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Supporting Information

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for

Design of Dual Emission Chemosensors for Ratiometric Detection of ATP Derivatives

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Synthesis of the Chemosensor (1-2Zn(II))

4,5-Dimethyl-9-chloroacridine (6a)

Compound **6a** was prepared from **5a** according to the procedure reported by Newman et al.[11] **5a** (4.0 g, 16 mmol) was dissolved in phosphoryl chloride (15 g),, and the mixture was refluxed for 2 h. After removal of phosphoryl chloride in vacuo, the residue was dissolved in THF and poured into cold aqueous 1N NaOH. The resulting mixture was extracted with AcOEt (x2). The combined organic phase was washed with water and brine followed by drying over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel (hexane : THF = 2 : 1) to give **6a** (2.14 g, 53%) as a bright yellow powder: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.94 (6H, s), 7.50-7.54 (2H, m), 7.64 (2H, d, *J* = 6.8 Hz), 8.27 (2H, d, *J* = 8.8 Hz); FAB-MS $C_{15}H_{12}ClN$: *m/e* 241 [M+H]$^+$.

4,5-Dibromomethyl-9-chloro-acridine (7a)

A suspension of **6a** (1.0 g, 4.14 mmol), *N*-bromosuccinimide (1.62 g, 9.10 mmol), and benzoyl peroxide (200 mg, 0.82 mmol) in CCl_4 (100 mL) was refluxed for 12 h with stirring. After removal of the solvent in vacuo, the residue was dissolved in CH_2Cl_2. The organic phase was successively washed with 1N NaOH (x2), water, and brine followed by drying over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel (hexane : CH_2Cl_2 = 3 : 1). The obtained solid was recrystallization from AcOEt to afford **7a** (511 mg, 31%) as yellow needles: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.41 (4H, s), 7.62 (2H, dd, *J* = 6.8, 8.8 Hz), 7.98 (2H, d, *J* = 6.8 Hz), 8.44 (2H, d, *J* = 8.8 Hz). FAB-MS $C_{15}H_{10}ClBr_2N$: *m/e* 399 [M]$^+$.

4,5-Bis[(2,2'-dipicolylamino)methyl]-9-chloro-acridine (1)

A mixture of **7a** (180 mg, 0.45 mmol), 2,2'-dipicolylamine (188 mg, 0.95 mmol), K_2CO_3 (187 mg, 1.35 mmol) in anhydrous DMF (4 mL) was stirred for 3 h at rt. After dilution with water, the resulting mixture was extracted with AcOEt (x2). The combined organic phase was washed water and brine followed by drying over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel (CH_2Cl_2 : MeOH : NH_3 = 400 : 10 : 1 → 300 : 10 : 1) to

give **1** (234 mg, 82%) as a yellow amorphous powder: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.95 (8H, s), 4.58 (4H, s), 7.21 (4H, t, *J* = 5.2 Hz), 7.64-7.72 (10H, m), 8.12 (2H, d, *J* = 6.0 Hz), 8.32 (2H, d, *J* = 8.8 Hz), 8.37 (4H, d, *J* = 5.2 Hz). FAB-MS $C_{39}H_{34}ClN_7$: *m/e* 636 [M+H]$^+$.

4,5-Bis[(2,2'-dipicolylamino)methyl]-9-chloro-acridine Zn(II) complex (1-2Zn(II))

To a solution of **1** (100 mg, 0.16 mmol) in distilled MeOH (10 mL) was added aqueous $Zn(NO_3)_2$ solution (300 mM; 1.05 mL, 0.31 mmol), and the mixture was stirred for 30 min at rt. After removal of the solvent in vacuo, the residue was dissolved in distilled water, and the solution was filtered through cellulose acetate filter (pore size; 0.45 μm) and then lyophilized. The obtained solid was washed with MeOH by filtration to afford **1**-2Zn(II) (69 mg, 42%) as a yellow powder: ^{1}H-NMR (400 MHz, CD_3OD) δ 3.85 (4H, d, *J* = 16.0 Hz), 4.15 (4H, d, *J* =16.0 Hz), 4.36 (4H, s), 6.98 (4H, d, *J* = 7.6 Hz), 7.63 (4H, t, *J* = 5.6 Hz), 7.79 (2H, dd, *J* = 6.8, 8.8 Hz), 7.88 (4H, t, *J* = 7.6 Hz), 8.04 (2H, d, *J* = 5.6 Hz), 8.56 (2H, d, *J* = 8.0 Hz), 8.71 (4H, d, *J* = 4.8 Hz). FAB-MS $C_{39}H_{34}ClN_7$•2Zn•3NO_3: *m/e* 949 [M-NO_3]$^+$. Anal Calcd for $C_{39}H_{34}ClN_7$•2Zn•4NO_3•H_2O: C, 45.35; H, 3.51; N, 14.92. Found: C, 45.38; H, 3.40; N, 14.83.

