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Supporting information

Effects of Nanoaggregation on Isoindigo-based Fluorophores for Near-infrared Bioimaging Applications

Nicholas E. Sparks,[‡] [a] Austin Dorris,[‡a] Sajith M. Vijayan,[a] Indika Chandrasiri,[a] Mohammad Farid Zia,[b] Alex Flynt,[b] Nathan I. Hammer,[*a] and Davita L. Watkins[*a]

[a]Department of Chemistry and Biochemistry, University of Mississippi, University, MS 38677, United States

[b]Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS, 39406, United States

Corresponding author: dwatkins@olemiss.edu; nhammer@olemiss.edu

General Summary

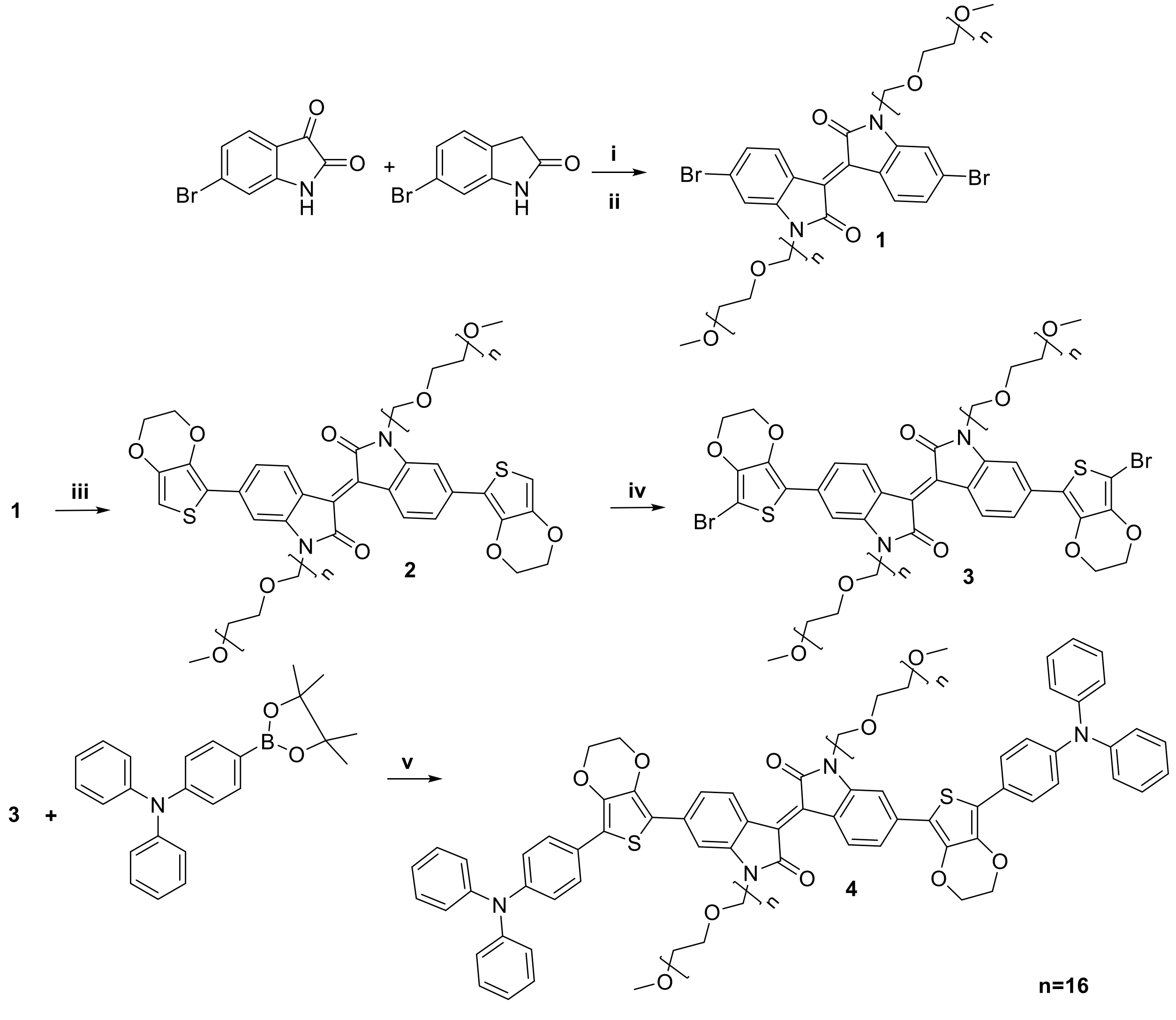
All materials and reagents were purchased from commercial sources and were used without further modification unless noted otherwise. Anhydrous solvents were obtained from a Glass Contour solvent purification system (Irvine, CA, USA). Thin-layer chromatography (TLC) was performed using SiO_2-60 F254 aluminum-backed plates with visualization by ultraviolet (UV) light while flash column chromatography was performed using a Purasil SiO_2-60, 230-400 mesh from Whatman. Size exclusion chromatography (SEC) was performed using Sephadex G-25. ^1H NMR spectroscopy was performed on a Bruker 400 MHz.

Optical Characterization

Solid state UV-Vis-NIR absorbance and emission spectroscopy was performed on a Varian Cary-500 spectrometer (Dorval, QC, Canada) with the help of an integrating sphere. Femtosecond transient absorption spectroscopy (fsTAS) was used to obtain the excited-state lifetimes for encapsulated **II-EDOT-TPA** and **PEG-II-EDOT-TPA** samples. Optical pulses of sub-100 fs bandwidth were generated using a Ti:sapphire regenerative amplified laser system (Coherent Astrella). The 800 nm fundamental output from the fs amplifier was tuned to the excitation wavelengths used for data acquisition inside an optical parametric amplifier (Light Conversion) before being routed into a transient absorption spectrometer (Ultrafast Systems Helios). Broadband probe pulses for measuring the transient absorption were generated inside the spectrometer by routing a portion of the 800 nm fs pulses through a CaF_2 crystal. Generating the probe pulses in this manner preserves the temporal characteristics of the fundamental pulses, for time-resolved measurements and the detection of short lived-transient species. Emission spectra were taken using a Horiba Quantamaster 8075 spectrofluorometer with dual excitation gratings and a photomultiplier tube detector. Spectra used for absolute quantum yields were taken using the integrating sphere included with the Quantamaster spectrofluorometer with excitation slits set to 4.4nm and emission slits at 5nm. The excitation was included in these spectra for use in calculating the ratio of peak intensity with and without the oxazine sample present in the integrating sphere. For relative quantum yields, slits were set to 8nm and 15nm for excitation and emission, respectively. Excitation wavelengths were not scanned across for relative quantum yields, allowing for the increased slit width. Oxazine was again analyzed under these conditions for direct comparison with the PEG-II-EDOT-TPA solutions. Photoluminescent lifetimes taken in solid and solution phase were acquired using a time-correlated single photon counting (TCSPC) setup. The beam from a picosecond diode laser is routed through an inverted microscope and focused onto the sample. Scattered emission is collected and the excitation laser is filtered out using a long-pass filter. Data were acquired using an avalanche photodiode (MPD Instruments) and the TimeHarp software from PicoQuant.

