Supporting information

Novel "Turn on-off" Paper Sensor Based on Nonionic Conjugated Polythiophene-Coated CdTe QDs for Efficient Visual Detection of Cholinesterase Activity

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Experimental

Materials and Reagents

Sodium borohydride ($NaBH_4$), tellurium powder, cadmium chloride ($CdCl_2$), glutathione (GSH), acetylcholinesterase (AChE), acetylthiocholine iodide(ATCh), 3-thiopheneacetic acid (98%), N-bromosuccinimide (NBS), polyethyleneglycol (Mn 2000 g mol-1), n-butyllithium (1.6 M in hexane), ethyl mercapto acetate, cupric oxide nanopowder, dicyclohexyl carbodiimide (DCC, 99%), 4-(N,N-dimethylamino)-pyridine (DMAP, 98%), N-hydroxysuccinimide (NHS, 98%), 2,5-thiophenediboronic acid (TDBA), tetrakis(triphenylphosphine) palladium(0), potassium carbonate, and sodium hydroxide were purchased from Sigma-Aldrich. All reagents and solvents were used as received without further purification. The distilled water used in all experiments had a resistivity higher than 18 $M\Omega \cdot cm^{-1}$ from a Milli-Q water purification system. Chromatography paper grade 1 was purchased from GE Healthcare UK Limited, Little Chalfont, UK.

Characterization

FT-IR spectra were recorded on an FT/IR-6300 Fourier Transform Infrared Spectrometer (Jasco, Japan). 1H-NMR spectra were recorded at 400 MHz using a Bruker NMR instrument. The fluorescence experiments were performed on an FP-6500 spectrofluorometer (Jasco, Japan) using a quartz cuvette with a 1-cm path length. The absorption spectra were obtained using an Agilent 8543 (Agilent, USA) UV/Vis spectrophotometer. TEM measurements were carried out using a JEM-2100F transmission electron microscope (JEOL, Tokyo, Japan) operating at 200 kV. The XRD powder pattern was obtained on an X'Pert PRO MPD X-ray diffractometer (Analytical, Netherlands) with Cu Kα (ratio $K\alpha_2/K\alpha_1=0.5$) radiation. Thermogravimetric analysis (TGA) was carried out using an SDT Q600 V20.9 Build 20 instrument at a heating rate of 10 °C/min with a constant N_2 flow rate of 20 mL/min within the temperature range of 35-800 °C. DLS analysis was carried out using a Zetasizer Nano ZS90 apparatus (Malvern Instruments, Worcestershire, U.K.). Zeta potentials were measured by Zeta potential analyzer.

Synthesis of (2,5-dibromo-thiophene-3-yl)-acetic acid (1)

Thiophene-3-acetic acid (0.5g, 3.5 mmol) and N-bromosuccinimide (0.64 g, 3.59 mmol) were dissolved in 30 mL dry DMF. The mixture was stirred for 24 h under nitrogen atmosphere at room temperature in the dark. The resulting mixture was rinsed with brine solution (3 times) and the aqueous layer was extracted with ethyl acetate. The organic layer was separated and dried over Na_2SO_4 and the solvent was concentrated under reduced pressure. The product was purified by column chromatography with hexane: ethyl acetate (8:2) to yield the desired product as a light yellow solid.

Yield: 86%; Mp: 121 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, see Fig. S1): 12.630 (s, 1H, COO**H**), 7.188 (s, 1H, thiophene), 3.57 (s, 2H, C**H**$_2$-COOH); ^{13}C NMR (400 MHz, DMSO-d_6, δ, see Fig. S2); 176.25 (CH_2), 129.08 (CH), 126.29 (C_d), 115.33 (C_a), 114.31 (C_b), 37.38 (C=O); FTIR (KBr, *υ,* see Fig. S3): 3430 cm^{-1} (O–H), 3100 cm^{-1} (=C–H), 2923 cm^{-1} (C–H), 1707cm^{-1} (C=O acid), 1414 cm^{-1} (C=C), 1126 cm^{-1} (C–O), 740 cm^{-1} (C–S), 626 cm^{-1} (C–Br); HRMS (ESI-MS, see Fig. S4) m/z calcd. for $C_6H_4Br_2O_2S$ $[M]^+$, 298.8178; found, 298.8199; Anal. calcd for $C_6H_4Br_2O_2S$: C, 24.53; H, 1.39; S, 10.69; Found: C, 24.66; H, 1.36; S, 10.65.

Synthesis of dibromothiophene-conjugated PEG (2)

2,5-dibromothiophene-3-acetic acid (**1**) (0.299 g, 1.0 mmol), DMAP (0.0122 g, 0.1 mmol), and PEG_{2000}–OH (10 g, 5 mmol) were dissolved in 20 mL of anhydrous CH_2Cl_2 and the mixture was stirred under nitrogen atmosphere. Then, (0.226 g, 1.1 mmol) of DCC (dissolved in 20 mL anhydrous CH_2Cl_2) was added in dropwise under nitrogen. After that, the mixture solution was stirred at room temperature for 48 h, and then the mixture was filtered and washed with 100 mL of CH_2Cl_2. The organic layer was washed with 10% $NaHCO_3$ and brine and then dried over Na_2SO_4. The solvent was removed under vacuum and the crude polymer was precipitated in cold diethyl ether and dried to yield **2**.

Yield: 85%; Mp: 59 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, see Fig. S5): 4.24 (OC**H**$_2$CH$_2$ in PEG unit), 3.75–3.4 (m, 4H, OCH$_2$C**H**$_2$ in PEG unit), 7.15 (s, 1H, thiophene), 3.54 (s, 2H, thiophene-C**H**$_2$), 1.2 (s, 1H, O**H**); FTIR (KBr, υ, see Fig. S6): 3468 cm^{-1} (O–H), 2886 cm^{-1} (C–H), 1735 cm^{-1} (C=O ester), 1472 cm^{-1} (C=C), 1106 cm^{-1} (C–O), 839 cm^{-1} (C–S), 618 cm^{-1} (C–Br); GPC (water, poly(ethylene oxide) standard), M_n :2.4 kDa; PDI: 1.24.

Synthesis of 4-bromothiophene-3-carbaldehyde (3)

3,4-dibromothiophene (2.4193 g□, 10.0 mmol) was dissolved in 25 mL of diethyl ether and stirred at −78 °C for 30 min under N_2 atmosphere, then n-butyllithium (0.6406 g, 10.0 mmol) was injected into the solution. After additional 30 min stirring, dimethylformamide (0.7310 g, 10.0 mmol) was dropped into the mixture and further stirred for two hours at room temperature.

Dark-orange liquid obtained after extraction with ether and vacuum evaporation was purified using column chromatography (hexane: ether = 3:1) to get 4-bromothiophene-3-carbaldehyde as yellowish oil.

Yield: 70%; ^{1}H NMR (400 MHz, DMSO-d6, δ, see Fig. S7): 7.36 (d, 1H, thiophene), 8.15 (d, 1H, thiophene), 9.75 (s, 1H, C**H**O); ^{13}C NMR (400 MHz, DMSO-d6, δ, see Fig. S8): 187.67 (C=O), 135.29 (C_d), 133.00 (C_b), 126.66 (C_a), 177.84 (C_c); Anal. calcd for C_5H_3BrOS: C, 31.44; H, 1.58; S, 16.78; Found: C, 31.51; H, 1.48; S, 16.75.

Synthesis of thieno[3,4-b] thiophene-2-carboxylic acid (4, 5)

To the 4-bromothiophene-3-carbaldehyde (0.4996 g, 2.615 mmol, dissolved in 25 mL of dimethylformamide), ethyl 2-mercaptoacetate (0.3455 g, 2.875 mmol), K_2CO_3 (0.5422 g, 3.923 mmol) and CuO nanopowder (0.0062 g, 0.0785 mmol) were added consecutively. The mixture was stirred at 80 °C for 12 h. After extraction with ether, evaporation of the solvent under vacuum, and purification using column chromatography (hexane: ether = 3:1), ethylthieno[3,4-b] thiophene-2-carboxylate (**4**, gray powder) was obtained with 81% yield. Hydrolysis of ethyl ester to carboxylic acid was carried out by heating an ethanol solution (50 mL) containing ethylthieno[3,4-b] thiophene-2-carboxylate (0.1698 g, 0.8 mmol) and excess amounts of LiOH (0.05748 g, 2.4 mmol) to 78 ºC for 2 hours. After cooling to room temperature, HCl (1.0 M) was added and stirred for another 30 min. The resulting suspension was extracted with ethyl acetate and dried over Na_2SO_4. The solvent was evaporated in vacuum to give pale pink solid (**5**), which was directly used in the next step without further purification.

Yield: 90%; ^{1}H NMR (400 MHz, CDCl3, δ, see Fig. S9): 13.39 (s, 1H, COO**H**),7.60 (s, 1H, thiophene), 7.32 (s, 1H, thiophene), 7.19 (s, 1H, 2-thiophene); ^{13}C NMR (400 MHz, $CDCl_3$, δ, see Fig. S10): 164.03 (C=O), 140.56 (C_f), 135.79 (C_b), 130.10 (C_d), 125.56 (C_c); 123.77 (C_a), 123.58 (C_g); Anal. calcd for $C_7H_4O_2S_2$: C, 45.64; H, 2.19; S, 34.80; Found: C, 45.58; H, 2.25; S, 34.92.

