Supporting Information

Fluorescence Turn-on Probe of Naphthalimide for Sensitive and Specific Detection of Iodide in Neutral Aqueous Solution and Real Samples

Dandan Li, Xinlei Chen, Yang Li, Tanyu Cheng, Weiping Zhu, Yufang Xu*, Xuhong Qian*

State key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Shanghai, 200237, China. Fax: +86 21 6425 2603; Tel: +86 21 6425 3589; *E-mail: xhqian@ecsut.edu.cn;yfxu@ecust.edu.cn.*

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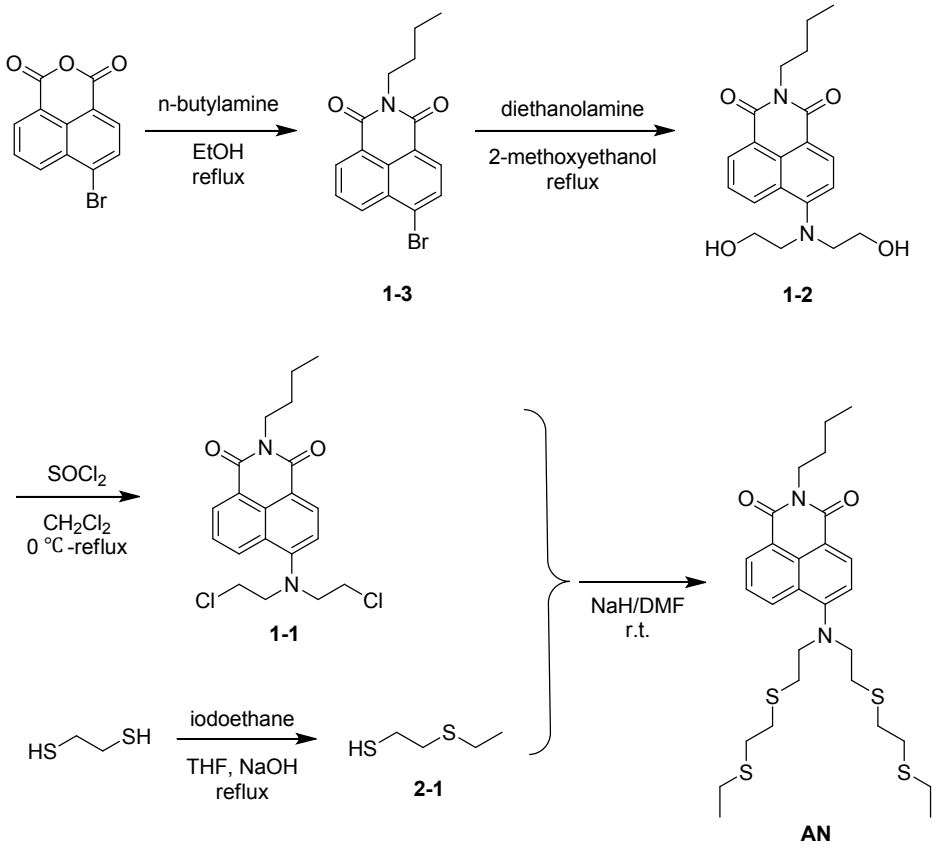
1. Materials and Instruments

Silica gel P60 (Qingdao) was used for column chromatography. All chemicals were purchased from TCI and Aladdin reagent Co without further purification except otherwise stated. All the organic solvents were of analytical grade. Acetonitrile were distilled by CaH_2 to remove the water before used. Water was purified by a Milli-Q system. The solutions of metal ions were prepared from $AgClO_4 \cdot H_2O$, $Cu(ClO_4)_2 \cdot 6H_2O$, $Hg(ClO_4)_2 \cdot 3H_2O$, $Zn(ClO_4)_2 \cdot 6H_2O$, $Cd(ClO_4)_2 \cdot 6H_2O$, $Mg(ClO_4)_2$, $Co(ClO_4)_2 \cdot 6H_2O$, $Ni(ClO_4)_2 \cdot 6H_2O$, $Ba(ClO_4)_2$, $Pb(ClO_4)_2 \cdot 3H_2O$, $Ca(ClO_4)_2 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$, $FeCl_3$, $Cr(ClO_4)_3 \cdot 6H_2O$, $LiClO_4$, $NaClO_4$ and $KClO_4$ in H_2O and $Cu^+(CH_3CN)_4PF_6^-$ in CH_3CN. The solutions of anions were prepared from KF, NaCl, NaBr, KI, $NaNO_3$, Na_2SO_4, $NaCO_3$, Na_3PO_4, CH_3COONa and NaHS in Mili Q water. Salt samples were purchased from local markets with the brand of china salt (1 deep well iodized salt, 2 refined salt without iodine, 3 tianshan lake salt, 4 deep well iodine-free salt).

