Chemistry, College of Science, Shanghai University, 99 Shangda Road, Shanghai, 200444, PR China*

[b] *Department of Urology, Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, 200011, P. R. China*

[c] *Center for Supramolecular Chemistry and Catalysis, Shanghai University, 99 Shangda Road, Shanghai, 200444, PR China*

[d] *Key Laboratory of Organosilicon Chemistry and Material Technology of Ministry of Education, Hangzhou Normal University, Hangzhou 311121, PR China*

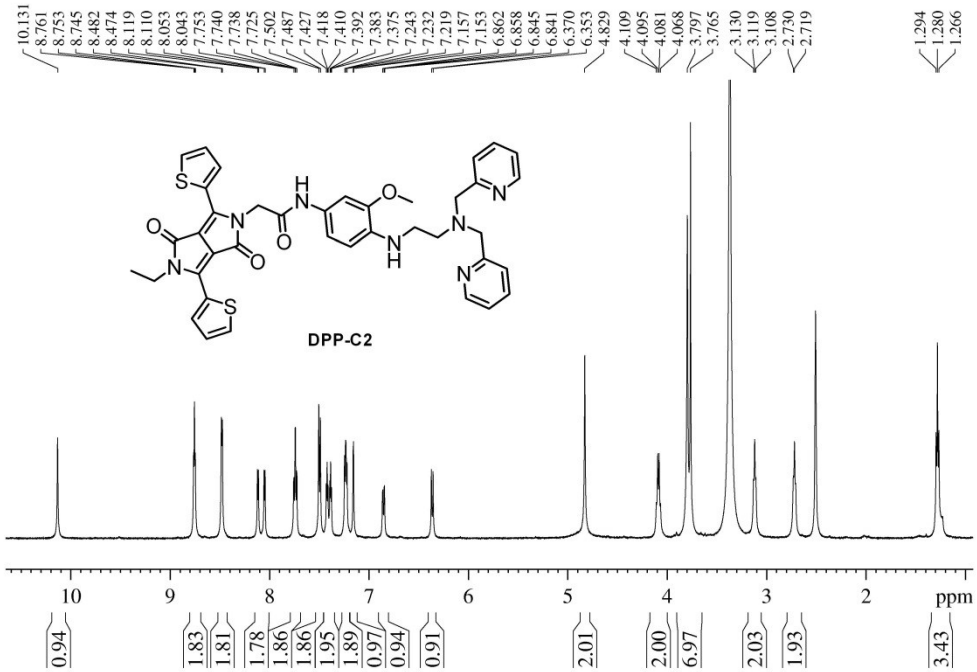

Figure S1. The ^{1}H NMR spectrum of **DPP-C2** in DMSO-d_6.

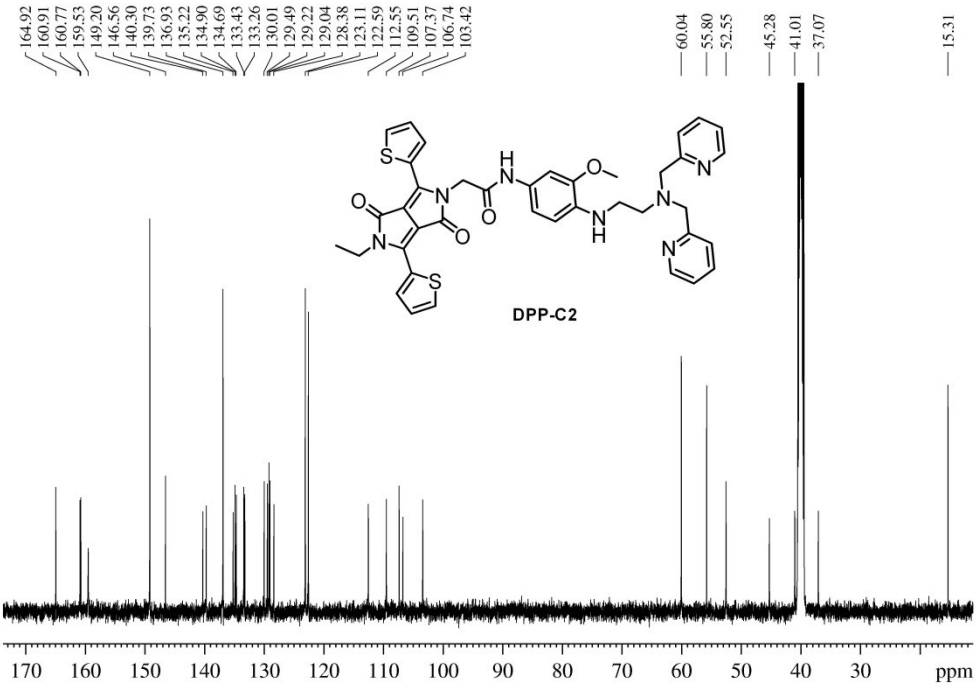

Figure S2. The ^{13}C NMR spectrum of **DPP-C2** in DMSO-d_6.

