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# A Near-Infrared Fluorescence Turn-On Sensor for Sulfide Anions

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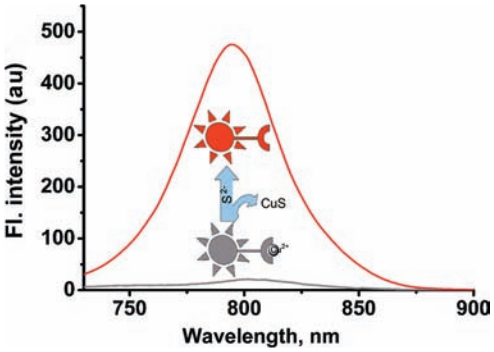
## ABSTRACT



**The first NIR fluorescent sensor for sulfide anions was constructed based on the displacement approach. The sensing ensemble is composed of a cyanine dye, a piperazine linker, an 8-aminoquinoline ligand, and copper. The favorable attributes of the sensor include a large NIR fluorescence turn-on signal in aqueous ethanol, high sensitivity, and high selectivity. The transition-metal-based displacement strategy may open an avenue for development of NIR fluorescent sensors for a wide variety of anion targets.**

Sulfide anions are generated not only as a byproduct in industrial processes but also in biosystems due to microbial reduction of sulfate by anaerobic bacteria and formation of sulfur-containing amino acids in meat proteins.[1] In addition, sulfide is employed in the conversion into sulfur and sulfuric acid, for the preparation of dyes and cosmetics. Exposure to a high level of sulfide can lead to irritation in mucous membranes, unconsciousness, and respiratory paralysis.[2] Therefore, the detection of sulfide anions is of high interest. So far, a few detection techniques have been developed for sulfide anions, such as titration,[3] inductively coupled plasma atomatic emission spectroscopy,[4] hydride generation atomic fluorescence spectrometry,[5] electrochemical methods,[6] ion chromatography,[7] spectrophotometry,[8] fluorimetry,[9] and chemiluminescence methods.[10]

Among these reported detection methods, fluorescent sensing has received great attention due to its simple operation and high sensitivity. There are a significant number of fluorescent sensors for metal ions and small biomolecules in aqueous media. However, the development of fluorescent sensors for anions in aqueous media is

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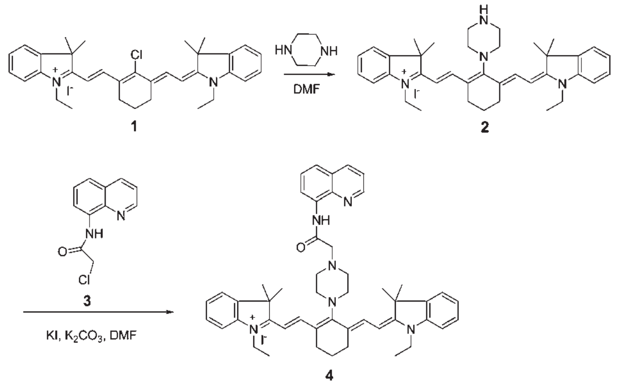
a challenging task owing to the strong hydration nature of anions, which weakens the interactions of the sensors with the target anions.[11] One way to tackle this hurdle is by employing the displacement method,[12] in which the dye–ligand–metal ion "ensemble" is nonfluorescent due to metal-ion-induced fluorescence quenching. However, the addition of anions may release the dye–ligand into the solution with revival of fluorescence.

Recently, a few attractive fluorescent chemosensors for sulfide anions have been constructed, but all of them have absorption and emission wavelengths in the visible range.[9,13] When compared to visible light, near-infrared (NIR) light (650–900 nm) is advantageous due to less photodamage, minimum fluorescence background, and less light scattering.[14] Thus, NIR fluorescent sensors are highly desirable. However, NIR fluorescent sensors for anions are relatively few. To our best knowledge, no small-molecule NIR fluorescent chemosensors for sulfide anions have been reported yet. In this work, we present the tricarbocyanine **4**-$Cu^{2+}$ ensemble (Scheme 1) as the first NIR fluorescent sulfide sensor.

