

SUPPORTING INFORMATION

Complex Structured Fluorescent Polythiophene Graft Copolymer as a Versatile Platform for Imaging, Targeted Delivery of Paclitaxel, and Radiotherapy

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Synthesis of 2,5-dibromothiophene-3-carboxylic acid (1). Thiophene-3-carboxylic acid (2.0 g, 15.6 mmol) and NBS (6.4 g, 35.9 mmol) were added to dry 50 mL of DMF under nitrogen atmosphere. The solution was stirred at 50 °C under nitrogen for 24 h. The reaction mixture was washed with brine. After that, the aqueous layer was extracted with CH₂Cl₂ and the organic layers were combined, dried over Na₂SO₄ and concentrated under reduced pressure to yield 1 (2.6 g, 60%, white powder). ¹H-NMR (CDCl₃, 500 MHz): δ 7.43 (s, 1H), 13.32 (s, 1H).

Synthesis of dibromothiophene-functionalized PEG macromonomers (DBT-PEG). 2,5-dibromothiophene-3-carboxylic acid (1) (4.1 g, 14.3 mmol), DMAP (174.7 mg, 1.43 mmol), Me-PEG₂₀₀₀-OH (25.74 g, 12.87 mmol) were dissolved in 100 mL of dry CH₂Cl₂ under nitrogen atmosphere. To this solution was added DCC (3.246 g, 15.7 mmol) in 20 mL dry CH₂Cl₂ drop-wise under nitrogen. Then, the reaction mixture was stirred for 5 days at room temperature. After that, the mixture was filtered and washed with 250 mL of CH₂Cl₂. The organic phase was quenched with 10% NaHCO₃ and brine, and then dried over Na₂SO₄. The solution was concentrated and passed through a silica-gel column using CH₂Cl₂ as eluent. After removal of solvent in *vacuo*, the polymer was precipitated in cold diethyl ether and dried to yield DBT-PEG (18 g, 85%, white powder). ¹H-NMR (CDCl₃, 500 MHz): δ 3.85–3.46 (broad), 3.38 (broad), 7.34 (s, 1H).

Synthesis of (2,5-dibromothiophen-3-yl)methanol (2). To the solution of thiophen-3-ylmethanol (6.45 g, 56.5 mmol), in 180 mL dry THF, NBS (22.13 g, 12.42 mmol) was drop-wisely added and stirred for 3 days at room temperature under nitrogen atmosphere. The solvent was evaporated in *vacuo*. Then the remaining crude material was washed with KOH (1.0 M, 30 mL), extracted with diethyl ether and purified by flash column chromatography (SiO₂, hexane: Ethyl acetate = 4:1, as eluent) to yield 2 (6.4 g, 41% brown oil). ¹H-NMR (CDCl₃, 500 MHz): δ 1.91 (broad, 1H), 4.55 (s, 2H), 7.0 (s, 1H).

Synthesis of 2,5-dibromo-3-(bromomethyl)thiophene (3). To the solution of 2 (6.4 g, 23.52 mmol), in 30 mL dry CH₂Cl₂ at 0 °C under nitrogen atmosphere, PBr₃ (4.5 mL, 47.88 mmol) was drop-wisely added and stirred at room temperature under nitrogen atmosphere for 24 h. Then, the solvent was evaporated in *vacuo* and oil residue treated with NaHCO₃ (30 mL, 10%) at 0 °C and extracted with CH₂Cl₂. The organic layer was evaporated and purified by flash column chromatography (SiO₂, hexane) to yield 3 (5.6 g, 71%, white solid). ¹H-NMR (CDCl₃, 500 MHz): δ 4.38 (s, 2H), 6.99 (s, 1H).

Synthesis of 2-((2,5-dibromothiophen-3-yl)methyl)isoindoline-1,3-dione (4). To the solution of 3 (4.0 g, 12 mmol) in 10 mL dry DMF, potassium phthalimide (3.3 g, 18 mmol) were added. After stirring for 24 h at 160 °C in a schlenk tube under nitrogen atmosphere, the reaction mixture was poured into water. The precipitate was washed with water, purified by flash column chromatography (SiO₂, CH₂Cl₂), precipitated in ethanol and dried under vacuum to yield 4 (3.2 g, 66%, white solid). ¹H-NMR (CDCl₃, 500 MHz): δ 4.77 (s, 2H), 6.99 (s, 1H), 7.93 – 7.69 (m, 2H).

