Supplementary Information for

Discrimination of adenine nucleotides and pyrophosphate in water by a zinc complex of an anthracene-based cyclophane

*Ping Hu, Shengjun Yang and Guoqiang Feng**

Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, People's Republic of China.
E-mail: gf256@mail.ccnu.edu.cn

Table of Contents

1. Fluorescence changes of cyclophane 1 toward addition of various metal ions.

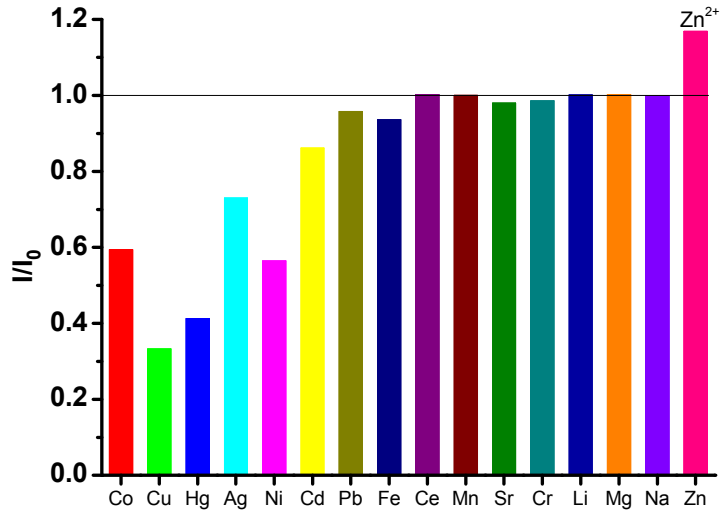


Fig. S1. The relative fluorescent intensity (I/I_0) of macrocyclic ligand **1** (10 μM) at λ_{em} = 420 nm upon the addition of two equiv. of metal anions. I_0 and I are the fluorescent intensity of **1** at λ_{em} = 420 nm before and after addition of metal anions (20 μM). Metal anions are Co^{2+}, Cu^{2+}, Hg^{2+}, Ag^{+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Fe^{2+}, Ce^{2+}, Mn^{2+}, Sr^{2+}, Cr^{2+}, Li^{+}, Mg^{2+}, Na^{+}, Zn^{2+}, and only elemental symbol showed in the figure. All the spectra were measured in pure aqueous solution of 10 mM HEPES buffer (pH 7.2) at 25 °C with λ_{ex} = 380 nm.

2. Facilitation of Zn^{2+} coordination to 1 by ATP, ADP and PPi.

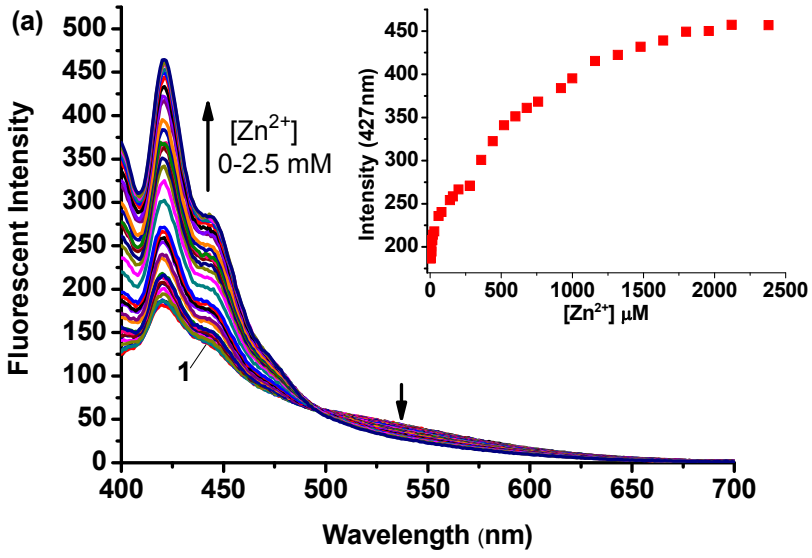


Fig. S2a Fluorescence spectra changes of cyclophane **1** (10 μM) upon titration of Zn^{2+} (0-2.5 mM) in pure aqueous solution of 10 mM HEPES buffer (pH 7.2) at 25°C with λ_{ex} = 380 nm.

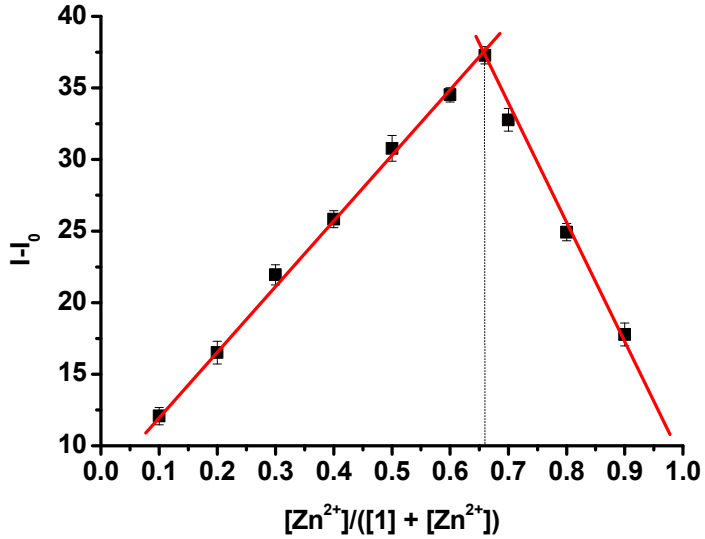


Fig. S2b Job's plot examined between ligand **1** and Zn^{2+} ([**1**] + [Zn^{2+}] = 10 μM) in 10 mM HEPES buffer (pH 7.2) at 25 ºC, λ_{ex} = 380 nm, λ_{em} = 427 nm. This experiment indicates a 1:2 binding mode between ligand **1** and Zn^{2+}; in other words, they formed a dinuclear zinc complex.

