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Supporting Information

for

A Calixpyridinium-Based Supramolecular Tandem Assay for Alkaline Phosphatase and Its Application to ATP Hydrolysis Reaction

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Table S1. Chemical Shift Changes ($\Delta\delta$, ppm) of Calixpyridinium Protons in the Presence of ATP, ADP, and AMP at pD 7.2[a,b]

	H_a	H_b	H_c	H_d	H_e
ATP	0.85	–0.14	–0.20	–0.11	0.03
ADP	0.20	–0.05	–0.07	–0.04	–0.03
AMP	0.17	–0.04	–0.05	–0.04	–0.03

[a] $\Delta\delta = \delta$(presence of 1 equiv of guest) – δ(free host). [b] The host and guest were mixed in a 1:1 stoichiometry at 5 mM.

Table S2. Chemical Shift Changes ($\Delta\delta$, ppm) of ATP, ADP, and AMP Protons in the Presence of Calixpyridinium at pD 7.2[a,b]

guests	H_1	H_2	H_3	H_4	H_5	H_6	H_7
ATP	–0.06	–0.06	–0.10	–0.01	–0.01	0.03	0.09
ADP	–0.04	–0.02	–0.07	–0.01	–0.03	0.01	0.05
AMP	–0.07	–0.08	–0.09	–0.02	–0.03	0.00	0.03

[a] $\Delta\delta = \delta$(presence of 1 equiv of host) – δ(free guest). [b] The host and guest were mixed in a 1:1 stoichiometry at 5 mM.

