Supporting Information

D-A-D organic small molecules with AIE effect for fluorescence imaging guided photothermal therapy

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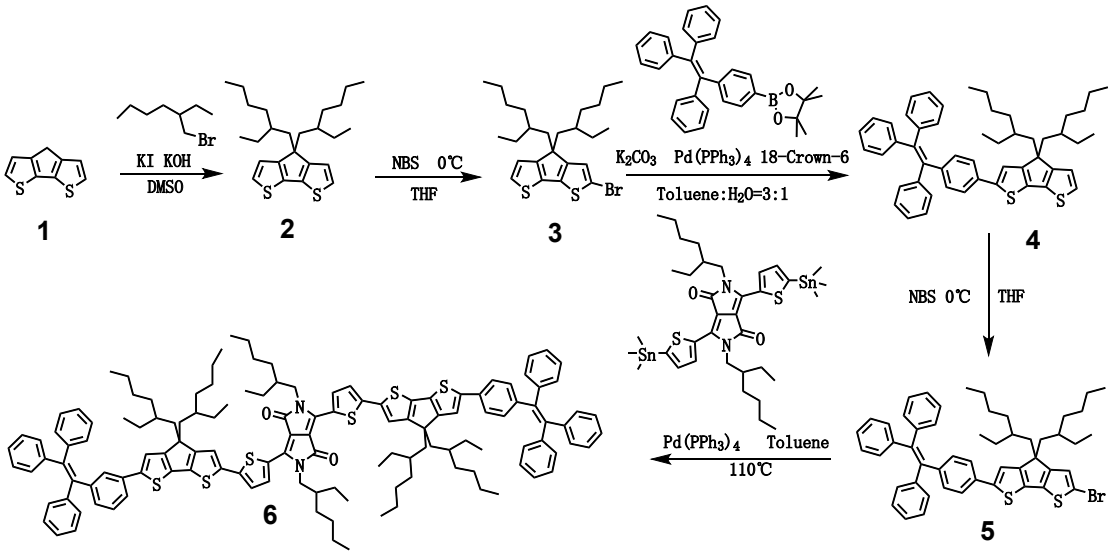

Figure S_1 TDA synthesis scheme.

1. Synthesis of 4,4-bis(2-ethylhexyl)-4H-cyclopentadiene[2,1-b:3,4-b'] dithiophene

Adding cyclopentadithiophene (CPDT) (1 mmol, 0.1779 g, 1 eq), bromoisooctane (2 mmol, 0.3863 g, 2 eq), and KI (0.2 mmol, 0.3320 g, 0.2 eq) into a 100 mL round-bottom flask. Then add 30ml anhydrous DMSO, vacuum the round bottom flask, fill it with N_2, and repeat 3-4 times. Finally, NaOH (4 mmol, 0.2244 g, 4 eq) was added and reacted at room temperature for 15 h. At the end of the reaction, 100 mL saturated salt water and n-hexane were used to extract 3-4 times, then anhydrous $MgSO_4$ was added to stand for 0.5 h, and then the light-yellow oil was obtained by filtration and vacuum distillation. 4,4 - bis (2-ethylhexyl)-4H-cyclopentadiene [2,1-b:3,4-b'] dithiophene (intermediate **2**) (0.9 mmol, 0.3620g) was obtained by silica gel column chromatography with n-hexane as eluent, and the yield was 90 %.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.13 (d, *J* = 4.9 Hz, 1H), 6.94 (dt, *J* = 4.9, 2.0 Hz, 1H), 1.94 – 1.83 (m, 2H), 1.34 – 1.23 (m, 2H), 1.07 – 0.83 (m, 12H), 0.80 – 0.74 (m, 3H), 0.60 (td, *J* = 7.4, 1.5 Hz, 4H).