^1H and ^{13}C NMR spectra were collected in $CDCl_3$ at 25 ºC on a Bruker AV-400 spectrometer at NMR Facility of East China University of Science and Technology (ECUST), from which chemical shifts reported in ppm (TMS as internal standard). Mass spectral analyses were carried out at the Analysis and Test Center of East China University of Science and Technology (ECUST).

2. Synthesis



Scheme 1 Synthesis of **AN**

***N*-butyl-4-bromo-1,8-naphthalimide 1-3[1]:** The solution of 4-bromo -1,8-naphthalic anhydride (5.0 g, 18.1 mmol), n-butylamine (2.64 g, 36.1 mmol) in 40 mL of ethanol was heated at reflux for 40 min and monitored by TLC. After the reaction was completed, the reaction mixture was cooled to complete the precipitation. The crude product was filtered, washed with water and recrystallized from ethanol to give 4 (4.26 g, yield: 71.06 %) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (d, *J* = 8.0 Hz, 1 H), 8.55 (d, *J* = 7.6 Hz, 1 H), 8.40 (d, *J* = 8.0 Hz, 1 H), 8.03 (d, *J* = 7.2 Hz, 1 H), 7.81-7.86 (m, 1 H), 4.17 (t, *J* = 8.0 Hz, 2 H), 1.68-1.75 (m, 2 H), 1.41-1.49 (m, 2 H), 0.98 (t, *J* = 7.2 Hz, 3 H)

***N*-butyl-4-(bis(2-hydroxyethyl)amino)-1,8-naphthalimide 1-2[2]:** Under an argon atmosphere, 1-3 (1.0 g, 3.0 mmol) and diethanolamine (5.0 mL, 52.0 mmol) were combined in 10 mL of 2-methoxyethanol and refluxed for overnight. Then the reaction mixture was poured into 50 mL water and extracted with ethyl acetate (50 mL × 3). The combined organic layer was dried and evaporated under reduced pressure. The residue was purified by column chromatography (silca gel, EtOAc as eluent) to give the yellow solid product (480.0 mg, Yield: 44.7 %). ^{1}H NMR (400 MHz ,$CDCl_3$): δ 8.87 (d, *J* = 8.0 Hz, 1 H), 8.56 (d, *J* = 7.2 Hz, 1 H), 8.49 (d, *J* = 8.0 Hz, 1 H), 7.69 (t, *J* = 7.2 Hz, 1 H), 7.40 (d, *J* = 8.0 Hz, 1 H), 4.16 (t, *J* = 7.2 Hz, 2 H), 3.85 (t, *J* = 4.8 Hz, 4 H), 3.61 (t, *J* = 5.2 Hz, 4 H), 1.74-1.66 (m, 2 H), 1.49-1.39 (m, 2 H), 0.97 (t, *J* = 7.6 Hz, 3 H)

***N*-butyl-4-(bis(2-chloroethyl)amino)-1,8-naphthalimide 1-1:** To a well-stirred solution of 1-2 (500 mg, 1.4 mmol) in CH_2Cl_2 (20 mL) was added a solution of $SOCl_2$ (1.5 g, 12.6 mmol) in 20 mL CH_2Cl_2 at 0 ℃.The reaction mixture was stirred for 30 min at 0 ℃and then heated to reflux. After cooled to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography (silca gel, petroleun ether /EtOAc= 4:1 as eluent) to give the yellow solid product (326.0 mg, Yield: 59.1 %) ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (d, *J* = 7.2 Hz, 1 H), 8.55 (t, *J* = 8.0 Hz, 2 H), 7.75 (t, *J* = 7.6 Hz, 1 H), 7.46 (d, *J* = 8.0 Hz, 1 H), 4.18 (t, *J* = 7.2 Hz, 2 H), 3.80 (t, *J* = 6.4 Hz, 4 H), 3.59 (t, *J* = 6.4 Hz, 4 H), 1.75-1.68 (m, 2 H), 1.48-1.42 (m, 2 H), 0.98 (t, *J* = 7.2 Hz, 3 H)。^{13}C NMR (100 MHz, $CDCl_3$): δ 164.3, 163.8, 152.1, 131.6, 131.5, 130.2, 130.1, 128.2, 126.4, 123.4, 119.7, 118.6, 55.8, 41.3, 40.2, 30.2, 20.4, 13.8。ESI-MS (m/z): 393 ($M+H^+$), mp：97.6 - 98.7 °C

2-(ethylthio)ethanethiol 2-1[3]: Under an argon atmosphere, to the mixture of dithioglycol (6 mL,71.5 mmol), NaOH (2.2 g, 55 mmol) in 70 mL of dry THF was added a solution of iodoethane (6 mL, 75.0 mmol) in 30 mL dry THF at reflux. After the addition was completed, the reaction mixture was refluxed for another 1 h. Then the reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the product as colorless oil.

