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Supplementary Information

Selective ATP recognition by boronic acid-appended cyclodextrin and fluorescent probe supramolecular complex in water

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Table of Contents

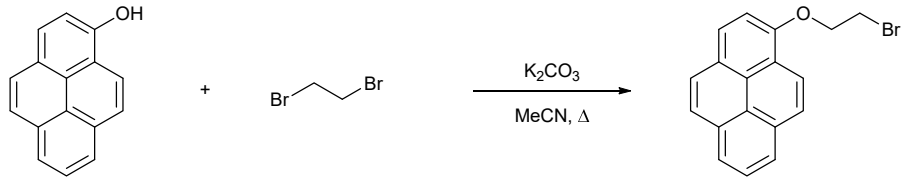
Experimental details

Reagents

1-Hydroxypyrene (Tokyo Chemical Industry), 1,2-diboromoethane (Tokyo Chemical Industry), potassium carbonate (Fujifilm Wako Chemicals), 2,2'-dipicolylamine (Tokyo Chemical Industry), sodium sulfate (Fujifilm Wako Chemicals), potassium iodide (Fujifilm Wako Chemicals), ammonium chloride (Fujifilm Wako Chemicals), 4-carbox-3-fluorophenylboroni acid (Fujifilm Wako Chemicals), benzoic acid (Tokyo Chemical Industry), *N*,*N*'-dicyclohexylcarbodiimide (DCC, Tokyo Chemical Industry), 1-hydroxybenzotriazole monohydrate (HOBt•H_2O, Tokyo Chemical Industry), 3A-amino-3A-deoxy-(2AS,3AS)-gamma-cyclodextrin hydrate (3-NH_2-γ-CyD, Tokyo Chemical Industry), acetonitrile (dehydrated, Kanto Chemical Industry), tetrahydrofuran (dehydrated, stabiliser free, Fujifilm Wako Chemicals), chloroform (Kanto Chemical Industry), dichloromethane (Fujifilm Wako Chemicals), ethanol (Fujifilm Wako Chemicals), *N*,*N*-dimethylformamide (DMF, dehydrated, Fujifilm Wako Chemicals), and acetone (Fujifilm Wako Chemicals) were used as received from the commercial resources. Deuterium oxide (D_2O, Kanto Chemical Industry), dimethyl sulfoxide-d_6 (DMSO-d_6, Kanto Chemical Industry), chloroform-d_1 ($CDCl_3$, Kanto Chemical Industry), 40% sodium deuteroxide solution (40% NaOD, Sigma Aldrich), and 35% deuterium chloride solution in D_2O (35% DCl, Fujifilm Wako Chemicals) were used as received for nuclear magnetic resonance (NMR) spectroscopic measurements. Dimethyl sulfoxide (DMSO, Luminasol®, Dojindo Laboratories), disodium hydrogenphosphate (Fujifilm Wako Chemicals), sodium pyrophosphate (Alfa Aesar), pentasodium triphosphate (Fujifilm Wako Chemicals), adenosine-5'-monophosphate sodium salt (Nacalai Tesque), adenosine-5'-diphosphate disodium salt hydrate (Tokyo Chemical Industries), adenosine 5'-triphosphate disodium salt hydrate (Tokyo Chemical Industries), guanosine 5'-triphosphate disodium salt (Fujifilm Wako Chemicals), cytidine 5'-triphosphate disodium salt n-hydrate (Fujifilm Wako Chemicals), uridine 5'-triphosphate trisodium salt n-hydrate (Fujifilm Wako Chemicals), γ-cyclodextrin (**γCyD**, Kanto Chemical Industry), β-cyclodextrin (**βCyD**, Kanto Chemical Industry), zinc nitrate hexahydrate 99.9% (Fujifilm Wako Chemicals), 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, Dojindo Laboratories), sodium hydroxide (Fujifilm Wako Chemicals), apyrase from potatoes (Sigma Aldrich), and Milli-Q water were used for spectroscopic measurement.

Synthetic procedure

*Synthesis of **1-Br***



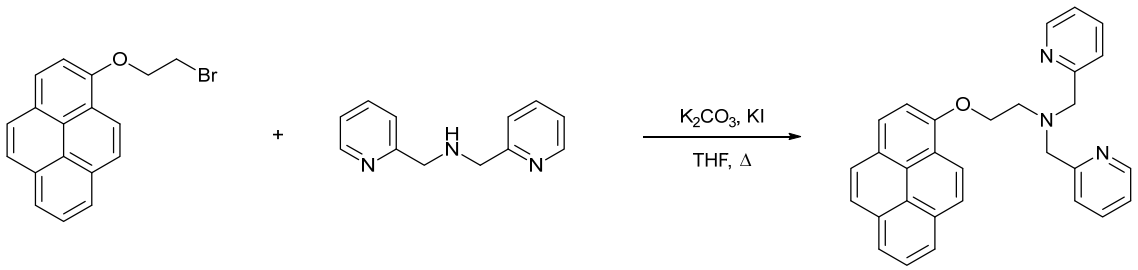
1-Br

1-Hydroxylpyrene (0.453 g, 2.08 mmol), 1,2-dibromoethane (2.035 g, 10.83 mmol), potassium carbonate (1.097 g, 7.937 mmol), and dehydrated acetonitrile (20 mL) were added into a round-bottom flask under an argon atmosphere. The suspension was refluxed at 85°C overnight. The reaction mixture was cooled to room temperature, subsequently the solvent was removed using a rotary evaporator. Chloroform (30 mL) was added into the residue, then the organic solution was washed with water (4 × 40 mL) using a separating funnel. The organic layer was dried over anhydrous sodium sulfate and filtered, then the filtrate was concentrated. Excess amount of ethanol was added into the concentrated solution, resulting in the precipitation of white solids. The solid was collected by filtration and washed with water and ethanol. The obtained solid was dried under reduced pressure. The desired product, **1-Br**, was yielded as a white solid (81.74 mg, 0.25 mmol, 12%). The production of **1-Br** was confirmed by the ^{1}H NMR spectra, which was identical with the literature data.[1]

^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.50 (d, J = 8.6 Hz, 1H), 8.14-8.06 (m, 4H), 7.99-7.91 (m, 3H), 7.53 (d, J = 8.0 Hz, 1H), 4.66 (t, J = 6.3 Hz, 2H), 3.86 (t, J = 6.3 Hz, 2H).

HRMS (m/z): (FAB) calcd. for $C_{18}H_{13}BrO$ [$M^{+\bullet}$]: 324.0174, found 324.0150.

*Synthesis of **1***



1

1-Br (81.7 mg, 0.251 mmol), potassium carbonate (0.253 g, 1.83 mmol), potassium iodide (0.231 g, 1.39 mmol), and dehydrated tetrahydrofuran (20 mL) were added into a round-bottom flask under

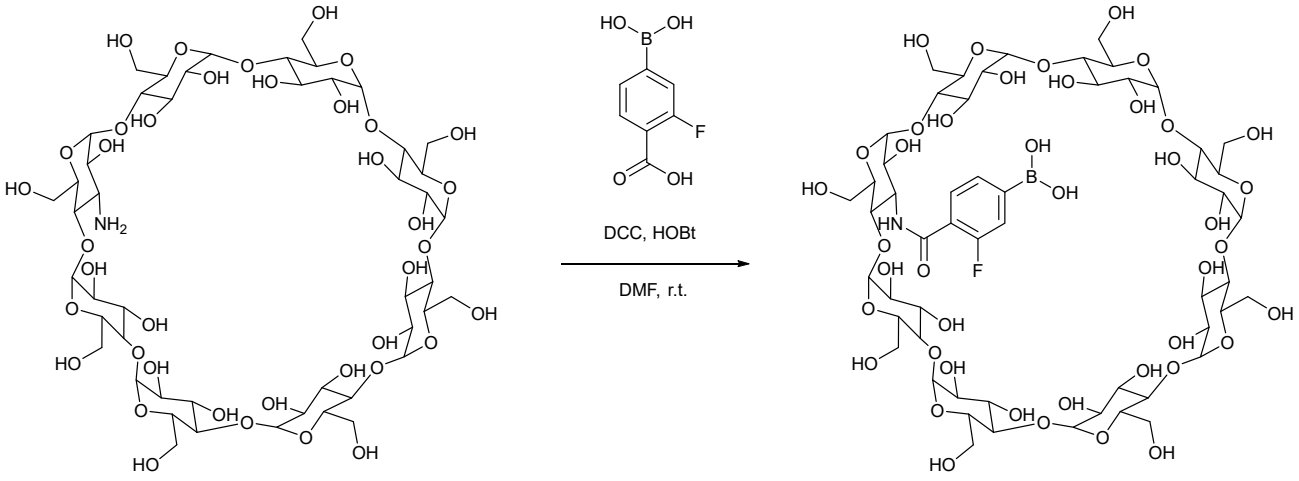
an argon atmosphere. Into the mixture, 2,2'-dipicolylamine (0.307 g, 1.54 mmol) dissolved in dehydrated tetrahydrofuran (5 mL) was added dropwise with stirring at room temperature. The suspension was refluxed at 70°C for 2 days. After cooling to room temperature, the reaction mixture was filtered to remove off insoluble salts. The organic solvent was removed from the filtrate using a rotary evaporator. The residue was dissolved in dichloromethane (30 mL), subsequently the organic solution was washed with 10% ammonium chloride aq. (2 × 20 mL) and water (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and filtered, then the filtrate was concentrated to dryness using a rotary evaporator. The residue was purified by size-exclusion chromatography using chloroform as an eluent. The desired product, **1**, was yielded as a brown solid (74.78 mg, 0.17 mmol, 67%).

^{1}H NMR ($CDCl_3$ + 1% D_2O, 500 MHz): δ 8.54-8.50 (m, 2H), 8.42 (d, J = 9.2 Hz, 1H), 8.09 (dd, J = 10.9, 7.4 Hz, 2H), 8.02 (dd, J = 8.6, 5.7 Hz, 2H), 7.94 (m, 2H), 7.87 (d, J = 9.2 Hz, 1H), 7.58 (m, 4H), 7.43 (d, J = 8.6 Hz, 1H), 7.12 (t, J = 5.7 Hz, 2H), 4.44 (t, J = 5.4 Hz, 2H), 4.11 (s, 4H), 3.31 (t, J = 5.4 Hz, 2H).

^{13}C{^{1}H} NMR ($CDCl_3$ + 1% D_2O, 125 MHz) δ 159.3, 152.8, 149.1, 136.7, 131.8, 131.7, 127.3, 126.4, 126.2, 125.9, 125.5, 125.4, 125.1, 125.0, 124.4, 124.2, 123.2, 122.2, 121.4, 120.4, 109.1, 67.0, 61.1, 53.6.

HRMS (m/z): (ESI) calcd. for $C_{30}H_{26}N_3O$ [M + H^+]: 444.20759, found 444.20603.

*Synthesis of **FBγCyD***



FBγCyD

FBγCyD was synthesised according to previously reported method.[2]

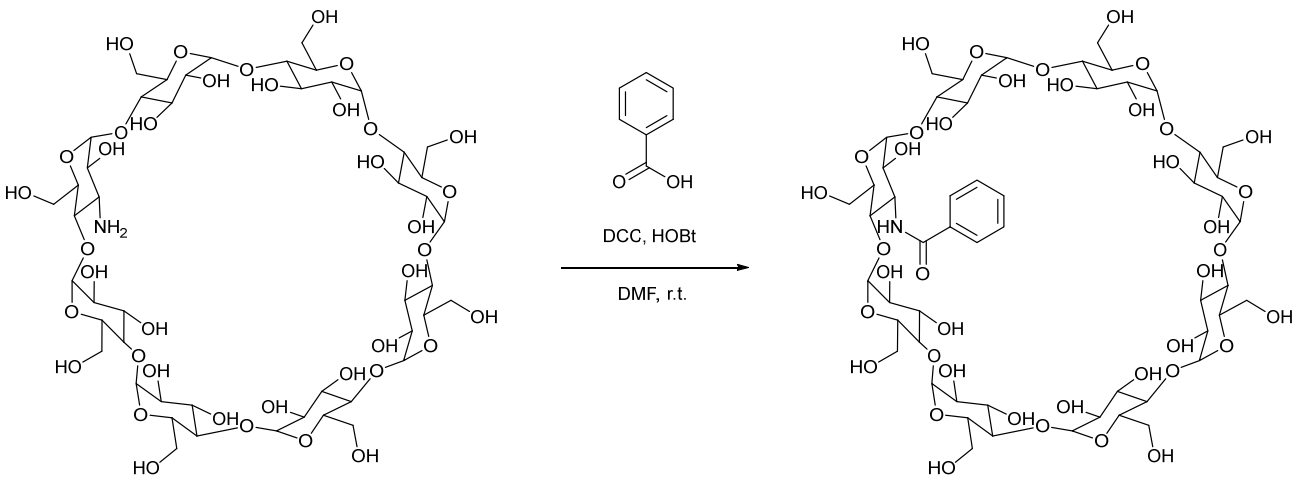
4-carboxy-3-fluorophenylboronic acid (0.044 g, 0.24 mmol), DCC (0.062 g, 0.30 mmol), and HOBt·H_2O (0.046 g, 0.30 mmol) were dissolved in 5 mL of dehydrated DMF and stirred in ice bath

for 30 min. 3-NH_2-γ-CyD (0.259 g, 0.20 mg) dissolved in 5 mL of dehydrated DMF was added into this suspension and stirred in an ice bath for 30 min and at room temperature for 20 hours. The solution concentrated by distillation under reduced pressure was stored in a refrigerator for more than 24 hours to precipitate a reaction byproduct. The floated byproduct was removed by cotton filtration, and the filtrate was poured into 800 mL of acetone while stirring. The suspension was subjected to suction filtration using a membrane filter (TYPE: JHWP). The obtained white solid was dissolved in 10 mL of deionised water and lyophilised under reduced pressure to obtain a white powder (0.250 g, 0.170 mmol, 85%). The ^{1}H NMR and HR mass spectra were identical to the reported data.[4]

^{1}H NMR (D_2O, 500 MHz): δ 7.68 (t, 1H), 7.49 (d, 1H), 7.40 (d, 1H), 5.05-4.91 (m, 8H), 3.88-3.34 (m, 48H).

