Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Cyclic-AMP-Dependent Protein Kinase (PKA) Activity Assay Based on the FRET between Cationic Conjugated Polymer and Chromophore-Labeled Peptide

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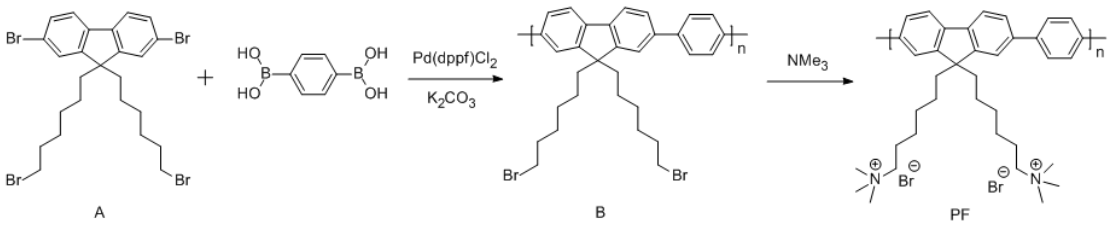
Supplementary Materials

Experimental

The water soluble CCP, poly (9, 9-bis (6′-*N*, *N*, *N*-trimethylammonium) hexyl) fluorenylene phenylene (PF) was synthesized according to the reported method.[1]



Scheme S1. Synthesis of PF.

Synthesis of compound B

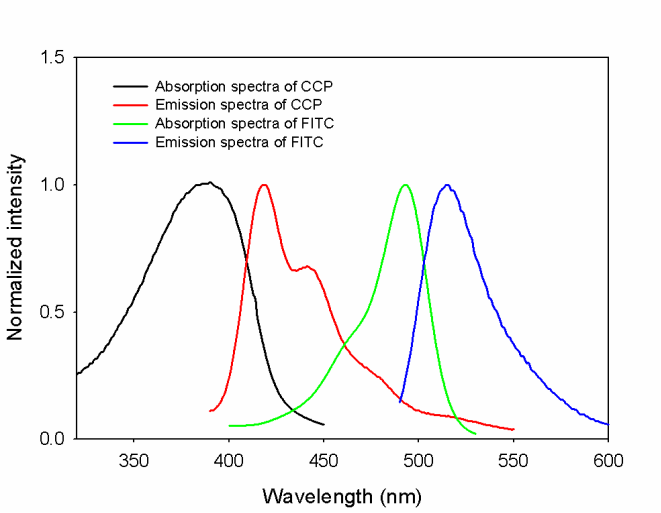
2, 7-Dibromo-9, 9-bis (6'-bromohexyl) fluorene (325 mg, 0.5 mmol), 1, 4-phenyldiboronic acid (82.9 mg, 0.5 mmol), potassium carbonate (830mg, 6 mmol) and Pd (dppf) Cl_2 (7 mg) were placed in a 50 mL round bottom flask. A mixture of THF (6 mL) and water (3 mL) was added to the flask, the reaction vessel was degassed and filled with argon. The mixture was stirred at 85 °C for 24 h. Cool down the reaction to room temperatureand, then precipitated into methanol. The polymer was filtered and washed with methanol and acetone, and then dried under vacuum for 24 h to afford compound B (197.5g, 70%), as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.84 (m, 5H), 7.70-7.60 (m, 4H), 7.5 (m, 1H), 3.31 (t, 4H), 2.11 (m, 4H), 1.70 (m, 4H), 1.26-1.15(m, 8H), 0.80 (m, 4H).

Synthesis of compound PF

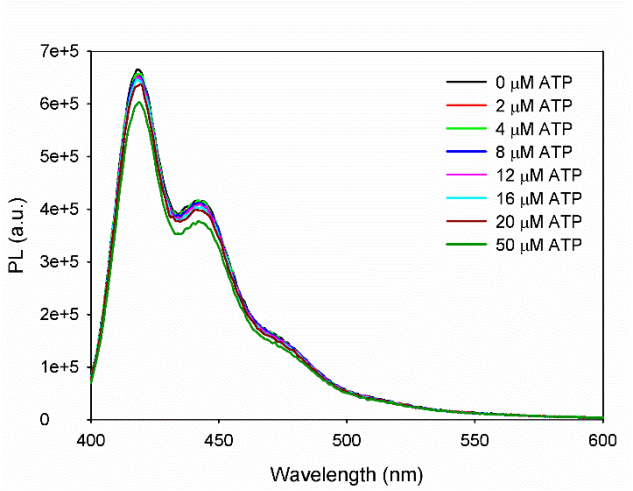
The neutral compound B (60 mg) and THF (10 mL) was placed in in a 25 ml round bottle flask, then condensed trimethylamine (2 mL) was added dropwise to the solution at -78 °C. Then the mixture was allowed to warm up to room temperature and react at

