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# A fast responsive chromogenic and near-infrared fluorescence lighting-up probe for visual detection of toxic thiophenol in environmental water and living cells

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ABSTRACT

Thiophenols as high toxic environmental pollutants are poisonous for animals and aquatic organisms. Therefore, it is indispensable to monitor thiophenols in the environment. Herein, a novel near-infrared fluorescent probe was developed for the detection of thiophenols, which was easily prepared by one-step coupling of 2,4-dinitrobenzenesulfonyl chloride with Nile blue. The probe showed a significant near infrared (~675 nm) fluorescence "turn-on" response to thiophenols with some good features including chromogenic reaction, high sensitivity and selectivity, fast response, near-infrared emission along with low detection limit (1.8 nM). The probe was employed to rapidly and visually determine thiophenols in several industrial wastewaters with good recoveries (90–110%). Moreover, this probe has been demonstrated good capability for imaging thiophenol in HeLa cells.

## 1. Introduction

Thiophenols (ArSH) as important industrial chemicals are extensively used to produce chemical intermediates, polymer, dyes and pigments [1–3]. Nevertheless, thiophenols are very harmful for human health, they can cause central nervous system damage, muscular weakness, wheezing, coma and even death [4–6]. Besides, thiophenols are also highly toxic for other animals and aquatic organisms. Studies disclosed that the median lethal concentration ($LD_{50}$) of thiophenols for mouse is around 46.2 mg $kg^{-1}$ [7,8]. In view of these harmful effects, ArSH have been listed as a category of the most important environmental pollutants by the United States Environment Protection Agency (EPA waste code P014) [8]. Therefore, it is indispensable to develop high an efficient method for monitoring thiophenols in the environment and living cells. The traditional detection methods mainly depend on GC-MS [9] and HPLC [10], which have good accuracy and reproducibility. Nevertheless, there are some limitations including high costs, long inspection times and high cell destruction, thus restrict their practical application for the rapid and *in situ* detection of thiophenols in environmental and biological samples.

Fluorescent probes have emerged as promising tools for the detection of some species due to their advantages of convenient operation, high selectivity and sensitivity, real-time detection, as well as non-invasive characteristics [11–13]. Wang et al. reported the first example of fluorescent probe for the detection of thiophenols based on thiolysis reaction of dinitrobenzenesulfonyl amides [14]. Since then, a lot of fluorescence "turn-on" type probes have been developed by utilizing 2,4-dinitrobenzenesulfonyl or dinitrophenyl ethers as recognition moieties [15–35], and various fluorophores as reporter such as 1,8-napthalimide [15,16], coumarin [17–19], BODIPY [20,21] and rhodamine [22]. However, most of them displayed short excitation/emission wavelengths. It has been recognized that near-infrared (NIR) fluorescent probes are suitable for biological analysis due to their low background fluorescence interference, minimal damage to biological samples and deep tissue penetration [36,37]. For environment monitoring, a fluorescent probe with significant chromogenic effect combined with near-infrared fluorescence turn-on signal towards analyte can minimize the interference from background, provides a visual manner to detect

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analyte with naked eyes. Up to date, a few of NIR fluorescent probes for thiophenols have been reported [38–42], and these probes only show changes in fluorescence intensity without chromogenic effect, thus cannot provide a visual manner to determine thiophenol. Therefore, it is desired to develop some high sensitive, chromogenic, fast responsive NIR fluorescence probes for detection of thiophenol in the living organisms and the environment.

Nile blue, a near-infrared fluorescent dye, has been widely used as a biocompatible imaging agent due to its high quantum yield, low cytotoxicity and high biocompatibility, near-infrared emission and good photochemical stability [43–45]. With these features in mind, we prepared a NIR fluorescent probe **NB-DN** by one-step coupling of Nile blue (**NB**) and 2,4-dinitrobenzenesulfonyl (**DN**) chloride. The probe **NB-DN** displayed remarkable chromogenic effect and fluorescence “turn-on” response to thiophenols with high sensitivity. The sensing mechanism of **NB-DN** to thiophenols was studied by $^{1}H$ NMR, HR-MS, HPLC, UV–vis absorption and fluorescence spectroscopy. Furthermore, **NB-DN** has been employed to determine thiophenols in industrial wastewater and utilized as proof of concept for imaging thiophenol in living HeLa cells.

## 2. Experimental section

### 2.1. Materials and instruments

All chemicals were of analytical grade and used as received if not specifically described. 4-Nitroaniline (98%), 1-naphthalenamine (99%) and 3-diethylaminophenol (98%) were purchased from Tianjin Heowns biochemical. 2,4-Dinitrobenzenesulfonyl chloride (97%) was purchased from Energy Chemical, China. Double-distilled water was used in all experiments. The solvents used for HPLC analysis, UV–vis absorption spectra and fluorescence measurements were of HPLC grade and supplied by Merck KGaA (Darmstadt, Germany).

