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An ICT Based Ultraselective and Sensitive Fluorescent Probe for Detection of HClO in Living Cells

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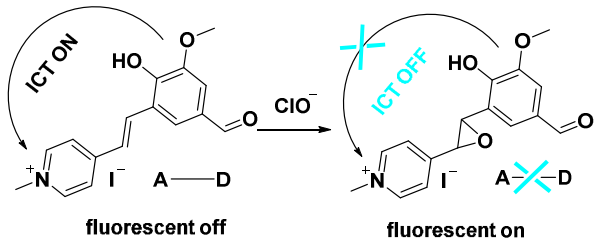
An ICT based ultraselective and sensitive probe for colorimetric and fluorescent detection of HClO *via* oxidative cleavage of an alkene linker to epoxide and then to aldehydes was developed through the conjugation of pyridinium with vanilline.

Hypochlorite (ClO^-) and its protonated form (HClO) as strong oxidizing agents are widely employed in various organic syntheses, disinfectants and bleaches.[1–3] In biological system, hypochlorous acid which is produced from peroxidation of chloride ions with the catalysis of heme enzyme myeloperoxidase (MPO) acts as an essential antimicrobial agent in organisms.[4] However, excess HClO can cause tissue damage and diseases such as hepatic ischemia-reperfusion injury, atherosclerosis, lung injury, rheumatoid and cardiovascular diseases, neuron degeneration, arthritis, and cancer.[5–9] Because of the biological and environmental importance of hypochlorous acid, chemists have made great efforts to measure the content of HClO as accurate as possible. Anteriorly, instrumental methods including potentiometry, polarography and coulometric have been applied in the hypochlorous acid detect.[10–12] Recently, optical probes have attracted significant interest because of its advantages such as high sensitivity and selectivity, speed, friendliness compared with traditional instrumental methods.

Up to now, the reported probes for HClO detection mainly include *p*-methoxyphenol oxidation to *p*-benzoquinone[13], oxime oxidtion to aldehydes[14], the chlorination of thiols and amines[15], *p*-aminophenol analog oxidation[16], thioether to sulfonate and selenide to selenoxide transformations[17, 18], the cleavage of carbon-carbon double bonds[19], oxidative processes in metal complexes and others[20–22] which have made great progresses. However, most of them might encounter the problems like poor water solubility and lineation, low quantum yield, tardy responses and interrogative selective. What's more, because of the strong oxidizability of HClO, most of the oxidation products displayed uncertain or unstable structure which obfuscated the detection mechanisms. To improve those imperfect factors, probes for HClO with sufficient properties still should be developed.

As a well know electron-withdrawing group, 1,4-dimethylpyridin-1-ium iodide moiety is always employed to improve the water solubility of the probe.[23] At the same time, systems with electron donor and acceptor moieties connected by conjugated C=C bond would generally exhibit non fluorescence through intramolecular charge transfer (ICT) process. However, the broken of the conjugation system by HClO would induce distinct fluorescent changes because of the interdiction of ICT process.[4, 24–26] With these considerations in mind, we designed and synthesized a new colorimetric and fluorescent probe with a vanillina moiety (electron donor) and methylpyridin-1-ium iodide moiety (electron acceptor) based on ICT process. This new fluorescent probe (probe **1** in Scheme 1) exhibit excellent turn-on fluorescent emission when encounter with HClO within 10s. With the characterization of the oxidative product, we confirmed the detection mechanism demonstrably. Furthermore, this probe was successfully applied in fluorescent imaging in living cells.

Scheme 1. Diagram of the Probe Design for ClO^- Detection



To exam the detection property of probe **1** towards HClO, we firstly measured the titration experiment in DMSO−HEPES buffer (3:1, v/v, pH 7.4) at 25 °C. As shown in Fig. 1a, probe **1** (50 μM) exhibited non fluorescent emission with the excitation at 373 nm in DMSO−HEPES buffer (3:1, v/v, pH 7.4). However, the addition of HClO induced a significant fluorescent emission at 468 nm gradually and further peaked with a fifty-fold fluorescent intensity increasing (Φ_f = 0.341 with quinine sulfate as reference) when the concentration of HClO reached 50 μM. The corresponding UV–vis spectral changes were also studied (Fig. S5a). The solution of 25 μM probe **1** in DMSO−HEPES buffer (3:1, v/v, pH 7.4) had two broad peaks centered at 470 nm and 345 nm. With addition of HClO (0−20

μM) to the above system, the original absorption intensity at both 470 nm and 345 nm decreased and the new peak at 269 nm increased synchronously and a well-defined isoabsorptive point was noted at 310 nm. Meanwhile, the color of the system changed from claybank to pale yellow. The ultraviolet absorption changes of the detection process mean the broken of the conjugation system. The kinetic analysis of **1** towards 10 equiv. of HClO displayed that the reaction was complete within 10 s (Fig. S6). These fast and distinct responses both in fluorescent emission and color changes promoted probe **1** to been used as a colorimetric and fluorescent probe for HClO.

