Electronic Supplementary Information (ESI)

High selective fluorescence imaging of cesium distribution in Arabidopsis using bis(trihydroxyphenyl)-appended fluorescent probe with turn-on system

Sung Ho Jung,[a] Tae Kyung Hyun,[b]* Jae-Yean Kim,[b] and Jong Hwa Jung[a] *

[a] Department of Chemistry and Research Institute of Natural Sciences, Gyeongsang National University, Jinju, South Korea. E-mail: *jonghwa@gnu.ac.kr*; Fax: *+82-055-758-6027*; Tel: *+82-055-772-1488*

[b] Division of Applied Life Science (BK21plus), Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju, South Korea.

Experimental Section

Characterization. ^{1}H and ^{13}C NMR spectra were measured on a Bruker DRX 300 apparatus. Mass spectra were obtained by a JEOL JMS-700 mass spectrometer. The optical absorption spectra of the samples were obtained at 298K using a UV–Vis spectrophotometer (Thermo Evolution 600). All fluorescence spectra were recorded in RF-5301PC spectrophotometer. Elemental analyses were performed with a Perkin Elmer 2400 series II.

SEM observations. For Scanning electron micrographs of the samples were taken with a field emission scanning electron microscope (FE-SEM, Philips XL30 S FEG). The accelerating voltage of SEM was 5–15 kV and the emission current was 10 μA.

Preparation of aggregated 1 with metal ions. Stock solutions of the **1** in DMF was diluted to 0.1 mM using water (fw = 99 vol %), followed by the addition of an aliquot of different metal ions. The final concentration of the metal ions was 1.0 mM unless specified. The solutions were mixed by Vortex and stood for 10 min prior to spectral measurement.

Method of UV-vis titration. Stock solutions of the **1** (2.5 x 10^{-5} M) was prepared in water/DMF (99/1, v/v%). The solution of the guest cation (Cs^+, 2.5 x 10^{-3} M) were prepared in water/DMF (99/1, v/v%). UV-vis spectra were initially recorded of **1** solution and adding increasing amount of guest cation solution to it. Binding constant was calculated according to the Benesi-Hildebrand equation. K_a was calculated following the equation stated below .[1]

$$1/(A\text{-}Ao) = 1/\{K(Amax\text{–}Ao)\,[Cs^+]_n\} + 1/[Amax\text{-}Ao]$$

Here Ao is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest cation, Amax is absorbance in presence of added $[Cs^+]$ max and K is the association constant (M^{-1}). The association constant (K) could be determined from the slope of the straight line of the plot of 1/(A-Ao) against $1/[Cs^+]_n$. The association constant (K_a) as determined by UV-vis titration method for **1** with Cs^+ is found to be 1.16 x 10^3 M^{-1}.

Plant growth conditions and imaging. Surface-sterilized seeds of *Arabidopsis thaliana* Col-0 were germinated and grown on the Murashige-Skoog (MS) medium containing 1% (w/v) sucrose, 0.8% (w/v) plant agar and 0.01 and 0.1 mM Cs_2CO_3. All plants were grown at 24°C under nine-days conditions with or without cesium. (light/dark regime of 16 h/8 h, white light, 100 μmol photons $m^{-2}s^{-1}$). Nine-day-old seedlings were vacuum-infiltrated with 0.1 mM probe 1 for 10 min, and then incubated in the dark condition for 80 min. The seedlings were washed with water, before fluorescence microscopy. Fluorescence was visualized using Olympus multiphoton laser scanning microscope (model FV1200MPE, Tokyo, Japan) with following filter setup: excitation 730 nm (two-photon) and emission BF 450-490 nm

Elemental analysis of Arabidopsis. Wild type (Col-0) seedlings were grown for 9 days then washed in deionized water and dried in an oven at 65 °C for 2 days. Approximately 2 mg of dried samples were extracted with 1 mL of 60% (v/v) HNO_3 at 125 °C for 1 h, then added of 1 mL of 30% (v/v) H_2O_2 to solution and diluted with ionized water to a total volume of 10 mL. A portion of this solution was filtered through a 0.45 μm PTFE membrane syringe filter. Cs^+ concentrations were measured using a inductively coupled plasma mass spectrometer (ELAN DRC II, Perkin Elmer) and calculated against the standard curve.