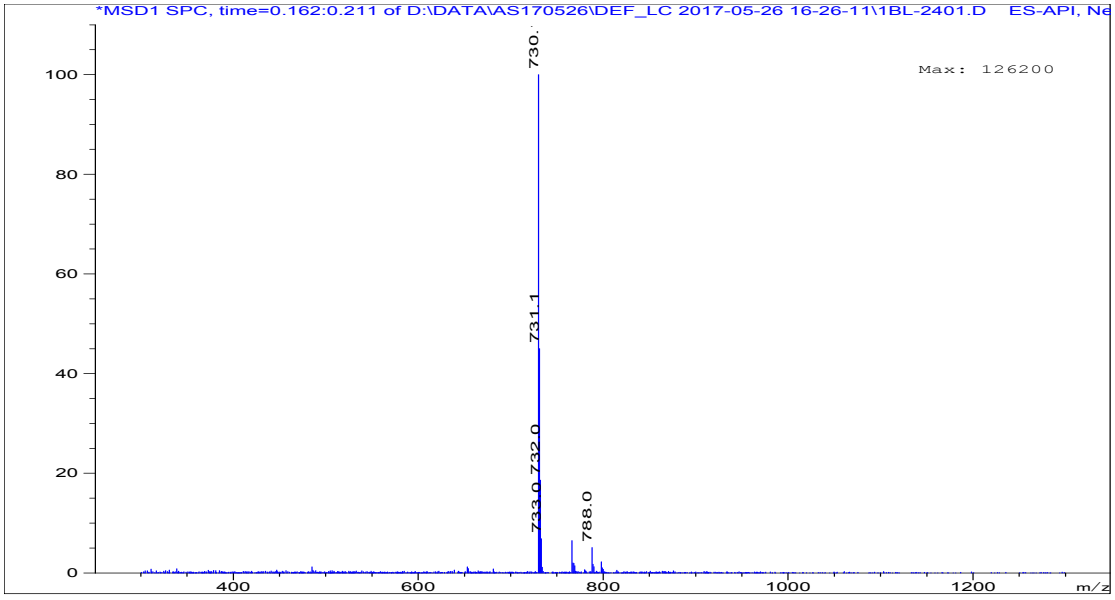

Figure S3. Mass spectrum of **DPP-C2**.

