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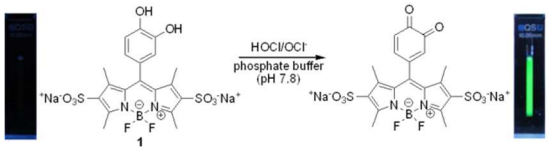
Graphical Abstract



A new, water-soluble BODIPY dye **1**, bearing sulfonate groups at the 2- and 6-positions was found to be a sensitive and selective fluorescent probe for the detection of HOCl/OCl$^-$ in aqueous buffer solution. The probe, which displays extremely weak fluorescence owing to efficient singlet excited state quenching by photoinduced electron transfer (PeT) from an electron-rich catechol group at a *meso*-position, responds to HOCl/OCl$^-$ through a dramatic enhancement of its fluorescence intensity.

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A Water-Soluble Sulfonate-BODIPY Based Fluorescent Probe for Selective Detection of HOCl/OCl$^-$ in Aqueous Media

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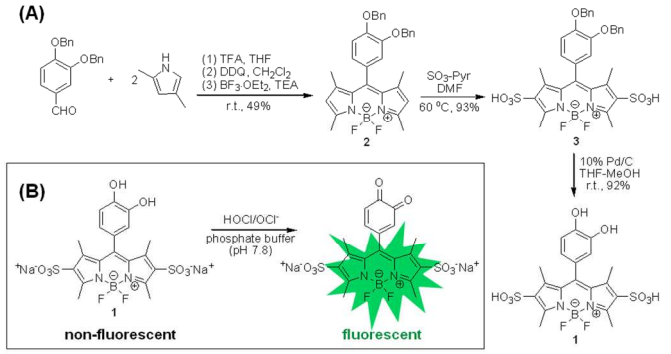
A new, water-soluble BODIPY dye 1, bearing sulfonate groups at the 2- and 6-positions was found to be a sensitive and selective fluorescent probe for the detection of HOCl/OCl$^-$ in aqueous buffer solution. The probe, which displays extremely weak fluorescence owing to efficient singlet excited state quenching by photoinduced electron transfer (PeT) from an electron-rich catechol group at a *meso*-position, responds to HOCl/OCl$^-$ through a dramatic enhancement of its fluorescence intensity.

Hypochlorous acid/hypochlorite (HOCl/OCl$^-$, pK_a 7.53) is a powerful oxidant employed in various organic transformations[1] and is used as a disinfectant, deodorant, and bleaching agent.[2] In living organism, HOCl/OCl$^-$ is formed by myeloperoxidase (MPO)-catalysed reaction of hydrogen peroxide (H_2O_2) with chloride ions (Cl^-), and it is abundantly present in activated neutrophils where it plays a crucial role in the human immune defence system.[3] In spite of the positive physiological role it plays in fighting microbial infection, HOCl/OCl$^-$ can have detrimental effects on host molecules by acting as an oxidant or an electrophilic halide donor, which bring about chemical modification of various biomolecules including proteins, membrane lipids, and nucleic acids.[4] Overproduction of these species leads to oxidative and/or chlorinative stress, which are closely linked with the induction of several diseases, including atherogenesis, cardiovascular disease, rheumatoid arthritis, neurodegenerative diseases, and cancer.[5] In addition, HOCl/OCl$^-$, which is a by-product of drinking water disinfection with chlorine, may cause anemia and nervous system problems.[6]

Because of these features, a great interest has arisen in the development of effective methods for accurate detection and quantification of HOCl/OCl$^-$ in both living systems and the environment. Fluorescence based approaches for this purpose are considered to be the most advantageous because of their high sensitivities, simplicities, and relatively low costs. As a result, a large number of fluorescent probes for the detection of HOCl/OCl$^-$ have been devised over the past decade.[7] Some of these probes utilize BODIPY,[8] fluorescein,[9] and rhodamine derivatives[10] as emitting species, and take advantage of the strong oxidative ability of HOCl/OCl$^-$ to modulate the fluorescence properties of these fluorophores. However, although these probes selectively distinguish HOCl/OCl$^-$ from other reactive oxygen species (ROS) in living organism, most operate only in aqueous media containing organic co-solvents and/or surfactants. Other drawbacks of these neutral organic probes are their tendency to form nonfluorescent dimers and higher aggregates or association complexes with hydrophobic biomolecules. These phenomena limit their use in both biological and environmental applications. Therefore, the availability of water-soluble HOCl/OCl$^-$ probes whose fluorescence properties are not perturbed in aqueous media would be highly advantageous. In the investigation described below, we have developed a water-soluble probe (**1**) of this type, which employs a catechol moiety as a redox-responsive receptor group for selective fluorescence 'turn-on' detection of HOCl/OCl$^-$ in aqueous media.

