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ESIPT-based fluorescent probe for sensitive detection of hydrazine in aqueous solution

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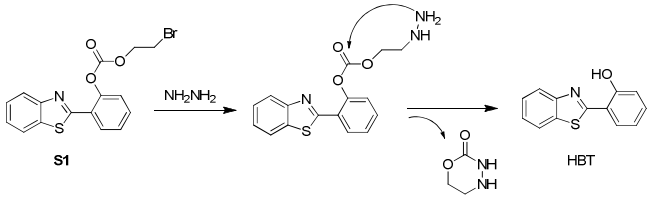
A fluorescent probe for sensitive detection of hydrazine based on ESIPT mechanism and substitution-cyclization-elimination cascade was developed. After the addition of hydrazine, an approximately 50-fold enhancement in fluorescence intensity at 465 nm was observed and the subsequent decrease at 375 nm was observed in 10 min with a detection limit of 0.147 μM. We also detected hydrazine in Hela cells successfully.

Hydrazine is a colorless liquid compound which has high alkalinity and strong reducing ability[1]. Hydrazine is widely used in missile and rocket propulsion systems and fuel cells owing to its explosive characteristics[2]. Hydrazine can be used as an important reactant in pharmaceutical preparation and a catalyst in chemical industries[3]. However hydrazine is proved to be highly toxic[4]. Humans are exposed to hydrazine by inhaling contaminated air, drinking polluted water and touching tainted dust[5]. Hydrazines have been found to be convulsants, hemolytic agents, cardiac depressants, hepatotoxins, neurotoxicants, local irritants, genotoxicants, and suspected carcinogens.[5] Different waste water has different components. The hydrazine propellant effluent mainly contains nitromethane, formaldehyde, hydrogen cyanide, dimethylamine and unsymhydrazone[6]. The industrial sewage, surface water and underground water often contains SO_4^{2-}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, glucose, urea and so on[7]. The highest hydrazine content in the industrial sewage is 3.125 μM/L. The highest hydrazine content in the drinking water is 0.3125 μM /L[7]. Therefore it is of vital importance to design a reliable method which can provide a sensitive and selective real-time recognition of hydrazine.

In recent years, fluorescent probes have been used extensively in the detection of important reactive molecules due to its sensitivity and selectivity, spatiotemporal resolution and noninvasiveness[8]. A lot of fluorescent probes for the detection of hydrazine have been reported[9]. For example, the probe reported by Xiaojun Peng[9c] made use of the reaction between arylidenemalononitrile and hydrazine. The resulting hydrazone led to ICT-induced absorption and fluorescence ratiometric responses. The detection system was acetate buffer (pH 3.7, 10 mM)-DMSO (1/9, v/v). Jong Seung Kim developed a fuorescence turn-on type probe[9d] which could react with hydrazine to obtain a five-membered ring. The detection system was the mixture of H_2O-CH_3CN (v/v, 1:9). Shyamaprosad Goswami reported a probe[9j] using Excited State Intramolecular Proton Transfer (ESIPT) mechanism and substitution-cyclization-elimination cascade. The detection system was CH_3CN:H_2O (2:3, v/v, and pH 7.4, 1 mM HEPES buffer). Suk-Kyu Chang reported a fluorescent probe[9e] using selective deprotection of levulinated coumarin by hydrazine. The detection system was the mixture of acetate buffer (pH 4.5, 10 mM) and DMSO(3:7, v/v). In this paper our aim is to develop a highly selective and sensitive ESIPT fluorescent probe with good solubility in water.

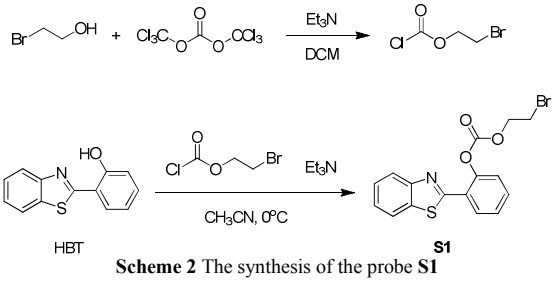
ESIPT has been used widely in fluorescence imaging.[10] Compared with the enol form, the keto form exhibits a large Stokes shift. This mechanism has been used to detect various species, such as palladium[11], MKP-6[12], and hydrogen sulfide[13]. We chose ESIPT mechanism because we wanted to observe both a "turn-on" signal and a red-shift since the dual channel fluorescence changes should be better than one.