The tricarbocyanine fluorophore is typically utilized for construction of NIR fluorescent sensors.[14] However, according to the Rehm–Weller equation,[15] it is very difficult to quench the fluorescence of tricarbocyanine NIR dyes by the photointroduced electron transfer (PET) approach due to their relatively long emission wavelength. It is known that heavy and transition metal ions have a strong fluorescence quenching character. We considered that if NIR dyes can be efficiently quenched by a metal species due to complexation, this may provide a basis for development of NIR fluorescence turn-on sensors for sulfide anions by the displacement method. With this in mind, 8-aminoquinoline was selected as the metal ion ligand, and it is incorporated into the tricarbocyanine fluorophore backbone through a piperazine linker (compound **4**, Scheme 1). The displacement approach may allow development of NIR fluorescent sensors with a high signal/noise ratio subjected to the efficient fluorescence quenching of the NIR dyes by the metal species.

**Scheme 1.** Synthetic Route to Compound **4**



The target compound **4** was readily synthesized in two steps as shown in Scheme 1. The starting compounds **1** and **3** were prepared according to the literature.[16] Reaction of compound **1** with piperazine in dry DMF under a nitrogen atmosphere gave compound **2**. The key intermediate, compound **2**, was then treated with compound **3** in the presence of a catalyzed amount of potassium iodide in dry DMF under a nitrogen atmosphere to afford product **4**. All the new compounds were well characterized by $^1H$ NMR, $^{13}C$ NMR, and HRMS.

With compound **4** in hand, we examined its optical properties in the absence or presence of various heavy and transition metal species. The free compound **4** exhibited an absorption band at around 694 nm in pH 7.0 HEPES buffer/ethanol (25 mM, 6:4 v/v) (Figure S1a). Upon addition of various heavy and transition metal ions such as $Hg^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Fe^{3+}$, $Co^{2+}$, and $Ni^{2+}$, no marked changes in the absorption profiles were noted. By contrast, introduction of $Cu^{2+}$ elicited a large red shift (78 nm) in the absorption from 696 to 774 nm, indicating that compound **4** binds with $Cu^{2+}$.

The free compound **4** is fluorescent with an emission peak at around 794 nm ($\Phi_{fl} = 0.11$).[17] However, addition of heavy and transition metal ions caused fluorescence quenching to a different extent (Figure S1b). In particular, the fluorescence of NIR dye **4** is almost completely quenched by $Cu^{2+}$ ions. Thus, we decided to further evaluate the $Cu^{2+}$-responsive nature of compound **4**. The compound was titrated with $Cu^{2+}$ ions in varying concentrations. The addition of increasing concentrations of $Cu^{2+}$ ions led to a gradual red shift of the absorption peak (Figure S2), and a distinct isosbestic at 732 nm was observed, suggesting that two species (compound **4** and the compound **4**–metal complex) are in equilibrium. As shown in Figure 1 and its inset, the fluorescence of NIR dye **4** is essentially completely quenched by 1 equiv of $Cu^{2+}$ ions. In good agreement with this finding, the Job plot also shows the formation of a 1:1 bonding mode between NIR

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dye **4** and $Cu^{2+}$ ions (Figure S3). Based on the 1:1 binding mode, the binding constant derived from the fluorescence titration data was found to be $3.0 \times 10^5\ M^{-1}$ (Figure S4).

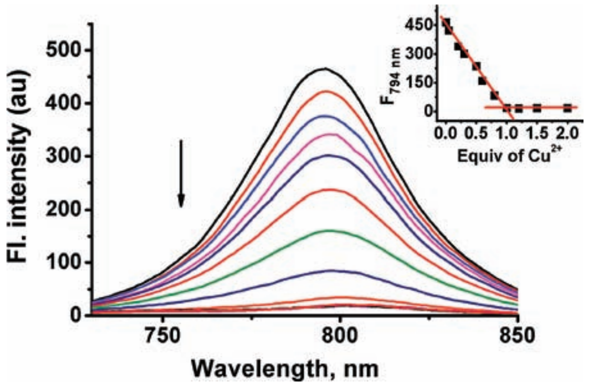

**Figure 1.** Fluorescence spectra of compound **4** (5 $\mu$M) with the increasing concentrations of $Cu^{2+}$ ions (0−2 equiv) in pH 7.0 HEPES buffer/ethanol (25 mM, 6:4 v/v). The inset shows the fluorescence intensity changes at 794 nm of compound **4** (5 $\mu$M) with the amount of $Cu^{2+}$ ions.

