Electronic Supplementary Information (ESI)

Mitochondria Targeted Self-assembled Ratiometric Fluorescence Nanoprobes for pH Imaging in Living Cells

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Table S1. Purification conditions of Ad-TPP in HPLC

Time (min)	Flow (mL/min)	Methanol %	Water %
0	1	30	70
10	1	45	55
20	1	45	55
30	1	55	45

* Column temperature set to 35 ^{o}C.

Figure S1 Mass Spectra of Ad-R and Ad-F

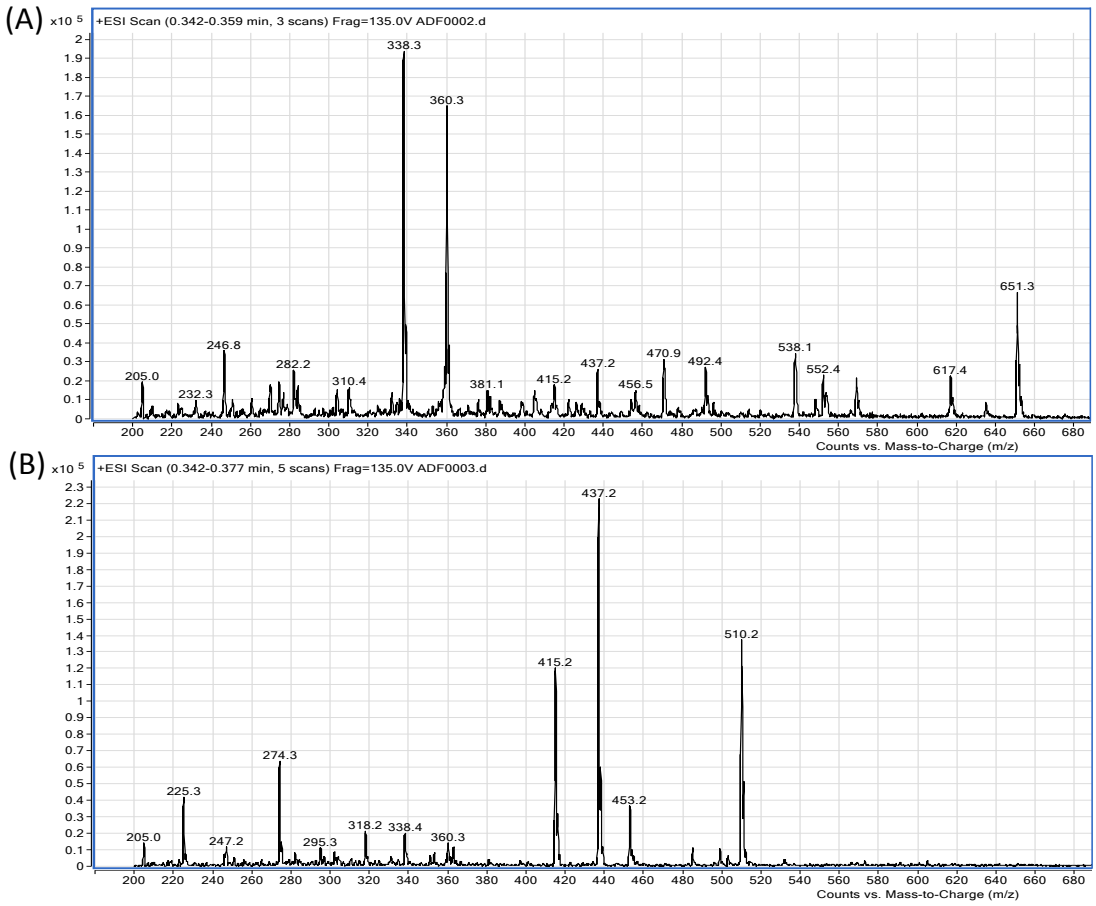
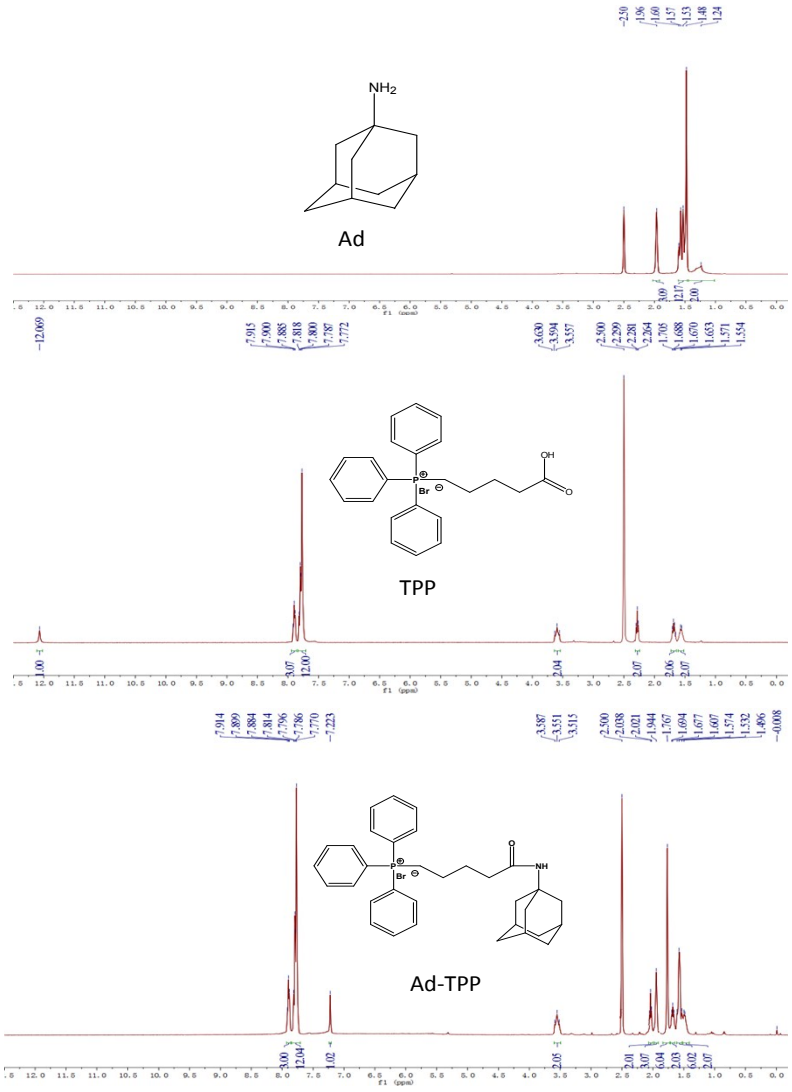