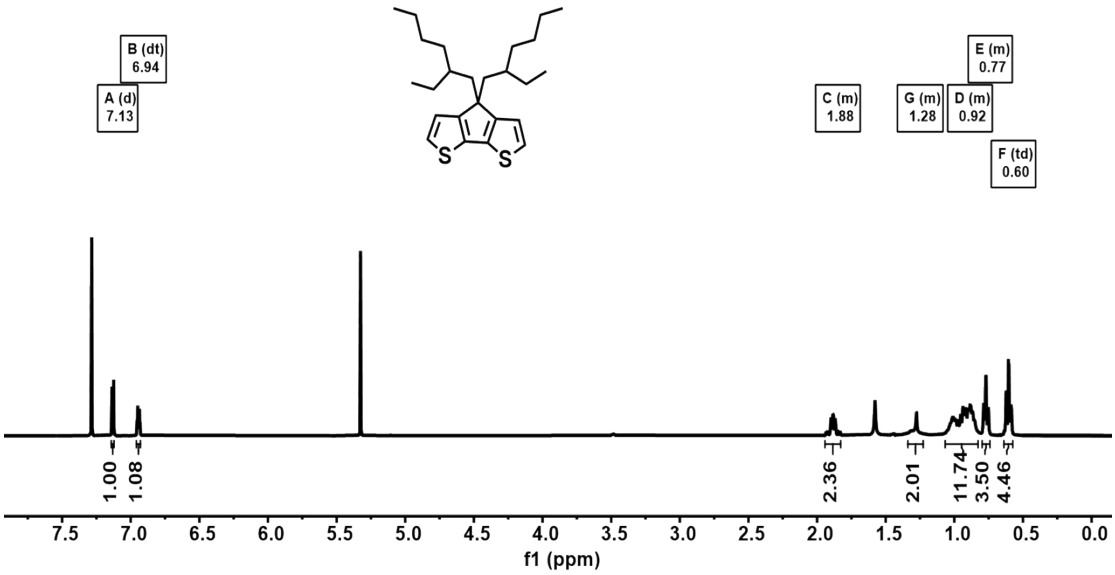

Figure S_2 NMR characterization of intermediate **2.**

2. Synthesis of 2-bromo-4,4-bis (2-ethylhexyl)-4H-cyclopentadiene [2,1-b:3,4-b'] dithiophene

The intermediate product **2** (0.9 mmol, 0.5070 g, 1 eq) was placed in a 100 mL round-bottom flask (dark, wrapped with foil). Then 25 mL THF was added and N_2 was introduced (15 min). NBS (0.9 mmol, 0.1601 g, 1 eq) was added in three batches under ice-water bath conditions (maintain N_2 environment during NBS addition). Finally, the reaction was conducted in dark for 12 h. After the reaction, 150 mL DCM and saturated salt water were used for extraction 3-4 times, and anhydrous $MgSO_4$ was added to stand for 0.5 h. Then the crude product was obtained by filtration and vacuum distillation. With DCM / n-hexane (1: 6) as the eluent, silica gel column chromatography was used to purify. Finally, a pale-yellow oily product 2 - bromo-4,4 - bis (2 - ethylhexyl) -4H-cyclopent -4H-cyclopentadiene [2,1-b:3,4-b’] dithiophene (intermediate product **3**) (0.765 mmol, 0.3673 g) was obtained in 85 % yield.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.14 (dd, *J* = 8.5, 4.9 Hz, 1H), 6.98 – 6.92 (m, 2H), 1.91 – 1.80 (m, 4H), 1.28 (s, 2H), 1.11 – 0.84 (m, 20H), 0.84 – 0.74 (m, 7H), 0.68 – 0.57 (m, 9H).

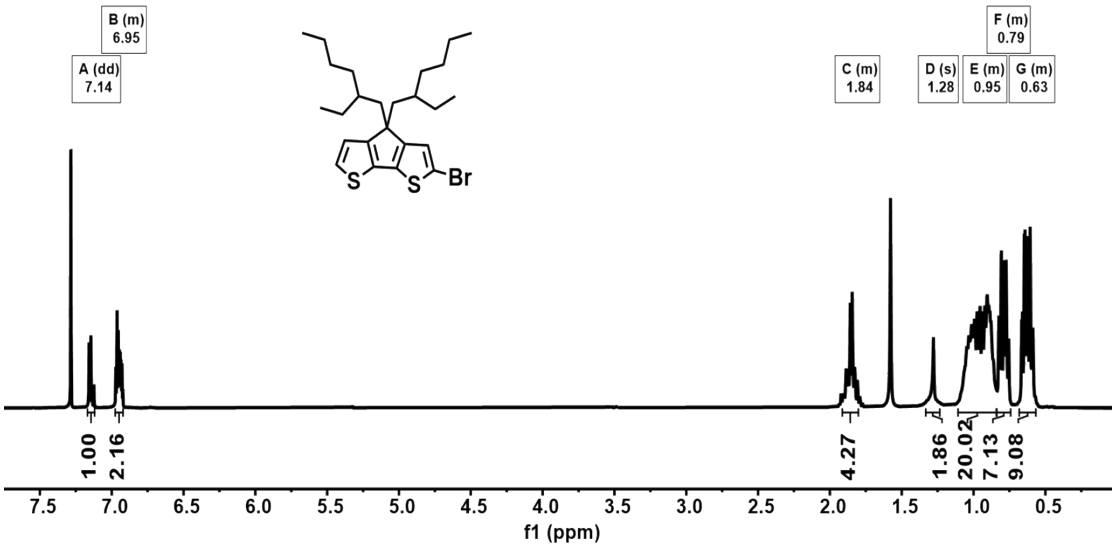

Figure S$_3$ NMR characterization of intermediate **3.**

3. Synthesis of 4,4 - bis (2-ethylhexyl)-2-(4-(1,2,2-triphenylvinyl) phenyl) -4H-cyclopentadiene [2,1-b:3,4 -b'] dithiophene

Intermediate products **3** (0.5 mmol, 0.2400 g, 1 eq), TPE-Bpin (0.6 mmol, 0.2749 g, 1.2 eq), K_2CO_3 (2 mmol, 0.2764 g, 4 eq), 18-crown-6 (0.015 mmol, 0.0040 g, 0.003 eq) were placed in a 100 mL round-bottom flask. Then add 30 mL toluene and 10 mL deionized water according to 3: 1, vacuum the round bottom flask, fill it with N_2, repeat 3-4 times, and finally reflux condensation at 85 °C for 24 h. After the reaction, 150 mL DCM and saturated salt water were used to extract 3-4 times, and then anhydrous $MgSO_4$ was added to stand for 0.5 h. Finally, the crude product was obtained by filtration and vacuum distillation. The white powder solid 4,4-bis (2-ethylhexyl) -2- (4-(1,2,4-triphenylvinyl) phenyl) -4H-cyclopentadiene [2,1-b: 3,4-b ‘] dithiophene (intermediate **4**) (0.45 mmol, 0.3296 g) was obtained by silica gel column chromatography with DCM / hexane (1: 3) as eluent, and the yield was 90 %.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 (dt, *J* = 8.5, 1.7 Hz, 2H), 7.21 – 7.00 (m, 19H), 6.94 (dt, *J* = 4.8, 1.7 Hz, 1H), 1.94 – 1.82 (m, 4H), 1.38 – 1.22 (m, 31H), 1.08 – 0.97 (m, 8H), 0.97 – 0.83 (m, 28H), 0.81 – 0.58 (m, 15H).

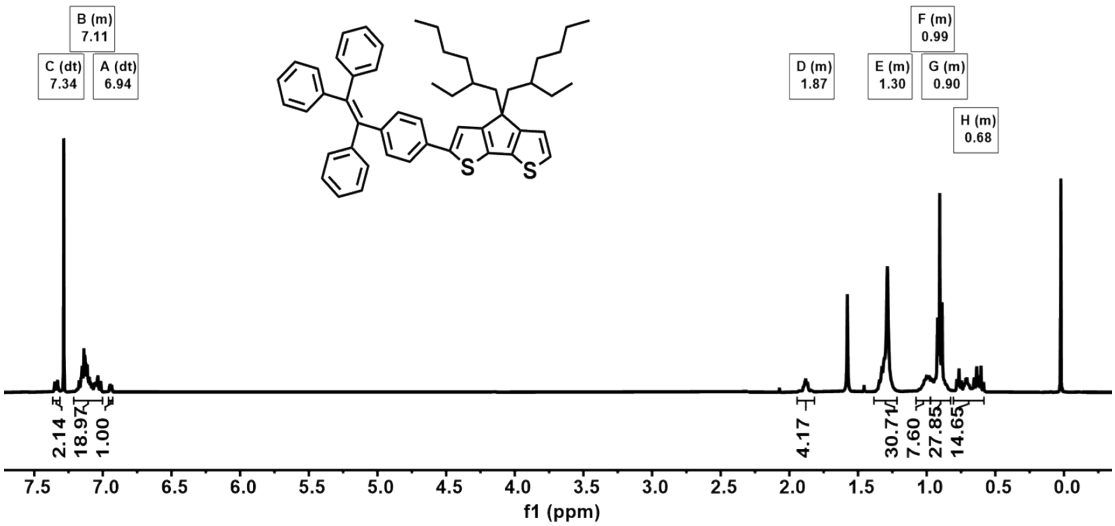

Figure S$_4$ NMR characterization of intermediate **4.**

4. Synthesis of 2-bromo-4,4-bis (2-ethylhexyl)-6-(4-(1,2,2-triphenylvinyl) phenyl)-4H-cyclopentadiene [2,1-b:3,4-b'] dithiophene

