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ESIPT-based fluorescence probe for the rapid detection of hypochlorite (HOCl/ClO⁻)†

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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 HClO/ClO⁻ through the attachment of a bioorthogonal dimethylthiocarbamate linker. TCBT-OMe was shown to rapidly detect HClO/ClO⁻ (<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 HClO/ClO⁻ in HeLa cells. Additionally, TCBT-OMe acts as a dual input logic gate for Hg^{2+} and H_2O_2. Interestingly, Hg^{2+} 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 HClO/ClO⁻ in water. Furthermore, TCBT-OMe was shown to detect exogenously added HClO/ClO⁻ in three different water samples with little interference thus demonstrating the effectiveness as a method for the detection of HClO/ClO⁻ in drinking water samples.

Hypochlorous acid (HOCl) is a biologically important reactive oxygen species (ROS), which partially dissociates to form its hypochlorite anion (ClO⁻) under physiological conditions. In biological systems, myeloperoxidase, an enzyme found in leukocytes produces HOCl/ClO⁻ by catalysing the reaction between $Cl + H_2O_2 \rightarrow HOCl$.[1] This vital ROS is used in immune defence systems due to its microbicidal properties.[1] Unfortunately, excessive production of HOCl/ClO⁻ can lead to the damage of a range of biological targets such as amino acids, proteins, carbohydrates and lipids.[2,3] As a consequence, HOCl/ClO⁻ has been associated with a number of diseases causing cell and tissue damage.[4]

In addition to its role in biological systems, HOCl/ClO⁻ is produced by the chlorination of water ($Cl_2 + H_2O \rightarrow HOCl$), which is the most common method for the treatment of water especially in public swimming pools.[5] NaOCl (Bleach) is also extensively used as a disinfectant for both domestic and industrial purposes. Unfortunately, over-exposure to HOCl/ClO⁻, results in swimming pool-associated asthma, irritation to the oesophagus, throat and spontaneous vomiting (http://www.who.int/water_sanitation_health/dwq/chlorine.pdf).[6] Additionally, there is an increased risk of bladder cancer associated with chlorinated by-products produced from chlorinated water.[7,8] Therefore, given the potential health hazard towards animals and humans, the development of an effective method for HOCl/ClO⁻ detection is required.

Within our research group, we are interested in developing reaction-based fluorescence sensors for the detection of biologically important analytes.[9–13] Small-molecule fluorescence probes are a particular attractive tool owing to their high sensitivity, selectivity and high spatial and temporal resolution.[14] 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.[15,16] Previously, we reported an ESIPT-based fluorescence probe for the detection of peroxynitrite (ONOO⁻) through the use of a benzyl boronic ester protecting group (Scheme 1).[15] This protecting group blocked the ESIPT process and therefore a low fluorescence intensity was observed. The addition of ONOO⁻, 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/ClO⁻ (see ESI,† S1).[17,18]

To obtain **TCBT-OMe** we first prepared **HBT-OMe** by the addition of a 2 : 1 H_2O_2–(30% in H_2O)/HCl solution to 2-aminothiophenol

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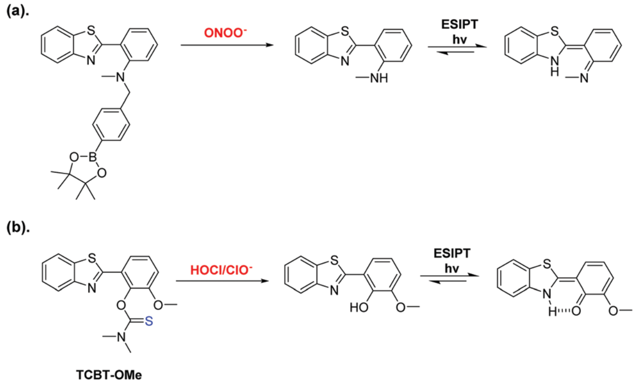

Scheme 1 (a) Our previously reported ESIPT probe for the detection of $ONOO^-$. (b) This work – a thiocarbamate linker-based ESIPT **TCBT-OMe** for the detection of HOCl/ClO^-.

