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

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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$_2$SO$_4$, 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>
<thead>
<tr>
<th>Sample</th>
<th>Integrated emission intensity ($I$)</th>
<th>Absorbance ($A$)</th>
<th>Refractive index of solvent ($n$)</th>
<th>Quantum yield ($Q$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine sulfate</td>
<td>41105</td>
<td>0.06</td>
<td>1.33</td>
<td>0.54 (known)</td>
</tr>
<tr>
<td>TPE-Acr</td>
<td>146151</td>
<td>0.18</td>
<td>1.33</td>
<td>0.64</td>
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<tr>
<td>Fluorescein</td>
<td>84367</td>
<td>0.07</td>
<td>1.33</td>
<td>0.925 (known)</td>
</tr>
<tr>
<td>TPE-Py</td>
<td>158831</td>
<td>0.23</td>
<td>1.33</td>
<td>0.53</td>
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<tr>
<td>Rhodamine B</td>
<td>94310</td>
<td>0.05</td>
<td>1.36</td>
<td>0.97 (known)</td>
</tr>
<tr>
<td>TPE-Quino</td>
<td>227927</td>
<td>0.19</td>
<td>1.33</td>
<td>0.59</td>
</tr>
</tbody>
</table>

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 ($\delta$) of the dyes in the wavelength range from 700-880 nm. We calculate $\delta$ 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 $\delta_1$ and $\delta_2$ 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.

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