Herein we report a fluorescent probe, which makes use of the off-on switch of ESIPT of the HBT (2-(2-Hydroxyphenyl) benzothiazole) moiety and a novel receptor. Upon the addition of hydrazine, the bromine atom would be substituted and then the amine group would attack the carbonate, forming a six-membered ring byproduct and leading to the release of the free HBT fluorophore (scheme 1).



Scheme 1 The deduced detection mechanism of the probe **S1**

The probe **S1** was conveniently prepared in two steps (scheme 2). Their structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (see the supporting information).



Scheme 2 The synthesis of the probe **S1**

First we conducted the pH titration of the probe. We found that when pH was larger than 8.5, the probe would be hydrolyzed, leading to an increase in fluorescence.

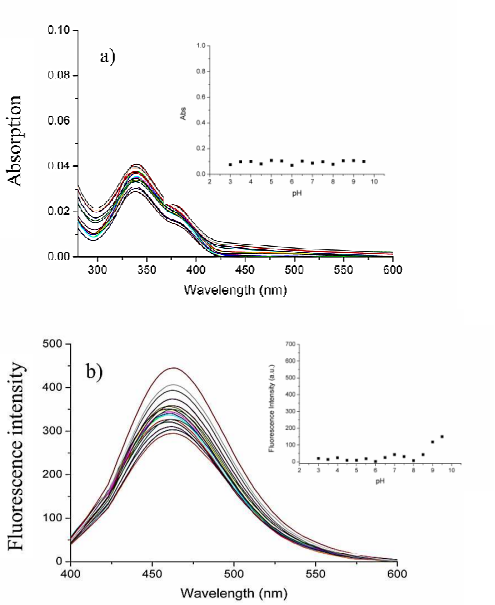
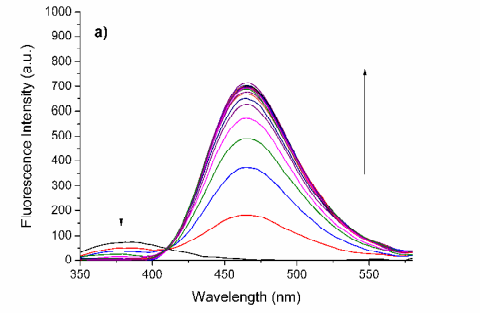


Fig.1 The pH titration of the probe **S1** (10 μM). (a) The absorption of the probe **S1** in response to different pH; (b) The emission of the probe **S1** in response to different pH. The excitation wavelength was 300 nm, the emission wavelength was 465 nm. Slit: 5 nm, 5 nm.

We examined the fluorescence properties of **S1** in the absence and presence of hydrazine. The probe (5 μM) was treated with 10 μM hydrazine and the fluorescence was recorded. The free probe **S1** displayed very weak fluorescence at 465 nm because the excited state intramolecular proton transfer was blocked. After the addition of hydrazine, the receptor was attacked by hydrazine twice and HBT was released. An obvious fluorescence enhancement at 465 nm was observed (Fig.1). The reaction was fast and the fluorescence could reach a plateau within 10 min. Finally, an approximate 50-fold increase in fluorescence intensity was obtained. We also tried different solvents as the test system, for example the mixture of PBS buffer and DMSO or the mixture of PBS buffer and CH_3CN (see the supporting information) respectively. The results suggested that PBS buffer with 1% ethanol as co-solvent was the most suitable detection system with the largest fluorescence enhancement.



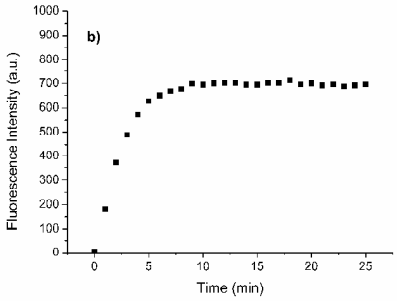


Fig.2 (a) The fluorescence responses of the probe **S1** (5 μM) to hydrazine (10 μM) in 25 min. (b) The fluorescence intensity at 465 nm after the addition of hydrazine (10 μM). The excitation wavelength was 300 nm, the detection system was PBS buffer (pH 7.4) with 1% ethanol as a cosolvent. Slit: 5 nm, 5 nm.

