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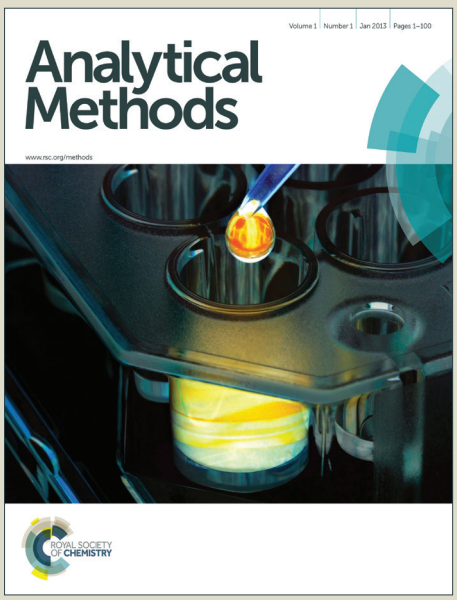

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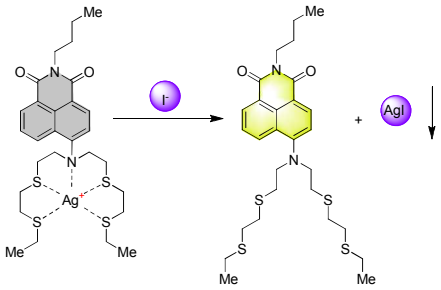
Fluorescence Turn-on Probe of Naphthalimide for Sensitive and Specific Detection of Iodide in Neutral Aqueous Solution and Real Samples

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



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

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A new naphthalimide-based fluorescence turn-on probe (ANAg) was developed for quantitative detection of iodide in aqueous solution. It exhibited low nanomolar detection limit and high selectivity over other potentially interfering anions and implied the potential for practical applications.

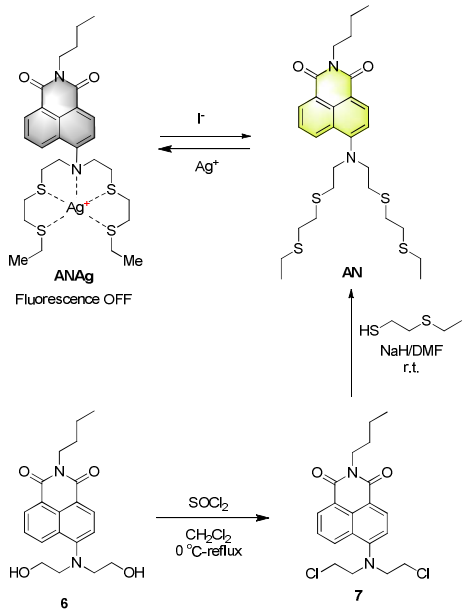
Iodine is an essential element to human and plays fundamental physiological roles at all stages of human development from being a fetus all the way to adulthood.[1] Iodine exists in human body predominantly in form of iodide (I^-), which is the bio-available form of iodine for thyroid.[2] Low iodine uptake often results in miscarriage[3], stillbirth[3], congenital anomalies[3], endemic goiter[4], hypothyroidism[5], cretinism[6], neurological disorders[7] or mental retardation[8]. Iodine was recently found to also display anti-inflammatory and anti-oxidative effects.[9] Natural food source of iodine include various seafood, milk and eggs. Iodine supplemented salt, in form of IO_3^-, is recommended for population who do not have sufficient dietary source of iodine. After ingestion, IO_3^- is reduced in the gut to I^- for absorption.[10] However, excessive uptake of iodine leads to adverse effects, such as degenerative, necrotic, and neoplastic lesions in thyroid gland, stomach or salivary glands.[11] Therefore, robust detection of iodide in aqueous solution has potentials for early stage disease diagnosis and is highly desirable.