Synthesis of compound 2: Hydrazine hydrate (10 ml, excess) was allowed to solution of **3** (4g, 1.8 mmol) in absolute ethanol (40 ml). The reaction mixture was then heated under reflux condition for 2-3 h. After cooling to room temperature, which was washed with distilled water and recrystallized form hot water to give colorless silky needled (2.82 g, 81 %). ^{1}H-NMR (300 MHz, DMSO-d_6) 10.64 (br s, 2H, -NH), 8.13 (s, 3H, Ar-H), 4.63 (br s, 4H, -NH$_2$); ^{13}C-NMR (125MHz, DMSO-d_6) 161.9, 148.3, 138.9, 123.7; ESI-MS: *m/z* 196.62 [M + H]$^+$; Anal. Calculated for $C_7H_9N_5O_2$ 195.1820, Found 195.1805.

Synthesis of ligand 1. A solution of **2** (0.195 g, 1.0 mmol) and 2,3,4-trihydroxybenzaldehyde (0.315 g, 2.0 mmol) in absolute ethanol (50 ml) was refluxed for 6h. Upon completion of reaction, yellow colored precipitates were obtained. The precipitates were filtered off, washed with ethanol and dried in vacuum oven (0.39 g, 83 %). ^{1}H-NMR (300 MHz, DMSO-d_6) 12.31 (br s, 2H, -OH), 11.25 (br s, 2H, -NH), 9.61 (s, 2H, -OH), 8.74 (s, 2H, -
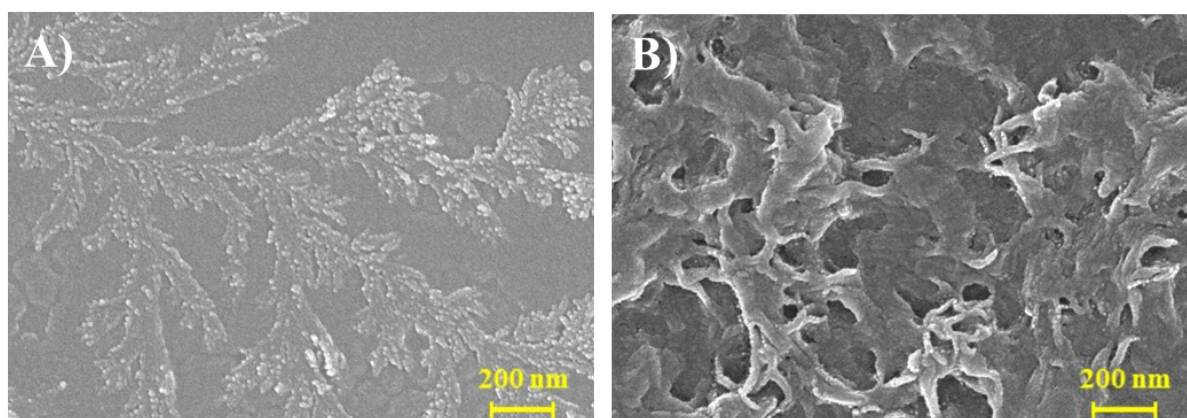
N=CH), 8.59 (s, 2H, -OH), 8.37-8.26 (m, 3H, Ar-H), 6.93 (d, 2H, Ar-H), 6.44 (d, 2H, Ar-H); ^{13}C-NMR (125 MHz, DMSO-d_6) 159.4, 152.3, 149.6, 148.5, 148.1, 140.5, 133.3, 125.9, 121.5, 111.4, 108.4; ESI-MS: *m/z* 468.25 [M+H]$^+$; Anal. Calculated for $C_{21}H_{17}N_5O_8$ 467.3940, Found 467.3962.
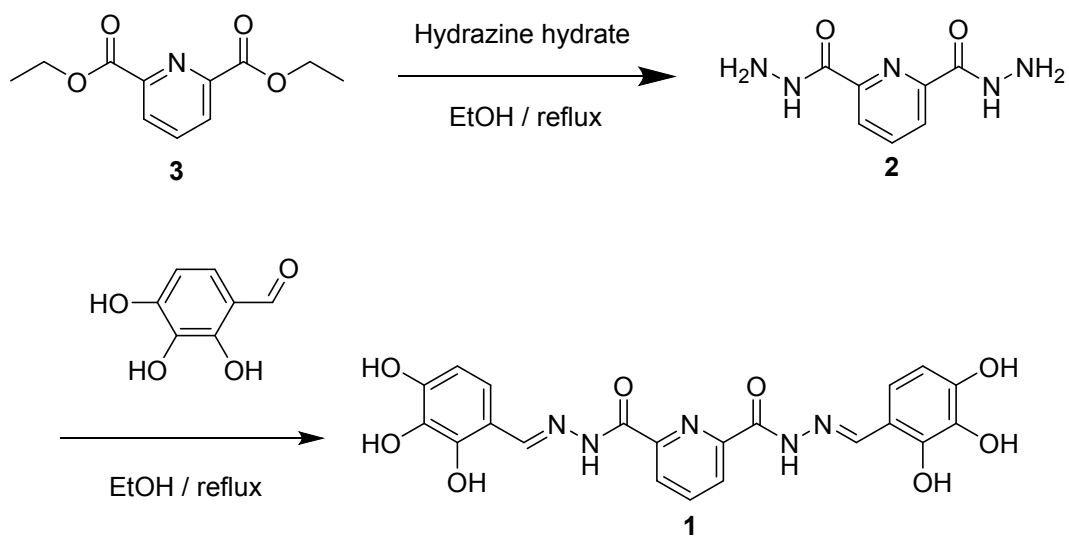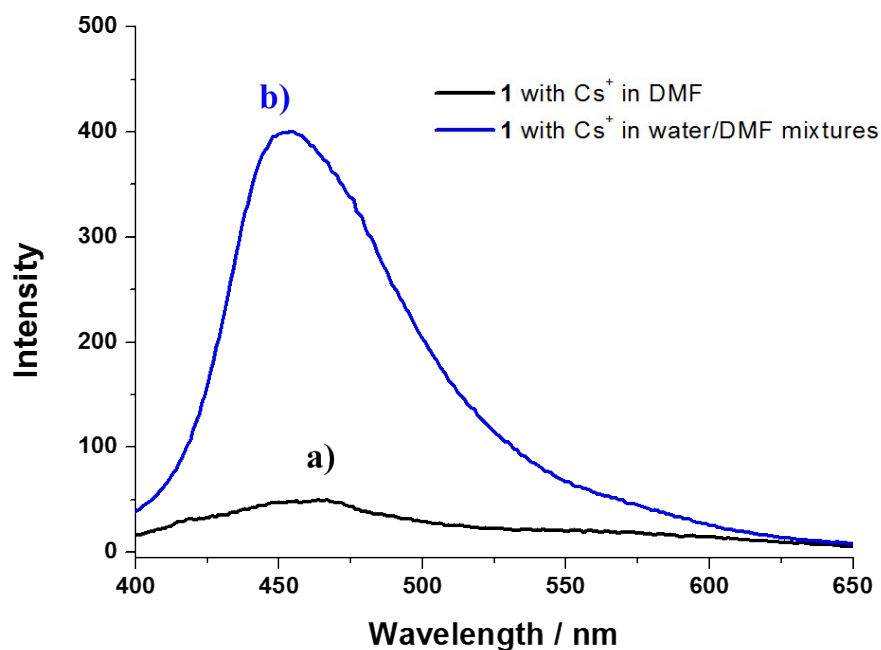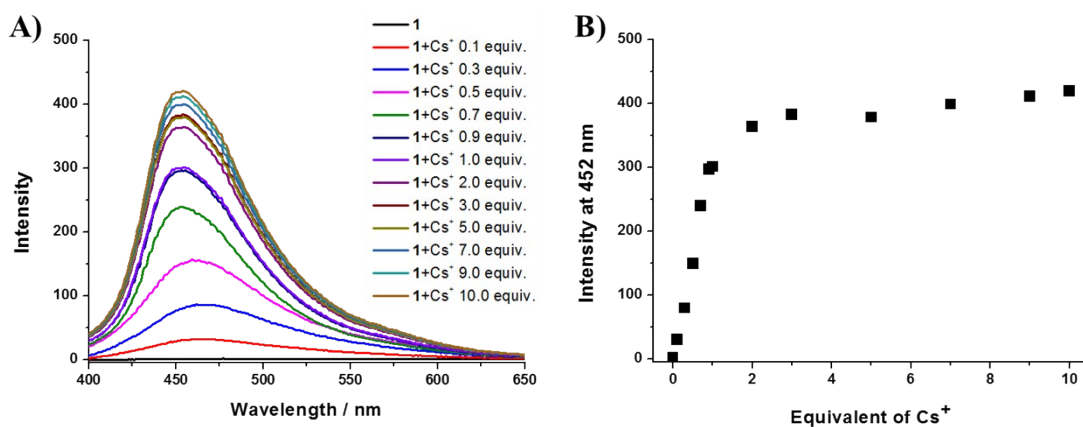

