



### ESIPT-based fluorescence probe for the rapid detection of hypochlorite (HOCl/CIO<sup>-</sup>)<sup>†</sup>

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ESIPT-based fluorescence probes are emerging as an attractive tool for the detection of biologically relevant analytes owing to their unique photophysical properties. In this work, we have developed an ESIPT-based fluorescence probe (TCBT-OMe) for the detection of HOCl/CIO<sup>-</sup> through the attachment of a bioorthogonal dimethylthiocarbamate linker. TCBT-OMe was shown to rapidly detect HOCl/CIO<sup>-</sup> (<10 s) at biologically relevant concentrations (LoD = 0.16 nM) and have an excellent selectivity towards others ROS/RNS and amino acids. Therefore, TCBT-OMe was tested in live cells and was successfully shown to be able to detect endogenous and exogenous HOCl/CIO<sup>-</sup> in HeLa cells. Additionally, TCBT-OMe acts as a dual input logic gate for Hg<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>. Interestingly, Hg<sup>2+</sup> alone gradually causes a fluorescence response but requires >30 min to produce a fluorescence response. Test strips containing TCBT-OMe were prepared and were demonstrated as an effective way to detect HOCl/CIO<sup>-</sup> in water. Furthermore, TCBT-OMe was shown to detect exogenously added HOCl/CIO<sup>-</sup> in three different water samples with little interference thus demonstrating the effectiveness as a method for the detection of HOCl/CIO<sup>-</sup> in drinking water samples.

Hypochlorous acid (HOCl) is a biologically important reactive oxygen species (ROS), which partially dissociates to form its hypochlorite anion (CIO<sup>-</sup>) under physiological conditions. In biological systems, myeloperoxidase, an enzyme found in leukocytes produces HOCl/CIO<sup>-</sup> by catalysing the reaction between Cl + H<sub>2</sub>O<sub>2</sub> → HOCl.<sup>1</sup> This vital ROS is used in immune defence systems due to its microbicidal properties.<sup>1</sup> Unfortunately, excessive

production of HOCl/CIO<sup>-</sup> can lead to the damage of a range of biological targets such as amino acids, proteins, carbohydrates and lipids.<sup>2,3</sup> As a consequence, HOCl/CIO<sup>-</sup> has been associated with a number of diseases causing cell and tissue damage.<sup>4</sup>

In addition to its role in biological systems, HOCl/CIO<sup>-</sup> is produced by the chlorination of water (Cl<sub>2</sub> + H<sub>2</sub>O → HOCl), which is the most common method for the treatment of water especially in public swimming pools.<sup>5</sup> NaOCl (Bleach) is also extensively used as a disinfectant for both domestic and industrial purposes. Unfortunately, over-exposure to HOCl/CIO<sup>-</sup>, results in swimming pool-associated asthma, irritation to the oesophagus, throat and spontaneous vomiting ([http://www.who.int/water\\_sanitation\\_health/dwq/chlorine.pdf](http://www.who.int/water_sanitation_health/dwq/chlorine.pdf)).<sup>6</sup> Additionally, there is an increased risk of bladder cancer associated with chlorinated by-products produced from chlorinated water.<sup>7,8</sup> Therefore, given the potential health hazard towards animals and humans, the development of an effective method for HOCl/CIO<sup>-</sup> detection is required.

Within our research group, we are interested in developing reaction-based fluorescence sensors for the detection of biologically important analytes.<sup>9–13</sup> Small-molecule fluorescence probes are a particular attractive tool owing to their high sensitivity, selectivity and high spatial and temporal resolution.<sup>14</sup> In particular, we are interested in using Excited State Intramolecular Proton Transfer (ESIPT)-based fluorescence probes due to their excellent photophysical properties, which include intense luminescence, photostability and a large Stokes shift.<sup>15,16</sup> Previously, we reported an ESIPT-based fluorescence probe for the detection of peroxyntirite (ONOO<sup>-</sup>) through the use of a benzyl boronic ester protecting group (Scheme 1).<sup>15</sup> This protecting group blocked the ESIPT process and therefore a low fluorescence intensity was observed. The addition of ONOO<sup>-</sup>, resulted in the fluorophore's deprotection and an increase in fluorescence intensity was observed.

