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# Surfactant-modulated discriminative sensing of HNO and $H_2S$ with a $Cu^{2+}$-complex-based fluorescent probe

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ABSTRACT

Recent investigations revealed the close relationship between $H_2S$ and HNO in biological systems. It is significant to develop an efficient fluorescent sensor to realize the discriminative sensing of HNO and $H_2S$. Herein, we designed and synthesized a novel fluorescent sensor (**CuHCD**) with hemicyanine-carbazole as fluorophore and bipyridine-triazole-$Cu^{2+}$complex as receptor, which enabled the selective recognition of HNO and $H_2S$, respectively via the non-covalent modulation of surfactant assemblies. The **CuHCD**/surfactant sensor system exhibited excellent selectivity for $H_2S$ at 5 mM SDS in HEPES buffer, and displayed high specificity for HNO at 1 mM SDS in HEPES buffer over other common anions and reactive species. Different detection mechanisms in sensing $H_2S$ and HNO were deeply investigated. The results showed that $H_2S$ seized $Cu^{2+}$ from the complex to recover the fluorescence of **HCD**, while HNO reduced $Cu^{2+}$ to $Cu^{+}$ in the complex to turn on the fluorescence. In different SDS micellar systems, **HCD** possessed different dispersity and binding capacity with $Cu^{2+}$, which led to the selective detection of $H_2S$ and HNO, respectively. The hypothesis was further confirmed by replacing SDS micelles with liposome under the same conditions.



## 1. Introduction

Hydrogen sulfide ($H_2S$), having the similar importance as carbon monoxide (CO) and nitric oxide (NO),[1] is considered as the third gaseous transmitter involving in many physiological and pathological processes including neurotransmission, vasodilatation, apoptosis, inflammation and $O_2$ sensing.[2] Recently, a few studies illustrated that $H_2S$ could interact directly or indirectly with sodium nitroprusside (SNP, a NO donor) to generate nitroxyl (HNO),[3] which is the one-electron reduced form of NO.[4] HNO is crucial in some cell signalling pathways due to its unique biological effects. For instance, existence of HNO could increase systolic force and decrease diastolic pressure in normal and failing heart, which occurs through direct modification of protein thiols to improve calcium sensitivity and the calcium cycling of myofilament.[5] These findings established a very close relationship between $H_2S$ and HNO.

In the past decades, to detect the two analytes individually, various fluorescence sensors have been developed,[6,7] among which $Cu^{2+}$-based complexes have excellent performance and attracted lots of attention. Generally, sensing of HNO is based on the mechanism of reducing Cu(II) to Cu(I) to affect the fluorescence.[6a–6d] For example, Lippard's group developed the first BODIPY-based fluorescent sensor to detect HNO, which had an excellent selectivity over other biologically relevant reactive nitrogen species (RNS), and the sensor was successfully utilized in detecting HNO in living cells.[6a] Afterwards, benzoresorufin-based copper complexes were synthesized for the detection of NO and HNO by changing the properties of the metal-binding sites.[6c] For the sensing of $H_2S$, the detection strategy always relies on the principle of seizing copper ion from the Cu(II) complex to recover the fluorescence.[7a–7c] For instance, Chang's group created a Cu(II)-dipicolylamine (DPA)-based fluorescence sensor for the sensing of sulfide under physiological and environmental conditions.[7a] Besides, Zeng's group developed another fluorescence sensor for sulfide anion based on the 8-hydroxyquinoline and Cu(II) complex to demonstrate that the chemosensor can detect sulfide anion in biological systems at a relatively low concentration.[7b]

It is noted that all of these sensors based on Cu(II)-complex were designed to detect either HNO or $H_2S$ without considering both of

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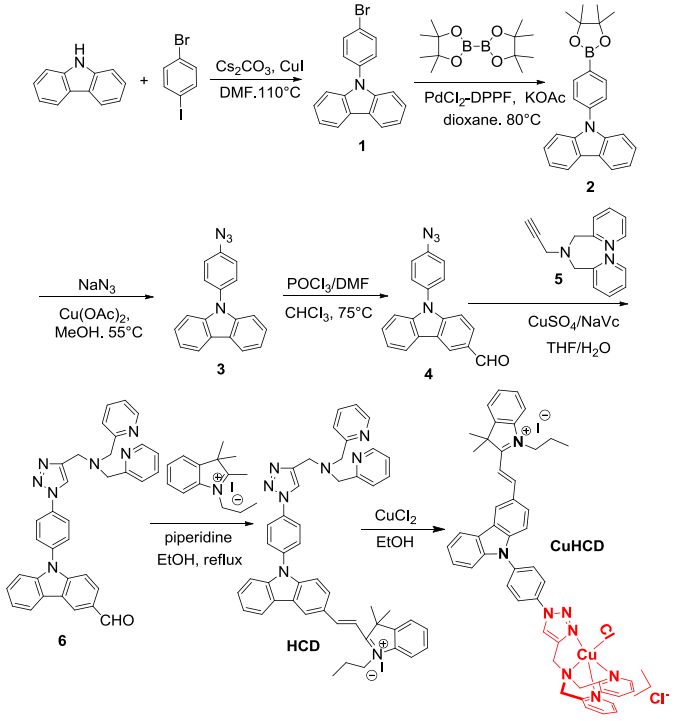
them. To the best of our knowledge, there were no fluorescent sensors to effectively and selectively discriminate the two reductive species. Therefore, it is imminent and challengeable to develop an efficient fluorescent analytic strategy to achieve the discriminative sensing of HNO and $H_2S$.

