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Single molecular multianalyte signaling of sulfide and azide ions by a nitrobenzoxadiazole-based probe

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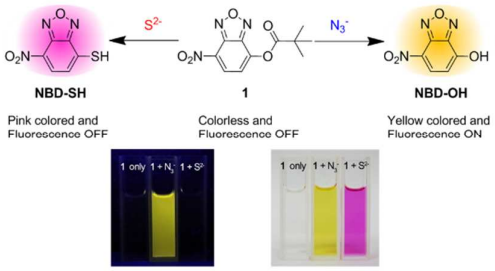
Sulfide- and azide-selective multianalyte optical signaling using nitrobenzoxadiazole-pivalate was realized by regioselective cleavage of the probe under the same conditions.



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Single molecular multianalyte signaling of sulfide and azide ions by a nitrobenzoxadiazole-based probe

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Sulfide- and azide-selective optical signaling using a single nitrobenzoxadiazole (NBD)-based probe was investigated. NBD-pivalate showed colorimetric signaling for sulfide via cleavage of the NBD–O bond to generate NBD–SH. It also showed chromogenic and fluorogenic signaling for azide by O–CO-Bu^t cleavage to yield NBD–OH under the same conditions.

The selective signaling or determination of chemically and environmentally important species is one of the most critical topics in modern chemical science.[1] Among various environmentally important targets, anionic species such as fluoride,[2] phosphates,[3] and cyanide[4] ions have attracted much research interest. Hydrogen sulfide is one of the most important industrial materials for the production of elemental sulfur, thiols, sulfides, and metal sulfides.[5] On the other hand, it is generated by degradation of organic matter, extraction of natural gas or oil, and refining of natural gas and crude oil.[6] However, hydrogen sulfide is known to be a toxic species.[7] According to the National Institute of Occupational Safety and Health (NIOSH), the concentration of H_2S immediately dangerous to life or health is 100 ppm, and the recommended exposure limit is 10 ppm for a maximum duration of 10 min.[8] For the analysis of H_2S, spectroscopic methods are undoubtedly most commonly employed ones, with electrochemical and chromatographic techniques holding the second and third positions, respectively.[9] In fact, a number of fluorescent probes have been developed for sensing and visualizing biological H_2S.[10] They are based on reduction of azides to amines, trapping via nucleophilic addition, and precipitation of metal sulfides.[11] However, by comparison, despite its industrial significance and adverse influence on human health in high concentration, H_2S determination in chemical and environmental samples has not been as actively investigated.[12]

Azide ion is also a toxic chemical species, possessing toxicity similar to that of cyanide.[13] However, it is widely used in industry, such as for preparation of biocides, in detonators for explosives, as a radical scavenger, and in anti-corrosion solutions.[14] It also has been used as a gas generant for automobile safety air bags.[15] However, azide-selective optical signaling systems have rarely been investigated. Fluorescence sensors based on a Cu^{2+} complex of a Schiff base[16] and formation of a molecular ring by indolyl-naphthalene[17] have been reported. More recently, a selective azide probe, based on the naphthalimide fluorophore and an alkyne receptor for click-activated ligation with azide, has been developed.[18] We recently reported an azide-selective dual signaling probe utilizing the selective deprotection of dichlorofluorescein chloroacetate.[19]

Recently, development of sensors or probes for multi-ion target has attracted much research interest. In particular, signaling of multiple analytes with a single molecular device is an ideal target for the chemical and biological sciences. Several systems have been successfully designed for the targeting of metal ions. Representative examples include differential detection of Hg^{2+} and Au^{3+} ions by a fluorescent probe based on the rhodamine-BODIPY platform,[20] Zn^{2+} and Cu^{2+} detection by a fluorescent chemodosimeter based on 1,8-naphthyridine,[21] multi-analyte chemosensing of Ba^{2+}, Hg^{2+} by a BODIPY and rhodamine conjugate,[22] and Zn^{2+} and F^- signaling by a multicomponent dansyl-NBD dyad.[23] However, most of these systems target metal ions, and we could not find previous literatures for the discrimination of anions by a single molecular probe.