A number of methods are available for iodide detection such as neutron activation analysis (NAA)[12], inductively coupled plasma-mass spectrometry (ICP-MS)[13], capillary electrophoresis (CE)[14] and iodide-selective electrodes (ISEs)[15], among which ICP-MS is recommended by the Association of Official Analytical Chemists (AOAC). Though reliable, the protocol based on ICP-MS requires expensive equipments, professional technicians and laborious detection procedures, and hence gives a moderate through-put. In comparison, fluorescence based detections are highly sensitive, versatile and have gained popularity.[16] In addition, instrumentations for fluorescence detection are facile and can be conveniently coupled to plate reader and robots to achieve high operational through-put.[17] Based on supramolecular recognition, several fluorescence probes coordinated to Cu^{2+} for I^- were reported (Fig. S1, ESI†).[18] Recently, a detection methodology for I^- emerged, which relies on I^- induced dissociation of Ag^+ from its fluorescent ligand.[17d, 19] These probes typically work in a solvent system with low water content, require an acidic pH or yield a turn-off signal.

Herein, we report an ensemble (**ANAg**) for fluorescent I^- detection. It operates in neutral aqueous media, yields a turn-on signal and displays superior detection limit. This probe was constructed by tethering a Ag^+-selective receptor to the 4-amino-1,8-naphthalimide, a fluorophore routinely used in templating a probe. I^- is expected to be able to sequester the Ag^+ ion from its ligand (**AN**) and concomitantly turn-on the fluorescence of the ligand because of its high affinity toward Ag^+ in aqueous media (Scheme 1).

The synthesis of the ligand was shown in scheme 1. The two hydroxyls in compound **6**[20] were converted to chloride in refluxing $SOCl_2$ to furnish **7**, which was further displaced by 2-(ethylthio)ethanethiol in anhydrous DMF to generate the ligand **AN.** The structure and purity of **AN** were verified by ^{1}H-NMR, ^{13}C-NMR and HRMS (Fig. S9, ESI†).

The **ANAg** complex was prepared by addition of Ag^+ into a solution of ligand **AN** in HEPES buffer (50 mM at pH = 7.4, 1% DMSO). Ligand **AN** has an maximum absorption at 420 nm. Upon photoexcitation, it gives an intense emission band with the maximum at 520 nm in buffered aqueous media (50 mM at pH = 7.4). This is attributed to the efficient intramolecular charge transfer (ICT) process from the dialkylamine group (at C-4) to the diimide moiety (at C-1 and C-8) of the fluorophore 1,8-



Scheme 1 Synthesis and I^- detection mechanism of **ANAg**

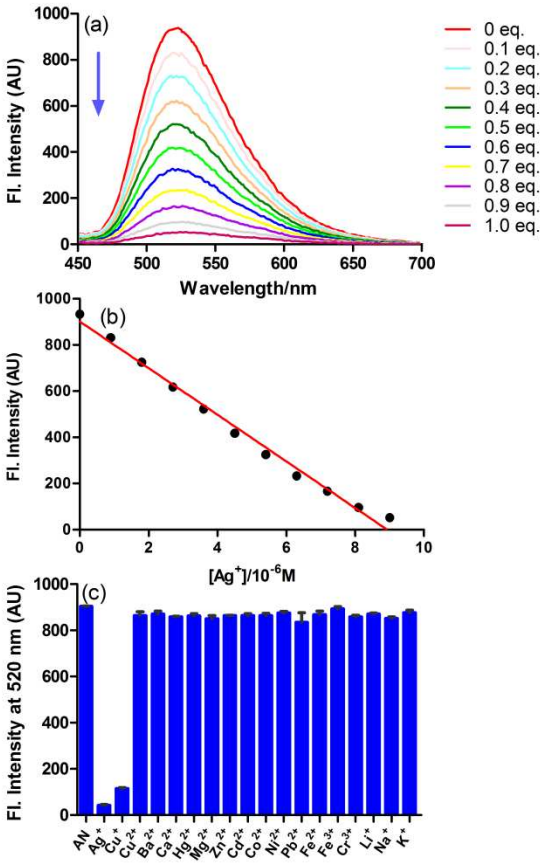


Fig.1 (a) Fluorescence spectra of **AN** (9 μM) in aqueous solution (HEPES, 50 mM, pH 7.4, 1% DMSO) in the presence of increasing concentration of Ag^+ (from 0 to 1.0 eq.). (b) linear relationship of fluorescence intensity at 520 nm as a function of Ag^+ concentrations. (c) Fluorescence responses of **AN** (9 μM) to metal ions (9 μM) in the aqueous buffer (HEPES, pH 7.4, 50mM, 1% DMSO). Excitation at 420 nm. Excitation and emission slit widths were both 5 nm