Nanoaggregate Preparation and Size Characterization

II-EDOT-TPA nanoaggregates were prepared by dissolving 1 mg of **II-EDOT-TPA** and 2 mg of the LDBC polymer in 200 µL of THF. This solution was added dropwise to 2 mL of Milli-Q water while sonicating. THF was removed under a gentle flow of nitrogen to give a 1 mg/mL solution of LDBC encapsulated **II-EDOT-TPA** nanoaggregates. To prepare the **PEG-II-EDOT-TPA** nanoaggregates, 1 mg of **PEG-II-EDOT-TPA** was dissolved in 3 mL of THF. The resulting solution was then added to 1 mL of Milli-Q water while sonicating. THF was then allowed to evaporate underneath a gentle flow of nitrogen overnight to afford a 1 mg/mL solution of **PEG-II-EDOT-TPA** nanoaggregates. Particle size and the polydispersity index (PDI) measurements were carried out on a Malvern Instrument Zetasizer Nano ZS using a He–Ne laser with a 633 nm wavelength, a detector angle of 173° at 25 °C. Dye loading (DL) and encapsulation efficiencies (EE) were obtained following a previously published protocol.[1]



Scheme S1: Synthesis **i:** AcOH, HCl, 24 h, reflux; **ii:** Cs_2CO_3, PEG_{900}-tosylate, DMF, 18 h, 110 °C; **iii:** K_2CO_3, $PdCl_2$, 1-adamantane carboxylic acid, EDOT, 2 h, 120 °C; **iv:** NBS, $CHCl_3$, 18 h, RT; **v:** N,N-diphenyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, $Pd(PPh_3)_4$, aq. Cs_2CO_3, Toluene:EtOH, 24 h, 100 °C.

Synthesis

Synthesis of (E)-6,6'-dibromo-[3,3'-biindolinylidene]-2,2'-dione (II-Br)[2]

6-Bromoisatin (2.43 mmol) and 6-bromooxindole (2.43 mmol) were added to a flame-dried round bottom flask (RBF) and was dissolved in 15 mL of acetic acid. Concentrated hydrochloric acid, HCl (3.40 mmol) was then added and the reaction mixture was allowed to heat at reflux for 24 hours. The reaction mixture was then allowed to cool to room temperature after which the reaction mixture was filtered. The resulting reddish-brown solid was washed with deionized water (50 mL), ethanol (50 mL), and finally ethyl acetate (50 mL). The solid was allowed to dry in a vacuum oven for 3 hours to afford 745 mg (73% yield) of dried product.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 11.09 (s, 1H), 8.99 (d, 1H), 7.19 (d, 1H), 7.02 (s, 1H)

Synthesis of PEG-II-Br

6,6'-dibromoisoindigo (1.70 mmol), cesium carbonate (1.70 mmol), and tetrabutyl ammonium bromide (0.17 mmol) were added to an inert flame-dried round bottom flask with a condenser and dissolved in 15 mL of anhydrous DMF. The reaction mixture was allowed to heat for 15 minutes before adding PEG$_{900}$-tosylate (4.25 mmol) which is dissolved in an inert vial in 5 mL of DMF. The reaction mixture was allowed to heat at 110 ºC for 18 hours. The reaction mixture was allowed to cool before adding ethyl acetate and filtering off the insoluble salts. The solvent was removed and the crude compound was purified by size exclusion chromatography to afford PEG-II-Br (40% yield).

^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm): 9.05 (d, 1H), 7.17 (s, 1H), 7.15 (d, 1H), 3.96 (t, 2H), 3.74 (t, 2H), 3.64 (m, 100H)

Synthesis of PEG-II-EDOT[3]

PEG-II-Br (0.387 mmol), K_2CO_3 (1.16 mmol), and $PdCl_2$ (0.077 mmol) were added to a flame-dried RBF and put under an inert atmosphere. DMAc was added and the solution was allowed to stir for 10 minutes. 1-adamantane carboxylic acid (0.116 mmol) was added under inert conditions and the solution was allowed to stir for 10 more minutes. 3,4-ethylenedioxythiophene (1.55 mmol) was then added and the RBF was heated to 120 ºC for 2 hours. The reaction mixture was then cooled to room temperature and to which EtOAc was added and any insoluble salts were filtered off. The solvent was then removed, and the product was purified using column chromatography with 3:2 MeOH:EtOAc as the eluent (44% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.04 (d, 1H), 7.35 (d, 1H), 7.28 (s, 1H), 6.36 (s, 1H) 4.29 (m, 4H), 4.00 (t, 2H) 3.75 (t, 2H) 3.61 (m, 100H)

Synthesis of PEG-II-EDOT-Br

PEG-II-EDOT (0.111 mmol) and NBS (0.278 mmol) were added to an inert flame dried round bottom flask and dissolved in 11 mL of MeCN. The RBF was covered in aluminum foil and the reaction mixture was allowed to stir at room temperature overnight. The crude NMR did not show the aromatic peak associate with EDOT after which MeCN was then removed and the product was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.02 (d, 1H), 7.10 (s, 1H), 7.08 (d, 1H), 4.32 (m, 4H), 3.97 (t, 2H), 3.73 (t, 2H), 3.63 (m, 100H)

Synthesis of PEG-II-EDOT-TPA

PEG-II-EDOT-Br (0.14 mmol), triphenylamine boronic ester (0.35 mmol), and palladium tetrakis (0.042 mmol) were added to an inert flame-dried round bottom flask with a condenser and were dissolved in a mixture of 12:7 mL toluene:ethanol. Cesium carbonate (0.35 mmol) was placed into an inert vial and dissolved in 4 mL of DI H2O before being added to the RBF. The reaction mixture was allowed to heat at 100 ºC for 24 hours. EtOAc was added to crash out any unreacted salts and the solvent was then removed and the crude product was purified using reverse phase chromatography with MeOH and 5% AcOH to afford **PEG-II-EDOT-TPA** (30% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.13 (d, 1H), 7.64 (d, 2H), 7.55 (d, 2H), 7.40 (d, 1H), 7.35 (s, 1H), 7.26-6.90 (m, 10H), 4.39 (m, 4H), 4.05 (t, 2H) 3.77 (t, 2H) 3.65 (m, 100H)

