Supplementary Information

A New Conjugated Polymers-Based Combination Probe for ATP Detection Using Multisite-Binding and FRET Strategy

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Experimental section

Materials and instruments. All chemicals were purchased from J&K Chemical Ltd. and Aladdin Industrial Corporation and used without further purification. 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), nucleoside triphosphate (NPPs), nucleoside bases, GSH, D-ribose and PPi were purchased from Sangon Biotech（Shanghai）Co., Ltd. Ultrapure Millipore water (18.6 MΩ) was used throughout the experiments. UV-vis absorption spectra, fluorescence spectra and fluorescence intensity ratios were recorded on a Molecular Devices SpectraMax M5. Dynamic light scattering (DLS) measurements were performed using a Brookhaven BI-90 Plus. Polymer molecular weights were measured by GPC-MALLS.

Synthesis of 4-(2,5-diiodo-4-methoxyphenoxy) butanoic acid (8). Iodine (427 mg, 1.7 mmol), potassium iodate (107 mg, 0.5 mmol) and compound 7 (300 mg, 1.3 mmol) were mixed in glacial acetic acid (5 mL). The mixed solution was heated at 120 ^{o}C for 3 h. The cooled mixture was poured into 10% aqueous $NaHSO_3$. The mixture was extracted with DCM, washed with distilled water, dried over Na_2SO_4 and finally got compound 8 (544 mg, 93%) by concentrated under vacuum. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (s, 2H), 4.01 (t, J = 6.0 Hz, 2H), 3.83 (s, 3H), 2.68 (t, J = 7.2 Hz, 2H), 2.14 (t, J = 6.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.94, 153.48, 152.45, 122.95, 121.44, 86.25, 85.39, 68.79, 57.13, 30.45, 24.25. HRMS (ESI): m/z: 460.8731 ($[M-H]^-$).

Synthesis of PPE-COOH. Compound 6 (90 mg, 0.2 mmol), Compound 8 (72 mg, 0.2mmol) and morpholine (5 mL) were mixed and degassed by purging with nitrogen gas for 15 min. Then $Pd(PPh_3)_4$ (12 mg, 0.01 mmol) and CuI (2 mg, 0.01 mmol) were added to the solution at room temperature under nitrogen gas. The mixed solution was heated at 60 ^oC for 48 h. The cooled mixture was dialyzed against 1 L deionized water for 2 days and lyophilized to afford an orange compound (101 mg, 62%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.09-6.86 (m, 4H), 4.21-4.24 (br, 4H), 4.12 (br, 2H), 3.87-3.91 (br, 7H), 3.80 (br, 4H), 3.63 (br, 8H), 3.53 (br, 4H), 3.36 (s, 6H), 2.67 (br, 2H), 2.17 (br, 2H). GPC: M_n= 16 500, M_w = 21 800, PDI = 1.32.

Synthesis of PPE-PBA. Under nitrogen, EDC (11 mg, 0.06 mmol), HOBt (8 mg, 0.06 mmol), 3-aminophenylboronic acid (7 mg, 0.05 mmol) and triethylamine (17 mg, 0.17 mmol) were added to the anhydrous DMF (6 mL) solution containing PPE-COOH (31 mg, 0.05 mmol). The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The final mixed solution was dialyzed against distilled water for 3 days to give PPE-PBA as an orange solid (21 mg, 56%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.08-6.86 (m, 5H), 4.23 (br, 4H), 4.13 (br, 2H), 3.92 (br, 7H), 3.80 (br, 4H), 3.63 (br, 8H), 3.52 (br, 4H), 3.35 (s, 6H), 2.59-2.64 (br, 2H), 2.18 (br, 2H). GPC: M_n= 21 400, M_w= 30 100, PDI = 1.41.

General Procedure for ATP Detection. In a 2 mL HEPES buffer (10 mM, pH 7.8), 2 μM PFP-NMe_3^+ (final concentration) and 1 μM PPE-PBA (final concentration) were added and incubated at room temperature for 10 min. Aliquot ATP solutions were

continuously added to the probe solution. After incubating, the fluorescence spectra were measured on a Molecular Devices SpectraMax M5 with an excitation of 380 nm.

