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A naphthalene-based two-photon fluorescent probe for selective and sensitive detection of endogenous hypochlorous acid

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ABSTRACT

An efficient naphthalene-based two-photon fluorescent probe for endogenous HClO has been reported in the present study, which consists of a 6-(2-benzothiazolyl)-2-naphthalenol fluorophore connected with a 4-aminophenol (the fluorescence quenching and response group). This probe exhibits a high selectivity and excellent sensitivity with a detection limit of 7.6 nM over other reactive oxygen species and analyte species, and the fluorescence intensity enhanced 103-fold when responsed. Furthermore, it was successfully used for two-photon imaging of endogenous HClO in live cells with high-resolution.

1. Introduction

Hypochlorous acid and its conjugate base ($HClO/ClO^-$) are essential oxygen metabolites in living systems [1], and endogenous HClO, which is produced from the reaction of chloride ion and hydrogen peroxide catalyzed by the enzyme myeloperoxidase (MPO) in leukocytes including macrophages, monocytes and neutrophils [2], plays important roles in human immune defense system and contributes to destroying the invading bacteria and pathogens [3]. However, over production of HClO and other ROS from the mitochondrial electron transport chain leads to oxidative stress [4,5], aberrant electron transport, disruption of calcium homeostasis, activation of apoptosis and tissue damage [6–8]. Accumulation of oxidative damage over time is related to debilitating human diseases, including Alzheimer's and some related neurodegenerative diseases, as well as cardiovascular disorders and cancer [9].

Recently, many efforts have been focused on maintaining the balance of HClO between health and disease, and studying its acting mechanism in the living system [10,11]. In order to achieve this goal, several fluorescent probes have been developed for HClO detection, most of which utilized the strong oxidation property of HClO in the design, for instance, the p-methoxyphenol [12], ethers [13–15], thioether [16–18], hydrazine [19–21], oximes [22,23], hydroxamic acid [24], selenide [25] and so on. However, most of them work under one-photon excitation, which limits their biological applications. An effective alternative to circumvent the drawbacks of one-photon excitation probing is the utilization of two-photon microscopy (TPM), which has more advantageous features over one-photon microscopy including reduction of photodamage and photobleaching, better three dimensional spatial localizations, deeper penetration depth, lower tissue autofluorescence and self-absorption [26,27]. To the best of our knowledge, few two-photon fluorescent probes have been reported to date for endogenous HClO detection [28,29].

Naphthalene derivative with a donor-π-acceptor (D-π-A) structure has a large two-photon active absorption cross-section and many other excellent characteristics, such as high fluorescence quantum yield and good photo-chemostability. Thus, naphthalene derivative has been employed extensively as an efficient two-photon (TP) platform for designing TP probes for various targets [30]. In this paper, we have developed a highly sensitive naphthalene-based two-photon fluorescent probe (**Np-ClO**, Scheme 1) for endogenous HClO detection. **Np-ClO** includes a D-π-A-structured naphthalene derivative and a 4-amino-phenol ether (the fluorescence quenching and response group) moiety. Since **Np-ClO** is able to be converted into 6-(2-benzothiazolyl)-2-naphthalenol fluorophore with electron-donor hydroxyl moiety by HClO, it could be *anti*cipated that the introduction of a photoinduced electron transfer (PET) group into the naphthalene platform would break its D-π-A structure and significantly diminish the TP emission of **Np-OH**. In aqueous solution, **Np-ClO** features good fluorescence selectivity for ClO^- over other ROS, and is also capable of detecting ClO^- production in the living cells with satisfactory results.

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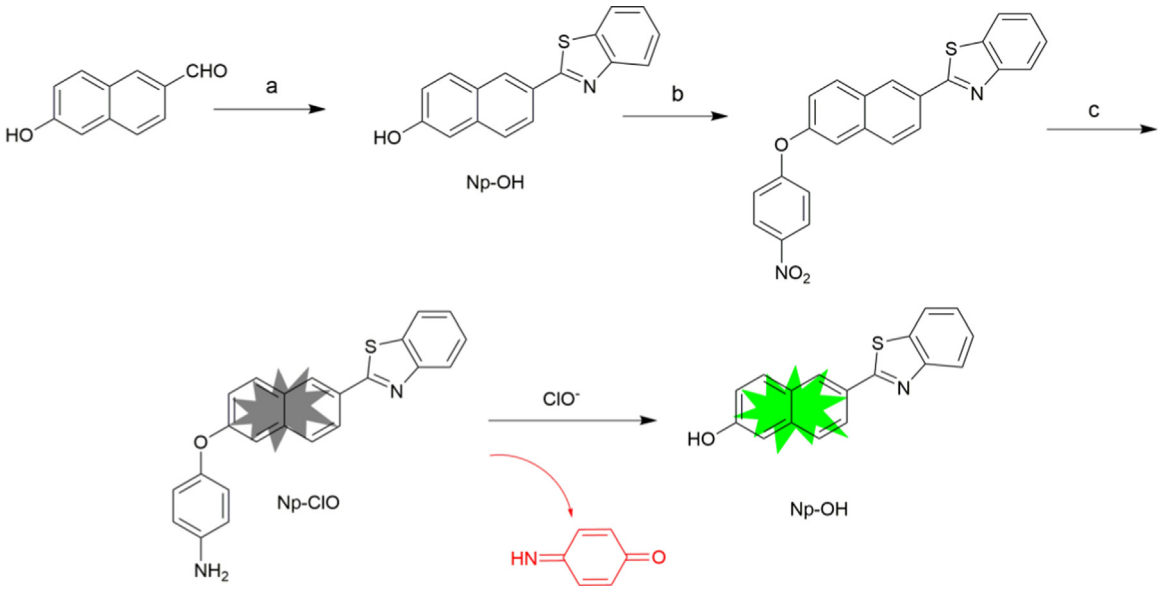

Scheme 1. Synthetic route for the two-photon fluorescent probe **Np-ClO** and the response mechanism for ClO^- (a. R: p-$MeC_6H_4SO_3H$, S: EtOH, 24 h, reflux, b. R: 1-fluoro-4-nitrobenzene, K_2CO_3, S: Dimethylformamide, 24 h, 70 °C, c. R: Fe, S: AcOH, 10 h, reflux.).

