Supporting Information for

Phenanthroline Bridged Bis(β-cyclodextrin)s/Adamantane-carboxylic Acid Supramolecular Complex as an Efficient Fluorescence Sensor to Zn$^{2+}$

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Contents

Scheme S1. Synthetic route of 1 .......................................................... S6

Figure S1-S3. Characterization data of compound 1. ............................ S7

Figure S4-S6. Characterization data of compound 2. ............................ S9

Figure S7. Relative fluorescence change of 1 with different metal cations. S9

Figure S8. Job’s plot of 1/AdCA/Zn$^{2+}$ system .................................. S10

Figure S9-S10. Binding constant and complex stoichiometry of 1/AdCA .... S11

Figure S11. Fluorescence emission spectra of 1 and 1/AdCA ................ S12

Figure S12. $^1$H ROESY spectrum of 1 .............................................. S13

Figure S13. $^1$H NOESY spectrum of 1/Zn$^{2+}$ complex ...................... S13

Figure S14. $^1$H NOESY spectrum of 1/AdCA/Zn$^{2+}$ complex .............. S14

Figure S15. Energy minimization structure of 1/AdCA/Zn$^{2+}$ complex ... S15

Figure S16. Dependence of observed rate constant $k_{obs}$ of 1/AdCA complex ... S15
Experimental Section

Materials. All chemicals were reagent grade unless noted. β-Cyclodextrin was recrystallized twice from water and dried in vacuo at 90 °C for 24 h before use. Crude N,N-dimethylformamide (DMF) was stirred with CaH₂ for 3 days and then distilled under reduced pressure prior to use. 2,9-Bis(hydroxymethyl)-1,10-phenanthroline¹, and mono-(6-deoxyl-6-azido)-β-cyclodextrin² were prepared according to the reported methods. Column chromatography was performed on 200-300 mesh silica gel.

Instruments. Elemental analysis was performed on a Perkin-Elmer-2400C instrument. NMR spectra were recorded on Bruker AV400 instruments. The fluorescent spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) on a Varian Cary Eclipse equipped with a Varian Cary single-cell peltier accessory to control temperature at 25 °C. Circular dichroism spectra were collected in a conventional quartz cell (10 × 10 × 45 mm) on a MOS-500 spectropolarimeter (Bio-Logic) at 25 °C. Fluorescence stopped-flow kinetics was measured using a Bio-Logic SFM-3000 (Bio-Logic) device equipped with the MOS-500 spectrometer and with a 150 W xenon-mercury lamp as excitation source at 25 °C. Three shots were performed successively for each mixing scenario and an average dynamic curve was obtained. Dynamic data were fitted using the Biokine software (Bio-Logic). The excited wavelength and slits were set as 272 nm and 8 nm, respectively. FC-08 flowing cell was used, and the typical dead time of the stopped flow is approximately 1.0 ms. The confocal fluorescent images were captured with a fluorescence-inverted microscope (Olympus
Synthesis of 2,9-dipropargyl-1,10-phenanthroline (2). To 20 mL dry DMF was added 2,9-bis(hydroxymethyl)-1,10-phenanthroline (481 mg, 2 mmol), and the solution was cooled to 0 °C, then NaH (4 mmol, 100 mg) was added into the solution. The mixture was stirred at 0 °C for 0.5 h, and then propargyl bromide (80% w/w solution in toluene, 500µL, 4 mmol) was added. The reaction mixture was stirred for 3 h in an ice bath. Then 20 mg NaH was added to complete the reaction, and the mixture was further stirred for another 12 h at room temperature. The reaction mixture was dried under reduced pressure to remove the solvent. The residue was dissolved in chloroform (100 mL) and washed with water (3 × 50 mL), then the organic phase was dried over MgSO₄. The solvent was removed under reduced pressure and compound 2 was obtained by column chromatography (silica gel) using dichloromethane/ethyl acetate (5:3 v/v) as the eluent to give pale yellow solid (198.5 mg, 31% yield). ¹H NMR (400 MHz, CDCl₃, ppm): δ = 2.51 (s, 2H, CH≡C-), 4.38 (d, J = 4 Hz, 4H, -CH₂-), 5.16 (s, 4H, -CH₂-), 7.79 (s, 2H, H of phenanthroline), 7.89 (d, J = 12 Hz, 2H, H of phenanthroline), 8.29 (d, J = 8 Hz, 2H, H of phenanthroline); ¹³C NMR (100 MHz, CDCl₃, ppm): δ = 58.5, 73.6, 75.0, 79.5, 121.0, 126.2, 128.1, 136.9, 145.1, 159.0 ppm; HR-MS (ESI), C₂₀H₁₆N₂O₂: [M + Na]⁺ m/z: calcd 339.1109, found: 339.1108.

Cell culture and confocal fluorescent imaging. Human cervical carcinoma (HeLa) cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and gentamicin (80 µg mL⁻¹) in 6-well plates (2 × 10⁴ cells mL⁻¹, 1 mL per well) for 24h. The cells were incubated with 0.1 mM
Zn(ClO$_4$)$_2$ for 0.5 h, and then washed with PBS buffer for three times and further incubated with 50 µM 1 or 1/AdCA for 3 h at 37 °C, respectively. The cells were washed twice with PBS buffer and then performed confocal fluorescent imaging.
Scheme S1. Synthetic route of 1.