N-butyl-4-(bis(2-((2-(ethylthio)ethyl)thio)ethyl)amino)-1,8-naphthalimide AN[3]:

To a mixture of NaH (100 mg, 4.17 mmol) in 5 mL of dry DMF was slowly added a solution of 2-1 (200 mg, 1.85 mmol) in 2 mL of dry DMF. After the addition is completed, the mixture was stirred for half an hour. Then a solution of 1-1 (350 mg, 0.88 mmol) in dry DMF was added to above mixture and stirred for overnight. The reaction solution was poured into water, extracted with CH_2Cl_2, washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography (silca gel, petroleun ether /EtOAc/methanol = 10:2:1 as eluent) to give the yellow oil product. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.59 (d, *J* = 6.4 Hz, 1 H), 8.51 (d, *J* = 8.0 Hz, 2 H), 7.72 (t, *J* = 7.2 Hz, 1 H), 7.33 (d, *J* = 8.0 Hz, 1 H), 4.17 (t, *J* = 7.6 Hz, 2 H), 3.61 (t, *J* = 7.2 Hz, 4 H), 2.73 (t, *J* = 7.2 Hz, 4 H), 2.64-2.61 (overlapped, 8 H), 2.46 (q, *J* = 7.2 Hz, 4 H), 1.74-1.66 (m, 2H), 1.49-1.39 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 6 H), 0.97 (t, *J* = 7.6 Hz, 3 H)。^{13}C NMR (100 MHz, $CDCl_3$): δ 164.3, 163.8, 153.0, 131.6, 131.4, 130.4, 130.1, 127.6, 126.0, 123.3, 118.1, 117.5, 53.6, 40.1, 32.4, 31.7, 30.2, 29.7, 26.6, 20.4, 14.7, 13.9. HRMS (ESI): Calcd for $C_{28}H_{40}N_2O_2S_4$ ($M+H^+$) 565.2051; Found, 565.2047

3. Methods

3.1 Spectroscopic materials and methods

Double distilled water was used to prepare all aqueous solutions. All spectroscopic measurements were performed in 50 mM HEPES buffer (pH 7.4, 1% DMSO) at room temperature. Absorption spectra were recorded using a Varian Cary100 Bio UV-Visible spectrophotometer. Fluorescence spectra were recorded using a Varian Cary Eclipse scanning spectrofluorometer equipped with a Xenon flash lamp. Samples for absorption and fluorescence measurements were contained in 1 cm×1 cm quartz cuvettes (3.5 mL volume).

3.2 Job plot

A series of solutions containing AN and Ag^+ were prepared such that the sum of the total metal ion and AN concentration remained constant (9 μM). The mole fraction (x) of metal ion was varied from 0.1 to 1.0. The corrected fluorescence (Y_0 (1-x)-Y) was plotted against the molar fraction of the metal ion solution, where Y_0 and Y represent the emission intensity at 520 nm in the absence and presence of Ag^+, respectively.

3.3 Detection of association constant

Association constants were investigated by fluorescence titrations. The concentration of Ag^+ ion was varied between zero and 1.8 μM. AN was maintained at 9 μM throughout the study. The fluorescence at 520 nm was monitored as the concentration of metal ion varied. The 1:1 complexation constant Ks was determined by a nonlinear least-squares analysis of Y versus C_M using the following equation as:

$$Y = Y0 + \frac{Ylim - Y0}{2}\left\{1 + \frac{CM}{CL} + \frac{1}{KsCL} - \left[\left(1 + \frac{CM}{CL} + \frac{1}{KsCL}\right)^2 - 4\frac{CM}{CL}\right]^{1/2}\right\}$$

Where Y_0 and Y represent the emission intensity at 520 nm in the absence and presence of metal ion, respectively, Y_{lim} is the emission response when no further change occurred upon continued addition of metals, C_L is the concentration of AN and C_M represents the total metal ion concentration.