We investigated the selectivity of the probe, choosing some common species in the human body. The results showed that the probe didn't respond to GSH, NaSH, Cys or Hcy. This indicated that the probe had the potential of detecting hydrazine in the biological system (seeing supporting information).

Then we decided to examine the sensitivity of the probe to hydrazine. The probe **S1** (5 μM) was treated with hydrazine (1-5 μM) for 25 min and a good linearity was found between the concentration of hydrazine and the fluorescence intensity at 465 nm (Fig.2). The detection limit was calculated to be 0.147 μM (S/N=3) according to the previous literature[14]. Therefore our probe is suitable for detecting hydrazine in the industrial waste water in which the highest permitted content of hydrazine is about 2 μM.

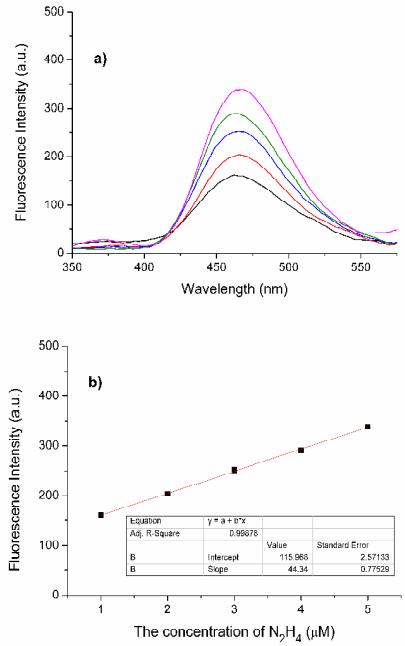


Fig.3 (a) The fluorescence responses of the probe **S1** (5 μM) to hydrazine (1-5 μM) after 25 min. (b) A liner calibration graph of the fluorescence intensity at 465 nm to hydrazine (1-5 μM). The excitation wavelength was 300 nm, the detection system was PBS buffer (pH 7.4) with 1% ethanol as a cosolvent. Slit: 5 nm, 5 nm.

Hydrazine is a strong reducing agent, and it can decompose in the presence of catalyst, such as Pt, Fe and so on[6]. The effluent containing hydrazine also includes other common species, such

as Cl^-, SO_4^{2-}, Ca^{2+}, Zn^{2+}, SO_3^{2-}, NO_3^-, Hg^{2+}, H_2S, glucose and urea[7]. The probe was treated with several anions and small molecules. Of all these species, only hydrazine could lead to an obvious fluorescence enhancement at 465 nm. This indicated that the probe showed good selectivity towards hydrazine.

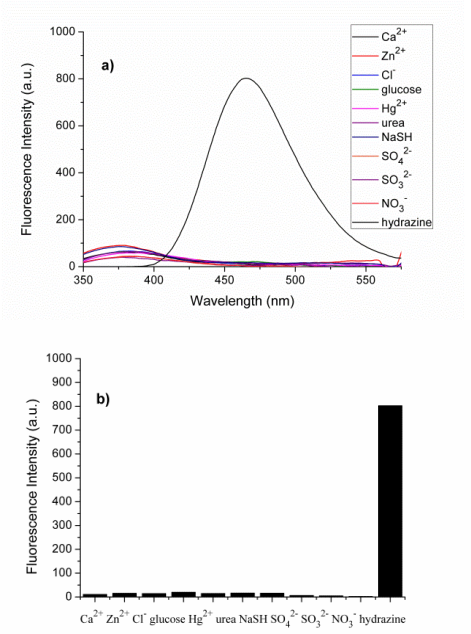
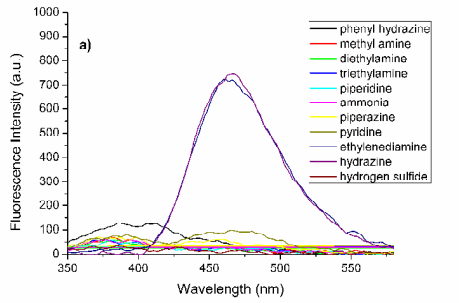