Selectivity Assay. In a 2 mL HEPES buffer (10 mM, pH 7.8), 2 μM PFP-NMe_3^+ (final concentration) and 1 μM PPE-PBA (final concentration) were added and incubated at room temperature for 10 min. Then various analytes were prepared in water and added to the probe solution. The final concentrations of analytes were fixed at 180 μM. After incubating, the fluorescence intensities at 496 nm and 420 nm were measured on a Molecular Devices SpectraMax M5 with an excitation of 380 nm.

Experimental conditions optimization:

CPs molar ratio optimization. Considering that the CPs molar ratio is crucial for the fluorescence intensity ratio as well as detection sensitivity, we investigated the fluorescence ratio of PPE-PBA to PFP-NMe_3^+ (R ($I_{496\ nm}/I_{420\ nm}$)) under different CPs molar ratios (PFP-NMe_3^+/ PPE-PBA). Herein, the CPs molar ratio of 2:1, 3:1, 1:1 and 1:2 are studied. As shown in **Fig. S2a**, when the CPs ratio is 2:1, the best fluorescence enhancement is obtained, which exhibits lower background signal and most sensitivity compared with others. Therefore, 2:1 as the optimized CPs molar ratio is used for the following studies.

pH optimization: It is noted that the pH value of the buffer strongly influences the formation of covalent bonding between PBA and 1,2 - diol.[1] Thus, some pH values from 6.0 to 8.5 were tested. As shown in **Fig. S2b**, when pH of the buffer is 7.8, the fluorescence intensity ratio reaches to the maximun after the formation of PFP-NMe_3^+/ATP/PPE-PBA complex, which indicates that the weak basic environment is favorable for the formation of the complex. Thus, pH 7.8 is chosen for our detection system.

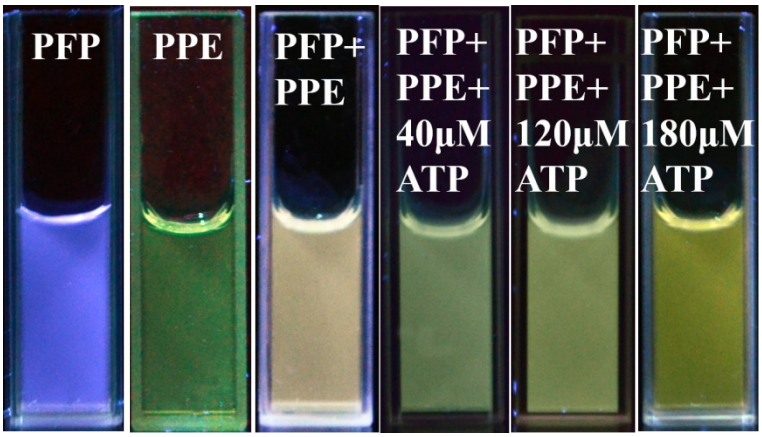

Fig. S1 Fluorescent photographs of polymers in the absence and presence of ATP under a hand-held UV lamp illumination (365 nm). [PFP-NMe_3^+] = 2 μM, [PPE-PBA] = 1 μM.