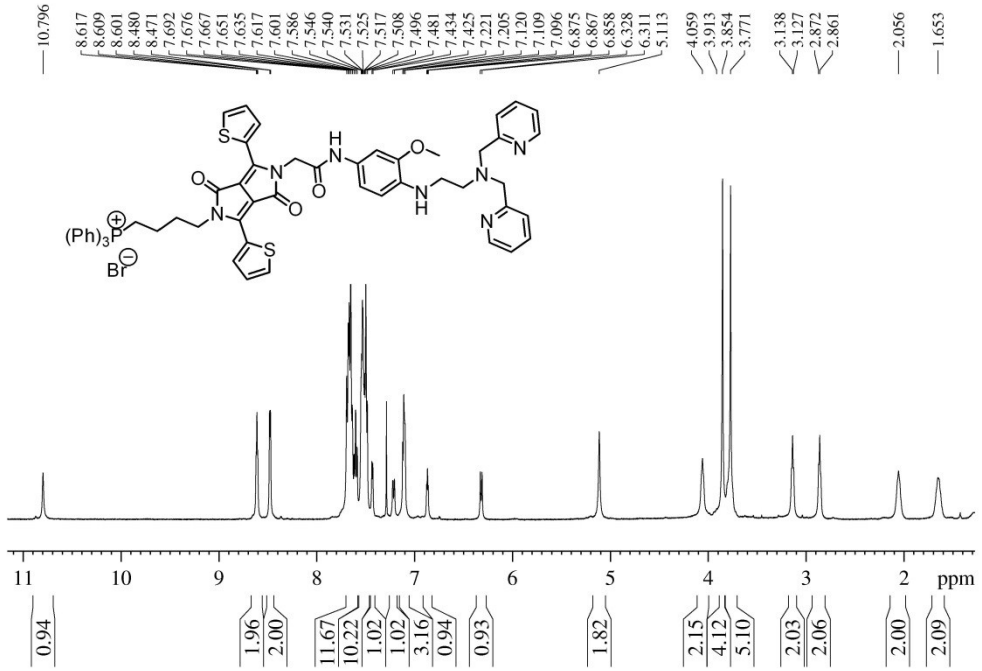

Figure S4. The ^{1}H NMR spectrum of **DPP-Mito** in $CDCl_3$.

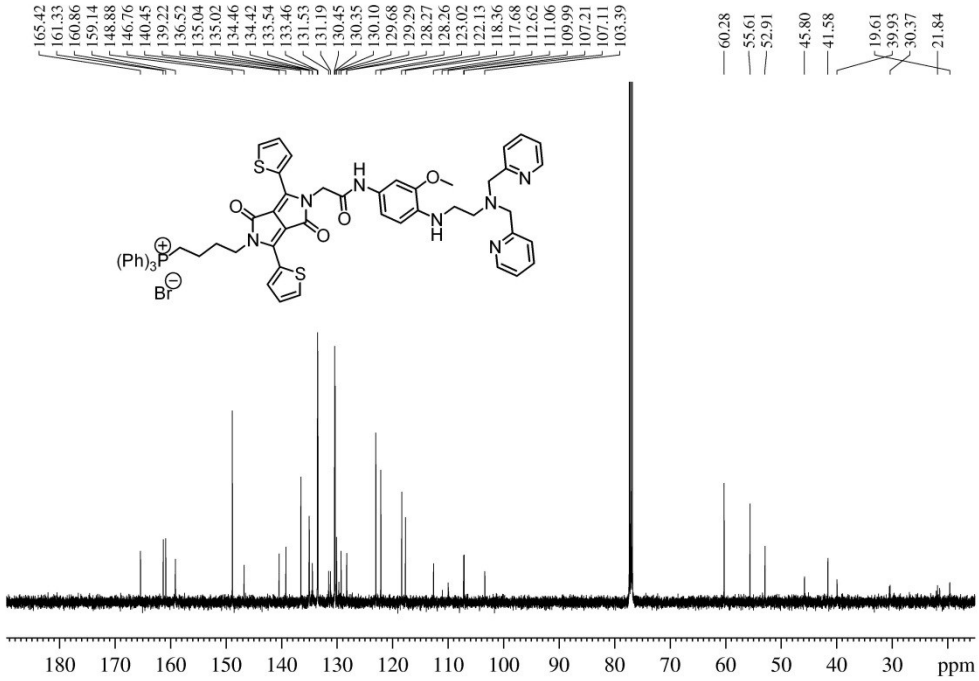

Figure S5. The ^{13}C NMR spectrum of **DPP-Mito** in $CDCl_3$.