Synthesis of the chemosensor 3-Zn(II)

The chemosensor **3**-Zn(II) was synthesized according to the same procedure descried for the synthesis of **1**-2Zn(II). The spectrum data of **3**-Zn(II) and the synthetic intermediates are as follows:

9-Chloro-4-methylacridine (6b): **6b** was synthesized from **5b** according to the procedure reported by Newman et al[11]: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.94 (3H, s), 7.52 (1H, dd, *J* = 6.6 , 8.6 Hz), 7.61-7.65 (2H, m), 7.77-7.81 (1H, m), 8.27 (1H, d, *J* = 8.8 Hz), 8.29 (1H, d, *J* = 8.8 Hz), 8.42 (1H, dm, *J* = 8.8 Hz). FAB-MS $C_{14}H_{10}ClN$: *m/e* 228 [M+H]$^+$.

4-Bromomethyl-9-chloro-acridine (7b): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.40 (2H, s), 7.61 (1H, dd, *J* = 6.8, 8.8 Hz), 7.65-7.70 (1H, m), 7.83-7.87 (1H, m), 7.96 (1H, d, *J* = 6.8 Hz), 8.31 (1H, d, J = 8.8 Hz), 8.43-8.46 (2H, m). FAB-MS $C_{14}H_9BrClN$: *m/e* 308 [M+H]$^+$.

4-[(2,2'-Dipicolylamino)methyl]-9-chloro-acridine (3): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.03 (4H, s), 4.65 (2H, s), 7.11-7.14 (2H, m), 7.61-7.66 (4H, m), 7.72 (2H, d, *J* = 7.6 Hz), 7.77-7.81 (1H, m), 8.17 (2H, t, *J* = 9.2 Hz), 8.35 (1H, d, *J* = 8.8 Hz), 8.42 (1H, d, *J* = 8.4 Hz), 8.53 (2H, d, *J* = 4.0 Hz). FAB-MS $C_{26}H_{21}ClN_4$: *m/e* 425 $[M+H]^+$.

4-[(2,2'-Dipicolylamino)methyl]-9-chloro-acridine Zn complex (3-Zn(II)): ^{1}H-NMR (400 MHz, CD_3OD) δ 4.12 (2H, d, *J* = 16.0 Hz), 4.59-4.63 (4H, m), 7.52 (2H, d, *J* = 8.0 Hz), 7.62 (2H, t, *J* = 6.4 Hz), 7.72 (1H, t, *J* = 7.6 Hz), 7.83 (1H, t, *J* = 7.6 Hz), 7.92 (1H, d, *J* = 6.8 Hz), 7.99-8.07 (2H, m), 8.12 (2H, t, *J* = 8.0 Hz), 8.51-8.56 (4H, m). FAB-MS $C_{26}H_{21}ClN_4$: *m/e* 550 $[M-NO_3]^+$. Anal Calcd for $C_{26}H_{21}ClN_4 \bullet Zn \bullet 2NO_3$: C, 50.83; H, 3.45; N, 13.68. Found: C, 50.88; H, 3.50; N, 13.46.

Synthesis of the chemosensor 2-2Zn(II)

3-(4,5-Dimethyl-acridin-9-yl)-benzoic acid ethyl ester (6c)

A mixture of **6a** (600 mg, 2.48 mmol), ethyl 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (1.04 g, 3.75 mmol), $Pd_2(dba)_3$ (114 mg, 0.124 mmol), 2-(di-*tert*-butylphosphino)biphenyl (90 mg, 0.30 mmol), and Cs_2CO_3 (2.42 g, 7.44 mmol) in anhydrous DMF (30 mL) was heated at 80 °C for 1 h under Ar. After dilution with water, the resulting mixture was extracted with AcOEt (x2). The combined organic phase was washed with water and brine followed by drying over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel (hexane : AcOEt = 40 : 1). The obtained solid was washed with hexane to afford **6c** (577 mg, 65%) as a pale yellow powder: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, *J* = 7.2 Hz), 3.00 (6H, s), 4.39 (2H, q, *J* = 7.2 Hz), 7.31 (2H, dd, *J* = 6.4, 8.8 Hz), 7.42 (2H, d, *J* = 8.0 Hz), 7.58-7.62 (3H, m), 7.67 (1H, t, *J* = 7.6 Hz), 8.10 (1H, m), 8.25 (1H, dm, *J* = 7.6 Hz). FAB-MS $C_{24}H_{21}NO_2$: *m/e* 356 $[M+H]^+$.

