Supporting Information

Experimental Details

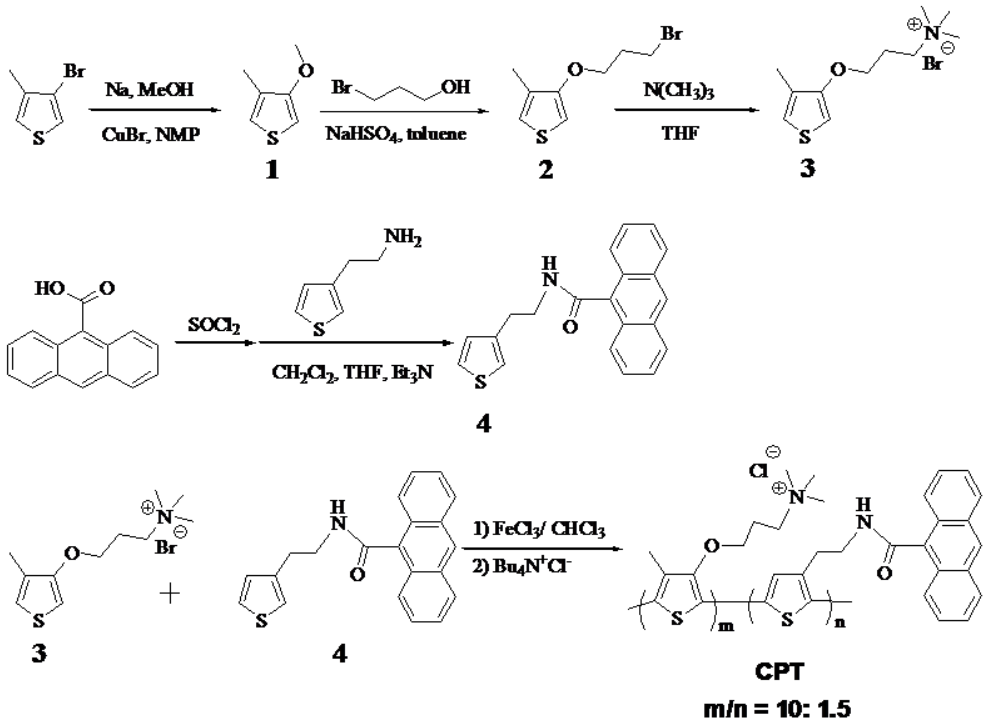
1. Materials

3-Bromo-4-methylthiophene and 9-Anthracenecarboxylic acid are purchased from TCI (shanghai) Development industry. 2-(3-thiophenyl)ethanamine is purchased from Feiyan Chemical. ATP, AMP, UMP, ADP and UTP were provided by Aladdin Reagent Company (Shanghai, China) for direct use. The water used throughout all experiments was purified by a Millipore filtration system. All solvents and reagents (analytical grade and spectroscopic grade) were obtained commercially and used as received unless otherwise mentioned. The polymer concentration was calculated based on the repeat unit.

2. Instruments

NMR spectra were collected on a Bruker Avance III-400 NMR spectrometer (400 MHz) using tetramethylsilane (TMS) as an internal standard. EI mass spectra were recorded on a VG ZAB-HS mass spectrometer (VG, U.K.). High-resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) source. Elemental analyses were performed on a Vanio-EL elemental analyzer (Analysensystem GmbH, Germany). GPC analysis was conducted with a Waters 2690 liquid chromatography system equipped with a Waters 996 photodiode detector and Phenogel GPC columns, using pullulan as the standard and H_2O as the eluent at a flow rate of 1.0 mL min^{-1} at 35 °C. 50 mL of 0.2 wt % of polymer in H_2O was injected into the columns. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter. Dynamic light scattering (DLS) measurements were performed using a Zetasizer Nano ZS90 (Malvern Instruments Co., UK) equipped with a He-Ne laser. All circular dichroism (CD) spectra were measured on a Jasco J-715 WI spectropolarimeter using a cylindrical quartz cell with a pathlength of 10 mm. The absorption spectrum was recorded on a Shimadzu UV-2550 spectrometer. Fluorescence measurements were carried out on a Hitachi F-4600 fluorescence spectrophotometer equipped with a xenon lamp excitation source. Fluorescence quantum yields were achieved by comparison with fluorescien in water as standard.

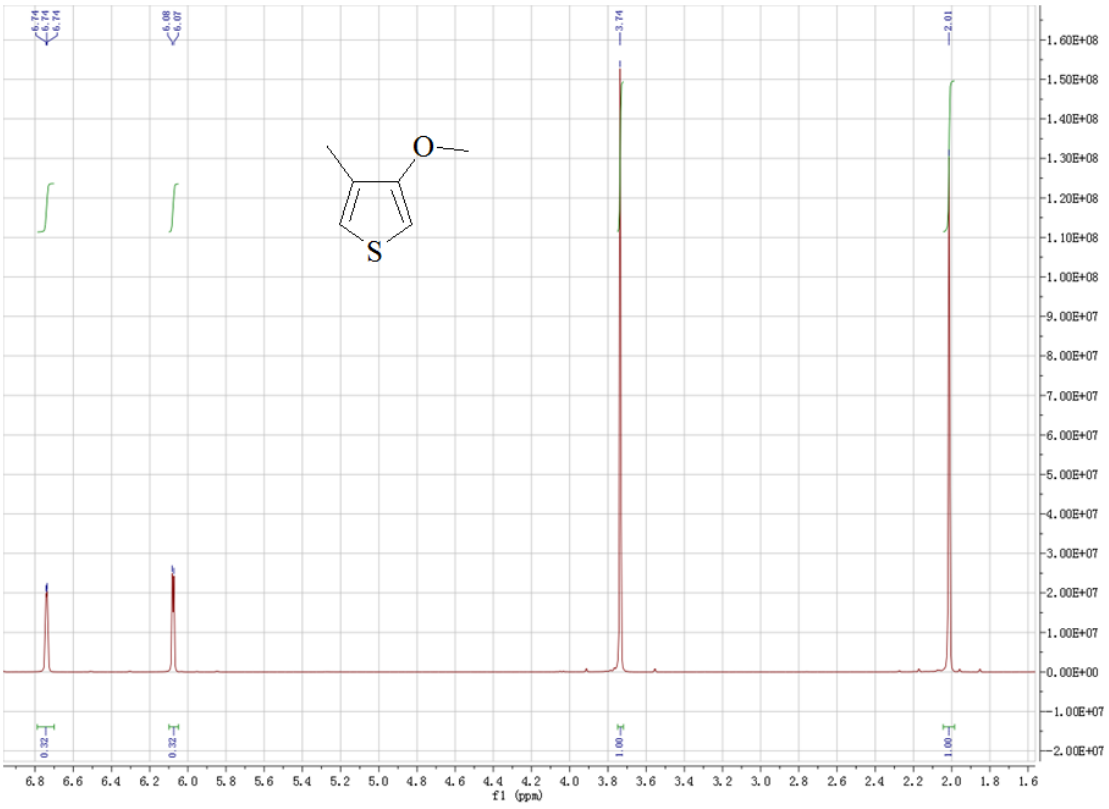
3. Synthesis of polythiophene derivatives



Scheme 1 Synthetic route of copolymer probe **CPT**.