We have devised a new multianalyte signaling probe for sulfide and azide ions based on an ester derivative of 4-hydroxy-7-nitrobenzoxadiazole (NBD–OH). Sulfide signaling was accomplished by sulfide-assisted cleavage of NBD ethers, such as NBD–OPh, NBD–SPh, and NBD–SePh.[24,25] On the other hand, azide signaling by the hydrolysis of phenol-acetate has been reported by us.[19] We have combined these two processes in a single molecule, NBD–pivalate **1**, expecting to achieve single molecular multianalyte signaling of sulfide and azide ions. The pivalate derivative of NBD **1** showed a prominent chromogenic

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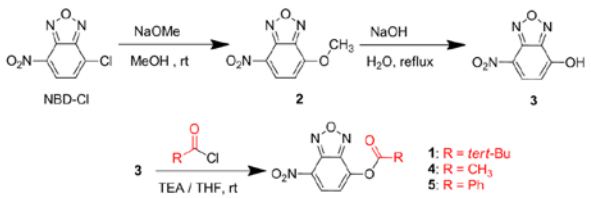
signaling behavior towards sulfide ions owing to the sulfide-selective cleavage of the NBD–O bond to generate pink colored NBD–SH. It also showed chromogenic and fluorescence turn-on type signaling by azide-assisted O–CO-Bu^t cleavage to yield yellow colored and fluorescent NBD–OH under the same conditions.

The NBD-based ester probes, NBD–pivalate **1,** NBD–acetate **4,** and NBD–benzoate **5**, were prepared by a three step reaction from NBD–Cl (Scheme 1). Reaction of NBD–Cl with sodium methoxide afforded methyl ether **2** (methanol, rt, 87%). Hydrolysis of **2** with NaOH resulted in an alcohol NBD–OH **3** (60%). Esterification of **3** with pivaloyl chloride yielded the pivalate **1** (THF, rt, 65%). Treatment of the alcohol **3** with acetyl chloride or benzoyl chloride gave the acetate **4** and benzoate **5**, respectively. Preliminary studies showed that the acetate **4** and benzoate **5** showed significant spontaneous hydrolysis to NBD–OH **3** under the signaling conditions (Fig. S1, ESI†). Therefore, all the experiments were carried out using the pivalate **1** in a 1:1 (v/v) mixture of acetonitrile and HEPES buffer solution (pH 7.1), where relatively well optimized signaling selectivity, signaling speed, as well as probe stability of pivalate **1** were observed.



Scheme 1 Preparation of ester derivatives of NBD **1**, **4**, and **5**.

NBD–pivalate **1** showed an absorption band at 340 nm in 50% aqueous acetonitrile, buffered at pH 7.1 (Fig. 1). Upon addition of various anions, the absorption spectra of **1** changed significantly only for two ions, sulfide and azide ions. An interesting observation is that the response of **1** was quite diagnostic for these two ions under the same measurement conditions. The band at 340 nm of **1** decreased and new stronger absorption bands appeared at 547 nm for sulfide and 464 nm for azide ions, respectively. Concomitantly, prominent solution color changes from colorless to pink for sulfide, or colorless to yellow for azide, were observed. The changes in the spectral profile, as well as the solution color change of **1**, are unique enough to differentiate the individual responses from the sulfide and azide ions independently.

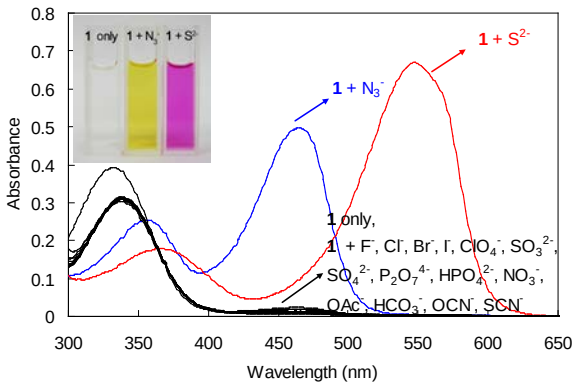


Fig. 1. UV–vis spectra of **1** in the presence of various anions (A^{n-}). Inset: Picture of **1** in the absence and presence of S^{2-} and N_3^- ions. [**1**] = 3.0 × 10^{-5} M, [A^{n-}] = 3.0 × 10^{-4} M. Absorption spectra were recorded in a 1:1 (v/v) mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM).