### 2.2. Equipments and methods

A Bruker AV-400 spectrometer was used to record the $^{1}H$ NMR and $^{13}C$ NMR spectra. High-resolution mass spectra were measured on a HP-1100 LC-MS spectrometer. A Hitachi FL 4500 fluorometer and a Hitachi UV 3310 spectrometer were employed to measure the fluorescence spectra and UV–Vis spectra, respectively. A Nikon A1 confocal laser-scanning microscope with a 100× objective lens was employed to acquire fluorescence images. The chromatography system consisted of a LC-10ATVP pump and SPD-10AVP UV–Vis detector (Shimadzu, Kyoto, Japan) with an injector (10 μL sample loop). The analysis was performed on an Optima Pak $C_{18}$ column (5 μm, 150 × 4.6 mm, RS tech Corporation, Daejeon, Korea) and Chromatography Data System N2000 (Surwit Technology, Hangzhou, China).

### 2.3. Synthesis of the probe **NB-DN**

Nile blue was prepared according to literature [45]. For the synthesis of **NB-DN**, Nile blue (100 mg, 0.31 mmol) and $K_2CO_3$ (260 mg, 1.88 mmol) were placed in a two-necked flask with 20 mL anhydrous acetonitrile. The flask was fixed in a ice-water bath, then 2,4-dinitrobenzenesulfonyl chloride (335 mg, 1.26 mmol) in 10 mL $CH_3CN$ was added dropwise under $N_2$ atmosphere for 4 h. When the reaction has finished, the solvent was removed by rotary evaporator and the residue was purified by silica gel column chromatography (eluent: $CH_2Cl_2$) to give probe **NB-DN** as a green solid (60 mg, 0.11 mmol, yield: 35.4%).

$^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ 8.88 (s, 1H), 8.74 (d, $J$ = 8.0 Hz, 1H), 8.60 (d, $J$ = 8.7, 1H),8.44 (d, $J$ = 8.7, 1H) 8.39 (d, $J$ = 8.0 Hz, 1H), 7.90–7.87 (m, 2H), 7.77 (m, 1H), 7.32 (s, 1H), 7.28 (d, $J$ = 8.7, 1H), 7.05 (s, 1H), 3.67 (q, $J$ = 7.0 Hz, 3H), 1.23 (t, $J$ = 7.0 Hz, 6H).

$^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 163.1, 153.9, 151.4, 148.4, 140.5, 135.1, 132.8, 132.0, 131.7, 131.8, 130.0, 129.8, 127.4, 126.1, 120.3, 115.4, 100.1, 96.5, 45.8, 13.0.

HRMS (EI) $m/z$ calcd for $[C_{26}H_{22}N_5O_7S]^+$: 548.1234, found: 548.1239.

### 2.4. Spectroscopic responses of the probe **NB-DN** towards various analytes

A stock solution of the probe **NB-DN** (1 mM) was prepared in HPLC grade DMF. The solutions of various testing species (1–10 mM) thiophenols were prepared in dimethylsulfoxide (DMSO), others species ($MgCl_2$, KCl, $FeCl_2$, $FeCl_3$, $CaCl_2$, $ZnCl_2$, $MnCl_2$, $BaCl_2$, $NiCl_2$, $CrCl_3$, NaF, NaCl, NaBr, $Na_2SO_4$, $NaHSO_3$, $NaNO_2$, $NaNO_3$, $Na_2S_2O_3$, $CH_3COONa$, $Na_2CO_3$, dithiothreitol (DTT), $NaN_3$, $Na_2SO_3$, GSH, Cys, Hcy, NaClO, $H_2O_2$) were prepared in double-distilled water.

### 2.5. Investigation of sensing mechanism of **NB-DN** toward thiophenol by HPLC and MS

For HPLC analysis, the standard solutions of probe **NB-DN** (10 μM), Nile blue (10 μM), ArSAr $(NO_2)_2$ (10 μM) and thiophenols (10 μM) were prepared in HPLC-grade acetonitrile, respectively. The probe **NB-DN** (10 μM) and thiophenols (10 μM) were mixed together and incubated for 12 min in HPLC-grade acetonitrile. Then these solutions (10 μL) were separately injected into $C_{18}$ column for analysis with methanol/water (85:15) as mobile phases, liquid phase changes were recorded by 254 nm.

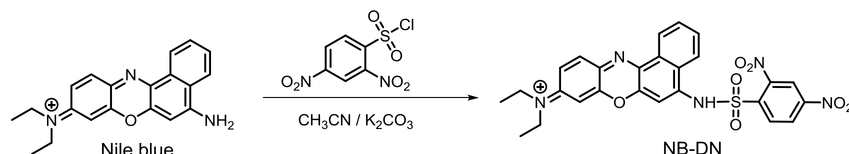
For MS analysis, the probe **NB-DN** (10 μM) and thiophenols (10 μM) were mixed and incubated for 12 min in HPLC-grade acetonitrile, high-resolution mass spectra (HRMS) of the product was measured on a HP-1100 LC-MS spectrometer.

