A fluorescent probe for hydrazine and its in vivo applications†

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In aqueous solution, probe 1 selectively reacts with hydrazine (N₂H₄), leading to a 30.5-fold turn-on fluorescence response at 560 nm. Probe 1 can detect hydrazine in both solution and gas state by color changes from readily prepared strips. Furthermore, it was found that probe 1 is able to detect hydrazine in Hela cells.

Hydrazine (N₂H₄), as an important reactive base and reducing agent, is widely used in the chemical, pharmaceutical and agricultural industries. It plays crucial roles in catalysts, corrosion, textile dyes, pharmaceutical intermediates, etc. Hydrazine is also often applied in missile and rocket propulsion systems as a propellant because of its flammable and detonable nature. Despite its usefulness, hydrazine has considerable toxic effects on potential environmental contamination during its manufacture, use, transport and disposal. In addition, it is documented that some nitrogen fixing bacteria may create hydrazine as a by-product. Hydrazine is readily absorbed by oral, dermal and inhalation routes of exposure, resulting in seriously neurotoxic and mutagenic damages to the liver, lungs, kidneys and the human central nervous system. Hydrazine has been classified as a probable human carcinogen. Therefore, it is highly desirable to explore reliable analytic approaches to detect a trace amount of hydrazine with satisfactory sensitivity and selectivity.

Fluorescent probe-based assay has gained increasing attention because of its high sensitivity and selectivity, spatiotemporal resolution, noninvasiveness, and more importantly, good compatibility for biosamples. A number of fluorescent sensors for hydrazine have been explored. Sessler et al. presented a fluorescent probe for hydrazine by a five-membered ring reaction based on trifluoracetyl acetonate naphthalimide derivative. Chang and other groups developed a series of turn-on hydrazine fluorescent probes by deprotection of levulinoyl or acetyl groups. Peng et al. reported a ratiometric fluorescent probe based on the reaction of hydrazine with arylidenemalononitrile. Xu et al. described a new probe based on the Gabriel mechanism for hydrazine detection. Very recently, Goswami selectively detected hydrazine by deprotection of the benzithiazole derivative, which is based on the recovery of excited state intramolecular proton transfer (ESIPT). Although these small molecule fluorescent probes have been applied in hydrazine detection, selectivity is a common problem for detection of hydrazine in the presence of competitive amines such as ammonia, diamines and relative bio-amines, which is crucial to effectively detect hydrazine in complex cellular milieus. Besides, most of them suffer from delayed response time (typically > 10 min) and complicated assay. Herein, we present a novel hydrazine fluorescent probe 1 (Scheme 1), where its 5'-hydroxyl functions to activate aldehyde group for hydrazine addition and capture hydrazine through inter- and intramolecular hydrogen bonding to fasten response. Probe 1 can

In this manuscript, we report a novel fluorescent probe for hydrazine, which could be used in both the gas and liquid states. The probe selectively reacts with hydrazine in aqueous solution, leading to a 30.5-fold increase in fluorescence intensity at 560 nm. The probe is capable of detecting hydrazine in both solution and gas states, and it is able to detect hydrazine in Hela cells. Hydrazine is a reactive base and reducing agent that is widely used in various industries, including catalysis, corrosion, textile dyes, pharmaceutical intermediates, etc. Despite its usefulness, hydrazine has significant toxic effects on the environment during its production, use, transport, and disposal. Hydrazine is readily absorbed through various routes of exposure, causing serious neurotoxic and mutagenic effects on the liver, lungs, kidneys, and the central nervous system. It is classified as a probable human carcinogen. Therefore, it is crucial to develop reliable analytical methods to detect trace amounts of hydrazine with high sensitivity and selectivity.

Fluorescent probe-based assays have gained increasing attention due to their high sensitivity, spatiotemporal resolution, noninvasiveness, and compatibility with biosamples. Numerous fluorescent sensors for hydrazine have been developed. Sessler et al. reported a fluorescent probe for hydrazine based on a five-membered ring reaction involving trifluoracetyl acetonate naphthalimide. Chang and other researchers developed a series of turn-on fluorescent probes for hydrazine by deprotection of levulinoyl or acetyl groups. Peng et al. reported a ratiometric fluorescent probe for hydrazine based on the reaction with arylidenemalononitrile. Xu et al. introduced a new probe based on the Gabriel mechanism for hydrazine detection. However, these small molecule fluorescent probes often suffer from delayed response times (typically > 10 min) and complicated assays.

