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Oxidized-morpholine dressing ratiometric fluorescent probe for specifically visualizing the intracellular glutathione

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ABSTRACT

A naphthalimide-based ratiometric fluorescent probe for determining glutathione (GSH) was constructed by installing two oxidized morpholine (e.g. thiomorpholine-S-dioxide and morpholine-N-oxide) components on the "off-to-on" GSH probe. This probe displayed high selectivity towards GSH. As well, the bioimaging application confirmed that this probe was capable of acting as an indicator to monitor the intracellular GSH. Thus, this work provides a promising strategy to construct the ratiometric fluorescent probe.

1. Introduction

Fluorescence bioimaging has developed to be a powerful tool for noninvasive tracking various bioactive species in living biological systems [1–7]. In recent years, numerous fluorescent probes based on organic small molecules have been reported [8]. The majority of them only displayed the change of single fluorescence signal upon the treatment of analytes and easily suffered from some environmental interferences such as solvent polarity, probe concentration, excitation intensity, instrumental efficiency and environmental conditions etc. [9] In comparison to the single emission signal, the ratiometric fluorescence could provide a built-in correction to weaken these interferences from environment and obtain an output of fluorescence signal with a higher signal-to-noise ratio [10]. Consequently, abundant efforts have devoted to the exploitation of ratiometric fluorescent probes [11].

Glutathione (GSH) as the most abundant intracellular nonprotein biothiol serves many cellular functions such as maintenance of intracellular redox equilibrium, xenobiotic metabolism, intracellular signal transduction and gene regulation [12–27]. In our recent works, we developed a type of sulfonamide-decorating naphthalimide that was able to selectively monitor the intracellular GSH along with an off-to-on signal output [28]. Probe **Np-NB** employed a sulfonamide moiety as the GSH-responsive group in Fig. 1A. Upon the treatment of GSH, the sulfonamide bond was cleaved and accompanied by a strong green emission, as a result of generating the GSH-linked product **Np-GSH**. In consideration of the demand of developing the ratiometric fluorescent probes and the reduction feature of the detected analyte GSH, we envisioned that a simple and easy-to-control oxidation approach towards probe **Np-NB** probably achieve the aim. Accordingly, we aimed to design an oxidized-morpholine decorating naphthalimide **Np-NSO$_2$-NO** on the basis of probe **Np-NB**. As presented in Fig. 1A, the benzyl group on probe **Np-NB** was firstly replaced by the thiomorpholine, and then the thiomorpholine and morpholine on the imide side were synchronously oxidized to form the target molecule **Np-NSO$_2$-NO**.

To verify the reasonability of molecular design, the time-dependent density functional theory (TD-DFT) calculations at the B3LYP/6-31G* level using the Gaussian 09 program were carried out. The absorption and the molecular orbitals containing the main electronic transitions with the largest oscillator strength of probe **Np-NSO$_2$-NO** were listed in Fig. 1B. Observations showed that the HOMO orbital was mainly assigned to the morpholine-N-oxide moiety whereas the electron density of the LUMO orbital predominantly distributed the naphthalimide scaffold. More, an

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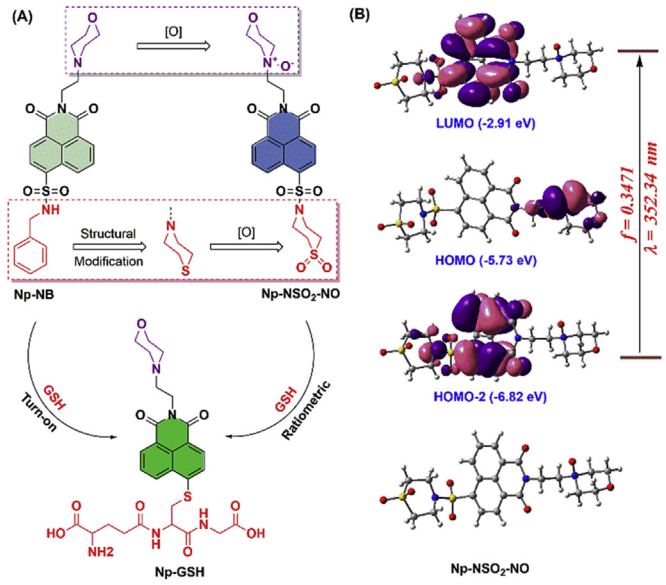

Fig. 1. (A) The design strategy and proposed sensing mechanism of probe **Np-NSO$_2$-NO** for GSH. (B) The calculated frontier molecular orbitals of **Np-NSO$_2$-NO** based on TD-DFT (B3LYP/6-31G*) calculations.

intense transition was predicted at about 352 nm with a larger oscillator strength of 0.3471 (HOMO-2 → LUMO), and the HOMO-2 was mainly delocalized the naphthalimide and extended to the sulfamide group, implying that probe **Np-NSO$_2$-NO** was fluorescent owing to a weak charge transfer.

Accordingly, we followed a general synthetic approach mentioned in our previous work [28]. The target probe **Np-NSO$_2$-NO** was obtained by treatment of 2-morpholinoethyl-4-sulfonylchloride-1, 8-naphthalimide with thiomorpholine, and then was directly oxidized by the excess *m*-chloroperoxybenzoic acid without further purification (In Scheme S1 ESI†). Its structure was well-characterized by NMR and mass spectrometry (see Supplementary data).