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 HOCl/ClO^- (10 μM), which resulted in the formation of a UV absorption peak at ∼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.[19,20] 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.[19,21–23] 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 HOCl/ClO^- by fluorescence in the presence of CTAB, 1 mM. As shown in Fig. 1a, **TCBT-OMe** was found to be very sensitive towards HOCl/ClO^- reacting with micromolar concentrations to produce a large increase in fluorescence (∼42 fold – Fig. S3, ESI†). **TCBT-OMe** was shown to rapidly react with HOCl/ClO^- 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). HOCl/ClO^- (35 μM) was added to **TCBT-OMe** at different pH values and a bell-shaped curve was observed. The largest fluorescence response was seen at the pK_a of HOCl/ClO^- = 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 HOCl/ClO^- therefore permitting its use as a fluorescence probe for the detection of HOCl/ClO^- in live cells. As shown in Fig. 2, **TCBT-OMe** was successfully used to visualise endogenously stimulated HOCl/ClO^- in HeLa cells using phorbol 12-myristate 13-acetate (**PMA**, which is a ROS stimulant that induces the production of HOCl/ClO^-). Separately, HeLa cells were also pretreated with 4-aminobenzoic acid hydrazide (**ABAH**, which is a specific inhibitor of MPO which suppressed the generation of HOCl) and as expected only weak fluorescence was observed. **TCBT-OMe** was also able to detect HOCl/ClO^- added exogenously to the HeLa cells.

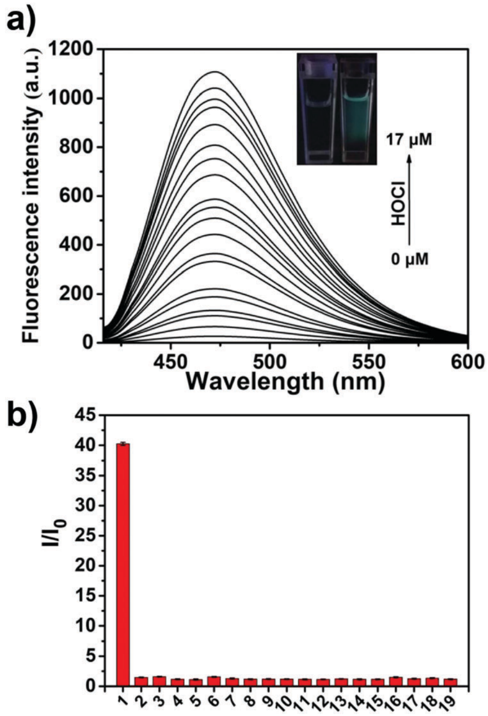

Fig. 1 (a) Fluorescence spectra of **TCBT-OMe** (5 μM) with increasing additions of HOCl/ClO^- (from 0 to 17 μM) in PBS buffer (pH 7.4, containing 1% DMSO, 1 mM CTAB). Measurements were taken after 1 min. λ_{ex} = 310 nm. Slit widths: ex = 6 nm em = 4 nm. (b) Selectivity bar chart of **TCBT-OMe** in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB with HClO (15 μM) and other interfering reagents (ROS/RNS and various amino acids). 1, HClO; 2, blank; 3, $ONOO^-$; 4, H_2O_2; 5, ROO•; 6, •OH; 7, •O_2^-; 8, $^1O_2$; 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 μM and 100 μM respectively, 30 min wait before measurement in buffer solution. λ_{ex} = 310 nm/λ_{em} = 472 nm error bars represent s.d. Measurements were taken after 30 min. λ_{ex} = 310 nm. Slit widths: ex = 6 nm, em = 4 nm.

The dimethylthiocarbamate linker of **TCBT-OMe** has previously been used in the construction of dual input molecular logic gate[24] for the detection of Hg^{2+} 'AND' H_2O_2 (see ESI† Scheme S2 for proposed mechanism).[25,26] Therefore, we evaluated the ability of **TCBT-OMe** to perform molecular logic with the input of Hg^{2+} and H_2O_2. The presence of solely H_2O_2 (120 μM) led to a small increase in fluorescence intensity (dashed line), however, with subsequent additions of Hg^{2+} (0–9 μM) a large fluorescence response was observed (Fig. 3a). To demonstrate that both analytes are required, Hg^{2+} was added first, followed by the addition of H_2O_2 (0–180 μM). As shown in Fig. 3b, the subsequent addition of H_2O_2 rapidly led to an increase in fluorescence intensity. **TCBT-OMe** was shown to be selective towards Hg^{2+} over other metal cations in the presence of H_2O_2 (see ESI,† Fig. S9). Interestingly, Hg^{2+} alone resulted in a slow increase in fluorescence intensity (see ESI,† Fig. S10). This is believed to be