HRMS (m/z): (ESI+) calcd. for $C_{55}H_{85}BFNO_{42}$ $[M + Na]^+$: 1484.4521, found 1484.4935.

*Synthesis of **PhγCyD***



PhγCyD

Benzoic acid (0.032 g, 0.26 mmol), DCC (0.063 g, 0.31 mmol), and HOBt·H_2O (0.047 g, 0.31 mmol) were dissolved in 5 mL of dehydrated DMF and stirred in ice bath for 30 min. 3-NH_2-γ-CyD (0.256 g, 0.20 mg) dissolved in 5 mL of dehydrated DMF was added into this suspension and stirred in an ice bath for 30 min and at room temperature for 20 hours. The solution concentrated by distillation under reduced pressure was stored in a refrigerator for more than 24 hours to precipitate a reaction byproduct. The floated byproduct was removed by cotton filtration, and the filtrate was poured into 800 mL of acetone while stirring. The suspension was subjected to suction filtration using a membrane filter (TYPE: JHWP). The obtained white solid was dissolved in 10 mL of deionised water and lyophilised under reduced pressure to obtain a white powder (0.231 g, 0.165mmol, 83%).

^{1}H NMR (D_2O, 500 MHz): δ 7.80 (d, J = 8.0 Hz, 2H), 7.62 (t, J = 7.2 Hz, 1H), 7.55 (t, J = 7.4 Hz,

2H), 5.17-5.05 (m, 8H), 4.61-3.56 (m, 48H).

^{13}C NMR (D_2O, 125 MHz): δ 171.6, 134.3, 132.9, 129.5, 127.8, 103.5, 102.3, 102.2, 102.1, 101.9, 101.8, 101.8, 81.1, 81.0, 80.8, 80.5, 80.1, 79.9, 73.8, 73.7, 73.6, 73.2, 73.0, 73.0, 72.9, 72.7, 72.5, 72.4, 72.2, 69.8, 61.0, 60.8, 60.7, 52.3.

HRMS (m/z): (ESI−) calcd. for $C_{55}H_{84}NO_{40}$ $[M - H]^-$: 1398.45696, found 1398.44560.

NMR spectra

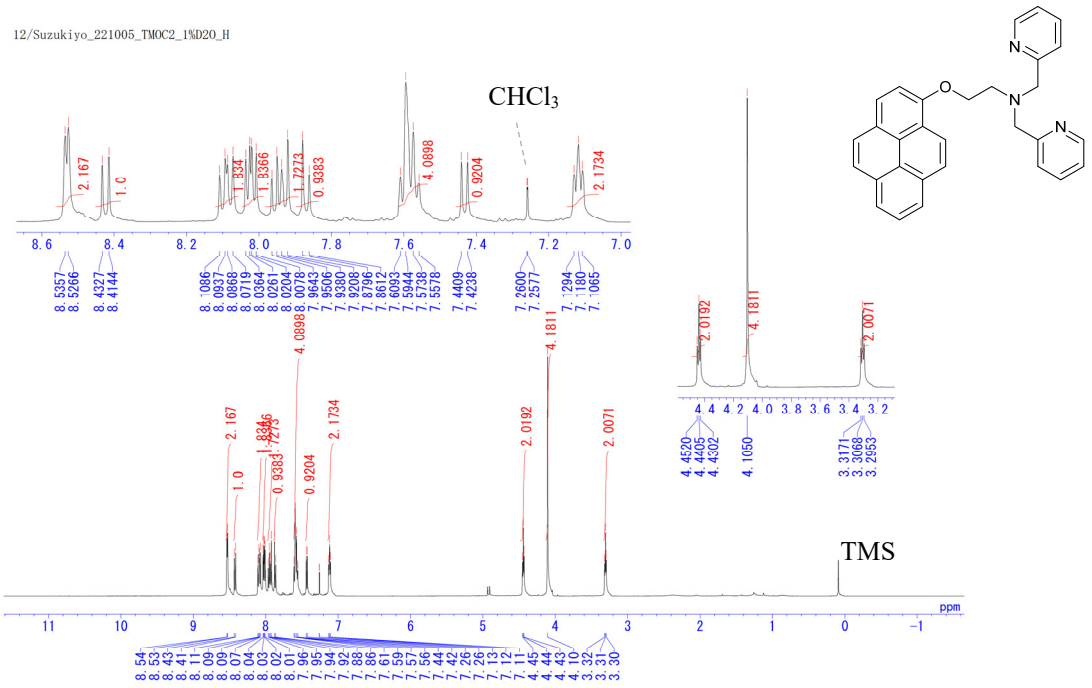


Figure S1. ^{1}H NMR spectrum of **1** in $CDCl_3$ + 1vol% D_2O.

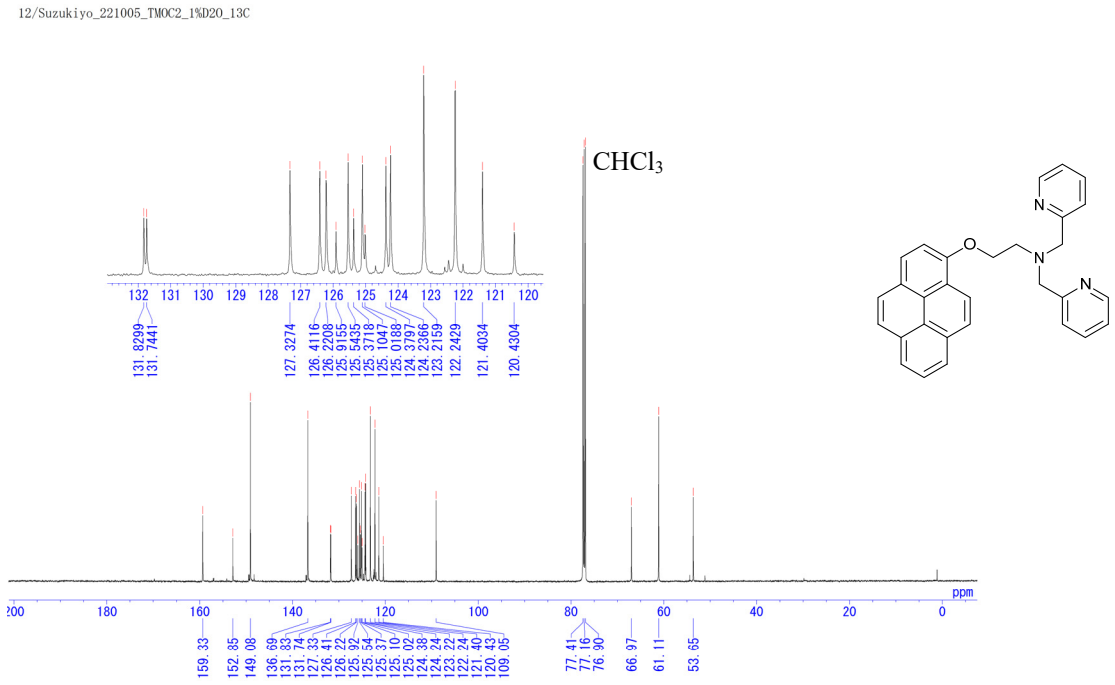


Figure S2. ^{13}C NMR spectrum of **1** in $CDCl_3$ + 1vol% D_2O.

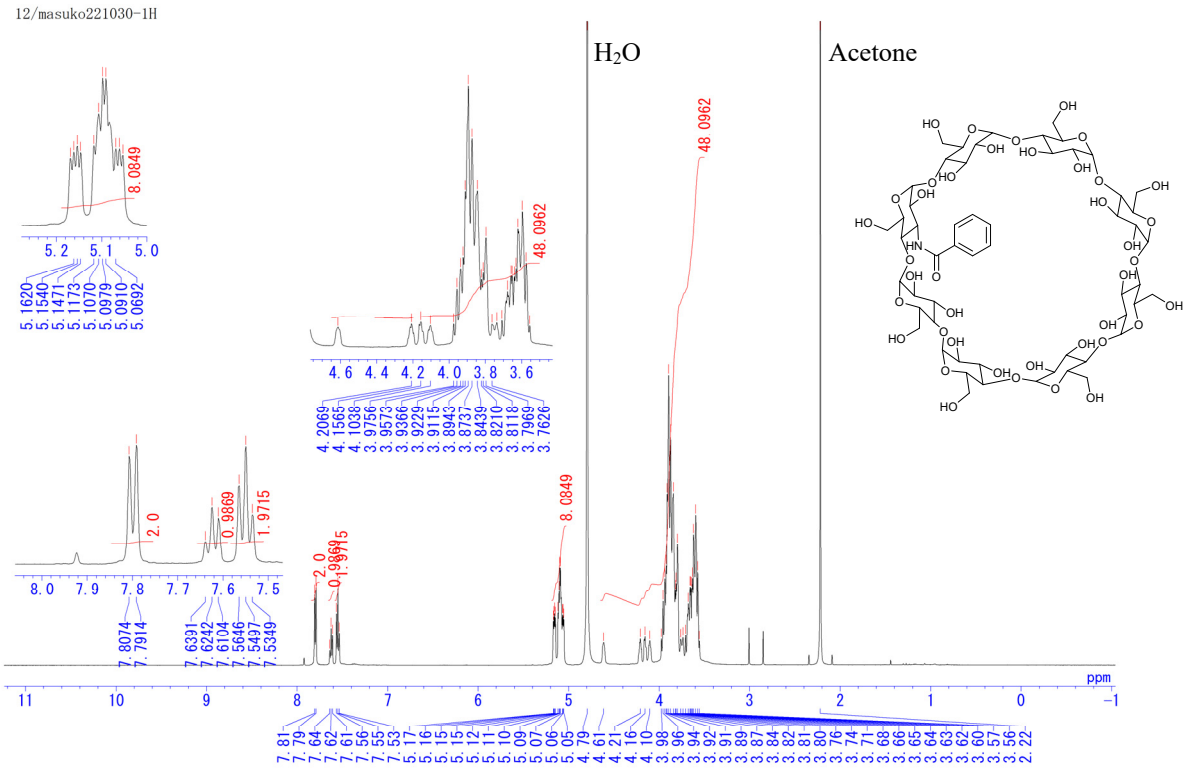


Figure S3. ^{1}H NMR spectrum of **PhγCyD** in D_2O. 3 μL of acetone was added as an internal standard.

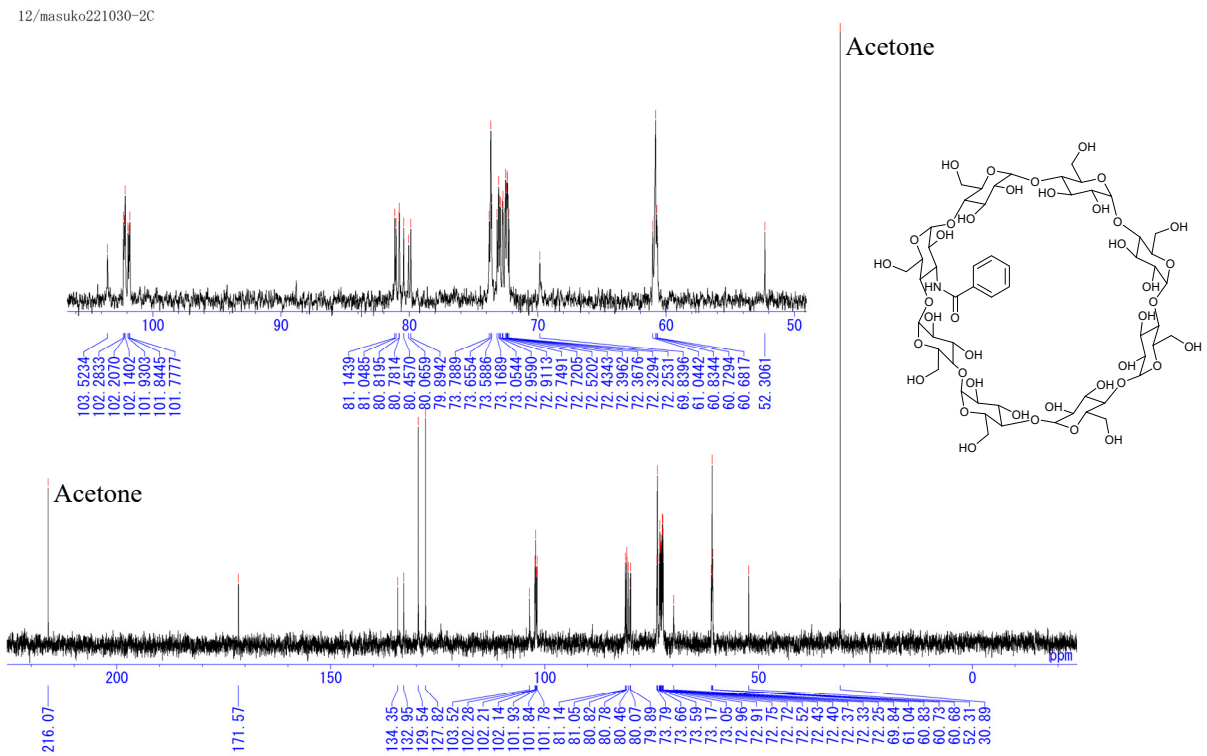


Figure S4. ^{13}C NMR spectrum of **PhγCyD** in D_2O. 3 μL of acetone was added as an internal standard.