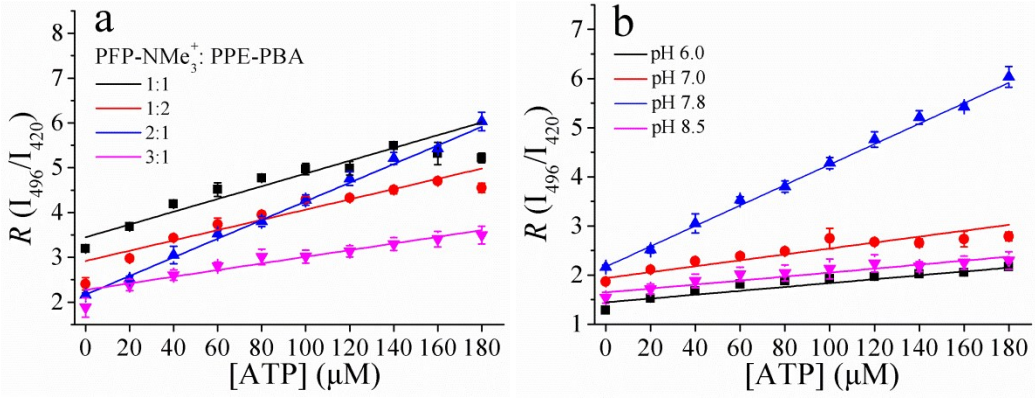

Fig. S2 (a) Fluorescence intensity ratios (I_{496}/I_{420}) of combination probe as a function of molar ratios at different concentrations of ATP. (b) Fluorescence intensity ratios (I_{496}/I_{420}) of combination probe as a function of pH at different concentrations of ATP. [PFP-NMe_3^+] = 2 μM, [PPE-PBA] = 1 μM. The excitation wavelength is 380 nm. The error bars represent the standard deviations based on three independent measurements.

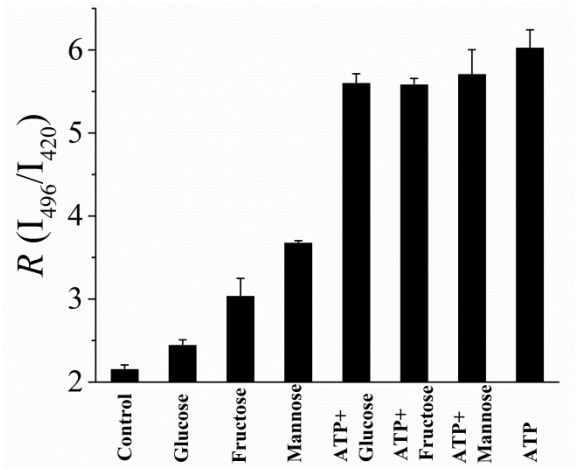

Fig. S3 The selectivity of combination probe toward ATP and saccharide in 10 mM HEPES buffer. [PFP-NMe_3^+] = 2 μM, [PPE-PBA] = 1 μM, [analytes] = 180 mM. λ_{max} = 380 nm.