Figure S1. $^1$H NMR (400 MHz) spectrum of 1 in D$_2$O at 25 °C.
Figure S2. $^{13}$C NMR (100 MHz) spectrum of 1 in D$_2$O at 25 °C.

Figure S3. MALDI-TOF mass spectrum of 1.
Figure S4. $^1$H NMR (400 MHz) spectrum of 2 in CDCl$_3$ at 25 °C.
Figure S5. $^{13}$C NMR (100 MHz) spectrum of 2 in CDCl$_3$ at 25 °C.
Figure S6. High-resolution mass spectrum of 2.

Figure S7. Relative fluorescence change (ΔF/F₀) of 1 at 377 nm in the presence of different metal cations in HEPES buffer (10 mM, pH = 7.2, [I] = 1.5 × 10⁻⁵ M, [AdCA] = 1.0 × 10⁻³ M, [Mᵢ⁺] = 3.0 × 10⁻⁵ M, λₑₓ = 272 nm).

Figure S8. Job’s plot of 1/AdCA/Zn²⁺ system in HEPES buffer solution (10 mM, pH = 7.2) at 25 °C ([I] + [Zn²⁺] = 2.0 × 10⁻⁵ M).
Determination of complex stoichiometry and binding constant of 1/AdCA system:³

In our case, the fluorescence intensity $F$ and other binding parameters obey Hill plot:

$$\log\left(\frac{F - F_{\text{min}}}{F_{\text{max}} - F}\right) = n \log[M] + B \ (B = \log \beta)$$

where $F$ is the fluorescence intensity of 1 in the presence of a certain concentration of AdCA; $F_{\text{max}}$ is the fluorescence intensity of 1 when the titration reaches equilibrium; $F_{\text{min}}$ is the fluorescence intensity of 1 without addition of AdCA; and $n$ is the binding stoichiometry of 1 with AdCA; and $\beta$ is the binding constant of 1 with AdCA.

**Figure S9.** Fluorescence intensity changes of 1 upon addition of AdCA in HEPES buffer solution (10 mM, pH = 7.2) at 25 °C ([1] = 1.5 × 10⁻⁵ M, $\lambda_{\text{ex}} = 272$ nm, and $\lambda_{\text{em}} = 368$ nm).
Figure S10. Linear fitting of $\log((F_{368} - F_{\text{min}})/(F_{\text{max}} - F_{368}))$ versus $\log[\text{AdCA}]$ ([I] = $1.5 \times 10^{-5}$ M, $F_{\text{max}} = 286$, and $F_{\text{min}} = 336$). From the slope ($n = 1.98$) and intercept ($\log \beta = 6.62$), it can be seen that the binding stoichiometry and $\log K_S$ value between I and AdCA are $2$ and $4.2 \times 10^6$ M$^{-2}$, respectively.

Figure S11. Fluorescence emission spectra of (a) I/AdCA complex with 90%
encapsulation ratio ([AdCA] = 1 \times 10^{-3} \text{ M}), (b) 1/AdCA complex with 50% encapsulation ratio ([AdCA] = 4 \times 10^{-5} \text{ M}), (c) free 1, (d) 1/AdCA complex in (a) with Zn^{2+}, (e) 1/AdCA complex in (b) with Zn^{2+}, and (f) 1/Zn^{2+} complex in HEPES buffer solution at 25 °C ([1] = 1.5 \times 10^{-5} \text{ M}, [Zn^{2+}] = 3 \times 10^{-5} \text{ M}, \lambda_{\text{ex}} = 272 \text{ nm}, \text{ and } \lambda_{\text{em}} = 377 \text{ nm}).

Figure S12. ROESY spectrum of 1 in D_2O at 25 °C. ([1] = 5 \times 10^{-3} \text{ M}).
Figure S13. NOESY spectrum of 1/Zn$^{2+}$ system in D$_2$O at 25 °C. [1] = 2.5 × 10$^{-3}$ M, [Zn$^{2+}$] = 5.0 × 10$^{-3}$ M).
**Figure S14.** NOESY spectrum of 1/AdCA/Zn$^{2+}$ system in D$_2$O at 25 °C. ([I] = 2.5 × 10$^{-3}$ M, [AdCA] = 7.4 × 10$^{-3}$ M, [Zn$^{2+}$] = 5.0 × 10$^{-3}$ M. Under this concentration, more than 99% of I and AdCA were converted to I/AdCA complex through a calculation based on the binding constant between CD and AdCA).
Figure S15. Energy minimization structure of 1/AdCA/Zn$^{2+}$ system obtained by molecular modeling study. The geometry of 1/AdCA/Zn$^{2+}$ complex was optimized by the molecular mechanics method with dreiding forcefield.

Figure S16. Dependence of observed rate constant $k_{obs}$ of 1/AdCA ([1] = 1.5 × 10$^{-3}$ M, [AdCA] = 2 × 10$^{-3}$ M) with different concentrations of Zn$^{2+}$ in HEPES buffer solution
(10 mM, pH = 7.2). Inset: Dynamic experiments of the rapid mixing of I/AdCA with different concentrations of Zn(ClO$_4$)$_2$ (0, 0.75, 1.5, 2.25, 3.0, and 3.75 × 10$^{-4}$ M). All concentrations mentioned above are the final ones after mixing.

References