We envisioned that transformation of a catechol to benzoquinone group under oxidation conditions would serve as the foundation for a fluorescence-based HOCl/OCl$^-$ probe. BODIPY was selected as the fluorophore in this probe because it possesses absorption maxima in the visible region with high absorption coefficients and emission maxima associated with high fluorescence quantum yields.[11] In addition, BODIPY derivatives typically have elevated photostabilities, and an ability to have their photophysical properties readily modulated by proper substitution. In Scheme 1 is shown the structure of the BODIPY-based probe **1**, designed on the basis of these features, and the mechanism by which it is proposed to sense HOCl/OCl$^-$. In order to achieve the desired water solubility and

to block detrimental aggregation in aqueous media, two sulfonate groups, inspired by the work of Boyer et al.,[12] are introduced at the 2- and 6-positions of the hydrophobic BODIPY ring system. The turn-on emission signaling mechanism of this probe utilizes the pendant catechol moiety as an electron transfer donor. Specifically, the catechol moiety in **1** has a higher HOMO energy than does the BODIPY moiety.[13] Consequently, emission from the BODIPY fluorophore should be efficiently quenched by reductive PeT from the catechol donor. Furthermore, HOCl/OCl$^-$ promoted oxidation of the catechol group in **1** will generate a corresponding benzoquinone moiety whose HOMO energy is significantly lower than that of the BODIPY fluorophore. As a result, this transformation will suppress the PeT quenching process and result in enhanced fluorescence from the BODIPY moiety. The design described above should enable the new probe to selectively monitor HOCl/OCl$^-$ through a fluorescence intensity increase that depends on the concentrations of this oxidant.



Scheme 1. (A) Synthesis and (B) proposed sensing mechanism of probe **1**.

The sulfonate containing BODIPY probe **1** was prepared by using the simple three-step route displayed in Scheme 1 (See ESI†). Condensation of 2,4-dimethylpyrrole and 3,4-dibenzyloxybenzaldehyde followed by electrophilic sulfonation with sulfur trioxide-pyridine complex and removal of the benzyl groups gave probe **1**. The sulfonate groups in **1** provide water solubility and reduce the aggregation tendency often seen in BODIPY dyes in aqueous solution (Fig. S4 and S5).

The absorption and fluorescence spectra of **1** in phosphate buffer (10 mM, pH 7.8) contain a sharp absorption band centered at 497 nm with high molar absorption coefficient (ε = 6.8 × 10^4 M^{-1}·cm^{-1}) and an emission maximum at 519 nm, respectively. Although observable, fluorescence from **1** is extremely inefficient (Φ_{FL} = 0.001), a phenomenon that is attributed to exergonic PeT process from the pendant catechol donor to the excited BODIPY fluorophore.

The fluorescence response of **1** to HOCl/OCl$^-$ in phosphate buffer (10 mM, pH 7.8, 25 °C) was investigated by monitoring changes in both the absorption and emission spectra. As the spectra included in Fig. 1 show, addition of 4 equiv of HOCl/OCl$^-$ to a solution of **1** induces slight bathochromic shifts of the dominant absorption and emission bands to 506 nm and 525 nm, respectively. A concomitant, up to *ca*. 80-fold increase in the fluorescence intensity at 525 nm takes place in a time dependent manner (Fig. 1B, inset). This result suggests that, as expected, the PeT quenching process in probe **1** is attenuated by HOCl/OCl$^-$ mediated oxidation.

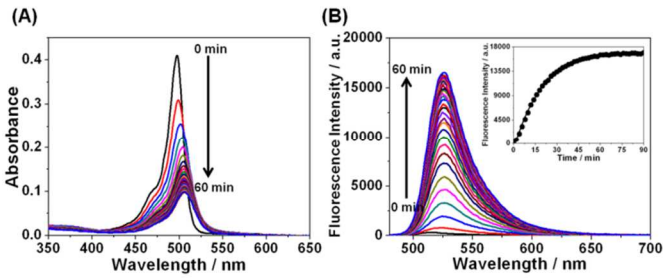


Fig. 1 (A) Absorption and (B) emission (excitation at 460 nm) spectra of **1** (10 μM) in phosphate buffer (10 mM, pH 7.8) upon treatment with NaOCl (40 μM) for different time periods (0 - 60 min) at 25 °C. Spectra were obtained every 2 min. Inset: A plot of fluorescence intensity at 525 nm as a function of incubation time.