Determination of the acid dissociation constant

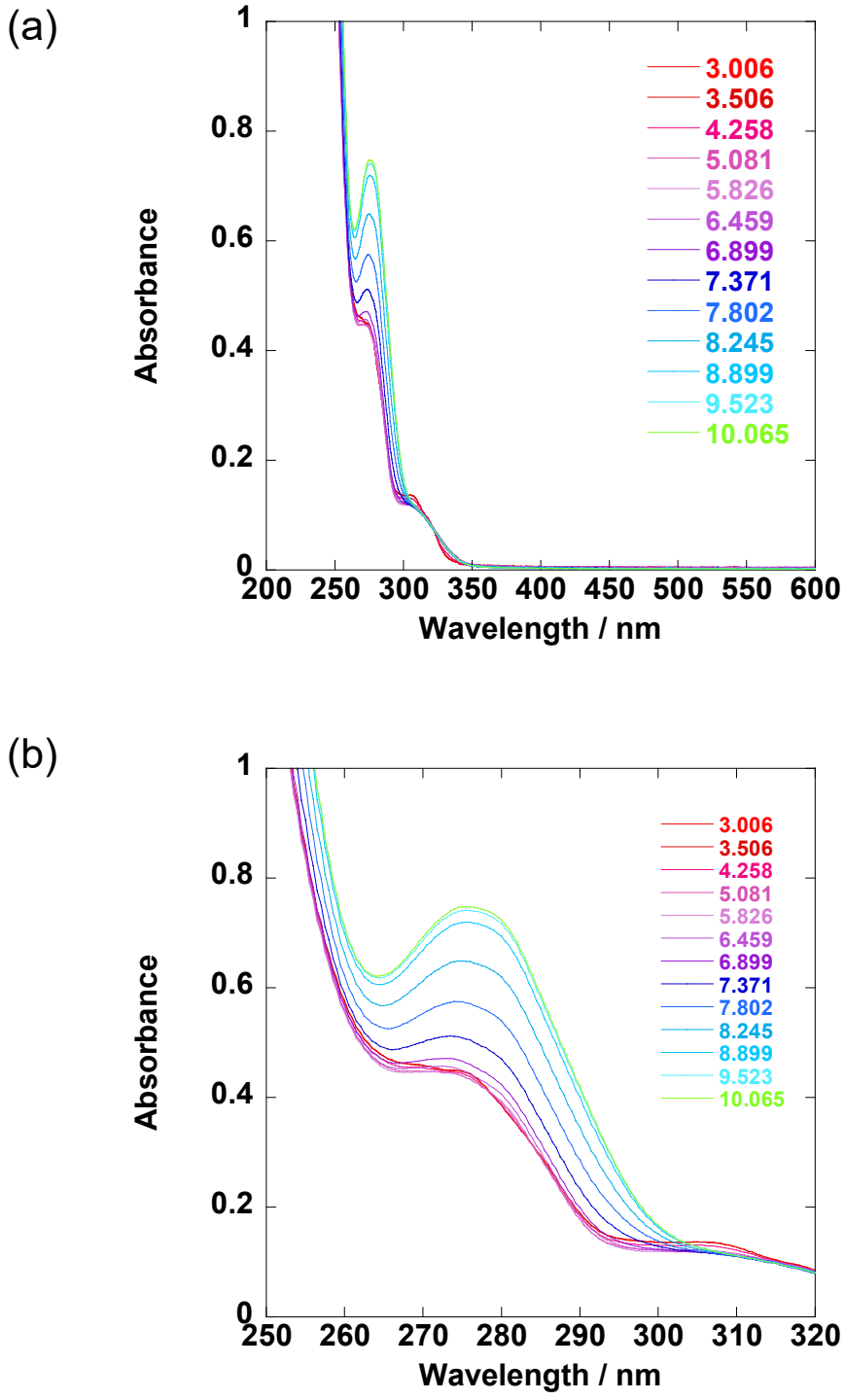


Figure S5. (a) UV-vis absorption spectra of 0.5 mM **FBγCyD** in water under various pH conditions: 5 mM phosphate buffer, and T = 25°C. (b) Enlarged spectra of (a).

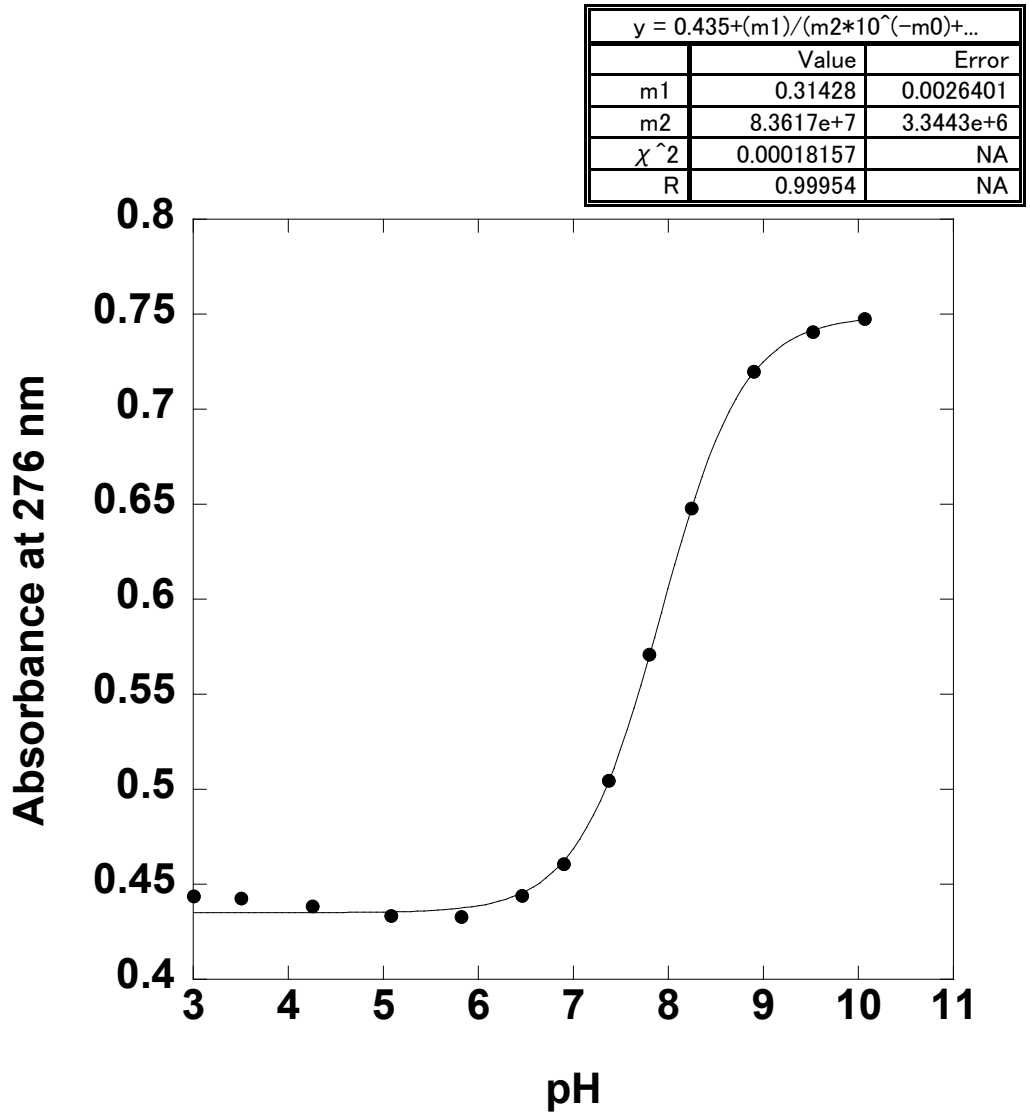
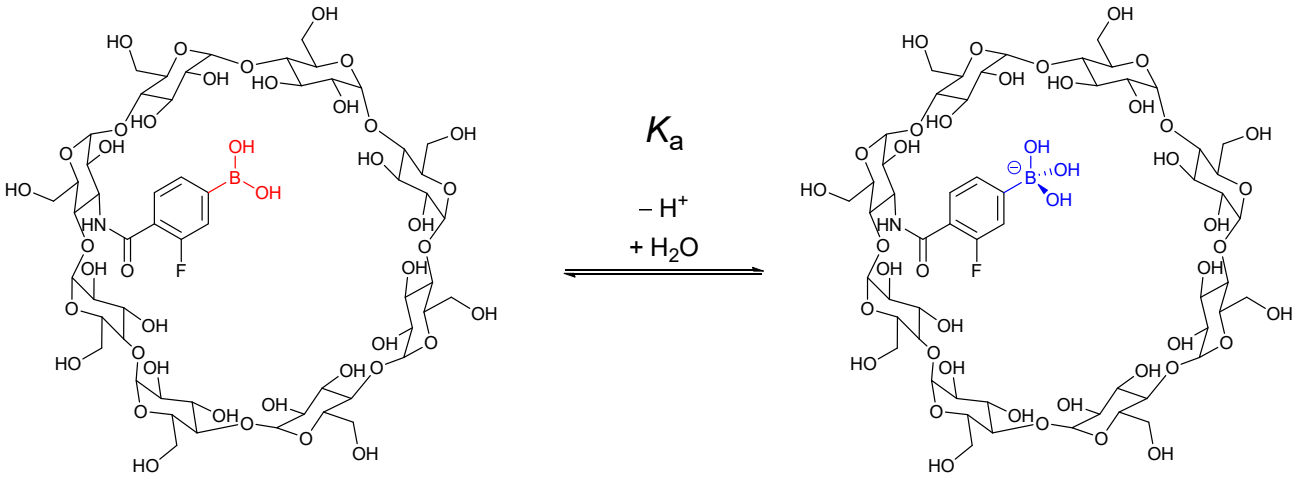


Figure S6. Absorbance at 276 nm of **FBγCyD** (0.5 mM) in water under various pH conditions: 5 mM phosphate buffer and T = 25°C. The plots were fitted with the theoretical equation of the acid dissociation model of monobasic acids.



FBγCyD

Scheme S1. Acid dissociation equilibrium of **FBγCyD** in aqueous solution.

According to Scheme S1, the total concentration of HA (C_A) and the acid dissociation constant (K_a) of **FBγCyD** are written as follows:

$$C_A = [HA] + [A^-] \quad \text{(S1)}$$

$$K_a = \frac{[A^-][H^+]}{[HA]} \quad \text{(S2)}$$

In Eqs. S1 and S2, [HA] and [A^-] denote the concentrations of **FBγCyD** and its conjugate base, respectively. Substitution of Eq. S2 into Eq. S1 affords Eq. S3.

$$\frac{C_A}{[A^-]} = \frac{[H^+]}{K_a} + 1 \quad \text{(S3)}$$

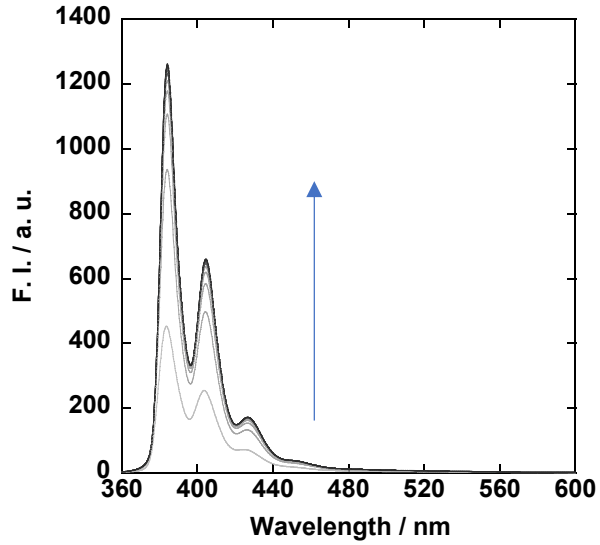
$$\therefore \frac{[A^-]}{C_A} = \left(\frac{[H^+]}{K_a} + 1 \right)^{-1} \quad \text{(S4)}$$

As pH = 7.40 and pK_a = 7.92,

$$\frac{[A^-]}{C_A} = 0.23$$

Fluorescence spectra at various FBγCyD concentrations

(a)



(b)

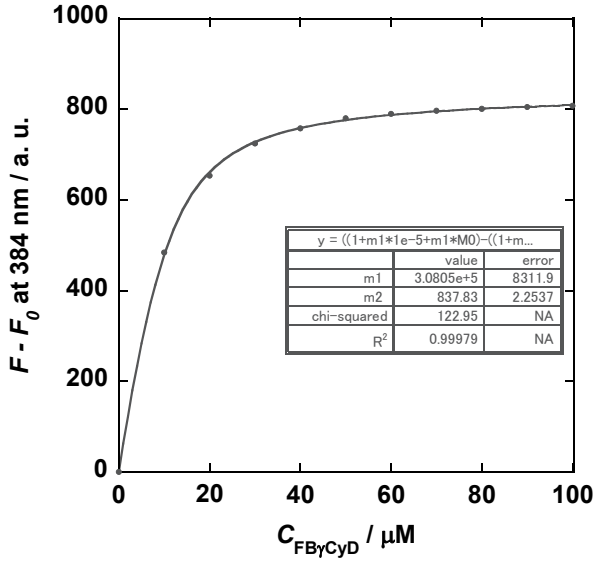


Figure S7. Fluorescence spectra (a) and the enhancement of the fluorescence intensity ($F - F_0$) of 384 nm (b) of **Zn-1** with increasing the concentration of **FBγCyD** in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm. The abbreviations F and F_0 denote the fluorescence intensity of **Zn-1** in the presence and absence of **FBγCyD**, respectively.