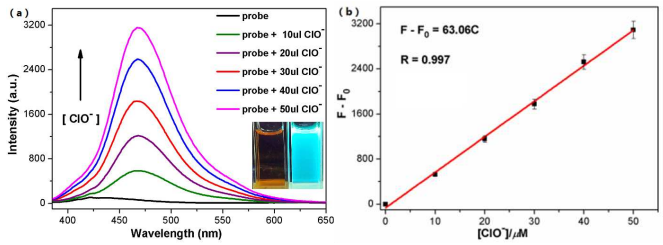


Fig. 1 (a) Fluorescent spectral changes of **1** (50 μM) upon addition of HClO (50 μM) in DMSO−HEPES buffer (3:1, v/v, pH 7.4) at 25 °C (Insert: the color of fluorescent responses of **1** towards HClO). (b) Working curve of **1** (50 μM) at 468 nm with HClO (50 μM) in DMSO−HEPES buffer (3:1, v/v, pH 7.4) at 25 °C. λ_{ex}= 373 nm, λ_{em}= 468 nm, slit: 5 nm/5 nm.

In order to investigate the sensitivity of probe **1** to HClO, the working curve was further measured upon treatment of 50 μM probe **1** with various concentrations of HClO (0−50 μM) in DMSO−HEPES buffer (3:1, v/v, pH 7.4). As shown in Fig. 1b, a linear calibration graph of the responses of the relative fluorescent intensity ($F - F_0$) at 468 nm to the HClO concentrations from 0 to 50 μM could be observed, which means that probe **1** could be potentially employed to detect HClO quantitatively. The detection limit, based on the definition by IUPAC (C_{DL} = 3 Sb/m), was found to be 0.068 μM from 10 blank solutions. This result demonstrated probe **1** to be a highly sensitive probe for HClO compared with other reported HClO chemosensors (Table 1).[27, 28, 29]

Table 1 Comparison table about the detection limits for HClO.

Method	Analyte	Solvent	Detection limit (M)
Ref. [20]	HClO	PBS−EtOH, 9:1	0.056×10^{-8}
Ref. [27]	HClO	PBS−CH_3CN, 4:1	3.6×10^{-7}
Ref. [28]	HClO	HEPES	3.5×10^{-7}
Ref. [29]	HClO	PBS	3.0×10^{-7}
Ref. [30]	HClO	PBS−EtOH, 1:1	1.6×10^{-8}
This work	HClO	HEPES−DMSO, 3:1	6.8×10^{-8}

To value the selectivity of probe **1** for HClO, various analytes including ClO^-, H_2O_2, $^1O_2$, NO, $O_2^{\bullet-}$, HO•, ClO_2^-, IO_4^-, $ONOO^-$, ROO•, CN^-, HS^-, HSO_3^- and Cys were measured in a solution of 50 μM **1** in DMSO−HEPES buffer (3:1, v/v, pH 7.4). Delightfully, even 100 equiv. of other anions and Cys did not induce any fluorescent increase (Fig. 2a) of the system except the introduction of HClO. At the same time, the competition experiments were measured to detect HClO by probe **1** with the presence of other anions and Cys. As shown in Figure 2b, all the competing anions did not interfere the detection of HClO. This result displayed highly selectivity of probe **1** towards HClO over other analytes mentioned above.