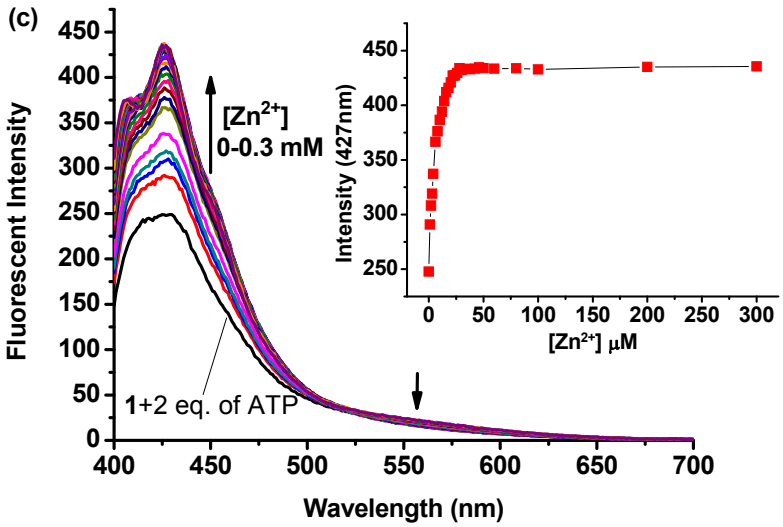


Fig. S2c Fluorescence changes of cyclophane **1** (10 μM) upon titration of Zn^{2+} in the presence of 2 equiv. of ATP. All experiments were measured in pure aqueous solution of 10 mM HEPES buffer (pH 7.2) at 25°C with λ_{ex} = 380 nm.

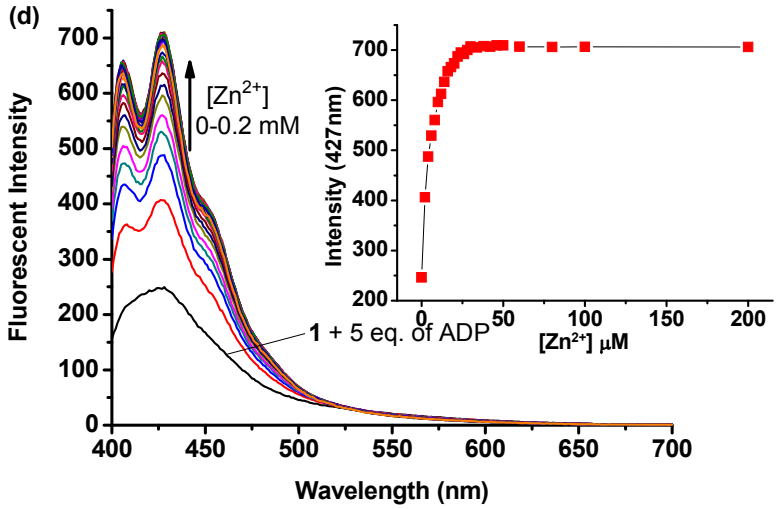


Fig. S2d Fluorescence changes of cyclophane **1** (10 μM) upon titration of Zn^{2+} in the presence of 5 equiv. of ADP. All experiments were measured in pure aqueous solution of 10 mM HEPES buffer (pH 7.2) at 25°C with λ_{ex} = 380 nm.