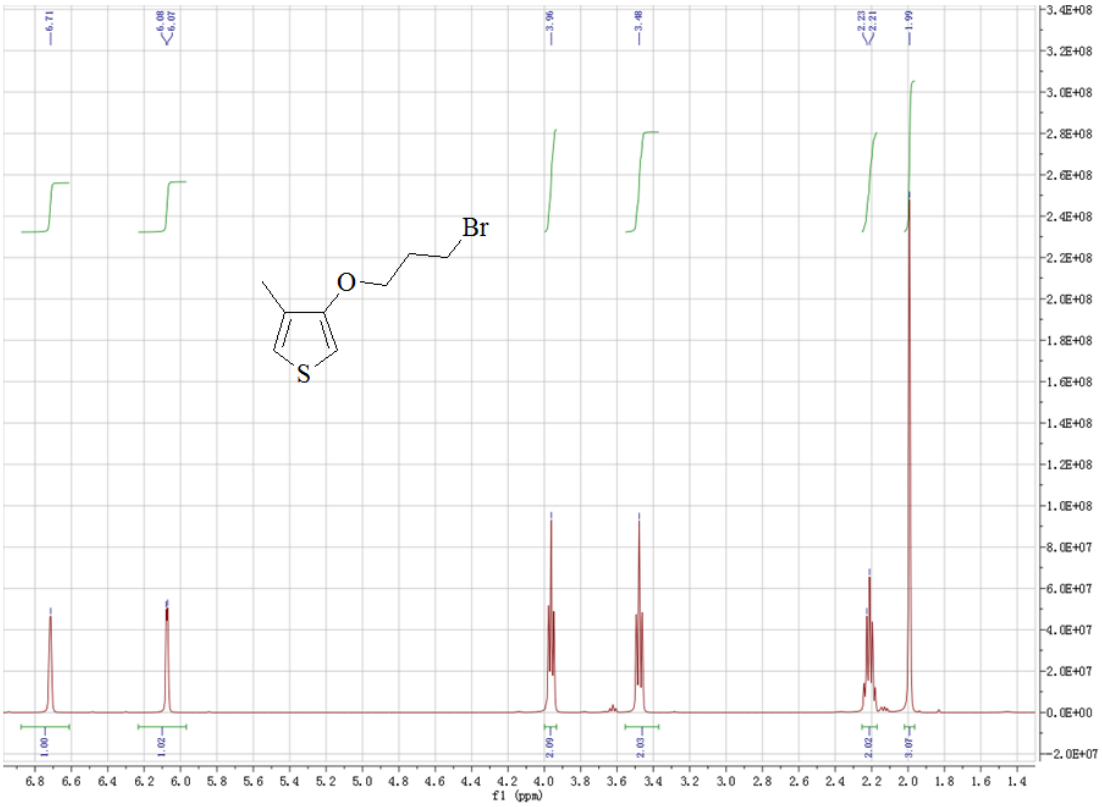
Synthesis of 3-Methoxy-4-methylthiophene (1) [1]

3-Bromo-4-methylthiophene (4.9 g, 27.6 mmol) and CuBr (2.5 g, 17.4 mmol) were added to a mixture of 18 mL of sodium methoxide (28% in methanol) and 7.0 mL NMP and refluxed for 3 days under N_2 atmosphere. After cooling, the solid was filtrated, and the filtrate was extracted three times with diethyl ether. The organic phase was dried with magnesium sulfate and then evaporated. The final product was purified by chromatography (silica gel, hexane) (3.25 g, 89 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.74 (s, 1H), 6.08 (d, 1H, J = 4.0 Hz), 3.74 (s, 3H), 2.01 (s, 3H). ESI MS: m/z $[M+H]^+$ = 127.92, calcd 128.03.



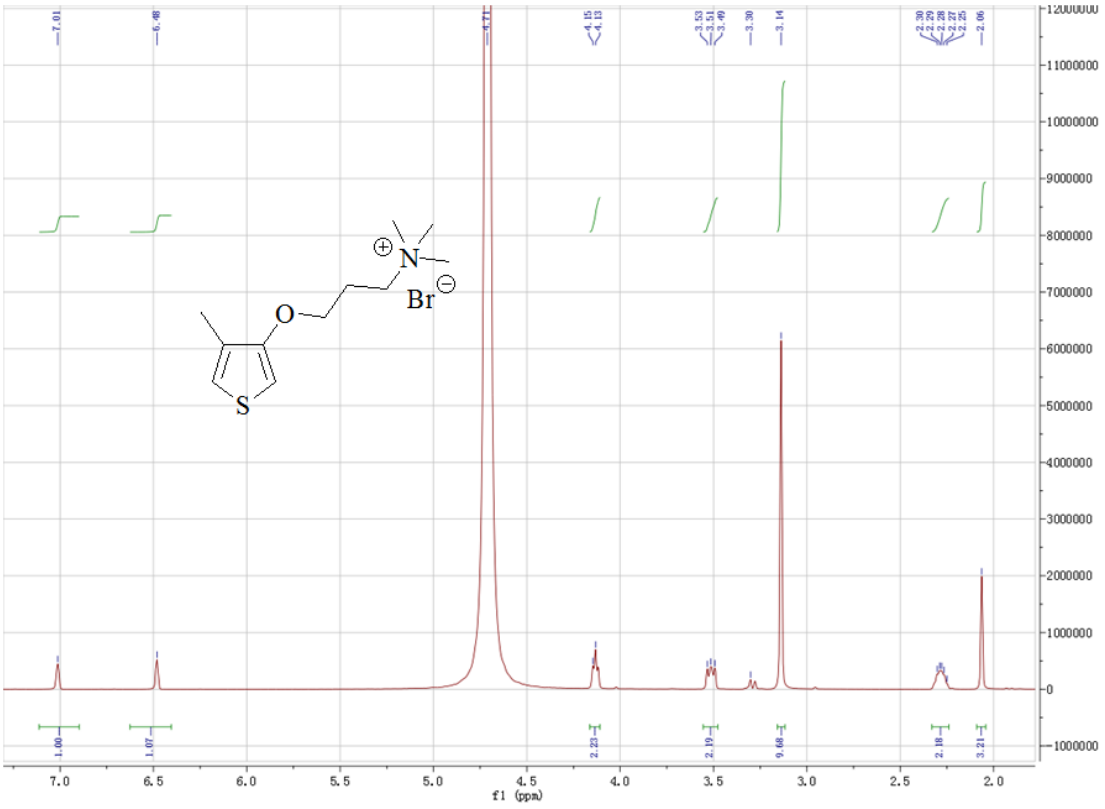
Synthesis of 3-(3-Bromo)propoxy-4-methylthiophene (2) [1]

3-Methoxy-4-methylthiophene **1** (2.88 g, 25.23 mmol), 3-bromo-1-propanol (7.71 g, 55.47 mmol), and $NaHSO_4$ (476.5 mg, 3.45 mmol) were added to 50 mL of toluene under N_2 atmosphere and heated at 100 °C until the produced methanol was distilled off. The reaction mixture was allowed to cool to room temperature and washed three times with water (30 mL each). The collected water phases were extracted with diethyl ether. The organic phases (i.e., toluene and diethyl ether phases) was combined and dried with magnesium sulfate, filtered and evaporated to dryness. The crude product was submitted to column chromatography (silica gel, hexane) to give 4.49 g (81%) **2** as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.72 (s, 1H), 6.08 (d, 1H, J = 4.0 Hz), 3.96 (m, 2H), 3.48 (m, 2H), 2.23 (m, 2H), 1.99 (s, 3H). ESI MS: m/z $[M+H]^+$ = 236.01, calcd 235.97.