3-(4,5-Dibromomethyl-acridin-9-yl)-benzoic acid ethyl ester (7c)

A suspension of **6c** (550 mg, 1.55 mmol), *N*-bromosuccinimide (607 mg, 3.41 mmol) in CCl_4 (40 mL) was refluxed for 2.5 h, during which time a catalytic amount of benzoyl peroxide (ca. 30 mg) was added each 30 min. After removal of the solvent

in vacuo, the residue was dissolved in AcOEt. The solution was successively washed with 1N NaOH (x2), water, and brine followed by drying over $MgSO_4$. The solvent was removed by evaporation, and the residue was purified by flash column chromatography on silica gel (hexane : CH_2Cl_2 = 3 : 1 → 2 : 1). The obtained solid was recrystallized from AcOEt to give **7c** (327 mg, 41%) as a pale yellow powder: ^{1}H-NMR (400 MHz, $CDCl_3$) d 1.38 (3H, t, *J* = 7.2 Hz), 4.40 (2H, q, *J* = 7.2 Hz), 5.47 (2H, d, *J* = 9.6 Hz), 5.51 (2H, d, *J* = 9.6 Hz), 7.41 (2H, dd, *J* = 6.8, 8.8 Hz), 7.59-7.61 (3H, m), 7.70 (1H, t, *J* = 7.6 Hz), 7.94 (2H, dd, *J* = 1.2, 6.8 Hz), 8.10 (1H, m), 8.28 (1H, dm, *J* = 7.6 Hz). FAB-MS $C_{24}H_{19}Br_2NO_2$: *m/e* 514 [M+H]$^+$.

3-[4,5-Bis[(2,2'-dipicolylamino)methyl] -acridin-9-yl]-benzoic acid ethyl ester (2)

This compound was synthesized from **7c** by the same procedure described for the synthesis of **1**: ^{1}H-NMR (400 MHz, $CDCl_3$) d 1.37 (3H, t, *J* = 7.2 Hz), 4.02 (8H, s), 4.39 (2H, q, *J* = 7.2 Hz), 4.69 (4H, s), 7.10-7.13 (4H, m), 7.41-7.47 (4H, m), 7.58-7.70 (10H, m), 8.08 (1H, m), 8.13 (2H, d, *J* = 5.2 Hz), 8.25 (1H, d, J = 7.6 Hz), 8.52 (4H, d, *J* = 4.8 Hz). FAB-MS $C_{48}H_{43}N_7O_2$: *m/e* 750 [M+H]$^+$.

3-[4,5-Bis[(2,2'-dipicolylamino)methyl]-acridin-9-yl]-benzoic acid ethyl ester Zn complex (2-2Zn(II))

This compound was synthesized from **2** according to the same procedure described for the synthesis of **1**-2Zn(II): *m/e* 1063 [M-NO_3]$^+$. Anal Calcd for $C_{48}H_{43}N_7O_2$•2Zn•4NO_3•2H_2O: C, 49.50; H, 4.07; N, 13.23. Found: C, 49.90; H, 4.12; N, 12.94.