Synthesis of (2,5-dibromothiophen-3-yl)methanamine (MA-DBT). The solution of 4 (3.2 g, 7.98 mmol) in 150 mL absolute ethanol with 25 mL hydrazine were stirred at 90 °C for 5 h in a schlenk tube. After allowing to cool to room temperature, the solvent was evaporated in *vacuo* and residue treated with NaHCO₃ (50 mL, 10%) and extracted with CH₂Cl₂ to yield MA-DBT (0.5 g, 74%, yellow oil). ¹H-NMR (CDCl₃, 500 MHz): δ 2.35 – 1.94 (m, 2H), 3.74 (s, 2H), 6.95 (s, 2H).

Synthesis of N⁶-Boc-L-lysine (LL(boc)). L-Lysine (5.0 g, 33.51 mmol) was dissolved in a 1.0 M NaHCO₃ solution (150 mL). CuSO₄·5H₂O (4.3 g, 17.22 mmol) in 40 mL H₂O was added to the solution, which subsequently turned dark blue. After further addition of 75 mL of a 1.0 M NaHCO₃ solution, Boc₂O (10.32 g, 47.3 mmol) dissolved in 40 mL acetone was

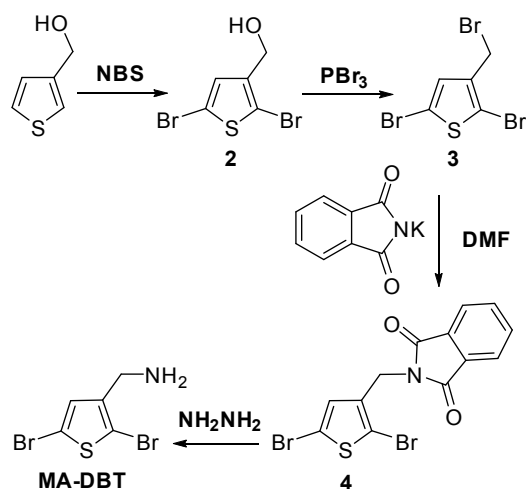
added and the mixture was stirred for 24 h at room temperature. 40 mL of methanol was added and the mixture was stirring for another 24 h. The blue slurry was filtered and the residue was dried in vacuum to yield the copper complex of L-lysine. Then, the copper complex (5.7 g, 10.23 mmol) was suspended in 400 mL H₂O and 8-Hydroxyquinoline (4.25 g, 29.3 mmol) was added. The suspension was stirred for 16 h turning from blue to yellow. The suspension was filtered, the residue discarded and the filtrate was washed with ethyl acetate (3 times with 200 mL). The aqueous phase was evaporated in *vacuo* and the residue again treated with ethyl acetate (100 mL) and filtrated. The residue was dried in vacuum to yield LL(boc) (3.9 g, 47%). ¹H-NMR (D₂O, 500 MHz): δ 1.36 – 1.22 (m, 11H), 1.45 – 1.35 (m, 4H), 3.00 – 2.91 (m, 2H), 3.66 – 3.56 (m, 1H).

Synthesis of *N*⁶-Boc-*N*²-(phenoxy carbonyl)-L-lysine (NCA-LL(boc)). To a stirred suspension of LL(boc) (1.66 g, 6.74 mmol) in methanol 10 mL, tetrabutylammonium hydroxide (40% in methanol) (4.37 g, 6.74 mmol) was slowly added at room temperature. After stirring for 1 h, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved into acetonitrile (10 mL), and then the resulting solution added dropwise to a stirred solution of diphenyl carbonate (DPC) (1.44 g, 6.74 mmol) in acetonitrile (10 mL) at room temperature. After stirring the solution for 1 h, the distilled water (75 mL) was added into the resulting mixture. The resulting mixture was acidified to pH 2.0-3.0 with 1.0 M HCl, and then extracted with ethyl acetate (3 × 30 mL). The combined organic layer was dried over Na₂SO₄, filtrated, and concentrated under reduced pressure. The crude products were purified with by column chromatography (SiO₂) in two steps using different elution solvents. First, the by-product PhOH was eliminated by eluting system of hexane: Ethyl acetate (4:1). Second, acetone was used as eluting solvent and then concentrated to give target compound NCA-LL(boc) (0.95 g, 40%). ¹H-NMR (D₂O, 500 MHz): δ 1.60 – 1.30 (m, 1H), 1.86 (m, 2H), 2.02 – 1.90 (m, 2H), 3.11 (m, 2H), 4.45 (d, *J* = 21.7 Hz, 1H), 6.18 –

