Supporting Information

Facilely prepared aggregation-induced emission (AIE) nanocrystals with deep-red emission for super-resolution imaging

Ruohan Xu,a Dongfeng Dang,*a Zhi Wang,a Yu Zhou,b,c Yanzi Xu,a Yizhen Zhao,b Xiaochi Wang,a Zhiwei Yang,b and Lingjie Meng*a,c

a School of Chemistry, Xi'an Key Laboratory of Sustainable Energy Material Chemistry, Xi'an Jiao Tong University, Xi'an, 710049, P. R. China.

b School of Physics, Xi'an Jiao Tong University, Xi'an, 710049, P. R. China.

c Instrumental Analysis Center, Xi'an Jiao Tong University, Xi'an, 710049, P. R. China.

Corresponding author:
Prof. Dongfeng Dang and Prof. Lingjie Meng
E-mail addresses: dongfengdang@xjtu.edu.cn and menglingjie@xjtu.edu.cn
**Synthetic Procedures**

The synthetic procedures to DTPA-BT-H, DTPA-BT-MO, and DTPA-BT-F are depicted in Scheme S1. Their molecular structures were also characterized by $^1$H NMR, $^{13}$C NMR and HR-MS.

**Synthesis of DTPA-BT-H**

To a 250 mL flask, compound 1 (4-Methoxy-N-(4-methoxyphenyl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)aniline, 2.5 mmol, 1.08 g), compound 2 (4,7-dibromo-2,1,3-benzothiadiazole, 1 mmol, 294 mg), ethanol (10 mL), Pd(PPh$_3$)$_4$ (Tetrakis(triphenylphosphine)palladium, 0.05 mmol, 57 mg) and K$_2$CO$_3$ (2 M, 12.5 mL) were added into toluene solution (70 mL) under a N$_2$ atmosphere. Then, the reaction mixture was stirred at 80 ℃ for 20 h. After cooling down, the mixture was extracted with dichloromethane (DCM) for three times (3×70 mL), and the obtained organic phase was washed with water (3×70 mL). Then, the solvent was removed under vacuum and the crude compound was purified by column chromatography (DCM/petroleum ether= 2/1, v/v) to get a pure DTPA-BT-H (535 mg, 72%). $^1$H NMR (600 MHz, Chloroform-d) δ 7.82 (d, $J$ = 8.82 Hz, 4H), 7.69 (s, 2H), 7.15 (d, $J$ = 8.94 Hz, 8H), 7.06 (d, $J$ = 8.76 Hz, 4H), 6.87 (d, $J$ = 8.94 Hz, 8H), 3.81 (s, 12H). $^{13}$C NMR (150 MHz, Chloroform-d) δ 156.09, 154.21, 148.78, 140.58, 132.02, 129.67, 129.09, 126.98, 119.80, 114.73, 55.49 ppm. HR-MS (TOF-ESI+): m/z calculated for [M]$^+$ C$_{46}$H$_{38}$N$_4$O$_4$S, 742.26138; Found, 742.26117. m/z calculated for [M]$^{2+}$ C$_{46}$H$_{38}$N$_4$O$_4$S, 371.13069; Found, 371.12916.

**Synthesis of DTPA-BT-MO**

The synthetic procedure to DTPA-BT-MO is similar to that in DTPA-BT-H. The only difference is that compound 2 is changed to be compound 3 (4,7-dibromo-5,6-methoxy-2,1,3-benzothiadiazole). The yield is 68% here (546...
mg. \textsuperscript{1}H NMR (600 MHz, Chloroform-\textit{d}) \( \delta \) 7.55 (d, \( J = 8.76 \) Hz, 4H), 7.17 (d, \( J = 8.88 \) Hz, 8H), 7.04 (d, \( J = 8.76 \) Hz, 4H), 6.87 (d, \( J = 8.94 \) Hz, 8H), 3.81 (s, 18H). \textsuperscript{13}C NMR (150 MHz, Chloroform-\textit{d}) \( \delta \) 156.11, 153.27, 152.35, 148.41, 140.59, 131.05, 127.20, 124.73, 123.58, 118.89, 114.72, 61.34, 55.48 ppm. HR-MS (TOF-ESI\textsuperscript{+}): \( m/z \) calculated for [M+H]\textsuperscript{+} \( C_{48}H_{42}N_4O_6S \), 803.29033; Found, 803.28715.