Synthesis of 3-(4-Methyl-3'-thienyloxy)propyltrimethylammonium bromide (3) [1]

Trimethylamine (10 mL) was added into a solution of 3-(3-Bromo)propoxy-4-methyl-thiophene (3 g, 11.4 mmol) in THF (100 mL). The mixture was stirred 24 h at room temperature, and then evaporated to dryness. The crude product was washed with THF to give 2.97 g (81 %). ^{1}H-NMR: (400 MHz, d_6-DMSO) *δ:* 7.01 (s, 1H), 6.48 (s, 1H), 4.15 (m, 2H), 3.51 (m, 2H), 3.14 (s, 9H), 2.30 (m, 2H), 2.06 (s, 3H). ^{13}C NMR (101 MHz, d_6-DMSO) *δ:* 154.73 , 129.16 , 120.77 , 98.40 , 66.72 , 59.51 , 56.38 – 51.18 (m), 22.64 , 11.79 . ESI MS: m/z $[M+H]^+$ = 294.83, calcd 295.04.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6.48
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4.13
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3.49
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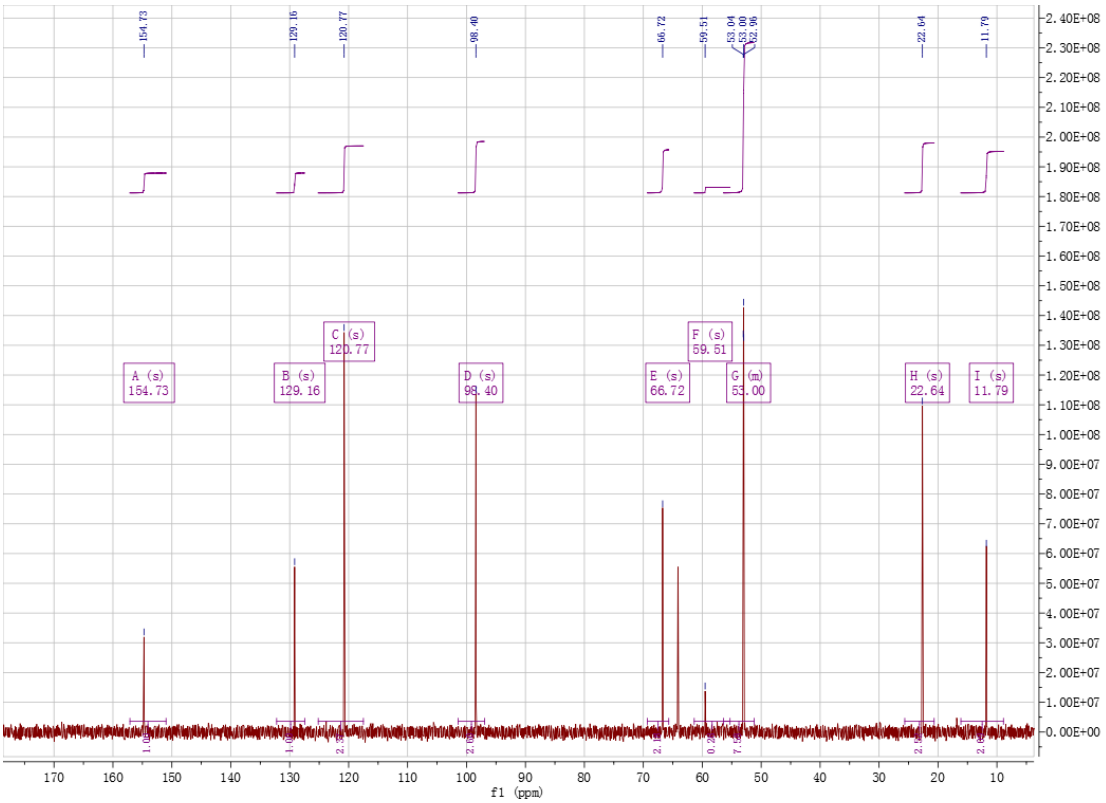
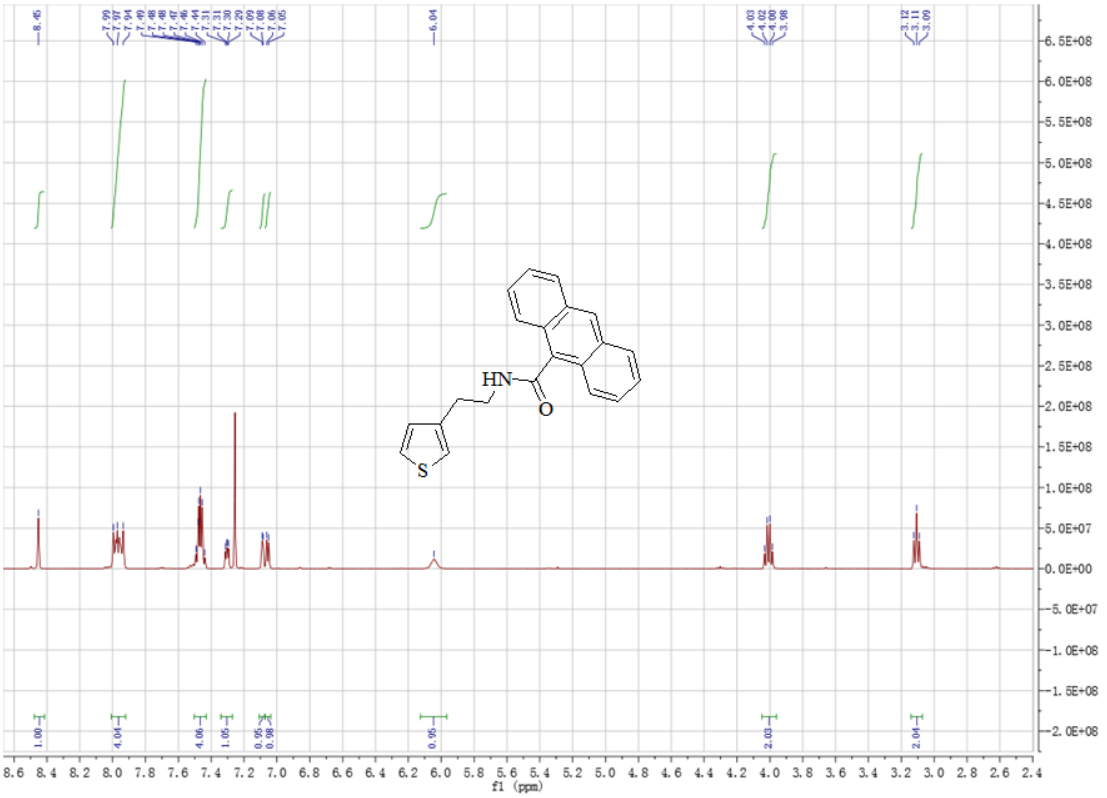
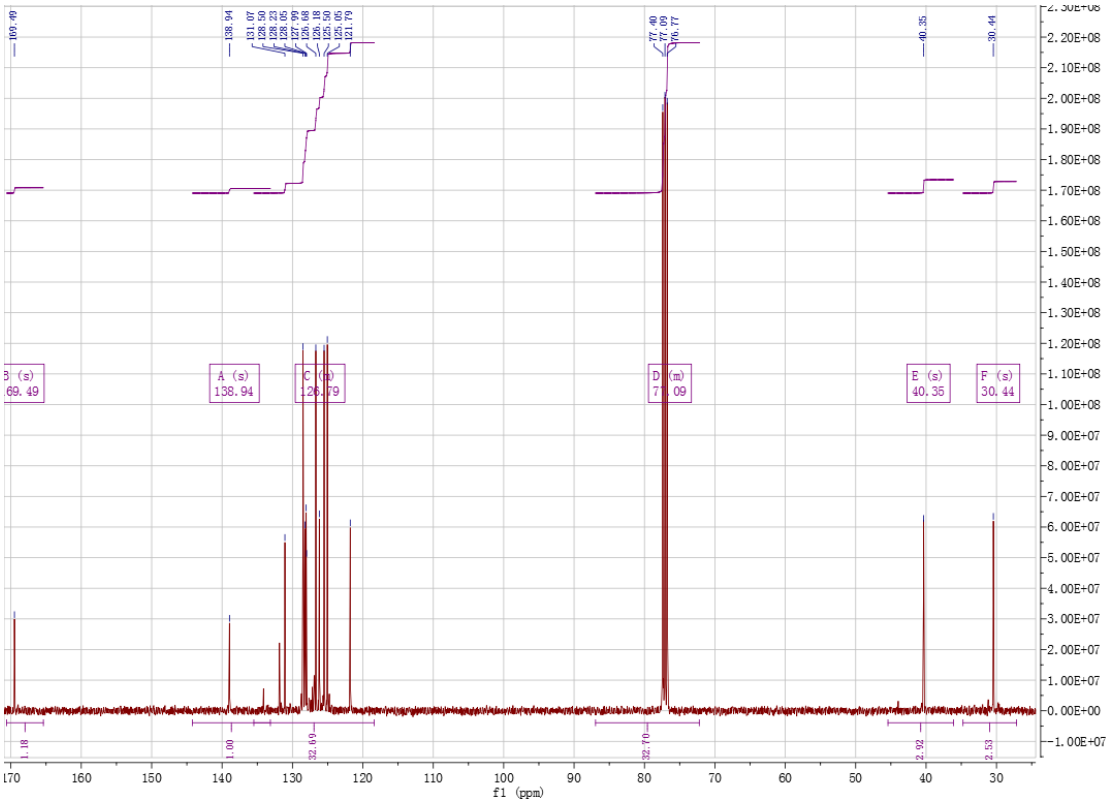
9.68
2.18
3.21
f1 (ppm)