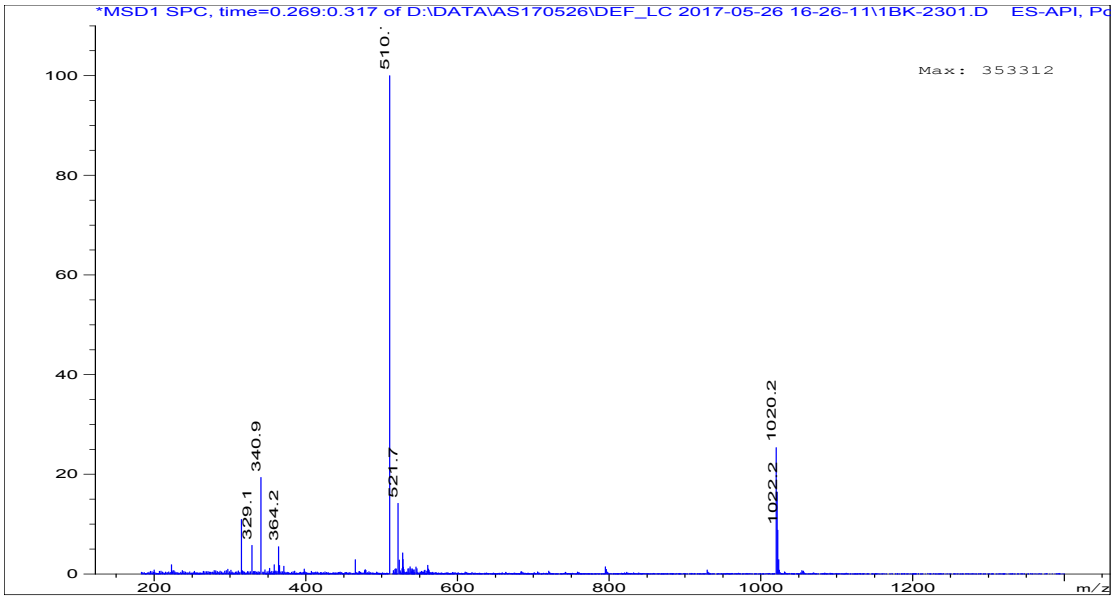

Figure S6. Mass spectrum of **DPP-Mito**.

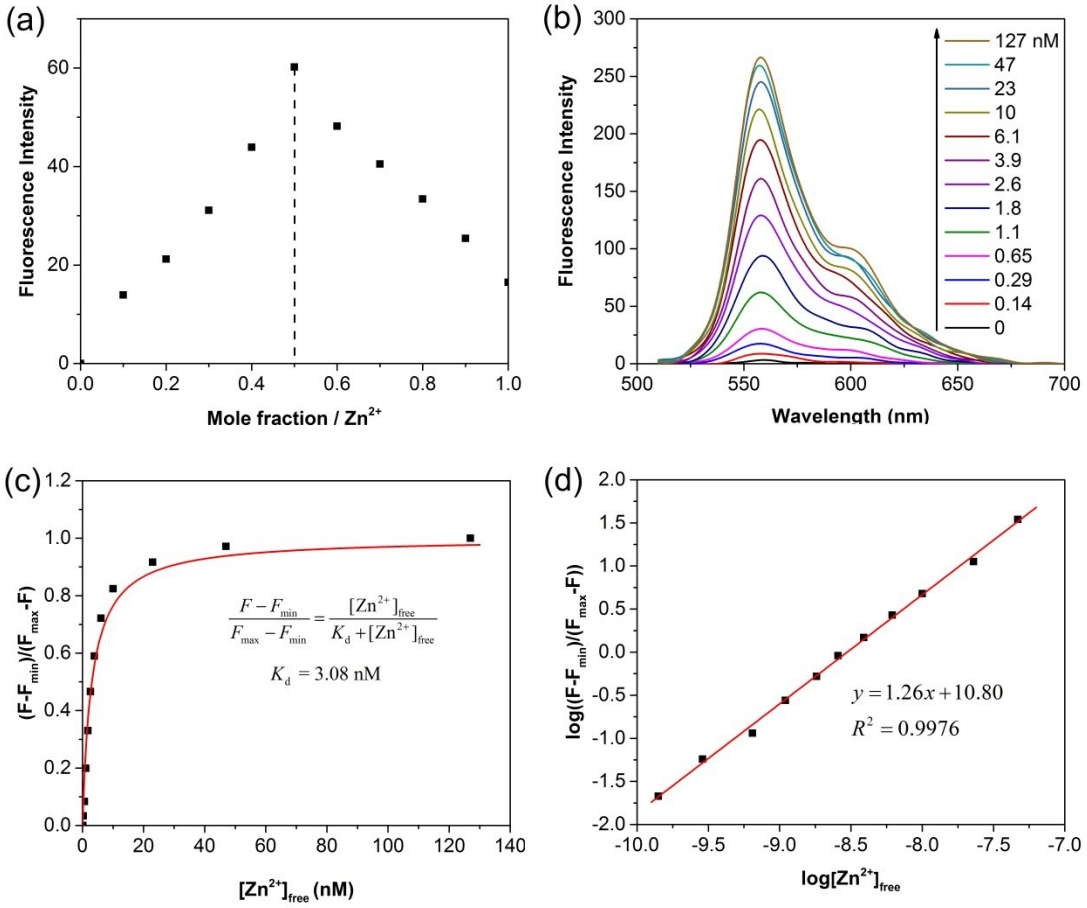

Figure S7. (a) Job's plot of **DPP-C2** in HEPES buffer (50 mM HEPES, 100 mM KCl, pH 7.2, 1% DMSO). The total concentration of **DPP-C2** and Zn^{2+} was maintained at 1μM. (b) Emission spectra of **DPP-C2** in HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, pH 7.2) with a series of $[Zn^{2+}]_{free}$ (0 ~ 127 nM). (c) The fluorescence titration curve of the complexation of **DPP-C2** with $[Zn^{2+}]_{free}$ (0 ~ 127 nM). (d) Hill plots for the complexation of **DPP-C2** with free $[Zn^{2+}]_{free}$. The excitation wavelength was 500 nm.