Fluorescence spectra of Zn-1 with γCyD

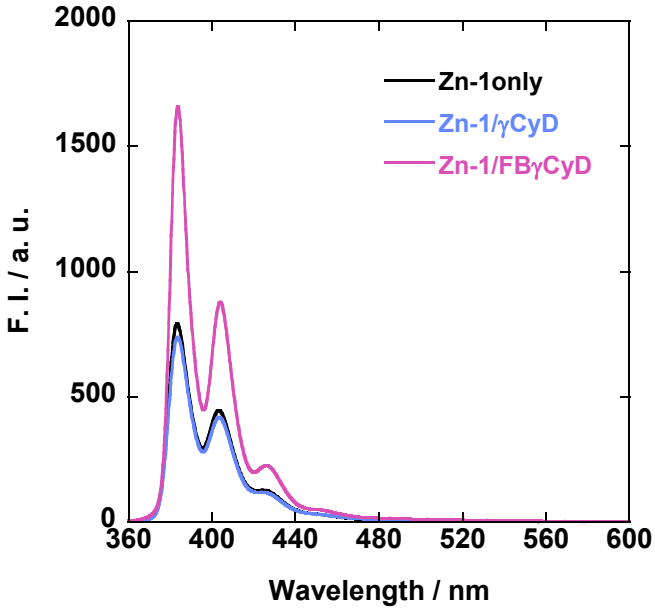


Figure S8. Fluorescence spectra of **Zn-1** in the absence and presences of **γCyD** and **FBγCyD** in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\gamma CyD}$ = 0.1 mM, $C_{FB\gamma CyD}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

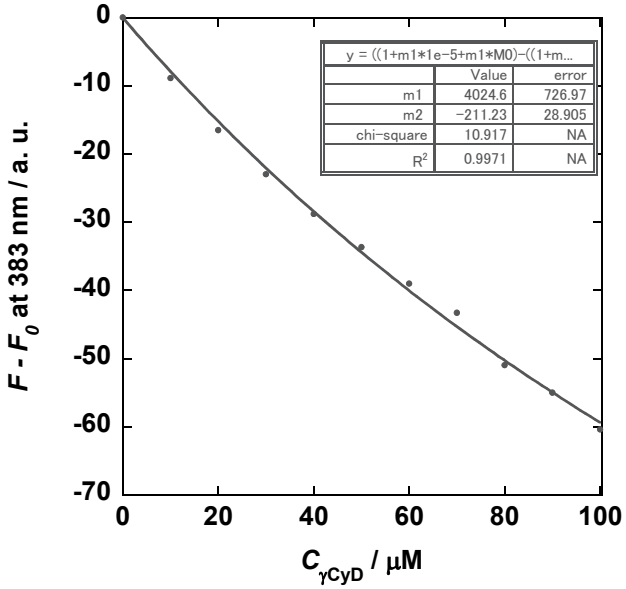
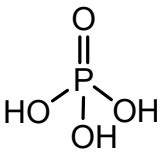
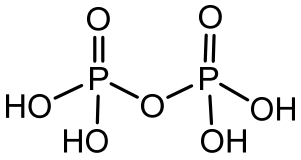


Figure S9. Change in the fluorescence intensity ($F - F_0$) of 383 nm of **Zn-1** with increasing the concentration of **γCyD** in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm. The abbreviations F and F_0 denote the fluorescence intensity of **Zn-1** in the presence and absence of **γCyD**, respectively.

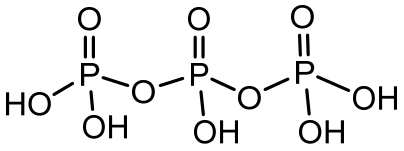
Structures of phosphate derivatives



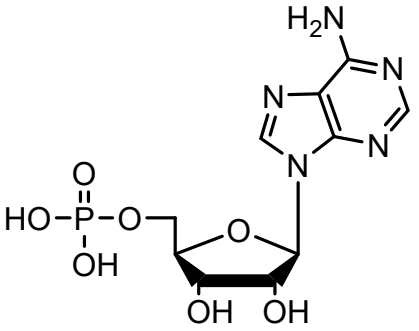
Monophosphate (Pi)



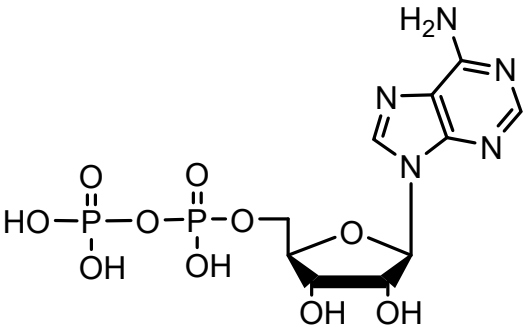
Pyrophosphate (PPi)



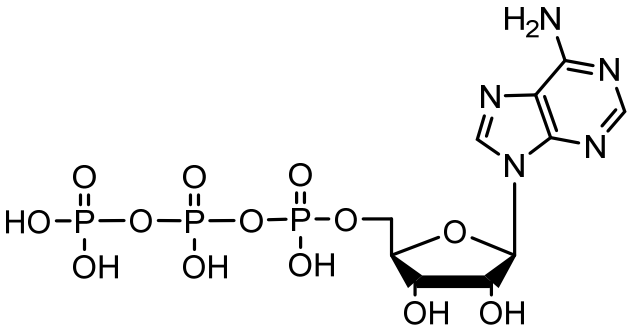
Triphosphate (Tri)



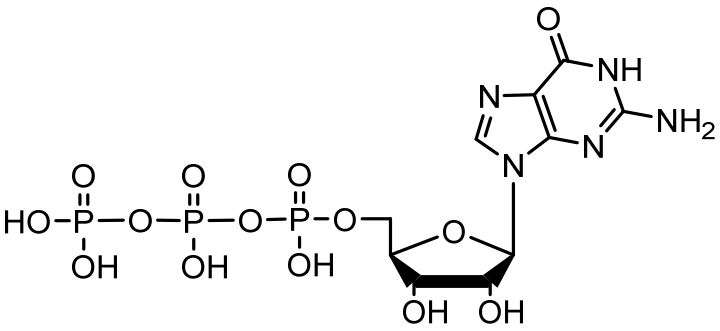
Adenosine-5’-monophosphate (AMP)



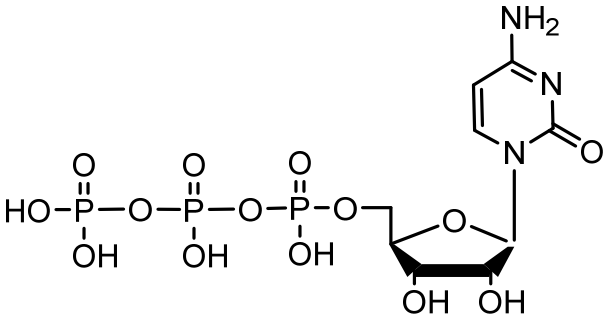
Adenosine-5’-diphosphate (ADP)



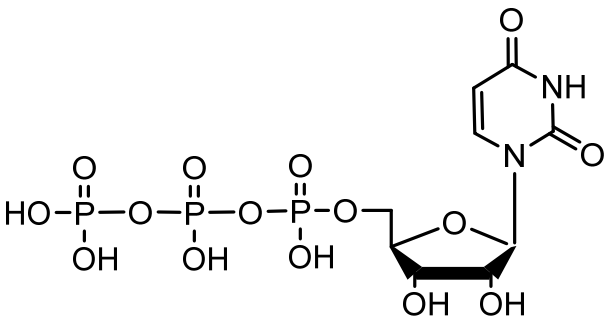
Adenosine-5’-triphosphate (ATP)



Guanosine-5’-triphosphate (GTP)



Cytidine-5’-triphosphate (CTP)



Uridine-5’-triphosphate (UTP)

Chart S1. The structures of phosphate derivatives.

UV-vis absorption spectra in the absence and presence of phosphate derivatives

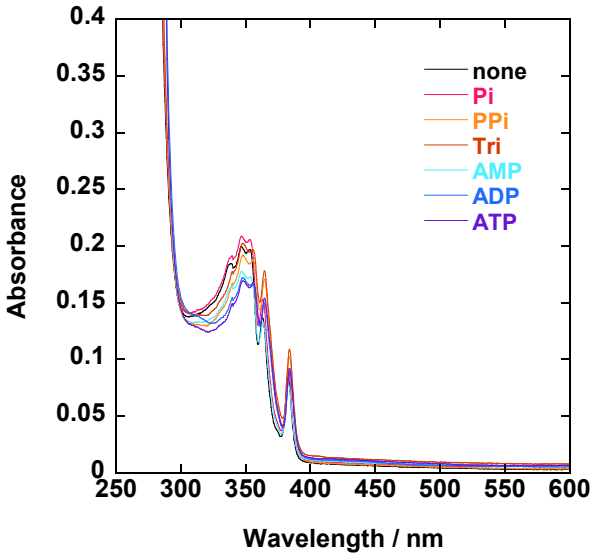


Figure S10. UV-vis absorption spectra of **Zn-1/FBγCyD** in the absence and presence of various 1 mM of phosphate derivatives in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, and T = 25°C.

Normalised fluorescence spectra of Zn-1/FBγCyD

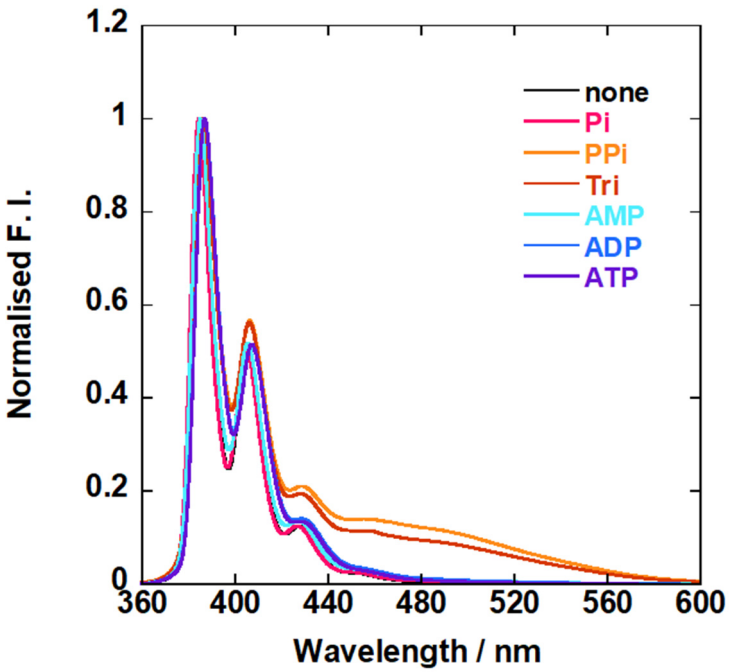
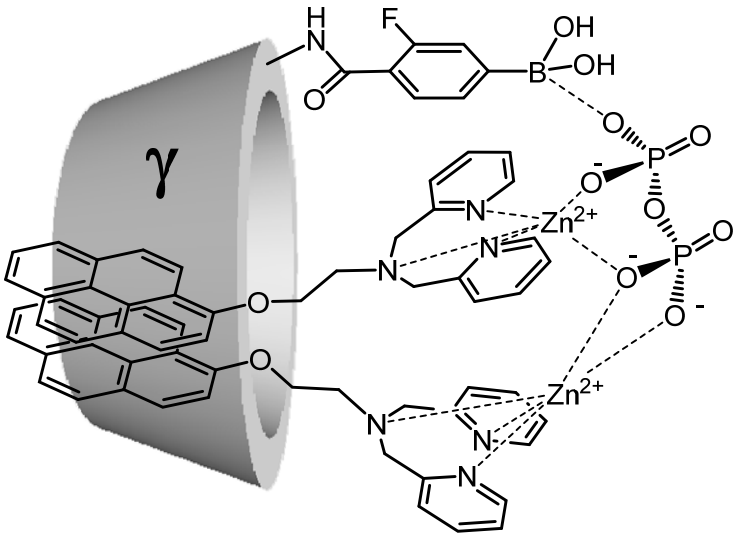


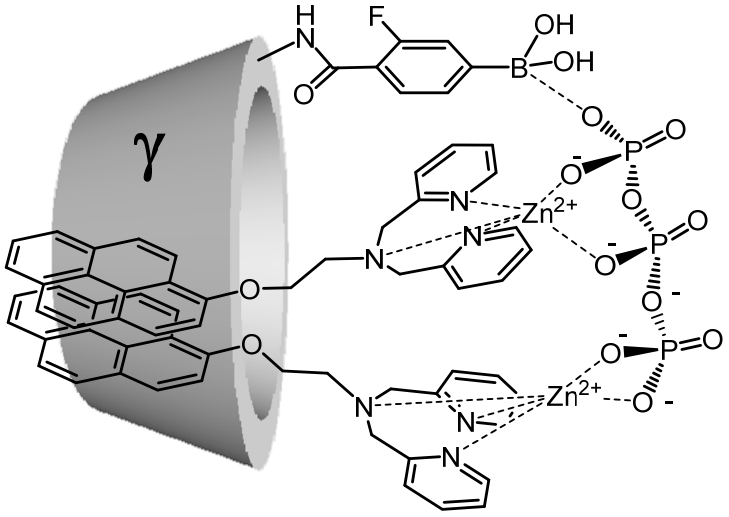
Figure S11. Normalised fluorescence spectra of **Zn-1/FBγCyD** in the absence and presence of various 1.0 mM of phosphate derivatives in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

(a)



PPi-(Zn-1)$_2$/FBγCyD

(b)



Tri-(Zn-1)$_2$/FBγCyD

Chart S2. Plausible structures of **PPi-(Zn-1)$_2$/FBγCyD** (a) and **Tri-(Zn-1)$_2$/FBγCyD** (b).