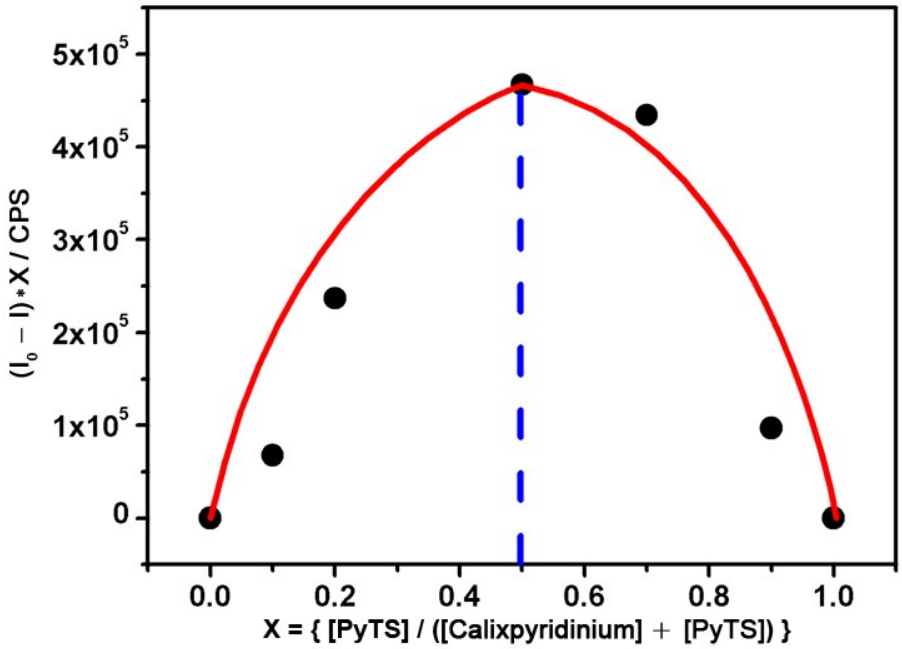
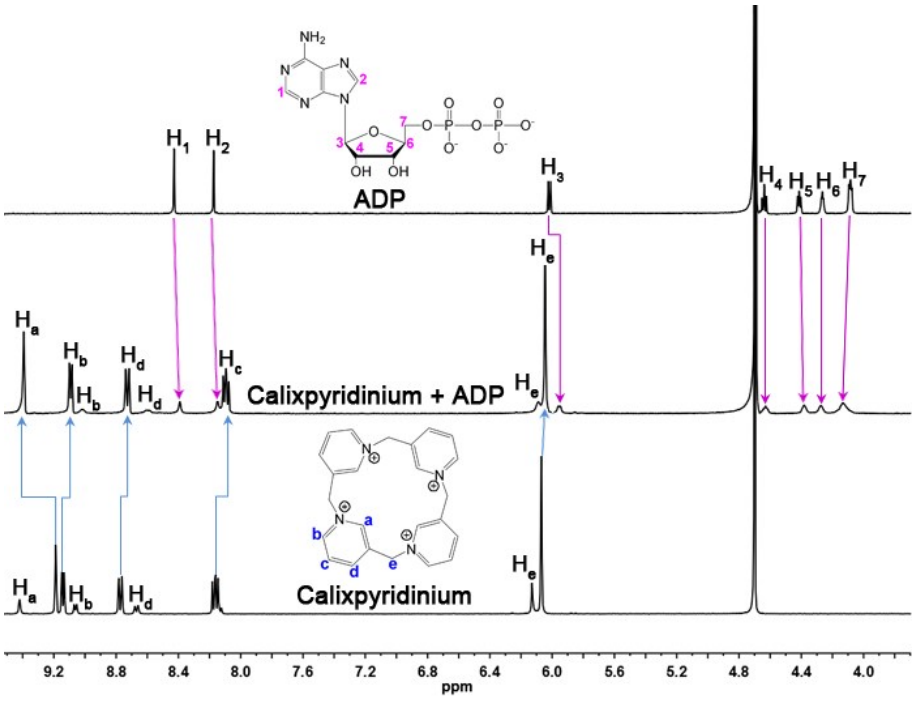
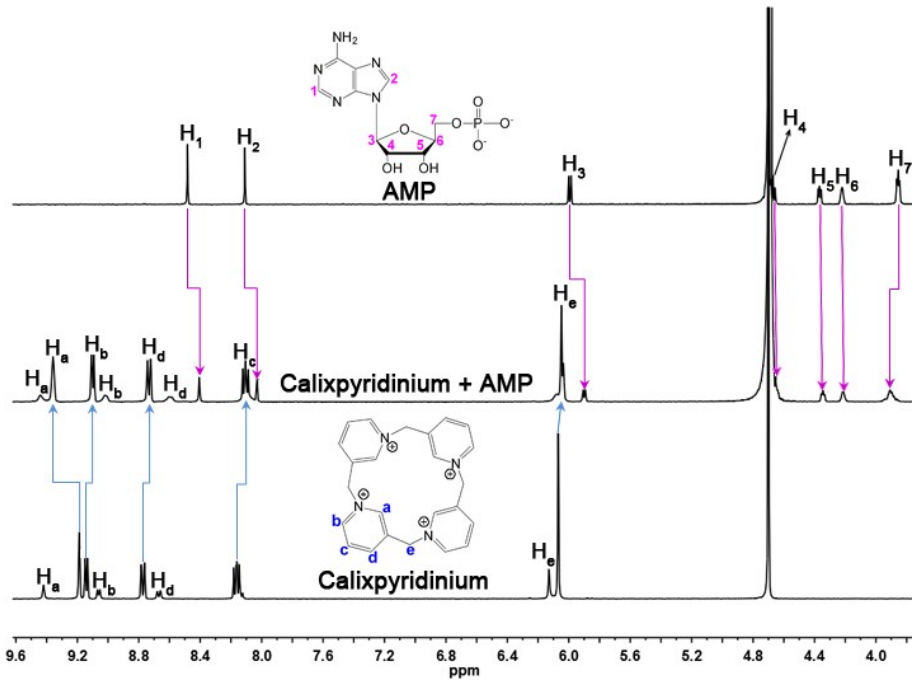


Figure S1. Job's plot for calixpyridinium and PyTS in 10 mM NaOAc solution at pH 7.2 (λ_{exc} = 339 nm, λ_{obs} = 385 nm), [calixpyridinium] + [PyTS] = 4 μM.