Synthesis of 4,6-dibromothieno[3,4-b] thiophene-2-carboxylic acid (6)

A solution of compound thieno[3,4-b] thiophene-2-carboxylic acid (0.184 g, 1.0 mmol) and NBS (0.356 g, 2.0 mmol) were dissolved in 20 mL dry DMF under a nitrogen atmosphere and stirring for 24 h at room temperature in the dark. After extraction with CH_2Cl_2, the organic phase was washed with brine, dried over Na_2SO_4. After evaporating the solvent, the brown solid was purified on silica-gel column chromatography to give a light-yellow product.

Yield: 80%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, see Fig. S10): 13.33 (s, 1H, COO**H**), 7.75 (s, 1H, 2-thiophene); ^{13}C NMR (400 MHz, CDCl3, δ, see Fig. S11): 164.19 (C=O), 139.02 (C_b), 133.46 (C_f), 128.56 (C_c), 127.72 (C_e); 116.02 (C_g), 98.42 (C_a); FTIR (KBr, υ, see Fig. S12): 3430 cm^{-1} (O–H), 3085 cm^{-1} (=C–H), 1705 cm^{-1} (C=O acid), 1483 cm^{-1} (C=C), 1185 cm^{-1} (C–O), 824 cm^{-1} (C–S), 594 cm^{-1} (C–Br); HRMS (ESI-MS, see Fig. S13) m/z calcd. For $C_7H_2Br_2O_2S_2$ $[M]^+$, 341.8372; found, 341.8375. Elemental analysis: Calcd for $C_7H_2Br_2O_2S_2$ Calcd: C, 25.58; H, 0.79; S, 18.75 Found: C, 25.35; H, 0.76; S, 18.62.

Synthesis of the nonionic conjugated polythiophene (CP)

The nonionic conjugated polythiophenes were synthesized through Suzuki coupling reaction,[1] as shown in **Scheme 1**. In a 100 mL flask, **2** (0.23 g, 0.10 mmol), **3** (0.342 g, 1.0 mmol) and 2,5-thiophenediboronic acid (0.206 g, 2.2 mmol) were dissolved in 15 mL of THF, and then 5 mL of aqueous 2.0 M K_2CO_3 solution was added. After that, the reaction mixture was degassed and purged with nitrogen several times before and after the addition of $Pd(PPh_3)_4$(0) (27 mg, 0.023 mmol). The solution was stirred at 70 °C for 48 h under nitrogen, and then the reaction mixture was cooled to room temperature. The resulting solution was extracted with CH_2Cl_2, washed with brine (3 times), and DI water (3 times), then dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The remaining solid residue was redissolved in chloroform (2 mL) and added slowly to methanol (50 mL). The precipitates were collected by filtration, washed with methanol, and dried in vacuum, offering a light yellow solid **CP**.

CP: Yield: 76%; ^{1}H NMR (400 MHz, CDCl3, δ, see Fig. S16): 13.40 (s, 1H, COO**H**), 8.11 (s, 1H, thiophene), 7.82 (d, 1H, thiophene), 7.55 (d, 1H, thiophene), 6.85 (s, 1H, thiophene), 4.30 (t, 2H, OC**H**$_2$CH$_2$ in PEG unit), 3.23-3.87 (m, nH, OCH$_2$C**H**$_2$ in PEG unit), 3.21 (t, 1H, O**H**); FT IR (KBr, υ, see Fig. S15): 3430 cm^{-1} (O–H), 2940 cm^{-1} (C–H_{as}), 2892 cm^{-1} (C–H_{sy}), 1748 cm^{-1} (C=O ester), 1705 cm^{-1} (C=O acid), 1637 cm^{-1} (C=C bond), 1470 cm^{-1} (CH_2 bending), 1115 cm^{-1} (C–O), 711 cm^{-1} (C–S); GPC (water, poly(ethylene oxide) standard), M_n :21.175 kDa; PDI: 1.28.

The photoluminescence quantum yield (PLQY)

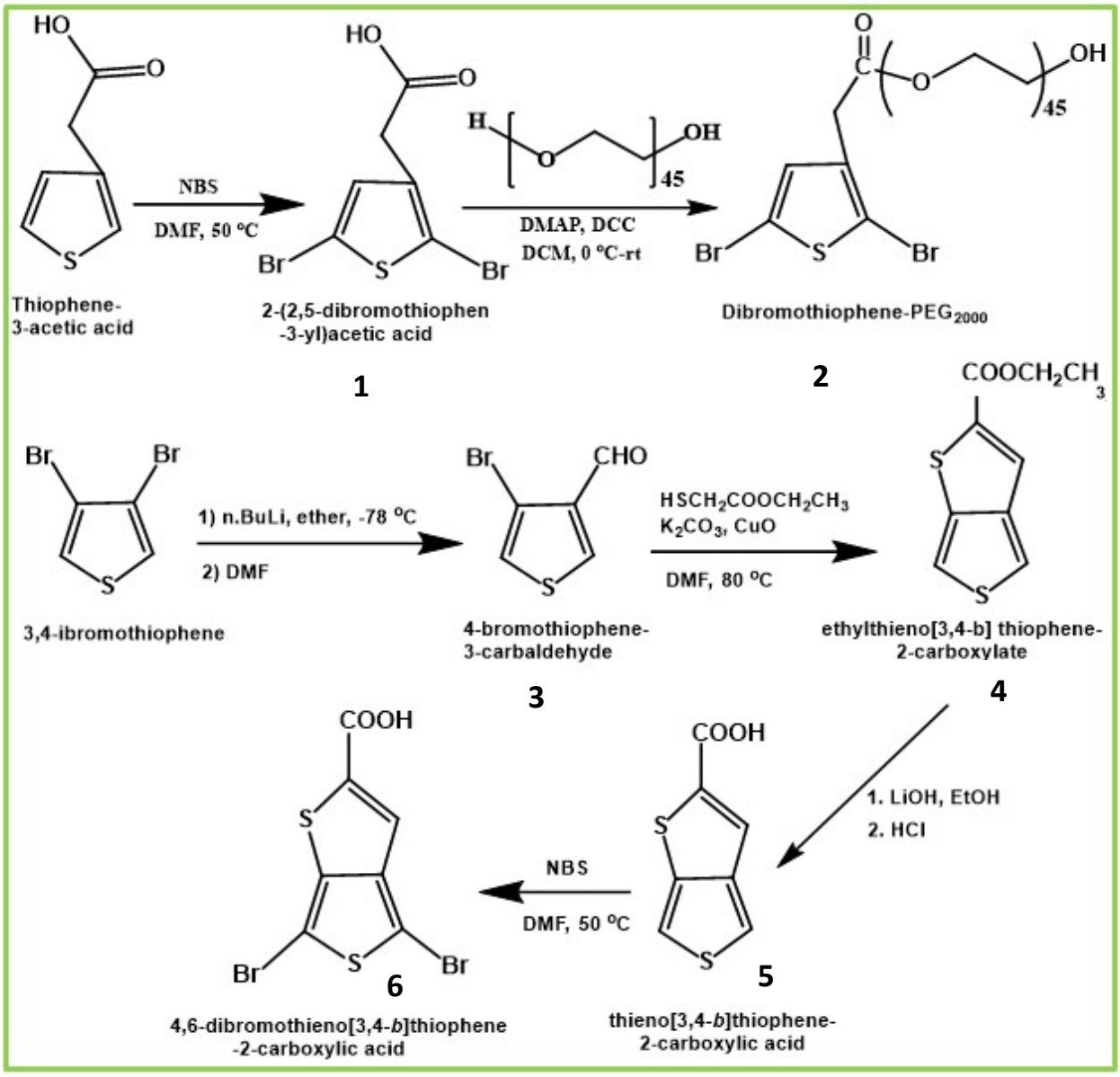
The photoluminescence quantum yield of **CP** was achieved by comparing the integrated fluorescence intensities, and the absorbance values of the CP with the reference, rhodamine B (Φ = 0.31), and the synthesized conjugated polythiophenes were measured in DI water (n = 1.33). The spectrophotometer was used to measure the emission value of the CP 435 nm excitation wavelengths. The excitation and emission slit width set at 3 nm. The quantum yield (QY) was determined using the equation (1) [2]

$$\Phi_x = \Phi_r \times \frac{I_x}{I_r} \times \frac{A_x}{A_r} \times \frac{n_x^2}{n_r^2} \quad (1)$$