Surfactant molecules are effective to enhance the stability, sensitivity and selectivity of a fluorescent sensor, and to facilitate the modulation of the sensory capability of a potentially bioactive system.[8] The main reason relies on the fact that amphiphilic molecules can form heterogeneous micelles and vesicles possessing hydrophobic cores in aqueous solutions.[9] Moreover, surfactants with surface charges are more efficient in increasing the detection sensitivity with the fluorescent sensor and analyte bearing converse charges due to the strong electrostatic interactions. For example, sodium dodecyl sulfate (SDS), an anionic surfactant, has been used in the detection of $Cu^{2+}$ and $Hg^{2+}$,[10] and cationic dodecyl trimethyl ammonium bromide (DTAB) was employed in sensing of anionic ions such as $S^{2-}$ and $F^-$.[11]

In the present work, we tend to construct a fluorescent sensor (**HCD**) based on Cu(II)-complex realizing the recognition of HNO and $H_2S$, respectively via the non-covalent modulation of surfactant assemblies. **CuHCD** contained carbazole as chromophore and Cu(II)-DPA complex as receptor.[7,12] The two moieties were bridged by a triazole linker formed by the click reaction. Hemicyanine, bearing a positive charge, having excellent photophysical properties with large molar extinction coefficient ($\varepsilon \approx 10^4$ $M^{-1}cm^{-1}$),[13] was introduced to expand the fluorophore conjugation system. The synthetic route for the hybrid molecule **HCD** (hemicyanine-carbazole-dipicolylamine) was shown in Scheme 1. Considering the positive charges in **CuHCD** complex, the anionic surfactant SDS was selected to modulate the fluorescent responses. Our studies have indicated that the selective detection of HNO and $H_2S$ with the same **CuHCD** complex can be achieved by changing the concentration of SDS in the micellar solutions.




**Scheme 1.** Synthetic route of **CuHCD**.

## 2. Result and discussion

### 2.1. Synthesis of HCD

Fluorescent sensor **HCD** was prepared through eight steps and the total yield was calculated to be ~16%. Briefly, the synthesis started from carbazole, which was modified by 1-bromo-4-iodobenzene through Ullmann reaction to obtain **1**.[14] Suzuki reaction was then carried out to yield corresponding borinic ester **2**.[15] An azide group was further introduced into the benzene ring via Chan-Lam reaction to afford **3**,[16] followed by Vilsmeier reaction to provide aldehyde **4**.[17] Next, 2, 2′-dipicolylamine was attached to the carbazole fluorophore by the copper(I)-catalytic click cycloaddition to yield **6**.[18] Finally, hemicyanine was incorporated into the fluorophore via Knoevenagel condensation to provide the desired probe **HCD**.[19] The structure of **HCD** and intermediates were determined by $^1H$, $^{13}C$ NMR and high resolution mass spectroscopies (*see* Supplementary data). In addition, the chelated complex **CuHCD** was facilely prepared by addition of aqueous $CuCl_2$ to **HCD** solution,[6b] and the 1:1 stoichiometry was confirmed by mass spectrometry and fluorescence titration, indicating that there was only one copper ion binding site in the sensor molecule.

### 2.2. UV–vis and fluorescence spectra of HCD

The UV–vis absorption spectrum of **HCD** with $Cu^{2+}$ was obtained in HEPES (10 mM, pH=7.4) buffer. **HCD** exhibits a strong band around 400 nm–550 nm with the maximum absorbance at 471 nm ($\varepsilon_{471}=1.38\times10^4$ $M^{-1}cm^{-1}$). Upon addition of $Cu^{2+}$ (1 equiv), the absorption band displayed no changes, while the absorption intensity decreased at 471 nm and increased at 231 nm (Fig. 1a), further confirming the coordination between $Cu^{2+}$ and **HCD**.

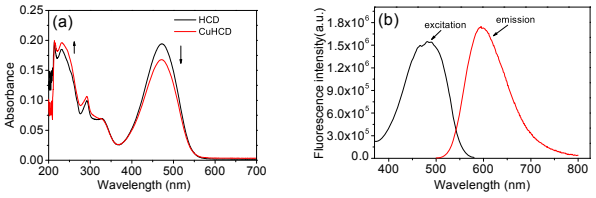



**Fig. 1.** (a) UV–vis absorption spectra of **HCD** and **CuHCD** (5 μM) in HEPES buffer (10 mM, pH=7.4). (b) Fluorescence excitation and emission spectra of **HCD** (10 μM) in HEPES buffer (10 mM, pH=7.4) solution.

The fluorescence spectrum of **HCD** in HEPES buffer exhibited a maximum excitation at 484 nm and maximum emission at 595 nm with a large Stokes shift of 111 nm (Fig. 1b). The fluorescence response (*on-off*) of **HCD** had a high specificity for $Cu^{2+}$ over other metal ions including alkaline earth metal ions ($Mg^{2+}$, $Ca^{2+}$) and transition metal ions ($Ag^+$, $Cd^{2+}$, $Co^{2+}$, $Cr^{3+}$, $Fe^{3+}$, $Mn^{2+}$, $Ni^{2+}$, $Pb^{2+}$, $Zn^{2+}$) (Fig. 2). Upon increasing the amount of $Cu^{2+}$ from 0 to 1 equiv, the emission intensity of **HCD** significantly decreased about 5-folds (Fig. S1). The fluorescence quenching could be attributed to the photoinduced electron transfer (PET) process from the chelated $Cu^{2+}$ to the hemicyanine-carbazole fluorophore. The emission peak no longer decreased with further addition of $Cu^{2+}$, indicating a 1:1 complexation between **HCD** and $Cu^{2+}$ (Fig. S1 inset). The association constant ($K_s$) of **HCD** and $Cu^{2+}$ was calculated to be $3.89\times10^5$ $M^{-1}$ based on the fluorescence titration experiments.