The above finding that the fluorescence of NIR dye **4** can be almost completely quenched by $Cu^{2+}$, and the fact that $Cu^{2+}$ can coordinate with sulfide anions to form the stable species, CuS (solubility product constant, $K_{SP} = 1.27 \times 10^{-36}$),[18] rendered us to speculate that the **4**-$Cu^{2+}$ ensemble is promising as a turn-on fluorescent sensor for sulfide anions. To test this idea, compound **4** was preincubated with $Cu^{2+}$, and the resulting ensemble was titrated with sulfide anions. As shown in Figure S5, addition of sulfide anions led to a big blue shift of the absorption band from 774 to 696 nm, which is essentially identical with the maximal wavelength of the absorption peak of the free dye **4**, indicating that addition of sulfide anions to the ensemble resulted in the release of the free dye **4**. Consistent with this observation, treatment of sulfide caused a significant fluorescence turn-on response at 794 nm (Figure 2), and up to a 27-fold fluorescence enhancement was observed, which is relatively large for NIR fluorescent anion sensors. Furthermore, the fluorescence intensities at 794 nm have an excellent linear relationship with the concentrations of sulfide anions from 0.5−8 $\mu$M (Figure S6), and the detection limit ($S/N = 3$) was calculated to 280 nM, indicating that the ensemble is highly sensitive to sulfide anions.

To examine the selectivity, the **4**-$Cu^{2+}$ ensemble (5 $\mu$M) was incubated with some representative anion species. As shown in Figure 3, 2000 equiv of $F^-$, $Cl^-$, $Br^-$, $NO_3^-$, $NO_2^-$, $N_3^-$, $SO_4^{2-}$, $SO_3^{2-}$, $CO_3^{2-}$, $PO_4^{3-}$, and $CH_3COO^-$ and 10 equiv of $I^-$ and $CN^-$ could not induce any marked fluorescence enhancement. By sharp contrast, 2 equiv of $S^{2-}$ elicited a large fluorescence enhancement in the NIR region. These results were further corroborated by the studies of absorption spectra of the ensemble in response to the various test anions (Figure S7). Thus, the spectroscopic studies suggest that the ensemble has a high selectivity for sulfide over other test anions, which may be attributed to the low solubility product constant of CuS.

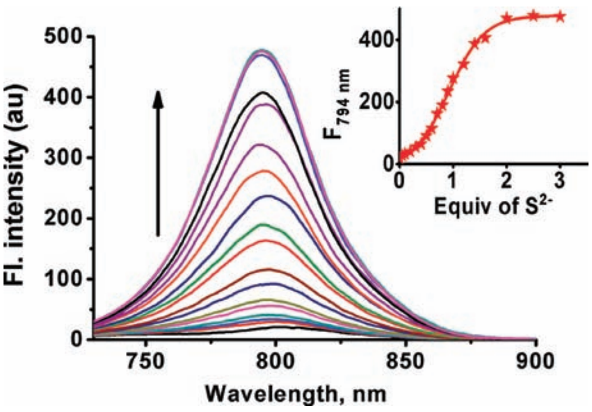

**Figure 2.** Fluorescence spectra of the **4**-$Cu^{2+}$ ensemble (5 $\mu$M) in pH 7.0 HEPES buffer/ethanol (6:4 v/v) in the presence of sulfide anions (0−3 equiv). The inset shows the fluorescence intensity changes at 794 nm of the ensemble (5 $\mu$M) in the presence of increasing sulfide concentrations (0−3 equiv).

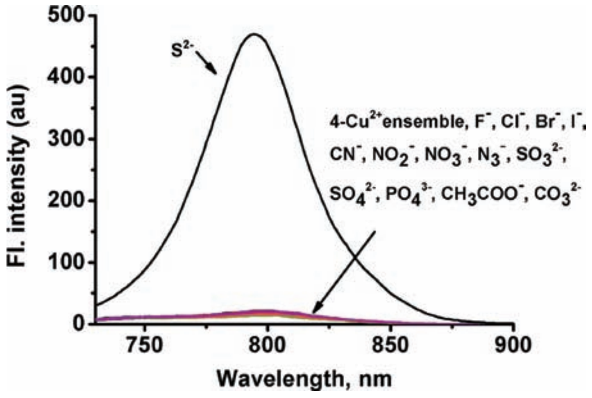

**Figure 3.** Changes of the fluorescence spectra of the **4**-$Cu^{2+}$ ensemble (5 $\mu$M) with anions (2000 equiv for $F^-$, $Cl^-$, $Br^-$, $NO_3^-$, $NO_2^-$, $N_3^-$, $SO_4^{2-}$, $SO_3^{2-}$, $CO_3^{2-}$, $PO_4^{3-}$, and $CH_3COO^-$; 10 equiv for $I^-$ and $CN^-$; 2 equiv for $S^{2-}$) in pH 7.0 HEPES buffer/ethanol (25 mM, 6:4 v/v).