### 2.6. Detection of thiophenols in industrial waste water by **NB-DN**

Three wastewater samples (50 mL) were collected from Tianjin Petrochemical Company (Wastewater A), Fine Chemical Company (Wastewater B) and Jinkang Pharmaceutical Co., Ltd. (Wastewater C) of Tianjin, China. These water samples firstly passed through a micro-filtration membrane and the pH values were adjusted to 7.4 with PBS buffer solution. Then 60% EtOH was mixed to form a test system. The probe and thiophenol stock solution at different concentrations (0, 2, 4, 6, 8 μM) were added to aliquots of water samples and incubated at room temperature for 12 min. Then, the fluorescence intensity of the solution was recorded at 675 nm.

### 2.7. Cell imaging of ArSH

HeLa cells (Human cervical cancer cell) were cultured in Dulbecco's modified Eagle's medium (DMEM) in a humidified atmosphere contain 5% $CO_2$ dioxide at 37 °C. The cells were planted in uncoated 35 mm diameter glass-bottomed dishes and incubated for 24 h. Then the cells were incubated with **NB-DN** (10 μM) for 30 min, washed thrice with




**Scheme 1.** Synthesis of probe **NB-DN**.

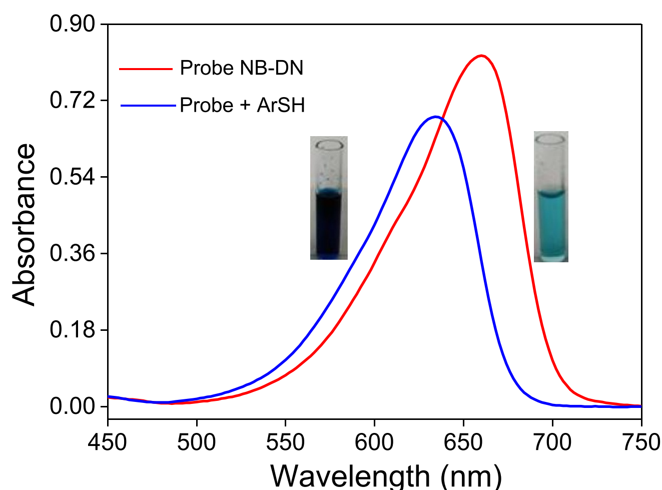



**Fig. 1.** UV/vis absorption spectra of **NB-DN** (10 μM) before and after addition of ArSH (24 μM). Inset: The color changes of probe **NB-DN** in presence of ArSH. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PBS, and mounted on the microscope stage. For imaging of ArSH, the above **NB-DN**-treated cells were incubated with different concentrations of ArSH (0, 20, 40, 80 μM) for 20 min and then ready for confocal fluorescence imaging.

## 3. Result and discussion

### 3.1. Preparation of probe NB-DN

Nile blue (**NB**), a water-soluble oxazine dye with near-infrared emission ($\lambda_{em}$ = 660–680 nm), was often used as a biocompatible dye for staining polyhydroxybutyrate (PHB) and nucleic acid (DNA) inside eukaryotic cells [46,47]. Keep these features in mind, in this work, we employed **NB** to prepare a fluorescent probe for ArSH. **NB** was readily coupled with 2,4-dinitrobenzenesulfonyl (**DN**) chloride to give probe **NB-DN** (see Scheme 1). Since a fluorescence quenching group **DN** was attached to **NB**, the probe **NB-DN** is almost non-fluorescent ($\Phi$ < 0.001) due to photo-induced electron transfer (PET). We envisioned that fluorescence quenching group **DN** could be removed by thiophenols through nucleophilic substitution reaction, thus releasing Nile blue and giving rise to a near-infrared fluorescence "turn-on" signal and chromogenic effect. The chemical structure of **NB-DN** was fully characterized by $^1$H NMR, $^{13}$C NMR and HR-MS (Figs. S6–8).

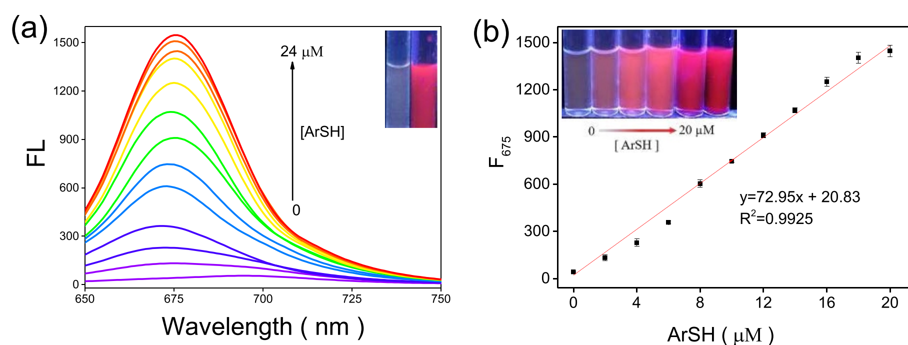
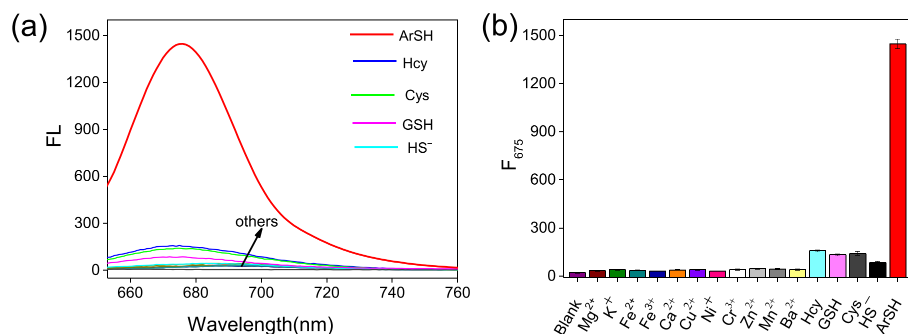