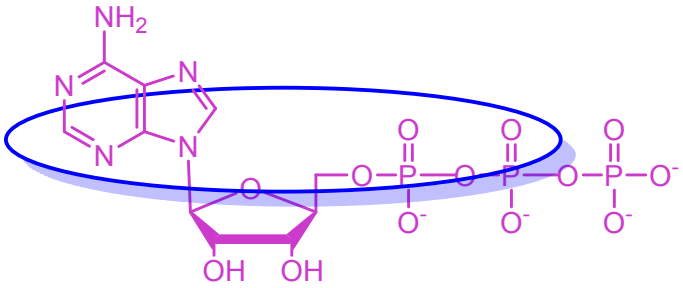


(a)

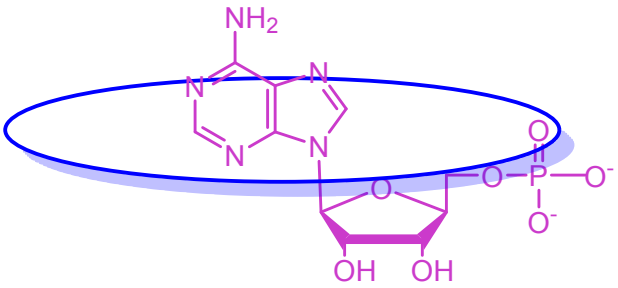


(b)

Figure S2. (a) ^{1}H NMR spectra of ADP, calixpyridinium, and calixpyridinium+ADP complex at pD 7.2; (b) ^{1}H NMR spectra of AMP, calixpyridinium, and calixpyridinium+AMP complex at pD 7.2. The host and guest were mixed in a 1:1 stoichiometry at 5 mM.



(a)



(b)

Figure S3. Deduced binding modes of calixpyridinium with ATP (a) and AMP (b) at pD 7.2 according to ^{1}H NMR spectra.