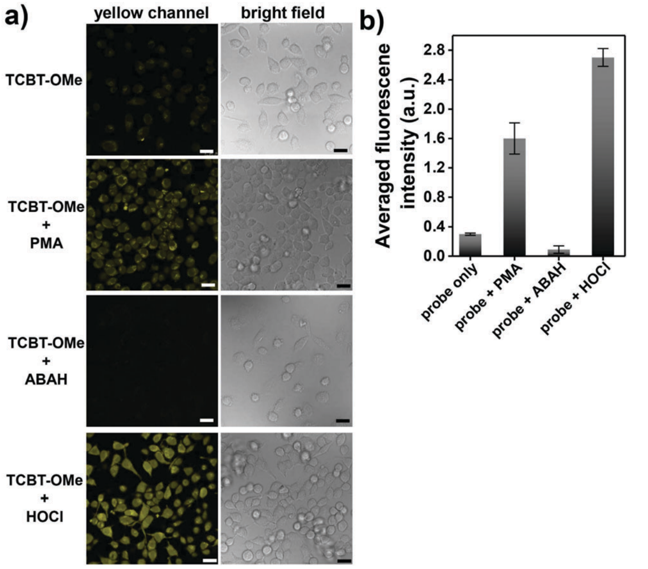

Fig. 2 (a) From top to bottom: HeLa cells were pretreated with **TCBT-OMe** (40 μM) for 30 min; HeLa cells pretreated with **TCBT-OMe** (40 μM) were then left for 30 min after preincubation with PMA (1.2 μg mL^{-1}) for 90 min; HeLa cells pretreated with **TCBT-OMe** (40 μM) were then left for 30 min after preincubation with 250 μM ABAH for 70 min; HeLa cells loaded with **TCBT-OMe** (40 μM) for 30 min followed by the exogenous addition of 8 μM NaOCl for 5 min. Scale bar: 25 μm λ_{ex} = 420 nm/λ_{em} = 420–590 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.

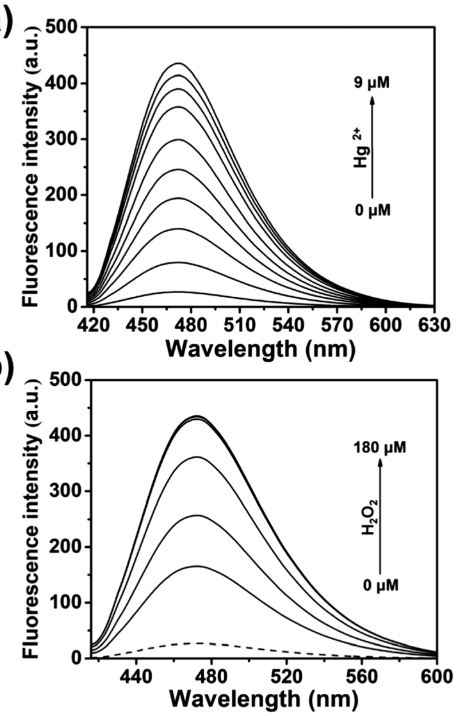

Fig. 3 (a) Fluorescence spectra of **TCBT-OMe** (5 μM) in the presence of H_2O_2 (120 μM) – (dashed line represent probe and H_2O_2) with increasing concentrations of Hg^{2+} (0–9 μM) in buffer solution pH 7.4, 1% DMSO, 1 mM CTAB 14 min wait between measurement. λ = 310 nm. Slit widths: ex = 6 nm em = 4 nm. (b) Fluorescence spectra of **TCBT-OMe** (5 μM) in the presence of Hg^{2+} (9 μM) – (dashed line represents probe and Hg^{2+}) with increasing concentrations of H_2O_2 (final concentration: 0, 20, 40, 80, 100, 120, 140 μM and 180 μM) in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB. 14 min wait between measurement in buffer solution. λ_{ex} = 310 nm. Slit widths: ex = 6 nm em = 4 nm.

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

Despite this interesting dual responsive reactivity of **TCBT-OMe**, this 'AND' logic requires minutes to fully react, whereas $HOCl/ClO^-$ reacts with **TCBT-OMe** within seconds. Therefore, due to the significantly greater reactivity of **TCBT-OMe** towards $HOCl/ClO^-$ over Hg^{2+}, we believed we could use it as an effective method for the detection of $HOCl/ClO^-$ 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 $HClO/ClO^-$ (0–200 μM). 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 $HClO/ClO^-$.