**Fig. 2.** (a) Fluorescence response of **NB-DN** (10 μM) toward ArSH (0–24 μM) in EtOH/PBS (v/v = 3/2, pH = 7.4). Inset: Fluorescence images of **NB-DN** (10 μM) after addition of ArSH (24 μM). (b) The linear correlation of fluorescence intensity and the concentration of ArSH (0–20 μM). $\lambda_{ex}$ = 620 nm. Slits: 5/5 nm. Error bars are ± SD, n = 3. Inset: Photograph of **NB-DN** solutions (10 μM) in the presence of ArSH (0–20 μM) under UV irradiation (365 nm).



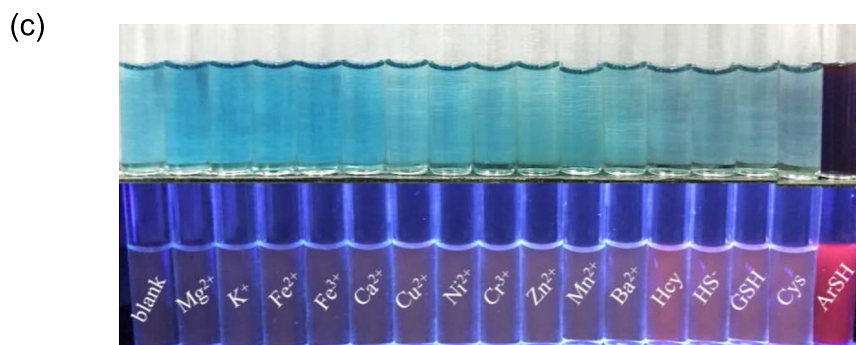




**Fig. 3.** Selectivity of **NB-DN** (10 μM) toward thiophenol and other analytes in PBS (10 mM, pH = 7.4). Metal ions (500 μM, $Mg^{2+}$, $K^+$, $Fe^{2+}$, $Fe^{3+}$, $Ca^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Cr^{3+}$, $Zn^{2+}$, $Mn^{2+}$, $Ba^{2+}$), thiols (500 μM, Hcy, NaSH, Cys, 1 mM GSH), and ArSH (24 μM). (c) Color changes (upper panel) and fluorescence (lower panel) photographs of probe **NB-DN** (10 μM) with various analytes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

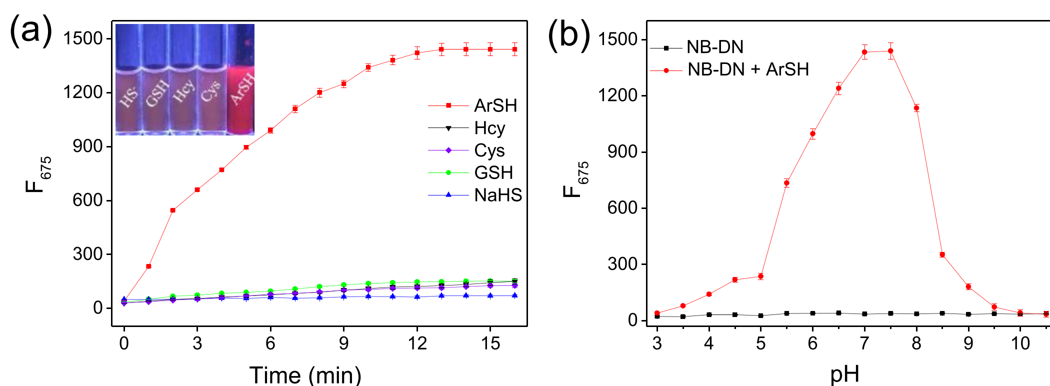



**Fig. 4.** (a) Time-coursed fluorescence responses of probe **NB-DN** (10 μM) to ArSH (24 μM), Cys (500 μM), Hcy (500 μM), NaSH (500 μM) and GSH (1 mM) in EtOH/PBS solution (v/v = 3/2, pH = 7.4), respectively. Inset: Fluorescence photographs of probe **NB-DN** (10 μM) in the presence of ArSH (24 μM), Cys (500 μM), Hcy (500 μM), GSH (1 mM) and NaHS (500 μM), respectively. (b) The pH-dependent fluorescence response of **NB-DN** (10 μM) towards ArSH (24 μM) in EtOH/PBS solution (10 mM, *v/v* = 3/2, pH = 7.4). $\lambda_{ex}$ = 620 nm, $\lambda_{em}$ = 675 nm. Slits: 5/5 nm.