154.73
129.16
120.77
98.40
66.72
59.51
53.04
53.00
52.96
22.64
11.79
A (s) 154.73
B (s) 129.16
C (s) 120.77
D (s) 98.40
E (s) 66.72
F (s) 59.51
G (m) 53.00
H (s) 22.64
I (s) 11.79
f1 (ppm)

Synthesis of 4-[9-Anthrylcarboxy(N-methylamino)]thiophene (4) [2]

9-Anthracenecarboxylic acid (3 g, 13.5 mmol) was heated in $SOCl_2$ solution (50 mL) to reflux for 5 h. The resulting solution was concentrated in *vacuo* to give a solid, which was subsequently dissolved in CH_2Cl_2 (120 mL) and added to a stirred solution of 2-(3-thienyl)ethanamine (552.0 mg, 2.9 mmol) in CH_2Cl_2 (20 mL) with 1.88 mL of triethylamine. After stirring at 35 °C for 2 days, the reaction mixture was poured into 1 N HCl (50 mL), washed with brine, dried over anhydrous $MgSO_4$, and then concentrated to give a brownish yellow solid, which was purified by column chromatography on silica gel by using the solvent system of n-hexane/ethyl acetate and recrystallized from the mixed solvent of n-hexane/dichloromethane. The compund **4** was obtained as orange rods (2.1 g, 49 %). ^{1}H NMR (400 MHz, $CDCl_3$) *δ:* 8.45 (s, 1H), 7.99 (m, 4 H), 7.49-7.43 (t, 4H), 7.31 (t, 1H), 7.09 (s,1H), 7.06 (d, 1H), 6.04 (s,1H), 4.03 (t, 2H), 3.12 (t, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ:* 169.49 , 138.94 , 131.85 , 131.07 , 128.50 , 128.35 – 127.93 (m), 126.68 , 126.18 , 125.50 , 125.05 , 121.79 , 40.35 , 30.44 . ESI MS: m/z $[M+H]^+$ = 331.03, calcd 331.10.





Preparation of copolymer CPT[1]

CPT were prepared according the procedure as following: 294 mg (1 mmol) of **3**, 49.7 mg (0.15 mmol) of **4** and 590 mg (3.64 mmol) of anhydrous $FeCl_3$ were mixed in 30 mL of dry $CHCl_3$, and stirred for 24 h at room temperature under N_2 atmosphere, then evaporated to dryness. The solid was washed by methanol, filtered. The crude product was washed by acetone/Soxhlet extraction for 24 h. The insoluble fraction of the polymer was dried and then dissolved in methanol by adding a few drops of hydrazine. The resulting polymer was precipitated by addition of a saturated solution of tetrabutylammonium chloride in acetone and washed again with acetone, and then dried under reduced pressure to give polymer **CPT** (331.6 mg, 53 %). GPC (H_2O, pullulan standard): M_w: 4.8 kDa, polydispersity index: 1.819. Anal. Calcd for **CPT** according to the feeding amounts of monomers of **3** and **4**: C 56.74%, H 7.59%, N 5.38%. Found: C 56.46%, H 7.89%, N 5.29%.

The integration of the characteristic peaks in the ^{1}H NMR spectra was used to calculate their chemical compositons.[3] The peaks at 3.21 and 4.09 ppm correspond to the methyl (N^+-C<u>H</u>$_3$) and methene (NH-C<u>H</u>$_2$) within the copolymer, respectively. The composition of the copolymer **CPT** was determined by the integration ratio of peak area of the proton signals from the methyl (N^+-C<u>H</u>$_3$) relative to that of the proton signals from the methene (NH-C<u>H</u>$_2$) in the copolymer. The calculated result showed the chemical composition of the copolymer is 10.0: 1.44, which is close to the original monomer compositions in the feed prior to polymerization.