3.4 Determination of quantum yield

The quantum yield of AN and ANAg+ were determined in 1% DMSO/H_2O according to the comparative method.[4]

$$\Phi T = \Phi ST\left(\frac{GradT}{GradST}\right)\left(\frac{\eta T^2}{\eta ST^2}\right)$$

Where Φ is the quantum yield. Grad is the slope of the line obtained from the plot of the integrated fluorescence intensity vs. absorbance. η refers to the refractive index of the solvent. The subscripts T and ST denote the tested samples and the standard sample, respectively. N-butyl-4-butylamino-1,8-naphthalimid was chosen as the standard sample, which has the quantum yield of 0.81 in $CHCl_3$.

3.5 Detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence enhancement of **AN** was dose-dependent with respect to I^-. The linear response ($y = 8.879\times10^7x + 26.80$ with $R^2 = 0.9972$) of fluorescent intensity (y) with respect to the concentration (x) of I^- was established. The lower detection limit (LDL) was calculated following equation. LDL = 3σ/slope (σ denotes the ratio signal and noise, which is the standard deviation of blank measurements, n = 10; slope refers to the slope of linear equation). The detection limit was determined to be 17.2 nM.

3.6 Detection iodide in real samples with ANAg

3g salt sample was treated with approximate 10 equiv. Vc in aqueous solution at room temperature, then the solvent was removed, and 30mL EtOH was added to extract the I^- for 4 hs. 6 mL extraction was concentrated and dissolved in 2 mL Mill-Q water. The solution was used for iodide test.

4. Data

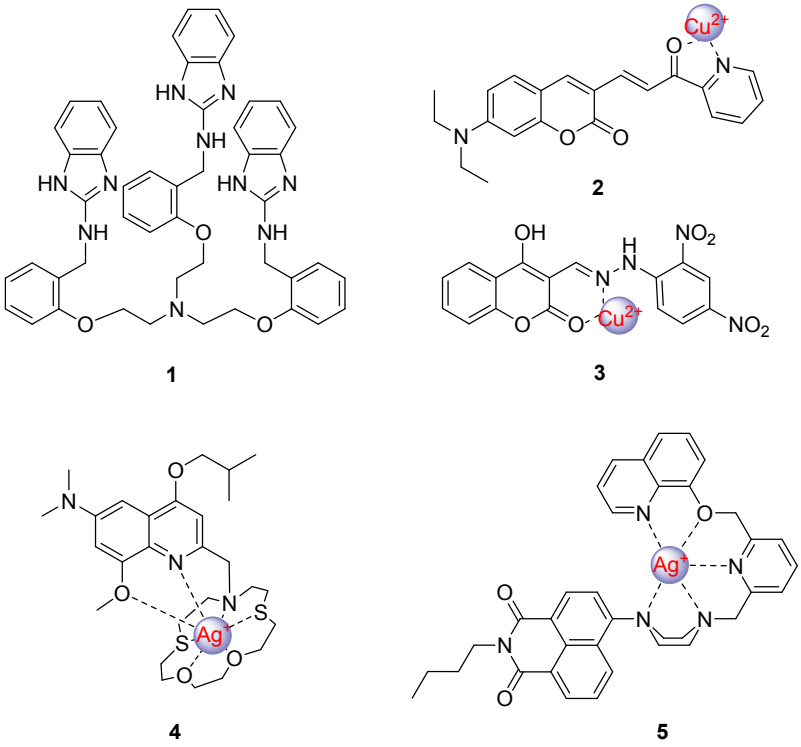


Fig. S1 Structures of reported probes for I^-

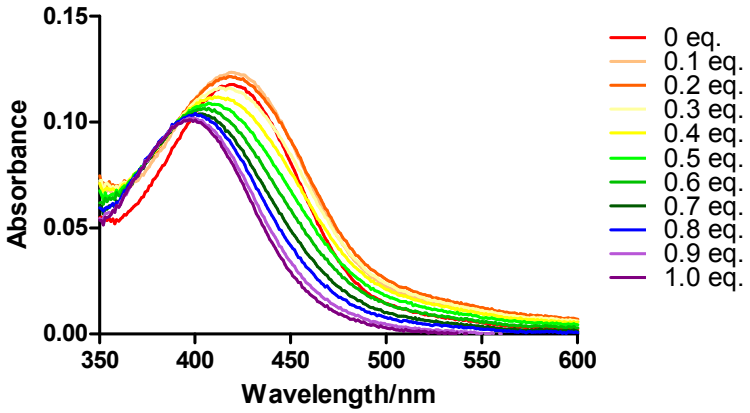


Fig. S2 The UV absorption spectra of probe **AN** (9 μM) upon addition of increasing concentration of Ag^+ (0 to 1.0 equiv) in HEPES buffer (pH 7.4, 50 mM, 1% DMSO).