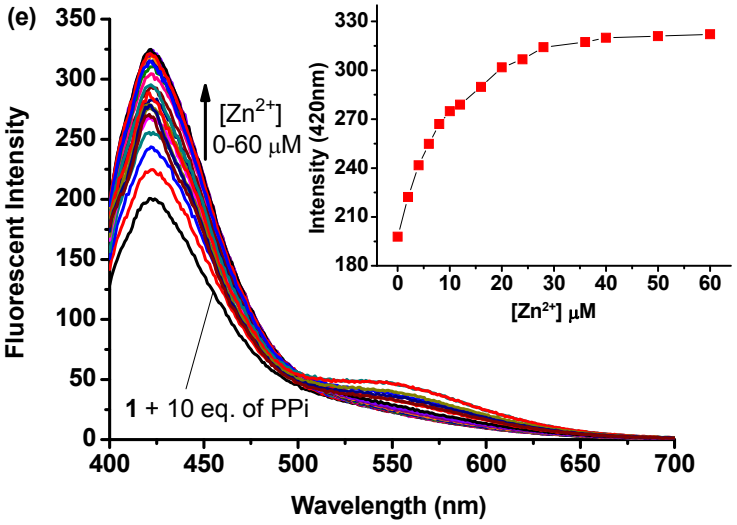
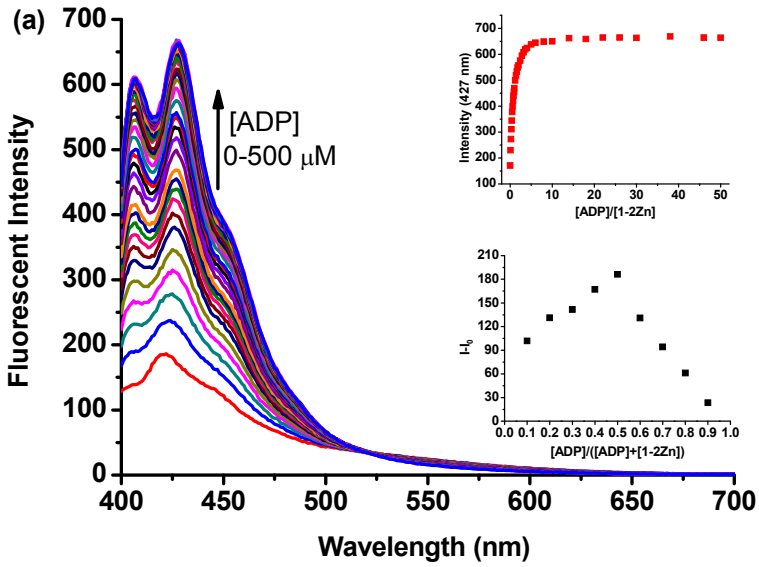
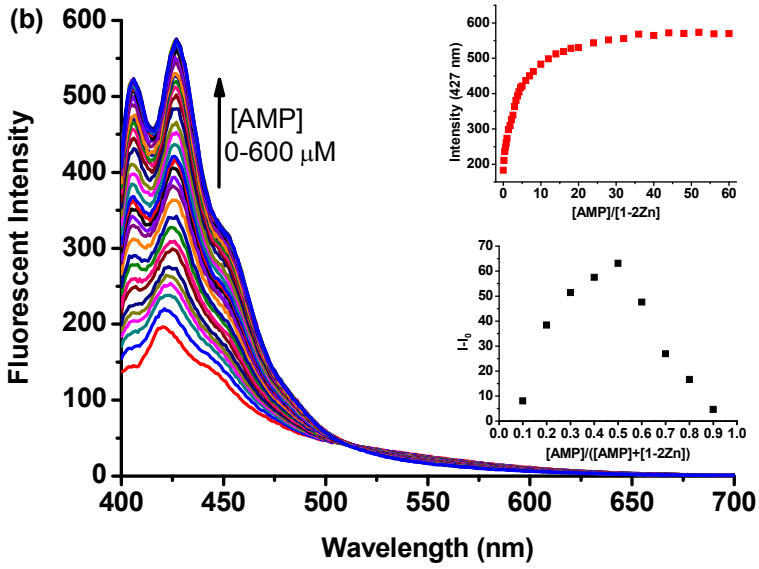
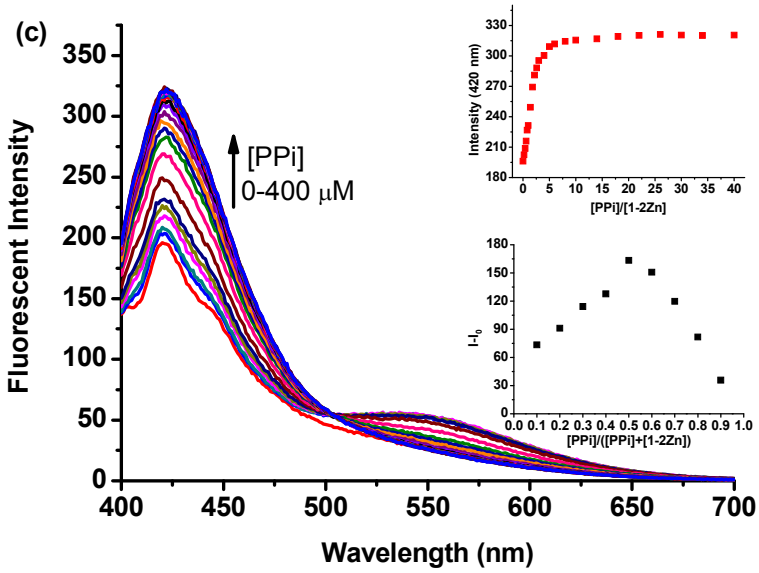


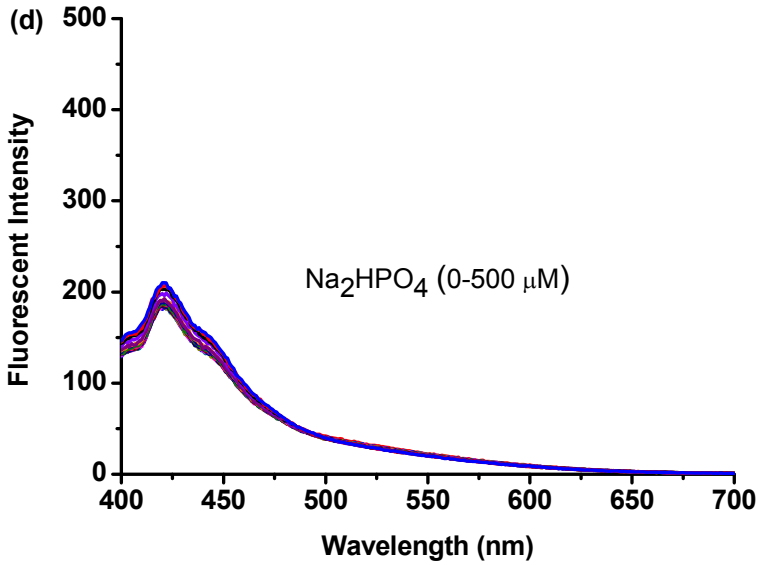
Fig. S2e Fluorescence changes of cyclophane **1** (10 μM) upon titration of Zn^{2+} in the presence of 10 equiv. of PPi. All experiments were measured in pure aqueous solution of 10 mM HEPES buffer (pH 7.2) at 25°C with λ_{ex} = 380 nm.