NMRs

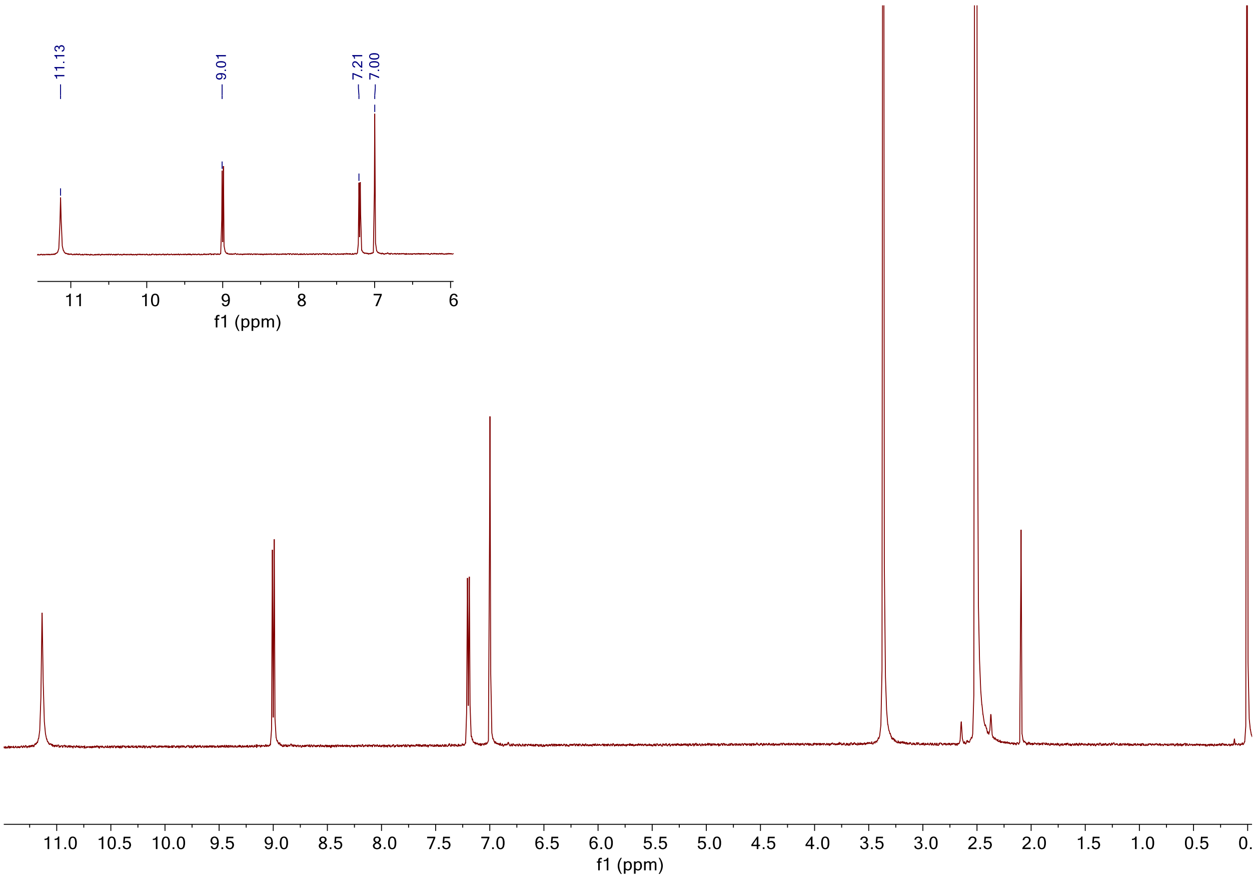


Figure S1: ^{1}H NMR of (E)-6,6'-dibromo-[3,3'-biindolinylidene]-2,2'-dione (**II-Br**), DMSO-d_6