Place intermediate **4** (0.3 mmol, 0.2197 g, 1 eq) in a 100 mL round-bottom flask (dark, tin foil wrapped), then add 20 mL THF, pass 15 min N_2. NBS (0. 3 mmol, 0. 0534 g, 1 eq) was added into the ice water bath in three batches (maintain N_2 environment during NBS addition) and reacted for 12 h in dark. After the reaction, 150 mL DCM and saturated salt water were used for extraction 3-4 times, then anhydrous $MgSO_4$ was added to stand for 0.5 h. The crude product was obtained by filtration and vacuum distillation. The white powder solid 2-bromo-4,4-bis(2-ethylhexyl)-6-(4-(1,2,2-triphenylvinyl) phenyl)-4H-cyclopentadiene [2,1-b:3,4-b'] dithiophene (intermediate **5**) (0.276 mmol, 0.2236 g) was obtained by silica gel column chromatography with DCM / n-hexane (1: 3) as eluent, and the yield was 92 %.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 (dt, *J* = 8.3, 1.9 Hz, 4H), 7.19 – 6.97 (m, 34H), 6.94 (t, *J* = 3.2 Hz, 1H), 6.82 (t, *J* = 3.1 Hz, 1H), 1.90 – 1.75 (m, 8H), 1.37 – 1.17 (m, 16H), 1.08 – 0.82 (m, 39H), 0.77 (t, *J* = 7.0 Hz, 8H), 0.65 (dtd, *J* = 29.4, 7.1, 3.1 Hz, 21H).

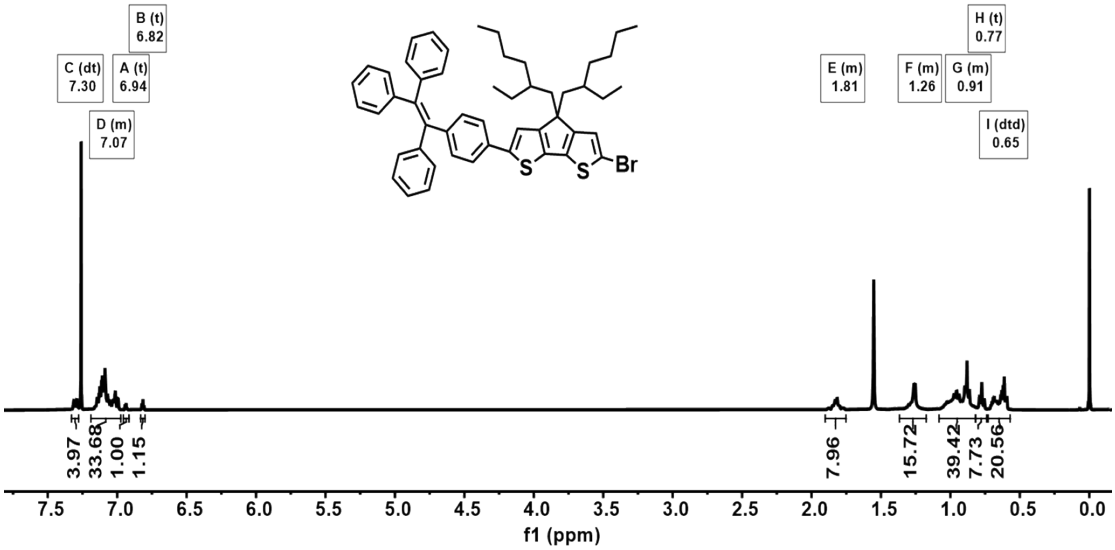

Figure S_5 NMR characterization of intermediate **5.**

6. Synthesis of TDA molecule

Intermediate product **5** (0.25 mmol, 0.2026 g, 2 eq) and DPP (0.125 mmol, 0.1065 g, 1 eq) were placed in a 50 mL round-bottom flask, and 20 mL anhydrous toluene was added. The round bottom flask was vacuumed and N_2 was introduced. Then Pd $(PPh_3)_4$ (0.0125 mmol, 0.0144 g, 0.05 eq) was added to the round-bottom flask and reacted at 110 ° C for 48 h under reflux. After the reaction, 200 mL ethyl acetate and saturated salt water were used to extract 3-4 times, and then anhydrous $MgSO_4$ was added to stand for 0.5 h. Finally, the dark green crude product was obtained by filtration and vacuum distillation. Then DCM / n-hexane (1:1) as eluent, silica gel column chromatography purification, removal of unreacted small molecular raw materials. The product in the silica gel column was further purified with ethyl acetate as eluent, and the purified product was obtained by filtration and vacuum distillation. Finally, the product was dissolved in 5 mL DCM, and then the DCM solution was dripped into 100 ~ 200 mL methanol to precipitate the product in methanol. The TDA molecule was obtained by centrifugation.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.12 – 8.92 (m, 1H), 7.36 (d, *J* = 7.7 Hz, 1H), 7.24 – 7.01 (m, 9H), 4.09 (q, *J* = 8.2 Hz, 3H), 1.94 (d, *J* = 15.3 Hz, 3H), 1.52 – 1.15 (m, 15H), 1.11 – 0.83 (m, 16H), 0.81 – 0.55 (m, 7H), 0.47 (d, *J* = 2.9 Hz, 2H).