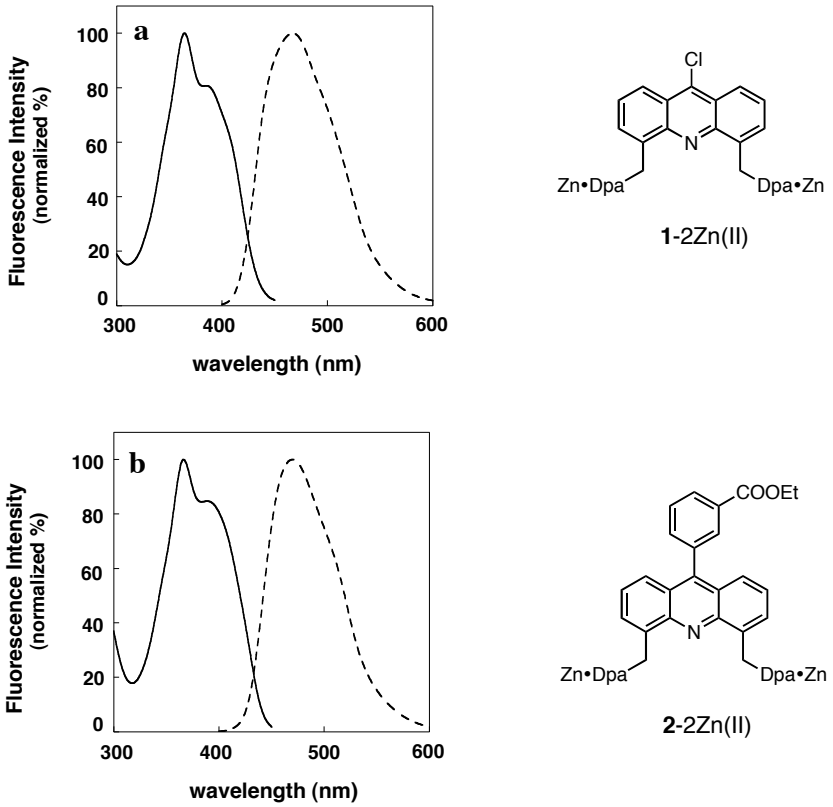

Figure S1. Excitation (solid line) and emission spectra (broken line) of the chemosensors: (a) **1**-2Zn(II) (10 μM, λ_{ex} = 368 nm, λ_{em} = 468 nm), (b) **2**-2Zn(II) (10 μM, λ_{ex} = 368 nm, λ_{em} = 468 nm). Conditions; 50 mM HEPES, pH 7.2, 20 °C.

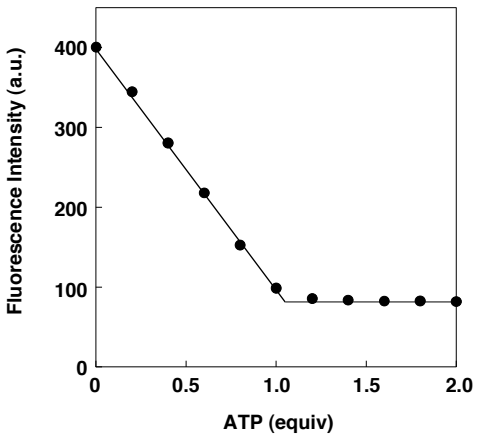

Figure S2. Molar ratio plot of the fluorescence emission (λ_{em} = 468 nm) of **1**-2Zn(II) (30 μM) with addition of ATP. Conditions; 50 mM HEPES, pH 7.2, λ_{ex} =368 nm, 20 °C.

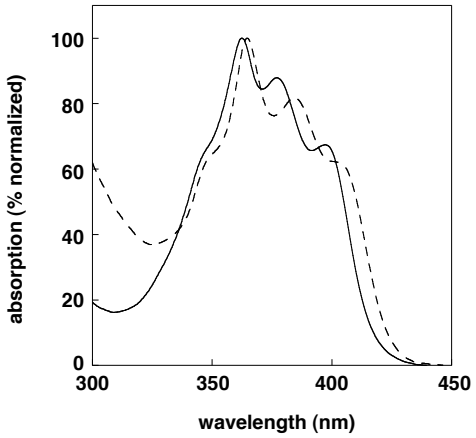

Figure S3. UV spectral of the ligand **1** under alkaline (solid line) and acidic (dashed line) conditions; [**1**] = 20 μM, aqueous 0.05 N NaOH or 0.1 N HCl solution / MeOH = 1 : 1.

