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Design of a ratiometric fluorescent probe for benzenethiols based on a thiol–sulfoxide reaction†

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A novel thiol–sulfoxide reaction based ratiometric probe for benzenethiols was synthesized and evaluated. The probe features a chemospecific reduction over a pH range of 1–10 by benzenethiols with a marked emission color change, enabling the highly selective detection and is promising for applications.

Construction of fluorescent probes for sensitive and selective sensing of thiols is of significant interest because of the simplicity, high sensitivity and high spatial resolution of fluorescence.[1,2] Most investigations have been focused on sensing of small-molecular-weight thiols including cysteine, homocysteine, and glutathione, which play significant roles in redox-related biological processes.[3] On the other hand, benzenethiols are important chemicals in the family of thiols, widely employed in the production of pesticides, polymers, and pharmaceuticals. In spite of their broad synthetic utility, benzenethiols are highly toxic and polluting compounds. It is well known that benzenethiols are more toxic than aliphatic alcohols. Exposure to benzenethiols can induce systemic injuries including shortness of breath, muscular weakness, nausea, vomiting, coma, and even death through targeting of the central nervous system, kidney, and liver.[4]

Most of the optical probes for thiols are chemodosimeters involving specific reactions between probes and thiols, especially utilizing the strong nucleophilicity of thiols. Therefore most of them exhibit poor selectivity toward benzenethiols and aliphatic thiols because aliphatic thiols and benzenethiols are a class of molecules with close chemical properties. Due to the similarity in reactivity, discrimination benzenethiols over aliphatic thiols is a challenging task. Only several fluorescent sensors that selectively respond to the widely existent benzenethiols have been constructed so far, utilizing the thiolysis of dinitrobenzenesulfonylamides, sulfonate ester or dinitrophenyl ethers by benzenethiols under neutral reaction conditions.[5] It is known that the strongly electron-withdrawing 2,4-dinitrobenzene group serves as an effective quencher, which significantly diminishes the fluorescence of the designed probes. In other words, most of the reported probes respond to benzenethiols with just a fluorescent turn-on signal. Unfortunately, single-intensity-based sensing is compromised by the local distribution of probes, drifts of light sources and detectors. New ratiometric fluorescent probes that can overcome the limitation of intensity-based probes and provide quantitative measurement are therefore in demand.

With the idea of developing a new ratiometric fluorescent probe that can discriminate between benzenethiols and aliphatic thiols, we undertook the effort of searching for an orthogonal reaction between the chemosensing agent and thiols, which can be used for careful design of probes to selectively sense benzenethiols. Accordingly, we became attracted to thiol–sulfoxide reactions, in which the sulfoxide functional group can be reduced to sulfide by acidic thiols accompanied by the oxidation state of sulfur changing from 0 to -2.[6] The changing oxidation state would have significantly effects on the photophysical properties. The electron-withdrawing nature of the sulfoxide would lead to hypsochromic shifts in absorption and emission wavelength compared to that of sulfide. More importantly, the ease of thiol oxidation by sulfoxide is markedly dependent on the acidity of the thiol. The observed ease of thiol oxidation is $ArSH > ArCH_2SH \gg RSH$, and aromatic thiols are about 10^3 to 10^4 times more reactive than aliphatic thiols.[7] In other words, aromatic thiols can be oxidized to their disulfides in high yields at room temperature while aliphatic thiols did not undergo any appreciable reaction in the same time period. On the base of these backgrounds and design considerations, we prepared the sulfoxide containing BODIPY analogue **BSFO**, reasoning that a reductive reaction with benzenethiols would generate highly fluorescent **BSFI** with red-shifted absorption and emission (Scheme 1). To this end, we synthesized the probe in two steps starting from 3-chloro-5,7-dimethyl-6-ethyl-8-phenyl-BODIPY prepared according to the literature.[8]

Fig. S1† shows the photophysical properties of **BSFO** and **BSFI** in a buffer solution (pH 6.1, 25 mM sodium phosphate buffer, 50% EtOH as co-solvent) at room temperature. The absorption and emission spectra of **BSFI** feature a similar shape to those of the classical BODIPY dyes. In the absorption spectrum, **BSFI** shows a strong band around 547 nm and a shoulder at the short wavelength side. Parallel to the absorption spectrum, the emission spectrum closely resembles the mirror image of the corresponding S_0–S_1 transition with a relative small Stokes shift

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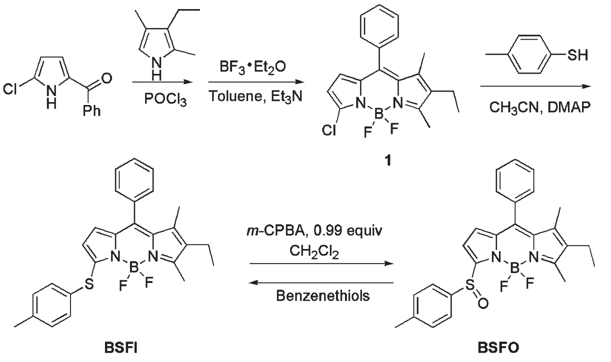

Scheme 1 Synthesis of **BSFO** and the conversion to **BSFI** by benzenethiols.