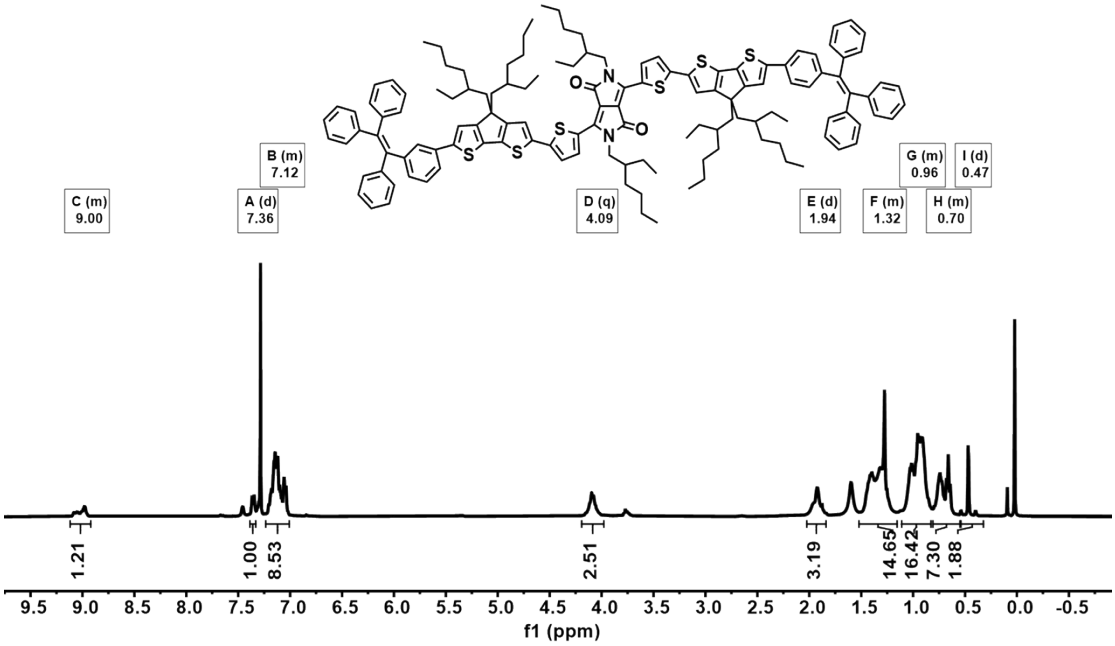

Figure S$_6$ NMR characterization of intermediate **6.**

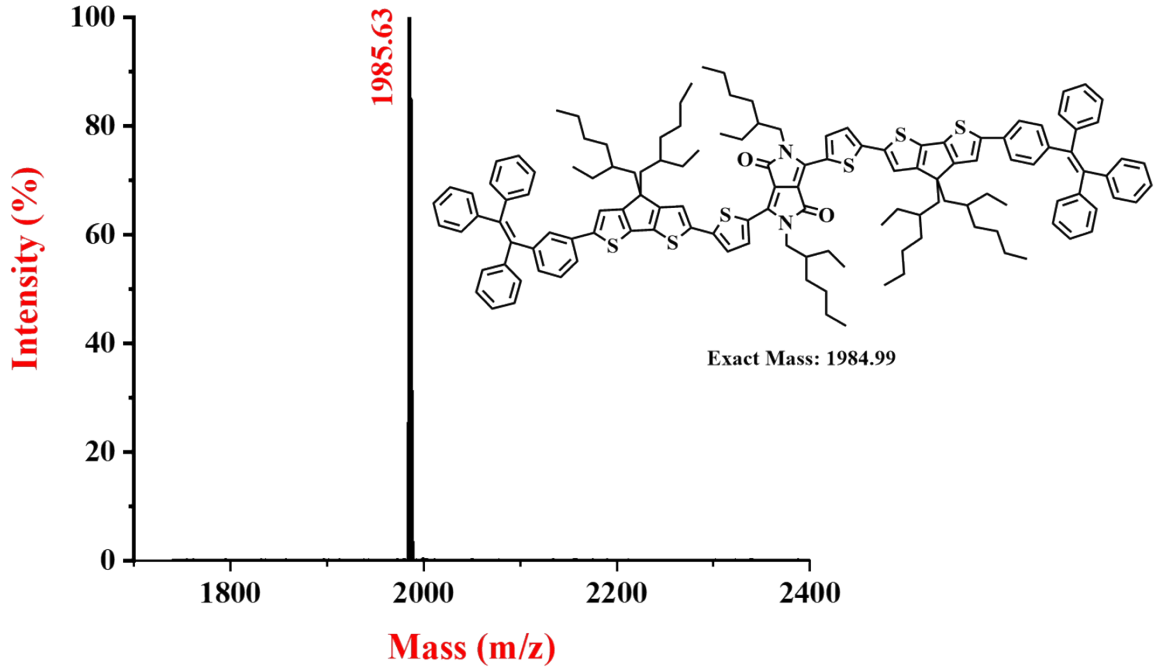

Figure S$_7$ Time of Flight Mass Spectrometry (TOF) aracterization of TDA molecules

7. Preparation and encapsulation efficiency calculation of TDA NPs

Preparation of TDA NPs by nanoprecipitation method: 1 mg TDA and 9 mg DSPE-mPEG$_{2000}$ were dissolved in 4 mL THF, and then 36 mL ultrapure water was placed in a 100 mL round-bottom flask. Under ultrasound, 4 mL THF solution was quickly transferred to water, and then ultrasound was continued for 5 min. After ultrasonication, 2 mL (500 μg·mL^{-1}) TDA NPs were obtained by vacuum distillation.

The above 1 mL TDA NPs (500 μg·mL^{-1}) were placed in a dialysis bag (molecular weight cut-off:5000), ultrasounded for 5 min, and then dialyzed for 48 h. The dialyzed solution was broken with an ultrasonic pulverizer, then freeze-dried and weighed (2.7mg). Finally, 10 mL ethyl acetate was dissolved and diluted, and the absorbance OD_{NPs} at 673 nm was 0.796.

Accurately weighing 1 mg TDA molecules, respectively, with ethyl acetate to prepare the concentration of 50,25,12.5,6.25,3.125,1.5625,0.78125 μg·mL^{-1} and 62.5,31.25,15.625,7.8125,3.9,1.95,0.9765 μg·mL^{-1} solution, and then with Uv-Vis-NIR were tested at 637 nm absorbance. The absorbance-concentration standard curve was established with concentration as abscissa and absorbance as ordinate. Substituting OD_{NPs} into the standard line, the concentration of TDA was measured to be 169.39 μg·mL^{-1}. Finally, the TDA in 1 mL TDA NPs solution was 0.169 mg, and DSPE-PEG$_{2000}$ was 2.531 mg.