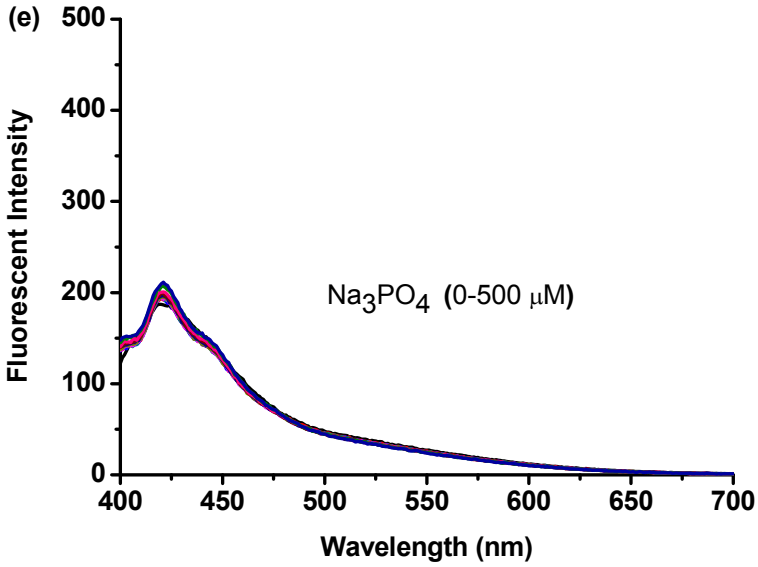
3. Fluorescence titration of 1-2Zn with ADP, AMP and other anions

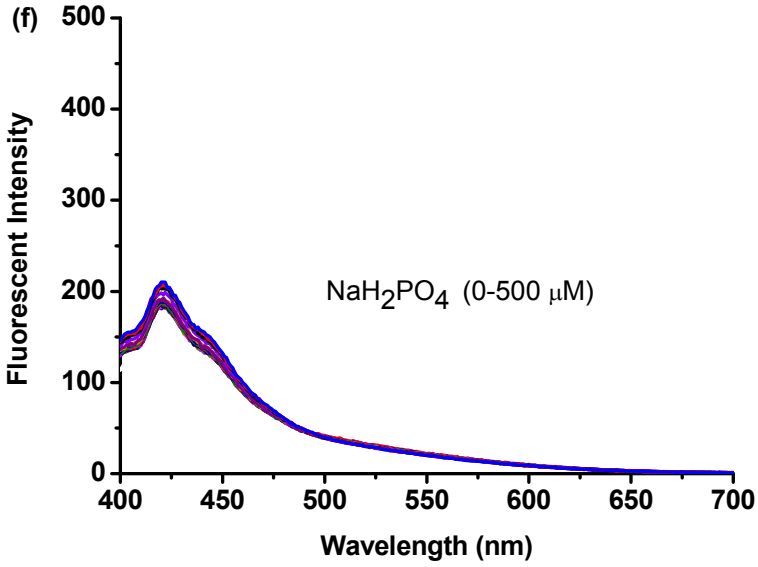





(c)
Fluorescent Intensity
Wavelength (nm)
[PPi]
0-400 μM
Intensity (420 nm)
[PPi]/[1-2Zn]
I-I0
[PPi]/([PPi]+[1-2Zn])


(d)
Fluorescent Intensity
Wavelength (nm)
Na2HPO4 (0-500 μM)


(e)
Fluorescent Intensity
Wavelength (nm)
Na_3PO_4 (0-500 μM)


(f)
Fluorescent Intensity
Wavelength (nm)
NaH_2PO_4 (0-500 μM)