Figure S1. Mass spectra of adamantane-labeled rhodamine (Ad-R) ESI-MS, m/z: 651.3 ($[M\text{-}Cl]^+$) (A) and adamantane-labeled fluorescein (Ad-F), ESI-MS, m/z: 510.2 ($[M\text{-}H]^-$).

Figure S2 ^{1}H-NMR spectra and mass spectra of Ad-TPP

Ad, TPP and Ad-TPP were characterized by ^{1}H NMR (Bruker 400MHZ Advance) (Figure S2A). Ad: ^{1}H NMR (400 MHz, DMSO, 25 ^{o}C) δ 1.96 (s, 3H), 1.60-1.48 (m, 12H), 1.24 (s, 2H).; TPP: ^{1}H NMR (400 MHz, DMSO, 25 ^{o}C) δ 12.07 (s, 1H), 7.92-7.89 (m, 3H), 7.82-7.77 (m, 12H), 3.59 (t, J = 14.4 Hz, J = 14.8 Hz, 2H), 2.28 (t, J = 6.8 Hz, J = 6.4 Hz, 2H), 1.70-1.65 (m, 2H), 1.58-1.55 (m, 2H); Ad-TPP:^{1}H NMR (400 MHz, DMSO, 25 ^{o}C) δ 7.91-7.88 (m, 3H), 7.81-7.77 (m, 12H), 7.22 (s, 1H), 3.55 (t, J = 14.4 Hz, J = 14.8 Hz, 2H), 2.04 (t, J = 6.4 Hz, J = 6.8 Hz, 2H), 1.94 (s, 3H), 1.77 (s, 6H), 1.70-1.66 (m, 2H), 1.61-1.53 (m, 6H), 1.53-1.48 (m, 2H). 1H NMR showed that Ad-TPP contains both the characteristic spectra of Ad and TPP. In addition. The molecular mass was characterized by LC-MS (1290/6460 Triple Quad). ESI-MS, m/z: 496.3 ($[M-Br]^{+}$) (Figure S-2B).

(A)



(B)

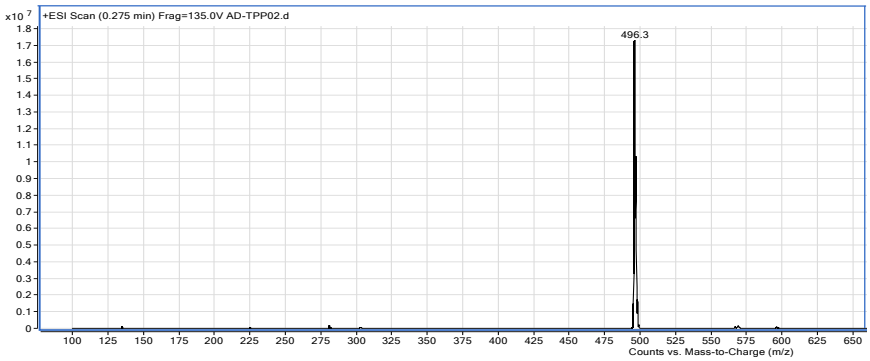


Figure S2. (A) ^{1}H-NMR spectra of 1-adamantanamine (Ad), 4-(carboxybutyl) triphenylphosphonium bromide (TPP) and adamantane-labeled TPP (Ad-TPP); (B) Mass spectra of Ad-TPP.

Figure S3 SEM imaging of T-SRFNPs.

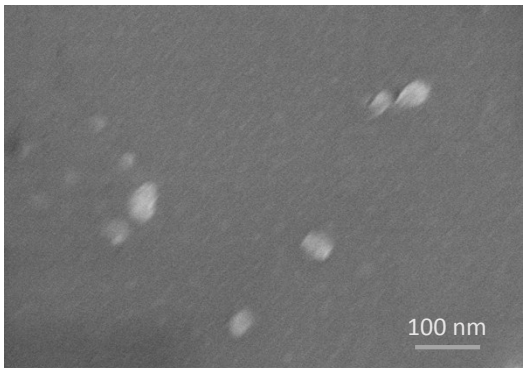


Figure S3. SEM imaging of T-SRFNPs.

Figure S4 Concentration optimization of Ad-TPP.