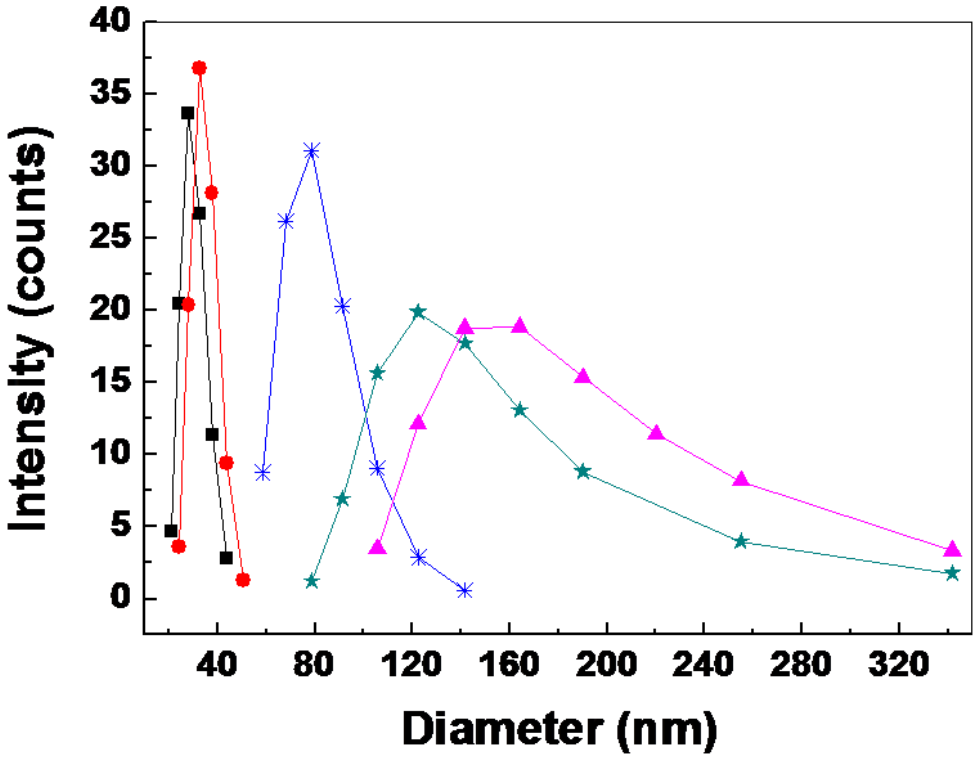


Fig. S1 Hydrodynamic diameter of **CPT** (100 μM) in Tris-HCl buffer solutions in the presence of ATP with various concentrations obtained from DLS. The concentrations of ATP from left to right are 0, 10, 30, 50, 90 μM, respectively. Three measurements were performed for each solution.

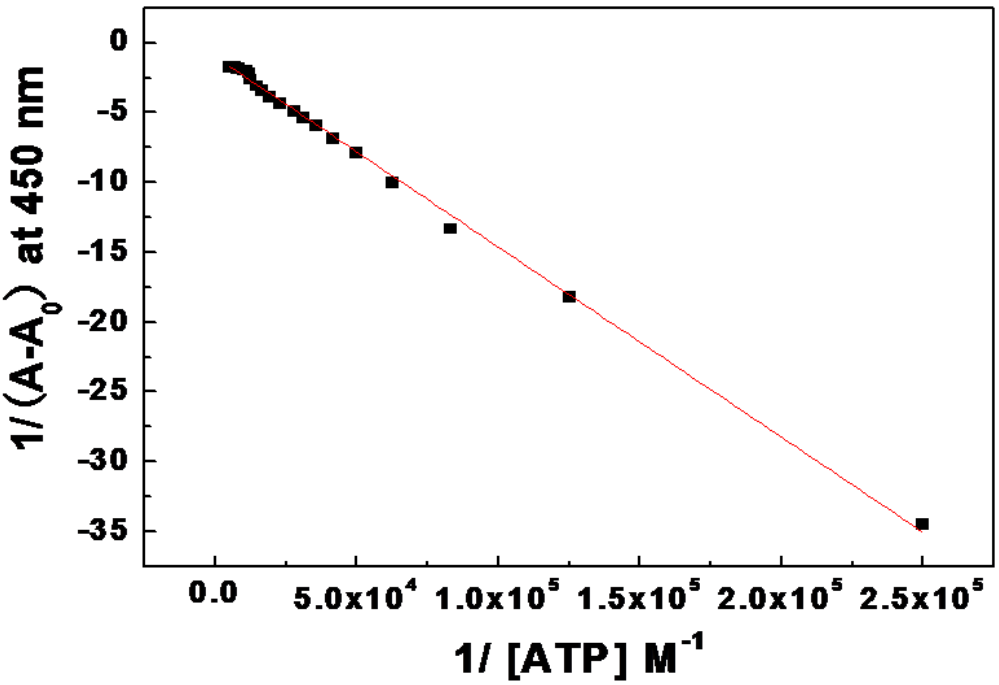


Fig. S2 Benesi-Hildebrand analysis.

The binding constant for **CPT/ATP** complexes was calculated according to the reported method. [4] The double reciprocal plot of $1/(A-A_0)$ versus 1/(ATP concentration) is linear with a correlation coefficient of 0.998 (Fig. S2), and the binding constant (K_{assoc}) can be estimated from the ratio of the intercept to the slope. A_0 is the initial absorbance of the free **CPT** at 450 nm and A is the recorded absorbance of complexes at different ATP concentrations. The overall binding constant for **CPT/ATP** complexes is estimated to be 7400 M^{-1}.

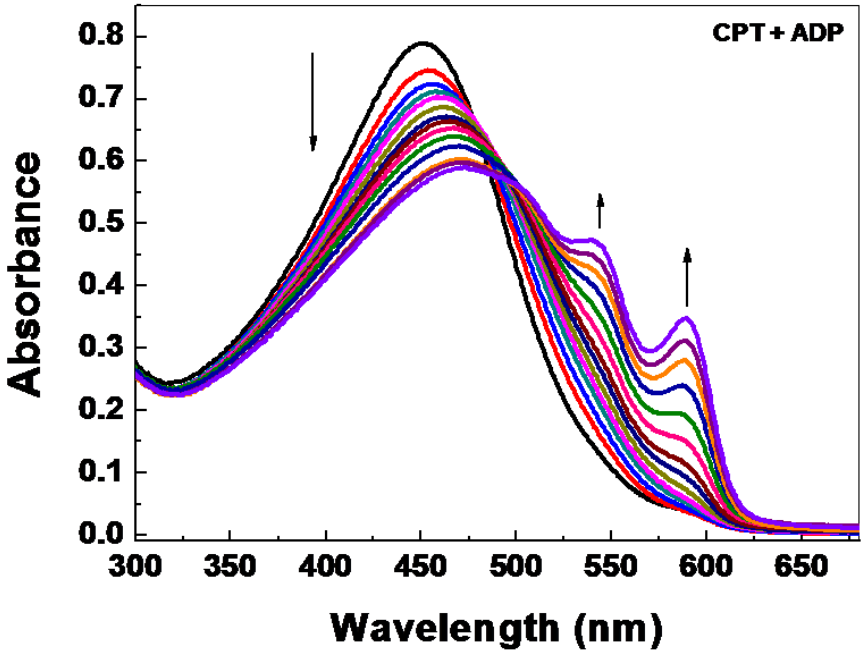


Fig. S3 UV-vis titration spectra of **CPT** (100 μM) in tris-HCl buffer solution with increasing amounts of ADP. [ADP] = 0, 8, 16, 24, 32, 44, 50, 60, 76, 96, 120, 152, 184, 200 μM from top to down.