Fig.4 (a) Fluorescence responses of the probe **S1** (5 μM) to various relevant species in the PBS buffer (with 1% ethanol) at room temperature. (b) The column chart of the selectivity of the probe. Excitation wavelength was 300 nm. Slit: 5 nm, 5 nm. Bars represent the final fluorescence intensity of **S1** after reacting with the following analytes, including Ca^{2+} (500 μM), Zn^{2+} (500 μM), Cl^- (500 μM), glucose (500 μM), Hg^{2+} (500 μM), urea (500 μM), NaSH (500 μM), SO_4^{2-} (500 μM), SO_3^{2-} (500 μM), NO_3^- (500 μM), and hydrazine (20 μM).

We also tested compounds that are less abundant but chemically more similar to hydrazine, such as ethylenediamine, piperazine, dimethylamine, triethylamine, pyridine and so on. According to the results, only ethylenediamine caused serious interference. Even phenyl hydrazine could not disturb the detection of hydrazine. We thought it was because that the benzene ring led to a large steric hindrance which prevent the second amine group reacting with the receptor.



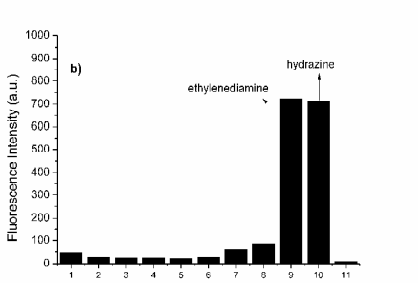


Fig.5 (a) Fluorescence responses of the probe **S1** (10 μM) to various relevant species in the PBS buffer (with 1% ethanol) at room temperature. (b) The column chart of the selectivity of the probe. Excitation wavelength was 300 nm. Slit: 5 nm, 5 nm. Bars represent the final fluorescence intensity of **S1** after reacting with the following analytes, including (1) phenyl hydrazine (200 μM), (2) methylamine (200 μM), (3) diethylamine (200 μM), (4) triethylamine (200 μM), (5) piperidine (200 μM), (6) ammonia (200 μM), (7) piperazine (200 μM), (8) pyridine (200 μM), (9) ethylenediamine (200 μM), (10) hydrazine (20 μM), and (11) hydrogen sulfide (200 μM).

At last, in order to investigate its ability of detecting hydrazine in the biological system, we chose Hela cells. After incubating Hela cells with the probe, we could only observe very weak fluorescence. However after adding hydrazine, the fluorescence increased a lot.

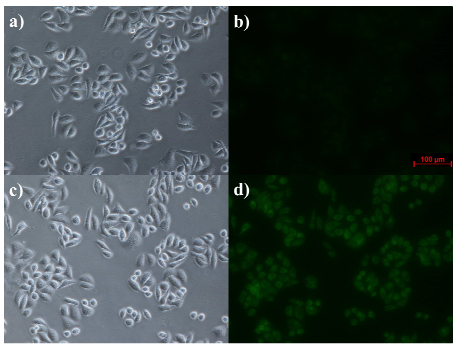


Fig.6 Fluorescence and bright-field images of HeLa cells. (a) Bright-field image of HeLa cells incubated with **S1** (10 μM) for 20 min. (b) Fluorescence image of HeLa cells incubated with **S1** (10 μM) for 20 min. (c) Bright-field image of HeLa cells first incubated with **S1** for 20 min and then with hydrazine (20 μM) added for another 40 min. (d) Fluorescence image of HeLa cells first incubated with **S1** for 20 min and then with hydrazine (20 μM) added for another 40 min.

In conclusion, we have developed a fluorescent probe for the detection of hydrazine based on the ESIPT mechanism and the dual nucleophilicity of hydrazine. A novel receptor was designed and it could react with hydrazine very fast with high sensitivity. A red-shift as well as an obvious fluorescence enhancement at 465 nm and a decrease at 370 nm could be observed with a detection limit of 0.147 μM. The probe had good solubility which made it possible to detect hydrazine in aqueous solution.

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