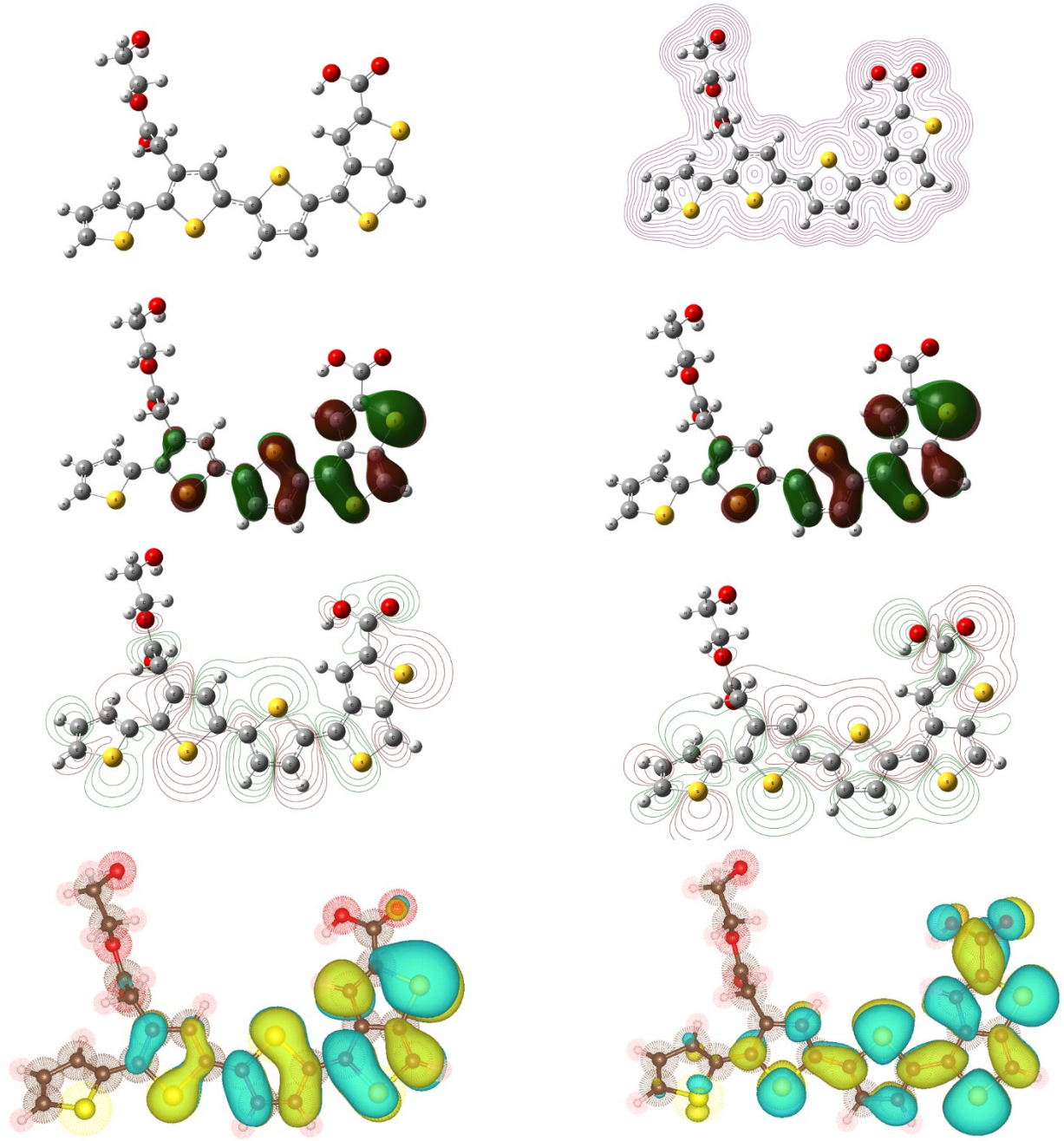
where Φ_x is the QY, I is the integrated fluorescence intensity, A is the absorbance, and n is the refractive index of the solvent; r denotes the standard, and x denotes the sample.

Optimizing conditions

As shown in Fig.S18a, the fluorescence gradually increased as the incubation time increased for incubation periods of less than 8 min; however, the fluorescence was gradually quenched after 8 min of incubation. This indicated that thiocholine first reacted with CP to enhance the fluorescence, whereas prolonged incubation produced a certain amount of thiocholine that was directly connected to the CdTe QDs, resulting in fluorescence quenching. An increase in the incubation time to 30 min did not cause any obvious change in fluorescence. A certain concentration of AChE has limited catalytic ability. Thus, we chose 30 min as the incubation time for the next experiment. The hydrolysis rate and capacity are strongly related to the ATCh concentration. As the concentration of ATCh increased, the fluorescence gradually decreased, as shown in Fig.S18b, 5 mM of ATCh achieved a high rate of enzymatic activity under this condition, and thus this concentration was chosen for the detection of enzymatic activity. As shown in Fig.S19, the fluorescence intensity of the CdTe QDs increased as the amount of CP increased, thereby confirming that CP can increase the fluorescence intensity of QDs.



Scheme S1. Reaction scheme for the synthesis of thiophene monomers



Scheme S2. Optimized geometries and molecular orbital surfaces of the HOMO and LUMO of CP2 polymer: HOMO = -5.702 eV; LUMO = -3.144 eV; Eg = 2.559 eV.

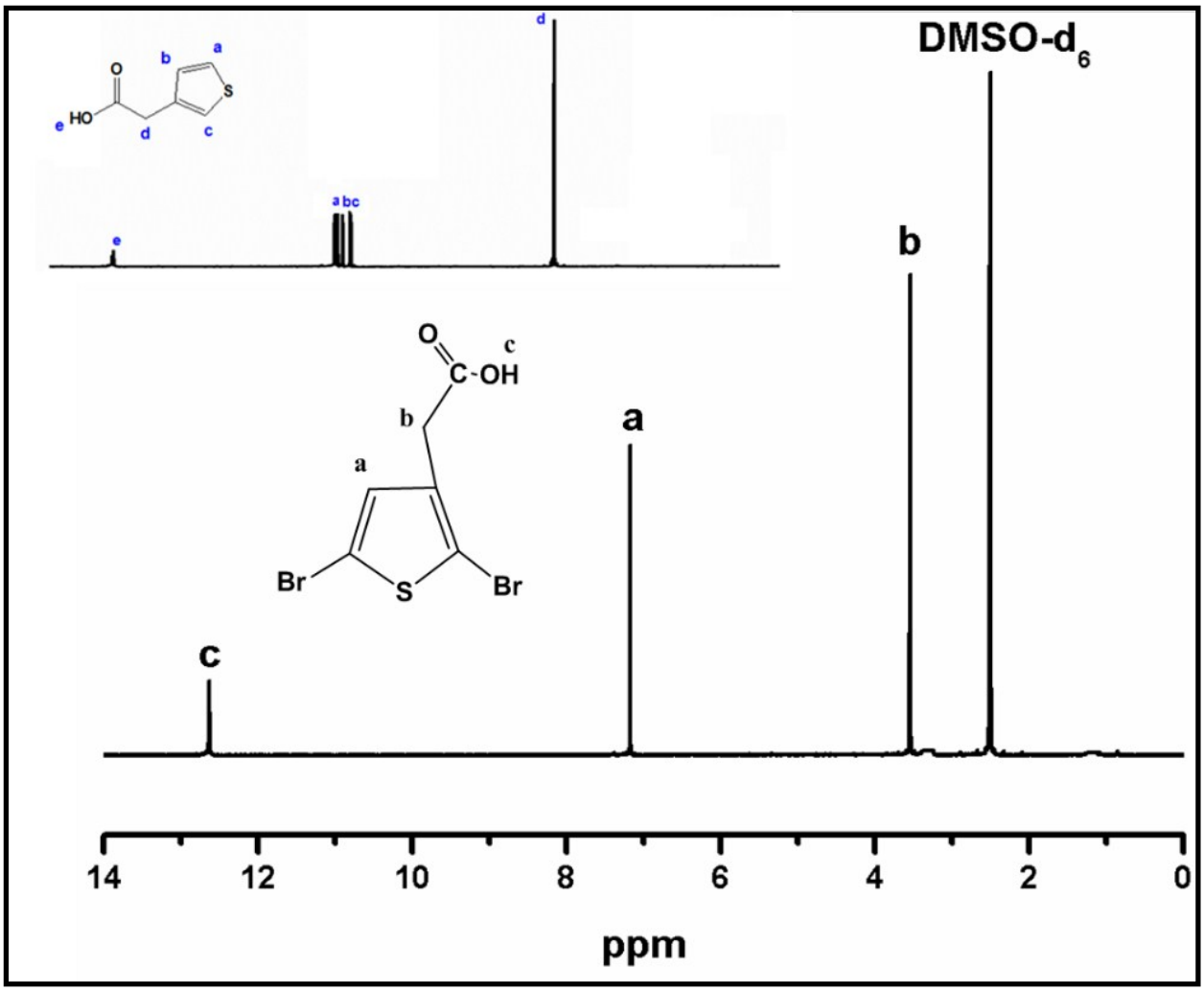


Fig. S1. ^{1}H-NMR spectra of (2,5-dibromo-thiophene-3-yl)-acetic acid, 1.