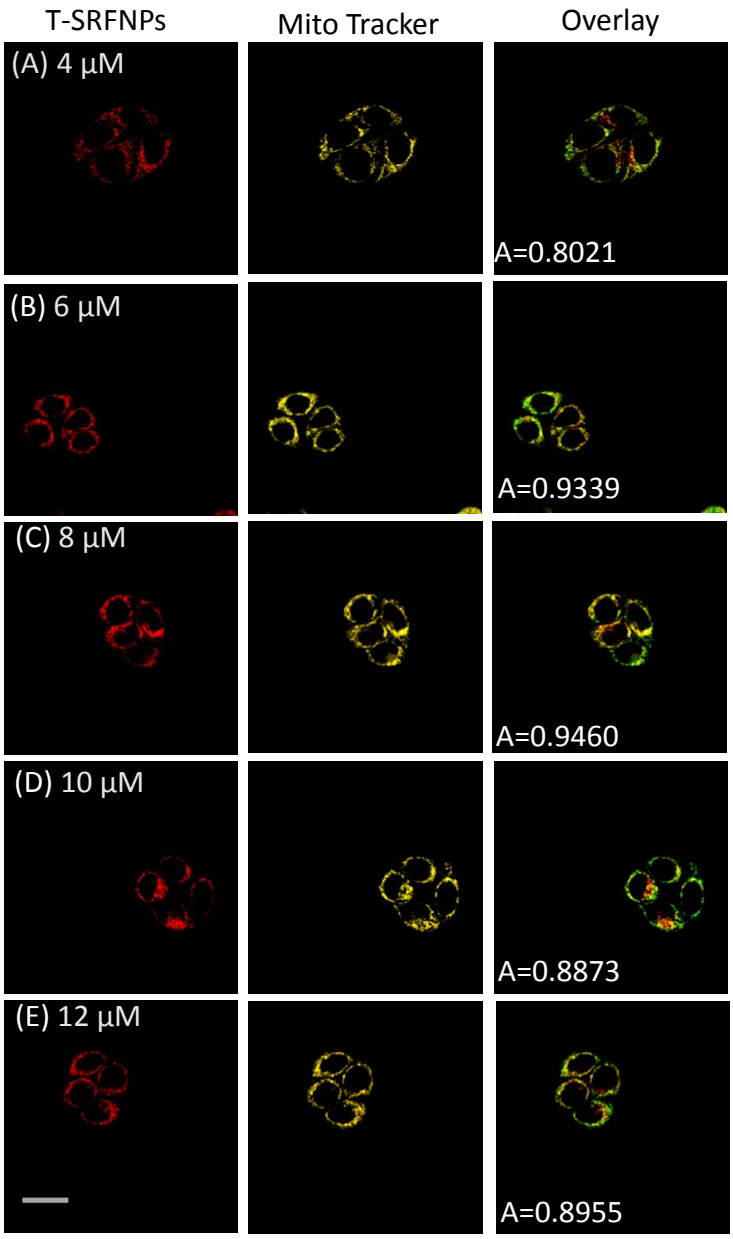


Figure S4. Concentration optimization of Ad-TPP. (A) - (E) Co-localization imaging of Hela cells with T-SRFNPs modified with different concentrations of Ad-TPP. The first row was the rhodamine channel (Ex= 488 nm, collection range: 560-620 nm, red channel); the second row was the Mito Tracker channel (Ex= 633 nm, collection range: after 660 nm, yellow channel); the third row was the overlay image of the first and the second rows. "A" represents the overlap efficiency; scale bar: 20 μm.

Figure S5. Stability of T-SRFNPs in complex systems.