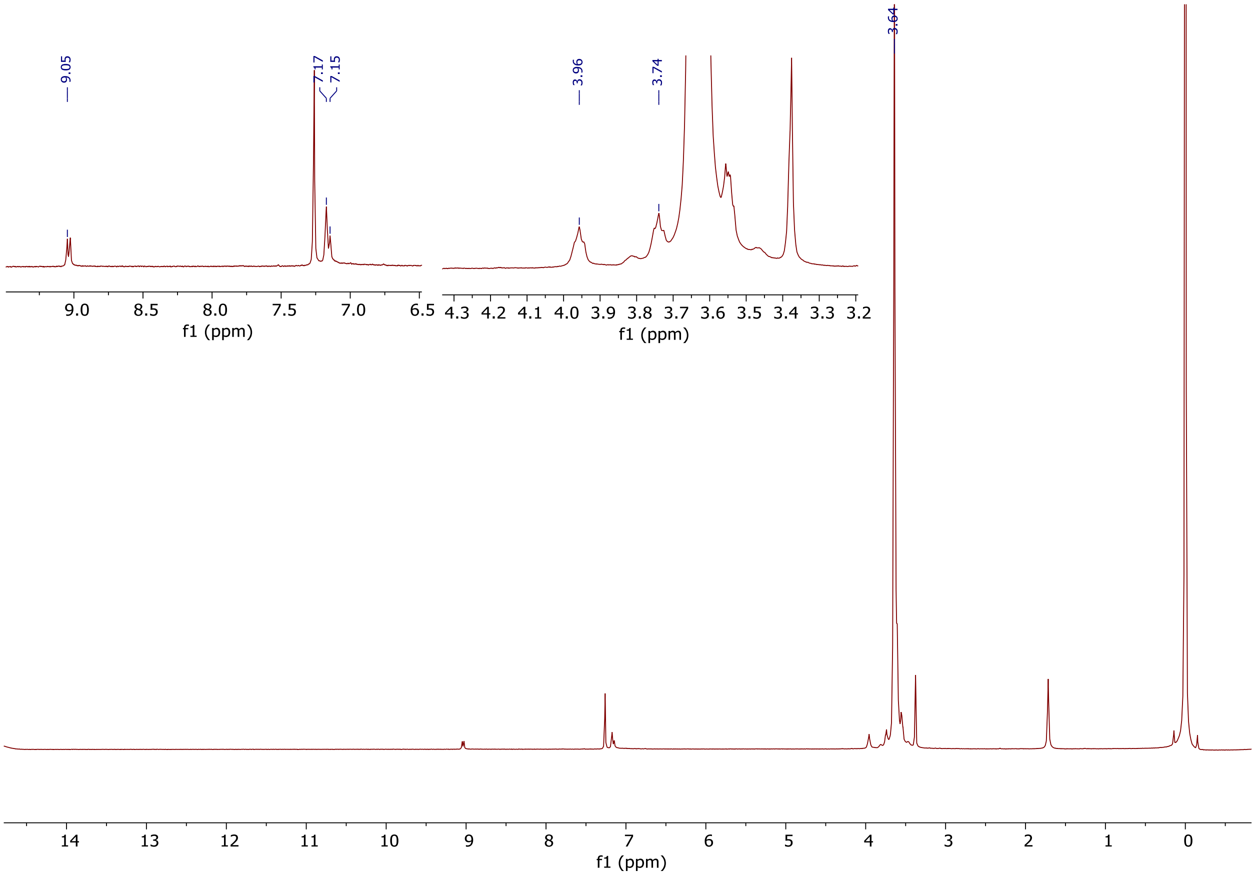


Figure S2: ^{1}H NMR of **PEG-II-Br**; CDCl$_3$

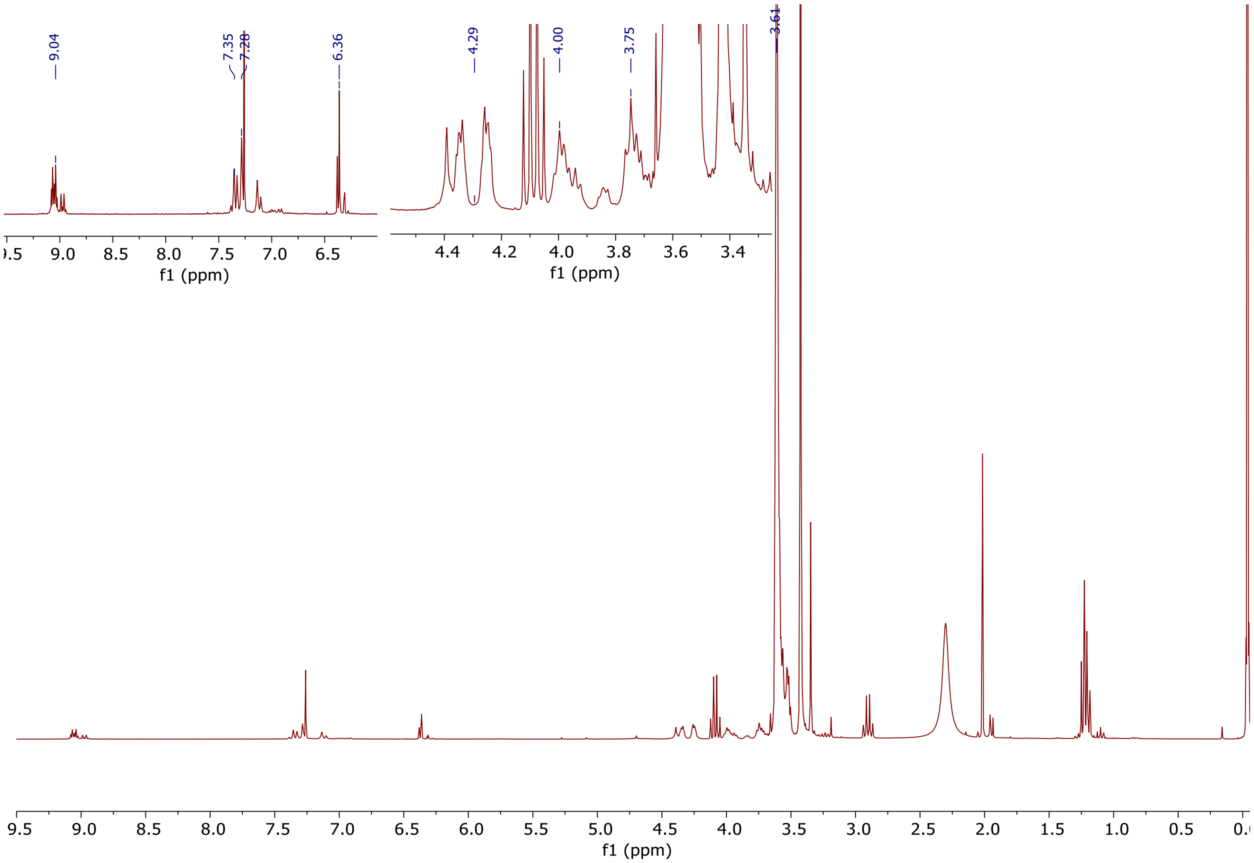


Figure S3: ^{1}H NMR of **PEG-II-EDOT** (residual ethyl acetate and ethanol); CDCl$_3$