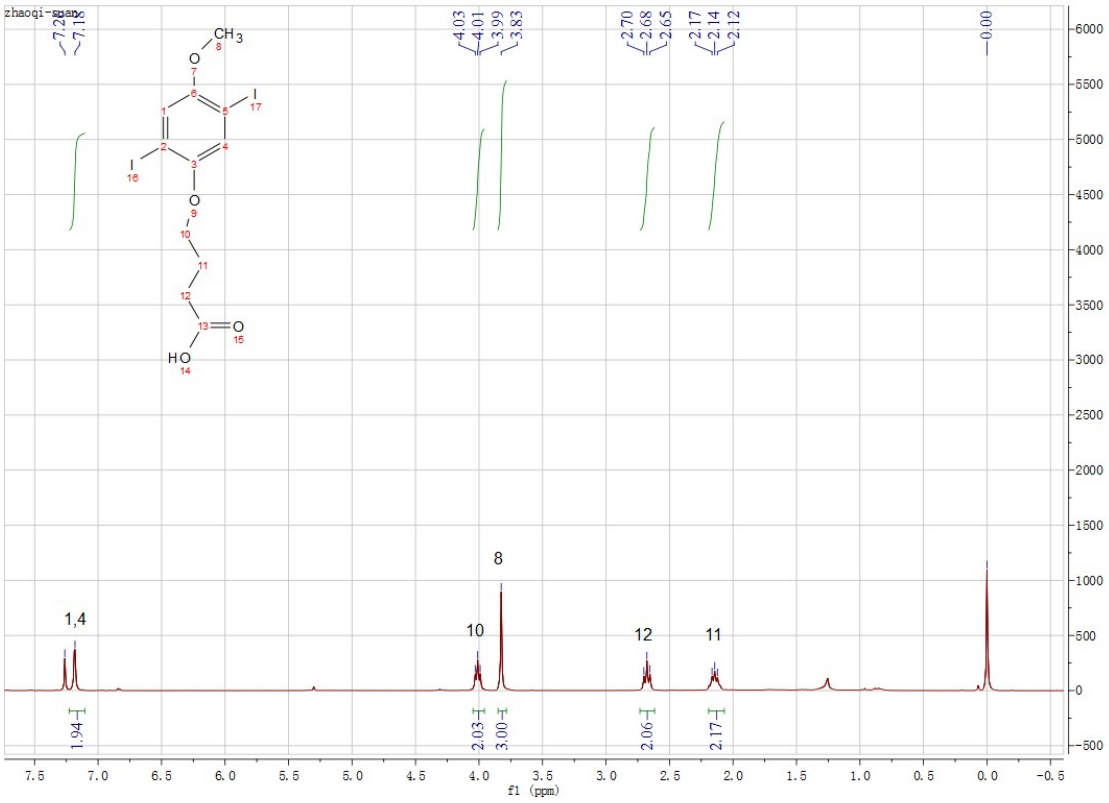

Fig. S4 ^{1}H NMR spectrum of compound 8 in $CDCl_3$.

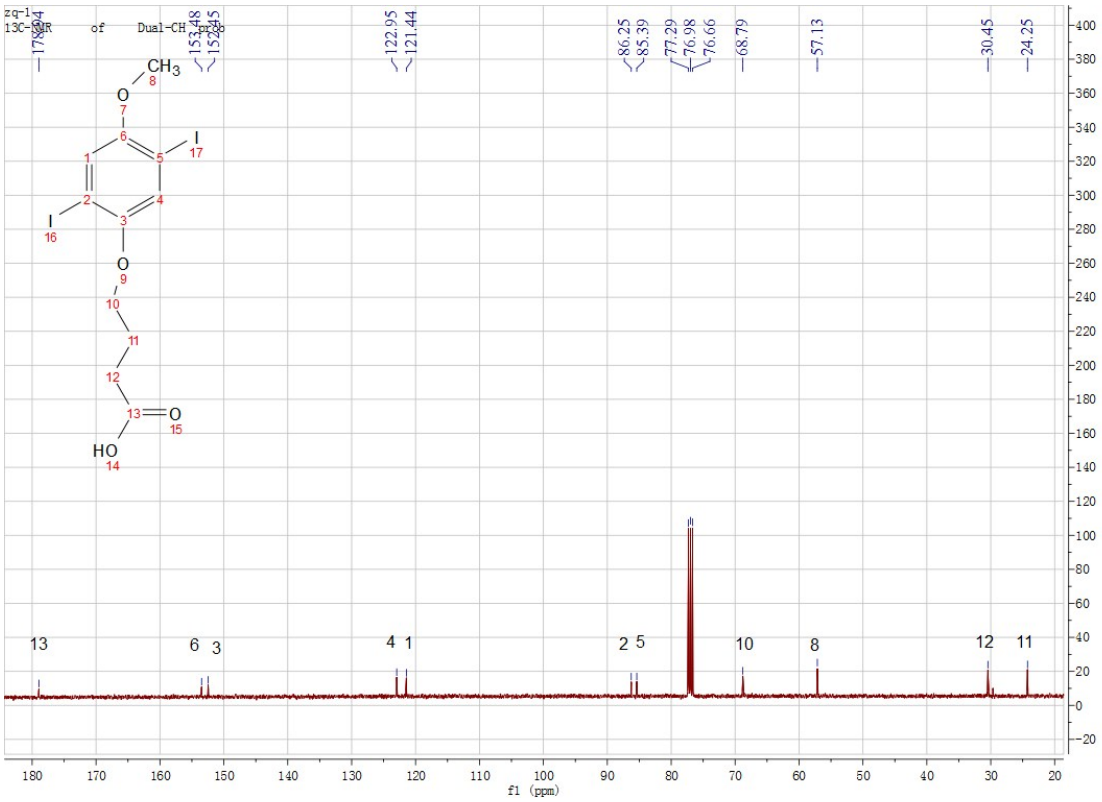

Fig. S5 ^{13}C NMR spectrum of compound 8 in $CDCl_3$.

