Supporting Information

for

A self-assembled amphiphilic imidazolium-based ATP probe

Jiang-Hua Zhu,‡a Chuan Yu,‡b Yong Chen,b Jinwoo Shin,c Qian-Yong Cao,*a
Jong Seung Kim*c

a Department of Chemistry, Nanchang University, Nanchang 330031, P. R. China. E-mail: cqyong@ncu.edu.cn
b Institute for Advanced Study, Nanchang University, Nanchang 330031, P. R. China
c Department of Chemistry, Korea University, Seoul 02841, Korea. E-mail: jongskim@korea.ac.kr
**Experimental Section**

**General methods**

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. \(N\)-(3-Imidazolyl)propyl dansylamide 3 was prepared by the literature method. Flash chromatography was carried out on silica gel (230-400 mesh). NMR spectra were recorded using Varian instruments (400 MHz). Chemical shifts were expressed in ppm and coupling constants \((J)\) in Hz. Surface tension measurements were carried out by a DCA 315 tensiometer (Cahn Instruments) using a platinum plate \((20 \times 15 \times 0.127 \text{ mm}^3)\) at room temperature.

**Calculation of the association constants**

The binding constants of the inclusion complex were obtained from the fluorescence titration data. According to the Benesi-Hildebrand method, the equations for 1:1 host:guest complexes are given below:

\[
\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{K(I' - I_0)[M]}
\]

In the equations, \(I_0\) is the intensity of fluorescence of \(\textbf{P1}\) without PPi, \(I\) is the intensity with a particular concentration of PPi, \(I'\) is the intensity of the fully complexed form at the highest concentration of PPi, and \(K\) is the binding constant.

**General UV-vis and fluorescence spectra measurements**

Stock solutions of anions (sodium or potassium salts, 0.01 M) were prepared in aqueous solution. Stock solutions of \(\textbf{1}\) (0.01 M) were prepared in DMSO-H\(_2\)O (1:1) solution. The concentration of \(\textbf{1}\) in the UV-vis and emission titrations was 0.02 mM in 100% aqueous solution. During the titration, anions solution ware added into a solution of \(\textbf{1}\) (2 mL) using a micro injector, and the whole volume of the final system can be considered constant because the volume of anions solution added is negligible compared to that of ligand’s solution.
**Cell culture and fluorescence imaging**

Liver cancer HEPG-2 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA) and penicillin (100 units/mL)-streptomycin (100 μg/mL) liquid (Invitrogen Corp., Carlsbad, CA) at 37 °C in a humidified incubator containing 5% CO₂ in air. The cells were incubated for 2 days on an uncoated 35 mm diameter glass-bottomed dish (D110100, Matsunami, Japan). Then, the cells were incubated with DMEM containing 10% PBS and 10 μM probe 1 at 37 °C for 30 min, washed twice with PBS, and mounted on the microscope stage. Fluorescence images were captured by a Nikon A1 confocal laser-scanning microscope equipped with a live cell workstation. Then the live HeLa cells were in situ treated with 1U mL⁻¹ apyrase 30 minutes, and then the same set of cells was used for confocal laser-scanning microscopy measurement.

**Cytotoxicity Assay by MTT Method**

Liver cancer HEPG-2 cells were seeded into 96-well plates and maintained overnight in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) (v/v), and then treated using various concentrations of probe 1 (0–30 μM) at 37 °C for 24 h. After the medium was poured out, 100 μL of freshly prepared 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 1 mg/mL in phosphate buffered saline [PBS]) was added to each well and the wells were incubated for 4 h. The supernatant was removed, and the cells were lysed by adding 100 μL of DMSO per well. The plate was gently shaken for 5 min, and then the absorbance of purple formazan at 520 nm was monitored using a Spectra MAX 340PC plate reader.
Synthesis of 1