### 2.3. Fluorescence spectra of CuHCD with $Na_2S$ and HNO

In HEPES buffer (10 mM, pH=7.4), the fluorescence intensity of **CuHCD** gradually increased up to 2-fold with addition of Angeli's salt[20] ($Na_2N_2O_3$, a HNO donor) from 0 to 50 equiv. Further addition

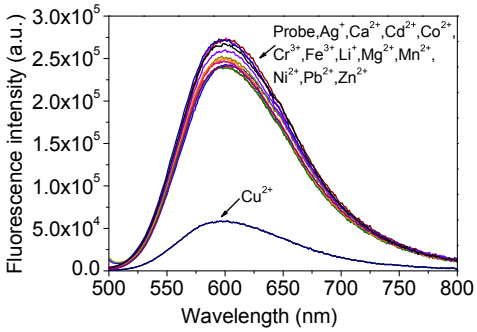



**Fig. 2.** Fluorescence responses of 5 μM **HCD** ($\lambda_{ex}$=484 nm) solution in the presence of $Cu^{2+}$ (5 μM) and some other metal ions (75 μM) in HEPES buffer (10 mM, pH=7.4).

of Angeli's salt led to no additional changes (Fig. S2a). However, the addition of $Na_2S$ can't result in any increasement in the fluorescence signal (Fig. S2b).

To our delight, the introduction of surfactant could enhance the fluorescence stability (Fig. S3a) and intensity (Fig. 3) significantly. Sodium dodecyl sulfate (SDS), dodecyl trimethyl ammonium bromide (DTAB), and triton X-100 (TX-100), which represent anionic, cationic and neutral surfactant, respectively, were used to investigate the surfactant effects. The surfactant concentration was controlled above their corresponding critical micelle concentration (CMC).[21a] As shown in Fig. 3, **HCD** exhibits a similar emission spectrum with that in neat buffer solution, and the maximum emission wavelength still was located around 595 nm in three kinds of micellar solutions. It is noted that the fluorescence intensity of **HCD** dramatically increased in micellar solutions compared with that in the neat HEPES buffer solutions. The largest enhancement of emission intensity (3.5-folds) was found in SDS micelles, followed by 3.2-folds in TX-100 and 1.6-folds in DTAB.

The main reason relies on the fact that **HCD** sensor with a hydrophilic hemicyanine and a hydrophobic carbazole is likely to aggregate in neat buffer solutions inducing fluorescence quenching, which can be relieved through dispersing in micelles system.[21b] This issue could also be further confirmed by the fluorescent difference of **HCD** in neat buffer and ethanol solution (Fig. S3b). The strong fluorescent intensity in SDS suggested that the **HCD** sensor was easier to be encapsulated by SDS micelles due to the electrostatic attractions between the positively charged hemicyanine fluorophore and the negatively charged SDS. On the contrary, DTAB bearing one positive charge had electrostatic repulsion with positively charged hemicyanine fluorophore, making it hard for DTAB micelles to encapsulate **HCD**. In addition, neutral TX-100 had no electrostatic interaction with **HCD**, causing a medium enhancement in fluorescent intensity only by hydrophobic interactions. Consequently, SDS was selected to construct the non-covalent modulation of surfactant assembly systems.

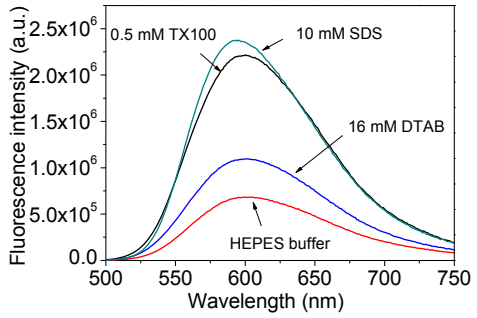



**Fig. 3.** Fluorescence emission spectra of **HCD** (10 μM) in HEPES buffer (10 mM, pH=7.4) and in the same buffer solution with addition of 0.5 mM TX100, 16 mM DTAB and 10 mM SDS ($\lambda_{ex}$=484 nm).

Then, we investigated the optimal SDS concentration in the response of $Na_2S$ and Angeli's salt in HEPES buffer (10 mM, pH=7.4). Much too surprising, with increasing of SDS concentration, the fluorescence showed dramatically opposite trends for Angeli's salt and $Na_2S$ (Fig. 4), respectively. Upon addition of $Na_2S$ (5 equiv), the fluorescence intensity of **CuHCD** gradually increased with SDS concentration varying from 1 mM to 3 mM, and then reached a plateau. However, the fluorescence had a maximum value in 1 mM SDS buffer solution containing Angeli's salt (550 equiv), and the emission signal gradually decreased with the SDS concentration changing from 1 mM to 5 mM. The results enable the sensor to selectively detect $S^{2-}$ and HNO through modulating SDS concentrations in HEPES buffer. As a consequence, the sensing behaviours of **CuHCD** to $S^{2-}$ and HNO were examined in HEPES buffer with 5 mM SDS and 1 mM SDS, respectively.

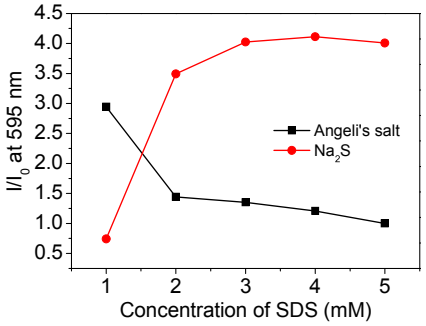



**Fig. 4.** Fluorescence responses of **CuHCD** (5 μM) in HEPES buffer (10 mM, pH=7.4) solution upon addition of SDS in the presence of Angeli's salt (550 equiv) or $Na_2S$ (5 equiv).

With 5 mM SDS in HEPES (10 mM, pH=7.4) buffer, we examined the selectivity of **CuHCD** toward various important anions in aqueous solution. The enhancement of fluorescence intensity was triggered by addition of $S^{2-}$ (5 equiv), while no obvious responses were observed in the presence of common anions (60 equiv) such as $NO_3^-$, $NO_2^-$, $S_2O_3^{2-}$, $HCO_3^-$, $CO_3^{2-}$, $CH_3COO^-$, $SO_4^{2-}$, $C_2O_4^{2-}$, $I^-$, $Br^-$, $Cl^-$, $F^-$, $H_2PO_4^-$ and $HPO_4^{2-}$, as well as the reactive oxygen/nitrogen species including $H_2O_2$, $ClO^-$, $ONOO^-$ and HNO (Fig. 5).