6.01 (m, 1H), 7.12 (d, $J = 7.0$ Hz, 2H), 7.19 (t, $J = 7.4$ Hz, 1H), 7.39 – 7.29 (m, 2H), 8.21 (broad, 1H).

Synthesis of primary amino functional dibromothiophene. Dibromothiophene functionalized macromonomer synthesis is one of the key steps in synthesis polythiophene graft copolymers due to fact that dibromothiophenes can be coupled with the thiophene diboronic acids and its derivatives by Suzuki coupling reaction. Therefore, (2,5-dibromothiophen-3-yl)methanamine (MA-DBT) that possessing both suitable functionalities for both polypeptide incorporation and Suzuki coupling processes was synthesized via Gabriel synthesis. This amino functional monomer was prepared via Gabriel synthesis based on transformation of primary alkyl halides into primary amines (1). The synthetic work presented in Scheme S1 started by thiophen-3-ylmethanol and followed by two different bromination techniques to reach 3. Then, the substitution reaction with the conjugate base of phthalimide gave the corresponding *N*-alkyl phthalimide, 2-((2,5-dibromothiophen-3-yl)methyl)isoindoline-1,3-dione (4). The yield of reaction was increased by using preformed potassium salt of phthalimide, instead of phthalimide, facilitating the reaction to be conducted in a polar protic solvent such as DMF under homogenous conditions. Reaction of the imide derivative, 4, with hydrazine in the presence of methanol results in the formation of final primary amine, MA-DBT, and phthalhydrazide as a by-product which was readily eliminated due to the low solubility in organic solvent.

Scheme S1. Chemical synthesis pathway of (2,5-dibromothiophene-3-yl)methaneamine.



The structure of MA-DBT was investigated by ¹H-NMR analysis presented in Figure S1. A proton in fourth position on thiophene ring shows singlet peak at 7.29 ppm and other slightly broad peak at 2.1 ppm was assigned to –NH₂ protons, which was further confirmed by the examination of the hydrogen–deuterium exchange reaction.

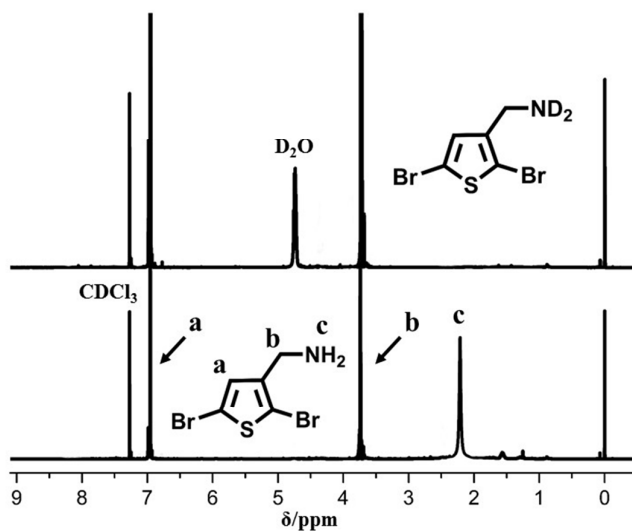
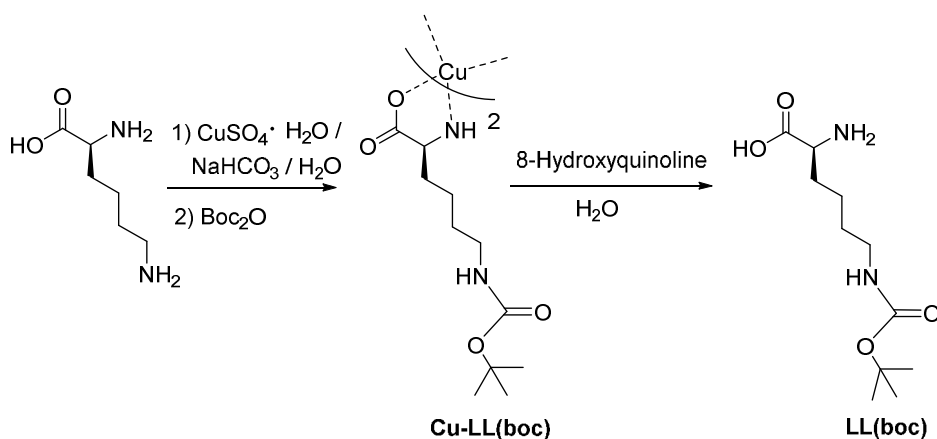