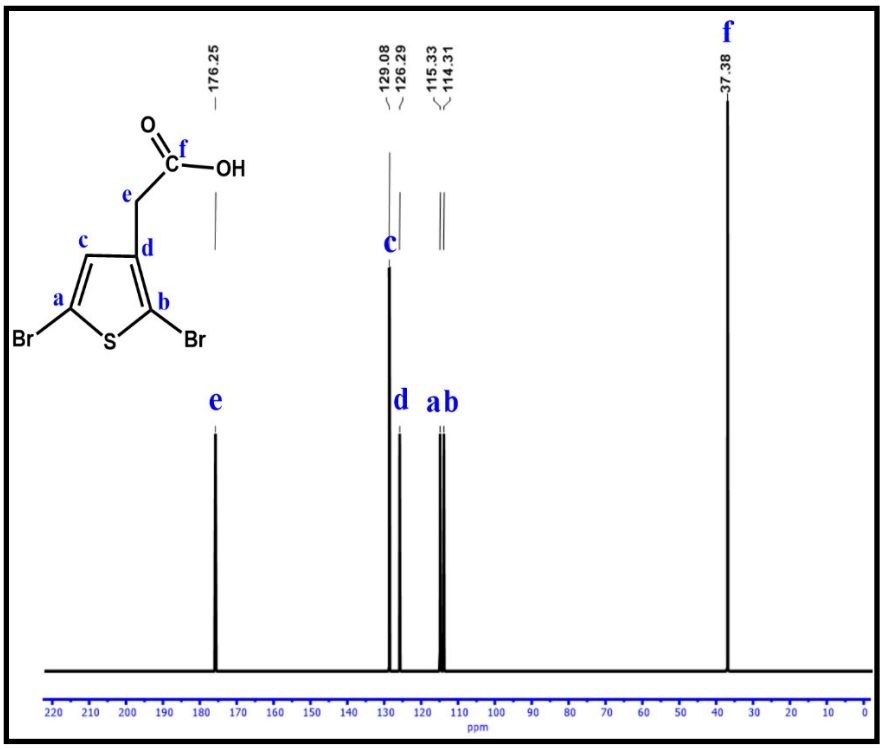


Fig. S2. ^{13}C-NMR spectra of (2,5-dibromo-thiophene-3-yl)-acetic acid, 1.

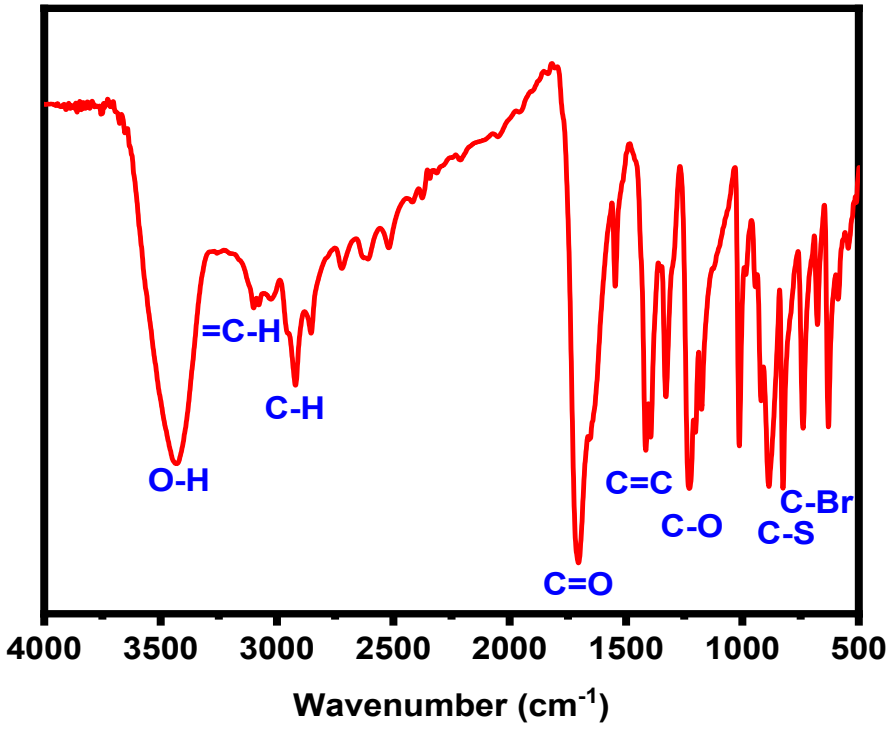


Fig. S3. FTIR spectrum of (2,5-dibromo-thiophene-3-yl)-acetic acid, 1.

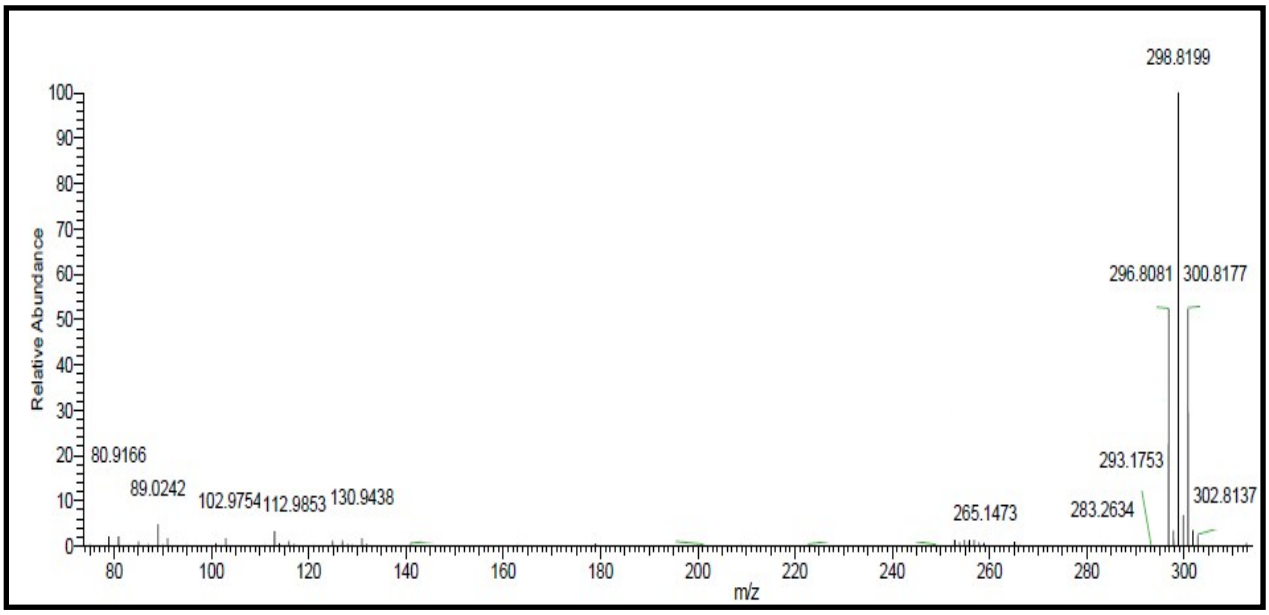


Fig. S4. ESI spectra of (2,5-dibromo-thiophene-3-yl)-acetic acid, 1.

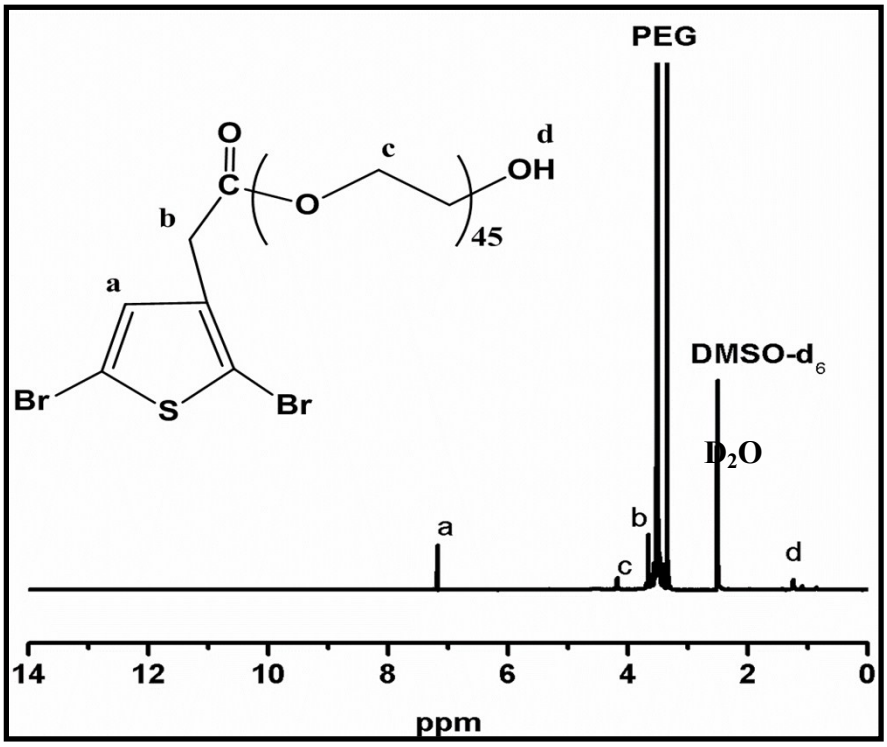


Fig. S5. ^{1}H-NMR spectrum of dibromothiophene-functional PEG (hydrophilic monomer, 2)