Scheme S1. Synthesis of ligand **1**.
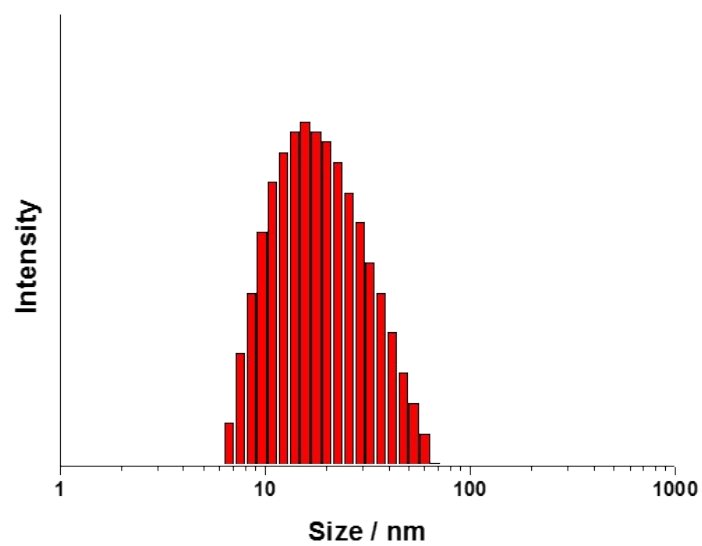
**Fig. S1** Particle size distribution of aggregated **1** with Cs$^+$ in water/DMF mixture (99:1 v/v%).

Fig. S2 SEM images of aggregated **1** with (A) K$_2$CO$_3$ and (B) Na$_2$CO$_3$ in water/DMF mixture (99:1 v/v%).
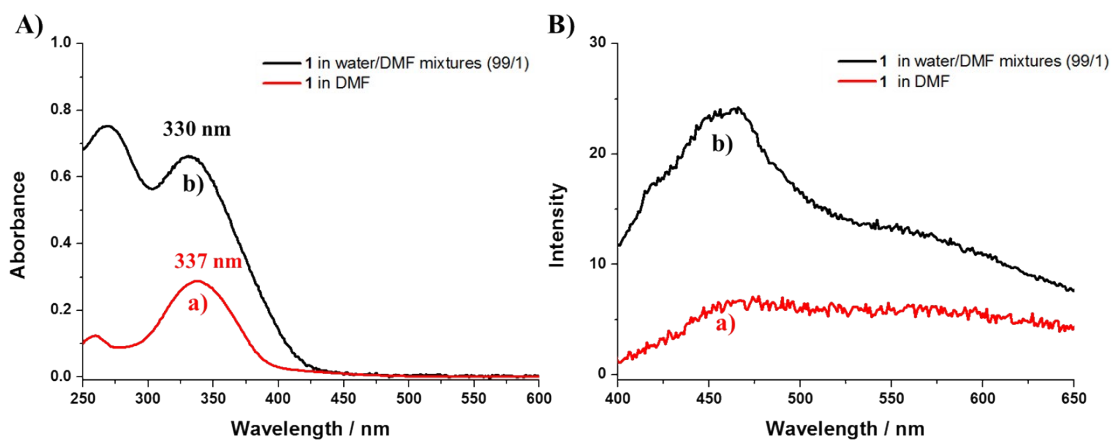
Fig. S3 Fluorescence spectra of **1** (0.1 mM) upon addition of Cs⁺ (10.0 equiv.) in (a) pure DMF and (b) a mixture of water/DMF mixture (99:1 v/v%).
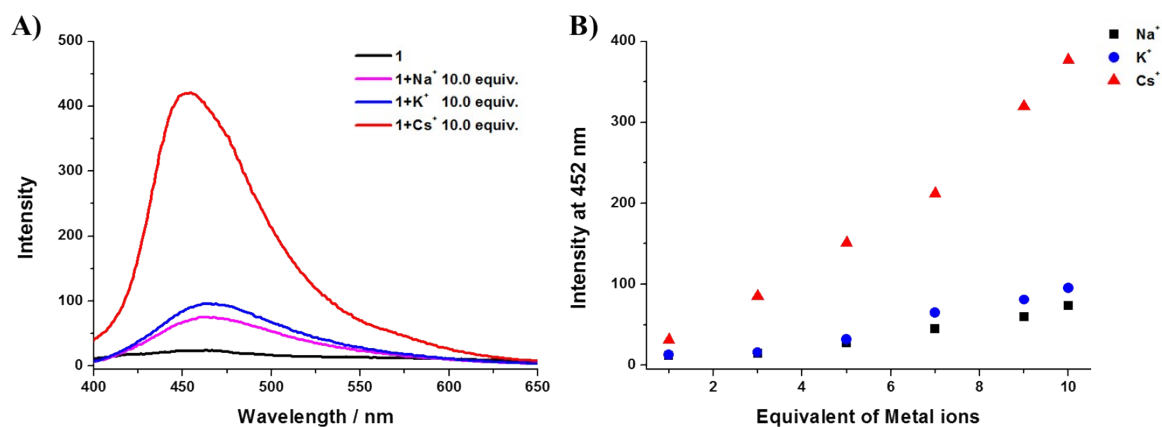