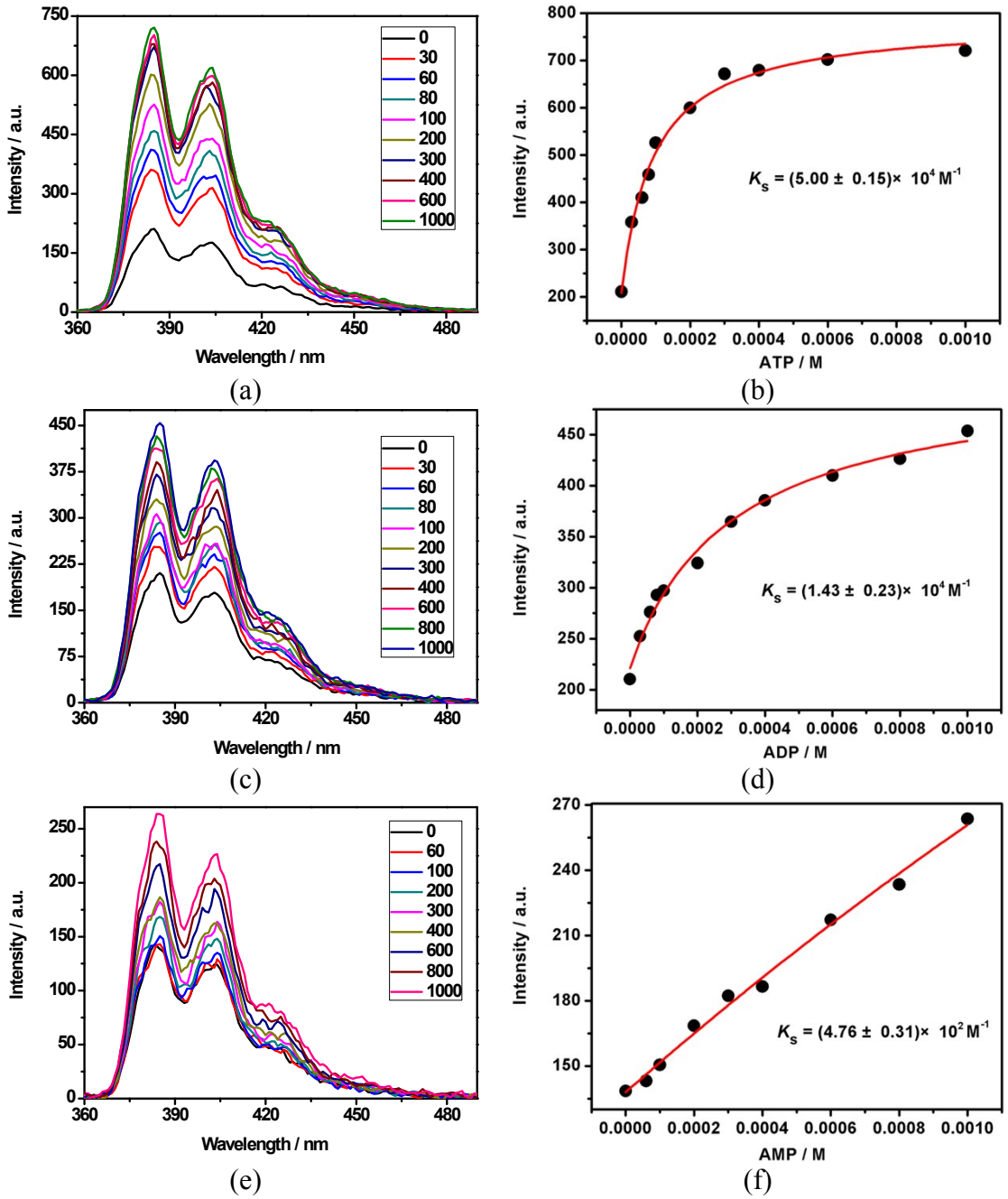


Figure S4. Competitive fluorescence titrations of ATP (a and b), ADP (c and d), and AMP (e and f) in the presence of PyTS (1.0 μM) and calixpyridinium (4.0 μM) in 10 mM NaOAc solution at pH 7.2 (λ_{exc} = 339 nm, λ_{obs} = 385 nm).

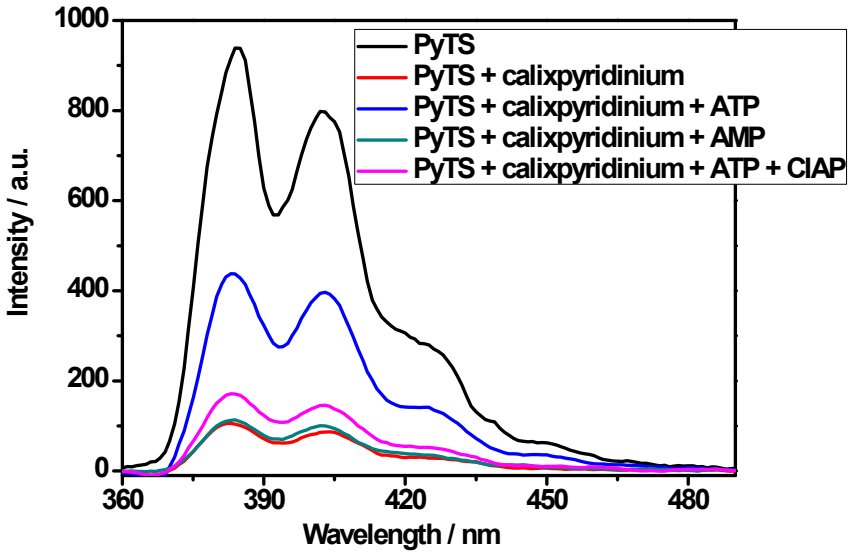


Figure S5. Fluorescence emission spectra of PyTS, PyTS+calixpyridinium, PyTS+calixpyridinium+ATP, PyTS+calixpyridinium+AMP, and PyTS+calixpyridinium+ATP+CIAP in 10 mM NaOAc solution at pH 7.2, excited at 339 nm.. The concentrations of PyTS, calixpyridinium, ATP, AMP, and CIAP are 1.0 μM, 4.0 μM, 60 μM, 60 μM, and 1.5 U/mL, respectively.