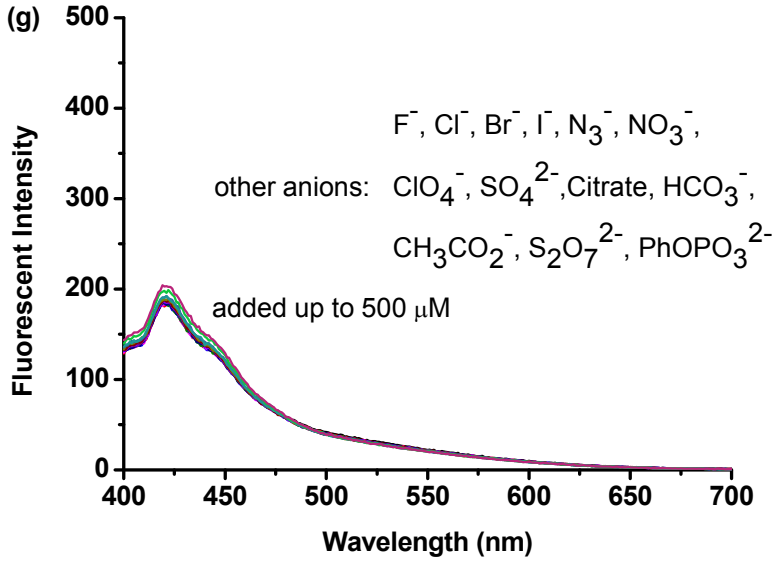
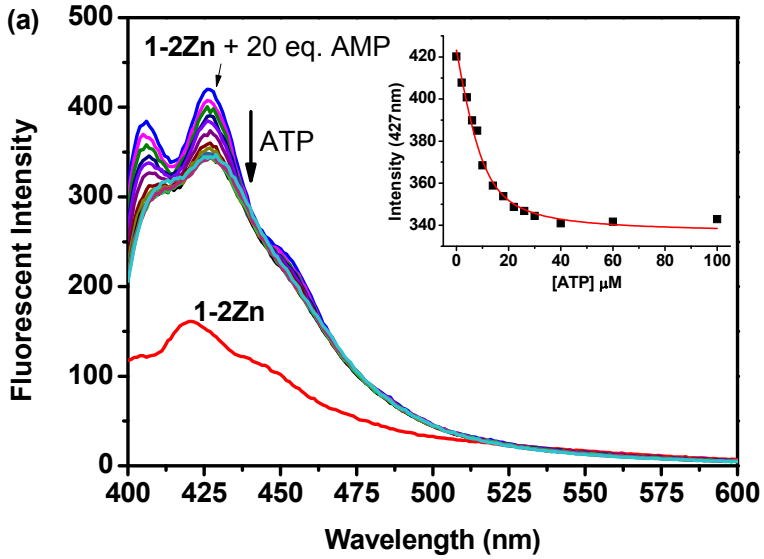


Fig. S3. Fluorescence spectra changes of **1-2Zn** (10 μM) upon the addition of various anions (as stated in each Figure) in 10 mM HEPES buffer (pH 7.2) at 25 ºC, λ_{ex} = 380 nm. Insert in (a)-(c): Fluorescent intensity changes against the phosphate concentrations and Job's plot examined between **1-2Zn** and phosphate anions ([**1-2Zn**] + [anion] = 10 μM).