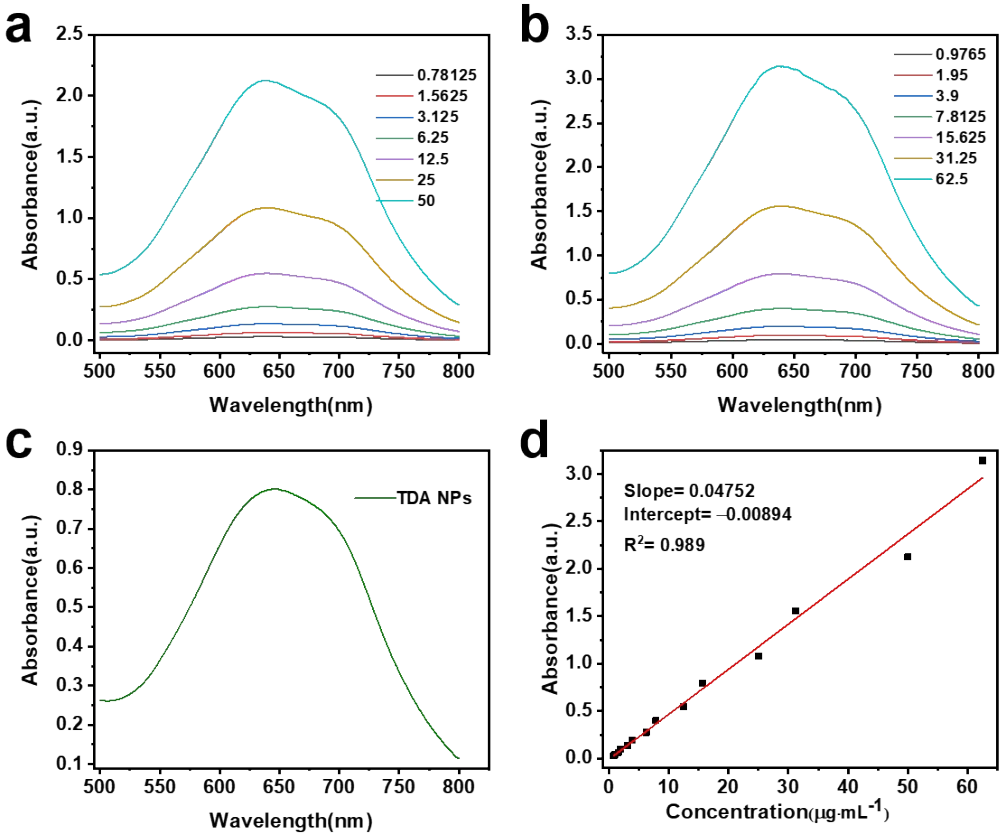

Figure S8 Characterization of encapsulation efficiency of TDA NPs: **(a) (b)** UV Absorption Spectra of Different Concentrations of TDA; **(c)** UV absorption spectra of dialyzed TDA NPs; **(d)** Regression equation obtained by linear regression of absorbance (y) of TDA molecule to concentration (x).

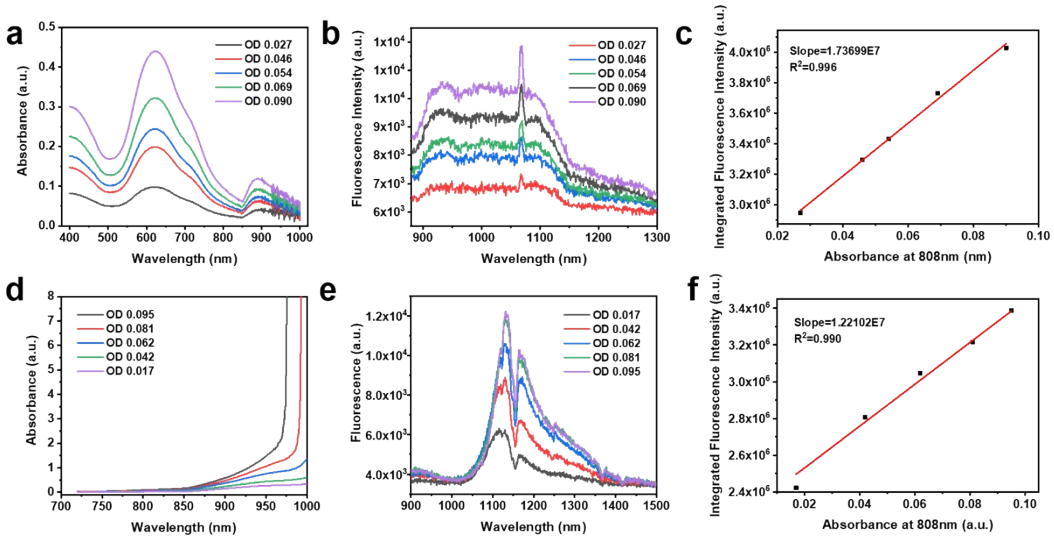

Figure S_9 Characterization of quantum yield: **(a)** Absorption spectra of TDA NPs at different concentrations; **(b)** Emission spectra of TDA NPs at different concentrations (excitation at 808 nm); **(c)** The fitting graph of absorbance and fluorescence intensity of TDA NPs; **(d)** Absorption spectra of IR26 at different concentrations (dissolved in DCM); **(e)** Emission spectra of IR26 at different concentrations (excitation at 808 nm); **(f)** IR26 absorbance and fluorescence intensity fitting diagram.