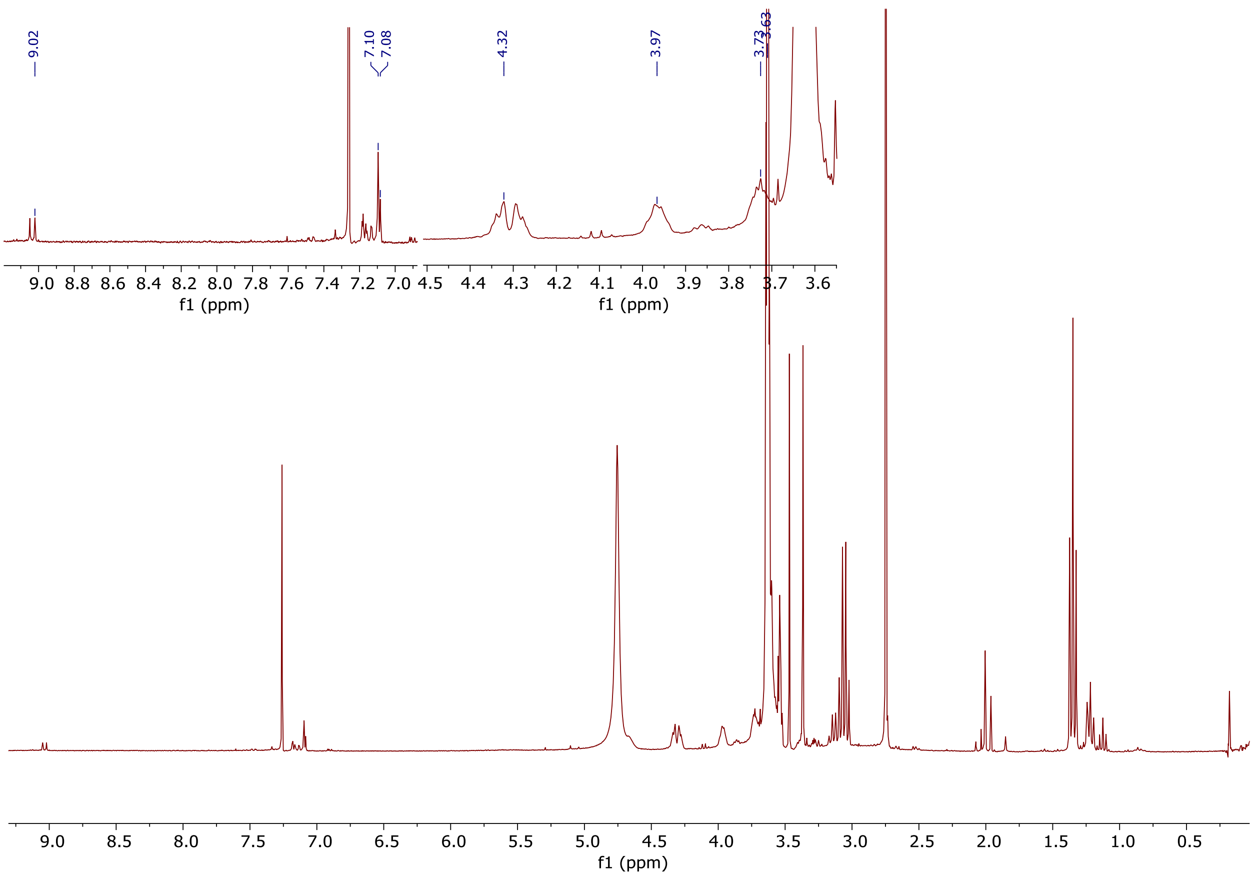


Figure S4: ^{1}H NMR of **PEG-II-EDOT-Br** (residual ethanol); CDCl$_3$

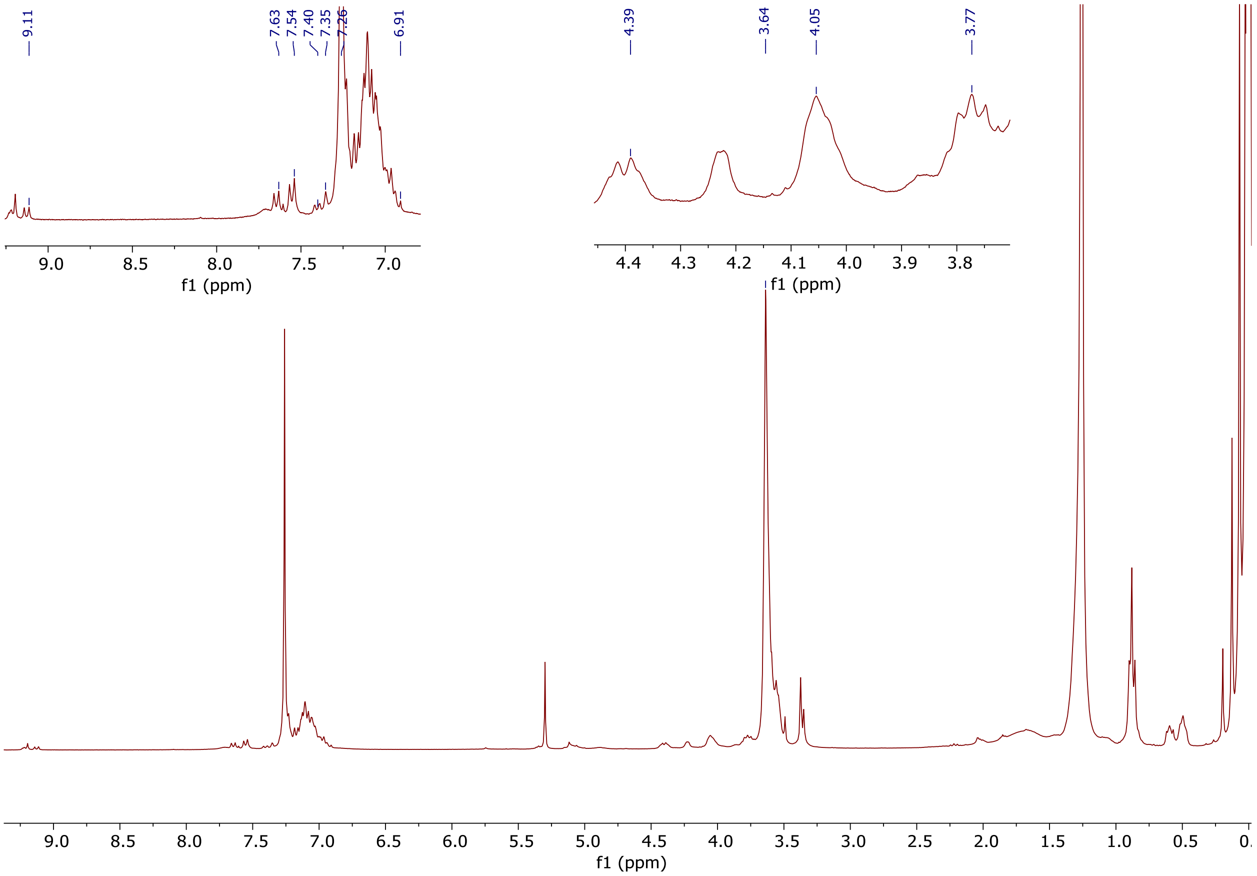


Figure S5: ^{1}H NMR of **PEG-II-EDOT-TPA** (residual DCM); $CDCl_3$

Photophysical Characterization

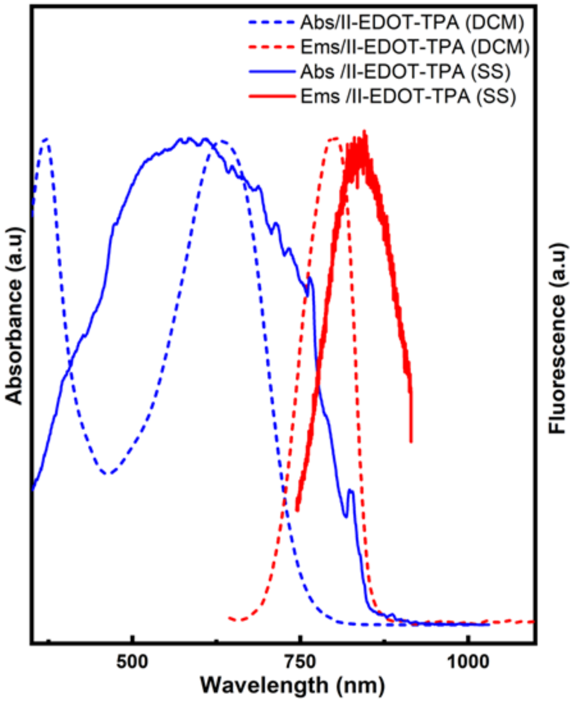


Figure S6: Optical properties of **II-EDOT-TPA**. Blue color indicates the absorbance whereas red indicates emission. Dashed lines indicate the optical properties of **II-EDOT-TPA** in solution state (in 6.0x10^{-4} M DCM) whereas solid lines indicate the solid state.

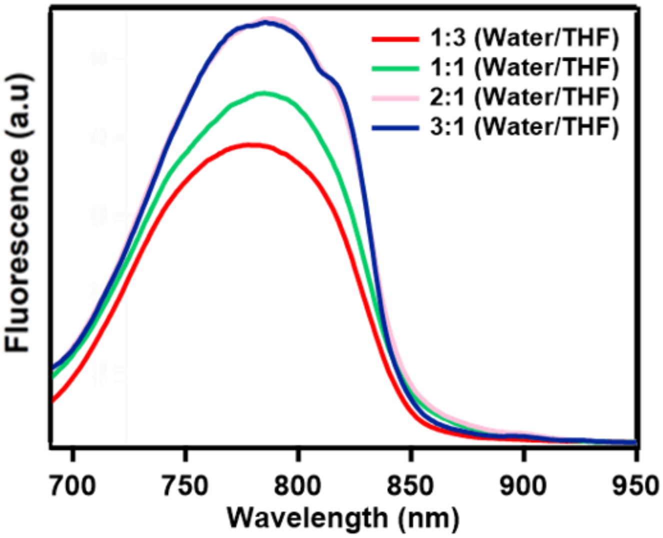


Figure S7: II-EDOT-TPA Emission in Water/THF mixtures.

Supporting Encapsulation Data for II-EDOT-TPA

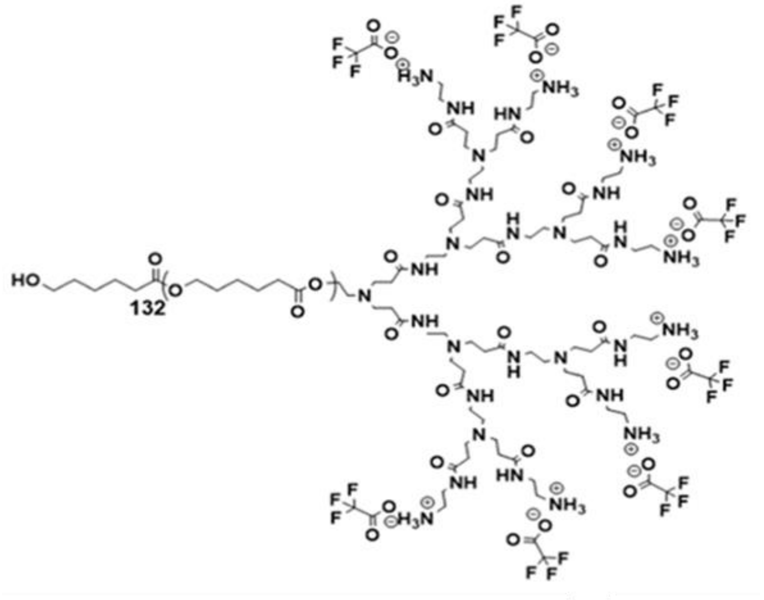


Figure S8: Linear dendritic block copolymer used to encapsulate **II-EDOT-TPA**[4]

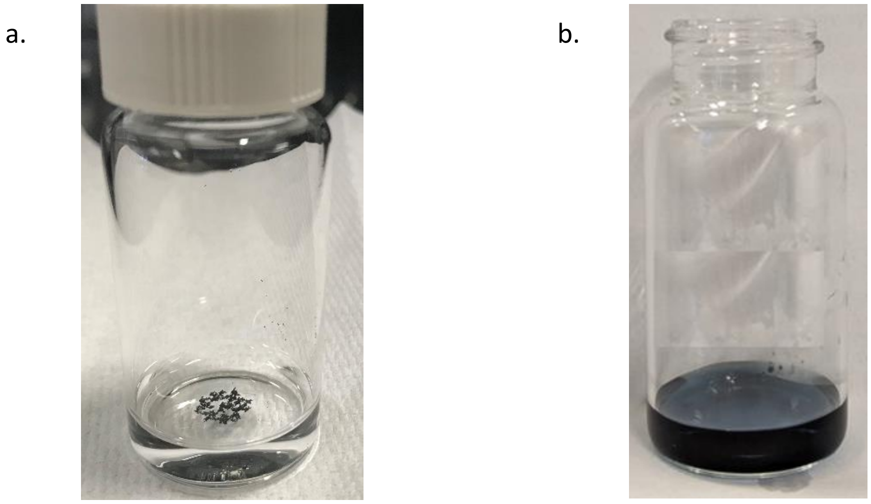


Figure S9: Image **II-EDOT-TPA** in aqueous solution before (a.) and after (b.) encapsulation into LDBC