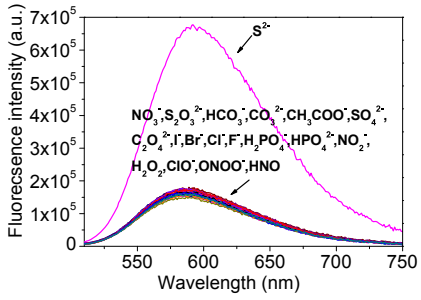



**Fig. 5.** Fluorescence responses of **CuHCD** (5 μM) in the presence of $S^{2-}$ (25 μM) and various anions (300 μM) in HEPES buffer with 5 mM SDS ($\lambda_{ex}$=484 nm).

The fluorescence titration experiments to $S^{2-}$ were then operated under the same condition. As shown in Fig. 6, the fluorescence intensity of **CuHCD** increased gradually upon the addition of $S^{2-}$ until it reached a plateau (5 equiv $S^{2-}$, Fig. 6 inset), and a fourfold fluorescence enhancement was obtained. The linear relationship of **CuHCD** for $S^{2-}$ was shown in Fig. S6a and the detection limit were determined to be 0.7 μM (3σ/κ). Both the peak pattern and the intensity of the enhanced emission spectra are closely matched to

those of **HCD** (Fig. S4), suggesting that the fluorescence of **HCD** was totally restored through the transformation from **CuHCD** (quenched) to free **HCD** (revived) (Scheme 2). Actually, $Cu^{2+}$ in **CuHCD** tends to form a quite stable precipitation CuS ($K_{sp}$=1.27×10$^{-36}$)[7a] with the targeted $S^{2-}$, which leads to the release of free **HCD**. The seizure of $Cu^{2+}$ by $S^{2-}$ was corroborated by mass spectrometry (Fig. S5). Two major peaks at *m*/*z* 868.14 and 733.28 were observed before and after the addition of $S^{2-}$, which corresponded to [**Cu$^{II}$HCD**Cl$_2$]$^+$ (868.24) and **HCD** (733.38). The results are consistent to the previous references,[7] which verified the excellent property of **CuHCD** as a sensor for $S^{2-}$ in SDS micellar solutions.

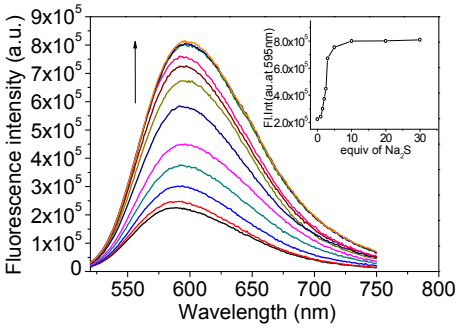
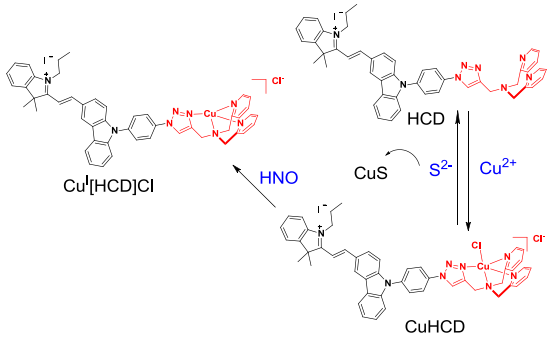



**Fig. 6.** Fluorescence titration of **CuHCD** (5 μM) in HEPES buffer (10 mM, 5 mM SDS, pH=7.4) in the presence of different amounts of $S^{2-}$. Inset: fluorescence intensity at 595 nm as a function of $S^{2-}$ concentration.




**Scheme 2.** Graphic of the mechanism of **CuHCD** in sensing HNO and $S^{2-}$.

For the detection of HNO, the experiments were performed in 1 mM SDS micellar solution (10 mM HEPES, pH=7.4). As performed in Fig. 7, with the concentration of $Na_2N_2O_3$ increasing, about 15-fold enhancement of fluorescence was obtained and the system reached saturation when $Na_2N_2O_3$ concentration up to 2000 equiv, The results indicated that [**Cu$^{II}$HCD**Cl$_2$]$^+$ can be complete reduced to [**Cu$^I$HCD**Cl]$^+$, and the supposition was further confirmed by mass spectrometry as well (Fig. S5). Two major peaks at *m*/*z* 868.23 and 831.39 corresponded to [**Cu$^{II}$HCD**Cl$_2$]$^+$ (868.24) and [**Cu$^I$HCD**Cl]$^+$ (831.27), respectively. In addition, the fluorescence response of **CuHCD** to HNO exhibited a curvilinear relationship (Fig. S6b) and detection limit was determined to be 23.0 μM (3σ/κ). Moreover, excellent selectivity towards Angeli's salt was also proved via screening various anionic ions and ROS specious (Fig. 8).

So far, it is clear that **CuHCD** can realize selective detection of HNO and $S^{2-}$ through modulating the SDS concentrations in micellar solutions. And the fluorescence responses of **CuHCD** to HNO and $S^{2-}$ were not affected by pH from 5.00 to 8.00 (Fig. S7). The detection mechanism of $S^{2-}$ seizing paramagnetic $Cu^{2+}$ from the sensor **CuHCD** to inhibit the fluorescence quenching and exported a 'off-on' signal. However, in the detection of HNO, **CuHCD** is reduced to **Cu$^I$[HCD]Cl** by the HNO donor $Na_2N_2O_3$, which also leads to a 'off-on' response in fluorescence (Scheme 2).

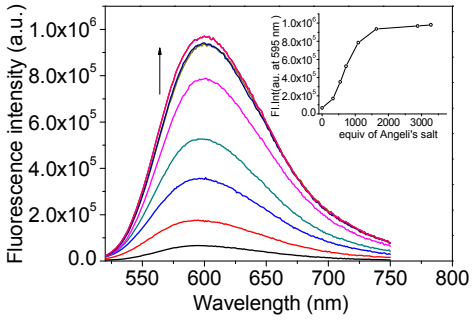



**Fig. 7.** Fluorescence titration of **CuHCD** (5 μM) in HEPES buffer (10 mM, 1 mM SDS, pH=7.4) in the presence of different amounts of Angeli's salt. Inset: fluorescence intensity at 595 nm as a function of Angeli's salt concentration.