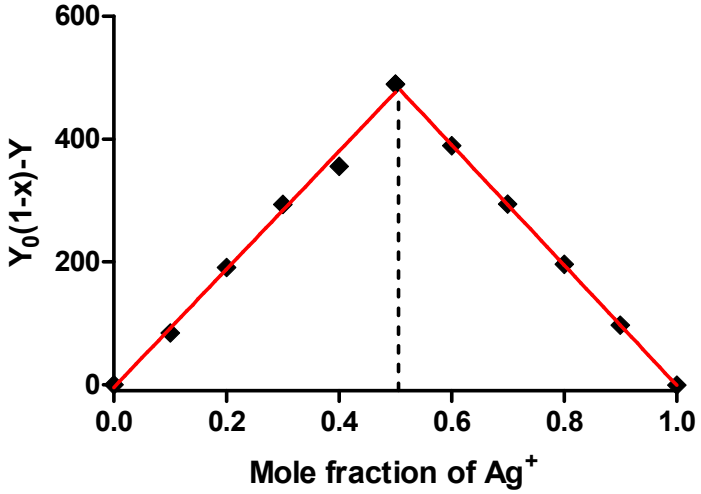


Fig. S3 Job's plot of **AN** and Ag^+ ([AN] + [Ag^+] = 10 μM) in aqueous solution (HEPES, pH 7.4, 50 mM, 1% DMSO). Excitation at 420 nm. Excitation and emission slit widths were both 5 nm.

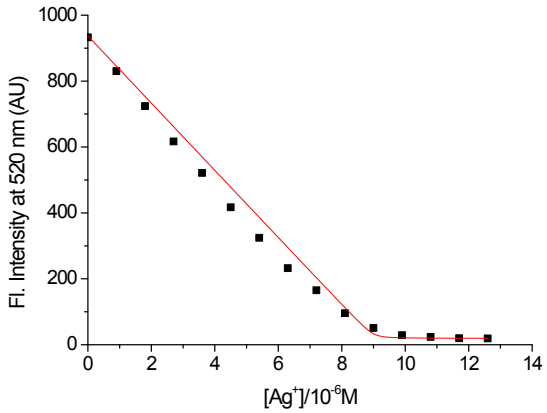


Fig. S4 Curve of fluorescence intensity at 520nm of **AN** versus increasing concentration of Ag+ in in HEPES buffer (pH 7.4, 50 mM, 1% DMSO). The concentration of **AN** was 9μM. Excitation at

420 nm. Excitation and emission slit widths were both 5 nm.

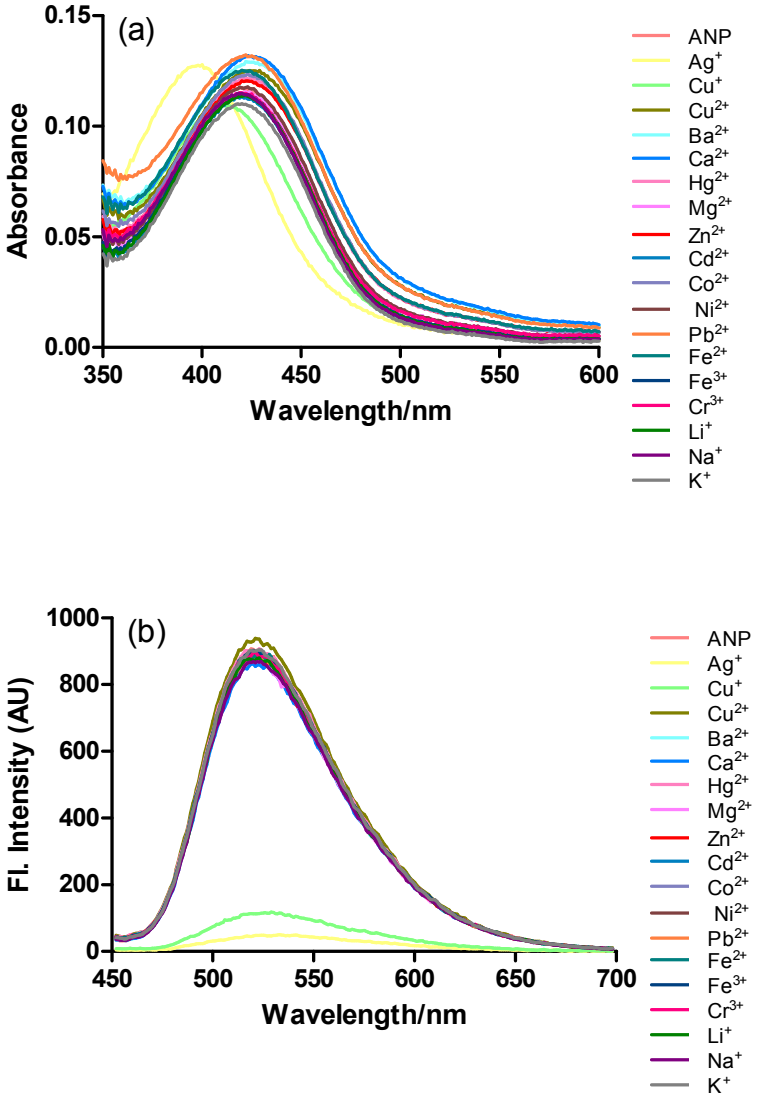
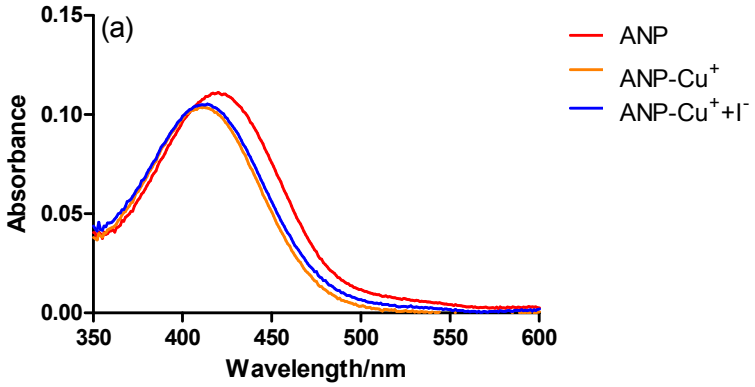