**Synthesis of DTPA-BT-F**

The synthetic procedure to DTPA-BT-F is similar to that in DTPA-BT-H. The only difference is that compound 2 is changed to be compound 4 (4,7-dibromo-5,6-fluorine-2,1,3-benzothiadiazole). The yield is 75\% here (584 mg). \textsuperscript{1}H NMR (600 MHz, Chloroform-\textit{d}) \( \delta \) 7.67 (d, \( J = 8.76 \) Hz, 4H), 7.17 (d, \( J = 8.94 \) Hz, 8H), 7.04 (d, \( J = 8.88 \) Hz, 4H), 6.88 (d, \( J = 8.94 \) Hz, 8H), 3.81 (s, 12H). \textsuperscript{13}C NMR (150 MHz, Chloroform-\textit{d}) \( \delta \) 156.41, 149.29, 140.22, 131.18, 127.42, 121.31, 118.68, 114.83, 55.51 ppm. HR-MS (TOF-ESI\textsuperscript{+}): \( m/z \) calculated for [M+H]\textsuperscript{+} \( C_{46}H_{36}F_2N_4O_4S \), 779.25036; Found, 779.24673.
Scheme S1. The synthetic route to DTPA-BT-H, DTPA-BT-MO, and DTPA-BT-F.

Figure S1. $^1$H NMR spectrum of DTPA-BT-H.
Figure S2. $^{13}$C NMR spectrum of DTPA-BT-H.

Figure S3. HR-MS spectrum of DTPA-BT-H.
Figure S4. $^1$H NMR spectrum of DTPA-BT-MO.

Figure S5. $^{13}$C NMR spectrum of DTPA-BT-MO.
Figure S6. HR-MS spectrum of DTPA-BT-MO.

Figure S7. $^1$H NMR spectrum of DTPA-BT-F.
Figure S8. $^{13}$C NMR spectrum of DTPA-BT-F.

Figure S9. HR-MS spectrum of DTPA-BT-F.
Figure S10. UV-vis spectra of DTPA-BT-H, DTPA-BT-MO and DTPA-BT-F in THF ([c] = 1×10^{-5} M).

Figure S11. Normalized UV-vis spectra (A-C) and normalized PL spectra (D-F) of DTPA-BT-H (A and D), DTPA-BT-MO (B and E) and DTPA-BT-F (C and F) in solvents with varied polarities.
Figure S12. Plot of Stokes' shift ($\Delta\nu$) vs. $\Delta f$ for DTPA-BT-H, DTPA-BT-MO and DTPA-BT-F in solvents with varied polarities.

Figure S13. PL spectra of DTPA-BT-H in THF/water mixtures with different $f_w$ ([c] = 1x10^{-5} M).
Figure S14. PL spectra of DTPA-BT-MO in THF/water mixtures with different $f_w$ ([c] = $1 \times 10^{-5}$ M).

Figure S15. PL spectra of DTPA-BT-F in THF/water mixtures with different $f_w$ ([c] = $1 \times 10^{-5}$ M).
Figure S16. Time-dependent fluorescence spectra of DTPA-BT-H in THF/water mixture ([c] = $2\times10^{-5}$ M, $f_w = 60\%$).

Figure S17. Time-dependent fluorescence spectra of DTPA-BT-MO in THF/water mixture ([c] = $2\times10^{-5}$ M, $f_w = 60\%$).
**Figure S18.** Time-dependent fluorescence spectra of DTPA-BT-F in THF/water mixture ([c] = 2×10^{-5} M, f_w = 60%).

**Figure S19.** Time-dependent PL spectra of DTPA-BT-F in THF/water mixture (three parallel experiments; [c] = 2×10^{-5} M, f_w = 60%; A-C); Plots of I/I_0 values and maximum emission wavelengths for DTPA-BT-F in THF/water mixture at different time (D); Colloidal size and the corresponding PDI values for DTPA-BT-F in THF/water mixture at different time (E).
Figure S20. Transient decay spectra of DTPA-BT-H in THF solution and in solids.

Figure S21. Transient decay spectra of DTPA-BT-MO in THF solution and in solids.
Figure S22. Transient decay spectra of DTPA-BT-F in THF solution and in solids.

Figure S23. XRD patterns of DTPA-BT-H, DTPA-BT-MO and DTPA-BT-F in pristine powder.
Figure S24. XRD patterns of DTPA-BT-H powder before and after grinding.

Figure S25. XRD patterns of DTPA-BT-MO powder before and after grinding.
**Figure S26.** XRD patterns of DTPA-BT-F powder before and after grinding.

**Figure S27.** TEM image of large sized precipitations by DTPA-BT-F in THF/water mixture ($f_w=60\%$) when stored for 6 d.
Figure S28. Particle size distribution of DTPA-BT-H NPs (A), DTPA-BT-MO NPs (B) and DTPA-BT-F NCs (C); Their corresponding colloidal stability of size and PDI values over 7 d (D-F).

Figure S29. TEM image of freshly-prepared DTPA-BT-F NCs (A and C) and room temperature-stored DTPA-BT-F NCs (B and D) in water (A and B) and cell growth media (C and D).
Figure S30. HOMO and LUMO energy levels of DTPA-BT-H, DTPA-BT-MO and DTPA-BT-F calculated by DFT at B3LYP/6-31G(d) level.