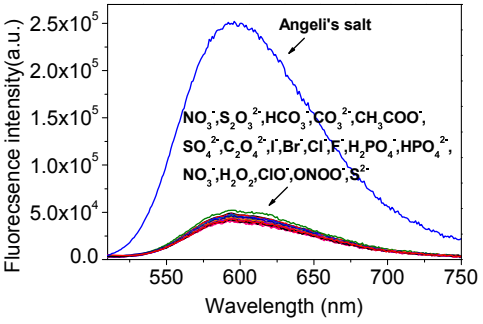



**Fig. 8.** Fluorescence responses of **CuHCD** (3 μM) in the presence of Angeli's salt (3 mM) and various anions (300 μM) in HEPES buffer with 1 mM SDS ($\lambda_{ex}$=484 nm).

The fluorescence changes of the sensor system to HNO and $S^{2-}$ were also visible under UV light (365 nm, Fig. 9). As shown in the picture, the orange emission of the **HCD** (15 μM) in 5 mM SDS micellar solutions (10 mM HEPES, pH=7.4) is remarkably quenched by the addition of $Cu^{2+}$ (2 equiv), and the orange emission soon recovered after the addition of $S^{2-}$ (10 equiv). Similar phenomenon was also observed in the system of Angeli's salt. It is noted that the fluorescence intensity is much weaker than that of $S^{2-}$.

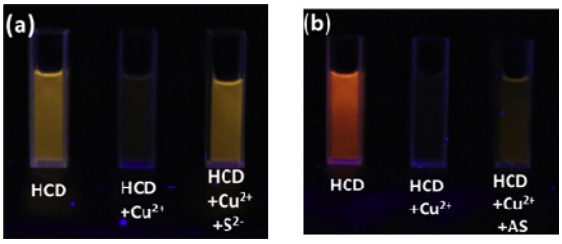



**Fig. 9.** (a) Colour changes of **HCD** solution (15 μM, containing 5 mM SDS) by introduction of $Cu^{2+}$ (2 equiv), followed by adding of $S^{2-}$ (10 equiv) (b) Colour changes of **HCD** solution (15 μM, containing 1 mM SDS) by introduction of $Cu^{2+}$ (2 equiv), followed by adding of Angeli's salt (AS, 1000 equiv).

## 2.4. Exploration of the detection mechanism

Considering the remarkable difference in fluorescence response with the SDS concentration changing from 1 mM to 5 mM, further investigation on the binding capacity of **HCD** with $Cu^{2+}$ was carried out to clarify the issue. From the titrations of **HCD** with $Cu^{2+}$ in the

three kinds of solution including neat HEPES buffer and the buffers with 1 mM or 5 mM SDS, the dissociation consistent ($K_d$) and Stern–Volmer quenching constant ($K_{sv}$) of **HCD** to $Cu^{2+}$ were measured. $K_d$ and $K_{sv}$, respectively represented the complexing capacity and sensing sensitivity of the fluorosensor to metal ions (see Supplementary data Fig. S8 for details).[22] As shown in Table 1, with the concentration of SDS changing from 0 to 5 mM, $K_{sv}$ increased while $K_d$ decreased to a certain extent, indicating the improved sensitivity and binding ability of **HCD** with $Cu^{2+}$. The results attributed to the strong electrostatic interactions between SDS micelles and $Cu^{2+}$,[8c] which leads to the accumulation of positive charged $Cu^{2+}$ on the aggregate surfaces when a higher SDS concentration (5 mM) is employed. Consequently, the existence of SDS micelles facilitated the interaction between **HCD** and $Cu^{2+}$.

**Table 1**
$K_{sv}$ and $K_d$ of **HCD** with $Cu^{2+}$ in three kinds of buffer solutions. $K_{sv}$ and $K_d$ were determined via direct titration of $Cu^{2+}$ to **HCD** and the titration data were fitted using Stern–Volmer equation and Benesi–Hildebrand equation, respectively

| Entry | Solution | $K_{sv}$ | $K_d$ (μM) |
|---|---|---|---|
| 1 | Neat HEPES | $(5.23\pm0.03)\times10^5$ | 2.57±0.08 |
| 2 | HEPES (1 mM SDS) | $(6.20\pm0.11)\times10^5$ | 1.77±0.39 |
| 3 | HEPES (5 mM SDS) | $(56.6\pm1.90)\times10^5$ | 0.50±0.01 |

In the detection of $S^{2-}$ and HNO, different mechanisms lead to the different interaction capacities of $Cu^{2+}$ within **CuHCD** complex. HNO with a weak reducing ability could only interact with the $Cu^{2+}$ in **CuHCD** when there is a weak binding force between **HCD** and $Cu^{2+}$ (Table 1, Entry 1 and 2). If the binding capacity is relatively strong, HNO could not reduce $Cu^{2+}$ effectively as shown in Table 1, Entry 3. However, the quite stable precipitation CuS ($K_{sp}=1.27\times10^{-36}$) enables $S^{2-}$ to seize $Cu^{2+}$ from **CuHCD** even there was a strong binding capacity between **HCD** and $Cu^{2+}$. Besides, the interaction between $S^{2-}$ and $Cu^{2+}$ was also hampered by the aggregation of **CuHCD**. As reported previously,[23] with the SDS concentration increasing, it became easier to capsulate **CuHCD** by SDS micelles via the electrostatic attractions between positively charged hemicyanine fluorophore and negatively charged SDS, which led to the sufficient dispersion of **CuHCD** directly. Since sensor molecules have better dispersity in solution with higher SDS concentration, $Cu^{2+}$ was more likely to be exposed and binded by $S^{2-}$ (Table 1, Entry 3). On the other hand, $S^{2-}$ could not snatch $Cu^{2+}$ effectively if **CuHCD** was not exposed in the solutions with poor dispersion as shown in Table 1, entries 1 and 2. In general, the binding ability and dispersion of **CuHCD** in the micellar systems jointly contributed to the response capability of HNO and $Na_2S$.

