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A Highly Selective Colorimetric and Ratiometric Two-Photon Fluorescent Probe for Fluoride Ion Detection

Jun Feng Zhang,[†,‡,§] Chang Su Lim,[†,§] Sankarprasad Bhuniya,[†] Bong Rae Cho,[*,†] and Jong Seung Kim[*,†]

Department of Chemistry, Korea University, Seoul 130-701, Korea, College of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming 650092, P.R. China

jongskim@korea.ac.kr; chobr@korea.ac.kr

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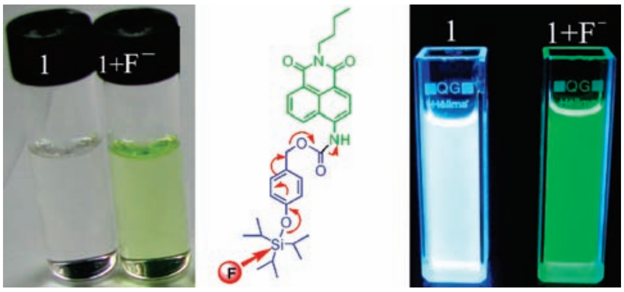
ABSTRACT



A naphthalimide-based highly selective colorimetric and ratiometric fluorescent probe for the fluoride ion displayed both one- and two-photon ratiometric changes. Upon reaction with the F^- (TBA^+ and Na^+ salts) anion in CH_3CN as well as in aqueous buffer solution, probe 1 shows dramatic color changes from colorless to jade-green and remarkable ratiometric fluorescence enhancements signals. These properties are mechanistically ascribed to a fluoride-triggered Si–O bond cleavage that resulted in a green fluorescent 4-amino-1,8-naphthalimide.

Fluorescent sensors for the detection and measurement of biologically important anions are actively investigated because of their roles in vital processes.[1] Among the anions, the fluoride ion (F^-) is of particular interest owing to its established role in dental care and clinical treatment for osteoporosis.[2] An acute intake of a large dose or chronic ingestion of lower doses of F^- can result in gastric and kidney disorders, dental and skeletal fluorosis, urolithiasis in humans, and even death.[3] For these reasons, an improved method for the detection and sensing of F^- with high selectivity is of current interest in the chemosensor research field.

A variety of fluorogenic chemosensors showing absorbance or fluorescence changes upon binding with F^- have been reported.[4] However, most of them show turn-off response in emission spectra and modest selectivity. For a quantitative measurement of F^-, a ratiometric sensor with high selectivity is required.[5] Although many ratiometric

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[†] Korea University.

[‡] Yunnan Normal University.

[§] These authors contributed equally to this work.

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fluorescent probes for cations have been reported,[6] very few ratiometric fluorescent sensors for F^- are known.[7] Thus, it still remains a challenge to develop a ratiometric fluorescence probe for F^-.

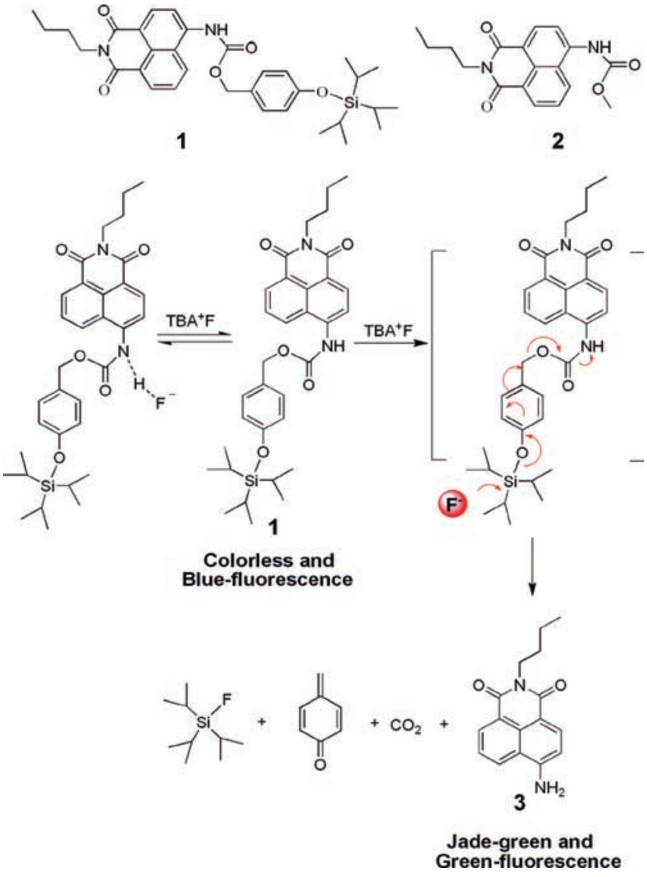
Recently, two-photon microscopy (TPM), utilizing two photons of lower energy for the excitation, has become a vital tool in biology. Compared to traditional fluorescence microscopy, TPM offers intrinsic 3D resolution combined with reduced phototoxicity, increased specimen penetration, and negligible background fluorescence. To make TPM more versatile tool in biology, it is crucial to develop a variety of two-photon (TP) probes for specific applications.[8] Although some TP probes have been reported, only a few of them had sufficient selectivity for anions.[9] Moreover, TP probes that can selectively detected analyte via ratiometric measurement are very rare.

