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An ortho-aldehyde modified probe to image thiols in living cells with enhanced selectivity

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ABSTRACT

Developing fluorescent probes to image thiols in the living system may provide powerful tools to study the functions of thiol-containing biological molecules. In this study, we report the design and evaluation of a novel turn-on fluorescent probe **NQNO** for selective detection of thiols in living cells. By introducing an ortho-aldehyde group to **NNO**, a conventional compound representing a class of thiol-imaging strategy, we obtained **NQNO** with enhanced selectivity for thiols over the major interferent hydrogen sulfide (H_2S). **NQNO** could be applied in phosphate-buffered saline (PBS), where the efficacy of **NNO** was usually weakened. Notably, **NQNO** demonstrated solid performance in imaging endogenous thiols in living cells without exerting cytotoxicity. In summary, **NQNO** has the potential to serve as a safe, sensitive and effective fluorescent probe for thiol imaging in biological systems.

1. Introduction

As important reactive sulfur species (RSS) in biological systems, biothiols play essential functions in regulating the oxidative stress through the equilibrium between reduced free thiols (RSH) and oxidized disulfides (RSSR) [1]. Typical endogenous biothiols include cysteine (Cys), homocysteine (Hcy) and glutathione (GSH). The concentrations of these thiols reflect the functional state of the associated enzymes and proteins, while an abnormal level of these thiols is associated with a variety of diseases such as cancer, acquired immune deficiency syndrome (AIDS), cardiovascular and Alzheimer's diseases [2–4]. Therefore, monitoring thiols in biological samples may provide important information for early detection of pathological changes. Among various detection techniques, fluorescent probes have become a powerful tool in visualizing the dynamic biological, because of its high sensitivity, non-invasive measurement and real-time imaging [5–10]. To design an appropriate probe for detecting thiols from biological samples, many strategies have been developed based on the strong nucleophilicity of the sulfhydryl group such as applying a Michael addition with maleimide group, the cleavage of S-S bonds or displacing the quenching group [11–13]. These probes have expanded our understanding of their formation and functions.

However, despite impressive progress in the development of thiol probes, challenges remain in distinguishing biothiols from other compounds in a complex biological environment. For instance, as a biological SH-containing molecule, hydrogen sulfide (H_2S) is a common interferent in real biological samples and has similar nucleophilicity with biothiols [14]. Also, H_2S is often a downstream mediator of these thiols in physiological processes. Thus, the level of H_2S can be tightly associated with that of the thiols in living systems [15]. Accurate detection of biothiols while avoid the interference from H_2S remained challenging.

Hence, we aimed to develop a novel probe for thiols over H_2S. Since recent probes for thiols mostly are based on the strong nucleophilicity of the sulfhydryl group, and since the sulfhydryl group can react with the aldehydes [16], we hypothesised that we could adding an aldehyde onto a probe and H_2S would react with the aldehyde first because of the latter's higher nucleophilicity [15]. Consequently, we modified the naphthalimides through Duff reaction to obtain a formylated product and used 2,4-dinitrophenyl as the quenching group to construct the probe **NQNO** for thiols (Scheme 1). In parallel, we synthesized the compound **NNO** as control – which not only is a conventional probe but also represents a class of strategy for thiol-imaging. Our data showed that the introduced ortho-aldehydes enabled **NQNO** to significantly resist the interference of H_2S in its selective detection of thiols, compared with **NNO**. Further, **NQNO** demonstrated a fast and sensitive imaging of thiols in living cells.

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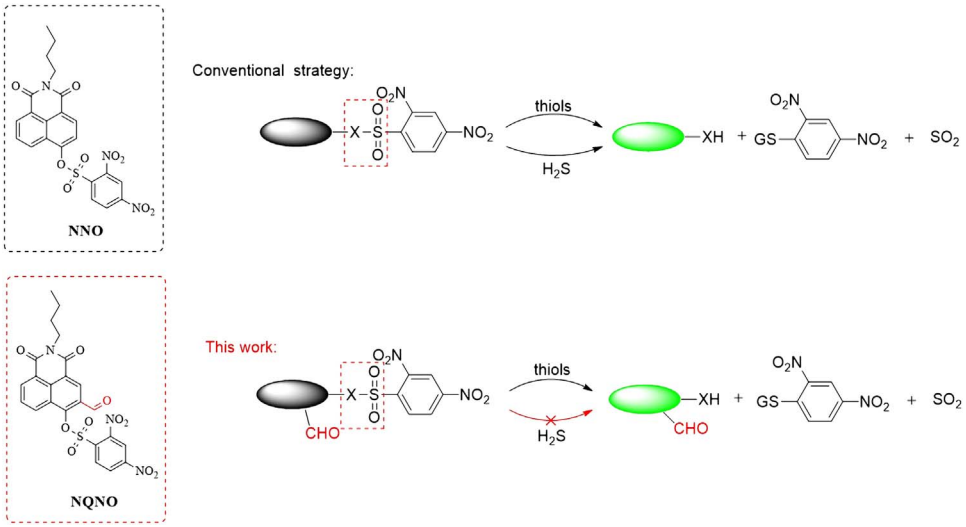

Scheme 1. The design scheme and structure of probes **NNO** and **NQNO**.