## 3. Fluorescence spectra of CuHCD with $Na_2S$ and HNO in liposomes

To further confirm the hypothesis above, Liposome system was established in current work. MPEG2000-DSPE (N-(carbonyl-methoxypolyethyleneglycol2000)-1, 2-distearoyl-*sn*-glycero-3-phosphoethanolamine, sodium salt) is a kind of amphiphilic polymer,[24] which tends to form liposome in aqueous solution because of the formation of hydrophilic centre, and is extensively used to construct nanoprobes with good membrane permeability.[25] In the liposome formed by MPEG2000-DSPE, fluorescence responses to HNO and $S^{2-}$ were quite different from those in micellar solutions. HNO and $S^{2-}$ led to the same fluorescence increasing trend with MPEG2000-DSPE concentration increasing from 5 μM to 70 μM (Fig. 10), and the optimal concentration of MPEG2000-DSPE was determined to be ≥20 μM. In agreement with the micellar system, the fluorescence titration of **CuHCD** to Angeli's salt and $S^{2-}$ was showed in Fig. S9. The Stern–Volmer quenching constant in HEPES buffer with 20 μM MPEG2000-DSPE was calculated to be $K_{sv}=(16.22\pm0.57)\times10^5$, which is between the values in buffer solutions with 1 mM and 5 mM SDS. The higher dispersion of **CuHCD** in MPEG2000-DSPE and the medium $K_{sv}$ value explained the fluorescence responses of **CuHCD** to HNO and $S^{2-}$ in liposomes, which further confirmed the previous hypothesis of detection mechanisms.

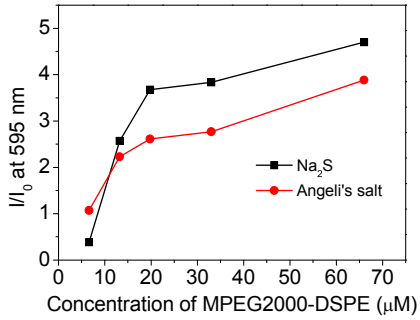



**Fig. 10.** Fluorescence response of **CuHCD** (5 uM) in HEPES buffer (10 mM, pH=7.4) upon addition of MPEG2000-DSPE in the presence of Angeli's salt (550 equiv) or $Na_2S$ (5 equiv).

## 4. Conclusions

In conclusion, we have successfully developed a $Cu^{2+}$-complex-based fluorescent sensor **CuHCD**, which is the first reported chemosensor capable of sensing $S^{2-}$ and HNO in different micellar systems. The **CuHCD**/surfactant sensor ensemble exhibited an excellent selectivity for $S^{2-}$ and HNO over other common anions and reactive oxygen/nitrogen species. **CuHCD** adopted different mechanisms in detecting $S^{2-}$ and HNO: the former seized $Cu^{2+}$ from the complex to recover the fluorescence while the latter reduced $Cu^{2+}$ to $Cu^{+}$. In different micellar systems, **HCD** exhibited different dispersity as well as different binding capacity with $Cu^{2+}$, which contributed to the selective detection of $S^{2-}$ and HNO.

## 5. Experimental section

### 5.1. General remarks

All of the materials were purchased as reagent grade and used without further purification. DMF was vacuum distilled over calcium hydride ($CaH_2$). $CHCl_3$ and piperidine were redistilled. Reactions were monitored with analytical thin-layer chromatography (TLC) on silica gel F254 glass plates and visualized under UV light (254 nm/365 nm). Flash column chromatography was performed on silica gel (200–300 mesh). $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectra were taken on a Bruker Avanced III spectrometer with tetramethylsilane as an internal standard and chloroform ($CDCl_3$) or methanol ($CD_3OD$) as the solvent. High resolution electrospray ionization mass spectra (HRMS-ESI) were recorded with a Waters LCT Premier XE mass spectrometer. Melting point was performed in X-4 precision microscopic melting point detector.

### 5.2. Synthesis

*5.2.1. 9-(4-Bromophenyl)-9H-carbazole (**1**).*[14] To a solution of 9-(4-bromophenyl)-9*H*-carbazole (800 mg, 4.8 mmol) in DMF (10 mL), 1-bromo-4-iodobenzene (1.765 g, 6.24 mmol) was added, followed by addition of CuI (274 mg, 1.44 mmol). The reaction mixture was stirred in a 110 °C of oil bath under argon for about 30 h until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature,

and the DMF was evaporated under vacuum. After removal of the solvent, the mixture was purified by column chromatography (Hexanes/EtOAc=150/1) to give compound 1 (1.299 g, 4.05 mmol, 84%) as yellow solid; mp: 144–145 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.14 (d, $J$=7.8 Hz, 2H), 7.74 (d, $J$=8.6 Hz, 2H), 7.46 (m, 2H), 7.40 (dd, $J$=13.5, 4.4 Hz, 4H), 7.32–7.28 (td, $J$=7.3, 1.32 Hz, 2H).

*5.2.2. 9-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl) phenyl)-9H-carbazole (**2**).*[15] A Schlenk flask was charged with compound **1** (600 mg, 1.87 mmol), then bis(pinacolato)diboron (522 mg, 2.06 mmol), $PdCl_2$DPPF (113 mg, 0.14 mmol), KOAc (550 mg, 5.61 mmol) and dry 1, 4-dioxane (15 mL) were added to the flask under argon. The mixture was preactivated for 1 h under room temperature followed by immersing in an oil bath at 80 °C with stirring for about 20 h until the starting material had completely disappeared as judged by TLC. The solvent was evaporated under reduced pressure and purified by column chromatography (Hexanes/EtOAc=200/1) to give compound **2** (546.4 mg, 1.48 mmol, 80%) as pale yellow solid; mp: 173–174 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.14 (d, $J$=7.7 Hz, 2H), 8.05 (d, $J$=8.2 Hz, 2H), 7.59 (d, $J$=8.2 Hz, 2H), 7.48–7.36 (m, 4H), 7.33–7.25 (m, 2H), 1.40 (s, 12H).