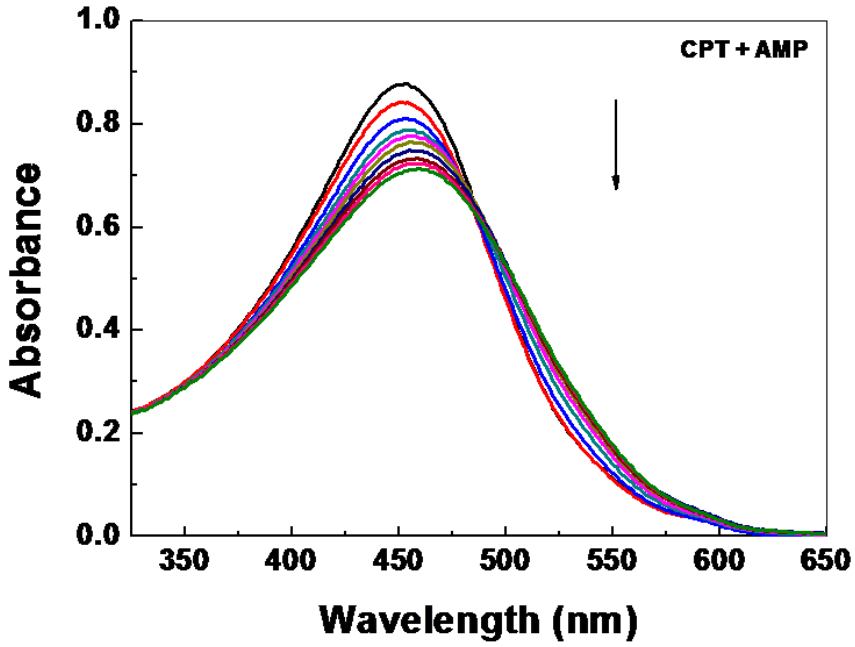


Fig. S4 UV-vis titration spectra of **CPT** (100 μM) in tris-HCl buffer solution with increasing amounts of ATP. [ATP] = 0, 4, 8, 15, 30, 40, 50, 60, 70, 80, 90 μM from top to down.

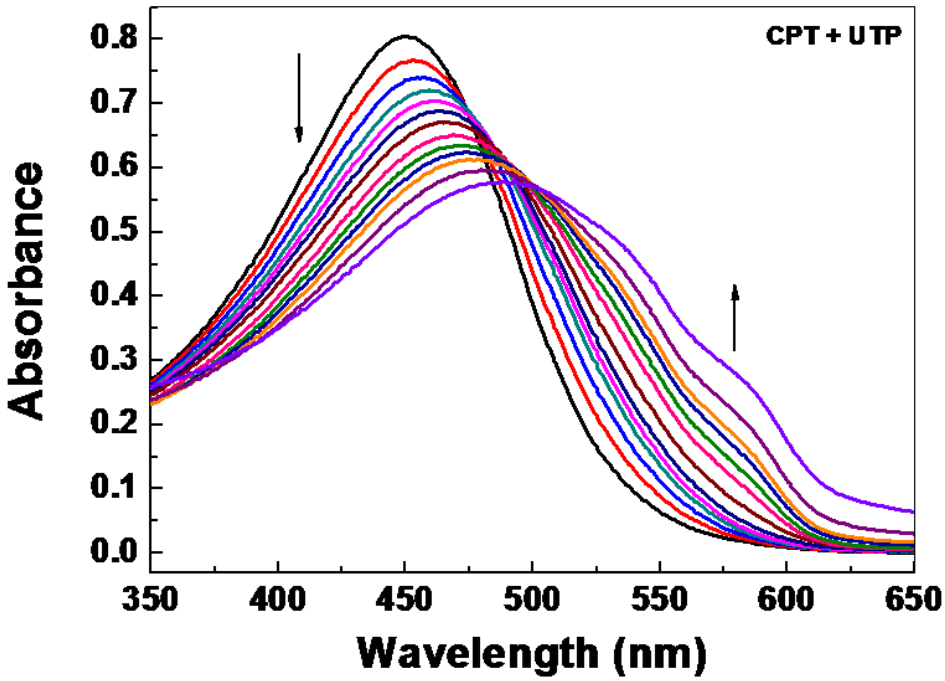


Fig. S5 UV-vis titration spectra of **CPT** (100 μM) in tris-HCl buffer solution with increasing amounts of UTP. [UTP] = 0, 4, 8, 12, 16, 20, 32, 40, 48, 60, 70, 90, 120 μM from top to down.

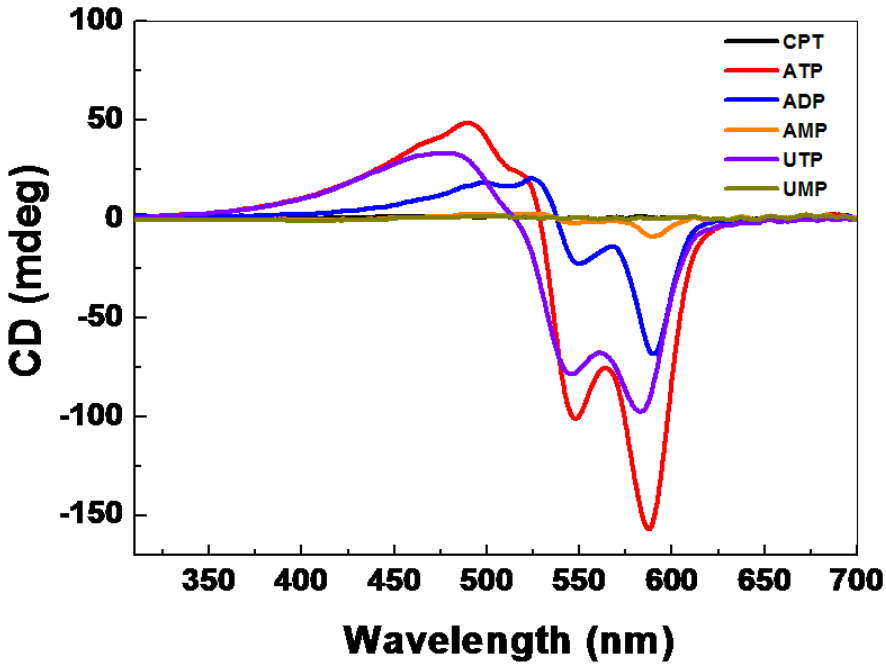


Fig. S6 CD spectra of **CPT** (100 μM) in water in the absence of anionic guests and in the presence of an equimolar amount of ATP, ADP, AMP, UTP and UMP.