### 3.2. Sensing properties of the probe NB-DN to thiophenols

In order to obtain the optimal buffer solution condition, the fluorescence response of probe **NB-DN** towards ArSH were investigated in different buffer solutions (Figs. S1–S2), the probe **NB-DN** exhibits the strongest fluorescence intensity, fastest response and good selectivity to ArSH (24 μM) in EtOH/PBS(v/v = 3/2) buffer solution, hence, the sensing properties in this work were studied in the optimized condition of EtOH/PBS(v/v = 3/2). As shown in Fig. 1, the **NB-DN** solution (10 μM) displayed a maximum absorption peak at 660 nm. This absorption peak gradually blue-shifted to 634 nm after incubation with thiophenol (24 μM) for 12 min, thus the color of **NB-DN** solution became into deep blue with significant chromogenic effect, providing a visual manner to determine thiophenol. Besides, **NB-DN** is nearly nonfluorescent in the buffer solution due to PET from **DN** to Nile blue. Upon the addition of thiophenol, an obvious fluorescence band appeared at 675 nm (Fig. 2a), which gradually enhanced with the concentration of ArSH increased from 0 to 24 μM and finally reached to the maximum value (400-fold enhancement). As a result, the fluorescence of the probe changed from colorless to red (Fig. 2b inset). Notably, the fluorescence intensity at 675 nm is linearly correlated to the concentrations of ArSH (0–20 μM) in PBS solution with low detection limit (1.8 nM), which is superior to most reported fluorescent probes for ArSH (Table S1). Therefore, the probe can be employed to quantitatively determine ArSH in the environment.

Then, we evaluate the selectivity for ArSH by examining the fluorescence response of **NB-DN** toward various cations (500 μM), anions (500 μM), some important biomolecules, thiophenols (24 μM) and aliphatic thiols Cys, Hcy (500 μM) and GSH (1 mM). As shown in Fig. 3 and Fig. S3, ArSH, *p*-F-$C_6H_4SH$, *p*-$CH_3$-$C_6H_4SH$ and 2-aminobenzenethiol lead to large fluorescence enhancements (27.9–46.1 fold) of the **NB-DN** at 675 nm, whereas the addition of Cys, Hcy, NaHS (500 μM) and GSH (1 mM) cause small fluorescence intensity enhancement. Besides, the probe did not exhibit any fluorescence enhancement after addition of cations ($K^+$, $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, $Fe^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Ba^{2+}$, $Cr^{2+}$, $Ni^{2+}$), anions ($F^-$, $Cl^-$, $Br^-$, $SO_4^{2-}$, $HSO_3^-$, $NO_2^-$, $NO_3^-$, $S_2O_3^{2-}$, $CH_3COO^-$, $CO_3^{2-}$, DTT, $ClO^-$, $N_3^-$, $SO_3^{2-}$), $H_2O_2$ and some important biomolecules. By contrast, **NB-DN** showed obvious chromogenic effect and fluorescence enhancement in the presence of ArSH (Fig. 3c and Fig. S3c). The result demonstrated that **NB-DN** is a highly selective fluorescent probe for thiophenols (especially for distinguishing thiophenols from aliphatic thiols/mercaptans) in aqueous solution.

### 3.3. Time-coursed and pH-dependent fluorescence response

The time-coursed fluorescence response of **NB-DN** toward ArSH, GSH, Cys, Hcy, and NaHS at 37 °C were investigated, respectively. The fluorescence intensity ($F_{675}$) of **NB-DN** increased to a plateau within 12 min after addition of ArSH (Fig. 4a), while Cys, Hcy, GSH, and NaHS caused much less fluorescence enhancement even at high concentrations (500–1000 μM). Moreover, the reaction kinetic constant of ArSH was much larger than that of biothiols and NaHS (Fig. S4), which was due to relatively low local softness ($S_{sulfur-}$) value of thiophenols [21,48] Therefore, **NB-DN** could rapidly and discriminatively determine ArSH from biothiols.