Figure S31. ESP of DTPA-BT-H, DTPA-BT-MO and DTPA-BT-F calculated by DFT at B3LYP/6-31G(d) level.

Figure S32. Dihedral angle distribution between phenyl rings a and b for DTPA-BT-H, DTPA-BT-MO and DTPA-BT-F in single molecular state (A) and aggregated state (B); The corresponding calculated FWHM values of dihedral angle distribution (C).
Figure S33. The cluster analysis of DTPA-BT-H simulated by AMBER18 package.

Figure S34. The cluster analysis of DTPA-BT-MO simulated by AMBER18 package.
**Figure S35.** The cluster analysis of DTPA-BT-F simulated by AMBER18 package.

**Figure S36.** UV-vis spectra of DTPA-BT-H NPs, DTPA-BT-MO NPs and DTPA-BT-F NCs ([c] = 20 μg·mL⁻¹).
Figure S37. PL spectra of DTPA-BT-H NPs, DTPA-BT-MO NPs and DTPA-BT-F NCs ([c] = 20 μg·mL⁻¹).

Figure S38. Fluorescence images of SiR (A) and DTPA-BT-F (B) thin film by UV lamp.
Figure S39. Time-dependent PL spectra for DTPA-BT-H NPs (A), DTPA-BT-MO NPs (B) and DTPA-BT-F NCs (C) under Xenon lamp irradiation over 1 h; Plots of $I/I_0$ values for DTPA-BT-H NPs (D), DTPA-BT-MO NPs (E) and DTPA-BT-F NCs (F) under Xenon lamp irradiation over 1 h.

Figure S40. Time-dependent PL spectra for SiR (A), DTPA-BT-F NCs (B) and their Plots of $I/I_0$ values (C, the inset is an enlarged view) under high power white laser (200 mW).
Figure S41. UV-vis spectra (A) and PL spectra (B) of DTPA-BT-F NCs dispersed in PBS solutions with different pH values \([c]= 20 \mu g \cdot mL^{-1}\).

Figure S42. The viability of HeLa and L02 cells after incubation with DTPA-BT-F NCs for 24 h under different concentration.
**Figure S43.** The viability of HeLa cells after incubation with DTPA-BT-F NCs for 24 h with or without light irradiation (400-800 nm; 100 mW·cm$^{-2}$, 5 min).

**Figure S44.** Relative fluorescence intensity of HeLa cells stained with LysoSensor Green DND-189 treated without (blank) and with DTPA-BT-F NCs.
Figure S45. CLSM images of HeLa cells co-stained with LysoTracker Green DND-26 (A), DTPA-BT-F NCs (B), and their overlapped images (C); The intensity profile of the whole region in A and B recorded by ImageJ software (D).

Figure S46. Relative fluorescence intensity of HeLa cells stained with DTPA-BT-F NCs incubated under 37 °C or 4 °C.
Figure S47. Relative fluorescence intensity of DTPA-BT-F NCs stained HeLa cells treated without (Blank) or with NH$_4$Cl (10 mM), CPZ (50 μM), genistein (150 μM) and amiloride (100 μM).

Figure S48. The viability of HeLa cells after incubation with DTPA-BT-H NPs and DTPA-BT-MO NPs for 24 h under different concentration.
**Figure S49.** CLSM images of the fixed cells (A-H) stained with DAPI (blue) and DTPA-BT-H NPs (red, B and D) or DTPA-BT-MO NPs (red, F and H); CLSM images of the live cells (I-P) stained with Hoechst 33342 (blue) and DTPA-BT-H NPs (red, J and L) or DTPA-BT-MO NPs (red, N and P).

**Figure S50.** Time-dependent fluorescent images of HeLa cells co-stained with LysoTracker Green DND-26 (A-F) and DTPA-BT-F NCs (G-L) under the irradiation; Plots of $I/I_0$ values for LysoTracker Green DND-26 and DTPA-BT-F NCs stained cells under the irradiation (M).
**Figure S51.** Time-dependent fluorescent images for FITC-Phalloidin (775 nm, 600 mW; A-F), Alexa Fluor488-Phalloidin (775 nm, 600 mW; G-L) and DTPA-BT-F NCs (775 nm, 600 mW; M-R) stained HeLa cells under the irradiation of STED beam.

**Figure S52.** Plots of $I/I_0$ values for FITC-Phalloidin, Alexa Fluor488-Phalloidin and DTPA-BT-F NCs stained HeLa cells under the irradiation of STED beam (775 nm, 600 mW).
**Figure S53.** Fluorescence image of the fixed HeLa cell co-stained with DAPI (blue), FITC-Phalloidin (green; depleted by 592 nm STED laser, 120 mW) and DTPA-BT-F NCs (red; depleted by 775 nm STED laser, 96 mW) via STED nanoscopy (A); Magnified fluorescence images in FITC-Phalloidin channel by CLSM (B), STED nanoscopy (C), and their corresponding PL intensity curves (D) in A (ROI 1).