To develop a ratiometric TP probe for F^-, we utilized 1,8-naphthalimide, a prototype intramolecular charge transfer (ICT) fluorophore, because of its advantageous optical properties, such as strong absorption and emission in the visible region, high photostability, large Stokes' shift, insensitivity to pH and a significant two-photon cross section.[10] A silyl ether has been introduced as the reaction site for F^-, because of the high affinity of F^- for silicon,[11] with the expectation that the reaction of F^- with **1** would trigger the cleavage of Si−O bond and release the green fluorescent 4-aminonaphthalimide (**3**) as shown in Scheme 1, thereby inducing the ratiometric changes in both color and fluorescence.

Herein, we report a new ratiometric TP fluorescent probe **1**, which shows a dramatic color change, remarkable ratiometric fluorescence enhancement, and significant ratiometric changes in the TP absorption and emission spectra upon addition of F^- in CH_3CN. For comparison, the photophysical properties of a reference compound **2** are also presented.

The synthesis of **1** is outlined in Scheme S1 (see Supporting Information). To synthesize **1**, *N*-butyl-4-amino-1,8-naphthalimide (**3**) was reacted with phosgene in the presence of 4-dimethylaminopyridine (DMAP) to obtain the carbamoyl chloride as an intermediate. Reaction of the carbamoyl chloride with [4-(triisopropylsilyoxy) phenyl] methanol (**4**) at 0 °C afforded **1** in 41% yield. Compound **2** was obtained in 59% yield by the same procedure except that methanol was used in place of **4**. The detailed experimental procedures and ^1H and ^{13}C NMR spectra are summarized in Supporting Information.

Scheme 1. Structures of Chemosensors **1** and **2**, and a Plausible Mechanism for the Spectroscopic Changes of **1** in the Presence of F^-



The reaction of **1** with tetrabutylammonium fluoride (TBAF) produced *N*-butyl-4-amino-1,8-naphthalimide (**3**) as the only product as revealed by the identical NMR spectrum of the reaction product compared to that of authentic **3** (Figure S1). This indicates that the reaction between **1** and TBAF most probably proceeds by the proposed mechanism as shown in Scheme 1.

When excess F^- (120 μM) was added to **1** in MeCN, the colorless solution turned jade-green; this was clearly recognizable by naked eyes. At a lower F^- concentration (< 40 μM) the absorption band at 365 nm decreased and a new band with a shoulder at 487 nm appeared (Figure 1). To assess the origin of this shoulder, we performed a proton NMR titration (Figure S1). When 3 equiv of F^- was added to **1**, the N−H signal of **1** at 7.46 ppm disappeared, whereas other protons shifted toward upfield. This indicates that the N−H bond is deprotonated by

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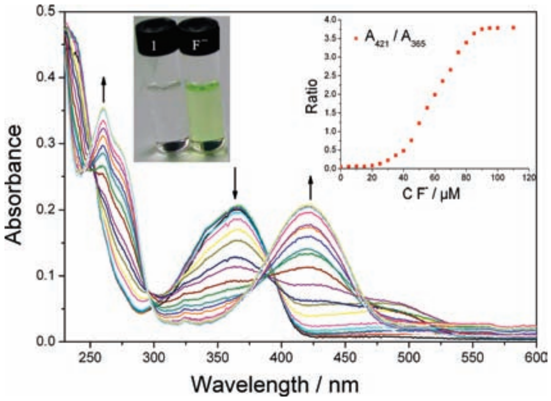

Figure 1. Absorbance spectra of **1** (10 μM) in CH_3CN in the presence of TBAF (0−110 μM). Insets: (left) color changes of **1** (10 μM) upon additions of TBAF (110 μM) in CH_3CN; (right) ratio of absorbance at 421 nm and absorbance at 365 nm as a function of F^- concentration.