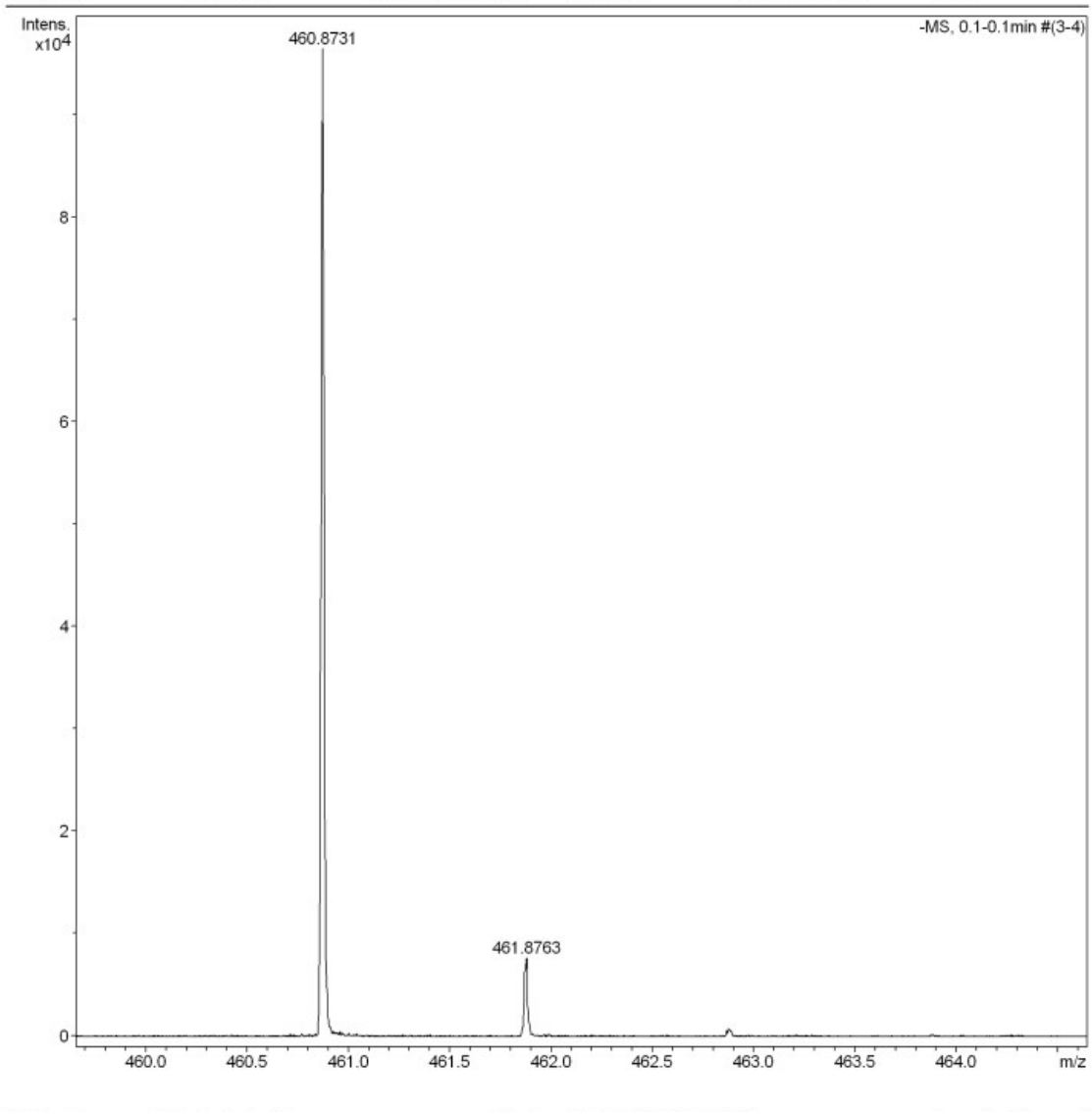

Fig. S6 HRMS spectrum of compound 8 in methanol.