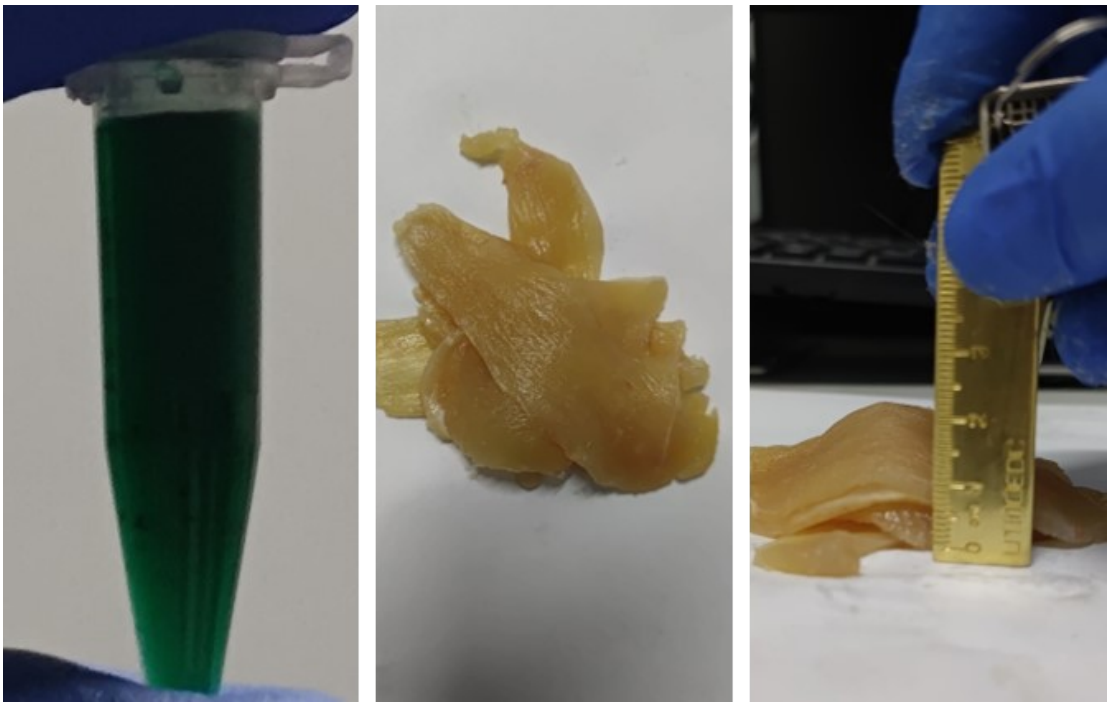

Figure S_{10} TDA solution and chicken breast meat for in vitro fluorescence test.

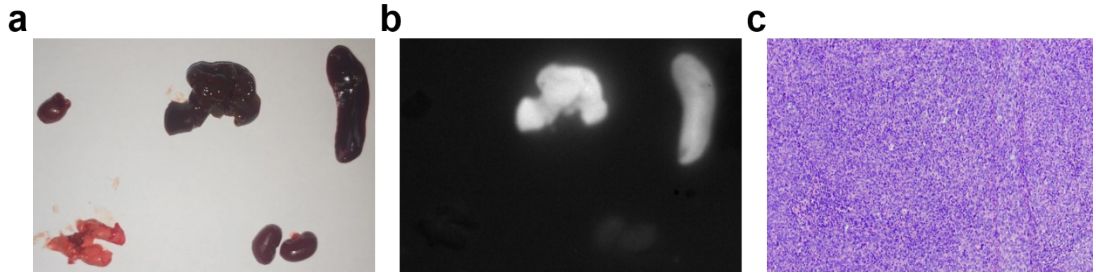

Figure S$_{11}$ Fluorescence characterization of organs after injection of 150 μL TDA NPs for 24 hours: **(a)** The heart, liver, spleen, lung, and kidney of nude mice under visible light; **(b)** The heart, liver, spleen, lung, and kidney of nude mice were stimulated by 808 nm; **(c)** Tumor sections.

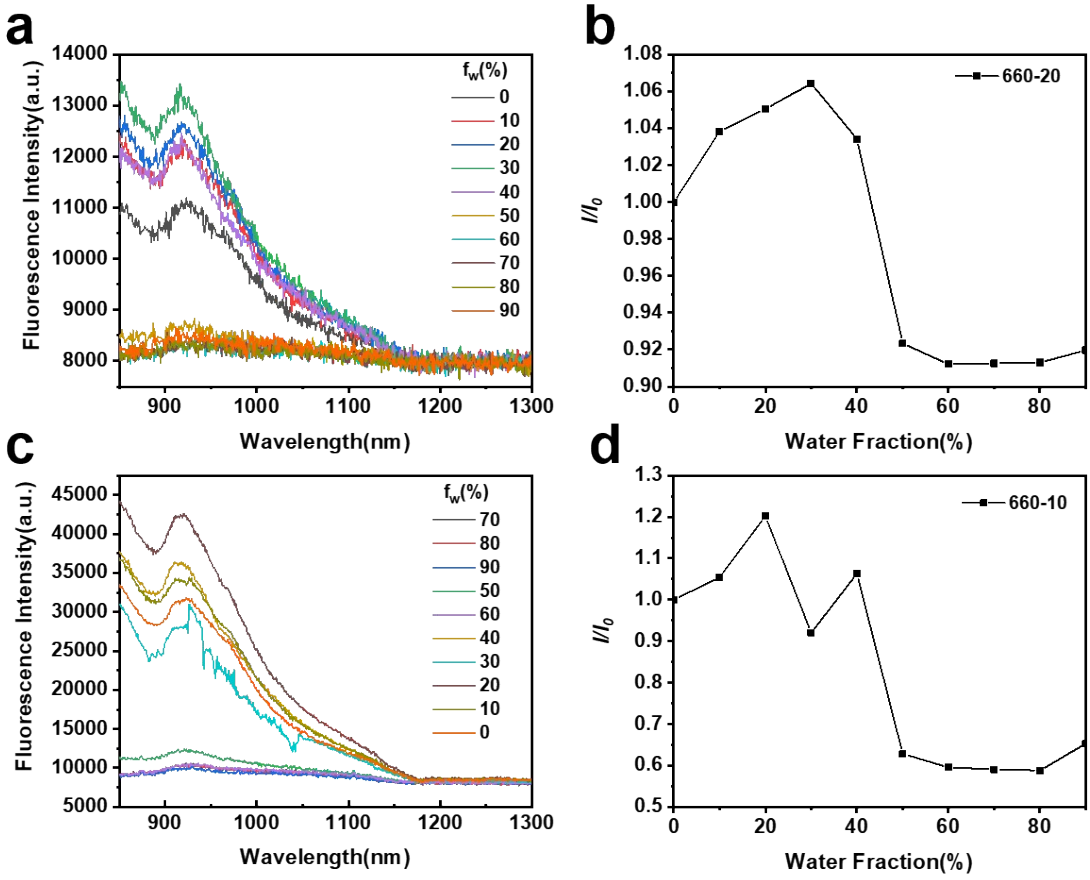

Figure S$_{12}$ Characterization of TDA AIE Performance: **(a)** Fluorescence emission spectra of 20 μg·mL^{-1} TDA under different water content (660 nm excitation); **(b)** The fluorescence intensity ratio (I / I_0) of 20μg·mL^{-1} TDA under different fw; **(c)** Fluorescence emission spectra of 10 μg·mL^{-1} TDA under different water content (660 nm excitation); **(d)** The fluorescence intensity ratio (I / I_0) of 10μg·mL^{-1} TDA under different fw.