Fig. S5 The UV absorption spectra and fluorescent emission spectra of probe **AN** (9 μM) upon addition of various metal ions (1.0 eq.) in HEPES buffer (pH 7.4, 50 mM, 1% DMSO). Excitation at 420 nm. Excitation and emission slit widths were both 5 nm.



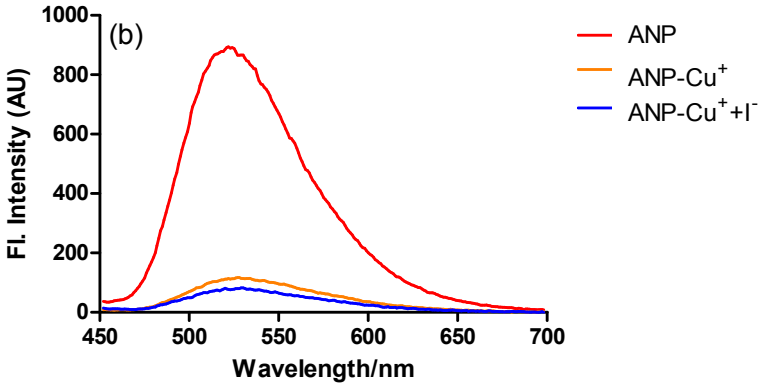


Fig. S6 The UV absorption spectra and fluorescent emission spectra of **AN** (9 μM), and **ANCu** (9 μM) upon addition of I^- (0 and 1.0 eq.) in HEPES buffer (pH 7.4, 50 mM, 1% DMSO). Excitation at 420 nm. Excitation and emission slit widths were both 5 nm.

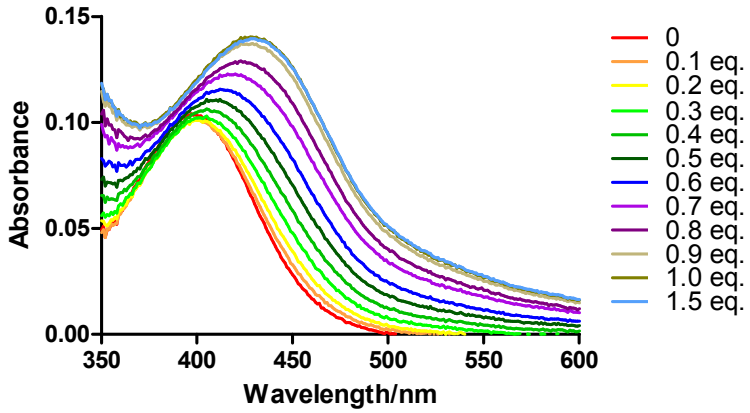


Fig. S7 The UV absorption spectra of probe **ANAg** complex (9 μM) upon addition of increasing concentration of I^- (0 to 1.5 eq.) in HEPES buffer (pH 7.4, 50 mM, 1% DMSO).