For the purpose of practical application, we also investigated the pH-dependent fluorescence sensing property of probe **NB-DN**. As shown in Fig. 4b, the fluorescence intensity of probe **NB-DN** is independent with the pH changes from 3 to 10.5. Upon the addition of thiophenol (24 μM), a noticeable fluorescence enhancement was observed under

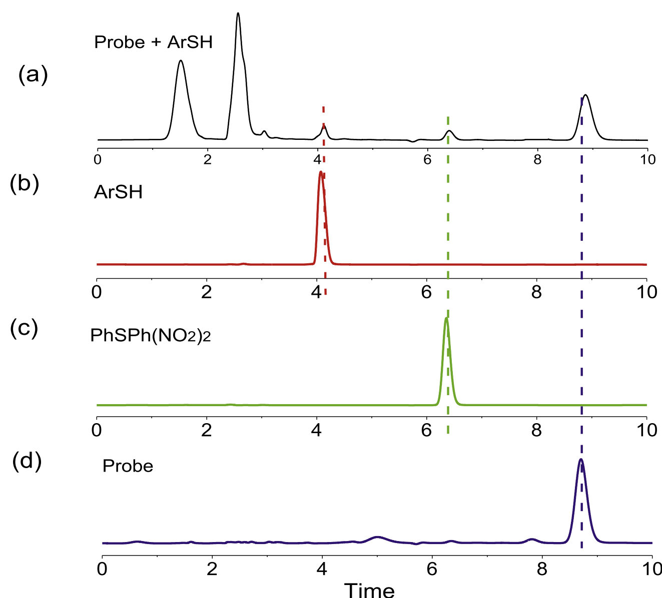
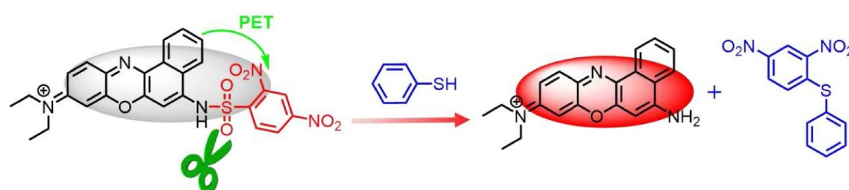



**Fig. 5.** HPLC profiles of (a) reaction mixture of probe (10 μM) with ArSH (10 μM), (b) ArSH (10 μM), (c) $PhSPh(NO_2)_2$ (10 μM), (d) probe. Eluent, methanol/$H_2O$ (v/v = 85/15), flow rate: 0.7 mL/min; detection wavelength: 254 nm; injection volume: 10 μL.