room temperature for 24 h. The precipitate was re-dissolved by the addition of water (10 mL). After the mixture was cooled down to -78 °C, extra trimethylamine (2 mL) was added and the mixture was stirred for 24 h at room temperature. After removing most of the solvent, acetone was added to precipitate compound PF (54 mg, 67%), as an off-white powder. ^{1}H NMR (400 MHz, CD_3OD): δ 8.07-7.51 (m, 10H), 3.31-3.22 (t, 4H), 3.04 (s, 18H), 2.25 (br, 4H), 1.57 (br, 4H), 1.18 (br, 8H), 0.77 (br, 4H).

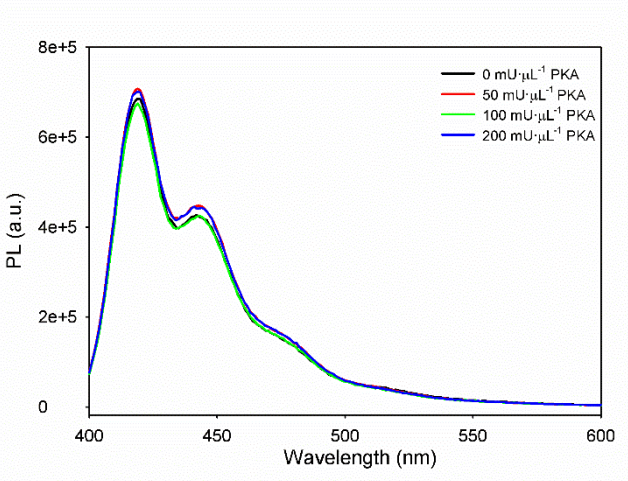
Results and discussion



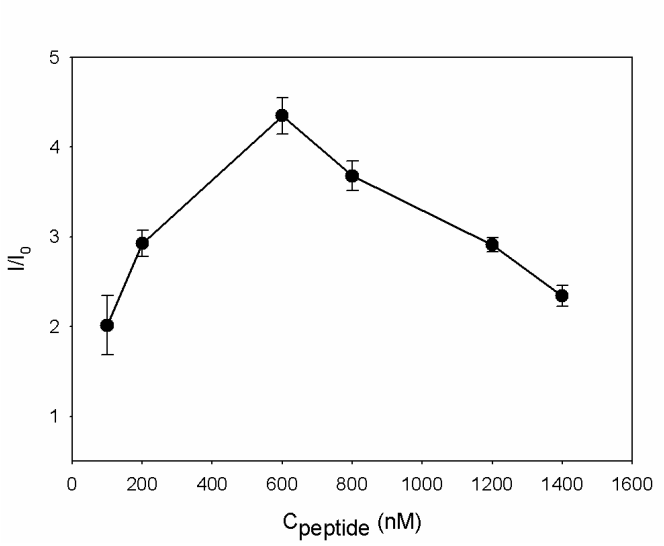
Supplementary Figure S1. The spectral properties of CCP and free FITC.



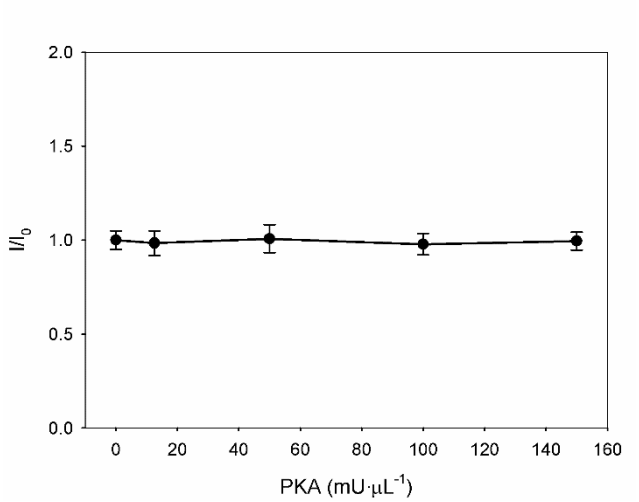
Supplementary Figure S2. Effects of concentration of ATP on the fluorescence intensity of CCP. Conditions: 2.95×10^{-6} M (in RUs) CCP.