F^- in MeCN, thereby increasing the negative charge density at the amide nitrogen. This would facilitate the intramolecular charge transfer (ICT) between the donor and acceptor and shift the emission maxima to a longer wavelength, as observed. The reference compound **2** showed similar behavior; the absorption band at 365 nm decreased while a new band at 487 nm appeared upon addition of F^-, AcO^-, and $H_2PO_4^-$. It is worth noting that the absorption band is not altered by the less basic anions such as Cl^-, Br^-, I^-, HSO_4^-, NO_3^-, PF_4^-, and ClO_4^- (Figures S3 and S4). These results provide strong evidence that the shoulder at 487 nm is probably due to the deprotonation of the amide N−H of **1**.[12]

As the F^- concentration was further increased (> 50 μM), the shoulder at 487 nm decreased and the absorption band at 425 nm increased, indicating that the F^--promoted cleavage of the silyl ether moiety liberating **3** has become the predominant reaction pathway under this condition (Scheme 1). Moreover, the ratios of the absorbance at 421 and 365 nm showed sigmoid dependence on the F^- concentration, with a 76-fold ratiometric enhancement (Figure 1, inset). This indicates the capability of **1** for quantitatively detecting F^- by absorption ratiometry.

We next assessed the ability of **1** to detect F^- by ratiometric fluorescence measurement. When F^- was added to **1**, the emission intensity at 449 nm decreased and that at 508 nm increased (Figure 2). Addition of F^-, AcO^-, and $H_2PO_4^-$ to **2** partially quenched the fluorescence without altering the emission maxima, whereas no change in the emission spectra were observed with less basic anions such as Cl^-, Br^-, I^-, HSO_4^-, NO_3^-, PF_4^-, and ClO_4^- (Figure S4). Hence, the initial decrease in the fluorescence intensity can be attributed, at least in part, to the fluorescence quenching due to the deprotonation of N−H bond of **1**.[12] When the F^- concentration was further increased, the deprotection of the silyl ether moiety to liberate **3** became the major reaction pathway and the fluorescence intensity at 508 nm increased. Moreover, the ratios of the emission intensities at 449 and 508 nm (F_{508}/F_{449}) showed sigmoid dependence on the F^- concentration, with a 220-fold ratiometric enhancement (Figure 2, inset). This indicates the capability of **1** for quantitatively detecting F^- by fluorescence ratiometry.

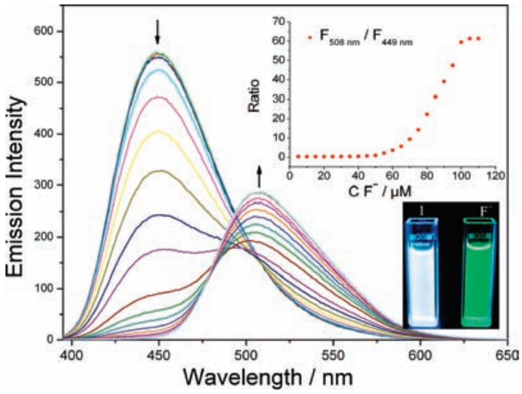

Figure 2. Fluorescence spectra of **1** (10 μM) in CH_3CN in the presence of TBAF (0−110 μM). Insets: (upper) ratios of fluorescent intensities at 508 and 449 nm as a function of F^- concentration; (lower) fluorescence images of **1** (10 μM) before and after additions of TBAF (110 μM) in CH_3CN. The excitation wavelength was 365 nm.

We then evaluated the selectivity of **1** for F^- by measuring the changes in the absorption spectra upon addition of excess amount of various anions. The unique absorption change with appearance of the jade-green color was observed only by the addition of F^-, which can be ascribed to the F^--promoted cleavage of the Si−O bond liberating **3**. When AcO^- and $H_2PO_4^-$ were added, the colorless solution of **1** turned pale yellow with appearance of a new absorption band centered at 487 nm. This is again due to the deprotonation of the amide N−H bond of **1** by the relatively basic AcO^- and $H_2PO_4^-$. On the other hand, no change in the UV spectra was noted with other anions such as Cl^-, Br^-, I^-, HSO_4^-, NO_3^-, PF_6^-, and ClO_4^- (Figure S5).