*5.2.3. 9-(4-Azidophenyl)-9H-carbazole (**3**).*[16] To a solution of compound **2** (228 mg, 0.617 mmol) in methanol (10 mL), $NaN_3$ (60.22 mg, 0.926 mmol) and $Cu(OAc)_2$ (12.32 mg, 0.0617 mmol) were added. The mixture was stirred in a 55 °C of oil bath under air for about 3 h until the starting material had been completely consumed as detected by TLC. The crude yellow oil was then diluted with EtOAc (150 mL), washed with saturated NaCl solution, and dried with $MgSO_4$. After removal of the solvent, the mixture was purified by column chromatography (Hexanes/EtOAc=150/1) to give **3** (126 mg, 0.44 mmol, 80%) as a yellow crystals; mp: 111–112 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.15 (d, $J$=7.7 Hz, 2H), 7.56 (d, $J$=8.8 Hz, 2H), 7.40 (td, $J$=8.0, 1.2 Hz, 2H), 7.35 (d, $J$=8.1 Hz, 2H), 7.30–7.25 (m, 4H).

*5.2.4. 9-(4-Azidophenyl)-9H-carbazole-3-carbaldehyde (**4**).*[17] A Schlenk flask was charged with dry DMF (5 mL), $POCl_3$ (5 mL) was then dropping to the flask in ice bathe. The mixture was stirred for 20 min at 0 °C and for another 1 h under room temperature. Then $CHCl_3$ (5 mL) and compound **3** (254.19 mg, 0.89 mmol) was added to reflux for about 10 h. The mixture was neutralized by NaOH solution in ice bath to pH=10. Then the crude product was diluted with DCM (150 mL), washed with saturated NaCl solution (30 mL), and dried with $MgSO_4$. After removal of the solvent by vacuum distillation, the mixture was purified by column chromatography (Hexanes/EtOAc=50/1) to give compound **4** (234 mg, 0.746 mmol, 83%). Dark yellow crystals; mp: 131–132 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.13 (s, 1H), 8.68 (d, $J$=1.2 Hz, 1H), 8.21 (d, $J$=7.7 Hz, 1H), 7.96 (dd, $J$=8.5, 1.5 Hz, 1H), 7.55 (d, $J$=8.7 Hz, 2H), 7.49 (t, $J$=7.1 Hz, 1H), 7.42–7.33 (m, 3H), 7.30 (d, $J$=8.7 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 191.64, 144.45, 141.84, 133.30, 129.58, 128.66, 127.53, 127.10, 123.82, 123.61, 123.24, 121.35, 120.75, 120.64, 110.21, 109.91, 77.37, 77.05, 76.73. HRMS (ESI): $m/z$ calcd for $C_{19}H_{13}N_4O$ $[M+H]^+$: 313.1089, found: 313.1095.

*5.2.5. (N, N-Bis(2-pyridylmethyl)-N-propargylamine) (**5**).*[6a] To a solution of Bis (2-picolyl) amine (BPA) (100 mg, 0.5 mmol) in THF (3 mL) was added propargyl bromide (80% in toluene) (0.1 mL, 0.78 mmol) followed by addition of $K_2CO_3$ (207 mg, 1.5 mmol). The mixture was stirred at room temperature until the starting material had completely disappeared as judged by TLC. After removing the solvent under reduced pressure, the residue was purified by column chromatography ($CH_2Cl_2$/MeOH=40:1) to give compound **5** (118 mg, 0.5 mmol, 100%) as yellow oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.57 (dd, $J$=4.9, 0.8 Hz, 2H), 7.67 (td, $J$=7.7, 1.8 Hz, 2H), 7.53 (d, $J$=7.8 Hz, 2H), 7.17 (dd, $J$=6.4, 5.1 Hz, 2H), 3.94 (s, 4H), 3.44 (d, $J$=2.3 Hz, 2H), 2.30 (t, $J$=2.4 Hz, 1H).

*5.2.6. 9-(4-(4-((Bis(pyridin-2-ylmethyl)amino)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-9H-carbazole-3-carbaldehyde (**6**).*[18] In a mixture of compound **4** (100 mg, 0.32 mmol) and compound **5** (91.3 mg, 0.385 mmol) in water and $t$-BuOH (v/v=3:1, 4 mL), freshly prepared sodium ascorbate (0.74 mL, 0.2 mol/L) was added, followed by the addition of $CuSO_4$ (1.28 mL, 0.05 mol/L) solution. The heterogeneous mixture was stirred vigorously overnight at room temperature for about 12 h. Then the raw product was washed with distilled water, extracted with DCM, and dried with $MgSO_4$. After removing the solvent with vacuum distillation, the mixture was purified by column chromatography ($CH_2Cl_2$/MeOH=20:1) to give compound **6** (82.8 mg, 0.168 mmol, 50%) as yellow solid; mp: 72–73 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.10 (s, 1H), 8.65 (s, 1H), 8.56 (d, $J$=4.1 Hz, 2H), 8.19 (d, $J$=7.7 Hz, 1H), 8.04 (d, $J$=8.5 Hz, 2H), 7.95 (d, $J$=8.5 Hz, 1H), 7.69 (dd, $J$=17.0, 8.0 Hz, 4H), 7.60 (d, $J$=7.7 Hz, 2H), 7.54–7.33 (m, 5H), 7.20–7.10 (m, 2H), 4.01 (s, 2H), 3.92 (s, 4H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 191.60, 158.94, 149.11, 145.37, 144.16, 141.53, 136.81, 136.61, 136.56, 129.80, 128.42, 127.70, 127.27, 123.83, 123.77, 123.47, 123.38, 122.20, 122.10, 121.62, 121.43, 120.83, 110.17, 109.90, 77.40, 77.08, 76.76, 59.59, 53.44, 48.49. HRMS (ESI): $m/z$ calcd for $C_{34}H_{28}N_7O$ $[M+H]^+$: 550.2355, found: 550.2354.