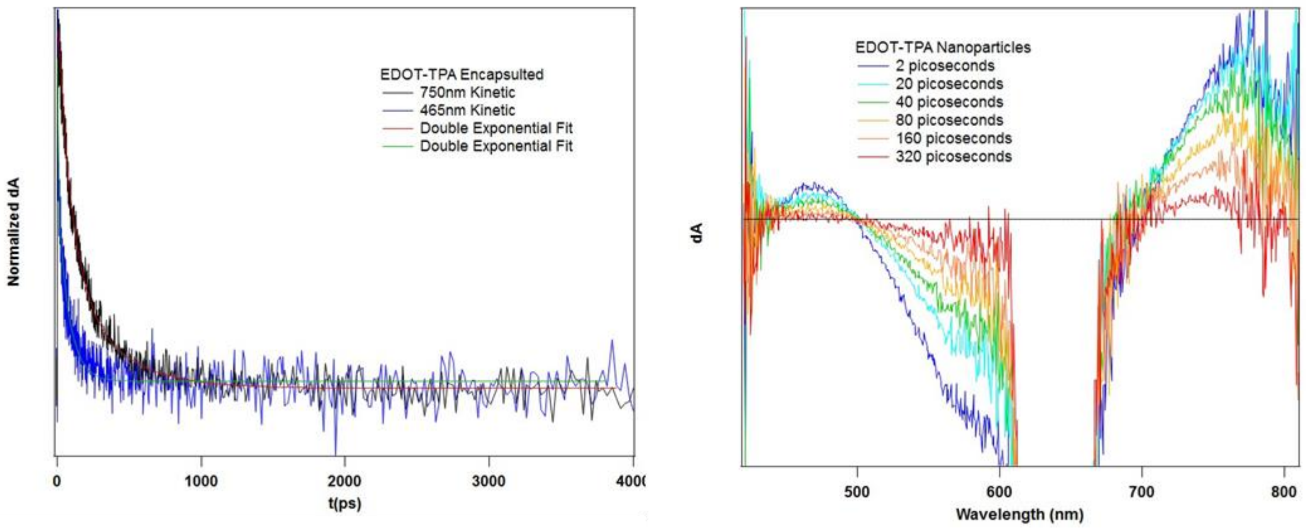


Figure S10: Excited state lifetimes (ps) and TAS spectra of LDBC encapsulated **II-EDOT-TPA** in 1 mg/mL aqueous solution

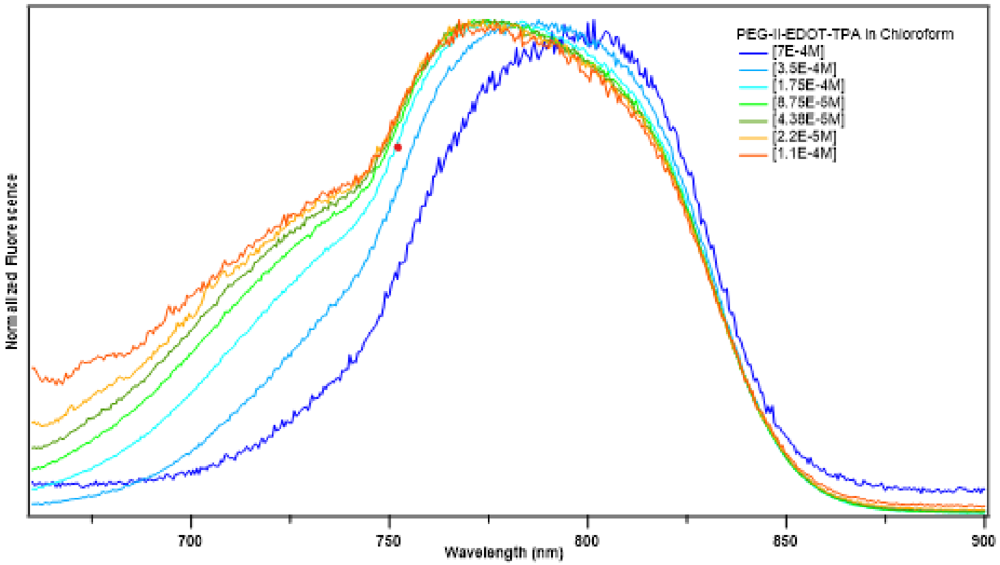


Figure S11: PEG-II-EDOT-TPA fluorescence at various concentrations in $CHCl_3$.

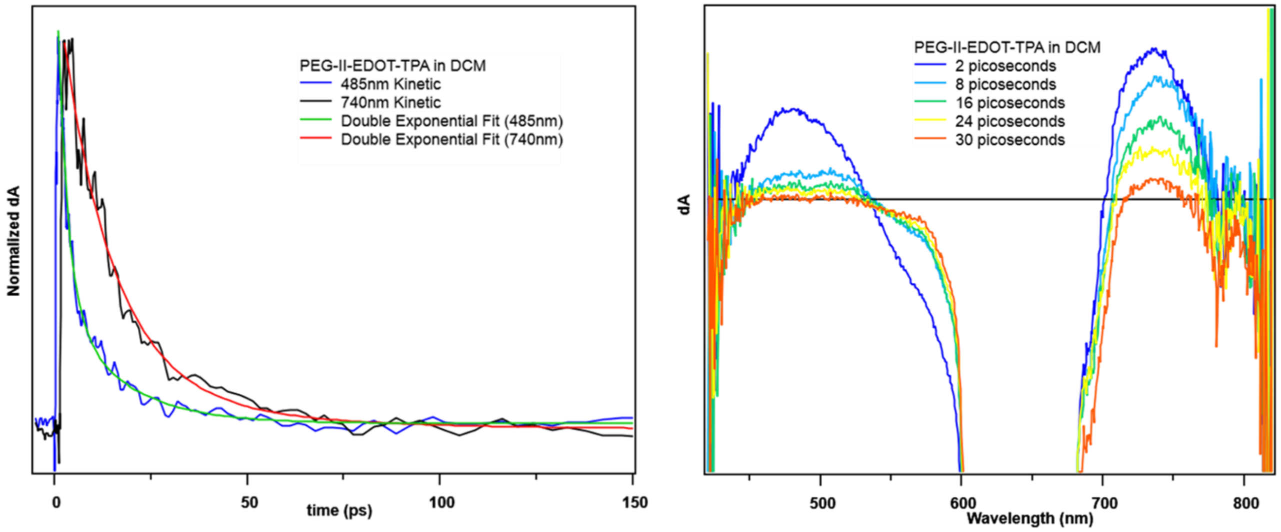


Figure S12: Excited State Lifetimes (ps) of **PEG-II-EDOT-TPA** in DCM (6.0x10^{-4} M) **(left).** TAS of **PEG-II-EDOT-TPA** in DCM (6.0x10^{-4} M) **(right).**