2. Experimental section

2.1. Materials and instruments

Unless otherwise stated in this paper, all reagents were purchased from the commercial suppliers and then utilized without further purification. And all reactions were accomplished under a nitrogen atmosphere by using the standard Schlenk techniques. ^{1}H NMR spectra were gauged with Bruker Mercury Plus 400 MHz in $CDCl_3$-d_1 with tetramethylsilane (TMS) as an internal reference, and mass spectrometer were collected on a tandem quadrupole (triple quadrupole) mass spectrometer (Waters) with ESI resource. UV/vis absorption spectra were measured with Hitachi U-3310 spectrophotometer, and fluorescence spectra admeasured on PerkinElmer-LS55 spectrophotometer.

2.2. Synthesis route

Synthesis of the compound **Np-NSO$_2$-NO**: The compound **Np-Cl** was synthesized on the basis of the procedures of our previously reported literature [28]. To a solution of the freshly prepared compound **Np-Cl** (0.9 g, 2.2 mmol) under a nitrogen atmosphere in anhydrous dichloromethane (50 mL) was added the triethylamine (1.5 mL) and thiomorpholine (0.2 mL, 2 mmol) respectively. The reaction mixture was stirred for 2 h at room temperature, and then added excess *m*-chloroperoxybenzoic acid (*m*-CPBA) (0.52 g, 3.0 mmol). After the reaction mixture stirred for another 2 h at room temperature, the residue was purified by the silica gel column chromatography (DCM: MeOH = 10: 1) to obtain a white solid **Np-NSO$_2$-NO** (190 mg, **18%**). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.90 (d, J = 12, 1H, Ar-H), 8.73 (d, J = 8, 1H, Ar-H), 8.68 (d, J = 8, 1H, Ar-H), 8.39 (d, J = 8, 1H, Ar-H), 7.96 (t, J = 16, 1H, Ar-H), 4.82 (t, J = 12, 2H, CH_2), 4.44–4.50 (m, 2H, CH_2), 3.87 (4H, CH_2), 3.79 (2H, CH_2), 3.65 (t, J = 12, 2H, CH_2), 3.38 (4H, CH_2), 3.21 (t, J = 8, 4H, CH_2). The ^{13}C NMR data were not obtained due to the poor solubility in the common D-substituted solvents. ESI-MS: m/z = 524.03 $[M+H]^+$, calculated exact mass = 523.11.

2.3. Detection

A stock solution of 1 mM **Np-NSO$_2$-NO** was prepared in HPLC grade N, *N*-Dimethylformamide (DMF) and a stock solution of 10 mM analytes was dissolved in distilled water, respectively, and used freshly. The various analytes mainly consisted of biothiols such as GSH, cysteine (Cys) and homocysteine (Hcy), biological amino acids such as alanine (Ala), tyrosine (Tyr), lysine (Lys), glutamic acid (Glu), serine (Ser), histidine (His), arginine (Arg), glycine (Gly), glutamine (Gln), phenylalanine (Phe) and methionine (Met) as well as common anions such as S^{2-}, HSO_3^-, HSO_4^-, HPO_4^{2-}, $S_2O_5^{2-}$, NO_3^-, OAc^- and Cl^-. For the typical spectral properties studies, a 3.0 mL 10 μM work solution comprising the **Np-NSO$_2$-NO** was

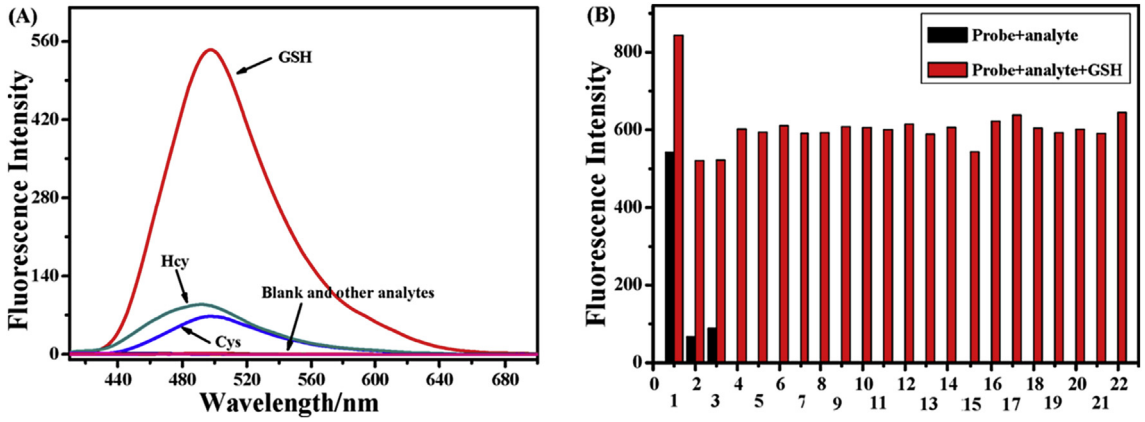

Fig. 2. (A) Fluorescence responses of **Np-NSO$_2$-NO** (10 μM) to GSH and other various analytes (100 μM) in PBS buffer solution (10 mM, pH = 7.4) containing 10% DMF. (B) Fluorescence responses of **Np-NSO$_2$-NO** (10 μM) with GSH (100 μM) in the presence of various analytes (100 μM) at 495 nm. Analytes including 1-GSH, 2-Cys, 3-Hcy, 4-Ala, 5-Tyr, 6-Lys, 7-Glu, 8-Ser, 9-His, 10-Arg, 11-Gly, 12-Gln, 13-Phe, 14-Met, 15-S^{2-}, 16-HSO_3^-, 17-HSO_4^-, 18-HPO_4^{2-}, 19-$S_2O_5^{2-}$, 20-NO_3^-, 21-OAc^-, 22-Cl^-. λ_{ex} = 390 nm, slit width: 15 × 4 nm.