4. Sensing of ATP in the presence of excess of AMP, ADP and PPi.



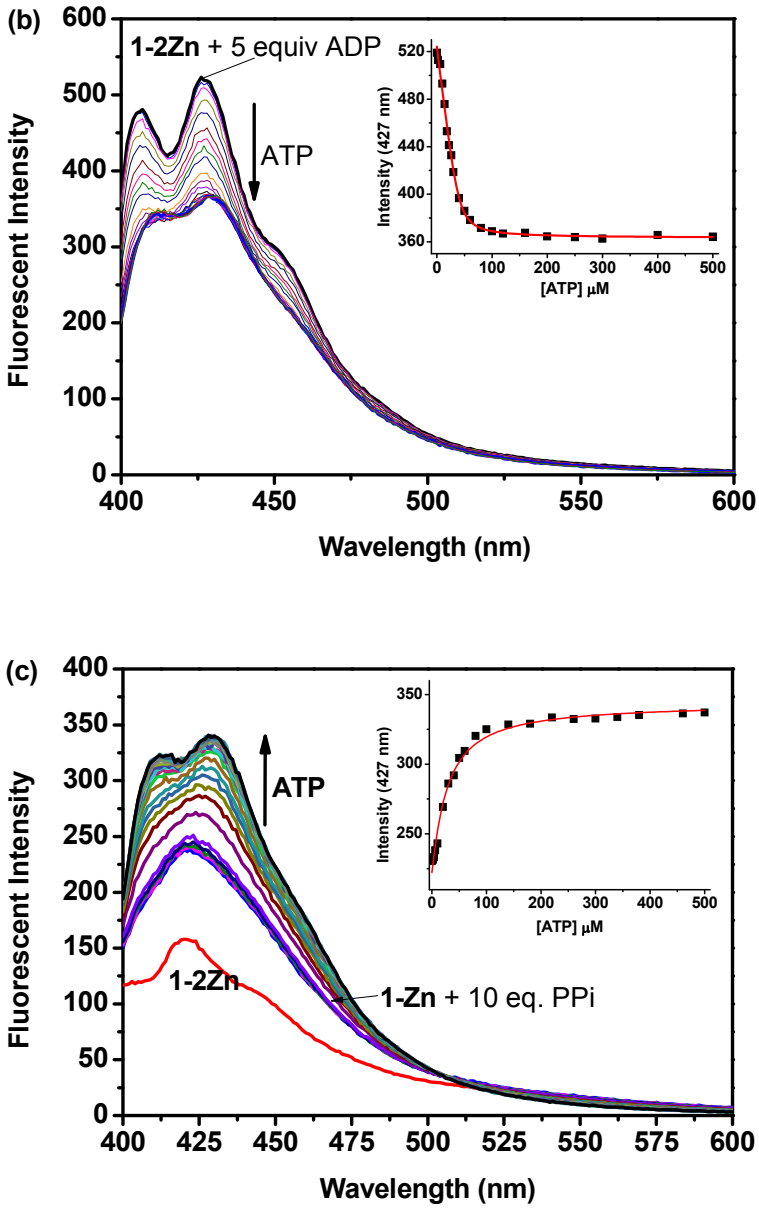
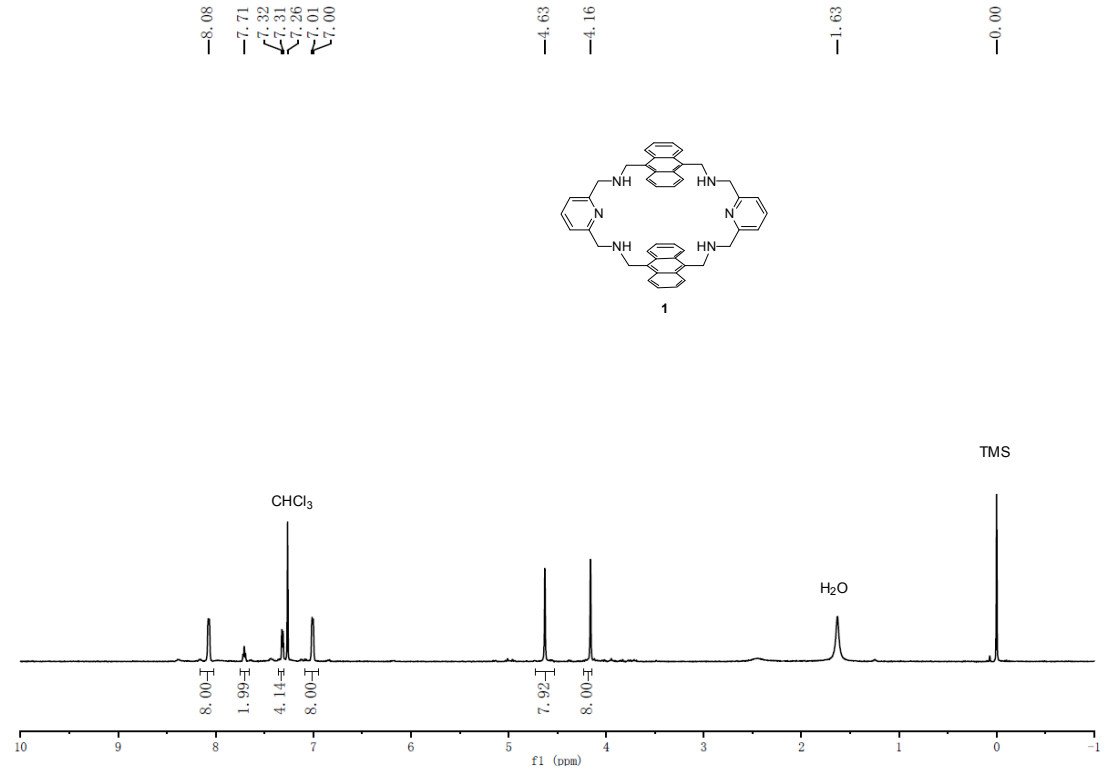
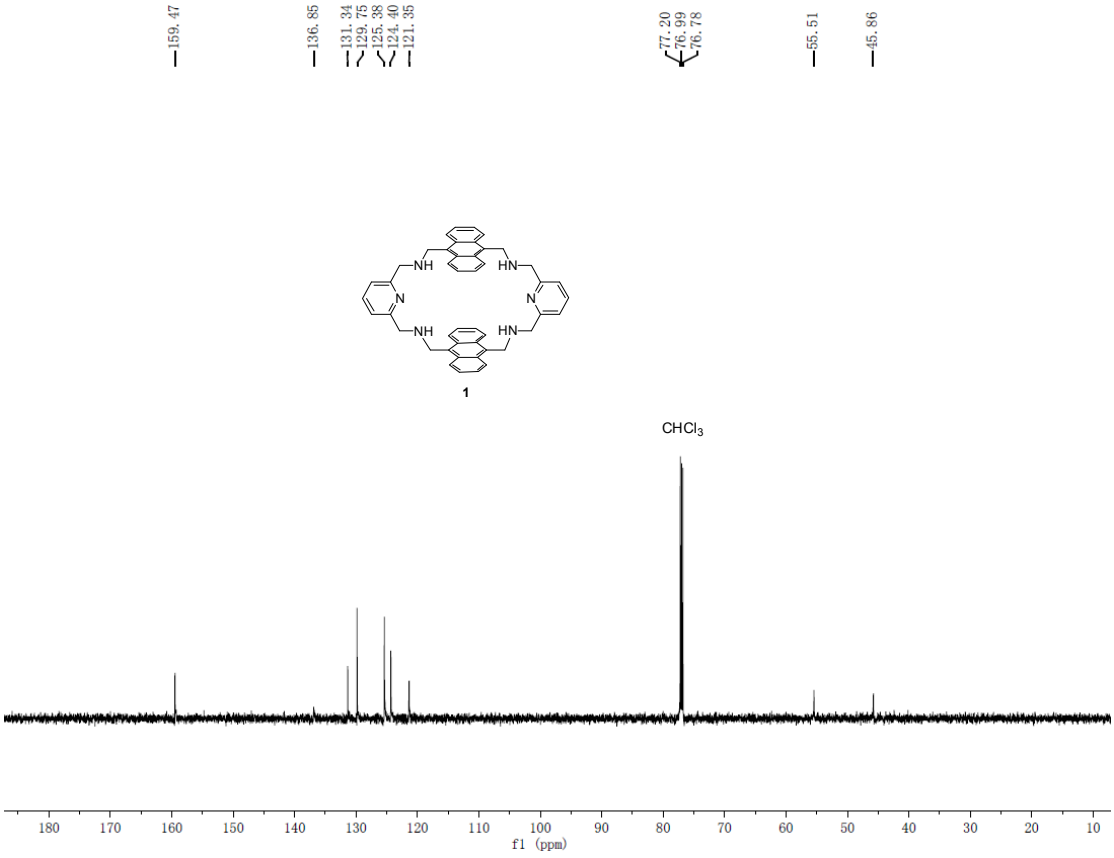