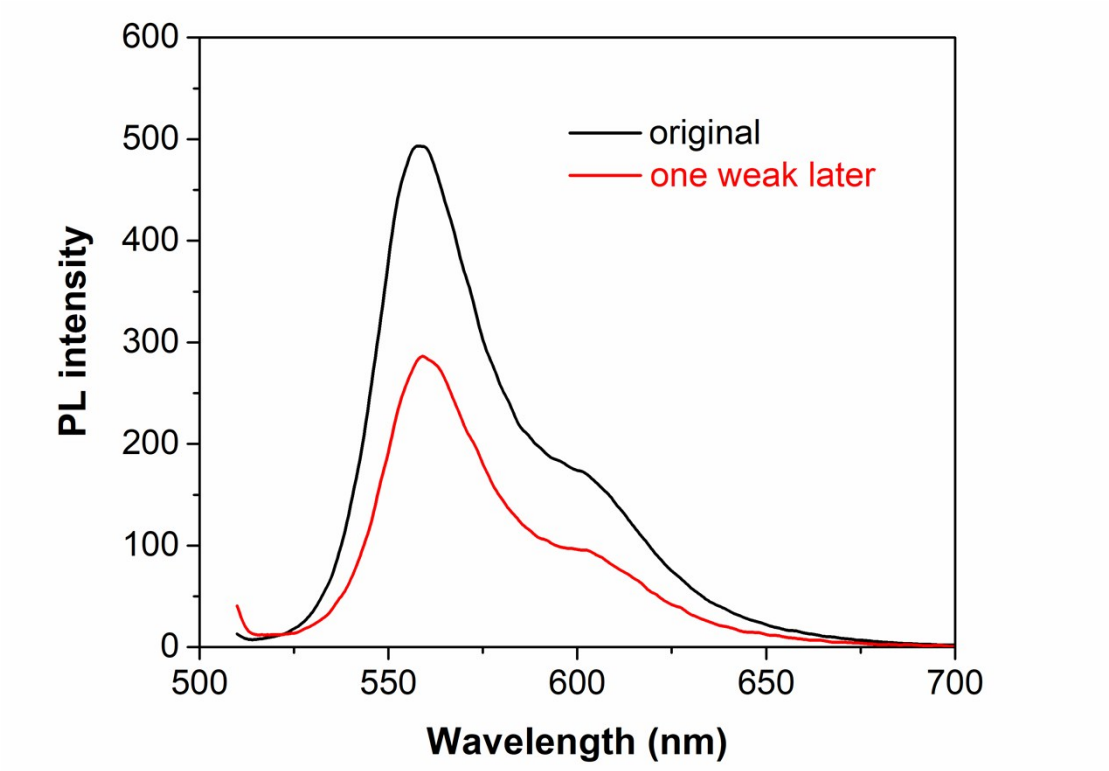

Figure S8. Fluorescence spectra of **DPP-Mito**/Zn^{2+} complex in buffer solution before and after exposure to natural light for one week.