Under a nitrogen atmosphere, N-(3-imidazolyl)propyl dansylamide 3 (200 mg, 0.56 mmol) and 1-bromohexadecane 4 (305 mg, 1.0 mmol) were dissolved in 30 ml acetonitrile. The solution was stirred at 90 °C for 10 h followed by evaporation of the solvent in vacuo. The crude was purified by column chromatography (CH₂Cl₂/MeOH, 98:2, v/v) to yield 1 as a yellow oil like liquid (316 mg, yield 85%). ¹H NMR (DMSO, 400MHz): δ = 9.60 (1 H, s), 8.39 (2 H, d, J = 8.4 Hz), 8.05 (1 H, d, J = 7.1 Hz), 7.74 (1 H, s), 7.64 (1 H, s), 7.48 (1 H, t, J = 8.1 Hz), 7.38 (1 H, t, J = 7.9 Hz), 7.24 (1 H, s), 7.04 (1 H, d, J = 7.4 Hz), 4.35 (2 H, s), 4.04 (2 H, d, J = 6.4 Hz), 3.06 (2 H, dd, J₁ = 7.2 Hz, J₂ = 14.3 Hz), 2.75 (6 H, s), 1.17–1.19 (33 H, m) ¹³C NMR (CDCl₃, 100 MHz): 150.7, 136.1, 133.7, 129.2, 128.8, 128.2, 127.9, 127.6, 122.1, 121.7, 120.5, 118.2, 118.5, 114.4, 30.9, 29.0, 28.7, 28.6, 28.5, 28.4, 28.0, 25.3, 21.7, 13.1. ESI-MS (M – Br⁻): 583.4021.

Synthesis of 2

Under a nitrogen atmosphere, N-(3-imidazolyl)propyl dansylamide 3 (200 mg, 0.56 mmol) and iodomethane (300 mg, 2.1 mmol) were dissolved in 30 ml acetonitrile. The solution was stirred at 90 °C for 10 h followed by evaporation of the solvent in vacuo to get the crude dark yellow oil-like liquid. The crude liquid was dissolved in 10 mL MeOH solution. Then NH₄PF₆ (500 mg, 3.1 mmol) was added and stirred for 2
h. The solvent was removed by evaporation under vacuum, and the crude was redissolved in DCM and filtered to remove the undissolvable aminium salts. Pure yellow solid 2 (235 mg, 0.45 mmol, 81% yield) was obtained after crystallization from hexane/DCM (1:1, v/v) solution. $^1$H NMR (DMSO, 400MHz): $\delta = 9.06$ (1 H, s), 8.53 (2 H, d, $J = 8.4$ Hz), 8.33 (1 H, d, $J = 8.4$ Hz), 8.13 (2H, t, $J = 7.6$ Hz), 7.65–7.72 (4 H, m), 7.32 (1 H, d, $J = 8.0$ Hz), 4.21 (2H, t, $J = 6.8$ Hz), 3.87 (s, 3H), 3.43 (6H, s), 2.83 (2H, dd, $J_1 = 7.2$ Hz, $J_2 = 12.4$ Hz), 1.94 (2H, t, $J = 6.8$ Hz). ESI-MS (M – BF$^-$$^-$$^-$): 373.1810.

Fig. S1 Job plot of 1-ATP according to the emission data
Fig. S2 Benesi-Hildebrand plot of 1-ATP according to the emission data

\[ Y = A + B \times X \]
\[ A = 8.96744 \times 10^{-4} \]
\[ B = 3.14723 \times 10^{-9} \]
\[ K = 2.8 \times 10^6 \]
\[ R = 0.99846 \]

Fig. S3 Emission intensity of 1 at 508 nm upon addition of different concentrations of ATP (0–5 μM) normalized between the minimum emission and the maximum emission intensity

\[ Y = A + B \times X \]
\[ A = 0.09108 \]
\[ B = 778639.25908 \]
\[ R = 0.99614 \]
\[ Df = A/B = 1.7 \times 10^{-7} \]
Fig. S4 The emission spectrum of 2 (10 μM) upon addition of various anions in aqueous solution.

Fig. S5 Size distribution of 1-ATP (10 μM) in aqueous solution measured by DLS.
Fig. S6 Competition experiment of I (0) H$_2$PO$_4^-$ (1) HSO$_4^-$ (2) ACO$^-$ (3) UTP (4)ADP (5) AMP (6) NO$_3^-$ (7) CO$_3^{2-}$ (8) F$^-$ (9) PPi (10) Cl$^-$ (11) Br$^-$

Fig. S7 Fluorescence intensities of I (10 μm) at 508 nm before and after the additional of ATP (1.0 equiv) at various pH values in H$_2$O solution
**Fig. S8** Cell viability results after incubation of HEPG-2 liver cancer cells with various concentrations of 1 in aqueous solution.

**Fig. S9** $^1$H NMR spectra of 1 in DMSO-d6 solution
Fig. S10 ¹³C NMR spectra of 1 in CDCl₃ solution

Fig. S11 ESI-TOF-MS spectra of 1
Fig. S12 $^1$H NMR spectra of 2 in DMSO-$d_6$ solution

Fig. S13 TOF-MS spectra of 2