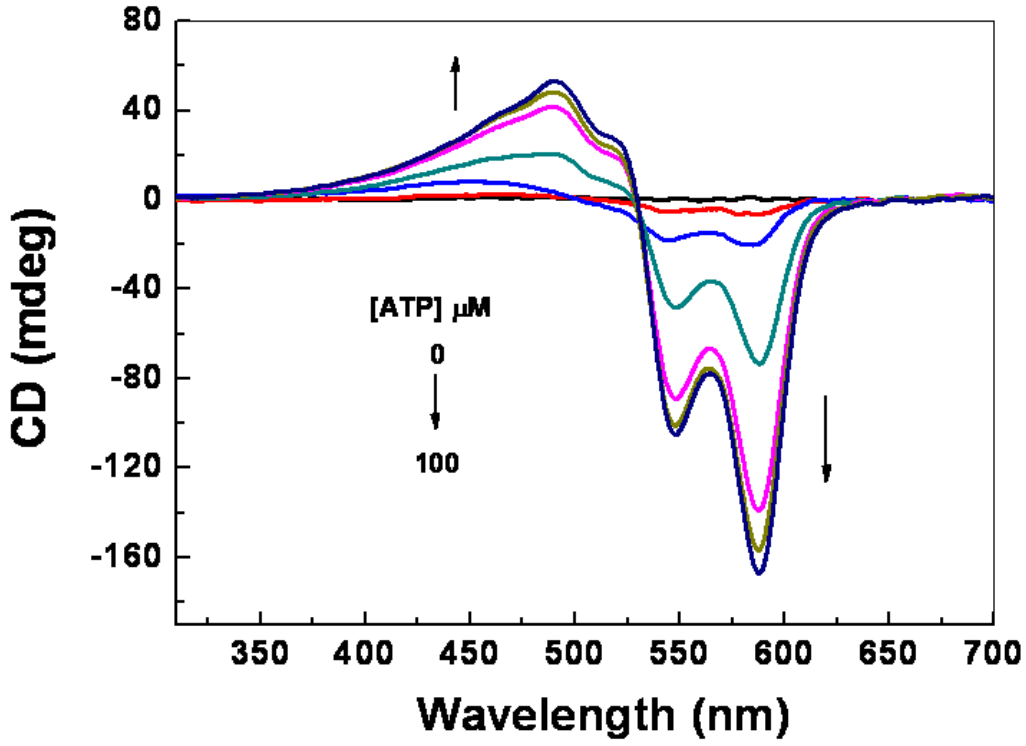


Fig. S7 CD spectra of **CPT** (100 μM) in water in the presence of various amounts of ATP. [ATP] = 0, 20, 30, 40, 50, 60, 80 μM.

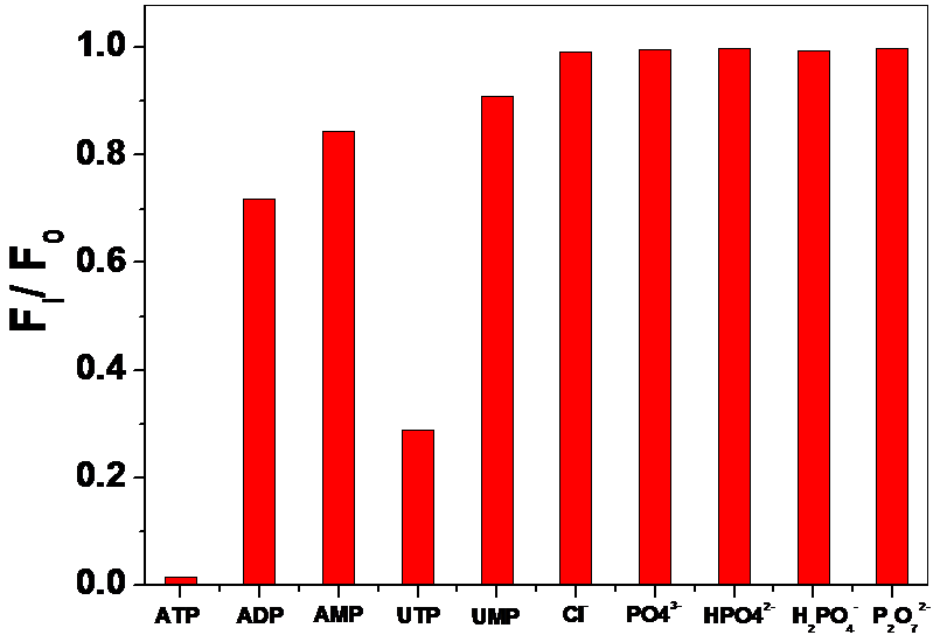


Fig. S8 Fluorescent spectra of **CPT** (100 μM) in water with an equimolar amounts of anion guests.

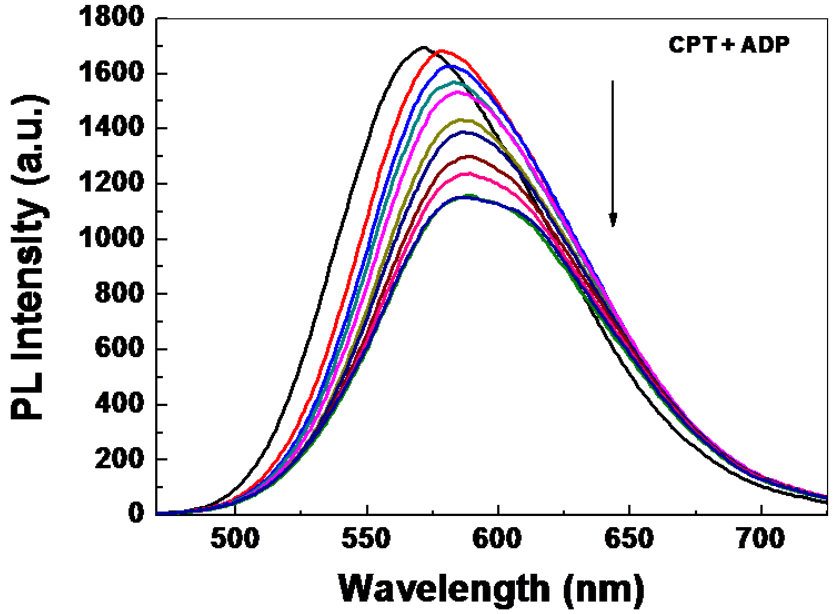


Fig. S9 Fluorescent titration spectra of **CPT** (50 μM) in tris-HCl buffer solution with different amounts of ADP. [ADP] = 0, 12, 20, 24, 38, 54, 70, 112, 128, 144, 164 μM.

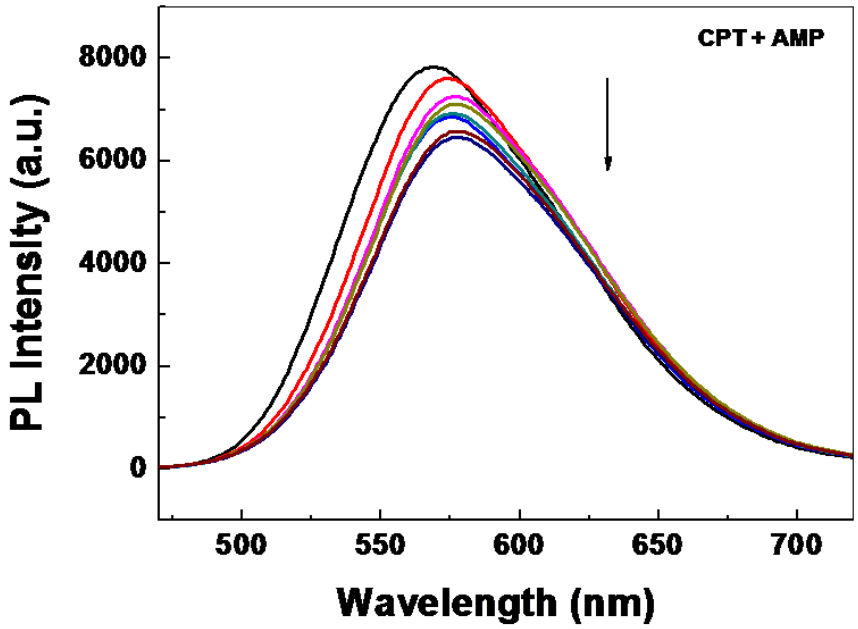


Fig. S10 Fluorescent titration spectra of **CPT** (100 μM) in tris-HCl buffer solution with different amounts of AMP. [AMP] = 0, 32, 42, 52, 62, 72, 82, 92 μM.