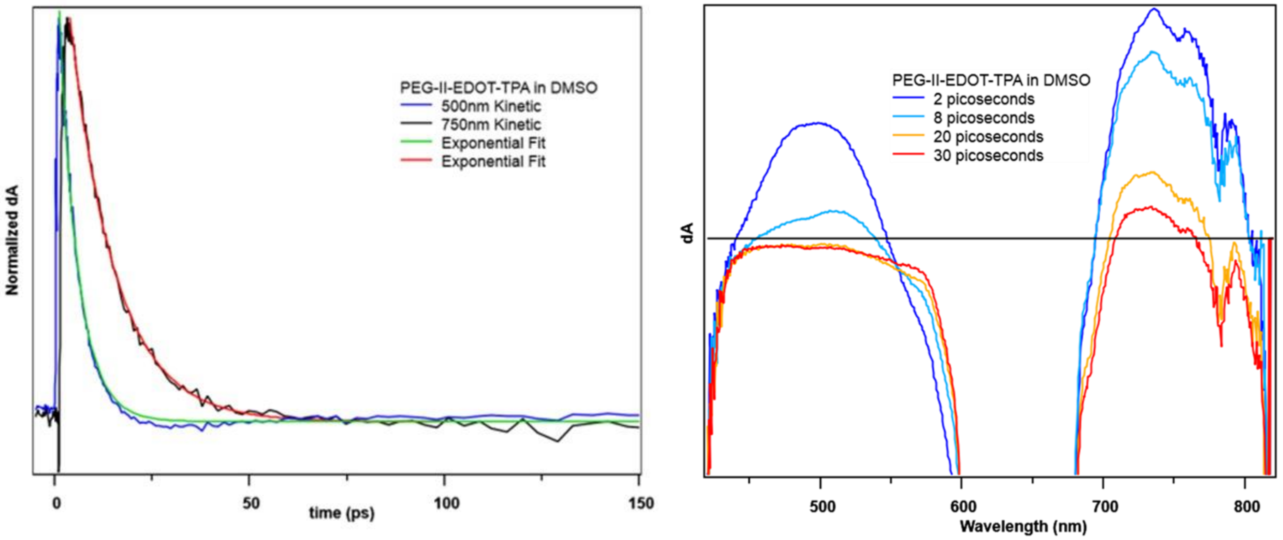


Figure S13: Excited State Lifetimes (ps) of **PEG-II-EDOT-TPA** in DMSO (6.0x10^{-4} M) **(left).** TAS of **PEG-II-EDOT-TPA** in DMSO (6.0x10^{-4} M) **(right).**

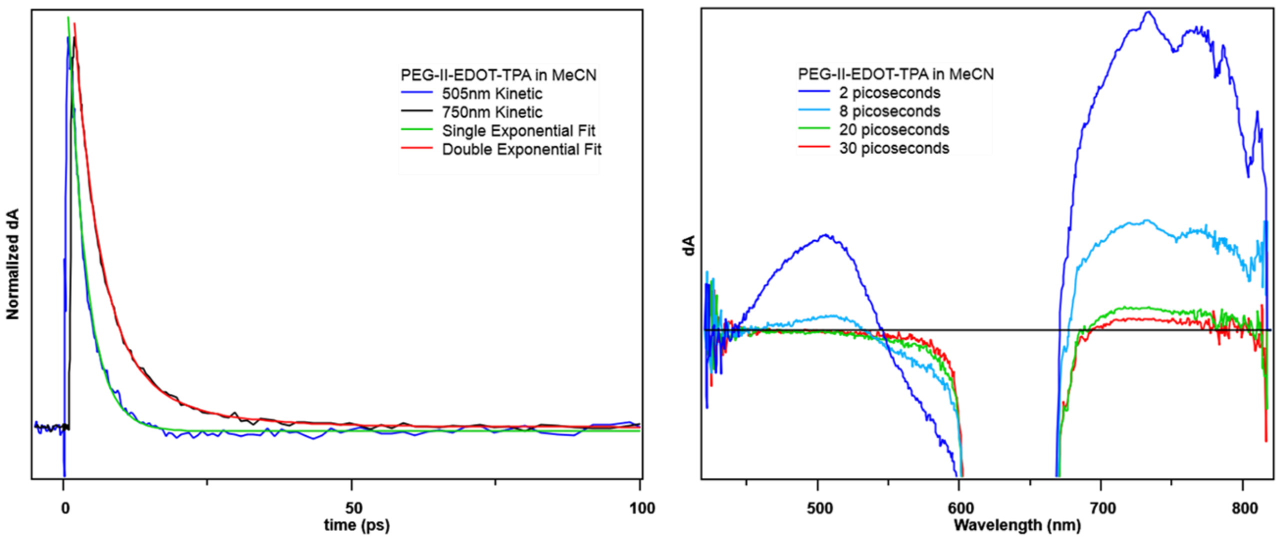


Figure S14: Excited State Lifetimes (ps) of **PEG-II-EDOT-TPA** in MeCN (6.0x10^{-4} M) **(left).** TAS of **PEG-II-EDOT-TPA** in MeCN (6.0x10^{-4} M) **(right).**

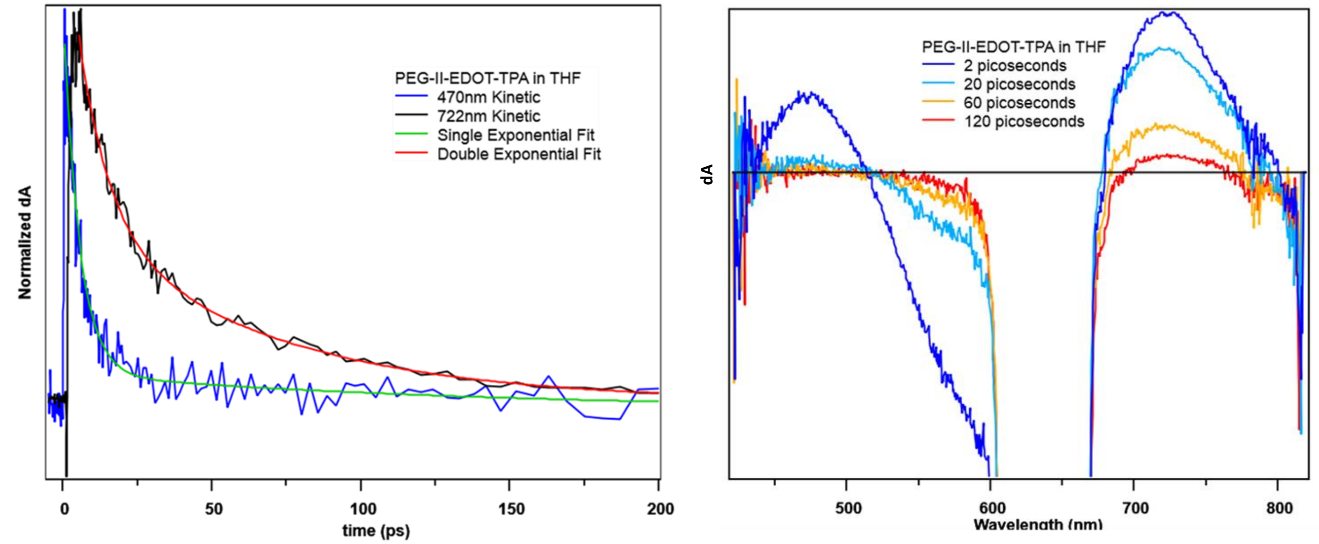


Figure S15: Excited State Lifetimes (ps) of **PEG-II-EDOT-TPA** in THF (6.0x10^{-4} M) **(left).** TAS of **PEG-II-EDOT-TPA** in THF (6.0x10^{-4} M) **(right).**