In this work, we believed a methoxy-hydroxybenzothiazole (HBT-OMe) fluorophore would provide an effective ESIPT fluorescence probe for the detection of HOCl/CIO<sup>-</sup> (see ESI,† S1).<sup>17,18</sup>

To obtain TCBT-OMe we first prepared HBT-OMe by the addition of a 2 : 1 H<sub>2</sub>O<sub>2</sub>–(30% in H<sub>2</sub>O)/HCl solution to 2-aminothiophenol

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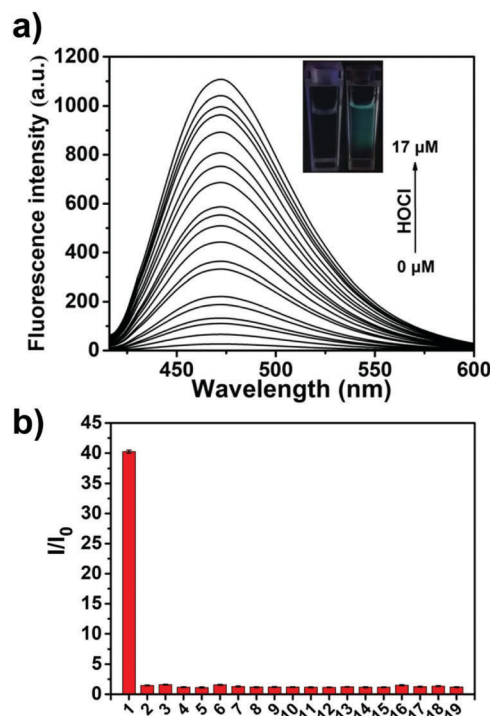


**Scheme 1** (a) Our previously reported ESIPT probe for the detection of  $\text{ONOO}^-$ . (b) This work – a thiocarbamate linker-based ESIPT **TCBT-OMe** for the detection of  $\text{HOCl/CIO}^-$ .

and *O*-vanillin in EtOH. This reaction proceeded quickly and smoothly, in a good yield (68%). With **HBT-OMe** in hand, four equivalents of dimethylthiocarbamoyl chloride was then added slowly to a solution of **HBT-OMe** in DCM. DIPEA was subsequently added dropwise to the reaction, which produced **TCBT-OMe** in excellent yield (72%).

We then evaluated the UV-Vis of **TCBT-OMe** with the addition of  $\text{HOCl/CIO}^-$  (10  $\mu\text{M}$ ), which resulted in the formation of a UV absorption peak at  $\sim 310$  nm (see ESI,† Fig. S1). Bhattacharyya *et al.* have reported that the fluorescence emission of the ESIPT process can be effected by intermolecular hydrogen bonding.<sup>19,20</sup> Therefore, evaluation of ESIPT-based fluorescence probes are commonly carried out in the presence of the surfactant cetyl trimethylammonium bromide (CTAB, 1 mM) or by using a large ratio of organic solvent.<sup>19,21–23</sup> It is believed that the formation of a micellar environment creates a hydrophobic pocket that aids the ESIPT process. Therefore, we evaluated the ability of **TCBT-OMe** to detect  $\text{HOCl/CIO}^-$  by fluorescence in the presence of CTAB, 1 mM. As shown in Fig. 1a, **TCBT-OMe** was found to be very sensitive towards  $\text{HOCl/CIO}^-$  reacting with micromolar concentrations to produce a large increase in fluorescence ( $\sim 42$  fold – Fig. S3, ESI†). **TCBT-OMe** was shown to rapidly react with  $\text{HOCl/CIO}^-$  producing a fluorescence response within less than 10 s (see ESI,† Fig. S4) and have a very low Limit of Detection (LoD) of 0.16 nM (see ESI,† Fig. S5).  $\text{HOCl/CIO}^-$  (35  $\mu\text{M}$ ) was added to **TCBT-OMe** at different pH values and a bell-shaped curve was observed. The largest fluorescence response was seen at the  $\text{p}K_a$  of  $\text{HOCl/CIO}^- = 7.53$  (Fig. S5, ESI†) suggestive of general acid–base catalysis being in operation. (see ESI,† Scheme S1 for proposed mechanism).