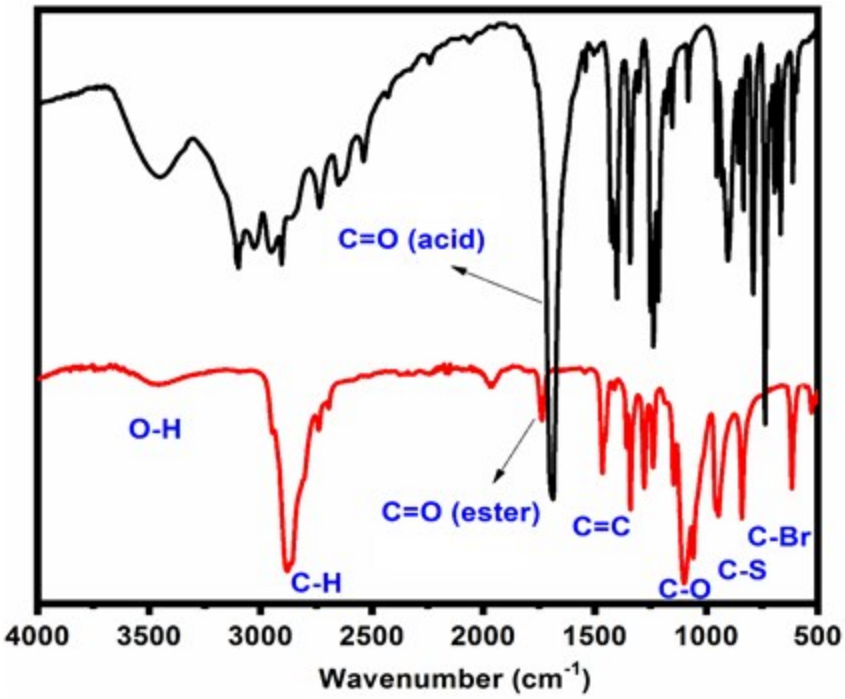


Fig. S6. FTIR spectra of nonionic monomer (a) 3-thiopheneacetic acid, (b) (2,5-dibromo-thiophene-3-yl)-acetic acid -PEG_{2000}, 2.

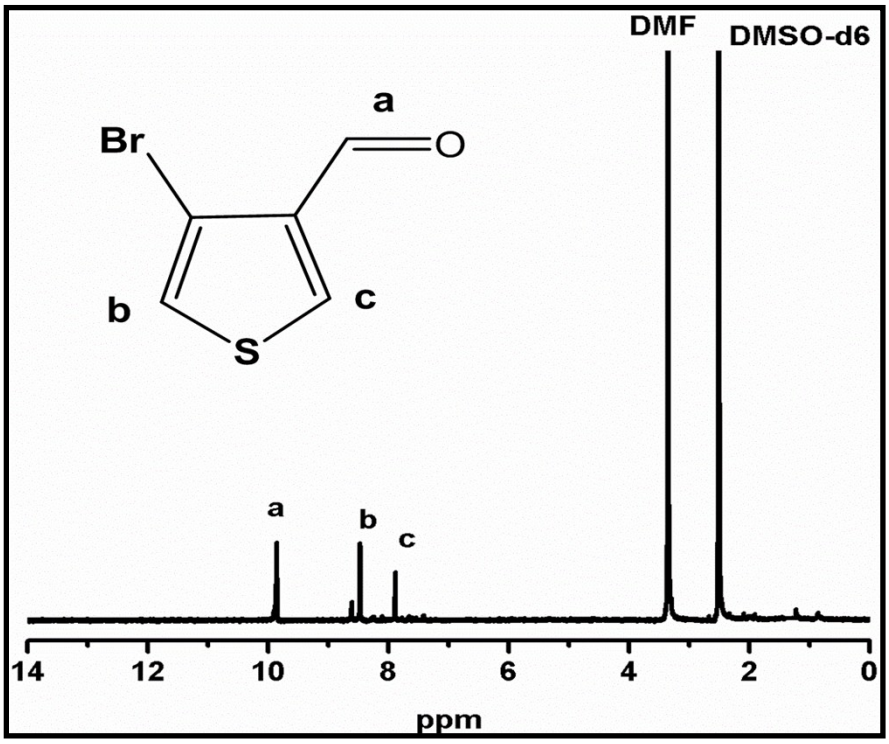


Fig. S7. ^{1}H NMR spectrum of 4-bromothiophene-3-carbaldehyde,3

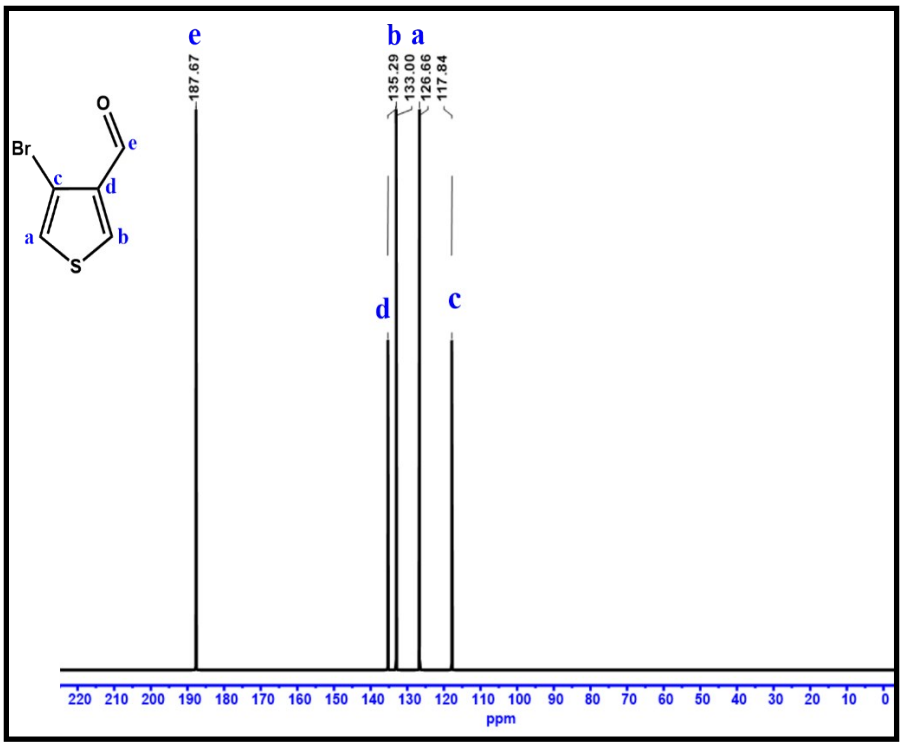


Fig. S8. ^{13}C NMR spectrum of 4-bromothiophene-3-carbaldehyde,3.

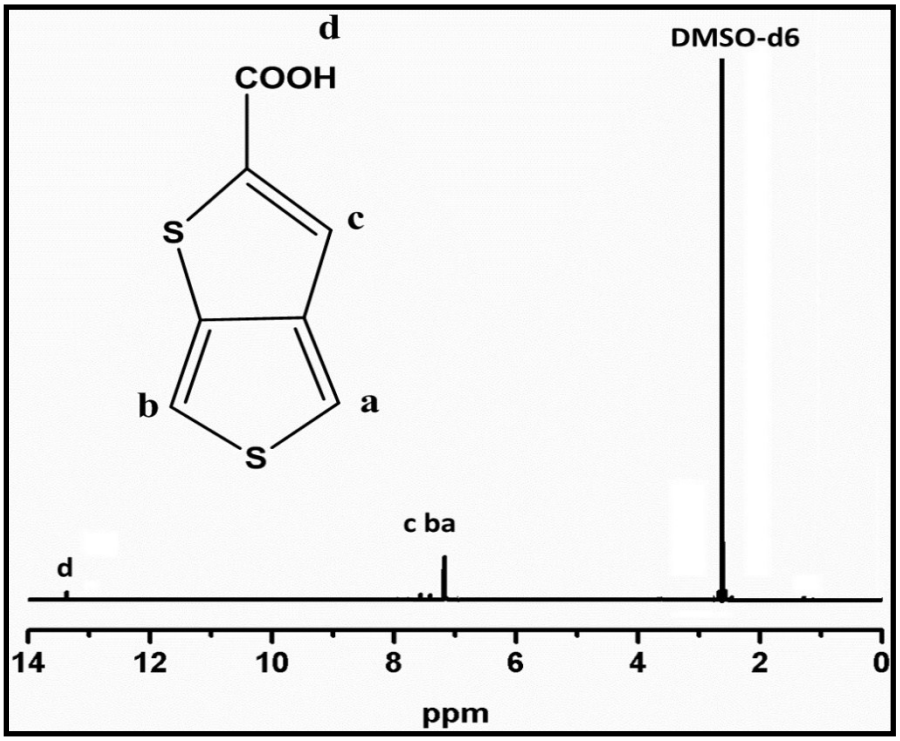


Fig. S9. ^{1}H NMR spectrum of thieno[3,4-b]thiophene-2-carboxylic acid,5.