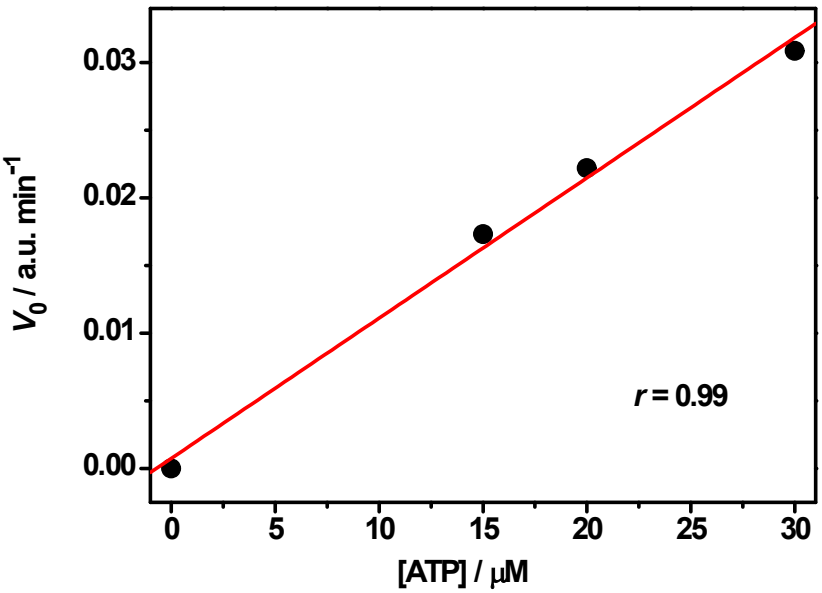


Figure S6. Linear relationship between the initial reaction rates and the concentrations of ATP (0−30 μM with 1.5 U/mL CIAP, 1.0 μM PyTS, and 4.0 μM calixpyridinium) in 10 mM NaOAc solution at pH 7.2 (λ_{exc} = 339 nm, λ_{obs} = 385 nm).

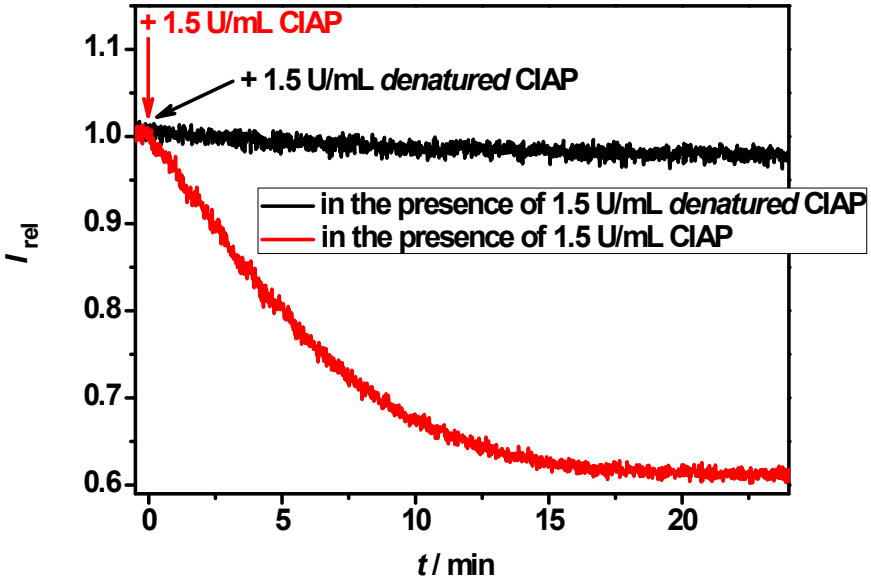


Figure S7. Continuous fluorescent enzyme assays for CIAP and *denatured* CIAP (1.5 U/mL) with the calixpyridinium/PyTS reporter pair (60 μM ATP, 1.0 μM PyTS, and 4.0 μM calixpyridinium in 10 mM NaOAc solution at pH 7.2; λ_{exc} = 339 nm, λ_{obs} = 385 nm).