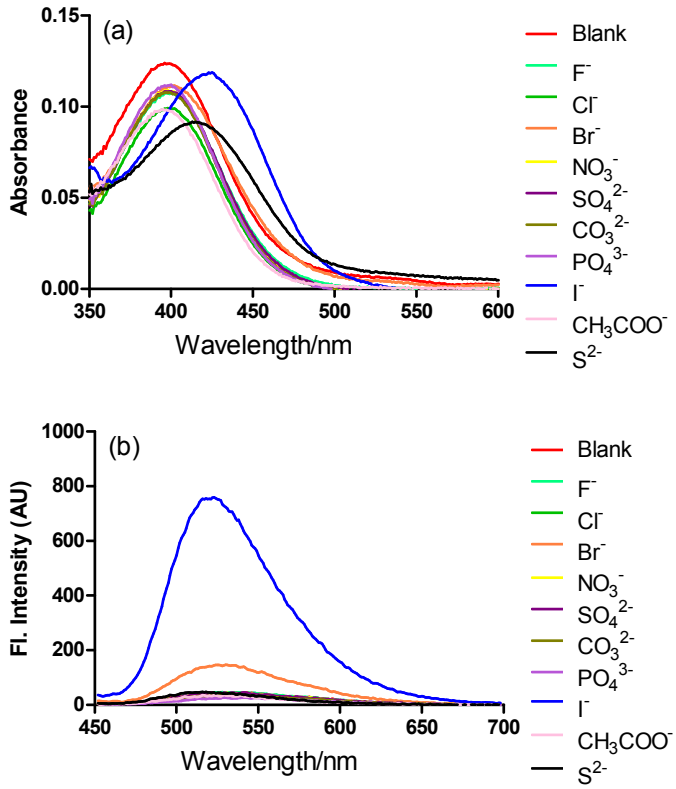
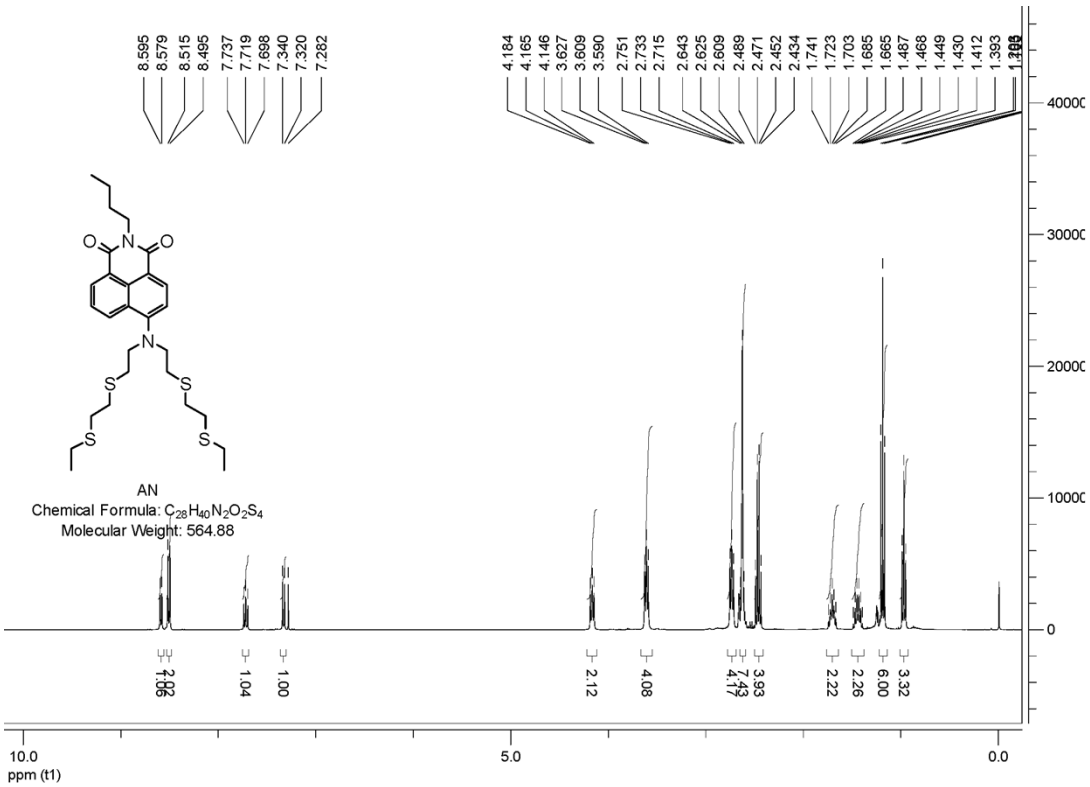


Fig. S8 The UV absorption spectra and fluorescent emission spectra of probe **ANAg** (9 μM) upon addition of various anions (1.0 equiv) in HEPES buffer (pH 7.4, 50 mM, 1% DMSO). Excitation at 420 nm. Excitation and emission slit widths were both 5 nm.