In this work, we introduce a novel hydrazine fluorescent probe 1 (Scheme 1), which selectively reacts with hydrazine through its 5'-hydroxyl functions to activate the aldehyde group. This reaction leads to a fast and efficient detection of hydrazine through inter- and intramolecular hydrogen bonding. The probe can be used in both solution and gas states, and it is capable of detecting hydrazine in Hela cells. This work demonstrates the potential of the fluorescent probe for hydrazine detection and its applications in various fields.
also detect hydrazine in both solutions and gas state by color changes from readily prepared strips.

Fluorescent sensors based on ESIPT mechanism, seen from 2-(2′-hydroxyphenyl)benzoxazole (HBO), have been attracted more interests because they endow ratiometric fluorescence change from the excited enol and keto tautomers, and large Stokes shift from tautomerization. However, this sensing process is often susceptible to microenvironment, especially in aqueous solution. Correspondingly, the accurate detection in biosystem through this method would be limited. To tackle this, we have tried the alternative strategy that analyte-induced attack on 4′/5′ instead of 2′ position of ESIPT molecules triggers turn-on fluorescence for detection. Based on this, we designed the fluorescent probe 1 that its 4′ and 5′ position was substituted by aldehyde and hydroxyl group, respectively, where the aldehyde group was used to selectively recognize hydrazine (usually aldehyde reacts with hydrazine to form Schiff base). With this design, we envisaged that probe 1 can selectively detect hydrazine with turn-on fluorescence response. For comparison, probes 2–4, lack of intramolecular hydrogen bonding with aldehyde group, were also synthesized. All these compounds were fully characterized by 1H NMR, 13C NMR and HRMS. The 1H NMR spectrum of probe 1 in DMSO-d6 is characterized by a singlet of –CHO peak at 10.6 ppm, and two singlet of –OH peaks at 10.5 and 10.3 ppm. The structure of probe 2 was further confirmed by its exclusive ESIPT emission character, where two emission peaks at 470 and 580 nm are assigned to enol and keto emission, respectively, and their fluorescence intensity ratio are dependent on the solvent polarity (Fig. S1†).

The UV-Vis absorption and fluorescence spectra of probe 1 in the absence and presence of hydrazine in CH3CN were initially investigated (Fig. S2†). Probe 1 displays two absorption bands at 316 (ε = 3.1 × 10^4 M⁻¹ cm⁻¹) and 406 nm (ε = 1.4 × 10^3 M⁻¹ cm⁻¹), respectively. While upon addition of hydrazine, the absorption at 425 nm gradually decreased, and a new absorption peak appeared at 380 nm with a well-defined isosbestic point at 406 nm. In fluorescence spectra excited at 390 nm, probe 1 exhibits two emission bans at 470 and 600 nm, which are attributed to the normal isomer (N* enol emission) and tautomer (T* keto emission), respectively. With the addition of hydrazine, the enol emission intensity gradually decreased, while the keto emission intensity at 560 nm (blue-shifted from 600 nm due to the intramolecular charge transfer effect induced by the addition of aldehyde with hydrazine) increased. Correspondingly, the fluorescence color was changed from carnation to yellow-green. The observed fluorescence change indicated that the hydrazine-addition to probe 1 is conductive to its tautomerization from enol to keto, providing the possibility for hydrazine detection.

Next, the spectra changes of the probe 1 with hydrazine were carried out in aqueous solution (CH3CN–HEPES buffer (1 : 2, v/v, pH 7.4)) to value the feasibility of real application. The spectra changes are similar to that in pure CH3CN (Fig. 1). Successive ratiometric absorption changes with an isosbestic point at 394 nm were observed upon increasing concentrations of hydrazine. In fluorescence spectra excited at 390 nm, the wavelength of maximal fluorescence intensity underwent blue-shift from 600 to 560 nm when probe 1 was treated with hydrazine. Meanwhile, the fluorescence intensity at 560 nm increased upon addition of hydrazine. The fluorescence quantum yield (φ) was increased from 0.002 to 0.08 and 30.5-fold fluorescence intensity increasing at 360 nm was correspondingly observed in the presence of 8 equivalents of hydrazine. The fluorescence intensity at 360 nm linearly increased with addition of hydrazine over a wide concentration range (0–120 μM, R² = 0.99) (Fig. 1b). The detection limit (3σ/slope) was calculated to be 8.42 × 10⁻⁶ M (2.7 ppb), which is much lower than that of TLV (10 ppb) recommended by EPA. All these results suggested that probe 1 could quantitatively detect hydrazine.