The fluorescence spectra showed different behavior. Compound **1** Showed strong emission at 449 nm upon excitation at 390 nm in CH_3CN. Addition of Cl^-, Br^-, I^-, HSO_4^-, NO_3^-, PF_6^-, and ClO_4^- did not change the emission spectra, although partial fluorescence quenching was observed upon the addition of AcO^- and $H_2PO_4^-$ (Figure S6). The unique emission red-shift was observed only with F^-, which further substantiated the excellent selectivity for F^- among all other anions. Furthermore, the unique absorbance and fluorescence bands resulting from the addition of the F^- ion were not influenced by the subsequent addition of other anions, including Cl^-, Br^-, I^-, HSO_4^-, NO_3^-, PF_6^-, ClO_4^-, AcO^-, and $H_2PO_4^-$ (Figures S7−S10). These results indicate the excellent

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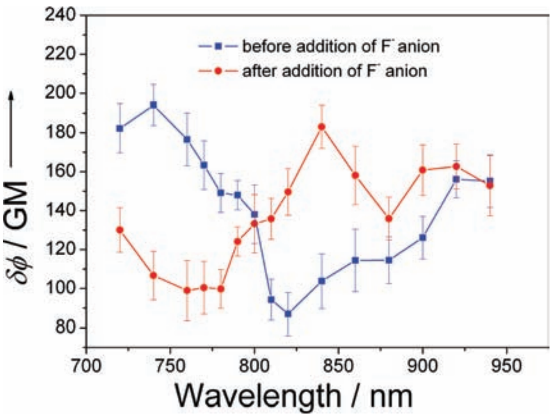

Figure 3. Two-photon action spectra of **1** (10 μM solution in CH_3CN) before (blue line) and after (red line) addition of TBAF (110 μM). Error bars represent the standard deviation from replicate experiments (n = 10).

selectivity of **1** for the F^- ion over other competitive anions.

Having established the utility of **1** by one-photon spectroscopy, we have investigated the TP properties of **1**. The TP action spectrum of **1** as determined by the fluorescence method showed the maximum TP action cross section ($\Phi\delta$) value of 195 GM at 740 nm. Upon addition of excess TBAF, the wavelength of the maximum $\Phi\delta$ value increased to 840 nm (Figure 3). The shape of the TP action spectra before and after addition of TBAF are qualitatively similar to those of corresponding one-photon absorption spectra. Moreover, the TP excited fluorescence spectra of **1** showed ratiometric emission profile upon addition of increasing amount of TBAF (Figure S11). Further, the ratios of the emission intensities at 515 and 465 nm (F515/F465) showed sigmoid dependence on the F^- concentration, with a 15-fold ratiometric enhancement (Figure S11, inset), thus offering a strong turn-on ratiometric response of the TP fluorescence emission. This indicates the capability of **1** for quantitatively detecting F^- by the TP fluorescence ratiometry.

We further explored the practical application of probe **1**. To this end, we have repeated all of the above experiments in CH_3CN-HEPES buffer solution (0.02 M, pH = 7.4) (8:2, v/v). In contrast to the almost instantaneous reaction in CH_3CN, the reaction of **1** with F^- required 3−8 h in CH_3CN-HEPES buffer depending on the reaction conditions. This can be attributed to the strong solvation of F^- by water, which would undoutedly have increased the activation barrier. Nevertheless, the reaction of **1** (10 μM) with NaF (20 mM) showed a ratiometric fluorescence changes in CH_3CN-HEPES buffer (Figure S12). Also, a high selectivity for F^- was retained in CH_3CN-HEPES buffer, as shown by the little change in the emission spectra in the presence of other anions. Moreover, the color of **1** changed from colorless to jade-green upon addition of excess NaF in CH_3CN-HEPES buffer, while other anions did not induce color changes. These results demonstrated that **1** can also be used in aqueous solution as a ratiometric fluorescent probe for F^-.

In summary, we have developed a novel colorimetric and ratiometric fluorescent sensor **1** for F^-. It displays a 49 nm red-shift of fluorescence emission, a dramatic color change from colorless to jade-green, as well as two-photon absorption and emission ratiometric changes upon addition of F^- in CH_3CN solution, as a result of F^--promoted Si−O bond cleavage liberating a green fluorescent 4-amino-1,8-naphthalimide. Moreover, as far as we are aware, **1** is the first naphthalimide-based dual spectroscopic ratiometric sensor for F^- that can be used both with one- and two-photon spectroscopy.

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Supporting Information Available. Experimental procedures, characterization data of compounds, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.