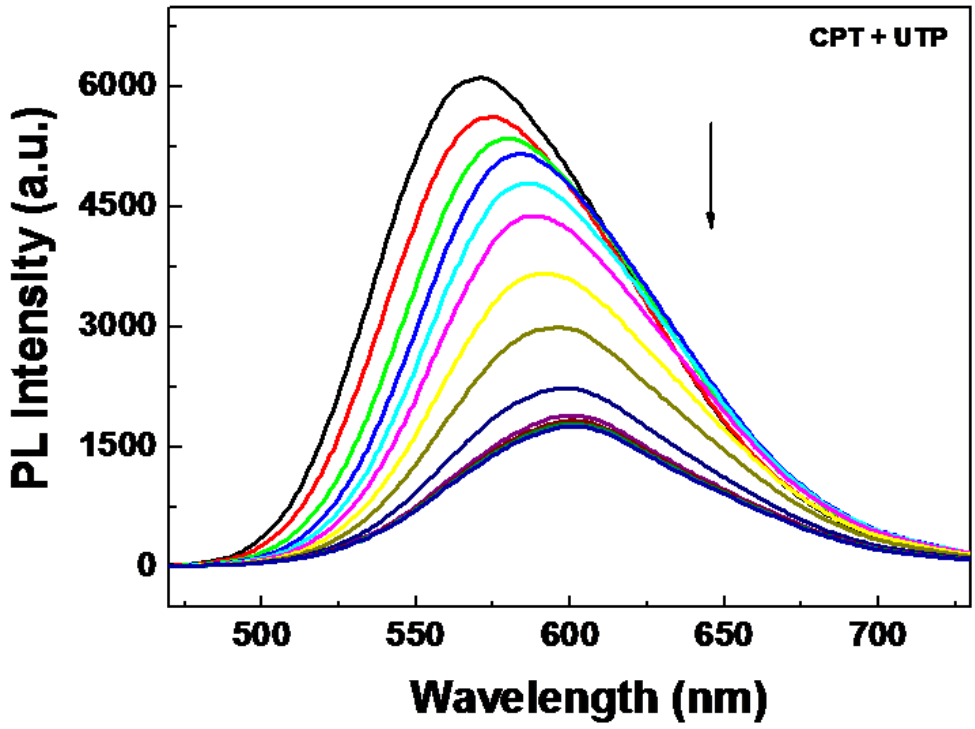


Fig. S11 Fluorescent titration spectra of **CPT** (100 μM) in tris-HCl buffer solution with different amounts of UTP. [UTP] = 0, 2, 6, 10, 14, 18, 22, 26, 30, 40, 50, 60, 80, 90 μM.

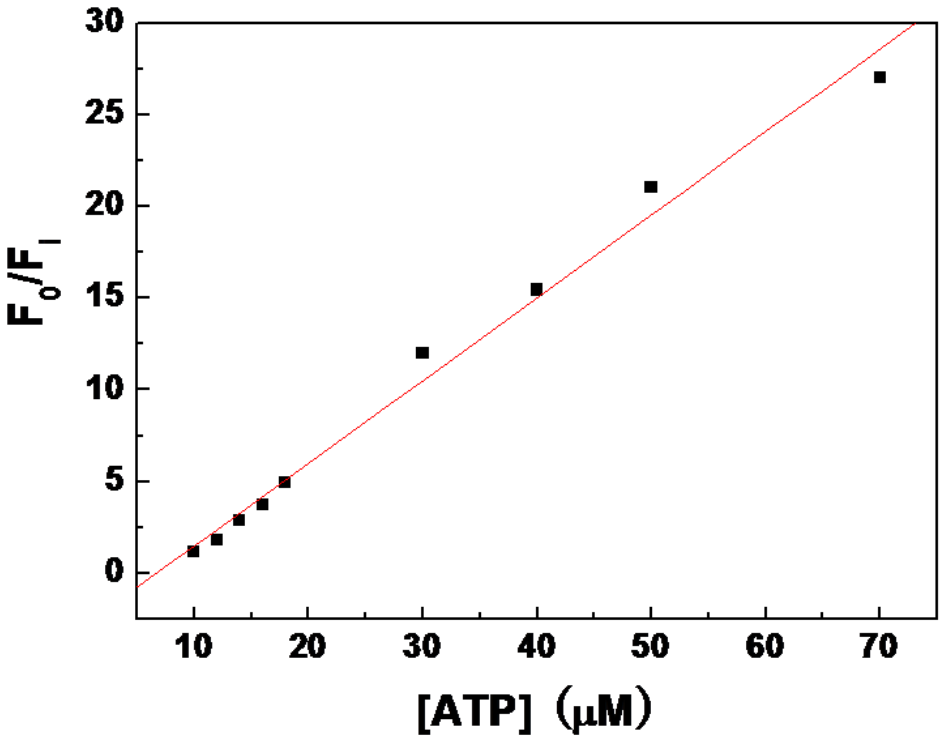


Fig. S12 F_0/F_i as a function of ATP concentrations. The data were extracted from the titration curves (Fig. 4). F_0 and F_i denote the emission intensity at 570 nm prior to and after addition of ATP, respectively.

Limit of detection

The detection limit was calculated based on the fluorescence titration according to the reported method. [5] In the absence of ATP, the fluorescence emission spectrum of probe CPT was measured by ten times and the standard deviation of blank measurement was achieved. To gain the slope, the ratio of the fluorescence intensity in the absence (F_0) and presence (F_i) of ATP at 570 nm was plotted as a concentration of ATP. So the detection limit was calculated with the following equation:

Detection limit = $3\sigma/k$

where σ is the standard deviation of blank measurement, k is the slope between the ratio of fluorescence intensity versus ATP concentration.

References:

[1] C. Li, M. Numata, A.-H. Bae, K. Sakurai and S. Shinkai, *J. Am. Chem. Soc.*, 2005, **127**, 4548.

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[3] H. Y. Liu, D. Avoce, Z. J. Song and X. X. Zhu, *Macromol. Rapid Commun.*, 2001, **22**, 675; Y. Lu, Y. Q. Han, J. H. Liang, H. X. Meng, F. L. Han, X D. Wang and C. X. Li, *Polym. Chem.*, 2011, **2**, 1866.
[4] H. Benesi and J. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703; K. T. Arun and D. Ramaiah, *J. Phys. Chem. A*, 2005, **109**, 5571.
[5] B. Zhu, F. Yuan, R. Li, Y. Li, Q. Wei, Z Ma, B. Du and X. Zhang, *Chem. Commun.*, 2011, **47**, 7098; Y. Che, X. M. Yang and L. Zhang, *Chem. Commun.*, 2008, 1413.