To explore the possible utility of probe 1 as fluorescent sensor for hydrazine, competitive experiments were carried out with 5 equivalents of various environmentally abundant metal ions, anions, cysteine (Cys), homocysteine (Hcy), glutathione (GSH), and other amines including triethylamine (TEA), ammonium hydroxide, thiourea, ethanediame, n-butylamine, hydroxylamine and dimethylamine. As seen in Fig. 2 and S3,† hydroxylamine induced very weak and slower fluorescence...
response, but all other interferents did not induce any appreciable fluorescence change at 560 nm. In the presence of these interferents, probe 1 still showed the same turn-on fluorescence response to hydrazine as that in the absence of these interferents except that Zn\(^{2+}\) and Ni\(^{2+}\) imposed few interferences. Moreover, the fluorescence intensity of probe 1 at 560 nm is independent of pH over a wide range from 4 to 10 (Fig. S4†). These results suggested that probe 1 can detect hydrazine with high selectivity even in complicated milieu.

To shed light on the interaction mechanism, the \(^1\)H NMR spectra titration of probe 1 with hydrazine in DMSO-\(d_6\) and \(D_2O\) (10 : 1, \(v/v\)) were investigated (Fig. S5†). In the absence of hydrazine, probe 1 exhibited a sharp singlet peak at 10.3 ppm, which is attributed to the –CHO group. Due to the existence of \(D_2O\), the –OH peaks at 10.6 and 10.5 ppm are too weak to be detected. Upon addition of hydrazine, the –CHO peak disappeared, suggesting that aldehyde has reacted with hydrazine.

In addition, the changes of chemical shifts for the aromatic protons of the benzoazole core were found. The reaction product of probe 1 with hydrazine was subjected to ESI-MS analysis and it was found to be (m/z = 270.0874 for \(C_{13}H_{11}N_3O_3, [M + H]^+\)) corresponding to calculation (m/z = 270.0879) (Fig. S6†). To further view the structure change of probe 1 before and after addition with hydrazine, density functional theory (DFT) and time dependent density functional theory (TDDFT) calculations with the B3LYP/6-31+G method using Gaussian 09,† the optimized geometry, the highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO) of the probe 1 and the possible product were shown in the Fig. S7 and S8.† From gas phase TDDFT calculations, a transition at 361.04 nm is close to the experimentally observed absorption at 371 nm (see Tables S1 and S2†).

Reaction time is an important factor to assess the feasibility for real time detection. Therefore, the time required for the addition with hydrazine was investigated in the identical condition in the presence of 10 equivalents of hydrazine. As shown in Fig. S9a,† the fluorescence intensity at 560 nm increased with time duration and then leveled off at about 7 min, which is more faster than that some hydrazine fluorescent probes recently reported."""10 From this result, it is concluded that probe 1 is suitable for rapid detection of hydrazine.

In probe 1, there are two kinds of hydroxyl groups, where one of them induces ESPI fluorescence for turn-on detection of hydrazine. An intriguing question is whether the other hydroxyl group listed on the ortho position of aldehyde plays important roles in the process of hydrazine sensing. To this end, probe 2, 3 and 4, lack of corresponding hydroxyl group, were studied in the same condition upon addition of hydrazine. In sharp contrast, probe 3 and 4 did not show any appreciable fluorescence response to hydrazine (Fig. S10†). Even though probe 2 exhibited exiguous fluorescence change to hydrazine, the reaction cannot be completed within 30 min (Fig. S9a†). From these results, the hydroxyl group activates the aldehyde through intermolecular hydrogen bonding to react with hydrazine. Interestingly, in anionic surfactant of sodium dodecyl sulfate (SDS) solution (10 mM, CMC = 8 mM) where micelles have formed, although probe 3 and 4 cannot react with hydrazine, the response of probe 2 to hydrazine was accelerated to complete within about 7 min, the same time as that probe 1 (Fig. S9b†). The negative charges on the micelle surface can help hydrazine gather around, enabling intermolecular reaction to perform more efficiently and enhancing the intermolecular reaction rates significantly.\(^{19}\)

Moreover, a cationic surfactant (CTAB, cetyltrimethyl ammonium bromide) with positive charges was also investigated in Fig. S11.\(^{†}\) In contrast, the reaction rates of probe 1 and 2 were suppressed because the repulsion between surface-positive micelle and hydrazine prevents the probes from reacting with hydrazine. Compared to probe 2, probe 1 showed fast reaction rate (7 min) in the solution even without micelle. It is presumed that the hydroxyl close to the aldehyde in probe 1 possibly sequesters hydrazine through intermolecular hydrogen bonding, altering the intermolecular reaction to intramolecular ones. This is the main reasons that probe 1 showed faster response to hydrazine (within about 7 min). The plausible recognition reaction was outlined in Scheme 2.