To investigate whether the **4**-$Cu^{2+}$ ensemble could still retain the sensing response to sulfide anions under the potential competition of other relevant anions, the ensemble (5 $\mu$M) was treated with sulfide anions (2 equiv) in the presence of various test anions (2000 equiv for $F^-$, $Cl^-$, $Br^-$, $NO_3^-$, $NO_2^-$, $N_3^-$, $SO_4^{2-}$, $SO_3^{2-}$, $CO_3^{2-}$, $PO_4^{3-}$, and $CH_3COO^-$; 10 equiv for $I^-$ and $CN^-$) in pH 7.0 HEPES buffer/ethanol (6:4 v/v). As displayed in Figures S8−S9, all the relevant anions tested have virtually no influence on the fluorescence detection of sulfide anions. Thus, the ensemble

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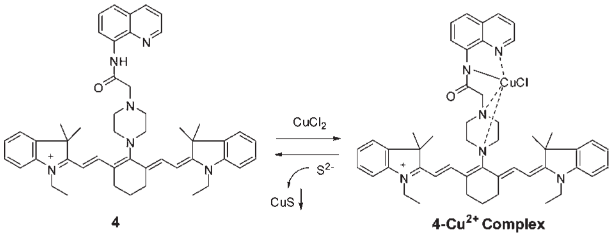
seems to be useful for selectively sensing sulfide even involving these relevant anions. The fluorescence responses of the ensemble toward sulfide anions were pH-dependent, and the maximal signal was observed in the pH range of 5–10 (Figure S10). This indicates that the ensemble can be employed to sense sulfide in a wide pH range.

To gain insight into the sensing mechanism, we decided to study the sensing process by both NMR and mass spectrometry. As shown in Figure S11b, the addition of 1 equiv of $Cu^{2+}$ ions to dye **4** in $CD_3OD$ rendered the $^1H$ NMR spectrum to be very broad due to the complexation of paramagnetic $Cu^{2+}$ to the dye. However, further addition of 2 equiv of sulfide anions to the ensemble resulted in the spectrum becoming resolved to some extent (Figure S11c). To further clarify the spectrum, the product of dye **4** + $Cu^{2+}$ + sulfide anions was isolated by a silica gel column and was then subjected to $^1H$ NMR analysis. The $^1H$ NMR of the resulting product is essentially identical to that of free dye **4**. In addition, after treatment of dye **4** with $Cu^{2+}$ in pH 7.0 HEPES buffer/ethanol (25 mM, 6:4, v/v), an intense peak at *m*/*z* 844.4 corresponding to (**4** + Cu + Cl − H)$^+$ is present in the ESI-MS spectrum (Figure S12). However, the further addition of sulfide anions led to the disappearance of the peak at *m*/*z* 844.4 and the formation of a new peak at 745.5, corresponding to (**4**)$^+$ (Figure S13). Thus, the studies of NMR, mass spectrometry, absorption spectrometry, and fluorescence spectrometry indicate that the sensor likely functioned by the displacement mechanism (Scheme 2). The deprotonation of the amide NH by $Cu^{2+}$ is precedent,[19] and $Cu^{2+}$ can form four-, five-, or six-coordinated complexes.[20] The binding of $Cu^{2+}$ to the nitrogen atom on the cyanine dye is in good agreement with the large absorption red shift[21] in the ensemble relative to the free dye **4**. However, the repeated efforts for crystallization of the **4**-$Cu^{2+}$ complex were not successful, and the detailed mechanism warrants further studies.

**Scheme 2.** Proposed Displacement Mechanism for Sensing of Sulfide Anions



In summary, we have constructed the first NIR fluorescent sensor for sulfide anions based on the displacement approach. The sensing ensemble is composed of a cyanine dye, a piperazine linker, an 8-aminoquinoline ligand, and copper. The favorable attributes of the sensor include excitation and emission in the NIR region, a relatively large NIR fluorescence turn-on signal in aqueous media, high sensitivity, high selectivity, and the ability to work well in a wide pH range. Given the fact that it is relatively challenging to switch off the fluorescence of NIR dyes, a transition-metal-based displacement strategy may open an avenue for the development of NIR fluorescent sensors for a wide variety of anion targets with a significant NIR fluorescence turn-on response.

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**Supporting Information Available.** Experimental procedures and some spectra of the sensor. This material is available free of charge via the Internet at http://pubs.acs.org.

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