Fluorescence spectra of Zn-1/FBγCyD with various phosphate concentrations

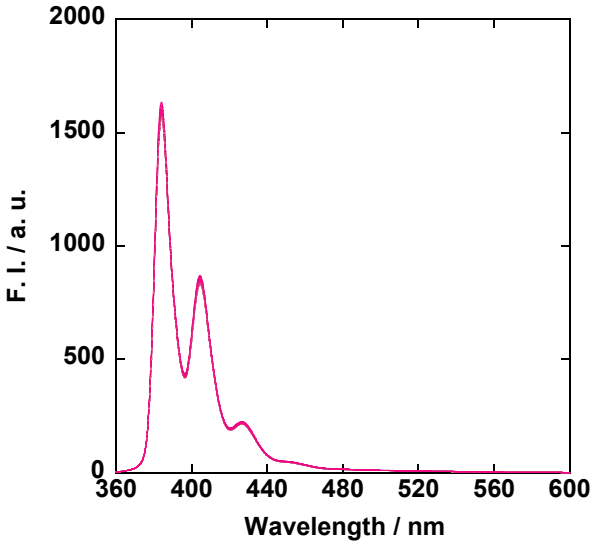


Figure S12. Change in fluorescence spectra of **Zn-1/FBγCyD** with increasing Pi concentration in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

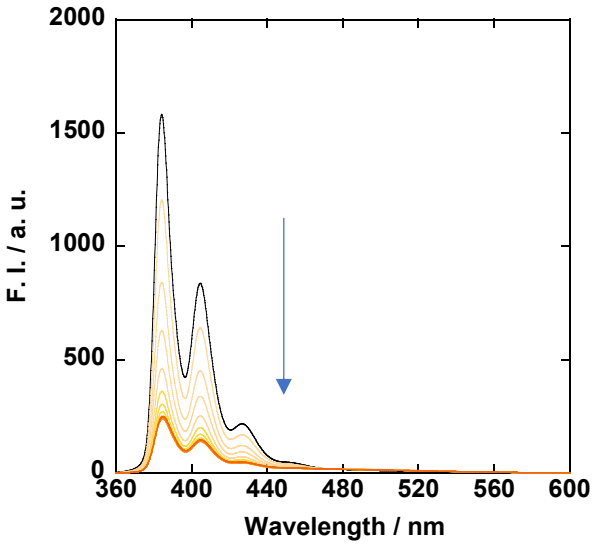


Figure S13. Change in fluorescence spectra of **Zn-1/FBγCyD** with increasing PPi concentration in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

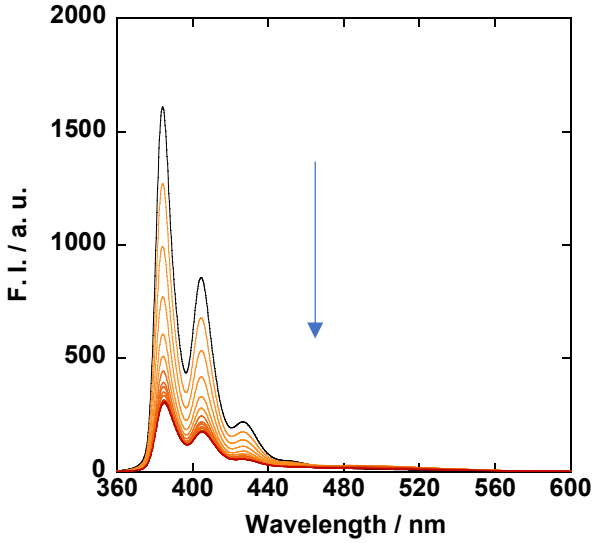


Figure S14. Change in fluorescence spectra of **Zn-1/FBγCyD** with increasing Tri concentration in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

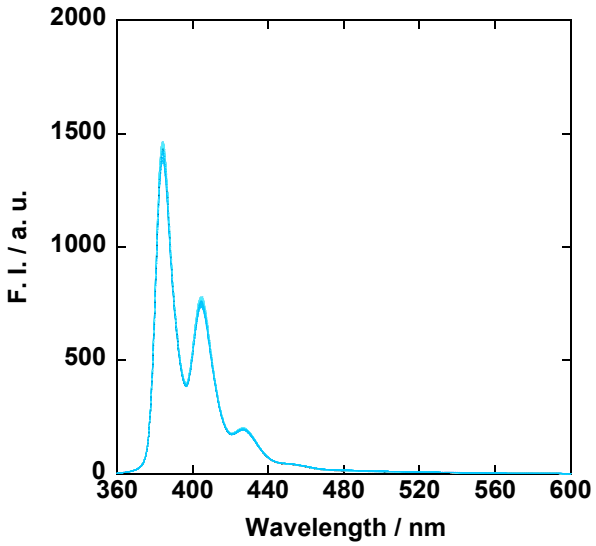


Figure S15. Change in fluorescence spectra of **Zn-1/FBγCyD** with increasing AMP concentration in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

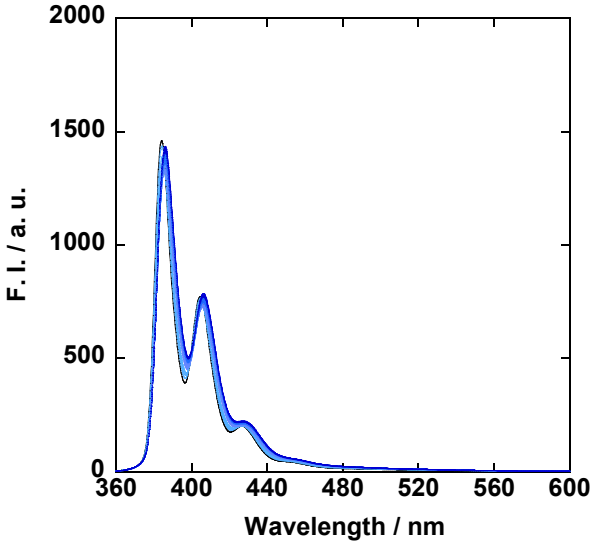


Figure S16. Change in fluorescence spectra of **Zn-1/FBγCyD** with increasing ADP concentration in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

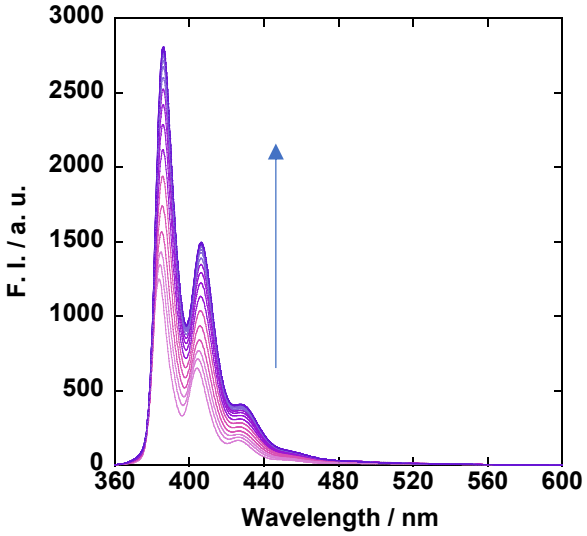


Figure S17. Change in fluorescence spectra of **Zn-1/FBγCyD** with increasing ATP concentration in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

Determination of conditional equilibrium constant

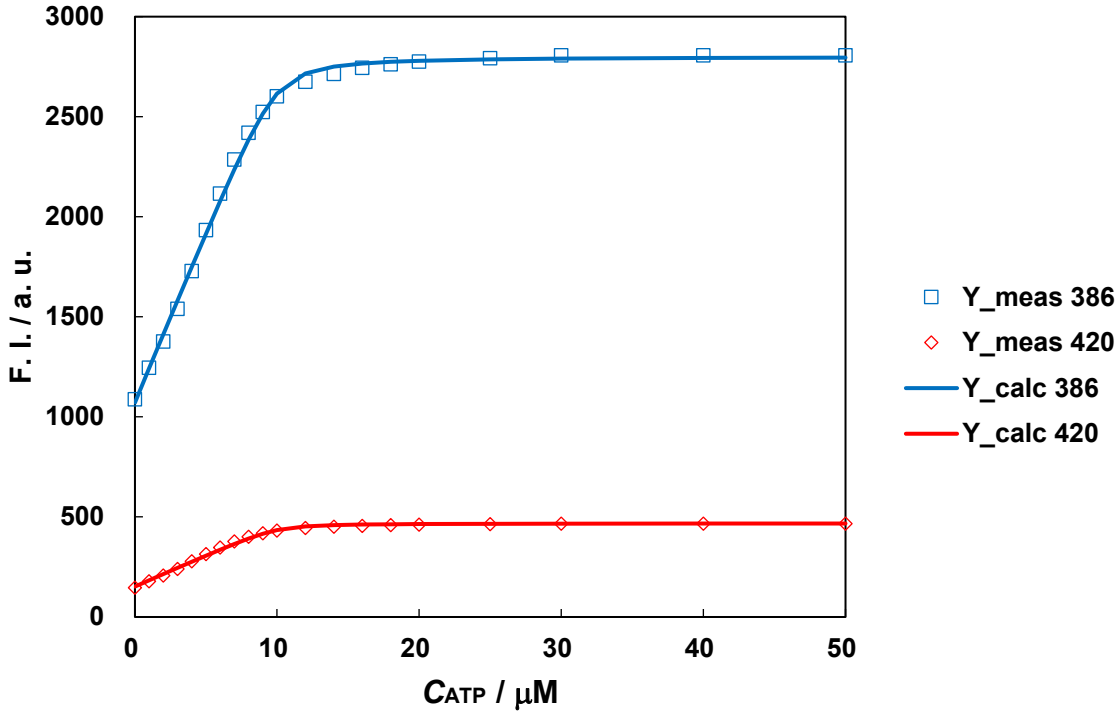


Figure S18. Fluorescence intensities of **Zn-1/FBγCyD** at various ATP concentrations in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm. The plots indicate experimental values. Solid lines indicate fitting values determined by the ReactLab program based on a 1:1 stoichiometric reaction model.

Determination of limit of detection

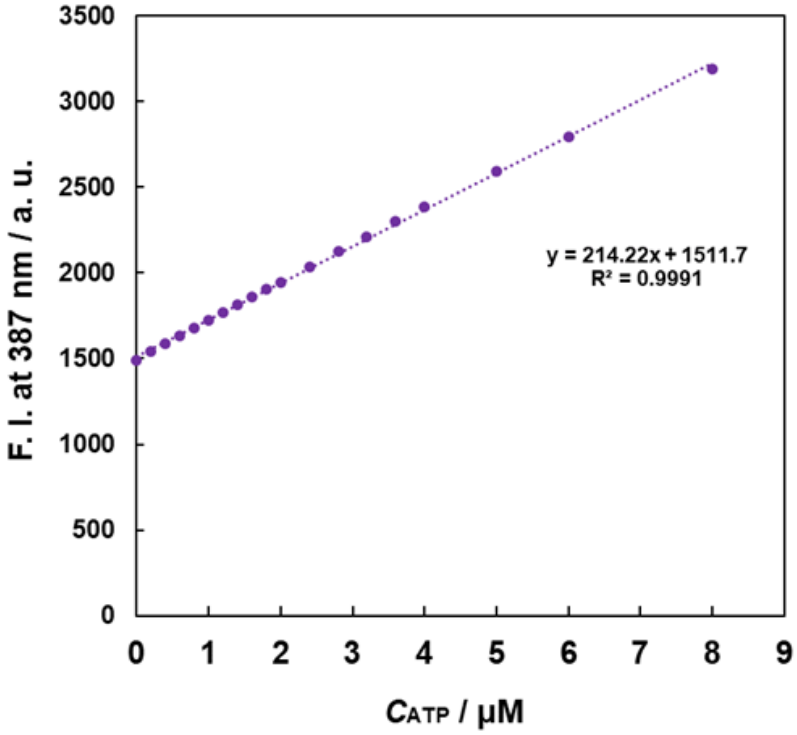


Figure S19. Calibration curve of **Zn-1/FBγCyD** for the detection of ATP in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

The limit of detection (LOD) of **Zn-1/FBγCyD** was calculated as the following equation.

$$\mathrm{LOD} = \frac{3\sigma}{a} \tag{S5}$$

In Eq. S5, a denotes the slope of the calibration curve for the quantification of ATP (Figure S19). The σ value indicates the standard deviation of the blank data corresponding to the fluorescence intensity of **Zn-1/FBγCyD** in the absence of ATP. The σ value was calculated according to Eq. S6.

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (I_{\mathrm{B,i}} - I_{\mathrm{B,ave}})^2}{n-1}} \tag{S6}$$

where $I_{B,i}$, $I_{B,ave}$ and n represent the fluorescence intensity of the blank sample, the average of $I_{B,I}$, and the number of repeated measurements (n = 10), respectively. The σ value was determined to be 1.350.

Fitting for the systems of the PPi and Tri additions

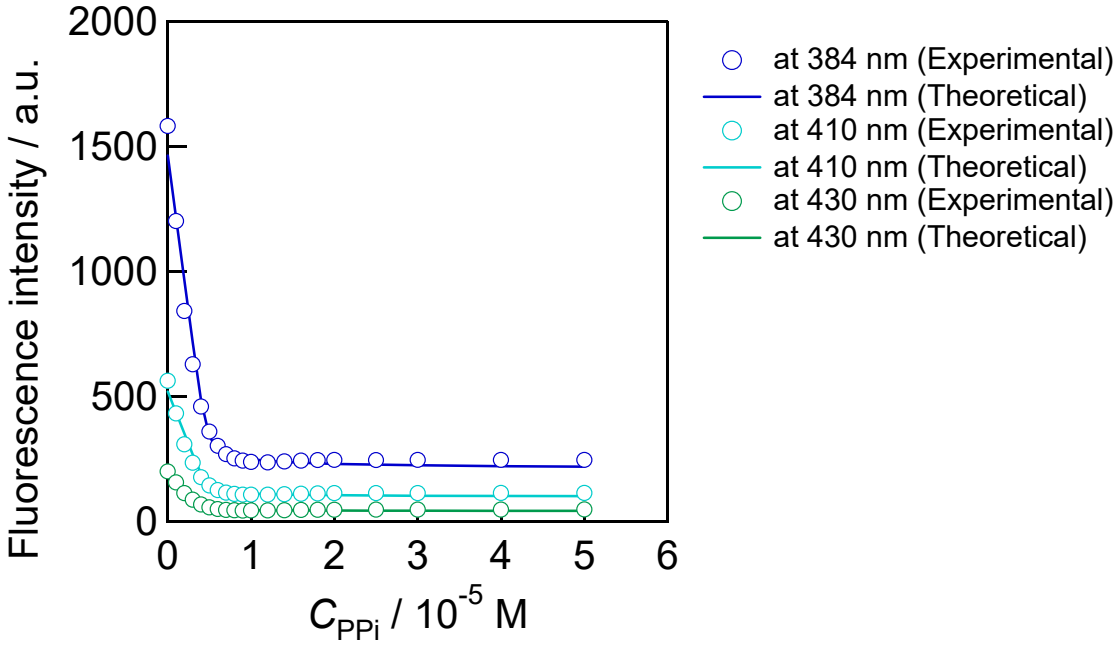


Figure S20. Fluorescence intensities of **Zn-1/FBγCyD** at various PPi concentrations in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm. The plots indicate experimental values. Solid lines indicate fitting values determined by the ReactLab program based on a 2:1 stoichiometric reaction model with the conditional equilibrium constant of (7.31 ± 0.24) × 10^{12} M^{-2}.