Fig. S4 (A) Fluorescence spectra of aggregated **1** (0.1 mM) with Cs⁺ (0-10.0 equiv.) in a mixture of water/DMF (99:1 v/v%). (B) Plot of Fluorescence intensity of aggregated **1** (0.1 mM) with Cs⁺ (0.1-10.0 equiv.) in water/DMF mixture (99:1 v/v%).
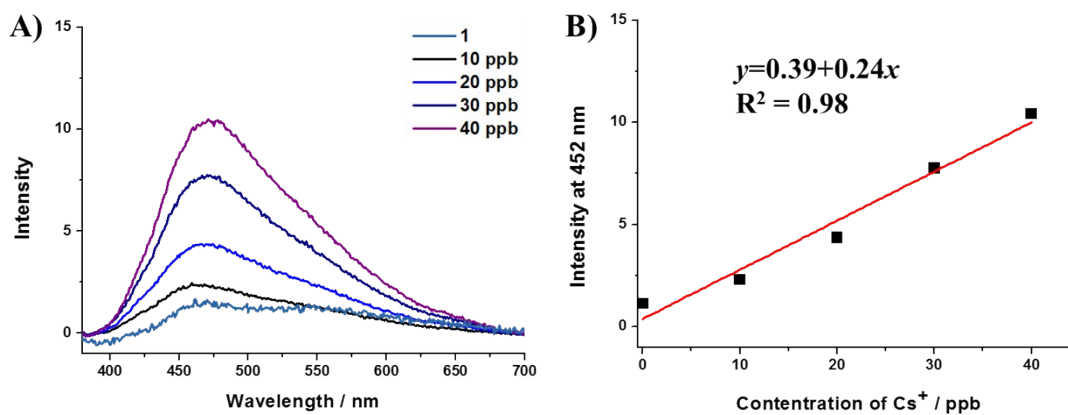
Fig. S5 (A) Fluorescence spectra of aggregated **1** with Cs^+ (0-40 ppb) in water/DMF mixture (99/1 *v/v*%). (B) Plot of fluorescence intensity.
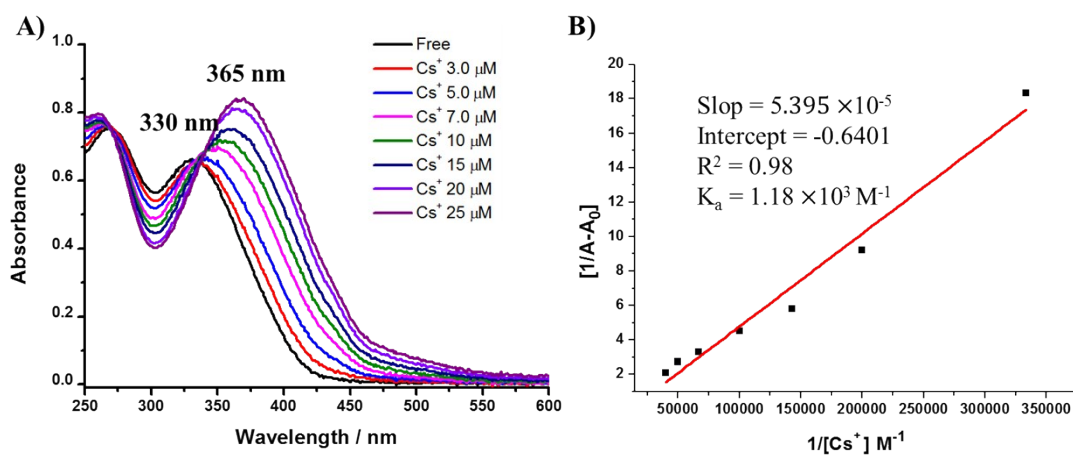