naphthalimide. Addition of Ag^+ induced a hypsochromic shift of the absorption band of **AN** from 420 nm to 400 nm (Fig. S1, ESI†). This suggests that coordination of nitrogen atom (at C-4) to Ag^+ occurred and the push-pull backbone of the naphthalimide fluorophore is affected. For this reason, fluorescent intensity of the solution decreased linearly with respect to the dose of added Ag^+ (Fig. 1a). Fluorescence quantum yield of the ligand **AN** dropped from 0.2 to 0.004 after Ag^+ association. It is likely that spin-orbit coupling of Ag^+ further contributed to the quenching of the excited state of **AN**.[21] A Job plot showed 1:1 stoichiometry between **AN** and Ag^+ (Fig. S3). The association constant between **AN** and Ag^+ was calculated to be $4.38 \times 10^8\ M^{-1}$ by least-square nonlinear analysis (Fig. S4, ESI†). We note that the ligand **AN** may also chelate Cu^+ with a similarly high association constant compared to Ag^+. This is not surprising since the tetrathia receptor was originally developed by Chang for Cu^+ recognition[22]. However, the affinity of Cu^+ toward I^- is much inferior ($Ksp_{(CuI)} = 1.1\times10^{-12}$, $Ksp_{(AgI)} = 8.3\times10^{-17}$) and therefore was not chosen in our study for iodide recognition (Fig. S6, ESI†). Other metal ions did not display an appreciable association constants to ligand **AN**, including Cu^{2+}, Ba^{2+}, Ca^{2+},Hg^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Li^+, Na^+ and K^+ (Fig. 1c and Fig. S5, ESI†).

Spectral responses of **ANAg** complex toward I^- were studied in HEPES buffer (50 mM at pH = 7.4) with 1% DMSO as a co-solvent (Fig. 2 and Fig. S7, ESI†). Upon addition of I^- to the **ANAg** complexes, Ag^+ was sequestered from the ligand **AN** and a bathochromic shift in absorption spectra (20 nm) and a fluorescence enhancement were observed with a maximum intensity at 520 nm, which was linearly increased in the range of 0.9 to 9 μM ($R^2 = 0.9972$) (Fig. 2b). A lower detection limit of 1.72×10^{-8} M was calculated with LDL = 3σ/slope. This renders probe **ANAg** more sensitive than the existing ones, to our knowledge.[18a, 18b, 19]

The spectral response of **ANAg** to various anions in HEPES buffer (pH 7.4, 50 mM, 1% DMSO) was performed to investigate the selectivity of this probe. Upon addition 1 equiv. of various anions, including F^-, Cl^-, Br^-, HCO_3^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, CH_3COO^- or S^{2-} respectively, none induced a noticeable fluorescence enhancement (Fig. 3a and Fig. S8, ESI†). The competition experiments were also conducted for ANAg. Fig 3b indicated that the fluorescence could not be recovered by I^- in the prescence of S^{2-}, other species had no obvious interference for I^- detection. Therefore, **ANAg** was a selective fluorescence "turn-on" switch probe for discrimination between I^- and potentially interfering anions in neutral aqueous solution.