We then evaluated the selectivity of **TCBT-OMe** towards other reactive oxygen/nitrogen species (ROS/RNS) and amino acids (Fig. 1b). Remarkably, **TCBT-OMe** had an excellent selectivity towards  $\text{HOCl/CIO}^-$  therefore permitting its use as a fluorescence probe for the detection of  $\text{HOCl/CIO}^-$  in live cells. As shown in Fig. 2, **TCBT-OMe** was successfully used to visualise endogenously stimulated  $\text{HOCl/CIO}^-$  in HeLa cells using phorbol 12-myristate 13-acetate (**PMA**, which is a ROS stimulant that induces the production of  $\text{HOCl/CIO}^-$ ). Separately, HeLa cells were



**Fig. 1** (a) Fluorescence spectra of **TCBT-OMe** (5  $\mu\text{M}$ ) with increasing additions of  $\text{HOCl/CIO}^-$  (from 0 to 17  $\mu\text{M}$ ) in PBS buffer (pH 7.4, containing 1% DMSO, 1 mM CTAB). Measurements were taken after 1 min.  $\lambda_{\text{ex}} = 310$  nm. Slit widths: ex = 6 nm, em = 4 nm. (b) Selectivity bar chart of **TCBT-OMe** in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB with  $\text{HClO}$  (15  $\mu\text{M}$ ) and other interfering reagents (ROS/RNS and various amino acids). 1,  $\text{HClO}$ ; 2, blank; 3,  $\text{ONOO}^-$ ; 4,  $\text{H}_2\text{O}_2$ ; 5,  $\text{ROO}^\cdot$ ; 6,  $\text{OH}^\cdot$ ; 7,  $\text{O}_2^{\cdot-}$ ; 8,  $^1\text{O}_2$ ; 9, NO; 10, glycine; 11, asparagine; 12, cysteine; 13, homocysteine; 14, glutathione; 15, arginine; 16, histidine; 17, serine; 18, glycine; 19, threonine. Note: the concentration of **TCBT-OMe** and each interfering species are 5  $\mu\text{M}$  and 100  $\mu\text{M}$  respectively, 30 min wait before measurement in buffer solution.  $\lambda_{\text{ex}} = 310$  nm/ $\lambda_{\text{em}} = 472$  nm error bars represent s.d. Measurements were taken after 30 min.  $\lambda_{\text{ex}} = 310$  nm. Slit widths: ex = 6 nm, em = 4 nm.

also pretreated with 4-aminobenzoic acid hydrazide (**ABAH**, which is a specific inhibitor of MPO which suppressed the generation of  $\text{HOCl}$ ) and as expected only weak fluorescence was observed. **TCBT-OMe** was also able to detect  $\text{HOCl/CIO}^-$  added exogenously to the HeLa cells.

The dimethylthiocarbamate linker of **TCBT-OMe** has previously been used in the construction of dual input molecular logic gate<sup>24</sup> for the detection of  $\text{Hg}^{2+}$  'AND'  $\text{H}_2\text{O}_2$  (see ESI† Scheme S2 for proposed mechanism).<sup>25,26</sup> Therefore, we evaluated the ability of **TCBT-OMe** to perform molecular logic with the input of  $\text{Hg}^{2+}$  and  $\text{H}_2\text{O}_2$ . The presence of solely  $\text{H}_2\text{O}_2$  (120  $\mu\text{M}$ ) led to a small increase in fluorescence intensity (dashed line), however, with subsequent additions of  $\text{Hg}^{2+}$  (0–9  $\mu\text{M}$ ) a large fluorescence response was observed (Fig. 3a). To demonstrate that both analytes are required,  $\text{Hg}^{2+}$  was added first, followed by the addition of  $\text{H}_2\text{O}_2$  (0–180  $\mu\text{M}$ ). As shown in Fig. 3b, the subsequent addition of  $\text{H}_2\text{O}_2$  rapidly led to an increase in fluorescence intensity. **TCBT-OMe** was shown to be selective towards  $\text{Hg}^{2+}$  over other metal cations in the presence of  $\text{H}_2\text{O}_2$  (see ESI,† Fig. S9). Interestingly,  $\text{Hg}^{2+}$  alone resulted in a slow increase in fluorescence intensity (see ESI,† Fig. S10). This is believed to be