In addition, the intensity of the fluorescence band at 525 nm increases upon exposure of probe **1** to HOCl/OCl$^-$ in a linear (correlation coefficient of 0.997) concentration dependent manner in the range of 2-20 μM (Fig. 2). This finding indicates that the probe can be utilized to detect HOCl/OCl$^-$ quantitatively. The detection limit was found to be 0.3 μM (S/N = 3). Owing to its nonfluorescent nature and significant fluorescence turn-on response, HOCl/OCl$^-$ detection by **1** can be clearly visualized using the naked eye (Fig. 2A inset). The pseudo-first order rate constant (k_{obs}) for the oxidation reaction, determined by treatment of **1** (1 μM) with HOCl/OCl$^-$ (100 μM) in 10 mM phosphate buffer (pH 7.8) at 25 °C (Fig. S15), was found to be 0.11 min^{-1}, which corresponds to a second order rate constant (k) of *ca* 2770 M^{-1}·min^{-1}.

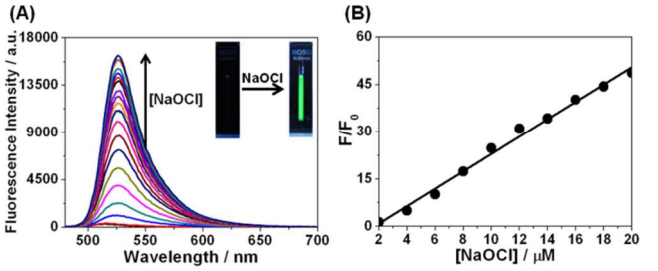


Fig. 2 (A) Fluorescence spectra (excitation at 460 nm) of **1** (10 μM) upon addition of HOCl/OCl$^-$ at different concentrations (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 μM) in phosphate buffer (10 mM, pH 7.8) at 25 °C. Each spectrum was obtained 60 min after incubation with HOCl/OCl$^-$. Inset: Photographs show fluorescence change of **1** in the absence (left) and presence (right) of HOCl/OCl$^-$ (40 μM) after incubation for 60 min under UV irradiation (365 nm). (B) A linear relationship between fluorescence intensity at 525 nm and concentrations of HOCl/OCl$^-$ (2 -20 μM). F_0 and F correspond to the fluorescence intensity of **1** in the absence and presence of HOCl/OCl$^-$, respectively. The emission intensity at 525 nm was determined after incubation at 25 °C for 60 min in presence of HOCl/OCl$^-$.

Next, the selectivity of probe **1** was evaluated by inspecting fluorescence changes that take place 1 h after addition of various reactive oxygen species (ROS) in phosphate buffer (10 mM, pH 7.8, 25 °C). Inspection of the graph given in Fig. 3

shows that **1** displays a positive fluorescence response to $HOCl/OCl^-$ and peroxynitrite ($ONOO^-$), and that little or no enhancement in its emission intensity at 525 nm occurs upon addition of other reactive species such as *tert*-butyl hydroperoxide (TBHP), hydroxyl radical (·OH), hydrogen peroxide (H_2O_2), *tert*-butoxy radical (·O^{t}Bu), and superoxide ($\cdot O_2^-$). Importantly, the response of **1** is seven times more sensitive to $HOCl/OCl^-$ than to $ONOO^-$. This selectivity is believed to be a consequence of the higher oxidation strength (more rapid oxidation rate) of $HOCl/OCl^-$ compared to other ROS.