To further demonstrate the potential of the probe **ANAg** for practical applications, we exemplified its detection of iodine content in commercial salts of various brands available in local markets. Results were compared side-by-side with concentration

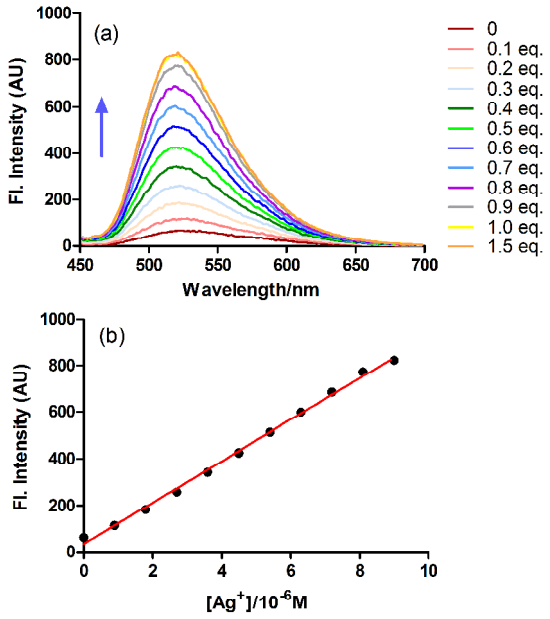


Fig. 2 (a) Fluorescence spectra of **ANAg** (9 μM) in aqueous solution (HEPES, pH 7.4, 50 mM, 1% DMSO) in the presence of increasing concentrations of I^- (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6,0.7, 0.8, 0.9, 1.0, and 1.5 equiv. respectively).(b) Linear relationship of fluorescence intensity at 520 nm as a function of I^- concentrations. Excitation at 420 nm. Excitation and emission slit widths were both 5 nm

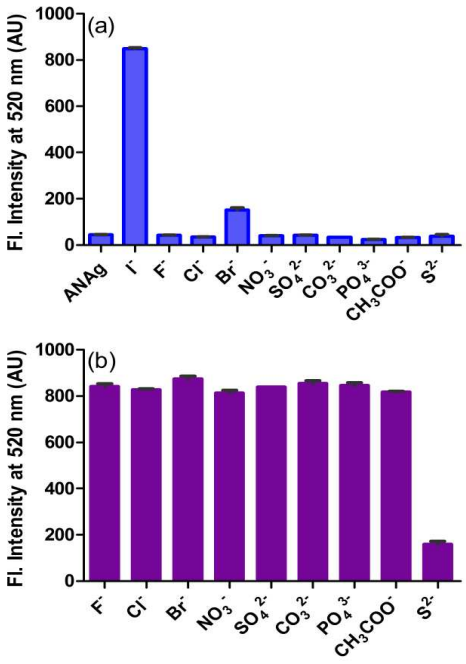


Fig. 3 (a) Fluorescence responses of the probe **ANAg** (9 μM) to anions (9 μM) in the aqueous buffer (HEPES, pH 7.4, 50mM, 1% DMSO). (b) Fluorescence responses of the probe **ANAg** (9 μM) to I^- in the presence of anions (9 μM) in the aqueous buffer (HEPES, pH 7.4, 50mM, 1% DMSO). Excitation at 420 nm. Excitation and emission slit widths were both 5 nm.

obtained by standard titration method.[23] We note that the IO_3^- in the salt was reduced to I^- prior to detection by treatment of ascorbic acid.[24] Obviously, results from these two methods are essentially identical (Table 1), suggesting that **ANAg** based protocol is highly reliable (seeing supporting information).

Table 1 Determination of iodine in real samples with different methods

Samples[a]	ANAg (mg/kg)	Method A[b] (mg/kg)
1	34.33±0.11[c]	33.84±0.21
2	0.22±0.09	0±0.21
3	30.11±0.24	30.46±0.21
4	0.28±0.03	0±0.21

[a] Sample 1 and sample 3 are iodised salts with different brands, Sample 2 and sample 4 are iodine-free salts with different brands.

[b] Standard titration method.

[c] Results are mean and standard deviation of three independent measurements.

In conclusion, we have presented that the complex **ANAg** is a robust probe for detecting I^- in neutral aqueous solution by sequestrating Ag^+ from **ANAg**. Its fluorescence intensity enhanced in a linear fashion with respect to the concentration of I^-. A low detection limit of 17.2 nM of I^- was obtained. We further exemplified its potential for practical applications by determination of iodine content in various commercial salt samples.

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Notes and references

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