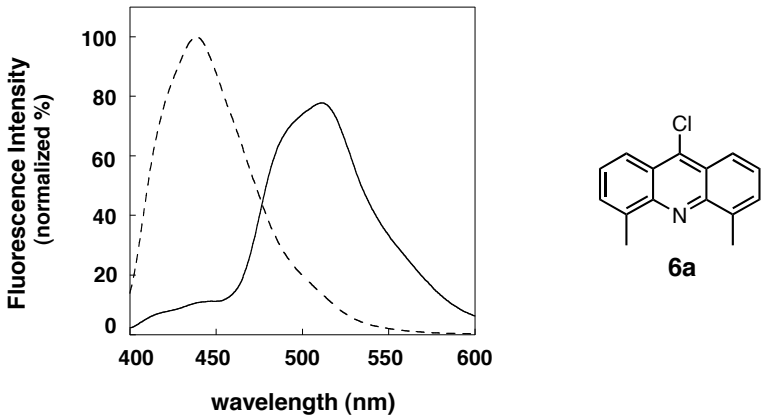

Figure S4. pH-dependent fluorescence spectra of **6a** (10 μM). The emission maximum is at 439 nm (broken line) under the alkaline conditions (0.05N NaOH / MeOH = 1 / 1 (v/v)) and at 510 nm (solid line) under the acidic conditions (0.1N HCl / MeOH = 1 / 1 (v/v)). 20 ˚C, λ_{ex} = 368 nm.

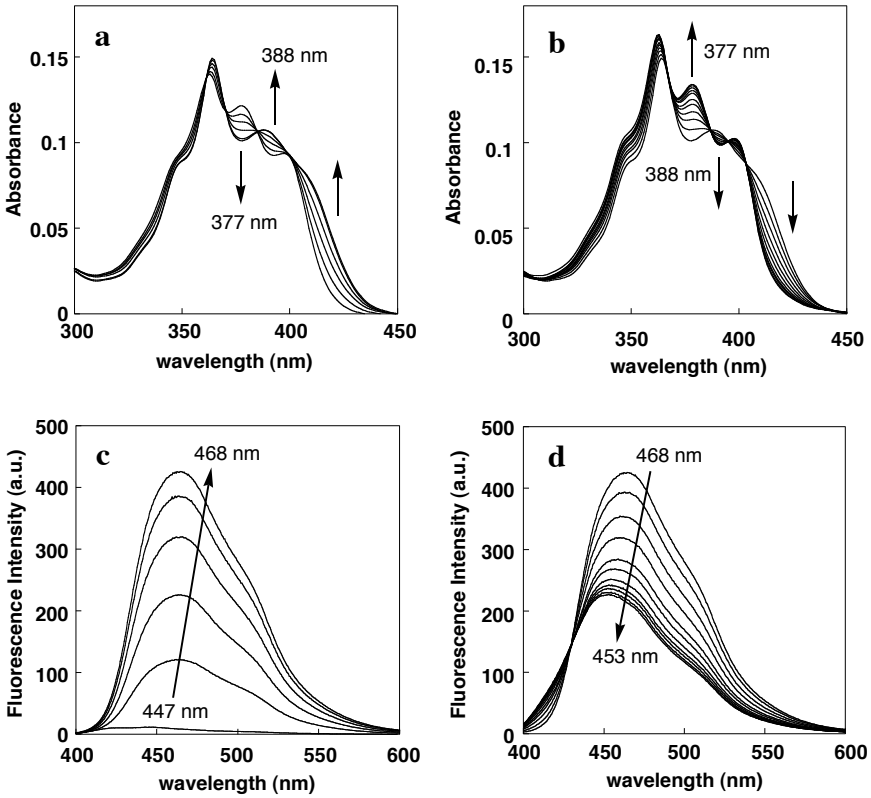

Figure S5. Zn(II)-induced UV absorption (a, b) and fluorescence emission change (λ_{ex} = 368 nm) (c, d) of the ligand **1** (20 μM) (a, c) [Zn(II)] = 0, 4, 8, 12, 16, 20 μM (0 ~ 1 equiv), (b, d) [Zn(II)] = 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60 μM (1 ~ 3 equiv) Conditions; 10 mM HEPES buffer (pH 7.2) / MeOH = 1 / 1 (v/v), 20 ˚C.