**Scheme 2.** Sensing mechanism of the fluorescent probe **NB-DN**.

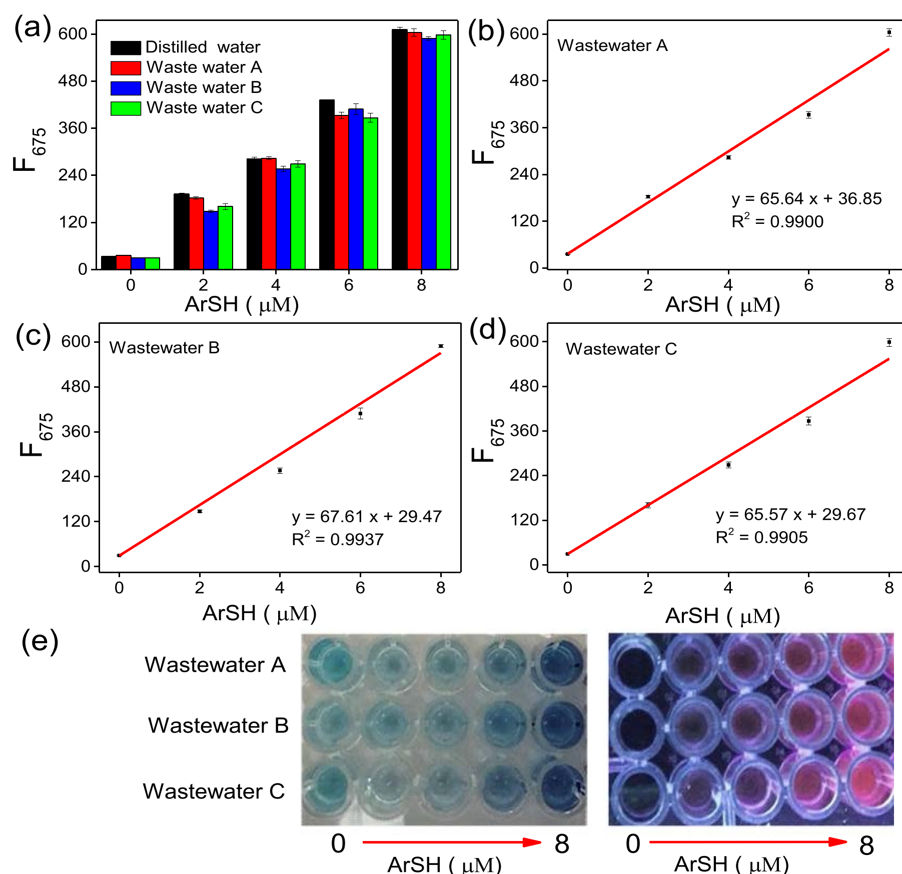



**Fig. 6.** Fluorescence intensity of **NB-DN** (10 μM) in distilled water, industrial Wastewater A, Wastewater B and Wastewater C spiked with various concentrations of ArSH (0, 2, 4, 6, and 8 μM). $\lambda_{ex}$ = 620 nm; Slits: 5/5 nm. (b–d) Linear plot of fluorescence intensity of **NB-DN** against the spiked concentrations of ArSH from 0 to 8 μM for each real water sample. Error bars are ± SD, n = 3. (e) Color changes (left) and fluorescence (right) photographs of probe **NB-DN** (10 μM) in the industrial Wastewater A, Wastewater B and Wastewater C spiked with various concentrations of ArSH (0 − 8 μM). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the pH 3–8, demonstrating that **NB-DN** might be utilized to determine thiophenols at a wide range of pH values.

### 3.4. *Proposed reaction mechanism of* ***NB-DN*** *toward thiophenol*

To explore the reaction mechanism, we analyzed the reaction products of probe **NB-DN** and thiophenol by HPLC and HR-MS. In the HPLC profile (Fig. 5), probe **NB-DN**, ArSH and $ArSAr(NO_2)_2$ showed retention time at 8.8 min, 4.1 min and 6.3 min, respectively. After **NB-DN** reacted with ArSH (Fig. 5a), a new peak at 6.3 min appeared, indicating that $ArSAr(NO_2)_2$ is one of the products. In addition, a predominant peak at $m/z$ = 318.3020 was observed from the HR-MS spectra of the mixture of **NB-DN** and ArSH (Fig. S5), which was identified as Nile blue. Based on the HPLC and HR-MS results, the sensing mechanism could be proposed as following (shown in Scheme 2): ArSH reacted with **NB-DN** through a nucleophilic substitution reaction ($SN_{Ar}$) to remove the DN group, affording $ArSAr(NO_2)_2$ and Nile blue. As a result, the probe exhibited a significant fluorescence “turn-on” response to ArSH.

**Table 1**
Determination of thiophenol in real water samples.

| Sample | ArSH Spiked (μM) | ArSH Recovered (μM) | Recovery (%) |
|---|---|---|---|
| Wastewater A | 0 | not detected | |
| | 2 | 2.20 ± 0.05 | 110 |
| | 4 | 3.76 ± 0.06 | 94 |
| | 6 | 5.40 ± 0.06 | 90 |
| | 8 | 8.51 ± 0.11 | 106 |
| Wastewater B | 0 | not detected | |
| | 2 | 1.98 ± 0.04 | 99 |
| | 4 | 3.56 ± 0.09 | 90 |
| | 6 | 5.92 ± 0.04 | 99 |
| | 8 | 8.27 ± 0.15 | 103 |
| Wastewater C | 0 | not detected | |
| | 2 | 1.99 ± 0.09 | 100 |
| | 4 | 3.71 ± 0.10 | 93 |
| | 6 | 5.44 ± 0.14 | 91 |
| | 8 | 8.53 ± 0.15 | 107 |

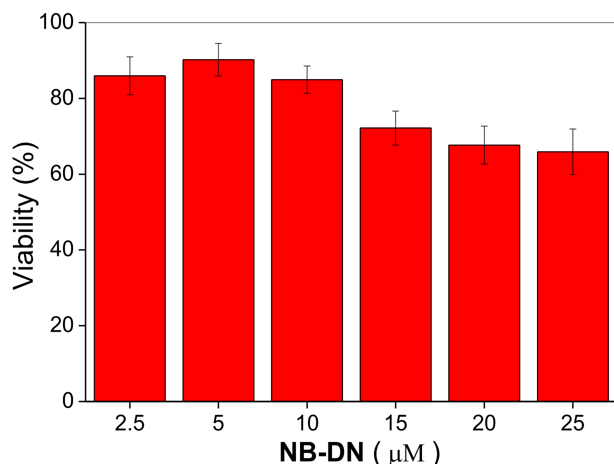



**Fig. 7.** Cell viability of HeLa cells after incubation with various concentrations of **NB-DN** (0–25 μM) for 24 h. Results are mean ± SD, n = 5.

### 3.5. Detection of thiophenols in environmental water samples

As its high toxicity for human being and environment, it is indispensable to monitor ArSH in the environment, especially in the discharged industrial wastewater. Herein, **NB-DN** was employed to determine thiophenol in environmental water samples. Three industrial wastewater samples were collected from the neighboring drains of Tianjin Petrochemical Company (Wastewater A), Fine Chemical Company (Wastewater B) and Jinkang Pharmaceutical Co., Ltd. (Wastewater C) of Tianjin, China. All water samples were first filtrated with filter membrane and the pH value was adjusted to 7.4. These water samples were spiked with different concentrations of ArSH (0, 2, 4, 6, 8 μM), and the fluorescence spectra of **NB-DN** in these water samples were measured with double-distilled water as the control. As shown in Fig. 6a, no obvious fluorescence enhancement was observed when **NB-DN** was added directly to the water samples, indicating no obvious ArSH pollution in these three wastewater samples. When these water samples were spiked with ArSH, the fluorescence intensity ($F_{675}$) increased linearly (Fig. 6b–d) with the spiked concentrations of ArSH (0–8 μM), and the recoveries were found to be 90%–110% (Table 1). Besides, the color and fluorescence of the solutions show obvious changes (Fig. 6e) in the presence of various concentrations of thiophenol. These results indicate that **NB-DN** can be employed as a reliable and visual analysis tool for determination of ArSH in real water samples.