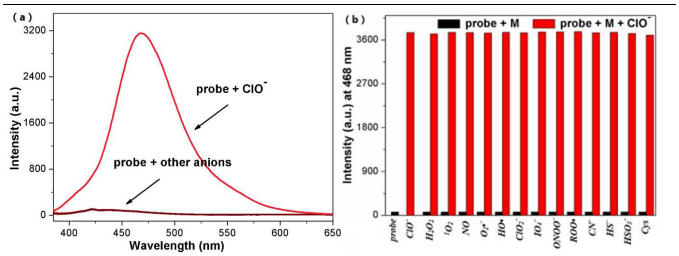
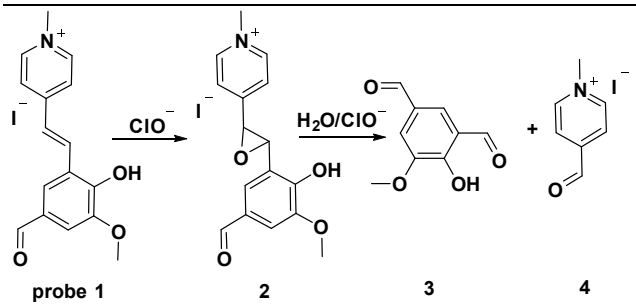


Fig. 2 (a) Fluorescent responses of probe **1** (50 μM) towards various anions and Cys (50 μM for HClO and 5000 μM for other anions and Cys) in DMSO−HEPES buffer (3:1, v/v, pH 7.4). (b) Competing responses of probe **1** (50 μM) towards various anions (50 μM for HClO and 5000 μM for other anions and Cys) in DMSO−HEPES buffer (3:1, v/v, pH 7.4)). λ_{ex}= 373 nm, λ_{em}= 468 nm, slit: 5 nm/5 nm.

To further confirm the detection mechanism, we measured the NMR titration experiment through adding HClO to a solution of probe **1** in DMSO-d_6. Compared with the ^{1}H NMR spectrogram of probe **1**, the signal of original alkene C=C proton at 7.65 ppm and 7.99 ppm disappeared gradually with the addition of HClO (Fig. s3). Those signal changes indicated the cleavage of the alkene linker in probe **1** caused by HClO. Moreover, the peak at 285.46 corresponding to $[\mathbf{2}]^+$ and 178.92 corresponding to [**3** - H]$^-$ and 122.83 corresponding to $[\mathbf{4}]^+$ in the MS spectrums further proved the mechanism mentioned above (Fig. S3). Thus, the sensing mechanism of probe **1** towards HClO was based on the cleavage of the alkene linker to epoxide and then to aldehydes as shown in Scheme 2.



Scheme 2. Proposed detection mechanism of probe **1** towards HClO.

To value the practical utilities of probe **1**, we further measured the cell experiments to detect exogenous HClO. Firstly, the cytotoxicity experiments displayed that probe **1** had only minimal cytotoxicity (Fig. S7). As shown in Figure 4a, HepG2 cells displayed non fluorescence when incubated with 30 μM probe **1** only for 30 min at 37 °C. However, incubating with HClO (10 μM, 20 μM and 30 μM respectively) would induce significant fluorescent emission of HepG2 cells which were preincubated with probe **1** (30 μM) (Fig. 4b, 4c and 4d). These results displayed excellent membrane permeability of probe **1** and further demonstrated that probe **1** could be applied to detect HClO in living cells.

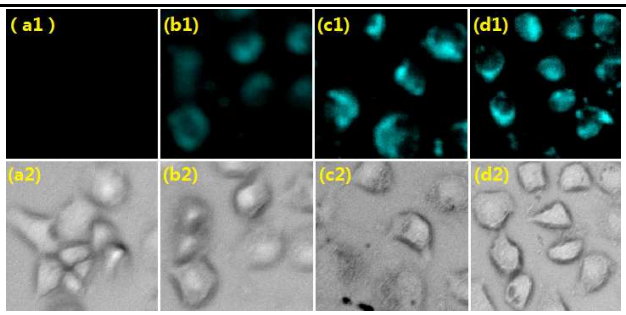


Fig. 4 Confocal fluorescence images of HepG2 cells: (a) fluorescence image of HepG2 cells after been treated with probe **1** (30 μM) for 30 min and its bright field; (b) (c) (d) fluorescence images of HepG2 cells preincubated with 30 μM probe **1** and further incubated with 10 μM HClO, 20 μM HClO and 30 μM HClO respectively and their bright fields.

In conclusion, we developed a new probe for colorimetric and fluorescent detection of HClO in aqueous solution. Based on the cleavage of an alkene linker in probe **1** to epoxide and then to aldehydes mechanism which break the ICT process with in the pyridinium (acceptor) and vanilline (donor) moieties, probe **1** displayed ultraselective and sensitive optical responses to HClO. Further cellular experiments displayed that probe **1** could be applied in bioimaging.

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Notes and references

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† Electronic Supplementary Information (ESI) available: ^{1}H NMR，^{13}C NMR, ESI-MS, crystallographic data and Supplementary figures. See DOI: 10.1039/c000000x/

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