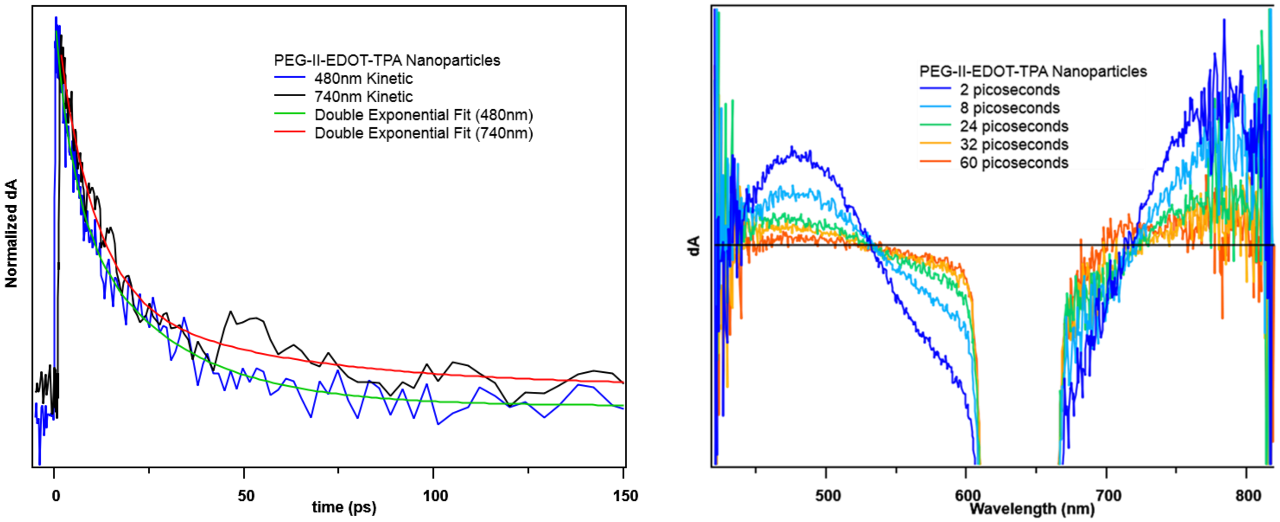


Figure S16: Excited State Lifetimes (ps) of **PEG-II-EDOT-TPA** nanoaggregates in 1 mg/mL aqueous solution (left). TAS of **PEG-II-EDOT-TPA** nanoaggregates in 1 mg/mL aqueous solution ($6.0\text{x}10^{-4}$ M).

Summary of Nanoparticle Data:

Table S1: DLS Spectra of Nanoaggregates

	DLS (nm)	PDI	STD (±)	TEM (nm)	STD±	ζ potential
II-EDOT-TPA NP	225.9	0.213	41.78	179.2	32.6	+30.6
PEG-II-EDOT TPA NP	124.3	0.253	53.57	212.8	29.8	+8.2

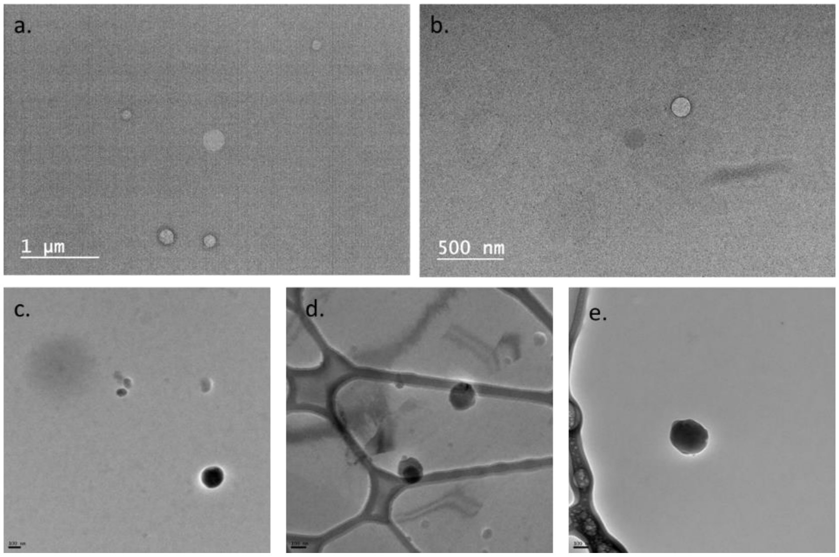


Figure S17: TEM images of LDBC encapsulated II-EDOT-TPA (a-b) and PEG-II-EDOT-TPA nanoaggregates (c-e)

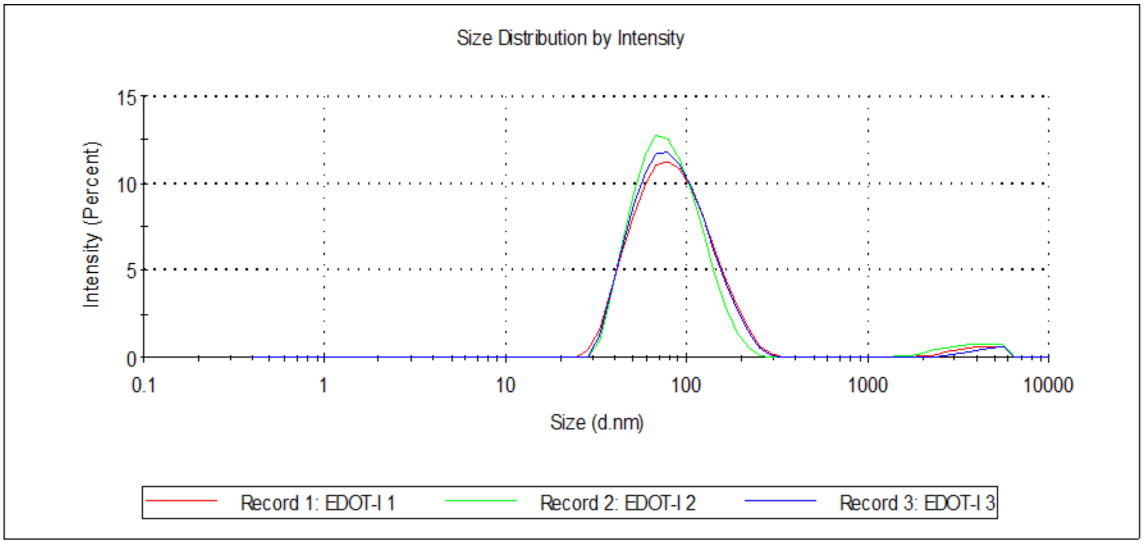


Figure S18: DLS curves of LDBC encapsulated **II-EDOT-TPA** in 1 mg/mL aqueous solution

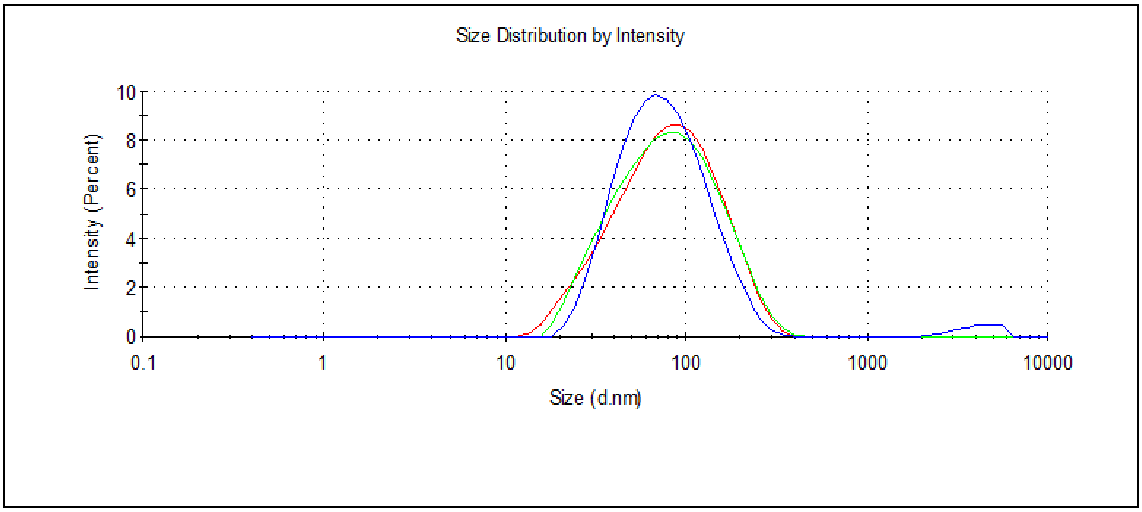


Figure S19: DLS curves of **PEG-II-EDOT-TPA** nanoaggregates in 1 mg/mL aqueous solution

References

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