*5.2.7. **HCD**.*[19] To a solution of compound **6**(38 mg, 0.07 mmol)in ethanol (3 mL) were added hemicyanine (36.2 mg, 0.11 mmol) and piperidine (0.1 mL) under argon. The mixture was refluxed for 15 h and a red suspension was obtained. Then the suspension was washed with NaI (0.1 mM) solution and extracted three times with DCM (30 mL). The organic phases were dried with $MgSO_4$. After removing the solvent with vacuum distillation, the residue was purified by column chromatography ($CH_2Cl_2$/MeOH=10:1) to get **HCD** (43 mg, 0.05 mmol, 73%) as red solid; mp: 230–235 °C; $^1$H NMR (400 MHz, MeOD): $\delta$ 9.11 (d, $J$=5.56 Hz, 2H), 9.06 (s, 1H), 8.84 (s, 1H), 8.70 (d, $J$=16.0 Hz, 1H), 8.36 (d, $J$=7.7 Hz, 1H), 8.22 (d, $J$=8.8 Hz, 3H), 8.11 (td, $J$=7.7, 1.6 Hz, 2H), 7.86 (d, $J$=8.8 Hz, 2H), 7.83–7.72 (m, 2H), 7.68–7.59 (m, 5H), 7.57–7.49 (m, 2H), 7.47–7.38 (m, 2H), 4.71 (t, $J$=7.3 Hz, 2H), 4.39 (s, 2H), 4.28 (d, $J$=17.4 Hz, 4H), 2.10–2.00 (m, 2H), 1.91 (s, 6H), 1.27 (m, 2H), 1.14 (t, $J$=7.4 Hz, 3H). $^{13}$C NMR (100 MHz, MeOD): $\delta$ 181.99, 156.25, 155.30, 148.79, 144.40, 143.93, 143.52, 141.50, 141.25, 140.98, 137.76, 135.90, 129.13, 128.87, 128.31, 127.41, 127.23, 124.84, 124.78, 124.62, 123.29, 122.85, 122.67, 121.83, 120.80, 114.42, 110.54, 110.10, 109.05, 56.85, 52.26, 25.64, 21.75, 9.95. HRMS (ESI): $m/z$ calcd for $C_{48}H_{45}N_8I$ $[M-I]^+$: 733.3767, found: 733.3766.

*5.2.8. **CuHCD**.*[6b] To a solution of **HCD** (20 mg, 0.023 mmol) in ethanol (1 mL) was added $CuCl_2 \cdot 2H_2O$ (4 mg, 0.023 mmol) solution in ethanol (1 mL). The mixture was stirred for about 30 min until the starting material had been completely consumed as detected by TLC. 6 mL of Hexane was added to the mixture and the complex was precipitated from solution. The product was obtained by filter as red-brown solid (18.4 mg, 0.018 mmol, 80%); mp: 235–240 °C; ESI-MS: $m/z$ calcd for $[M-I]^+$ 868.2421, found: 868.2425.

### 5.3. General fluorescence measurements

Stock solution of **CuHCD** at the concentration of $5\times10^{-3}$ M was prepared in methanol and stored in a cold place. Absorption spectra were recorded by Shimadzu UV2600 spectrophotometer and fluorescence spectra were obtained by FS5 fluorescence spectrophotometer. All metal ions stock solutions are prepared from their nitrate, chloride, perchlorate or sulfate ($AgNO_3$, $CaCl_2$, $CdSO_4 \cdot 8H_2O$, $CoCl_2 \cdot 6H_2O$, $CrCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $FeCl_3 \cdot 6H_2O$, LiCl, $Mg(ClO_4)_2$, $MnSO_4 \cdot H_2O$, $Ni(ClO_4)_2 \cdot 6H_2O$, $PbCl_2$, $Zn(ClO_4)_2 \cdot 6H_2O$) and all

anions stock solutions are obtained from their sodium salts ($NaNO_3$, $Na_2S_2O_3$, $NaHCO_3$, $Na_2CO_3$ $CH_3COONa$, $NaHSO_3$, $Na_2SO_4$, $Na_2C_2O_4$, NaI, NaBr, NaCl, NaF, $NaH_2PO_4{\cdot}H_2O$, $Na_2HPO_4{\cdot}12H_2O$, $NaNO_2$, NaClO). Peroxynitrite solution was synthesized as reported.[26] Sodium trioxodinitrate ($Na_2N_2O_3$, Angeli's salt) was used as nitroxyl donor. Angeli's salt was prepared by the method of King and Nagasawa and stored under −20 °C before use.[20b] All cuvette experiments were carried out at room temperature.

### 5.4. Determination of $K_s$, $K_d$ and $K_{SV}$

The association constant ($K_s$) and dissociation consistent ($K_d$) were calculated based on the titration curve of the **HCD** with $Cu^{2+}$ and were determined by the following equation.[22b]

$$\log\frac{F-F_{min}}{F_{max}-F} = logK_s + nlog[X] \quad (1)$$

$$\log\frac{F-F_{min}}{F_{max}-F} = -logK_d + nlog[X] \quad (2)$$

where, $F$, $F_{min}$ and $F_{max}$, are observed, minimum, and maximum fluorescence intensity, respectively.

The Stern–Volmer quenching constant ($K_{sv}$) were obtained by the titration data of **HCD** to $Cu^{2+}$, and then count by the following equation.[22a]

$$\frac{F}{F_0} = 1 + K_{SV}[Cu] \quad (3)$$

$F_0$ and $F$ being the fluorescence intensities of **HCD** in the absence and presence of quencher (metal ion).

### 5.5. Measurement of detection limit

The detection limit was determined by fluorescence titration and then calculated with the following equation.[27]

$$\text{Detection limit} = 3\sigma/\kappa$$

where σ is the standard deviation of measurements of blank simple for 10 times, κ is the slope between intensity versus sample concentration.

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## Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.07.039. These data include MOL files and InChiKeys of the most important compounds described in this article.

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