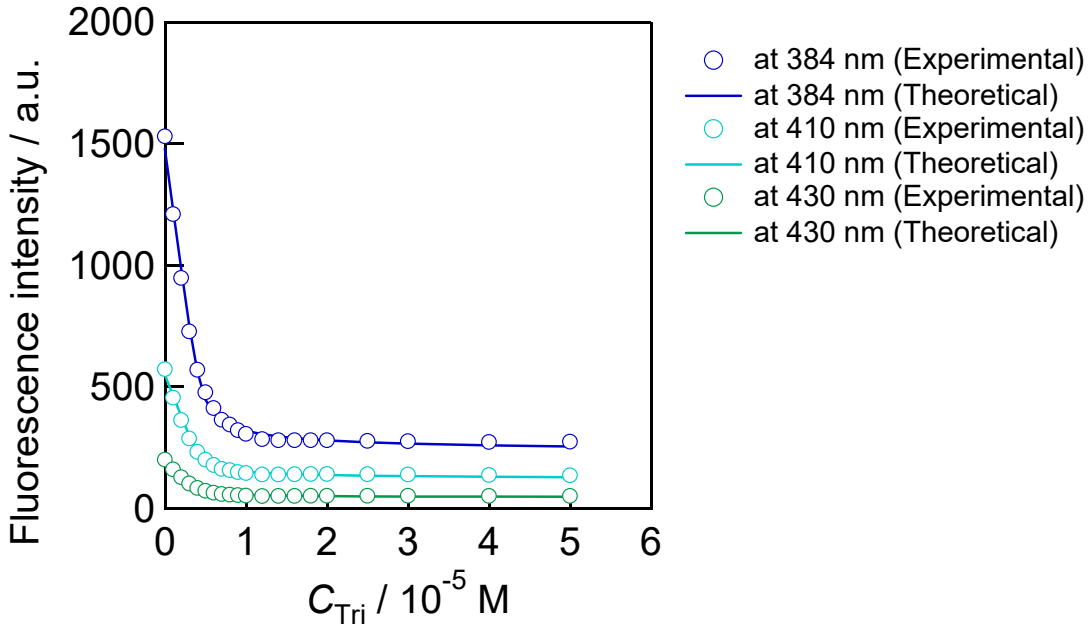


Figure S21. Fluorescence intensities of **Zn-1/FBγCyD** at various Tri concentrations in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm. The plots indicate experimental values. Solid lines indicate fitting values determined by the ReactLab program based on a 2:1 stoichiometric reaction model with the conditional equilibrium constant of (1.36 ± 0.01) × 10^{12} M^{-2}.

Competitive experiment

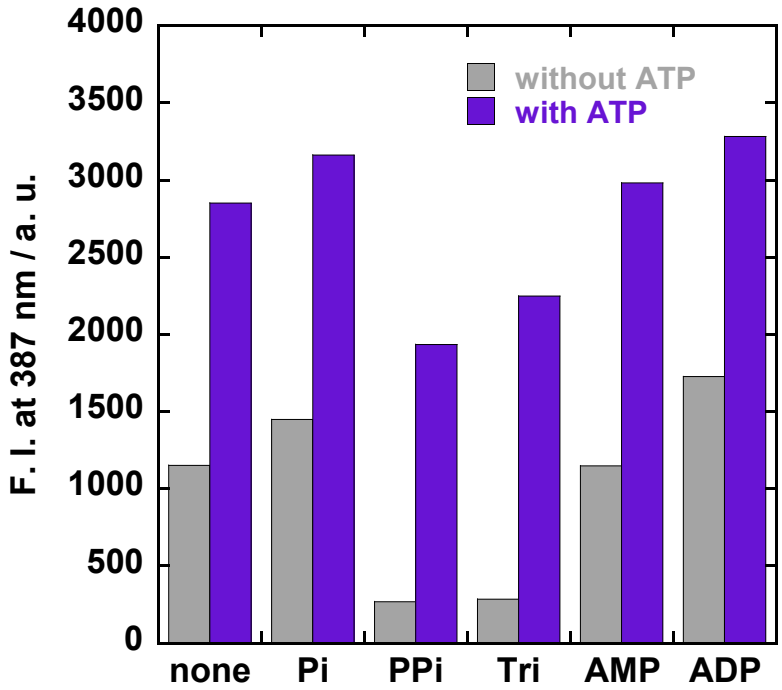


Figure S22. Fluorescence intensity at 387 nm of **Zn-1/FBγCyD-ATP** in the absence and presence of various 0.10 mM of phosphate derivatives in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{ATP} = 0.10 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

Fluorescence response of Zn-1 for phosphate derivatives

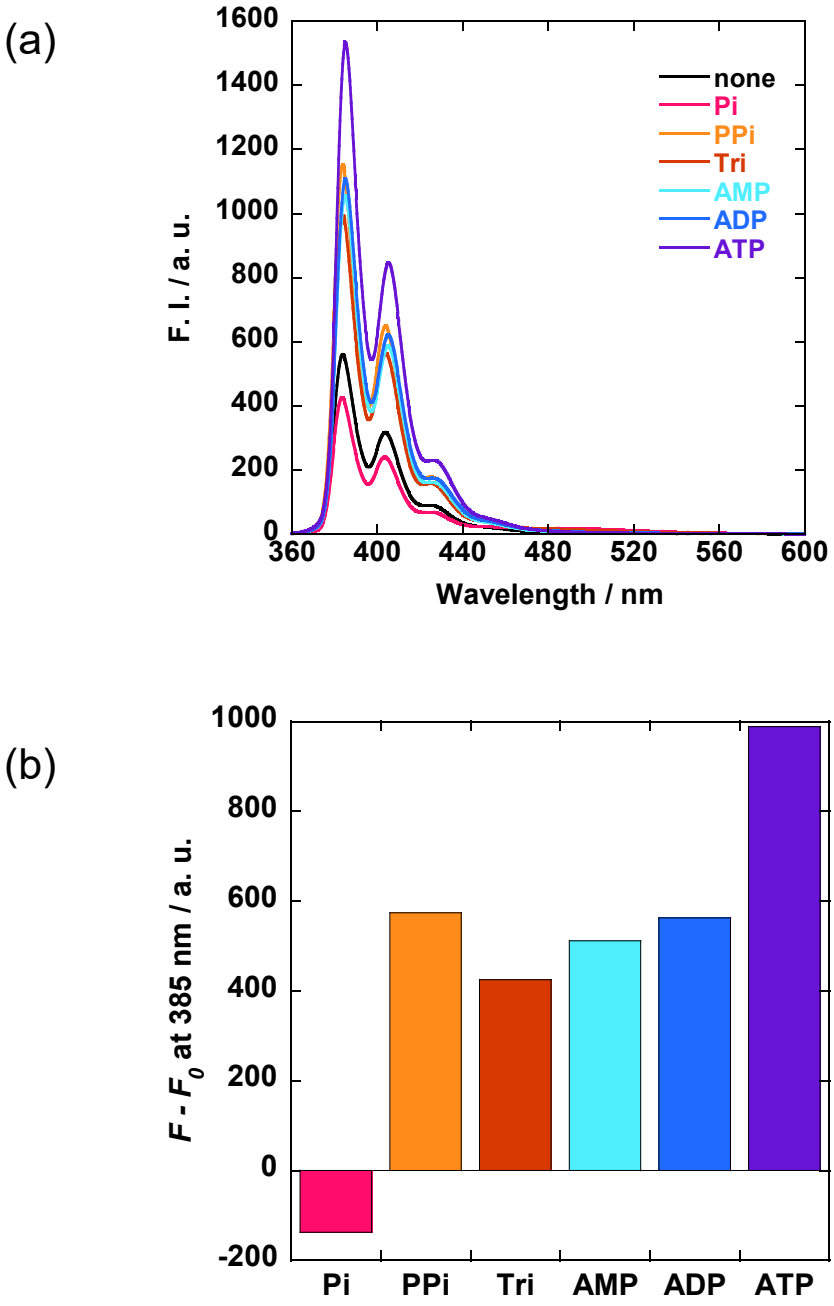


Figure S23. Fluorescence spectra of **Zn-1** in the absence and presence of various 1.0 mM of phosphate derivatives in DMSO/water (1/99 in vol.) (a) and the difference of the fluorescence intensity at 385 nm of **Zn-1** before and after the addition of the phosphate derivatives (b): C_1 = 10 μM, C_{Zn} = 10 μM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

Fluorescence response of Zn-1/γCyD for phosphate derivatives

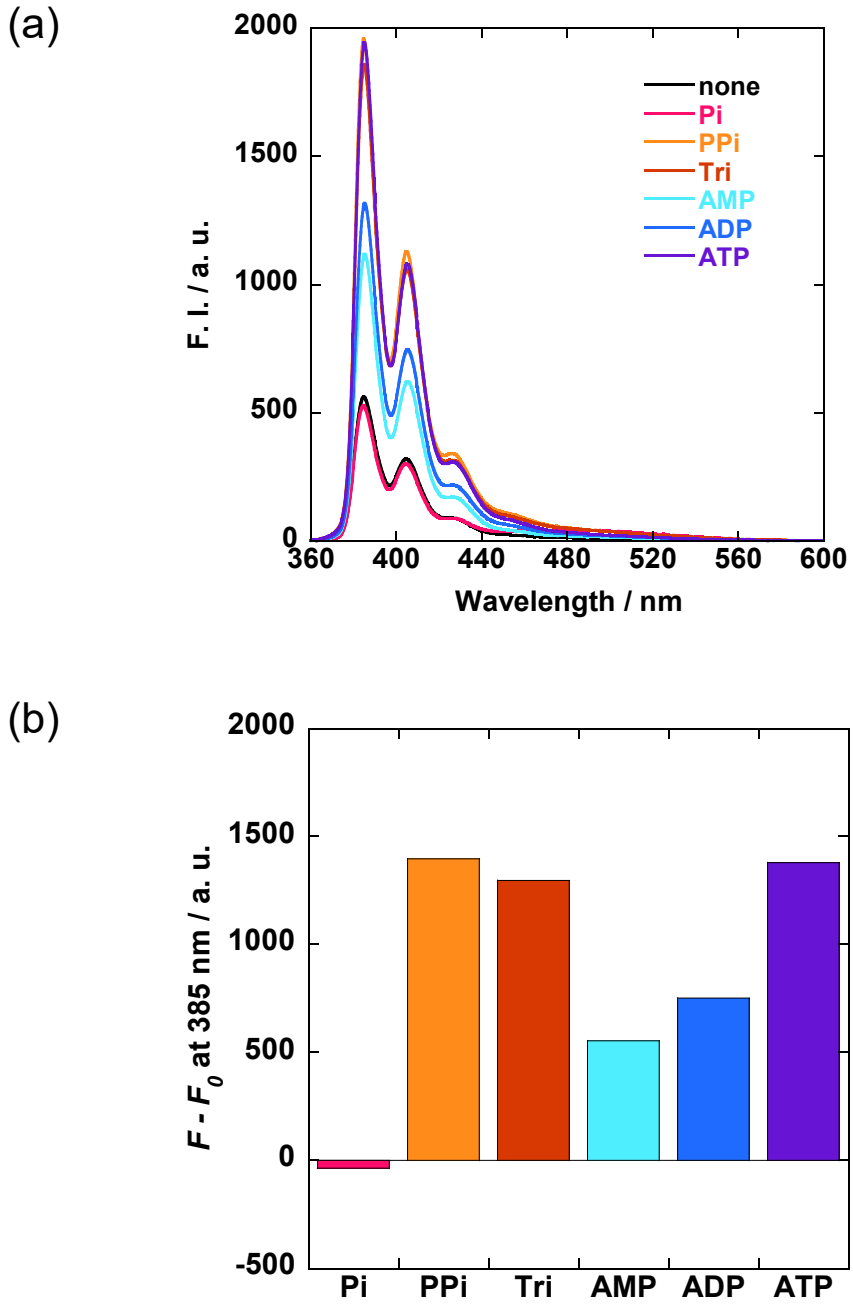


Figure S24. Fluorescence spectra of **Zn-1/γCyD** in the absence and presence of various 1.0 mM of phosphate derivatives in DMSO/water (1/99 in vol.) (a) and the difference of the fluorescence intensity at 385 nm of **Zn-1/γCyD** before and after the addition of the phosphate derivatives (b): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\gamma CyD}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