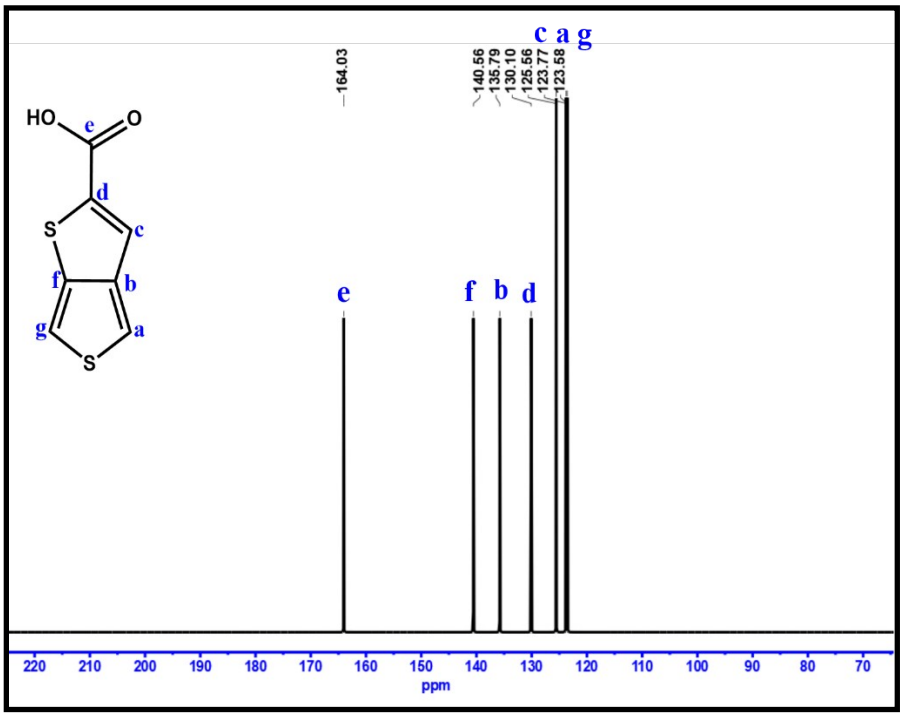


Fig. S10. ^{13}C NMR spectrum of thieno[3,4-b]thiophene-2-carboxylic acid, 5.

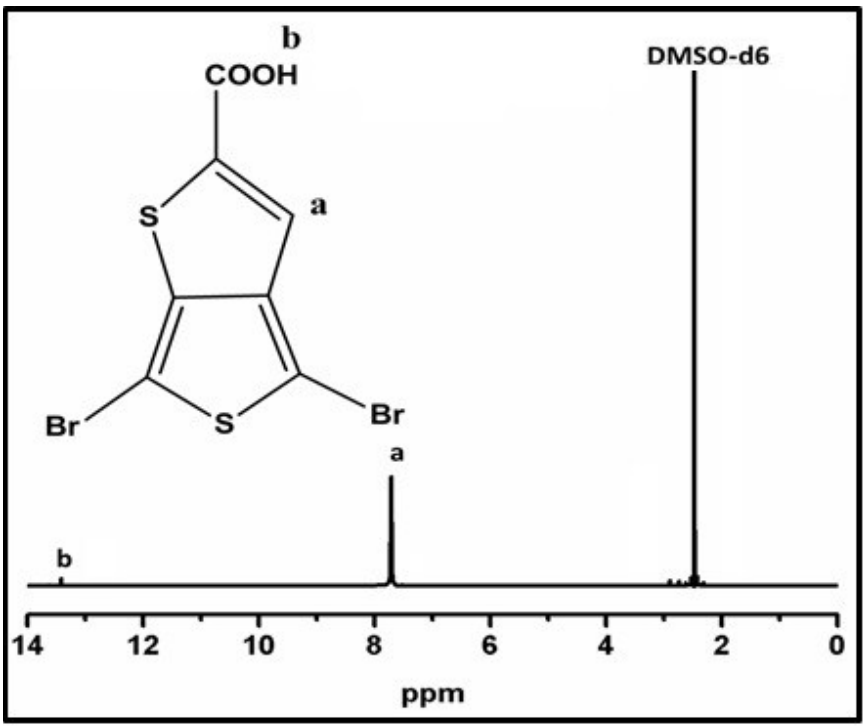


Fig. S11. ^{1}H NMR spectrum of 4,6-dibromothieno[3,4-b]thiophene-2-carboxylic acid, 6.

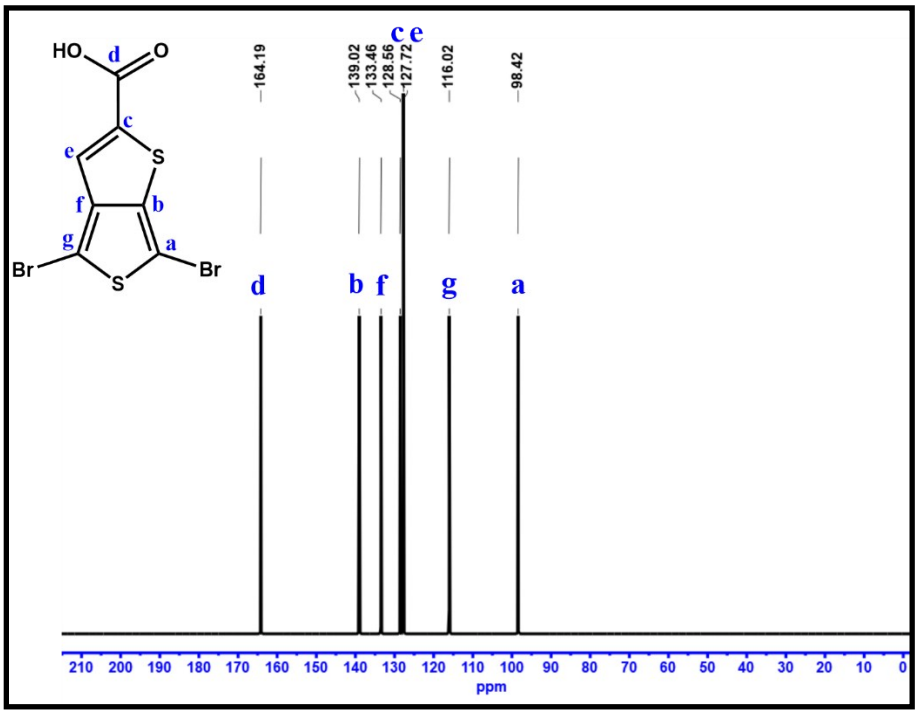


Fig. S12. ^{13}C NMR spectrum of 4,6-dibromothieno[3,4-b]thiophene-2-carboxylic acid, 6.

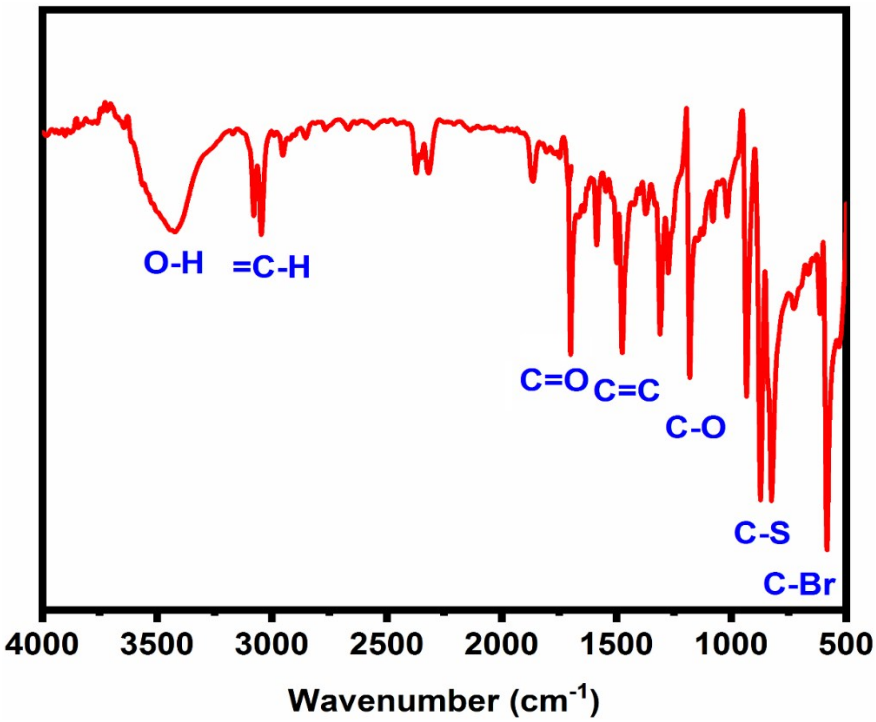


Fig. S13. FTIR spectrum of 4,6-dibromothieno[3,4-b]thiophene-2-carboxylic acid, 6.