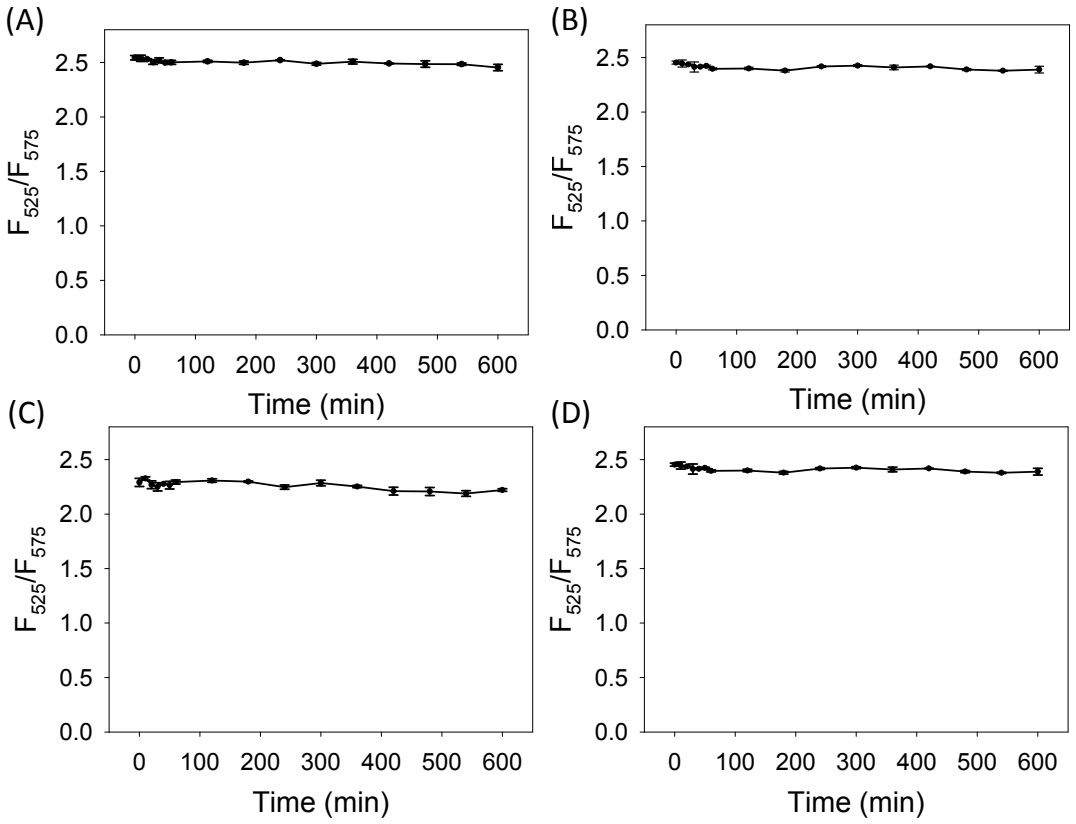


Figure S5. Stability of T-SRFNPs in complex systems. The fluorescence ratios changed with time of T-SRFNPs in PBS buffer (10 μM, pH=7.4) (A); human serum (15% v/v) (B); HeLa cell lysate (C) and cell culture medium containing 10% fetal bovine serum (D), respectively. F_{525} and F_{575} represent the fluorescence intensity at 525 nm and 575 nm, respectively. The error bar represents the standard deviation of three experiments.

Figure S6. Selectivity of T-SRFNPs.

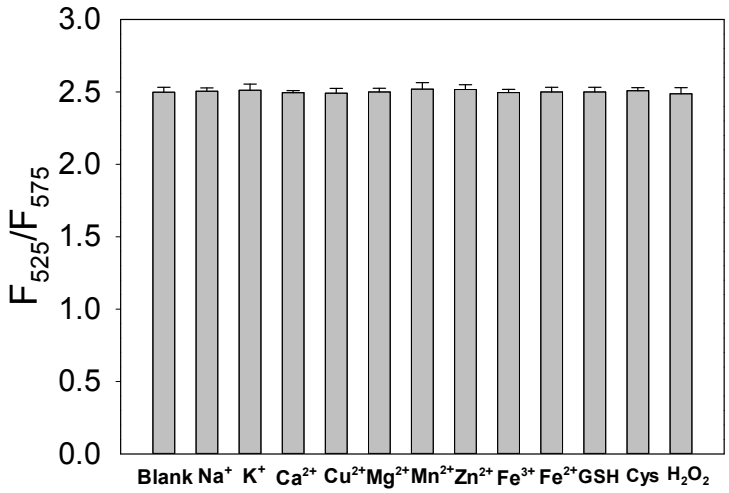


Figure S6. Selectivity tests of the nanoprobes toward various metal ions and oxidative-stress-associated redox chemicals. The error bar represents the standard deviation of three experiments.