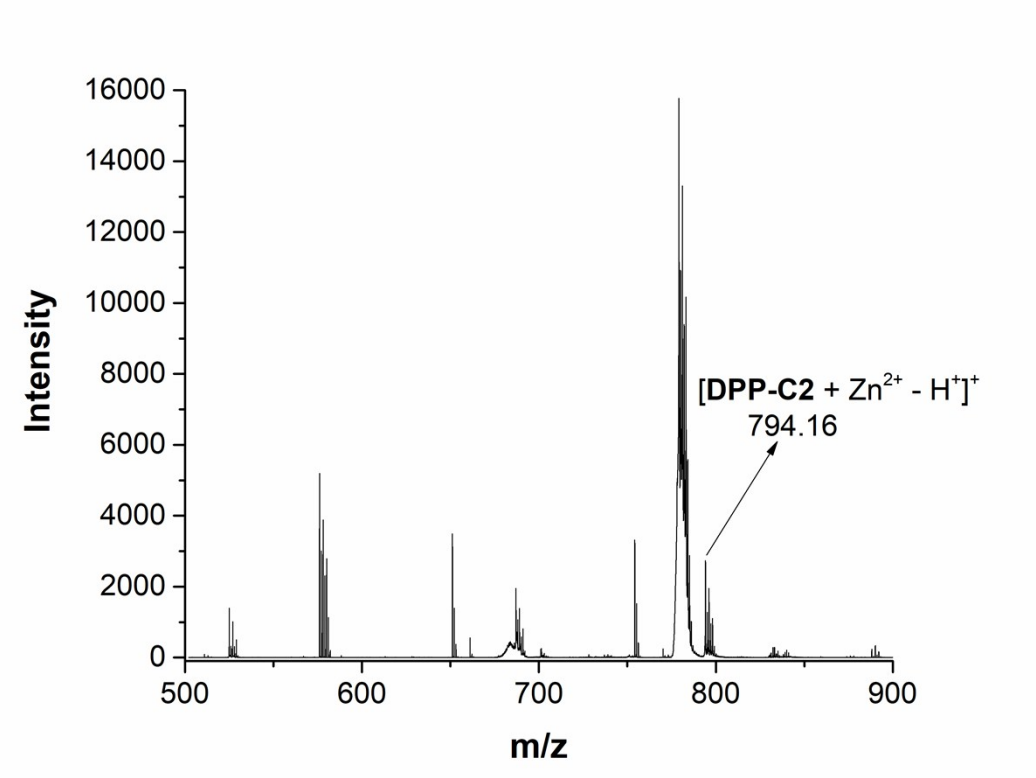

Figure S9. MS (MALDI-TOF) spectrum of **DPP-C2**/Zn^{2+} complex.

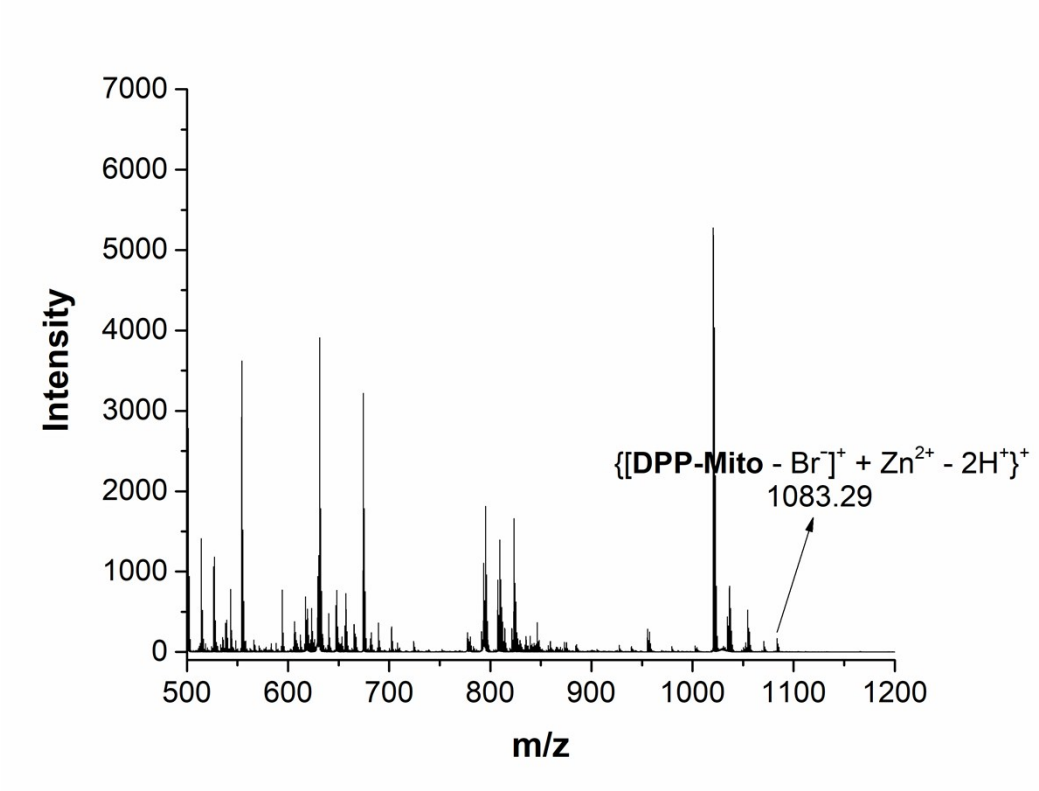

Figure S10. MS (MALDI-TOF) spectrum of **DPP-Mito**/Zn^{2+} complex.