### 3.6. Cytotoxicity of NB-DN and cellular imaging of thiolphenol

Prior to cell imaging experiment, the cytotoxicity of **NB-DN** (2.5, 5, 10, 15, 20, 25 μM) in HeLa cells was evaluated using a CCK-8 assay. As shown in Fig. 7, the cell viability remained above 85% after incubation with 10 μM **NB-DN** for 24 h, demonstrating that probe **NB-DN** has low cytotoxicity. With these features in mind, we then employed the probe **NB-DN** to image ArSH in living cells. HeLa cells were incubated with **NB-DN** (10 μM) for 30 min and then washed thrice with PBS for fluorescence imaging. As shown in Fig. 8, obscure red fluorescence was observed from the red channel after incubation with **NB-DN** (10 μM). These cells were further incubated with different amounts of thiophenol (0, 20, 40, 80 μM) for 20 min, and the fluorescence images were recorded. Fig. 8 showed a clear cell profile with observable red fluorescence after these HeLa cells were further treated with 20 μM ArSH, and the red fluorescence became much brighter after treatment with 40 μM ArSH. Whereas, incubation with 80 μM ArSH led to cell morphology change (membrane contract) and weakened red fluorescence, which might be due to the cytotoxicity resulted from high concentration of thiophenol. Therefore, the probe would be served as a good indicator for imaging ArSH in living cells.

## 4. Conclusions

In summary, we have developed a novel near infrared fluorescent probe **NB-DN** for the detection of thiophenols, which exhibited remarkable chromogenic reaction and NIR (675 nm) fluorescence "turn-on" response toward thiophenols with high selectivity, fast response (12 min), and an extremely low limit of detection (1.8 nM). Moreover, **NB-DN** has been successfully employed for monitoring the level of thiophenols in industrial wastewater with good recoveries (90–110%). **NB-DN** was also utilized for imaging thiophenol in HeLa cells. Therefore, this work provides a good manner for ultrasensitive determination of thiophenol in environmental water and imaging thiophenols *in vitro*.

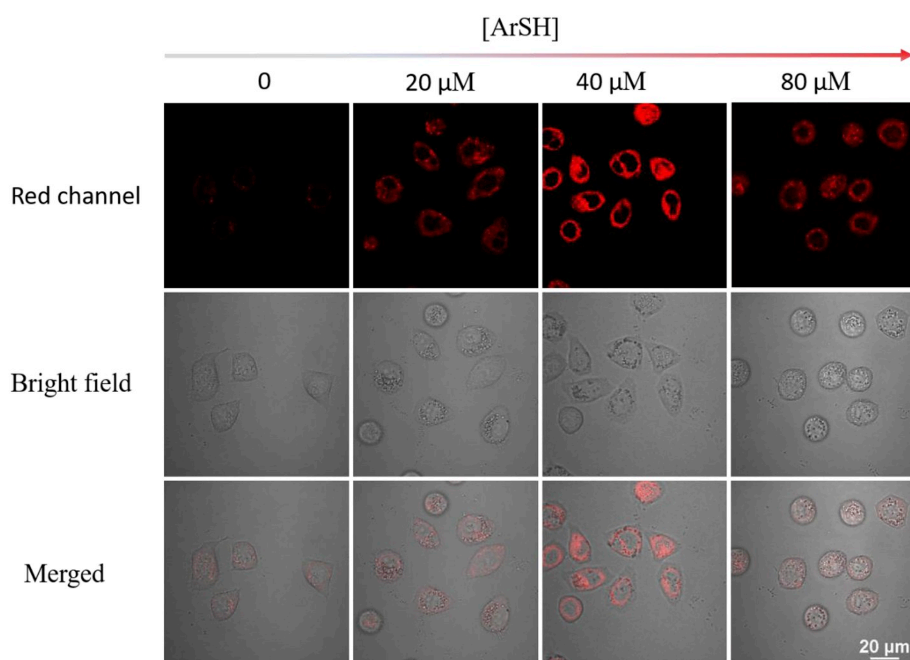



**Fig. 8.** Confocal fluorescence images of ArSH in living HeLa cells. HeLa cells were incubated with probe **NB-DN** (10 μM) at 37 °C for 30 min, and then further treated with various concentrations of ArSH (0, 20, 40, 80 μM). Fluorescence images of HeLa cells from red channel ($\lambda_{ex}$ = 638 nm, $\lambda_{em}$ = 640–705 nm). Scale bar: 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.03.113.

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