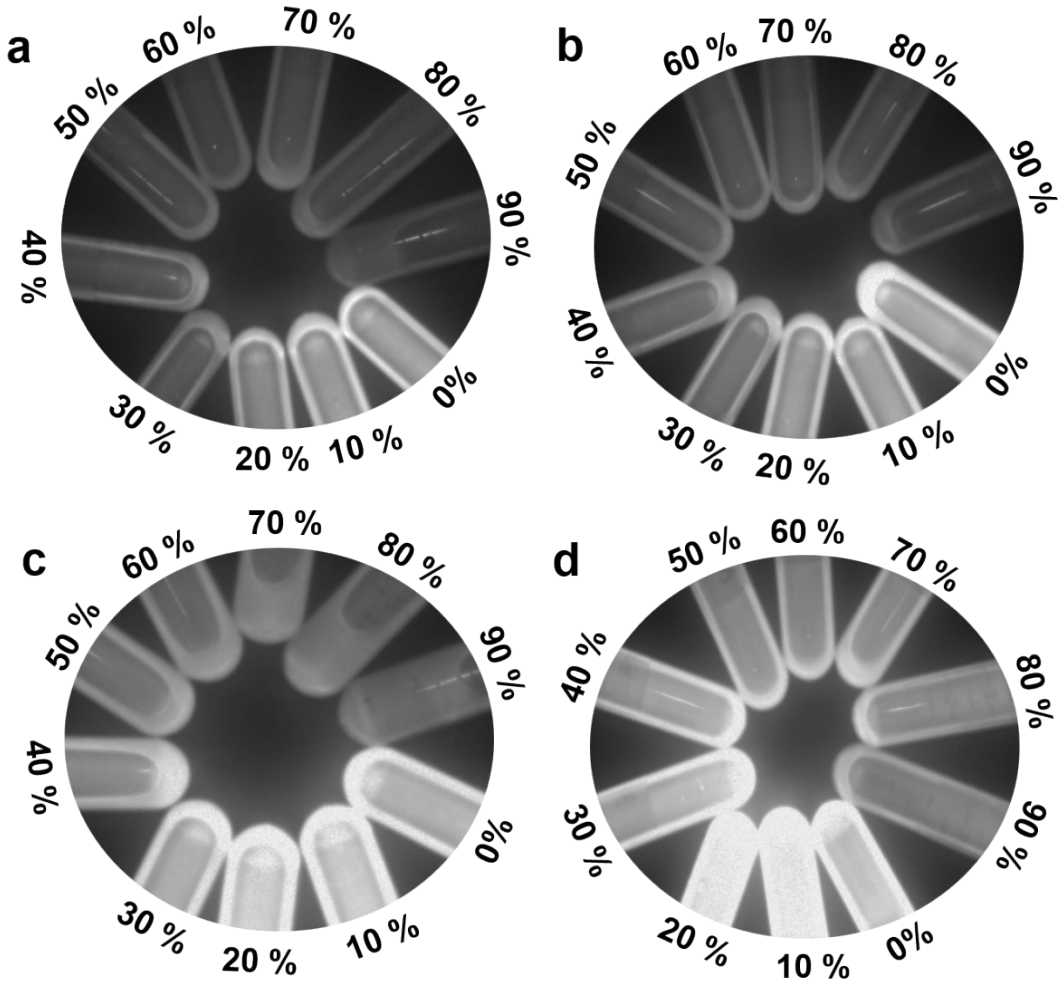

Figure S_{13} Characterization of TDA fluorescence properties at different concentrations and fw: **(a) (b) (c) (d)** 10μg·mL^{-1}, 20μg·mL^{-1}, 50μg·mL^{-1},100μg·mL^{-1} (808 nm, 850 nm long-pass filter). It can be seen from **(a) (b)** that the concentration of TDA NPs is low, the TDA molecules are relatively small, the ACQ effect dominates, and the fluorescence intensity is low; It can be seen from **(c) (d)** that with the increase of TDA NPs content, TDA molecules are relatively more, AIE effect dominates, and the fluorescence intensity gradually increases The curve trend in the (I / I_0) plot can be intuitively explained by the fluorescence imaging shown above, thus validating the relationship between AIE and ACQ in the mixed solution.