Fluorescence response of Zn-1/PhyCyD for phosphate derivatives

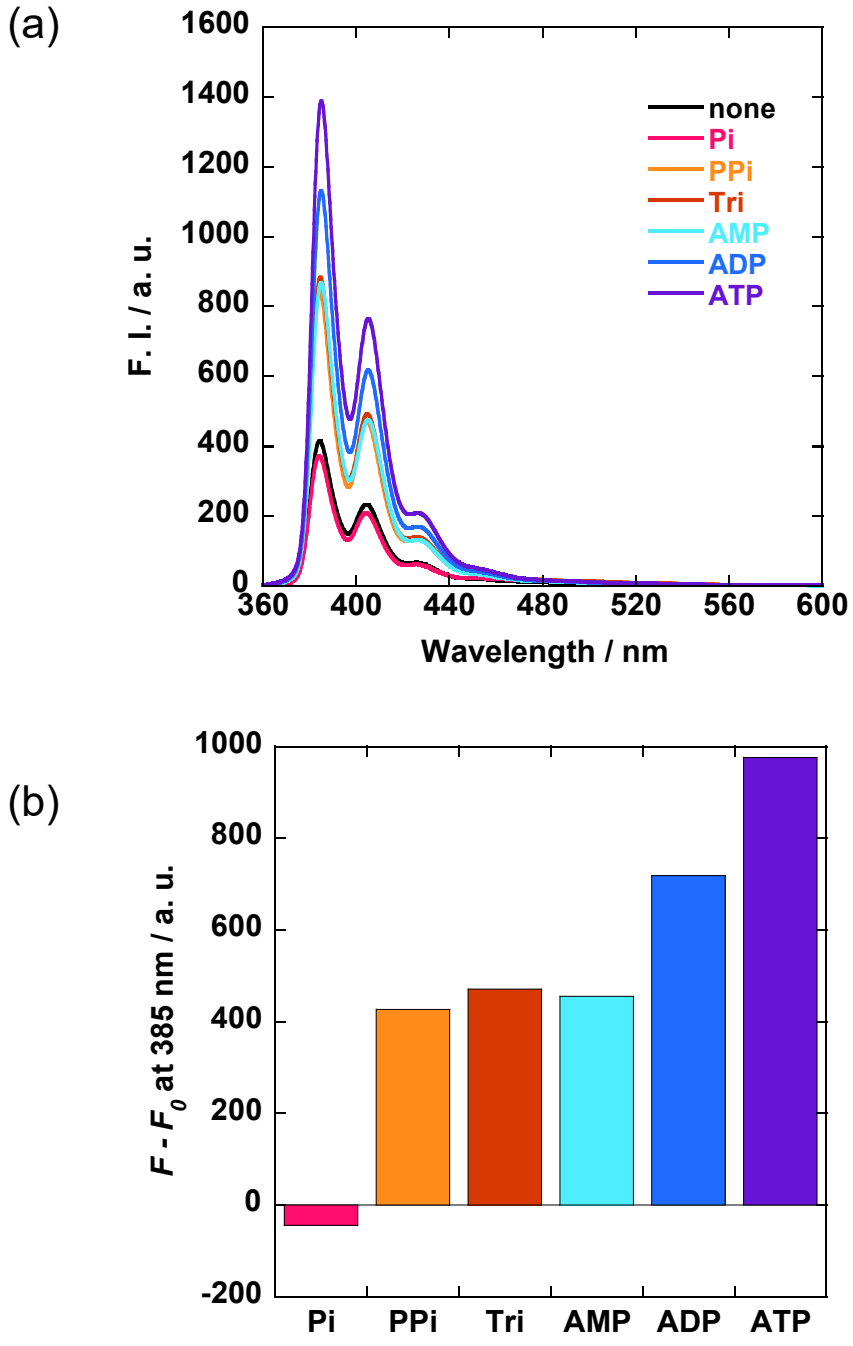


Figure S25. Fluorescence spectra of **Zn-1/PhγCyD** in the absence and presence of various 1.0 mM of phosphate derivatives in DMSO/water (1/99 in vol.) (a) and the difference of the fluorescence intensity at 385 nm of **Zn-1/PhγCyD** before and after the addition of the phosphate derivatives (b): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{PhγCyD}}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

Comparison of fluorescence spectra in the presence of ATP

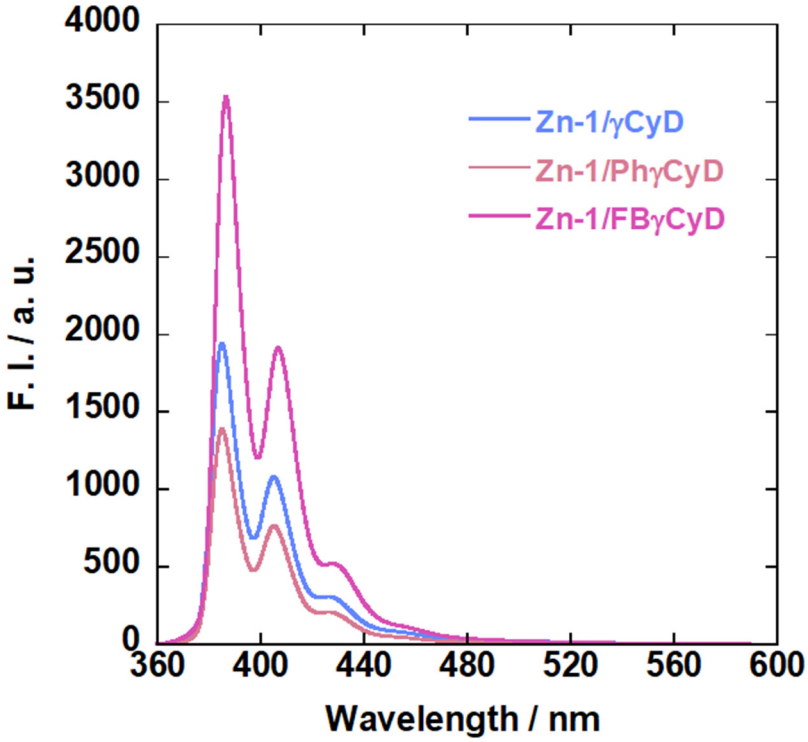
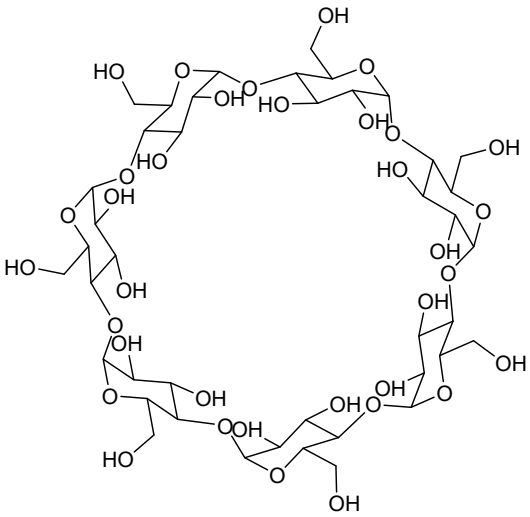
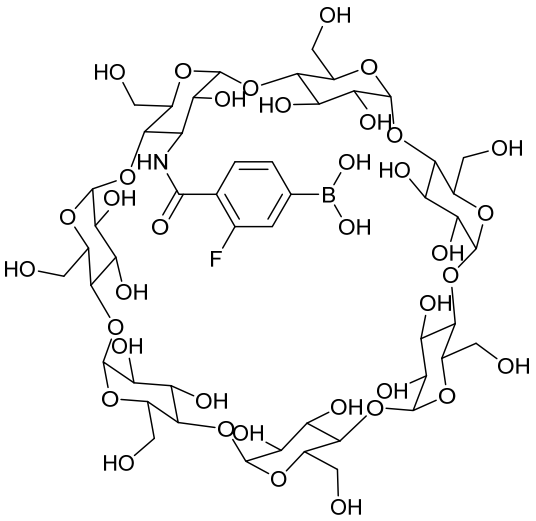


Figure S26. Fluorescence spectra of **Zn-1/γCyD**, **Zn-1/PhγCyD**, and **Zn-1/FBγCyD** in the presence of ATP (1 mM) in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{CyD}}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

Effect of CyD ring size on selectivity



βCyD



FBβCyD

Chart S3. The structures of **βCyD** and **FBβCyD**. **FBβCyD** was synthesised according to our previous report.[2] The synthesis was confirmed by ^{1}H NMR spectrum and HRMS-ESI(−).

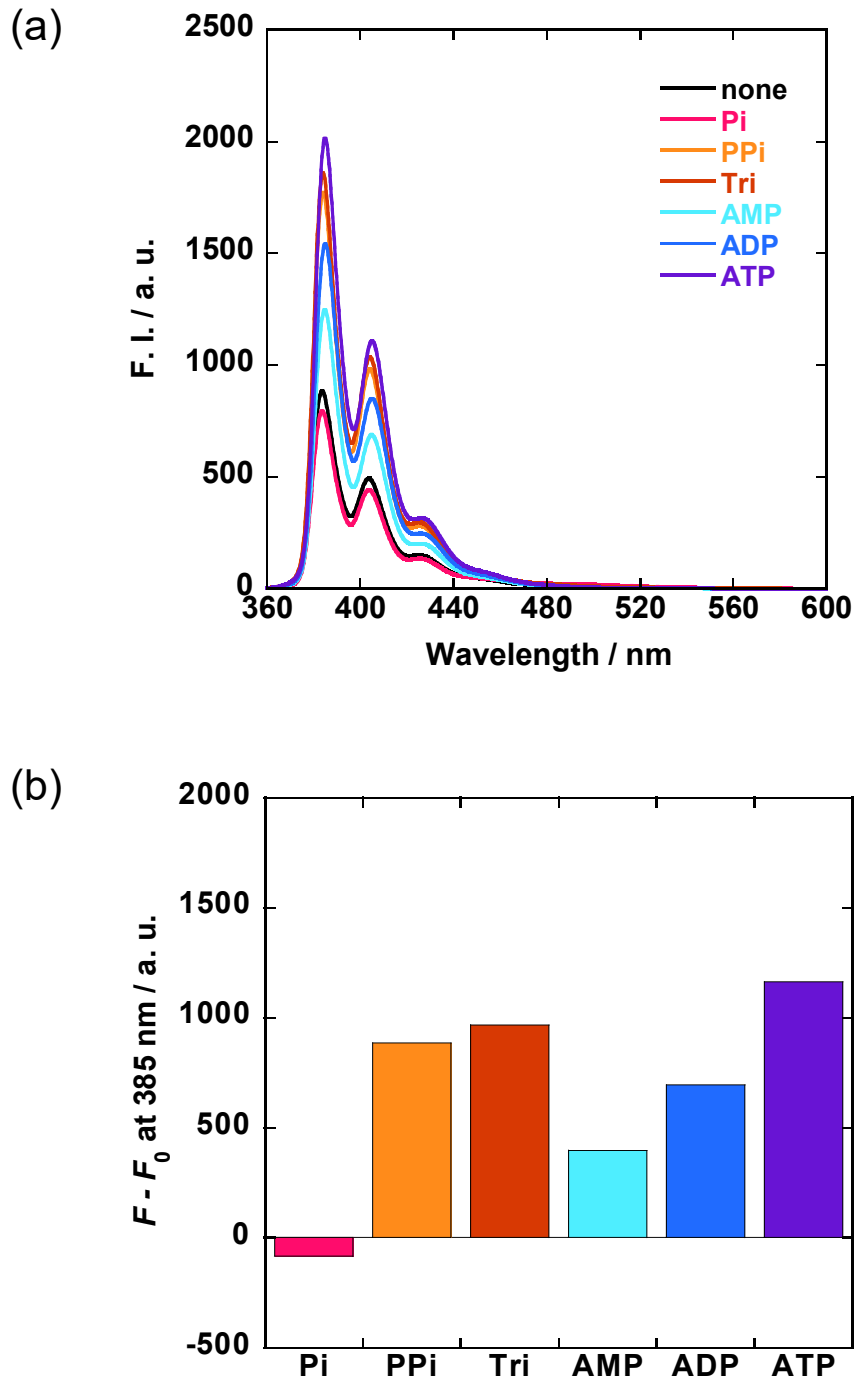


Figure S27. Fluorescence spectra of **Zn-1/βCyD** in the absence and presence of various 1.0 mM of phosphate derivatives in DMSO/water (1/99 in vol.) (a) and the difference of the fluorescence intensity at 385 nm of **Zn-1/βCyD** before and after the addition of the phosphate derivatives (b): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\beta CyD}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