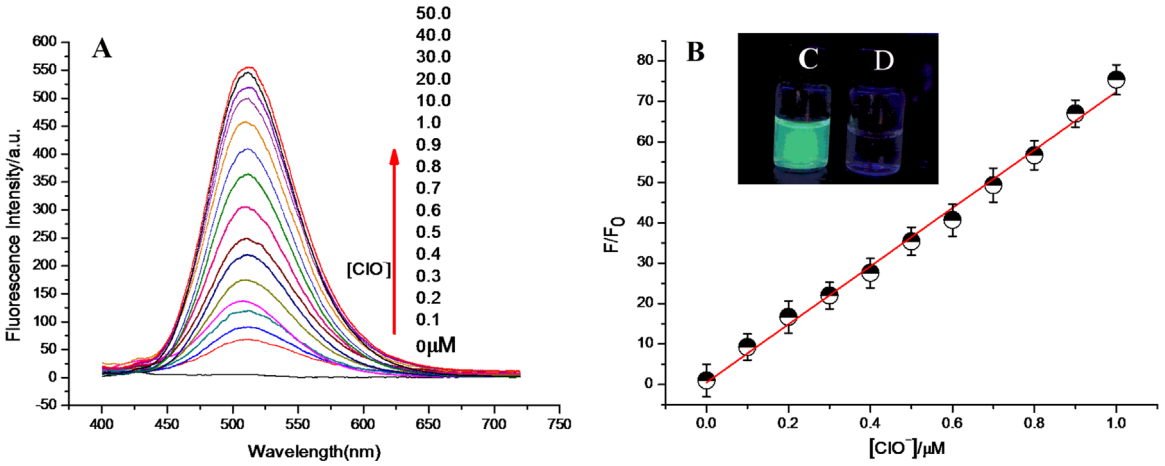

Fig. 1. (A) Fluorescence emission spectra of **Np-ClO** (1 μM) in PBS buffer (10 mM) at different ClO^- concentrations (0-50 μM); (B) Calibration curve of **Np-ClO** to ClO^- concentrations (0-1.0 μM); (C and D) Change in the fluorescence of the probe after and before the addition of 50 μM ClO^- in 1 μM of **Np-ClO** under excitation by 365 nm UV.

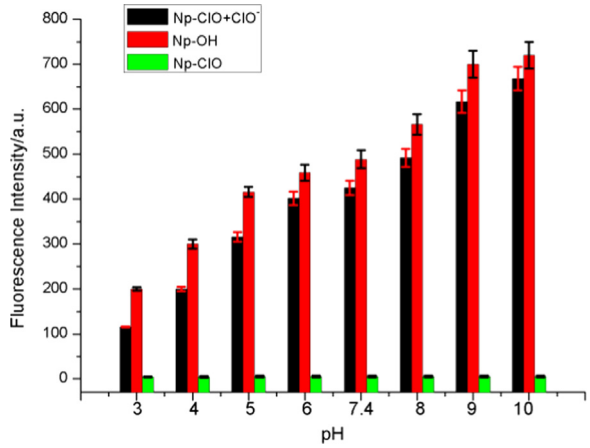

Fig. 2. The fluorescent emission intensities at 508 nm of **Np-ClO** (1 μM), **Np-OH** (1 μM) and **Np-ClO** (1 μM) in the absence or presence of ClO^- (40 μM) at various pH values, λ_{ex}=372 nm.

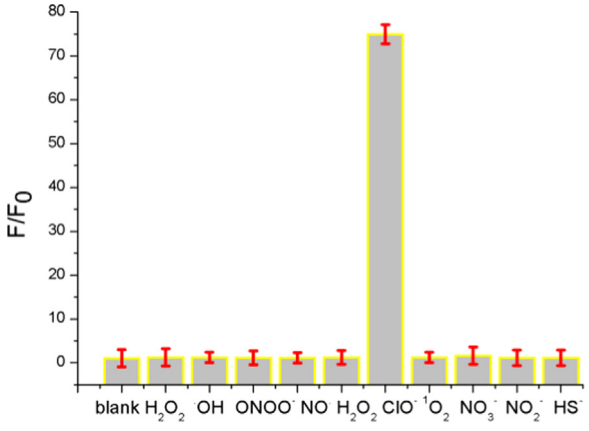

Fig. 3. The fluorescence intensity of probe **Np-ClO** (1 μM) to various analytes (blank, 50 μM .OH, 50 μM $ONOO^-$, 50 μM O_2^-, 50 μM H_2O_2, 50 μM $^1O_2$, 5 μM ClO^-, 50 μM NO_3^-, 50 μM NO_2^- and 50 μM HS^-) in PBS buffer (10 mM, 1% DMSO, pH 7.4), λ_{ex}=372 nm.

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