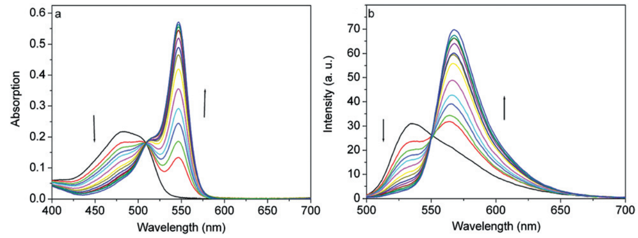

Fig. 1 (a) Absorption and (b) fluorescence response of 10 μM **BSFO** to 1 mM benzenethiols. Spectra were acquired before and 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, and 70 min after benezenethiols were added. Data were acquired at 40 °C in 25 mM sodium phosphate, pH 6.1, with excitation λ_{ex} = 484 nm.

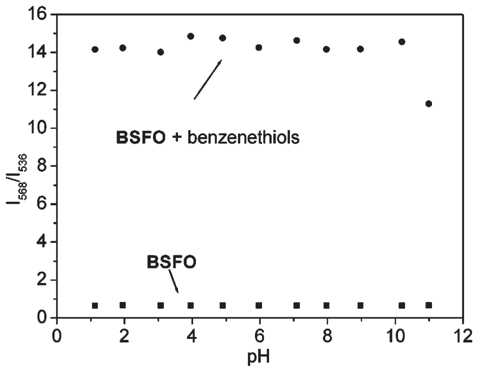

Fig. 2 Fluorescence emission ratios (I_{568}/I_{536}) of **BSFO** (10 μM) as a function of pH values in the absence or presence of benzenethiols (100 equiv.). The excitation wavelength was 484 nm.

and a high fluorescence quantum yield (0.3 in DMSO). A large hypsochromic shift of 63 nm and 32 nm can be observed in absorption and emission, respectively, when comparing **BSFO** to **BSFI** upon changing the substituent in the 3-position from the electron withdrawing character to the electron donating character. This blue shift in emission is accompanied by a decrease in Φ_F (0.08 in DMSO), which is consistent with the general behavior of other aromatic sulfur-containing compounds.[9] The observation that the emission spectrum of **BSFI** has a 32 nm redshift in comparison with that of **BSFO** indicates that sulfoxide-containing **BSFO** is a promising ratiometric fluorescent probe for benzenethiols provided that **BSFO** could be reduced by benzenethiols to generate sulfide-containing **BSFI**.

We first investigated whether **BSFO** could react with benzenethiols in pH 6.1 buffer–EtOH (1 : 1) at 40 °C. When **BSFO** (5 mM) was mixed with *p*-thiocresol (500 mM) and stirred for 1 hour, a stable fluorescent product was observed clearly on the TLC plate. HRMS analysis of the reaction mixture revealed two signals (Fig. S2†). In addition to the signal from unreacted **BSFO** (*m*/*z* = 461.1445), the other signal (*m*/*z* = 445.1648) is consistent with the formulation of **BSFI**. The reaction product was isolated and subjected to the ^{1}H NMR characterization (Fig. S3†), which supported the formation of **BSFI**.

We then assessed the sensory response of **BSFO** to benzenethiols in a buffer solution (pH 6.1, 50% EtOH as co-solvent). In the absence of benzenethiols, **BSFO** displays one major absorption band centered at 484 nm with a corresponding fluorescence maximum at 536 nm. Reaction of **BSFO** with benzenethiols triggered redshifts of the absorption and emission spectra (Fig. 1), resulted from the generation of **BSFI** as identified by its absorption and emission spectra, respectively. In absorption, upon addition of benzenethiols, a decrease in the absorption band at 484 nm and a concomitant increase of a new band at 547 nm were observed, with a distinct isosbestic point at 508 nm, resulting in the color of the solution turned from yellow to pink (Fig. S4†). Notably, the ratio of the absorbance at 547 and 484 nm increases over 348-fold, allowing the possible colorimetric and ratiometric detection of benzenethiols even with naked eyes. The ratio of **BSFI** to **BSFO** emission intensities (I_{568}/I_{536}) upon excitation at 484 nm varies from 0.65 in the absence of benzenethiols to 14.17 after complete conversion to **BSFI**, a *ca.* 22-fold emission ratio change, establishing that **BSFO** can serve as a ratiometric fluorescent probe for benzenethiols. The detection limit for benzenethiols using **BSFO** was found to be 7.41 × 10^{-7} (Fig. S5†).