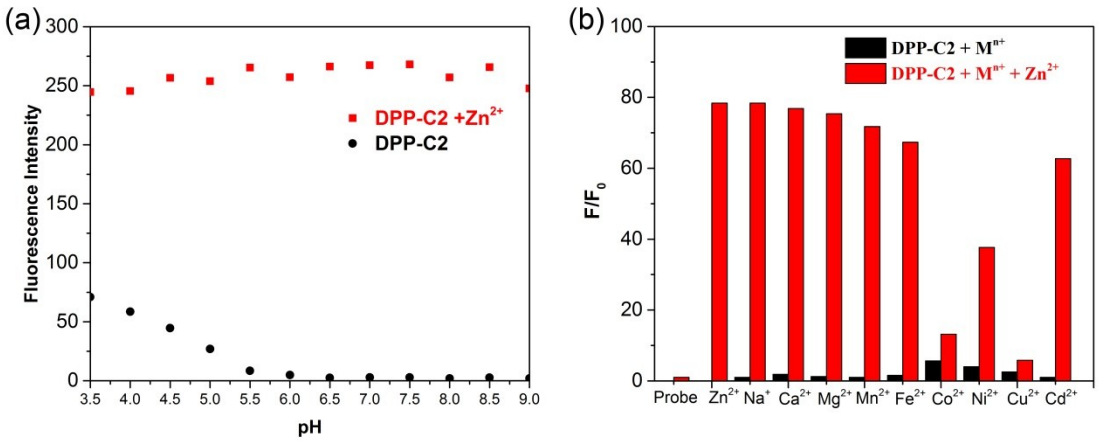

Figure S11. (a) The influence of pH on **DPP-C2** with or without Zn^{2+} in HEPES buffer. (b) Metal ion selectivity profiles of **DPP-C2** (1 μM) in HEPES buffer solutions (50 mM HEPES, 100 mM KCl, 10 mM EGTA, pH 7.2, 1% DMSO). Black bars represent the relative fluorescence intensities (F/F_0) of **DPP-C2** with various metal ions. Red bars represent the relative fluorescence intensities (F/F_0) of **DPP-C2** in the presence of the indicated metal ions, followed by Zn^{2+}.