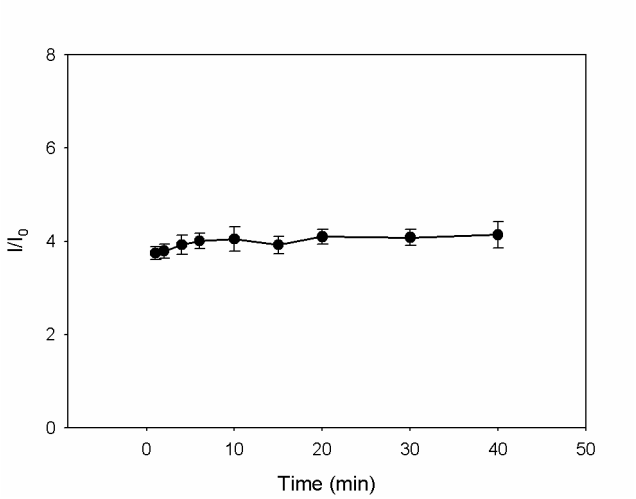
Supplementary Figure S3. Effects of concentration of PKA on the fluorescence intensity of CCP. Condition: 2.95×10^{-6} M (in RUs) CCP.



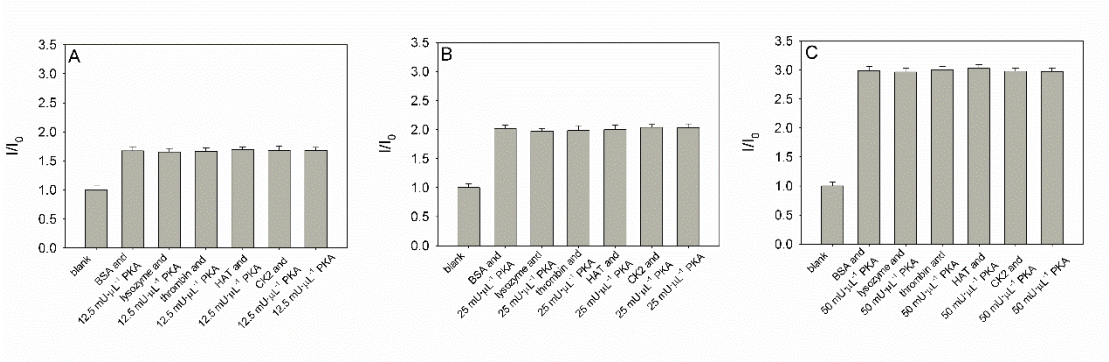
Supplementary Figure S4. Optimization of the F-S-peptide concentration. Conditions: 2.95×10^{-6} M (in RUs) CCP, 4 μM ATP, 50 mU·μL^{-1} PKA, 100, 200, 600, 800, 1200, 1400 nM F-S-peptide.



Supplementary Figure S5. Effect of concentration of PKA on the FRET between CCP and F-S-peptide. Conditions: 2.95×10^{-6} M (in RUs) CCP; 4 μM ATP; 0, 12.5, 50, 100, 150 mU·μL^{-1} PKA; 600 nM F-S-peptide.



Supplementary Figure S6. Optimization of the incubation time between F-P-peptide and CCP. Conditions: 2.95×10^{-6} M (in RUs) CCP, 4 μM ATP, 50 mU·μL^{-1} PKA, 600 nM F-S-peptide. Start timing after mix F-P-peptide and CCP.



Supplementary Figure S7. The effect of other proteins on PKA. Conditions: 2.95×10^{-6} M (in repeat units (RUs)) CCP, 4 μM ATP, the concentration of each kind of protein was 0.83 nM, 50 mU·μL^{-1} PKA is about 0.26 nM.

Reference

1. B. Liu, S. Wang, G. C. Bazan and A. Mikhailovsky, *J. Am. Chem. Soc.*, 2003, 125, 13306-13307.