2. Experimental

2.1. Materials

All chemicals and reagents were from commercial suppliers and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 600 MHz AVANCE III spectrometer with chemical shifts reported in ppm at room temperature. Mass spectra were obtained with Thermo Scientific MSQ Plus mass spectrometer (USA). Absorption spectra were collected by using HACH DR6000 UV/VIS Spectrophotometer (USA). Fluorescence spectra were measured with a Thermo Scientific Lumina Fluorescence Spectrometer (USA).

2.2. Synthesis of NQNO and NNO

NNO and **NQNO** were synthesized according to reported literature [17]. The naphthalimides or formylated naphthalimides (1 mmol) was dissolved in 5 mL dichloromethane. Triethylamine (155 μL, 1.1 mmol) was added into the solution, stirring for 10 min. Then 2,4-Dinitrobenzenesulfonylchl (295 mg, 1.1 mmol) was added to the mixture above, stirred for another 6 h. After these, the mixture was washed with 1 M hydrochloric acid, dried over anhydrous $MgSO_4$ and removed the solvent to obtain a crude product. **NQNO** and **NNO** were prepared by silica gel column chromatography using dichloromethane/petroleum ether (2/1) as the elution.

NQNO (71% yield) ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.24 (t, 1 H), 9.01 (s, 1 H), 8.84 (d, J =7.0 Hz, 1 H), 8.78 (d, J = 7.2 Hz, 1 H), 8.70 – 8.57 (m, 2 H), 8.37 (d, J = 7.6 Hz, 1 H), 7.95 (t, 1 H), 4.25 – 4.17 (m, 2 H), 1.78 – 1.66 (m, 2 H), 1.52 – 1.42 (m, 2 H), 0.99 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 186.47, 186.42, 163.04, 162.25, 151.42, 149.81, 149.13, 134.58, 133.74, 133.54, 131.73, 130.65, 129.81, 129.38, 127.22, 126.78, 126.66, 123.25, 123.18, 121.01, 40.64, 30.10, 20.33, 13.81. HRMS (ESI): m/z calculated for $C_{23}H_{18}N_3O_{10}S^+$, 528.0713. Found 528.0655.

NNO (46% yield) ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.89 (s, 1 H), 8.85 (d, J = 7.2 Hz, 1 H), 8.69 (dd, J = 7.3 Hz, 2 H), 8.66 (dd, J = 7.3 Hz, 1 H), 8.56 (dd, J = 7.3 Hz, 1 H), 8.53 (d, J = 7.3 Hz, 1 H), 7.90 (dd, J = 7.3 Hz, 1 H), 4.16 (t, 2 H), 1.73 – 1.67 (m, 2 H), 1.48 – 1.40 (m, 2 H), 0.98 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.24, 161.96, 151.27, 151.06, 148.98, 140.67, 135.78, 133.88, 132.48, 129.16, 128.98, 128.74, 127.42, 127.05, 123.17, 123.05, 120.97, 88.76, 40.57, 30.11, 20.32, 13.81. HRMS (ESI): m/z calculated for $C_{22}H_{18}N_3O_9S^+$, 500.0764. Found 500.0779.

2.3. Fluorometric analysis

The probes were dissolved in tetrahydrofuran (THF) to obtain a 5 mM stock solution. Each analyte was dissolved in deionized water to prepare a 10 mM stock solution. Then the probe solutions were diluted in 3 mL PBS buffer (10 mM, pH 7.4) for **NQNO** or 3 mL PBS buffer (10 mM, pH 7.4) containing 10% THF for **NNO** to the required concentrations for measurements, different analytes were added. All fluorescence measurements were measured at room temperature.

2.4. Cell culture and fluorescent imaging

HeLa cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (V/V) Fetal Bovine Serum (FBS, Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO_2. Then cells were transferred to culture dishes. Cell imaging was carried out after washing the cells with PBS (10 mM, pH 7.4). Cells were imaged by confocal scanning microscopy.

3. Results and discussion

3.1. Design and synthesis

4-hydroxyl-N-butyl-1,8-naphthalimide was prepared from the starting material, 4-bromo-1,8-naphthalic anhydride, according to reported literature [18]. The formylated product was obtained through the Duff reaction that introduced the ortho-aldehyde group to the 3′ position of 4-hydroxyl-N-butyl-1,8-naphthalimide. Followed by a substitution reaction between hydroxyl groups and 2,4-dinitrobenzene sulfonyl chloride, the two probes **NNO** and **NQNO** were obtained (confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS spectroscopy, ESI).

3.2. Reaction kinetics towards thiols and H_2S

The reaction kinetics of the two probes towards thiols and H_2S were measured. As shown in Fig. 1a, upon the addition of GSH, Hcy and Cys (100 μM for each), the probe **NQNO** (5 μM) exhibited enhanced fluorescence intensity at 525 nm (excitation was 450 nm), which was attributed to the cleavage of benzenesulfonate moieties, resulting in the fluorescent release of the formylated naphthalimides (Fig. S14). And the addition of 100 μM H_2S (NaHS as the donor) brought about no significant changes. While for **NNO** (5 μM), the addition of GSH, Hcy, Cys and H_2S (100 μM for each) resulted in 4.3, 3.5, 3.3 and 2.6 folds enhancement after 30 min, respectively (Fig. 1b). As such, H_2S can also displace the benzenesulfonate moiety, leading to a significant