Fig. S6 (A) UV-vis spectra of **1** (2.5×10^{-5} M) in (a) DMF and (b) water/DMF mixture (99/1 v/v %). (B) Fluorescence spectra of **1** (0.5 mM) in (a) DMF and (b) water/DMF mixture (99/1 v/v %).
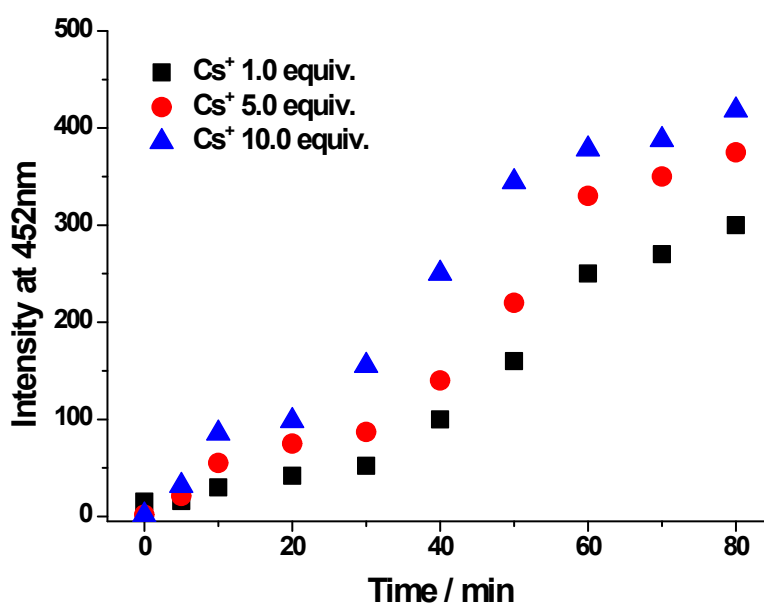
Fig. S7 A) Fluorescence spectra of aggregated **1** (0.1 mM) with Na+, K+ and Cs+ (10.0 equiv.) in water/DMF mixture (99:1 v/v%). B) Plot of fluorescence intensity.
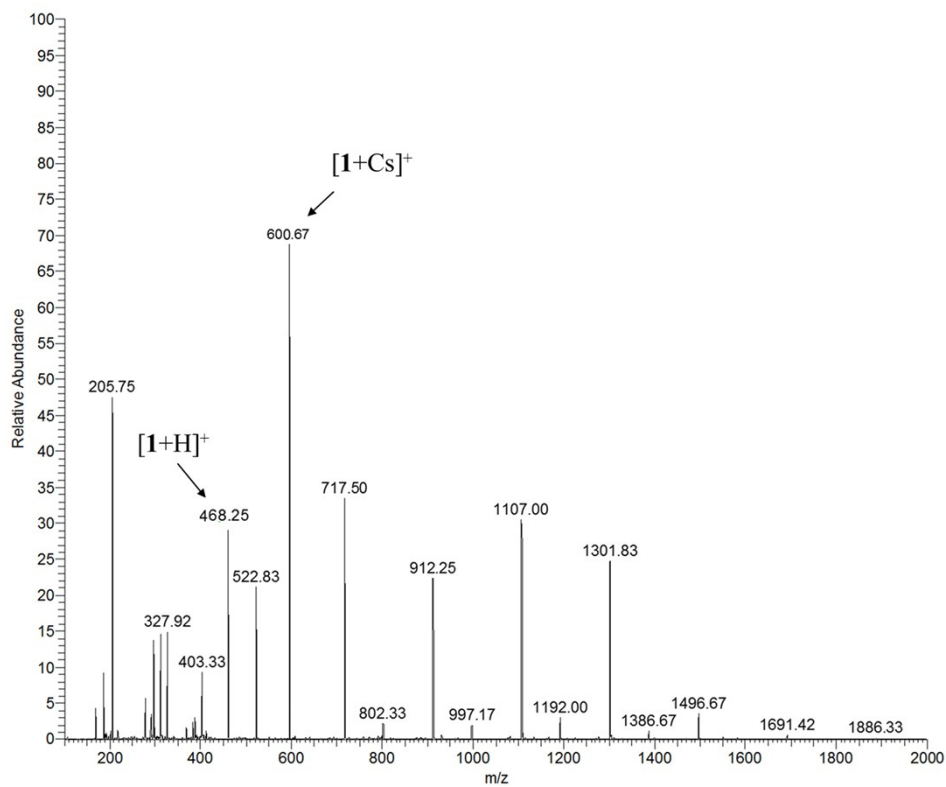
**Fig. S8** ESI-mass spectrum of aggregated **1**-Cs+ complex.