The pH of the solution was also of great importance to the reduction. In the absence of benzenethiols, the probe was stable over a wide range of pH values from 1 to 10 (Fig. 2). However, the ratiometric responses of the probe toward benzenethiols were pH-dependent. With increasing pH value, the probe becomes more sensitive with the increase of rate constant under pseudo-first-order reaction conditions. Under pseudo-first-order reaction conditions (0.01 mM **BSFO** and 1 mM thiol), the observed rate constant at pH 6.1 and 40 °C is found to be 0.20 min^{-1}, 0.71 min^{-1} at pH 7.2, and 1.96 min^{-1} at pH 8.3, respectively (Fig. S6†). Notably, the ratio of emission intensities (I_{568}/I_{536}) upon excitation at 484 nm exhibited marked increase in the region of pH 1–10. Unlike the reported probes which function well at a pH value higher than the pK_a value of benzenethiols (~6.5), **BSFO** can work better at a pH value lower than the pK_a value of benzenethiols, that is, in acidic conditions. The observation that **BSFO** functions over a much wider pH range than the 2,4-dinitrobenzene-based fluorescent probes and **BSFO** has a maximal sensing response at pH 1–10 values suggests the probe is more promising for applications.

Moreover, the ratiometric emission response of **BSFO** is highly selective for benzenethiols over other related species in a buffer solution (Fig. 3). No comparable ratiometric response was observed from the representative amino acids, sugar, vitamins, aromatic alcohols and amines. Because the detection is based on

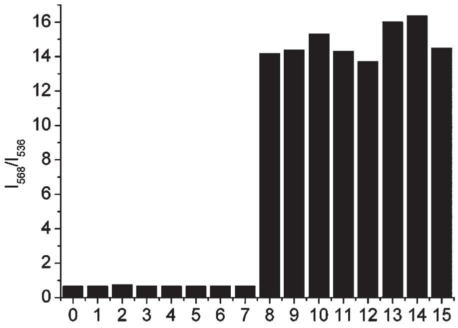

Fig. 3 Fluorescence ratiometric response of **BSFO** to 50 equiv. of various species (left part from 0 to 8 represents **BSFO** only, $CH_3(CH_2)_{11}SH$, $PhCH_2SH$, cystein, glycine, $PhNH_2$, PhOH, D-xylose, and *p*-thiocresol respectively) and response to 50 equiv. of *p*-thiocresol in the presence of 50 equiv. of other analysts (right part from 9 to 15 represents $CH_3(CH_2)_{11}SH$, $PhCH_2SH$, cystein, glycine, $PhNH_2$, PhOH, D-xylose respectively). Bars represent emission intensity ratios I_{568}/I_{536} at 30 min after addition of analysts. Data were acquired at 40 °C in 25 mM sodium phosphate buffer (50% ethanol as co-solvent), pH 6.1, with λ_{ex} = 484 nm.

the reducing properties of sulfoxide by thiols, aliphatic thiols were also tested. Under the same experimental conditions (in pH 6.1 PB buffer–EtOH (1 : 1) at 40 °C), aliphatic thiols did not trigger appreciable response in the assay time period. Only addition of benzenethiols such as *p*-thiocresol, 4-chlorobenzenethiol and 4-bromobenzenethiol, induced a significant enhancement of the fluorescence ratio. Introduction of benzenethiols with strong electron withdrawing or donating group at 4-position, such as 4-nitrothiophenol and 4-aminobenzenethiol, elicited much less enhancement. Although aliphatic thiols are known to be able to induce reduction of sulfoxides, aliphatic thiols are about 10^3 to 10^4 times less reactive than aromatic thiols. The reduction by aliphatic thiols must proceed at high temperature and high pH value. Thus, this high selectivity could be attributed to reaction time and conditions required for the reduction of sulfoxides by different thiols. We also examined the ratiometric response to benzenethiols in the presence of the potential competition of relevant analytes. All the relevant analytes tested have virtually minimum influence on the fluorescent detection. Taken together, these selectivity assays demonstrate that the chemoselective reduction of a sulfoxide to a sulfide can be used for ratiometric fluorescence detection of benzenethiols in aqueous media.

A linear relationship is always important for accurate analysis. When the ratiometric emission response (I_{568}/I_{536}) of **BSFO** was plotted against benzenethiols concentration, a calibration curve revealing a good linear relationship (*R* value = 0.99) in the concentration range of 0–0.3 mm was obtained (Fig. S5†), indicating that **BSFO** can be potentially employed to quantitative determination of benzenethiol concentrations. Encouraged by the promising linear results, we applied this probe to measure benzenethiol concentrations in water samples from Huangpu River. The internal standard method was employed in the experiment. Four water samples with different amount of added benzenethiols were studied. The data obtained using probe **BSFO** are very similar to the added benzenethiol concentration (Table S1†). This confirms that **BSFO** is effective for quantitative detection of benzenethiol in water samples.

In summary, we have presented the synthesis and evaluation of a novel reduction-sensitive fluorescence probe for benzenethiols. This probe features a marked absorption and emission redshift upon reduction with benzenethiols to give sulfide. More importantly, unlike the reported probes, **BSFO** functions over a much wider pH range and can work better at acidic conditions. Notably, this thiol-sulfoxide reaction has scarcely previously used in fluorescent probe design. Furthermore, the probe was found to be very selective for benzenethiols in aqueous media. The good linear relationship makes this probe suitable for accurate quantitative determination of benzenethiol concentrations in water samples.

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