Figure S1. ¹H-NMR spectra of MA-DBT before (bottom) and after (top) proton exchange.

Synthesis of N^6 -Boc- N^2 -(phenoxy carbonyl)-L-lysine. Chemical synthesis of polypeptides can be accomplished via ring-opening polymerization (ROP) of corresponding NCA in presence of initiator (2). In this study, L-lysine based NCA precursor namely N^6 -Boc- N^2 -(phenoxy carbonyl)-L-lysine was chosen in order to synthesize poly(L-lysine). Firstly, L-lysine copper (II) complex was formed in order to block the reactivity of the α -amine. Then, side amine of L-lysine was protected by boc group. To remove copper from N^6 -Boc-L-lysine. To remove copper, 8-hydroxyquinoline was used as chelating agent. (Scheme S2)⁴⁹.

Scheme S2. Synthesis of N^6 -Boc-L-lysine (LL(boc)).



Incorporation of Boc group into L-lysine structure was investigated by ^1H -NMR analysis (Figure S2). Proton peaks of methyl groups (a) clustered in the region of 1 to 2 as expected. While singlet proton peaks of methyl that bounded with $-\text{NH}_2$ (c), and $-\text{NH}(\text{C}=\text{O})-$ (b) appear 3.6, and 3 ppm, respectively.

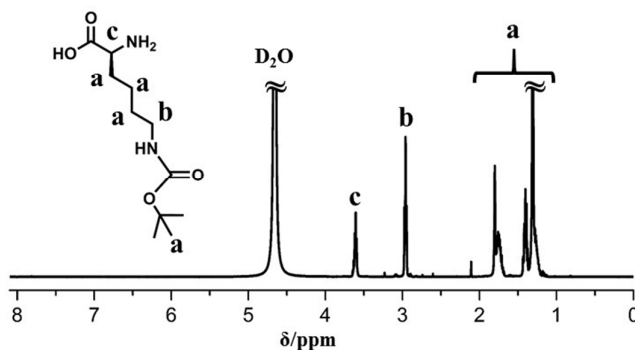
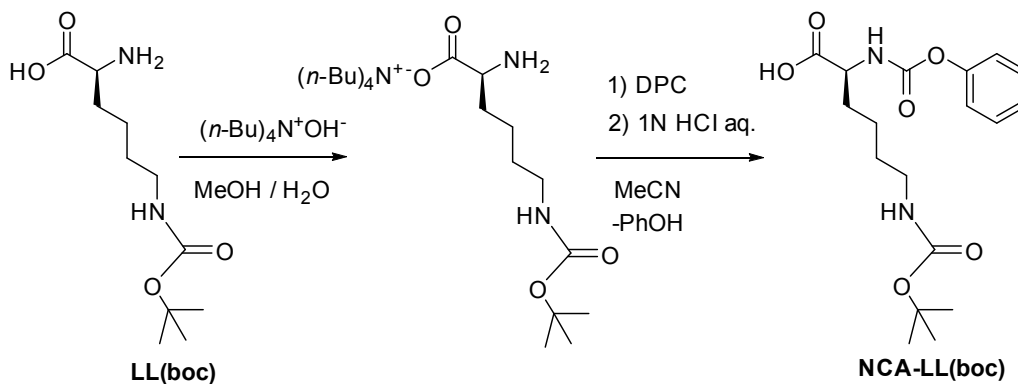


Figure S2. ^1H -NMR spectrum of N^6 -Boc-*L*-lysine.