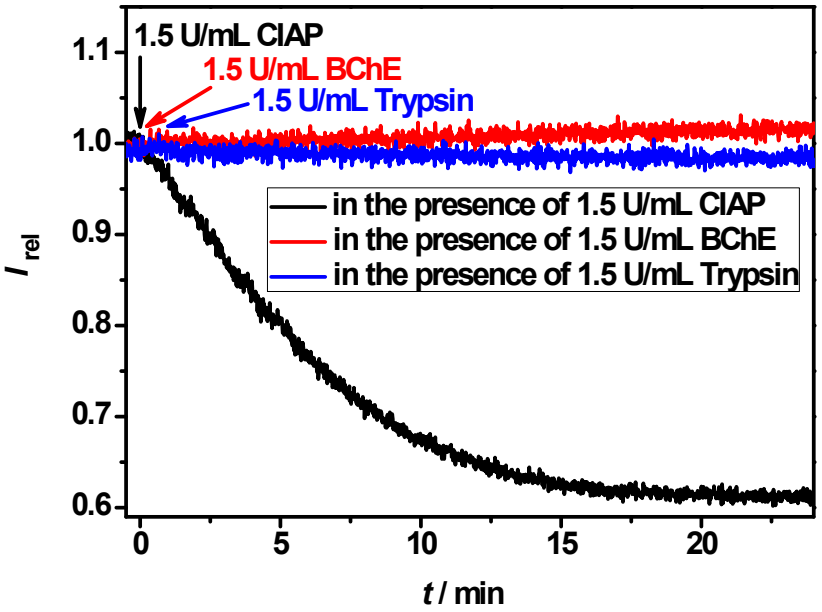


Figure S8. Continuous fluorescent enzyme assays for CIAP, BChE, and Trypsin (1.5 U/mL) with the calixpyridinium/PyTS reporter pair (60 μM ATP, 1.0 μM PyTS, and 4.0 μM calixpyridinium in 10 mM NaOAc solution at pH 7.2; λ_{exc} = 339 nm, λ_{obs} = 385 nm).

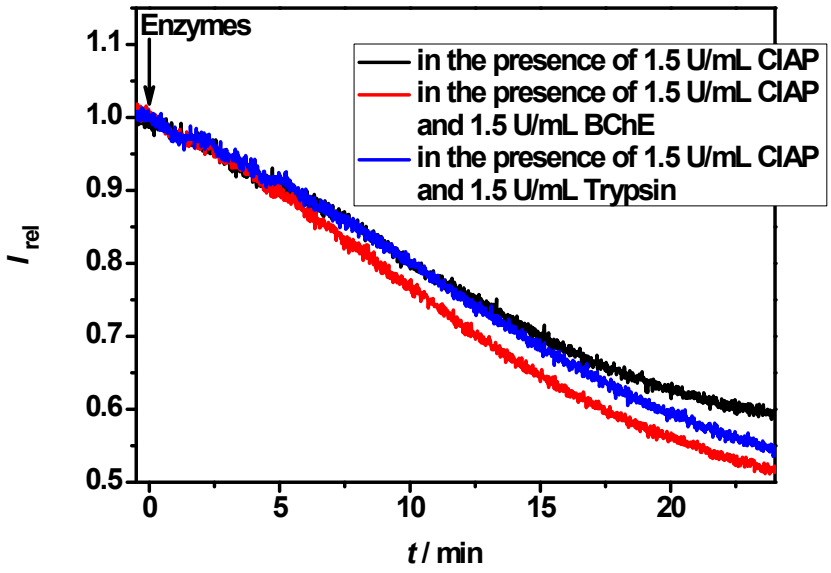


Figure S9. Continuous fluorescent enzyme assays for CIAP (1.5 U/mL) in the absence and presence of BChE (1.5 U/mL) or Trypsin (1.5 U/mL) with the calixpyridinium/PyTS reporter pair (60 μM ATP, 1.0 μM PyTS, and 4.0 μM calixpyridinium in 10 mM NaOAc solution at pH 7.2; λ_{exc} = 339 nm, λ_{obs} = 385 nm).