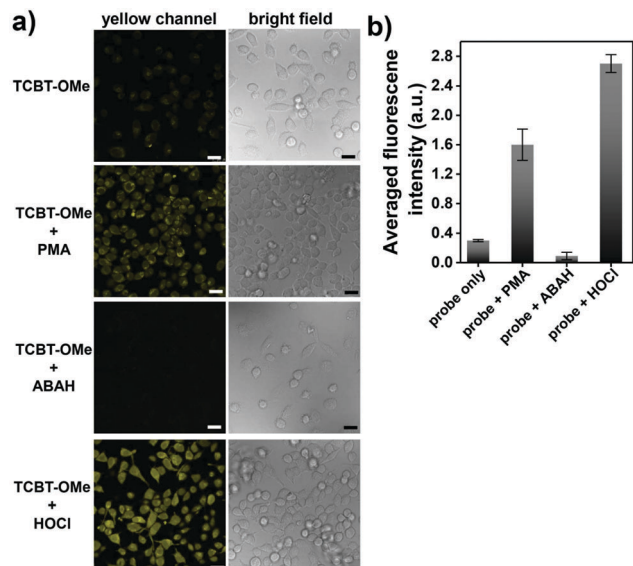


Fig. 2 (a) From top to bottom: HeLa cells were pretreated with **TCBT-OMe** (40  $\mu\text{M}$ ) for 30 min; HeLa cells pretreated with **TCBT-OMe** (40  $\mu\text{M}$ ) were then left for 30 min after preincubation with PMA (1.2  $\mu\text{g mL}^{-1}$ ) for 90 min; HeLa cells pretreated with **TCBT-OMe** (40  $\mu\text{M}$ ) were then left for 30 min after preincubation with 250  $\mu\text{M}$  ABAH for 70 min; HeLa cells loaded with **TCBT-OMe** (40  $\mu\text{M}$ ) for 30 min followed by the exogenous addition of 8  $\mu\text{M}$  NaOCl for 5 min. Scale bar: 25  $\mu\text{m}$   $\lambda_{\text{ex}} = 420 \text{ nm}$ / $\lambda_{\text{em}} = 420\text{--}590 \text{ nm}$ . (b) The histogram shows the semi-quantitative calculation of averaged fluorescence intensity (FI) of each fluorescence panel in the displayed images by ImageJ software.

due to the instability of the dimethylcarbonate formed from the reaction of **TCBT-OMe** with  $\text{Hg}^{2+}$ .

Despite this interesting dual responsive reactivity of **TCBT-OMe**, this 'AND' logic requires minutes to fully react, whereas  $\text{HOCl}/\text{ClO}^-$  reacts with **TCBT-OMe** within seconds. Therefore, due to the significantly greater reactivity of **TCBT-OMe** towards  $\text{HOCl}/\text{ClO}^-$  over  $\text{Hg}^{2+}$ , we believed we could use it as an effective method for the detection of  $\text{HOCl}/\text{ClO}^-$  in drinking water sources.

We produced test strips by simply soaking a commercially available test strip in water containing **TCBT-OMe** (0.8 mM). After drying, test strips impregnated with **TCBT-OMe** were placed in water containing  $\text{HClO}/\text{ClO}^-$  (0–200  $\mu\text{M}$ ). As shown in Fig. 4, there is a clear colour/intensity difference in the test strips that have been dipped into water containing various concentrations of  $\text{HClO}/\text{ClO}^-$ .

In addition to detecting  $\text{HClO}/\text{ClO}^-$  in water, **TCBT-OMe** was added into three different water samples containing 1 mM CTAB (Sample A, tap water from University of Bath; Sample B, water from the Avon River (Bath); Sample C, water from Roman spa in Bath). Interestingly, little interference was observed for the exogenous addition of  $\text{HClO}/\text{ClO}^-$  to each water sample (>95% recovery) – see ESI,† Table S1.