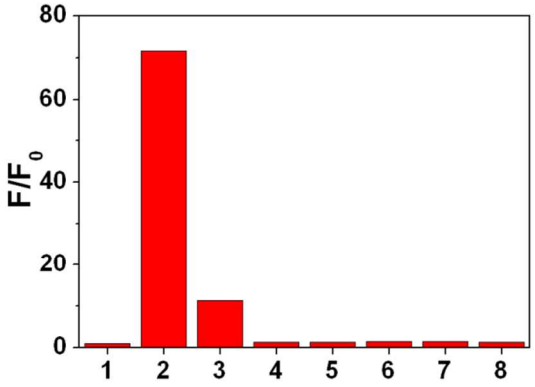


Fig. 3 Relative fluorescence responses of **1** (10 μM) to various ROS (40 μM $HOCl/OCl^-$, 40 μM $ONOO^-$, and 100 μM for others) in phosphate buffer (10 mM, pH 7.8) at 25 °C. All data were obtained after incubation with each analyte for 60 min. The intensities of the emission signals were determined at 525 nm (excited at 460 nm). 1: only probe **1**, 2: $HOCl/OCl^-$, 3: $ONOO^-$, 4: TBHP, 5: H_2O_2, 6: $\cdot O_2^-$, 7: ·OH, 8: ·O^{t}Bu. Bars represent the final (F) over the initial (F_0) integrated emission.

To identify the product formed in the process that produces the fluorogenic response, 1 mg of **1** was oxidized with NaOCl in water. The structure of the highly fluorescent major product was determined by using LC/MS (Fig. S16). The mass spectrum of the product contains a peak found at 547 (m/z) $[M]^-$ that corresponds to the chloroquinone containing BODIPY ion. This observation suggests that the increase in emission intensity brought about by exposure of **1** to $HOCl/OCl^-$ is caused by the fact that the electron-deficient chlorinated *o*-quinone moiety formed in this process participates less efficiently in PeT quenching of the excited BODIPY fluorophore. It is important to note that HOCl mediated oxidative conversion of 1,2-dihydroxybenzene to the corresponding halogenated *o*-quinones was reported earlier by Bull et. al.[14]

Photochemical and chemical stabilities are critical requirements of the new probe, ensuring that it does not undergo spontaneous oxidation in aqueous media. A photostability study was performed by carrying out a continuous irradiation (λ_{ex} = 460 nm) of **1** (10 μM) under the aerobic assay conditions using a 150 W steady-state Xe lamp while simultaneously monitoring the fluorescence spectrum (Fig. S6). The results show that the intensity of emission from **1** remains constant over a 60 min irradiation period (F/F_0 = 1.78). In addition, the chemical stability of the probe is demonstrated by the observation that negligible changes in fluorescence spectra take place when **1** is incubated in buffers with pH values ranging from 3-9 at 25 °C for 60 min. In contrast, the fluorescence producing reaction of **1** is highly pH-dependent, having a maximum rate in the neutral or slightly alkaline pH range (Fig. 4) The stability of probe **1** over this broad pH range, coupled with its high sensitivity and selectivity, suggests that it is suitable for the investigation of biological events.

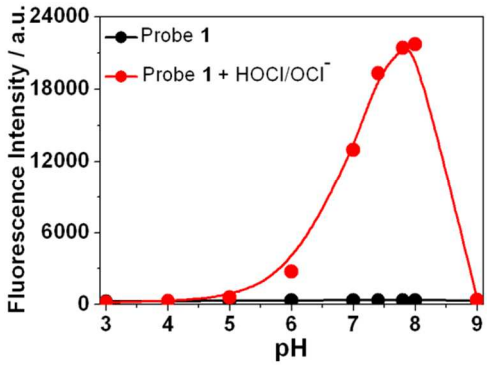


Fig. 4 Relative fluorescence intensities at 525 nm of **1** (excitation at 460 nm) in the absence (black) and presence (red) of $HOCl/OCl^-$ (Incubation time = 60 min) under different pH conditions (25 °C). [**1**]= 10 μM. [$HOCl/OCl^-$] = 40 μM.

In order to evaluate whether the photophysical properties of **1** are perturbed by interactions with macromolecules and surfaces in cellular environments, its absorption and emission spectra in the presence of bovine serum albumin (BSA) were measured (Fig. S13). Addition of 0.5% BSA to a solution of **1** in phosphate buffer (10 mM, pH 7.8, 25 °C) does not induce any spectral changes, indicating that no interference would arise by its association with hydrophobic biomolecules when **1** is employed as a biosensor.[15]

In summary, the results described above show that the BODIPY-based fluorescent probe **1** is a selective and sensitive sensor for $HOCl/OCl^-$ in aqueous buffer solution. Probe **1** possesses excellent water solubility and displays a selective "off−on" fluorescence-based response when reacted with HOCl in aqueous solution.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details and characterization and additional absorption and emission spectra. See DOI: 10.1039/c000000x/

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15 In the presence of BSA, the less significant fluorescence responses of probe **1** upon reaction with HOCl/OCl^- were observed, which might be due to high reactivity of HOCl/OCl^- with thiols and amines of BSA.