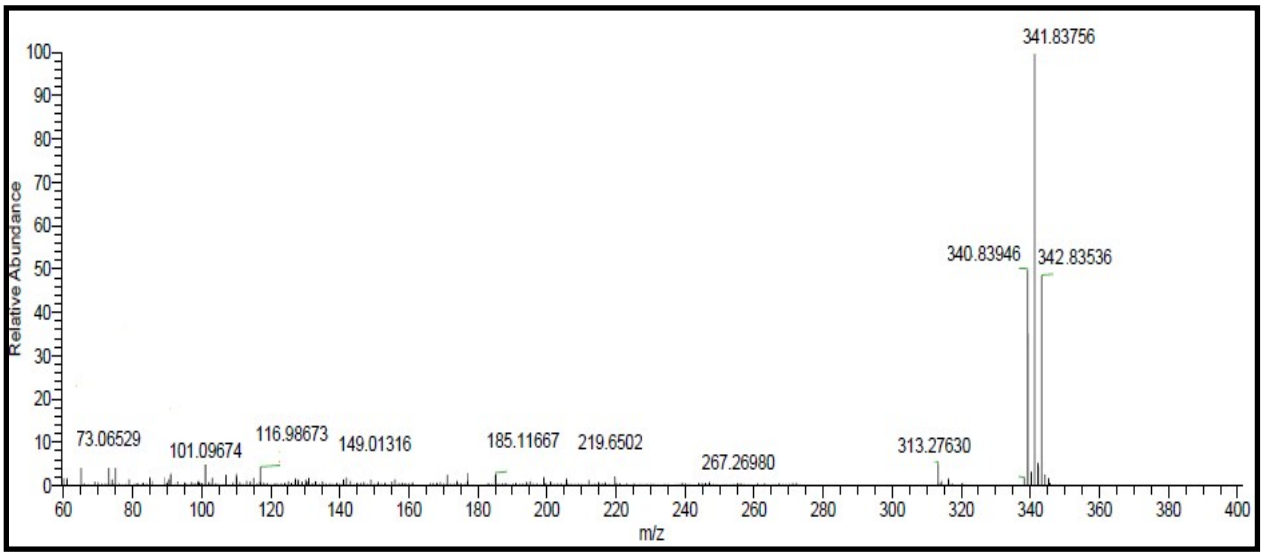


Fig. S14. ESI spectrum of 4,6-dibromothieno[3,4-b] thiophene-2-carboxylic acid, 6.

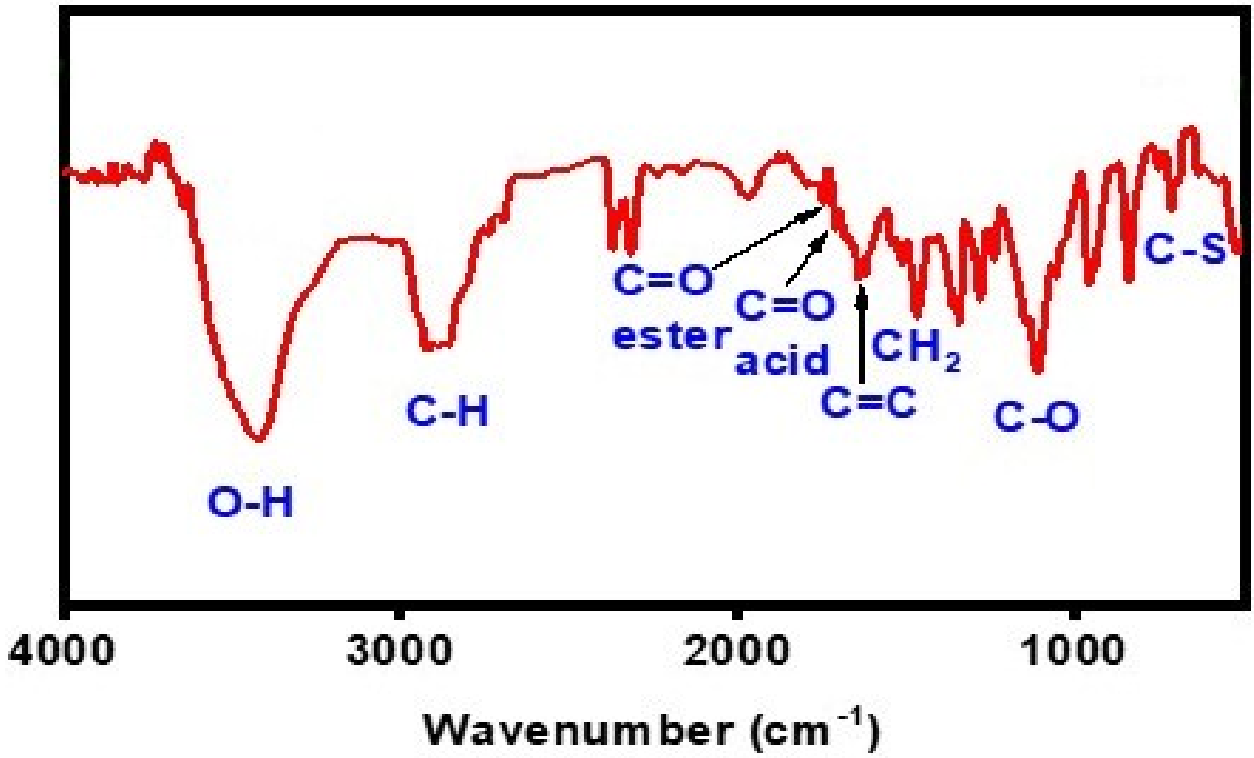


Fig. S15. FTIR spectra of nonionic conjugated polythiophene, CP.

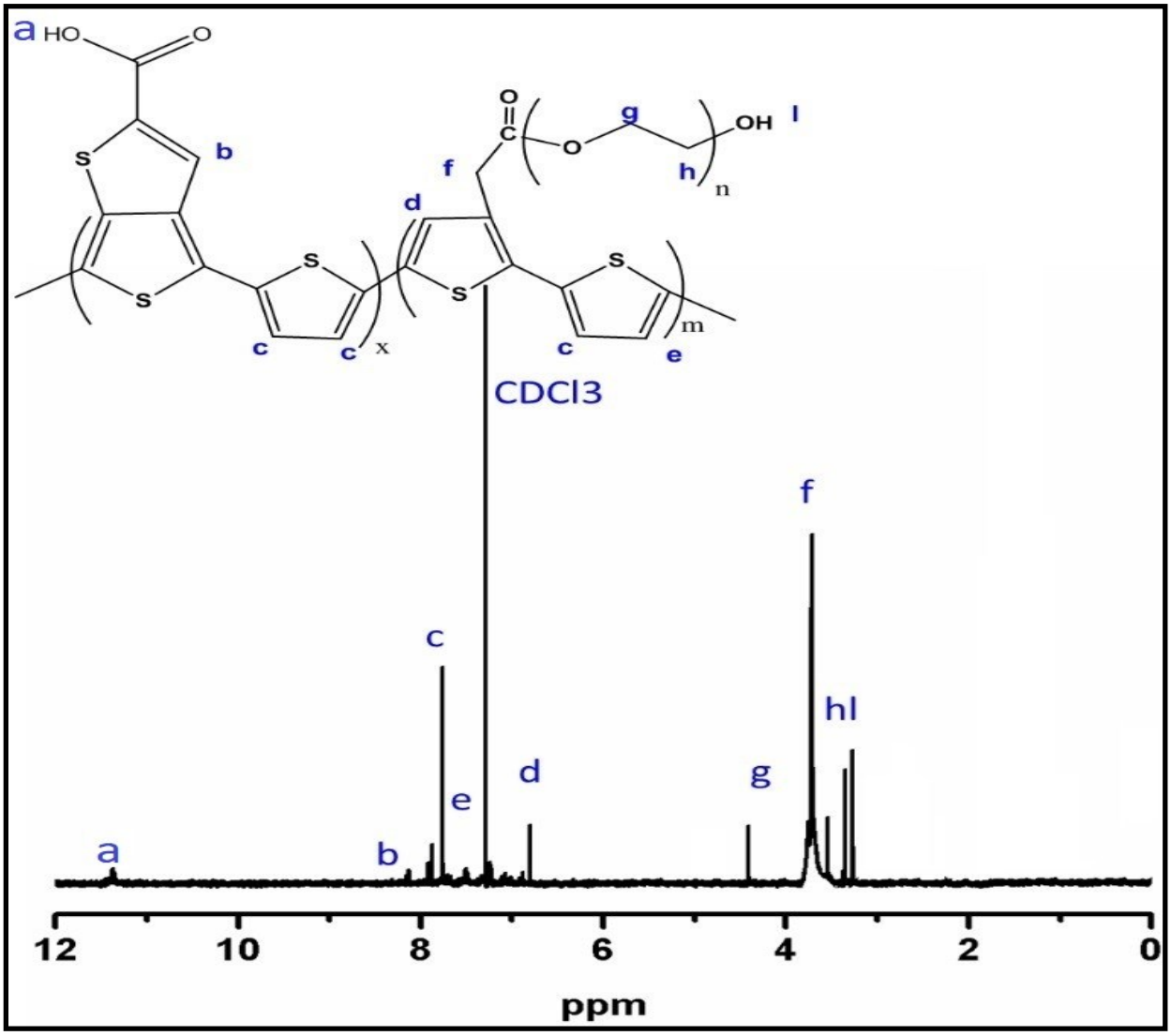


Fig. S16. ^{1}H NMR spectrum of nonionic conjugated polythiophene (CP).