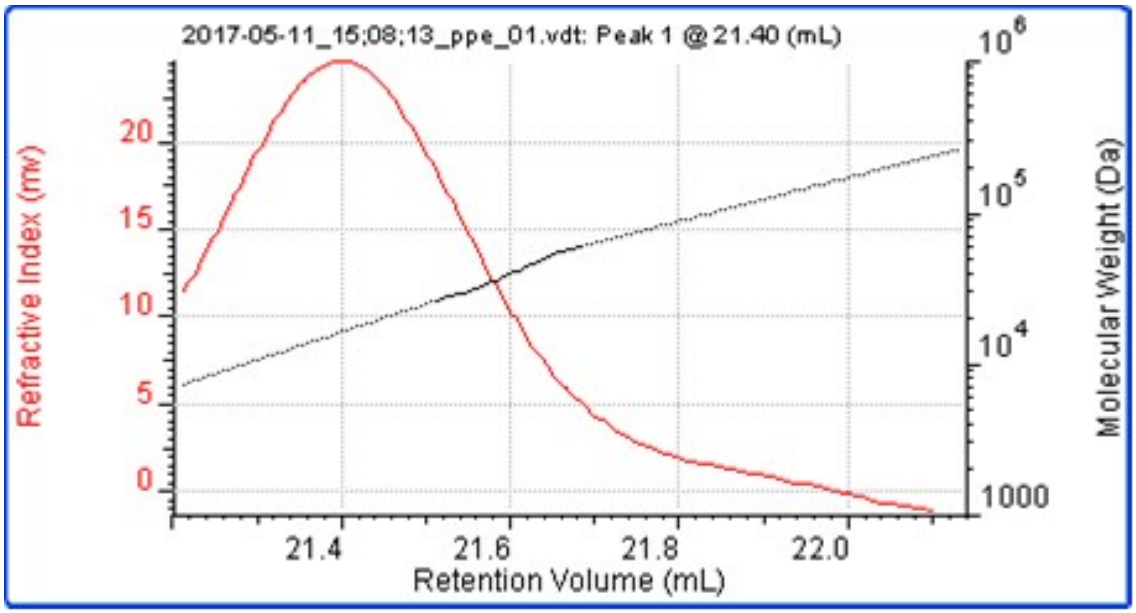

Fig. S7 GPC curve of PPE-COOH.

