Organic molecular nanostructure probes for two-photon imaging of mitochondria and microbes with emission between 430 nm to 640 nm

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This SI includes the following contents:

Quantum Yield Measurements

We calculate the quantum yield (Q) of TPE-dots with the following equation. We choose quinine sulfate in 0.1 M H_2SO_4, fluorescein in water, and rhodamine B in ethanol as standards of TPE-Acr, TPE-Py, and TPE-Quino, respectively. Since Q is the quantum yield, I is the measured integrated emission intensity, n is the refractive index, and A is the optical density. The subscript R refers to the reference fluorophore of known quantum yield.

$$Q = Q_R \frac{I}{I_R} \frac{A_R}{A} \frac{n^2}{n_R^2}$$

Table S1: Quantum yields of the TPE dots.

Sample	Integrated emission intensity (I)	Absorbance (A)	Refractive index of solvent (n)	Quantum yield (Q)
Quinine sulfate	41105	0.06	1.33	0.54 (known)
TPE-Acr	146151	0.18	1.33	0.64
Fluorescein	84367	0.07	1.33	0.925 (known)
TPE-Py	158831	0.23	1.33	0.53
Rhodamine B	94310	0.05	1.36	0.97 (known)
TPE-Quino	227927	0.19	1.33	0.59

Two-photon absorption (TPA) cross-section

We study the two-photon absorption (TPA) spectra of the TPE-dots using a two-photon-induced fluorescence (TPIF) technique with a femtosecond pulsed laser source. A mode locked Ti: Sapphire laser is excitation source with pulses of 100 fs and a repetition rate of 80 MHz. We measure TPA cross-sections (δ) of the dyes in the wavelength range from 700-880 nm. We calculate δ from the following equation:

$$\frac{\delta_2}{\delta_1} = \frac{F_2 Q_1 c_1 n_1}{F_1 Q_2 c_2 n_2}$$

Where δ_1 and δ_2 are the TPA cross-sections, F_1 and F_2 are the TPIF intensities, Q_1 and Q_2 are the fluorescence quantum yields, c_1 and c_2 are the concentrations, n_1 and n_2 are the refractive index of solvents.

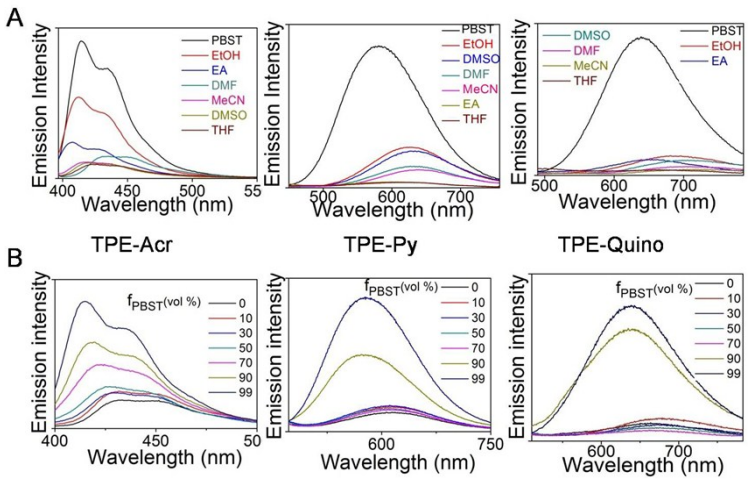

Fig. S1. AIE ability of TPE-Acr, TPE-Py, and TPE-Quino. (A) Emission spectra of TPEs in different solvents. Solution concentration：20 μM. Abbreviation: DMF: dimethylformamide, THF: tetrahydrofuran, EA: ethyl acetate, MeCN: acetonitrile, EtOH: ethanol, DMSO: dimethyl sulfoxide, PBST: phosphate-buffered saline (PBS) with 0.1% Tween 20. (B) Emission spectra of TPEs in THF-PBST mixtures with different PBST fractions (f_{PBST}). Excitation wavelength: 380 nm, 390 nm and 405 nm, respectively.