Because the spectral changes are quite large, ratiometric analysis of the signaling behavior of NBD–pivalate **1** towards anions was attempted. Ratiometry using the changes in absorbance ratio of pivalate **1** at 547 and 340 nm (A_{547}/A_{340}), clearly showed the prominent selectivity toward sulfide ions (A_{547}/A_{340} = 4.74) (Fig. S2, ESI†). The ratio for other ions varied between a narrow range of 0.01 for the rest of the tested anions and 0.02 for azide ions. Azide signaling selectivity of **1** was also pronounced and only the sulfide ions interfere considerably. The absorbance ratio of **1** at the two characteristic wavelengths of 464 and 340 nm (A_{464}/A_{340}) clearly showed the prominent selectivity toward azide ions (A_{464}/A_{340} =2.36) (Fig. S3, ESI†). In this case, sulfide ions showed interference in the selective signaling of azide by **1** with a considerable A_{464}/A_{340} ratio of 0.67. Other ions showed little interference, revealing ratios between 0.02 for SO_4^{2-} and 0.08 for F^-.

NBD–pivalate **1** showed weak fluorescence emission at 561 nm in 50% aqueous acetonitrile, buffered at pH 7.1 (Fig. 2 and Fig. S4, ESI†). Among the tested anions, only azide revealed significant fluorescence enhancement at 549 nm (I/I_0 = 62.6). Contrary to the distinctive colorimetric signaling behavior of **1** for the two anions of sulphide and azide, fluorescence signaling was possible only for azide ions. Sulfide ions did not induce any useful fluorescence changes due to the rather weak fluorescence of the signaling product NBD–SH. Often, the NBD derivatives with substituents containing bridging O, N, or S atom at the 4 position showed very weak emission.[26] Other common anions showed negligible responses: the I/I_o at 549 nm varied in a narrow range between 1.0 (for NO_3^-) and 4.8 (for F^-).

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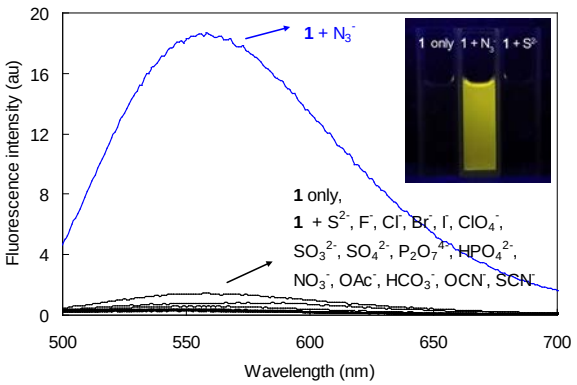

Fig. 2. Fluorescence spectra of **1** in the presence of various anions. Inset: Picture of **1** in the absence and presence of S^{2-} and N_3^- ions under a UV lamp. [**1**] = 1.5 × 10^{-5} M, [A^{n-}] = 1.5 × 10^{-4} M. Fluorescence spectra were measured in a 1:1 (v/v) mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), λ_{ex} = 355 nm.

The observed signaling is due to the reaction of **1** with sulfide and azide ions in completely different modes (Scheme 2). Nucleophilic reaction of pivalate **1** with a sulfide ion was regioselectively carried out at the C-4 atom of the NBD moiety, whereas that with azide ion was exclusively performed at the carbonyl carbon of the pivaloyl group. The NBD–SH for sulfide ions or NBD–OH for azide ions, thus generated, showed their characteristic signaling behaviors. The suggested transformation from **1** to NBD–SH or NBD–OH, by sulfide or azide ions, respectively, was confirmed by ^{1}H NMR and UV–vis measurements. The ^{1}H NMR spectrum of **1** in the presence of sulfide or azide ions was identical to that of NBD–SH and NBD–OH, respectively (Fig. 3 and Fig. S5, ESI†). After treatment with 10 equiv of sulfide or azide ions, the UV–vis spectrum of **1** transformed to those of NBD–SH and NBD–OH (Fig. S6 and S7, ESI†). The signaling speed of **1** was relatively fast and completed within 5 min for both sulfide and azide ions (Fig. S8, ESI†). Furthermore, signaling of sulfide and azide ions was not significantly affected by the solution pH between pH 3.6 and 9.0 (Fig. S9, ESI†).