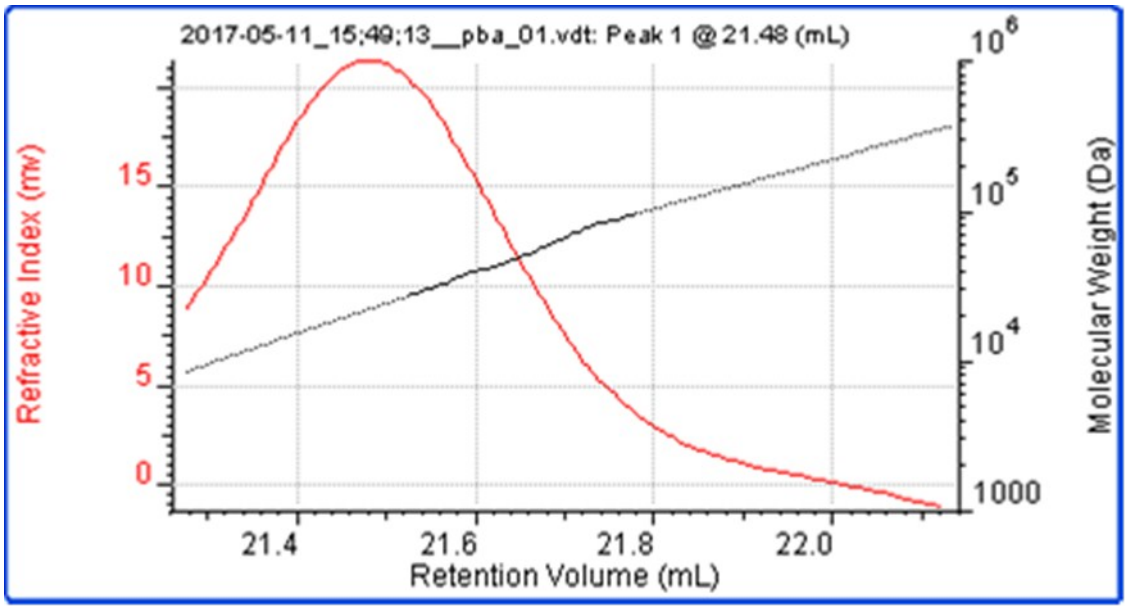

Fig. S8 GPC curve of PPE-PBA.

Table S1 Absolute quantum yield (Φ_F) and average lifetime (τ) of PFP-NMe_3^+ and PPE-PBA in the absence and presence of ATP.

Samples	Fluorescence Lifetime (ns) [a]	Fluorescence Quantum Yield (%) [b]
PFP-NMe_3^+	1.38 (λ_{ex} = 405 nm)	6.13 (λ_{ex} = 380 nm)
PPE-PBA	1.79 (λ_{ex} = 405 nm)	2.26 (λ_{ex} = 380 nm)
PPE-PBA	0.70 (λ_{ex} = 435 nm)	3.42 (λ_{ex} = 440 nm)
PFP-NMe_3^+ + PPE-PBA + ATP	0.61 (λ_{ex} = 405 nm)	- [c]
PFP-NMe_3^+ + PPE-PBA + ATP	1.56 (λ_{ex} = 405 nm)	5.40 (λ_{ex} = 380 nm)

[a] The average lifetimes were calculated from the decay curves of PFP-NMe_3^+ and PPE-PBA at 420 nm and 496 nm, respectively.

[b] The absolute quantum yields Φ_F were determined using a spectrofluorometer equipped with an integrating sphere. The emission spectral range for PFP-NMe_3^+ and PPE-PBA were calculated from 400 nm - 450 nm and 450 nm - 650 nm, respectively.

[c] The quantum yield can not be detectable.

Table S2. Comparison of This Work with Other Methods for Detecting ATP

Method	System	Linear range	Detection limit	Distinguish from	Ref.
FRET and colorimetric	Coumarin modified cationic polythiophene	0-6 μM	29 nM	ADP, AMP	2
aggregation-enhanced intra- and intermolecular FRET	1,4-dithienylbenzothiadiazole doped cationic polyfluorene	30-70 μM	0.1 nM	ADP, AMP	3
fluorescence turn-off and colorimetric	anthracene modified cationic polythiophene	10-70 μM	2.3 nM	ADP, AMP and UTP	4
fluorescence turn-off	cationic polythiophene	0-750 nM	36 pM	ADP, AMP	5
fluorescence turn-off and colorimetric	cationic poly (p-phenylene ethynylene terthiophene)	-	-	ADP, AMP	6
ratiometric surface-enhanced Raman scattering	Rox-labeled complementary DNA modified AuNP and Cy5-labeled ATP-binding aptamer	0.1-100 nM	20 pM	NPPs	7
fluorescence turn-off	aptamer DNA-templated silver nanoclusters	0-4 mM	0.44/0.65 mM	NPPs	8
fluorescence turn-off	diethylenetriamine modified Rhodamine	0.1-10 mM	0.033 mM	ADP, AMP and NPPs	9
fluorescence turn-on	phenylboronic acid modified Rhodamine B	0.5-10 mM	-	ADP, AMP and NPPs	10
FRET	PFP-NMe_3^+ and phenylboronic acid modified PPE-PBA as combination probe	0-180 μM	2.5 μM	ADP, AMP, NPPs and A, C, G, U, saccharides	this study

Reference:

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