Then, N^6 -Boc-*L*-lysine was transformed into corresponding ammonium salt by ion exchange reaction, and then ammonium salt-protected *L*-lysine was carbamylated at $N\alpha$ by nucleophilic attack of active primary amino group to the carbonyl group of diphenyl carbonate (Scheme S3). The obtained NCA precursor can be stored in room temperature for several months (3).

Scheme S3. Synthesis of N^6 -Boc- N^2 -(phenoxy carbonyl)-*L*-lysine (NCA-LL(boc)).



The structure of N^6 -Boc- N^2 -(phenoxy carbonyl)-*L*-lysine was investigated by ^1H -NMR analysis presented in Figure S3. Clusters of phenyl proton peaks (f) around 7 indicates the successful synthesis of LL(boc) to NCA-LL(boc). Proton peaks of methyl appeared between 1 and 2 while proton peaks of amide at position 2 (e), and side chain amide (d) were observed around 8.1, and 6, respectively.

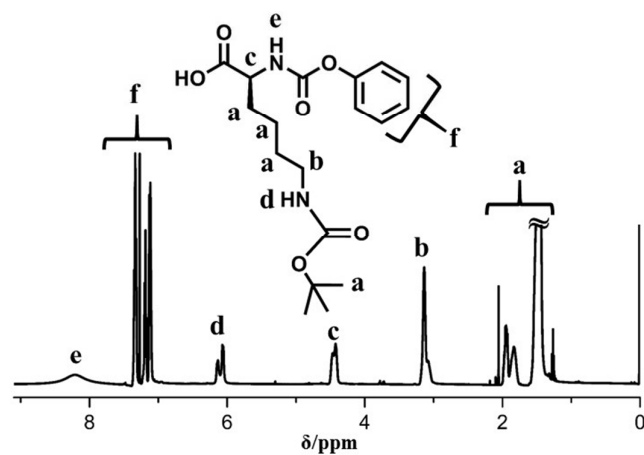


Figure S3. ^1H -NMR spectrum of N^6 -Boc- N^2 -(phenoxy carbonyl)-L-lysine.

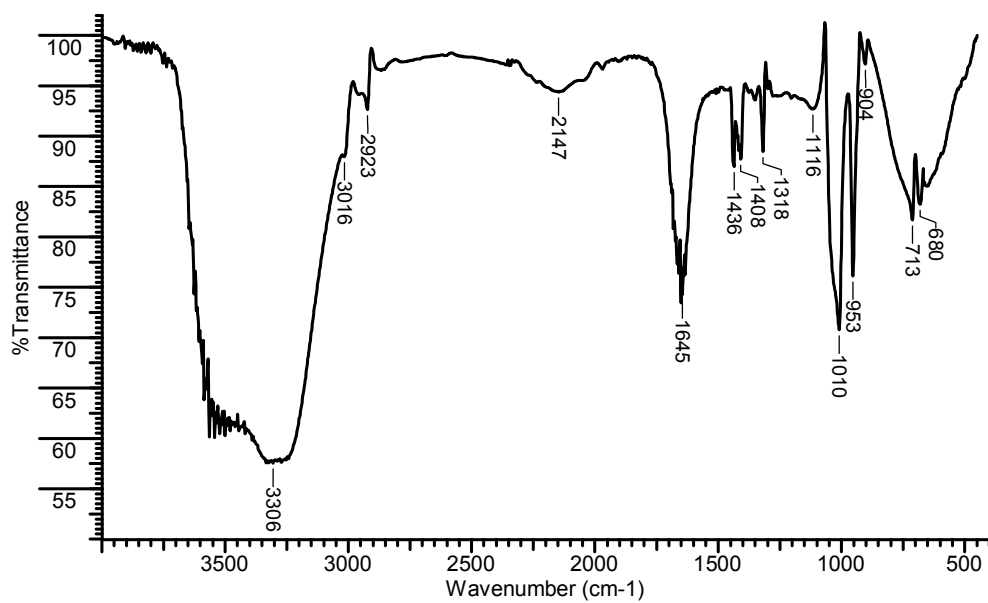


Figure S4. FT-IR spectrum of PT-g-PLL-PEG.