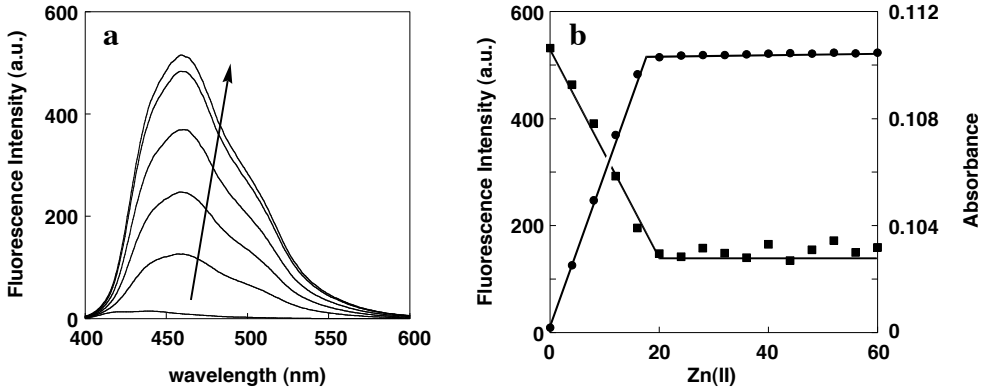

Figure S6. (a) Zn(II)-induced fluorescence emission change of the ligand **3** (20 μM, λ_{ex} = 368 nm)); [Zn] = 0, 4, 8, 12, 16, 20 μM. (b) plot of fluorescence emission (λ_{em} = 460 nm, ●) and UV absorption (375 nm, ■) change of the ligand **3** (20 μM) in the Zn(II) titration. Conditions; 10 mM HEPES buffer (pH 7.2) / MeOH = 1 / 1 (v/v) at 20 ˚C.

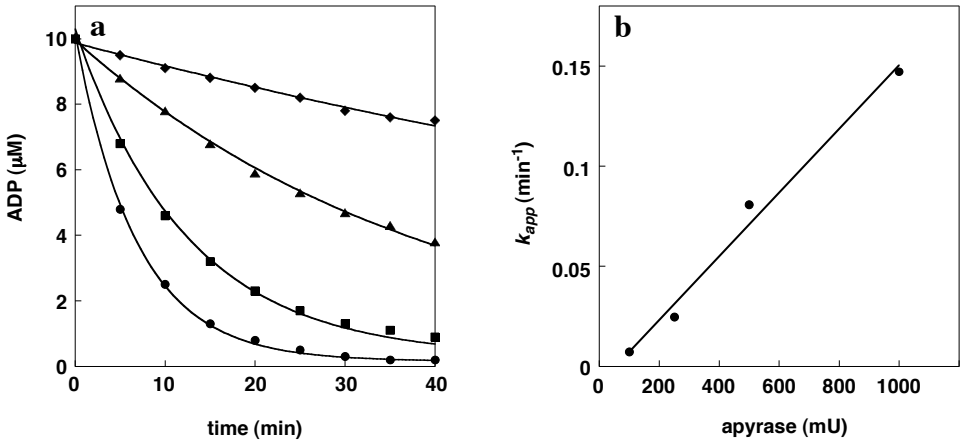

Figure S7. Fluorescence real-time detection of the apyrase-catalyzed ADP hydrolysis using **2**-2Zn(II). (a) kinetic analysis of the ADP degradation based on first order reaction kinetics in the reaction using 1000 (●), 500 (■), 250 (▲) and 100 (◆) mU of apyrase. Each time-trace plot is fitted to the following equation; [ADP] = 10exp(-k_{app}• t) + x, where k_{app} represents ADP degradation rate (min^{-1}), t is reaction time (min), and x is the residual ADP concentration at the end of the reaction. (b) plot of the ADP hydrolysis rate (k_{app}, min^{-1}) obtained from the kinetic analyses of the data in (a).

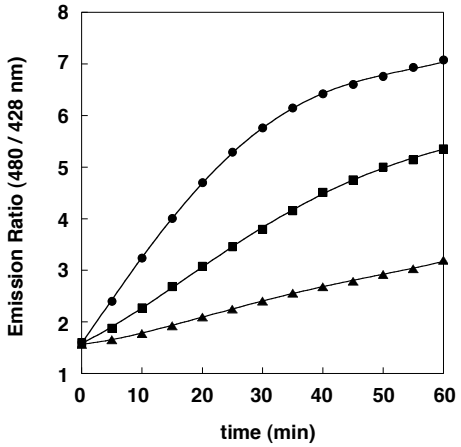

Figure S8. Fluorescence real-time detection of the apyrase-catalyzed ATP hydrolysis using **2**-2Zn(II). Time trace plot of ATP hydrolysis using 1000 (●), 500 (■), and 250 mU (▲) of apyrase by the emission ratio (480/428 nm). Conditions; [**2**-2Zn(II)] = 10 μM, [ATP] = 10 μM, 50 mM HEPES buffer, 10 mM NaCl, pH 7.2, 25 ˚C, λ_{ex} = 368 nm.