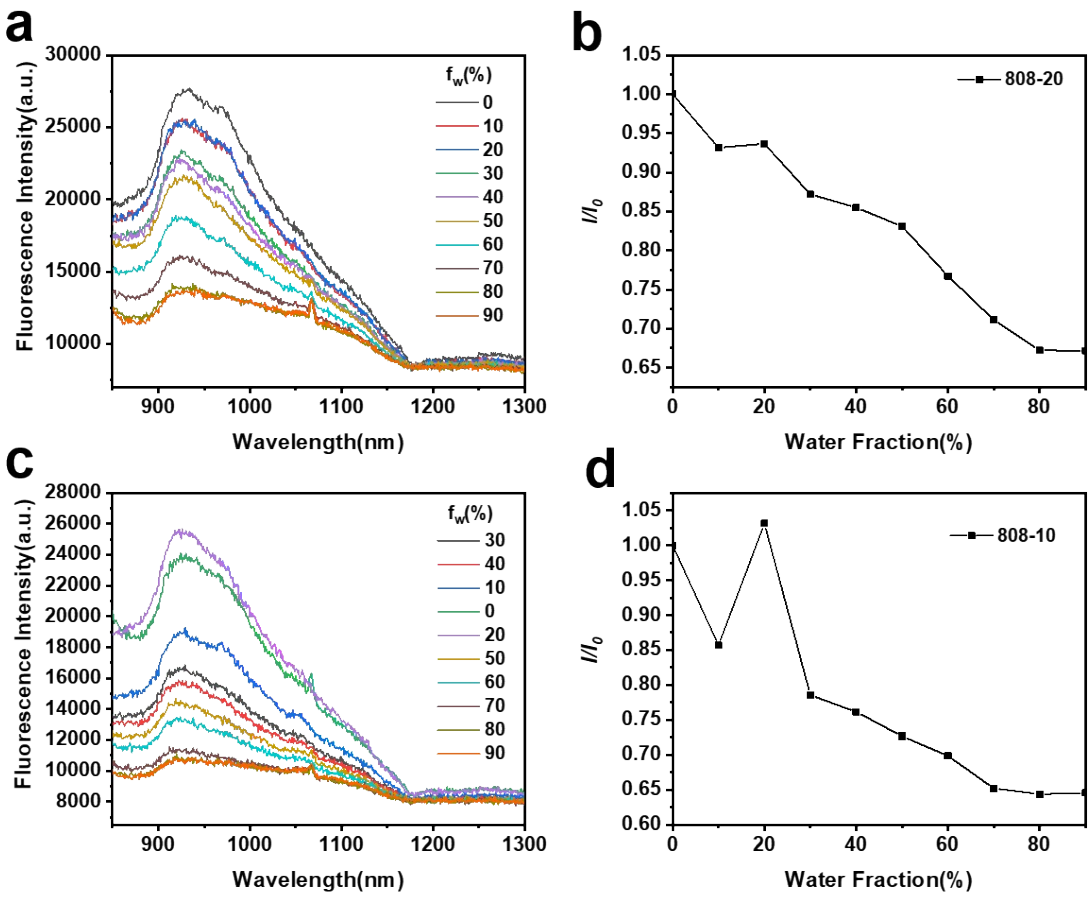

Figure S_{14} Characterization of TDA AIE Performance: **(a)** Fluorescence emission spectra of 20 μg·mL^{-1} TDA under different water content (excitation at 808 nm); **(b)** The fluorescence intensity ratio (I / I_0) of 20μg·mL^{-1} TDA under different fw; **(c)** Fluorescence emission spectra of 10 μg·mL^{-1} TDA under different water content (excitation at 808 nm); **(d)** The fluorescence intensity ratio (I / I_0) of 10μg·mL^{-1} TDA under different fw. Through the curve trend on I / I_0, it can be further demonstrated that TDA NPs have a certain AIE effect, thus confirming the speculative relationship between AIE and ACQ in the mixed solution.

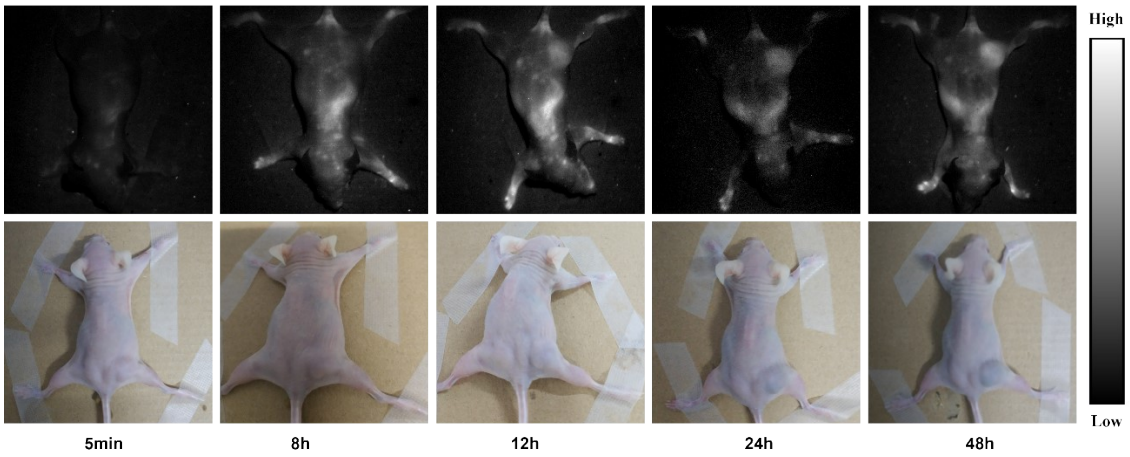

Figure S_{15} Fluorescence imaging of tumor-bearing nude mice at different times after injection of TDA NPs.

Material:

n-hexane (AR, 97%), DCM (AR, 99.5%), methanol (AR, 99.5%), ethyl acetate (AR, 99.5%), THF (AR, 99.0%), DMSO (AR, 99%), 2-bromofluorene (97%), 1,4-2 bromine butane (99%), potassium iodide (99%), sodium chloride (99.5%), 18-crown-6 (99%), TPE-Bpin(97%), EDOT(99%), n-butyl lithium (1.6 M in hexane), NBS(99%), DSPE-mPEG2000(Mw 2000 Da), MTT(98%), $Pd(PPh_3)_4$ (Pd ≥8.9%), DPP(97%) are purchased from Aladdin; Toluene(AR, 99%), acetone(AR, 99%), ether (AR, 99%) are purchased from China Pharmaceutical Chemical Reagent Co. Ltd. Trimethyltin chloride solution (1.0 M in hexane) is purchased from China Reagent Network.

Laboratory apparatus:

NMR Spectrometer (NMR), AvanceNeo400; Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS), AutoFlexMax, Bruker. Laser particle size and potential analyzer equipment (DLS), ZeTASIzer Nano ZS90, Malvin Instruments Co. Ltd. Transmission electron microscope (TEM), JEM-2100Plus， Japan Electronics Company. UV-Vis near-infrared spectrometer (Uv-Vis-NIR), UH4150, Hitachi Hi-Tech Company. Steady/transient fluorescence spectrometer， FLS-1000, Edingburgh. Enzyme calibration, SpectraMax M3, Molecular Equipment Co. Ltd. Nano near infrared small animal real-time imaging system, Uninano NIR-II, Huijia Biological Instrument. Thermal imager, Fotric226,

Shanghai Thermo-Electromechanical Technology Co. Ltd. Photoacoustic equipment, 600 OPO 532-20, InnoLas Germany.