Scheme 2 Signaling of sulfide and azide ions by regioselective cleavage of NBD–pivalate **1**.

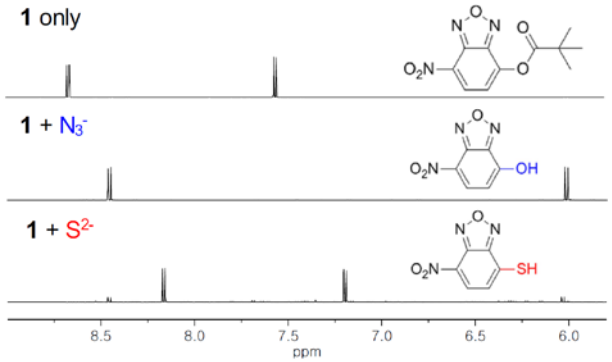

Fig. 3. Partial ^{1}H NMR spectra of **1** alone and **1** in the presence of N_3^- and S^{2-} ions. [**1**] = 5.0 mM in CD_3OD. Spectra for **1**+N_3^- and **1**+S^{2-} were obtained after purification of the signaling product by column chromatography.

Next, competitive signaling behavior of **1** for sulfide and azide ions was elucidated. In the presence of other anions as background, responses of **1** toward sulfide ions are not significantly affected (Fig. 4, grey bar). Only sulfite ions interfered significantly with the signaling of sulfide ions. That is due to the direct reaction of targeted sulfide ions with background sulfite ions. In neutral to alkaline solutions (pH ≥ 7) sulfite is known to react with sulfide to yield thiosulfate.[27] On the other hand, azide-selective signaling of **1** was also not influenced by the presence of common anions except sulfite ions (Fig. 4, black bar). Azide ions are known to form a complex with sulfur dioxide having a composition of $3N_3^-$–$4SO_2$.[28] Therefore, considerably diminished azide signaling of **1** was observed due to the formation of the complex with sulfur dioxide that was in equilibrium with the sulfite ions in the aqueous solution.[29] In addition, the possible mutual interference of azide and sulfide ions in the selective signaling of each individual ion was examined. The signaling of targeted ions by probe **1** was found to be not significantly influenced by the presence of each other ion (Fig. S10 and S11, ESI†).

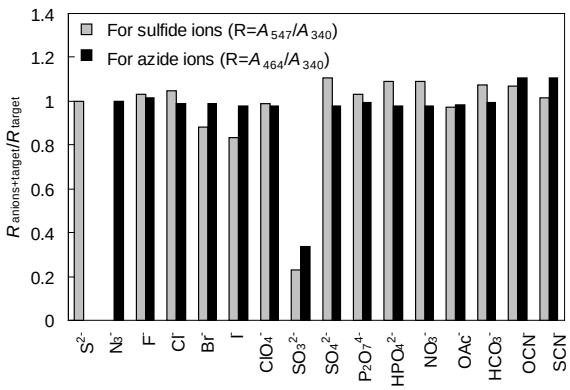

Fig. 4. Competitive signaling of sulfide (grey bar) and azide (black bar) ions by **1** in the presence of other anions with background. [**1**] = 3.0 × 10^{-5} M, [S^{2-}] = [N_3^-] = [A^{n-}] = 3.0 × 10^{-4} M. The absorption spectra were recorded in a 1:1 (v/v) mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM).