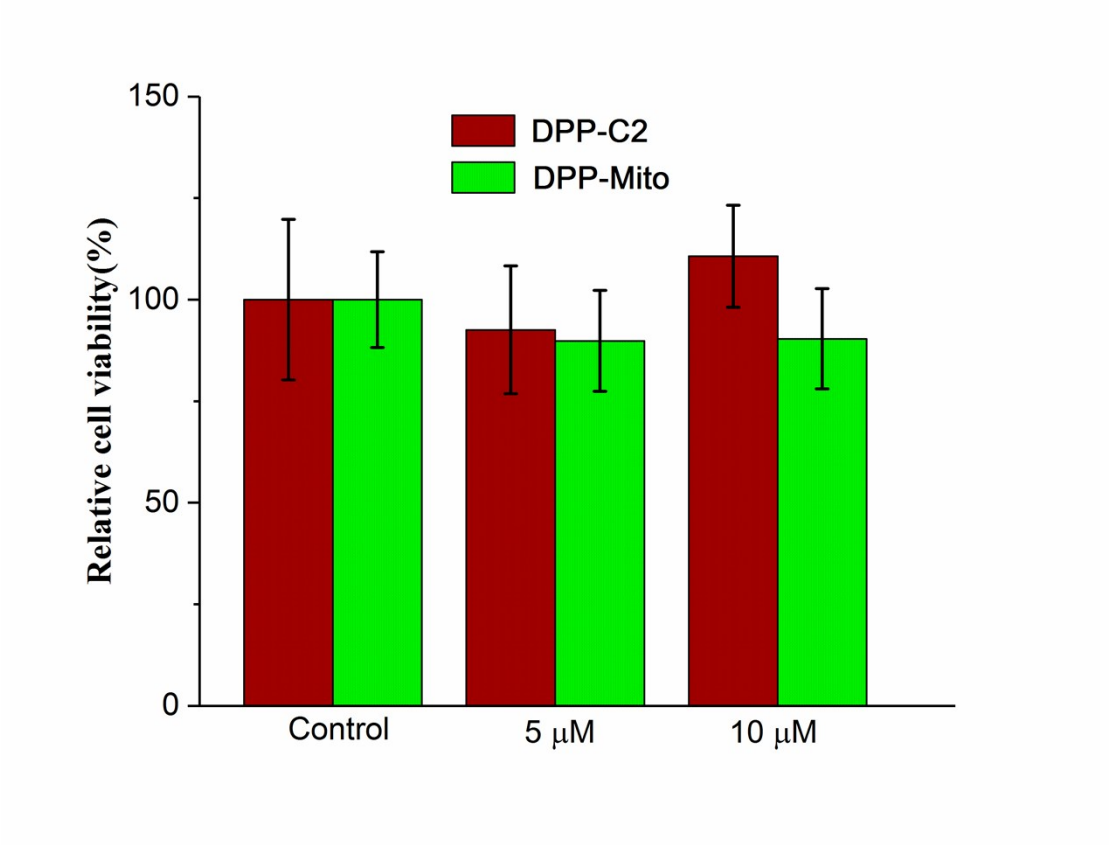

Figure S12. Viability of Hela cells after incubation in cell culture medium containing 5 and 10 μM probes for 24 h as measured by CCK-8 cell-viability assay.

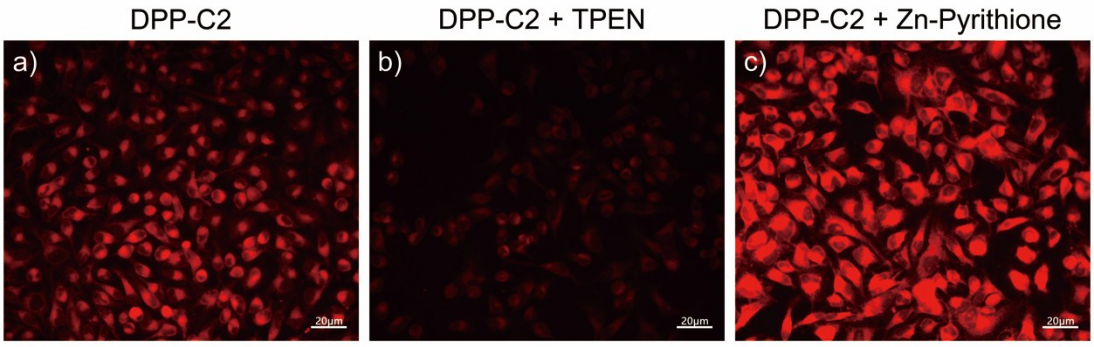

Figure S13. Fluorescent microscopy images of Hela cells (a) the cells were incubated with **DPP-C2** (10 μM) for 24 h; (b) the cells were incubated with **DPP-C2** (10 μM) for 24 h, followed by further incubation with TPEN (50 μM) for 10 min; (c) the cells were incubated with **DPP-C2** (10 μM) for 24 h, followed by further incubation with a mixture of 50 μM zinc pyrithione for 10 min.

Table S1. Spectroscopic properties of probes with/without Zn^{2+} in various solvents.[a]

Compound	Solvent	λ^{abs}_{max} (nm) [b]	λ^{fl}_{max} (nm) [c]	Φ [d]
DPP-C2	DCM	543	562	0.03
	DMSO	547	564	0.0144
	Ethanol	536	557	0.025
	Buffer	519	558	0.0036
DPP-C2+Zn^{2+}	DCM	543	563	0.1544
	DMSO	548	564	0.2829
	Ethanol	538	557	0.2887
	Buffer	526	558	0.1330
DPP-Mito	DCM	543	563	0.0271
	DMSO	548	565	0.02
	Ethanol	539	559	0.0154
	Buffer	515	560	0.0086
DPP-Mito+Zn^{2+}	DCM	542	562	0.2418
	DMSO	548	564	0.2825
	Ethanol	539	559	0.2863
	Buffer	510	560	0.1562

[a] The probes were measured in HEPES buffer (50 mM HEPES, 100 mM KCl, 10 mM EGTA, pH 7.2, 1% DMSO) with or without 10 mM Zn^{2+}. [b] The maximum absorption wavelength in solution. [c] The maximum emission wavelength in solution. The excitation wavelength was 500 nm. [d] Fluorescent quantum yields were determined in reference to rhodamine 6G (Φ_F = 0.89, in CH_2Cl_2).