**Figure S54.** Fluorescence images of DTPA-BT-F NCs-stained cell captured by CLSM (A), STED nanoscopy under different beam powers (B-E), and their corresponding FWHM values in the ROI marked by yellow circle (F-I); Plot of lateral spatial resolution in B-E under STED nanoscopy versus STED power (J). The lateral spatial resolution is defined as $R/R_0$ value here, where $R_0$ is the FWHM difference between CLSM and STED mode at initial power (24 mW) and $R$ is the FWHM difference at other powers (48, 72, and 96 mW).
**Figure S55.** Power dependent depletion intensity for DTPA-BT-F NCs in STED nanoscopy.

**Figure S56.** Theoretical resolution of DTPA-BT-F NCs under different STED power by the equation:

\[
\Delta x \approx \frac{\lambda}{2NA\sqrt{1 + \frac{I}{I_{\text{sat}}}}}.
\]
**Figure S57.** The live HeLa cells stained with Hoechst 33342 (blue) and DTPA-BT-F NCs (red) captured by CLSM (A) and STED nanoscopy (B) in 2D view.

**Figure S58.** CLSM image of the live HeLa cell (A, the inset is the enlarged view of ROI marked by dashed square); Time-dependent STED images in ROI, and their corresponding intensity profiles along the dashed lines by CLSM and STED nanoscopy (B-F).
Figure S59. Normalized emission intensity of DTPA-BT-F NCs stained HeLa cells under different excitation wavelength (A); Fluorescent images of HeLa cells stained with DTPA-BT-F NCs (E₅: 830 nm; B) in TPF microscopy.
Table S1. Photo-physical properties of DTPA-BT-H, DTPA-BT-MO and DTPA-BT-F.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Stokes' shifts (nm/cm(^{-1}))</th>
<th>(\Phi^{[a]}/\Phi^{[b]}) (%)</th>
<th>(\tau^{[a]}/\tau^{[b]}) (ns)</th>
<th>(k_r^{[a]}) ((\times10^7) s(^{-1}))</th>
<th>(k_r^{[b]}) ((\times10^7) s(^{-1}))</th>
<th>(k_n^{[a]}) ((\times10^7) s(^{-1}))</th>
<th>(k_n^{[b]}) ((\times10^7) s(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>DTPA-BT-H</td>
<td>196/6019</td>
<td>24.06/33.96</td>
<td>3.11/5.34</td>
<td>7.74</td>
<td>6.35</td>
<td>24.41</td>
<td>12.38</td>
</tr>
<tr>
<td>DTPA-BT-MO</td>
<td>204/6984</td>
<td>36.26/2.49</td>
<td>3.75/6.63</td>
<td>9.67</td>
<td>0.38</td>
<td>17.00</td>
<td>14.70</td>
</tr>
<tr>
<td>DTPA-BT-F</td>
<td>254/7597</td>
<td>4.03/36.49</td>
<td>1.03/4.22</td>
<td>3.91</td>
<td>8.65</td>
<td>93.17</td>
<td>15.04</td>
</tr>
</tbody>
</table>

\(^{[a]}\) Measured in THF solution; \(^{[b]}\) Measured in solids. Radiative decay rate: \(k_r = \Phi/\tau\); Non-radiative decay rate: \(k_n = 1/\tau - k_r\).

Table S2. Photo-physical properties of DTPA-BT-H, DTPA-BT-MO and DTPA-BT-F in THF solution and in aggregated NPs or NCs state.

<table>
<thead>
<tr>
<th>Samples</th>
<th>(\varepsilon) ((\times10^3) M(^{-1})·cm(^{-1}))</th>
<th>(\Phi^{[a]}) (%)</th>
<th>(L^{[b]}) ((\times10^3) M(^{-1})·cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NPs or NCs</td>
<td>Solution</td>
<td>NPs or NCs</td>
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<tr>
<td>DTPA-BT-H</td>
<td>6.94</td>
<td>24.06</td>
<td>24.40</td>
</tr>
<tr>
<td>DTPA-BT-MO</td>
<td>6.39</td>
<td>36.26</td>
<td>14.18</td>
</tr>
<tr>
<td>DTPA-BT-F</td>
<td>13.10</td>
<td>4.03</td>
<td>27.06</td>
</tr>
</tbody>
</table>

\(^{[a]}\) Photoluminescence quantum yield; \(^{[b]}\) Fluorescence brightness (L), \(L = \varepsilon \cdot \Phi\).