Fig. S4. Fluorescence spectra changes of **1-2Zn** (10 μM) upon the addition of ATP in the presence of (a) 200 μM AMP, (b) 50 μM ADP, (c) 100 μM PPi in 10 mM HEPES buffer (pH 7.2) at 25 ºC, λ_{ex} = 380 nm. Insert: Fluorescent intensity changes at 427 nm against the concentration of ATP added.

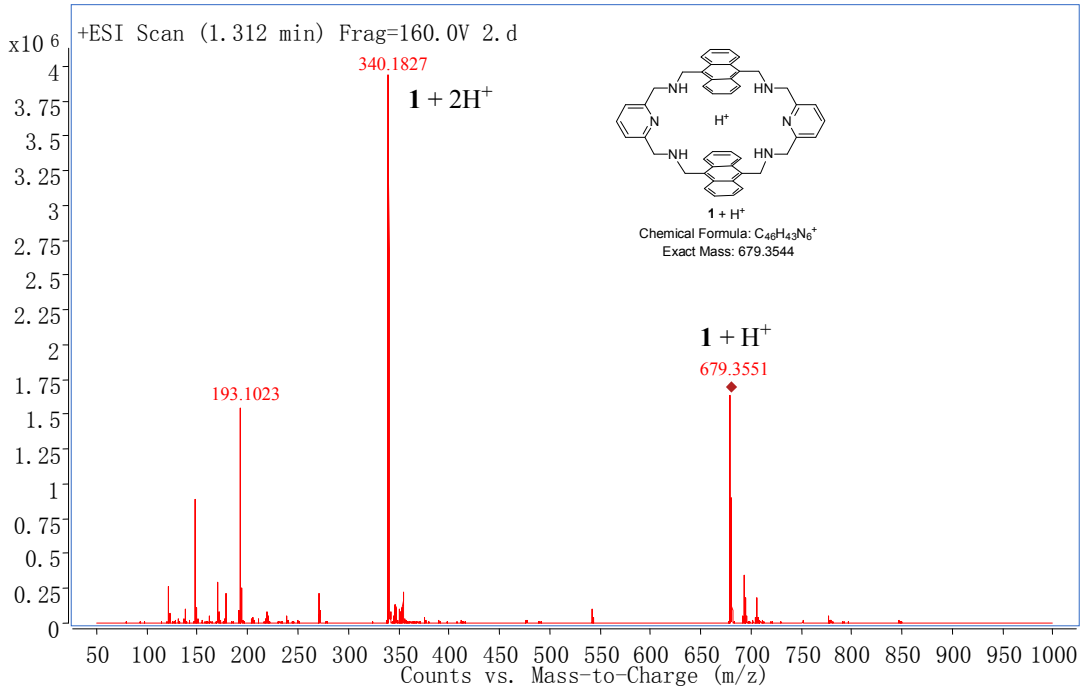
5. NMR and Mass spectra of cyclophane compound 1 and 4.



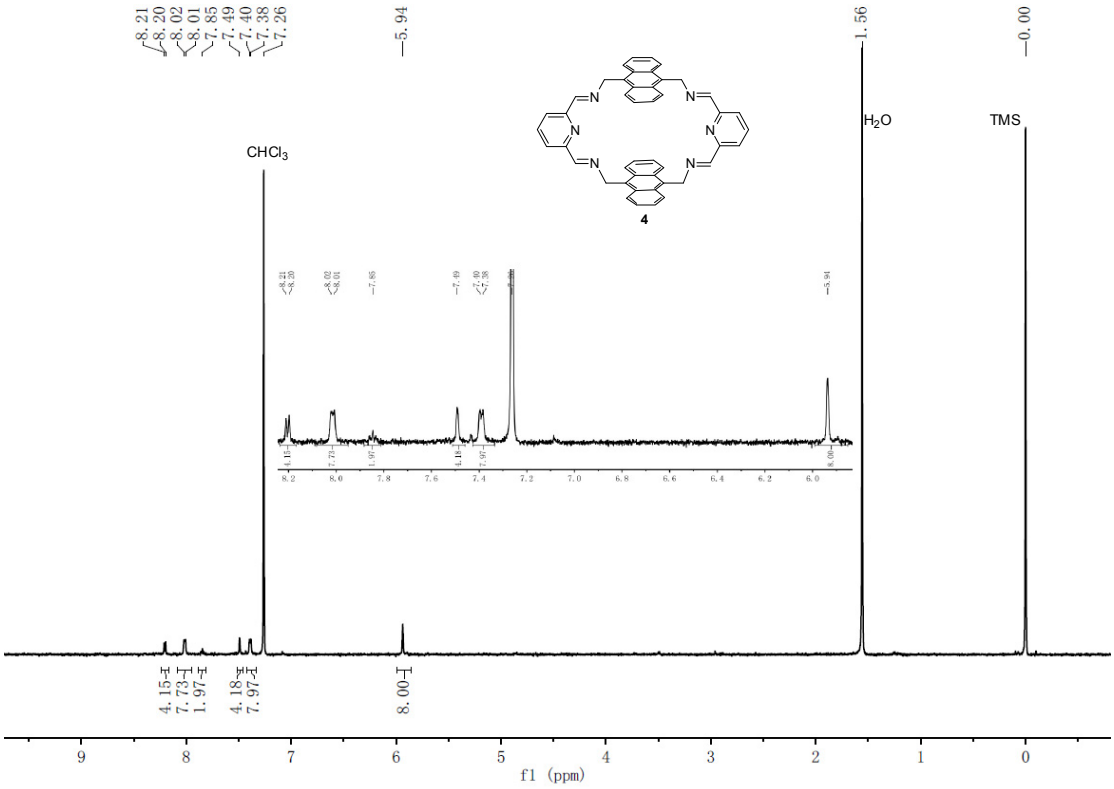
(a) ^{1}H NMR spectrum of cyclophane compound **1** in $CDCl_3$.