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The concentration dependence of the signaling behavior of **1** was assessed by titration with sulfide or azide ions. As shown in Fig. 5, the absorbance ratio (A_{547}/A_{340}) at 547 nm and 340 nm increased steadily with the increases in sulfide concentration up to 3.0×10^{-4} M. On the other hand, titration of **1** with azide ions also provided a nice calibration plot up to 3.0×10^{-4} M of azide ions (Fig. S12, ESI†). From these concentration dependent signaling behaviors, the detection limits of **1** for the determination of sulfide and azide ions were estimated as 2.9×10^{-5} M (1.8 ppm) and 1.6×10^{-5} M (0.8 ppm), respectively.[30] To check the possibility for the signaling of sulfide and azide ions in actual samples, determination of analytes in simulated wastewater[31] was carried out. As can be seen from the Fig. S13 and S14 (ESI†), signaling of sulfide and azide ions by **1** in simulated wastewater was possible with a satisfactory calibration plot. These observations imply that the probe **1** could be useful for the optical signaling of sulfide and azide ions in practical samples.

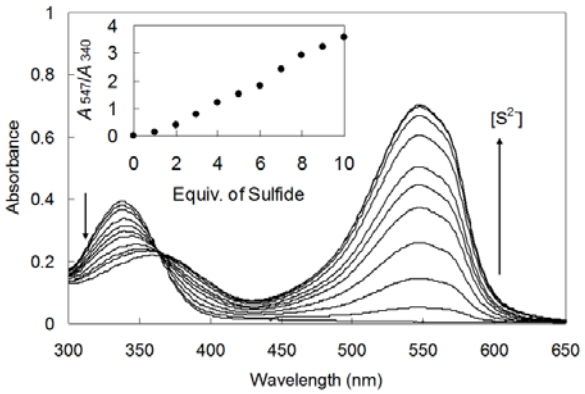

Fig. 5. Concentration-dependent UV–vis signaling of sulfide ions by **1**. [**1**] = 3.0×10^{-5} M, [S^{2-}] = from 0 to 3.0×10^{-4} M. Absorption spectra were recorded in a 1:1 (v/v) mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM).

Finally, application of the probe **1** as a practically useful test strip was attempted (Fig. 6 and S15, ESI†). Upon treatment of the strip with varying amounts of analytes, pink colored spot (in sulfide signaling) or yellow-colored fluorescent spot (in azide signaling) was developed. The intensity profiles of the resulting spots were analysed by Adobe Photoshop. The results imply that the prepared test strip could readily signal sulfide and azide ions down to 10^{-4} M range without any sophisticated instruments.

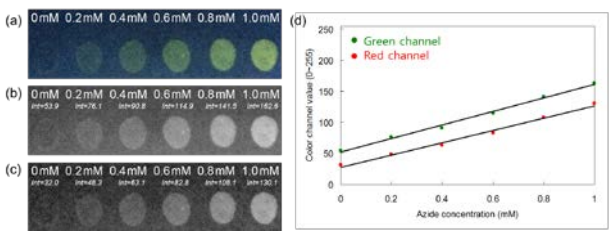

Fig. 6. Pictures of changes in (a) original, (b) green channel, (c) red channel of **1** in the presence of varying amounts of azide ions and (d) changes in green and red channel intensity of **1** as a function of azide ions. Intensities of green and red channel were obtained by recording color channel values (0–255) of image in Adobe Photoshop. [N_3^-] = from 0 to 1.0×10^{-3} M in distilled water.

In summary, a novel reaction-based probe having selective and discriminative signaling behavior towards sulfide and azide ions was investigated. The probe, based on the single molecule NBD–pivalate, exhibited multianalyte signaling behavior by totally different reactions involving cleavage of NBD–O and O–COR bonds for sulfide and azide ions, respectively. Thus generated NBD–SH exhibited a pink color for sulfide, while NBD–OH showed a yellow color with a prominent fluorescence enhancement. The developed probe works in a unique signaling mode, and could be useful for single molecular multianalyte signaling of sulfide and azide ions in aqueous environments.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details, NMR spectra, and chemosignaling behavior of the probe are reported. See DOI: 10.1039/c000000x/

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