5 The characterization data of AN



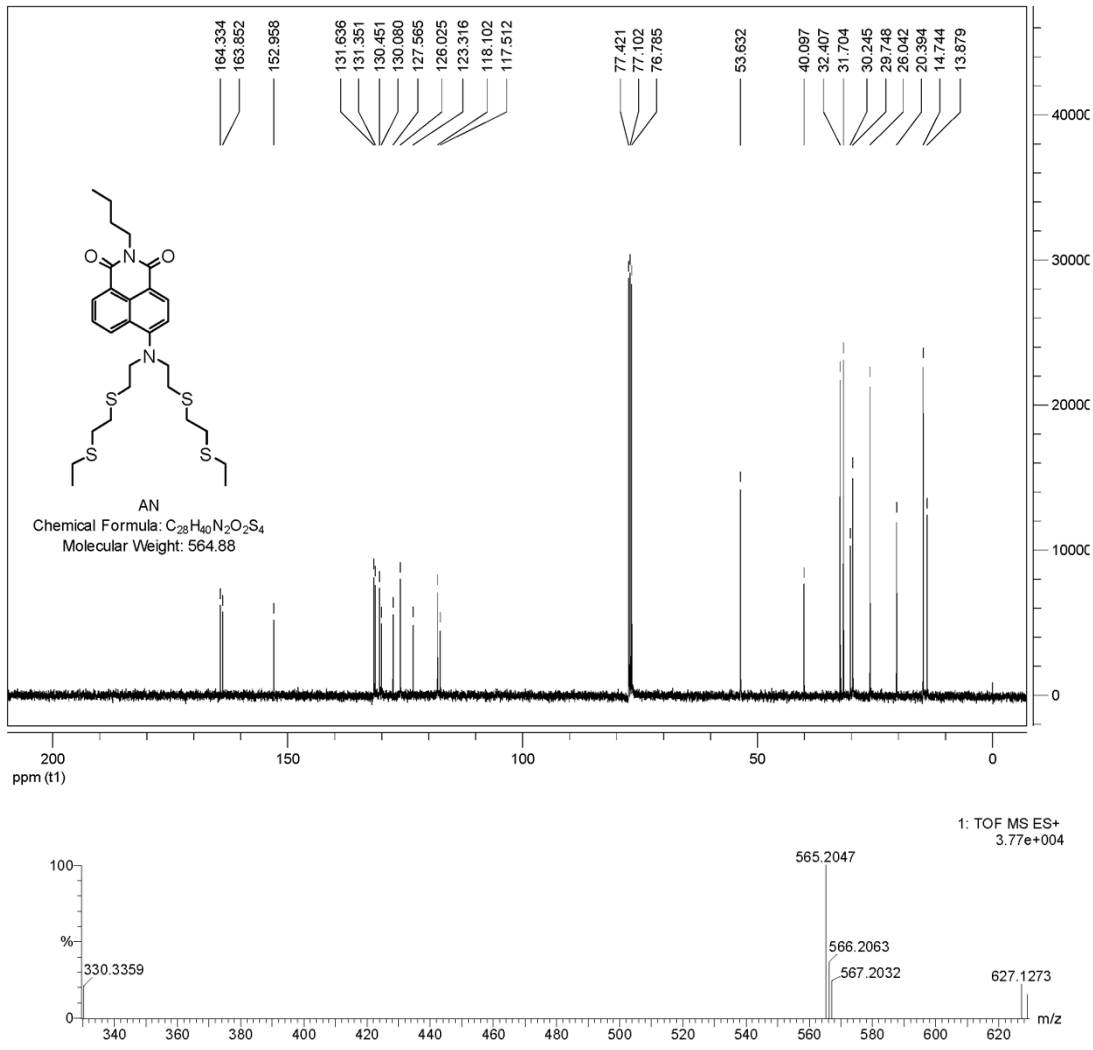


Fig. S9 The ^{1}H-NMR spectra, ^{13}C-NMR spectra and HRMS spectra of **AN**

6. References

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