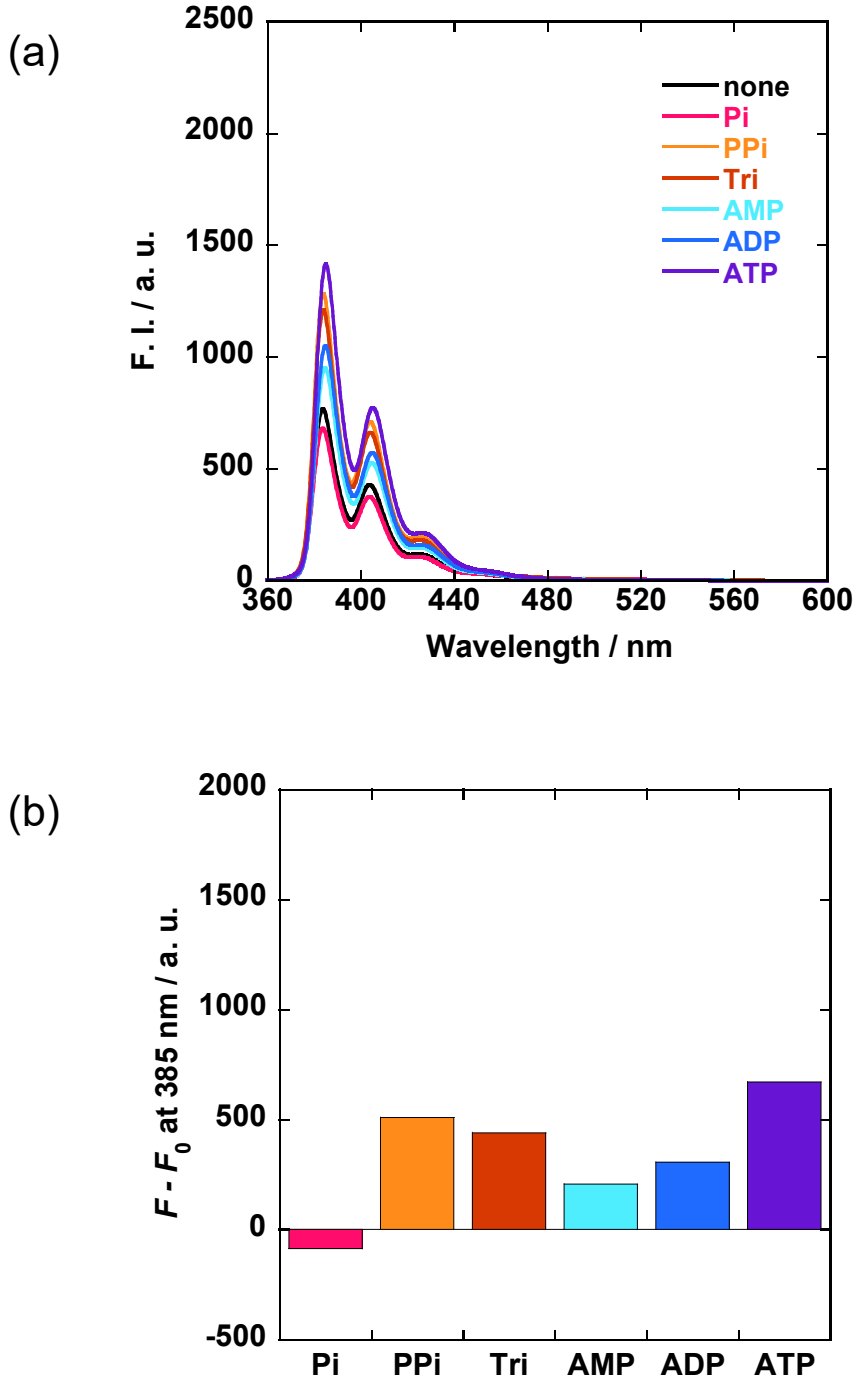


Figure S28. Fluorescence spectra of **Zn-1/FBβCyD** in the absence and presence of various 1.0 mM of phosphate derivatives in DMSO/water (1/99 in vol.) (a) and the difference of the fluorescence intensity at 385 nm of **Zn-1/FBβCyD** before and after the addition of the phosphate derivatives (b): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\beta CyD}}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

ICD spectra

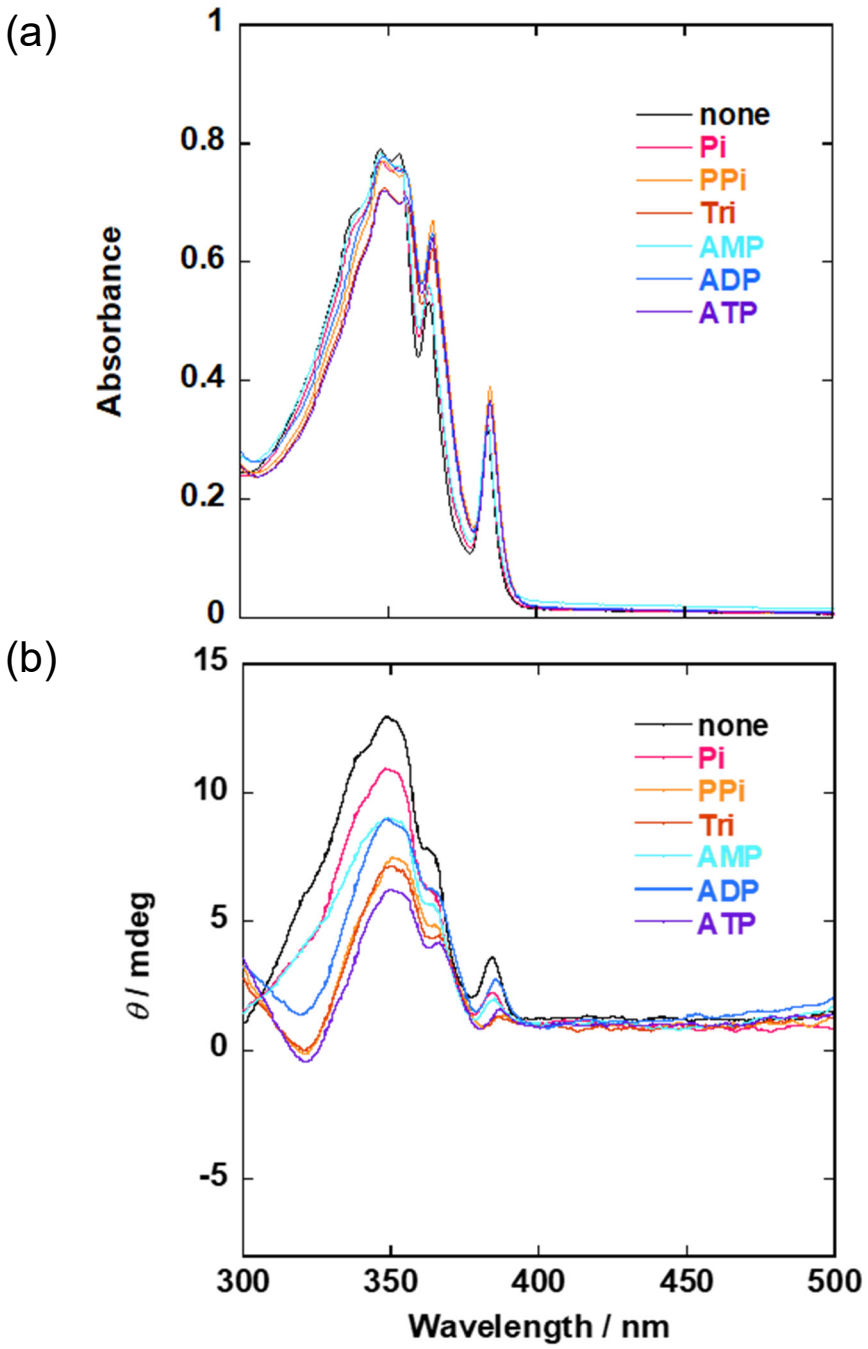


Figure S29. UV-vis absorption (a) and ICD (b) spectra of **Zn-1/FBγCyD** in the absence and presence of various 1.0 mM of phosphate derivatives in DMSO/water (1/99 in vol.): C_1 = 40 μM, C_{Zn} = 40 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.5 mM, C_{HEPES} = 5 mM, pH 7.4, and T = 25°C.

Robustness against apyrase

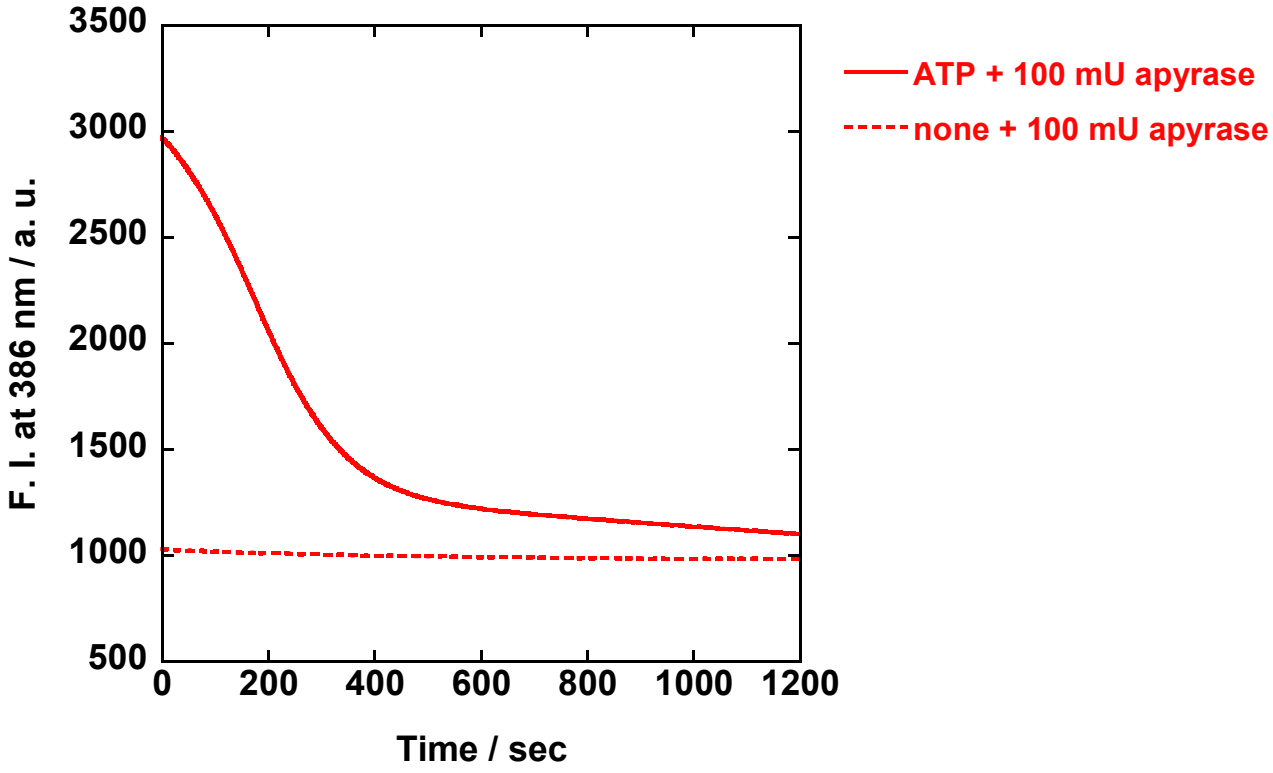


Figure S30. Changes in the fluorescence intensity at 386 nm of **Zn-1/FBγCyD** in the absence and presence of ATP (30 μM) after the additions of apyrase (100 mU) in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

Fluorescence after the apyrase addition

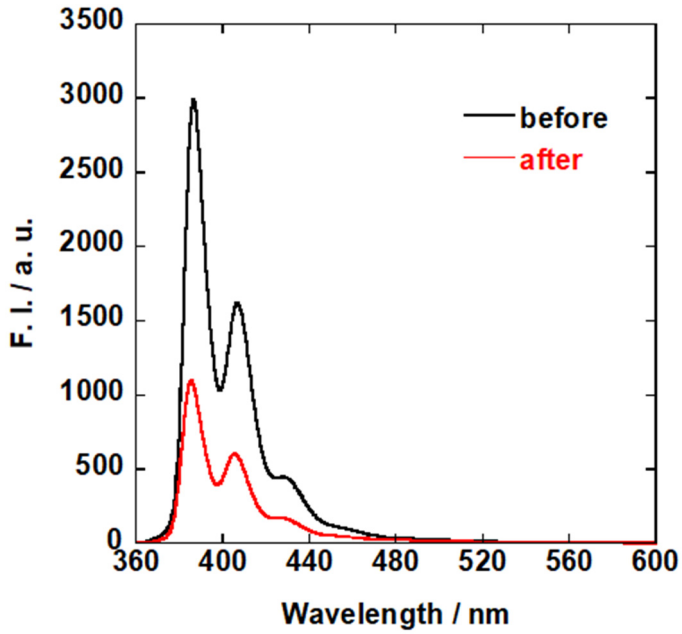


Figure S31. Fluorescence spectra of **Zn-1/FBγCyD** with ATP before and 1200 seconds after the addition of apyrase (100 mU) in DMSO/water (1/99 in vol.): C_1 = 10 μM, C_{Zn} = 10 μM, C_{ATP} = 30 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, T = 25°C, and λ_{ex} = 350 nm.

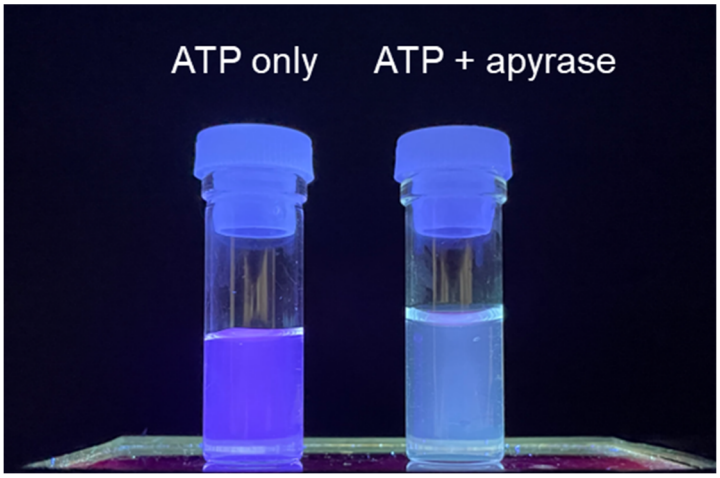


Figure S32. Photograph of the fluorescence of **Zn-1/FBγCyD** with ATP before and 1200 seconds after the addition of apyrase (100 mU) in DMSO/water (1/99 in vol.) with 365 nm UV light: C_1 = 10 μM, C_{Zn} = 10 μM, C_{ATP} = 30 μM, $C_{\mathbf{FB\gamma CyD}}$ = 0.1 mM, C_{HEPES} = 5 mM, pH 7.4, and T = 25°C

1 H. Liang, Z. Yao, W. Ge, Y. Qiao, L. Zhang, Z. Cao and H. C. Wu, Selective and sensitive detection of picric acid based on a water-soluble fluorescent probe, *RSC Adv.*, 2016, **6**, 38328–38331.

2 K. Aoki, R. Osako, J. Deng, T. Hayashita, T. Hashimoto and Y. Suzuki, Phosphate-sensing with (di-(2-picolyl)amino)quinazolines based on a fluorescence on-off system, *RSC Adv.*, 2020, **10**, 15299–15306.