In summary, we have developed an ESIPT-based fluorescence **TCBT-OMe** for the detection of  $\text{HClO}/\text{ClO}^-$ . **TCBT-OMe** was shown to have a very high sensitivity and selectivity towards  $\text{HClO}/\text{ClO}^-$  fully reacting within 10 s and having a LoD of 0.16  $\mu\text{M}$ . Significantly, **TCBT-OMe** was able to detect endogenous and

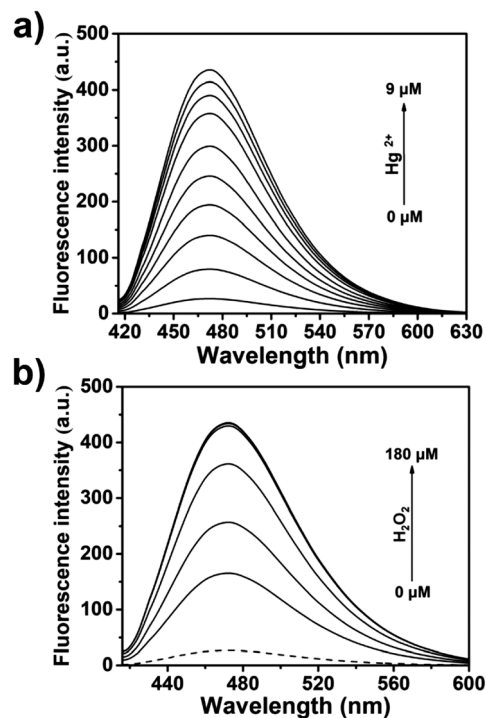


Fig. 3 (a) Fluorescence spectra of **TCBT-OMe** (5  $\mu\text{M}$ ) in the presence of  $\text{H}_2\text{O}_2$  (120  $\mu\text{M}$ ) – (dashed line represent probe and  $\text{H}_2\text{O}_2$ ) with increasing concentrations of  $\text{Hg}^{2+}$  (0–9  $\mu\text{M}$ ) in buffer solution pH 7.4, 1% DMSO, 1 mM CTAB 14 min wait between measurement.  $\lambda = 310 \text{ nm}$ . Slit widths:  $\text{ex} = 6 \text{ nm}$   $\text{em} = 4 \text{ nm}$ . (b) Fluorescence spectra of **TCBT-OMe** (5  $\mu\text{M}$ ) in the presence of  $\text{Hg}^{2+}$  (9  $\mu\text{M}$ ) – (dashed line represents probe and  $\text{Hg}^{2+}$ ) with increasing concentrations of  $\text{H}_2\text{O}_2$  (final concentration: 0, 20, 40, 80, 100, 120, 140  $\mu\text{M}$  and 180  $\mu\text{M}$ ) in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB. 14 min wait between measurement in buffer solution.  $\lambda_{\text{ex}} = 310 \text{ nm}$ . Slit widths:  $\text{ex} = 6 \text{ nm}$   $\text{em} = 4 \text{ nm}$ .

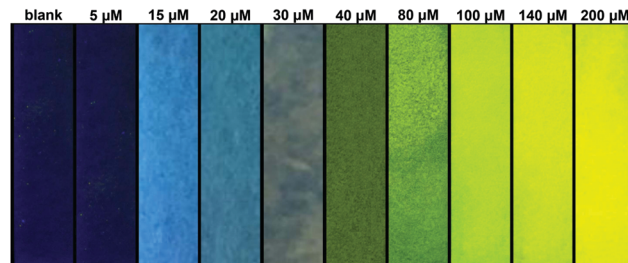


Fig. 4 Photograph showing the colour changes of **TCBT-OMe** impregnated test strips after addition to water samples containing different concentrations of  $\text{HClO}/\text{ClO}^-$  under UV light (365 nm).

exogenous  $\text{HClO}/\text{ClO}^-$  in HeLa cells. Additionally, **TCBT-OMe** was shown as a dual input logic gate with  $\text{Hg}^{2+}$  and  $\text{H}_2\text{O}_2$  as inputs. Interestingly,  $\text{Hg}^{2+}$  alone gradually produced a fluorescence response but required >30 min to produce a significant fluorescence response. Test strips containing **TCBT-OMe** were developed and shown to be an effective way to detect  $\text{HClO}/\text{ClO}^-$  in water. Furthermore, **TCBT-OMe** was shown to detect exogenously added  $\text{HClO}/\text{ClO}^-$  in three different water samples with little interference demonstrating its effectiveness as a method to detect  $\text{HClO}/\text{ClO}^-$  in drinking water samples.



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## Conflicts of interest

No conflicts of interest.

## Notes and references

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