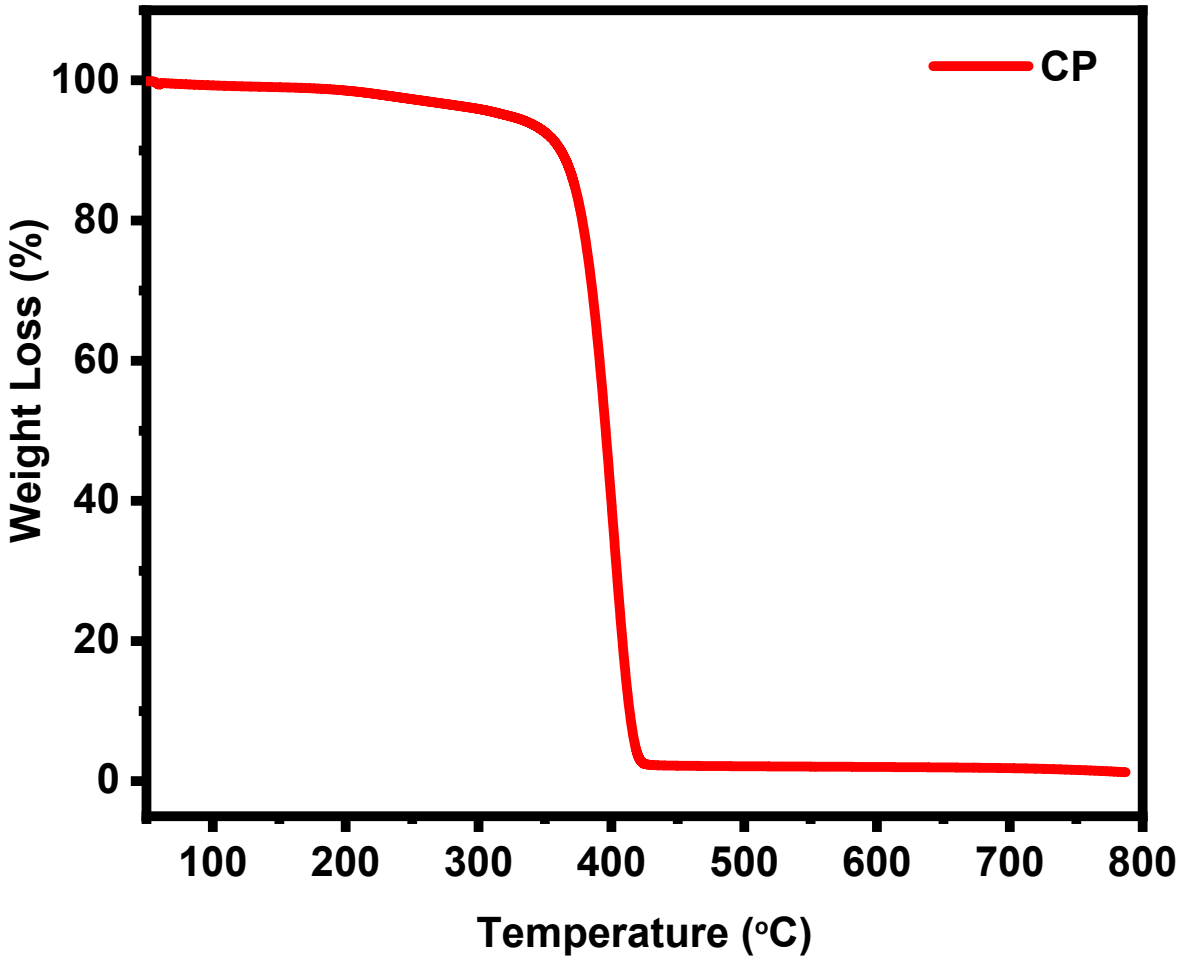


Fig. S17. Thermogravimetric analysis (TGA) of nonionic conjugated polythiophene (CP) in the range 30–800°C at a heating rate of 10 °C/min.

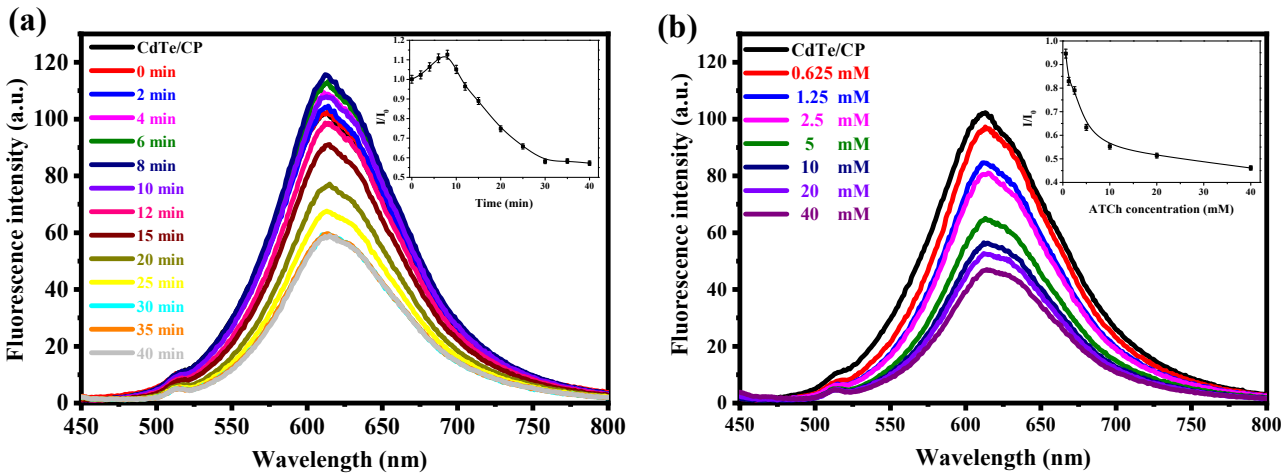


Fig. S18. Photoluminescence (PL) spectra (a) after various incubation times (ATCh concentration: 5 mM, AChE concentration: 0.1333 U/mL) and (b) for various ATCh concentrations (AChE concentration: 0.1333 U/mL, incubating time: 30 min); EX: 5 EM: 5, Excitation wavelength: 435 nm, solutions were diluted 10 times

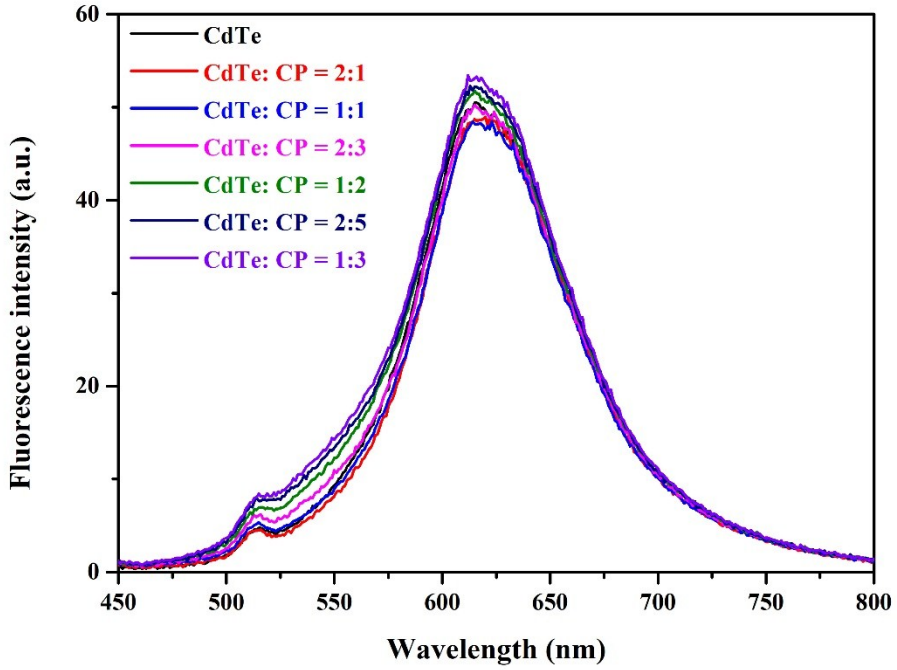


Fig. S19. Different mixed ratio of CdTe and CP, solution diluted 10 times, fixed the concentration of CdTe. EX:5 EM:5, CdTe:50µL.

Table S1. Characterization and optical properties of the CP

Polymer	Yield %	Mn kDa[a]	PDI	λmax,abs nm[b]	λmax,em nm[b]	PLQY %	λonset nm[c]	Egopt eV[d]	EHOMO/ELUMO eV[e]	Eg eV[f]
CP	76	21.17	1.28	435	577	55	506	2.45	−5.702/−3.144	2.559

[a] Measured by GPC.

[b]Measured in 1.0 mg mL^{-1} DI water.

[c]Obtained from the onset wavelength of the polymer.

[d]Evaluated by $E_g^{opt} = 1240/\lambda_{onset}$.

[e]$E_{HOMO} = -(E_{ox} + 4.8\ eV)$ and $E_{LUMO} = -(E_{red} + 4.8\ eV)$.[3]

[f]Calculated according to $E_{LUMO} - E_{HOMO}$.

PLQY: PL quantum yields

Table S2. Detection of AChE in human serum samples

Sample	Spiked (U/mL)	Found (U/mL)	Recovery (%)	RSD (%)
1	0.0010	0.0011	107	3.32
2	0.0023	0.0026	112	4.88

Notes and references

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