Fig. S9 (A) UV-vis titration of aggregated **1** (2.5 x10^{-5} M) upon addition of Cs$^+$ (3.0-25 μM) in water/DMF mixture (99:1 v/v%). B) Association constant curve of aggregated **1** with Cs$^+$ by UV-vis method.

Fig. S10 Plot of the change of fluorescence intensity of aggregated **1** with different concentration of Cs$^+$ (1.0, 5.0, 10.0 equiv.) in water/DMF mixture (99/1 *v/v%*) as a function of time (min).
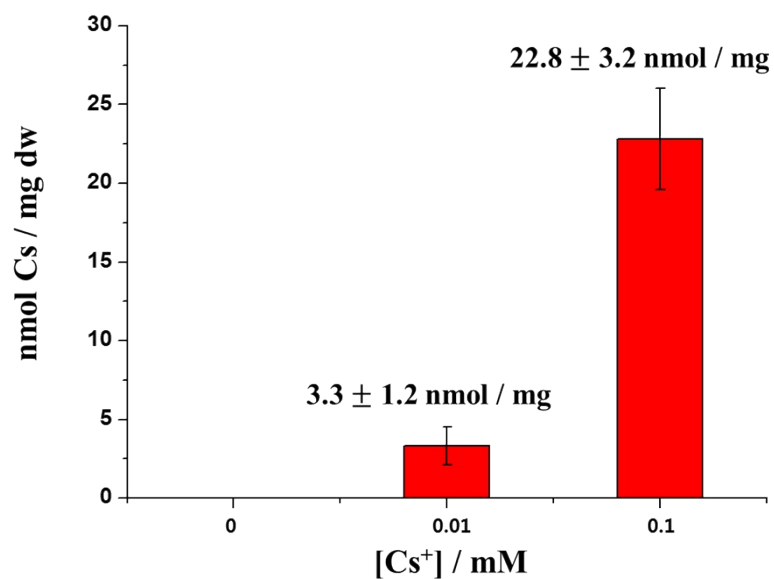
Fig. S11 Cesium concentrations in Arabidopsis according to inductively coupled plasma mass spectroscopy (ICP-MS). Arabidopsis seeds were germinated and grown for 9 days on medium containing Cs_2CO_3 (0, 0.01 and 0.1 mM).

References

1. (a) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc*., 1949, **71**, 2703-2707; (b) K. A. Conners, Binding Constants, The Measurement of Molecular Complex Stability; Wiley: New York, **1987**.