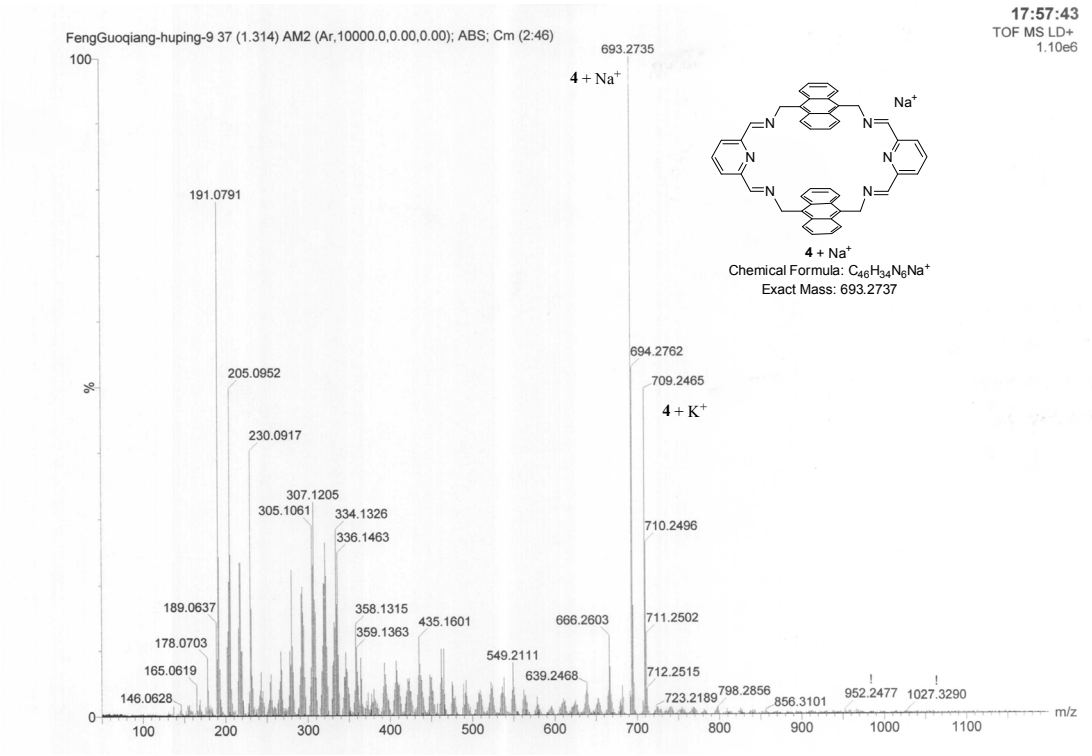
(b) ^{13}C NMR spectrum of cyclophane compound **1** in $CDCl_3$.



(c) HR-MS spectrum of cyclophane compound **1**.



(d) ^{1}H NMR spectrum of compound **4** in $CDCl_3$.



(e) HR-MS spectrum of Schiff base compound **4**.

Figure S5. NMR and Mass spectra of compound **1** and **4**.

6. Equation used for the determination of the apparent association constants (K_a)

$$R + A \underset{K_d}{\overset{K_a}{\rightleftharpoons}} C \qquad (1)$$

$$R = \text{Receptor};\ A = \text{Anion};\ C = \text{Complex};\quad K_a = \frac{1}{K_d}$$

$$\frac{I}{I_0} = 1 + 0.5 * m * \left\{([A] + [R]_0 + K_d) - \sqrt{([A] + [R]_0 + K_d)^2 - 4[R]_0[A]}\right\} \qquad (2)$$

R is the receptor (**1-2Zn** in this work), A is anion, C is the resulting 1:1 binding complex (Equation 1). Equation (2) is used for a nonlinear fitting of the titration data in Fig.3b to obtain K_d values, where [A] is the concentration of anions added, $[R]_0$ is the initial concentration of **1-2Zn** (set as $[R]_0$ = 10 μM), K_d is the dissociation constants. (Ref: K. A. Connors, *Binding Constants*, Wiley, New York, 1987.)