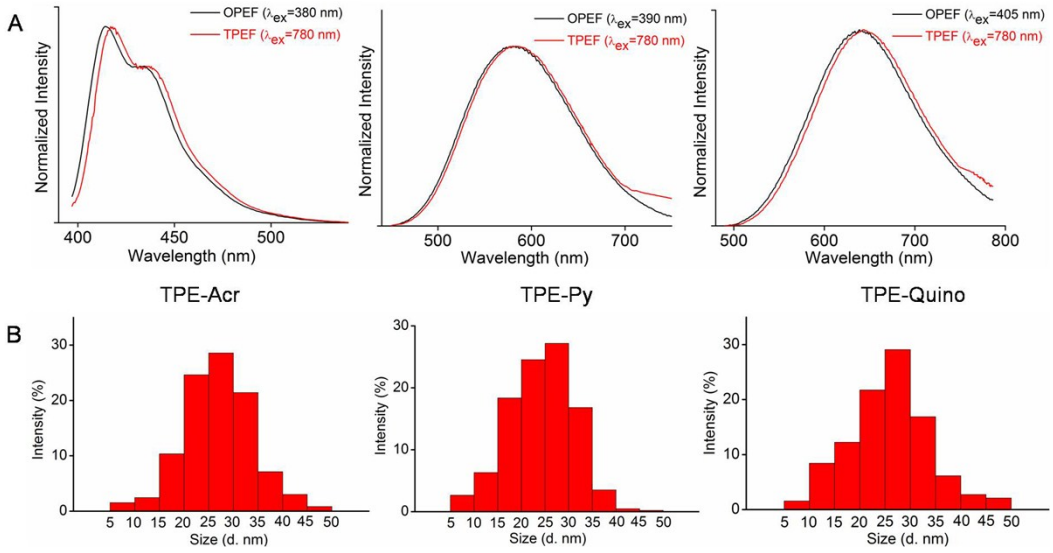

Fig. S2. One-photon excited fluorescence (OPEF) spectra with two-photon excited fluorescence (TPEF) spectra of TPE-Acr, TPE-Py, and TPE-Quino dots (A); dynamic light scattering (DLS) analysis of particle sizes (B).

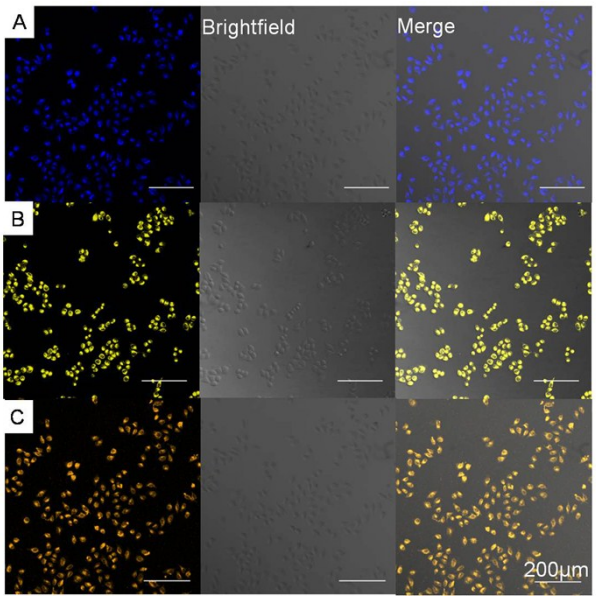

Fig. S3. HeLa cells images with (A) TPE-Acr, (B) TPE-Py, and (C) TPE-Quino dots using one-photon excitation.

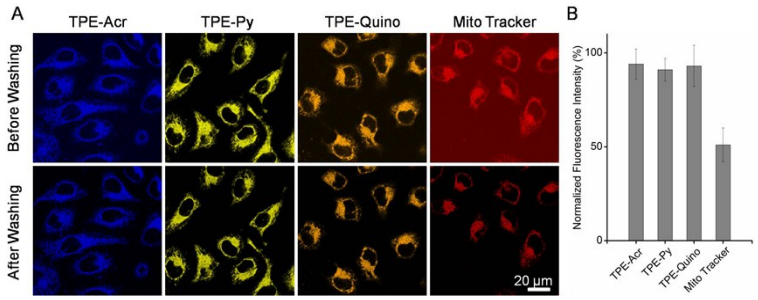

Fig. S4. Washing is not required for TPE dots staining. (A) Fluorescent images of HeLa cells stained by TPE-dots and Mito Tracker Red before and after washing. (B) fluorescence intensity of HeLa cells after washing.

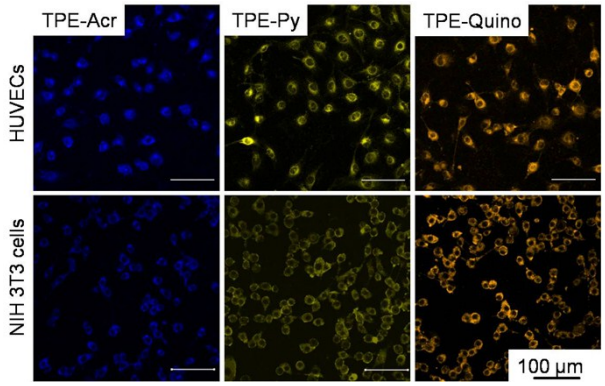

Fig. S5. HUVECs and NIH 3T3 cells images with AIE dots using one-photon excitation.