To make the detection experiments easy to operate and more practical, probe 1 was applied for the detection of gas-state hydrazine and discriminate their aqueous solution with different concentrations. Test strips prepared by dipping the filter paper with probe 1 solution and then being dried were covered on the top of jars containing different concentration hydrazine solution for 10 min at r.t. before they were observed. The fluorescence color was observed using a hand-held UV lamp with excitation at 365 nm. As shown in Fig. 3, the color of strips changed from olive-green to celadon, and the fluorescence color changed from purple to nearly colorless with increasing the concentration of hydrazine solution. The concentration of hydrazine in aqueous solution was easy to distinguish by naked eye, providing the facile and visual detection of hydrazine. Moreover, the test strips loaded probe 1 can response to the hydrazine gas or other vaporized organic materials (i.e., ammonia, water, triethylamine, dimethylamine, formaldehyde, HCl, methylamine, CO\(_2\) and \(n\)-butylamine), where the vapors were considerably excess relative to probe 1. As shown in Fig. S12, upon exposure to hydrazine vapor, distinctive fluorescence color change (from grass green to light brown) was observed. Although burnt umber and mauve occurred in the presence of dimethylamine and \(n\)-butylamine, respectively, these colors were distinguishable from that induced by hydrazine vapor. Moreover, we have proved that aqueous solution containing these amines cannot trigger any fluorescence change of probe 1 (Fig. 2). These findings suggest that probe 1

![Scheme 2 Proposed detection mechanism of probe 1 towards hydrazine.](https://example.com/scheme2.png)
can selectively detect hydrazine vapors by naked eyes. As far as we know, this is the first probe that can detect hydrazine in both solution and gas state by color changes of readily prepared strips.

It has been reported that some fluorescent probes bearing aromatic aldehyde structure can selectively recognize mercaptan amino acids such like Cys and GSH which are abundant in living cells through specific aldehyde cyclization reaction.20 Although probe 1 reacts with hydrazine through aldehyde addition, it showed no fluorescence response to these amino acids but selectively to hydrazine. Based on this, probe 1 was examined to detect hydrazine in living cells. In this study, Hela cells were incubated with probe 1 and then treated with hydrazine. Fluorescence images were acquired via confocal microscopy. As shown in Fig. 4, probe 1 displays no fluorescence signal in cells, which can efficiently avoid the interference of background. In contrast, strong fluorescence signature was seen in the presence of hydrazine. These cell experiments indicated that probe 1 could provide a selective response of hydrazine in living cells.

In summary, probe 1 undergoes a selective aldehyde addition with hydrazine to give a ratiometric absorption spectra change and a turn-on fluorescence response at 560 nm in aqueous solution. The fluorescence changes at 560 nm are linearly proportional to the increasing concentration of hydrazine over a wide range (0–120 μM). The detection limit of probe 1 to hydrazine was found to be 2.7 ppb, which falls below the 10 ppb limit set by EPA. Competition experiments demonstrated that probe 1 selectively reacts with hydrazine, even in the presence of other interferents including cations, anions, amino acids and other relative amines. Based on the facts that probes 2–4 showed no obvious fluorescence response to hydrazine in buffer solution and SDS micelles enhance the reaction rates of probe 2 to hydrazine, the hydroxyl adjacent to aldehyde group plays two roles during the detection process, which contain activation of aldehyde group for hydrazine addition through intramolecular hydrogen bonding and hydrazine capturing through intermolecular hydrogen. The response of probe 1 to hydrazine is fast within about 7 min. In addition, probe 1 can detect hydrazine in both solution and gas state by color changes of readily prepared strips. Finally, it was found that probe 1 is able to detect hydrazine in Hela cells, which is proved the great potential of the probe for practical application.

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Notes and references

18 See in the ESI.