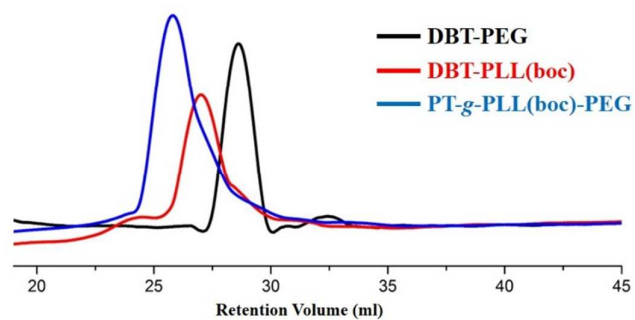


Figure S5. GPC traces of the macromonomers and graft copolymer.

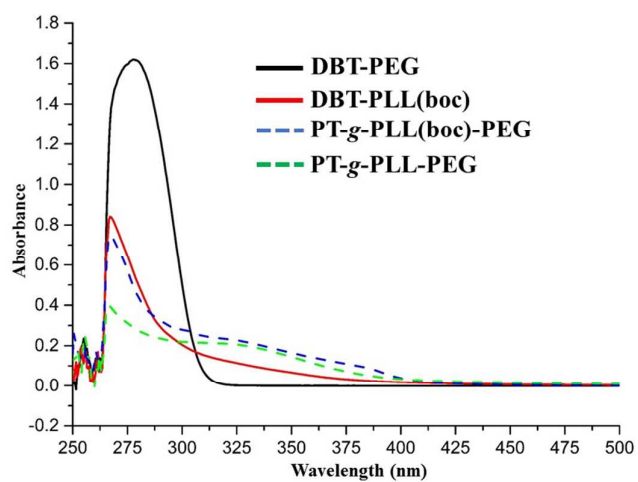


Figure S6: UV-Vis absorption spectra of the macromonomers and graft copolymers

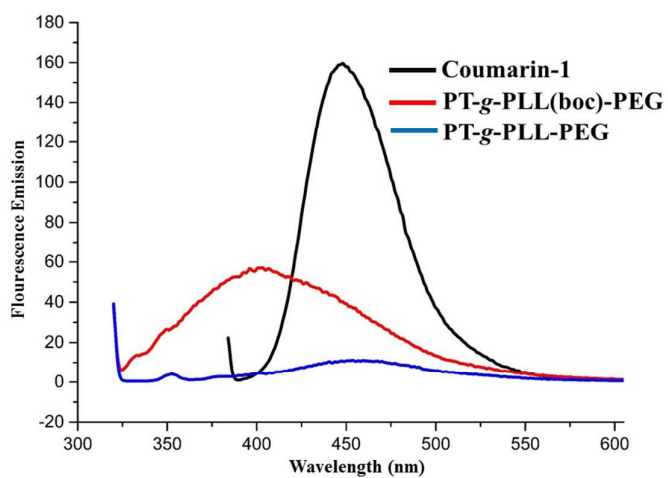


Figure S7: Fluorescence emission of Coumarin-1, PT-g-PLL(boc)-PEG and PT-g-PLL-PEG

Table S1: Photophysical properties of DBT-PLL(boc), DBT-PEG, and PT-g-PLL(boc)-PEG

Polymer	$\epsilon(\text{L.mol}^{-1}.\text{cm}^{-1})$	Φ_f in Water	Φ_f in DMF
DBT-PLL(boc)	84106 ^a	-	-
DBT-PEG	2060 ^b	-	-
PT-g-PLL(boc)-PEG	32647 ^c	-	0.45 ^d
PT-g-PLL-PEG	-	0.08 ^d	-

Measured at ^a268 nm, ^b 277 nm, ^c327 nm in DMF solutions. ^dDetermined according to Coumarine reference