In addition to detecting $HClO/ClO^-$ 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 $HClO/ClO^-$ 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 $HClO/ClO^-$. **TCBT-OMe** was shown to have a very high sensitivity and selectivity towards $HClO/ClO^-$ fully reacting within 10 s and having a LoD of 0.16 μM. Significantly, **TCBT-OMe** was able to detect endogenous and exogenous $HClO/ClO^-$ in HeLa cells. Additionally, **TCBT-OMe** was shown as a dual input logic gate with Hg^{2+} and H_2O_2 as inputs. Interestingly, 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 $HClO/ClO^-$ in water. Furthermore, **TCBT-OMe** was shown to detect exogenously added $HClO/ClO^-$ in three different water samples with little interference demonstrating its effectiveness as a method to detect $HClO/ClO^-$ in drinking water samples.

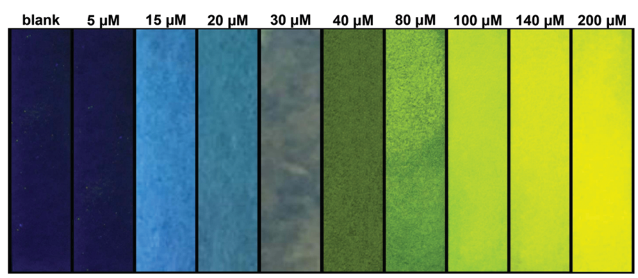

Fig. 4 Photograph showing the colour changes of **TCBT-OMe** impregnated test strips after addition to water samples containing different concentrations of $HClO/ClO^-$ under UV light (365 nm).

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Conflicts of interest

No conflicts of interest.

Notes and references

1 A. Strzepa, K. A. Pritchard and B. N. Dittel, *Cell. Immunol.*, 2017, **317**, 1–8.
2 M. J. Davies, *J. Clin. Biochem. Nutr.*, 2011, **48**, 8–19.
3 S. J. Klebanoff, *J. Leukocyte Biol.*, 2005, **77**, 598–625.
4 B. S. van der Veen, M. P. J. de Winther and P. Heeringa, *Antioxid. Redox Signaling*, 2009, **11**, 2899–2937.
5 L. Kunigk, R. Gedraite and C. J. Kunigk, *Environ. Eng. Manage. J.*, 2018, **17**, 711–720.
6 C. Zwiener, S. D. Richardson, D. M. De Marini, T. Grummt, T. Glauner and F. H. Frimmel, *Environ. Sci. Technol.*, 2007, **41**, 363–372.
7 K. P. Cantor, R. Hoover, P. Hartge, T. J. Mason, D. T. Silverman, R. Altman, D. F. Austin, M. A. Child, C. R. Key, L. D. Marrett, M. H. Myers, A. S. Narayana, L. I. Levin, J. W. Sullivan, G. M. Swanson, D. B. Thomas and D. W. West, *J. Natl. Cancer Inst.*, 1987, **79**, 1269–1279.
8 C. M. Villanueva, K. P. Cantor, S. Cordier, J. J. K. Jaakkola, W. D. King, C. F. Lynch, S. Porru and M. Kogevinas, *Epidemiol.*, 2004, **15**, 357–367.
9 A. C. Sedgwick, R. S. L. Chapman, J. E. Gardiner, L. R. Peacock, G. Kim, J. Yoon, S. D. Bull and T. D. James, *Chem. Commun.*, 2017, **53**, 10441–10443.
10 C. M. Lopez-Alled, A. Sanchez-Fernandez, K. J. Edler, A. C. Sedgwick, S. D. Bull, C. L. McMullin, G. Kociok-Kohn, T. D. James, J. Wenk and S. E. Lewis, *Chem. Commun.*, 2017, **53**, 12580–12583.
11 A. C. Sedgwick, H. H. Han, J. E. Gardiner, S. D. Bull, X. P. He and T. D. James, *Chem. Commun.*, 2017, **53**, 12822–12825.
12 D. Wu, A. C. Sedgwick, T. Gunnlaugsson, E. U. Akkaya, J. Yoon and T. D. James, *Chem. Soc. Rev.*, 2017, **46**, 7105–7123.
13 E. V. Lampard, A. C. Sedgwick, X. L. Sun, K. L. Filer, S. C. Hewins, G. Kim, J. Yoon, S. D. Bull and T. D. James, *ChemistryOpen*, 2018, **7**, 262–265.
14 J. Chan, S. C. Dodani and C. J. Chang, *Nat. Chem.*, 2012, **4**, 973–984.
15 A. C. Sedgwick, X. L. Sun, G. Kim, J. Yoon, S. D. Bull and T. D. James, *Chem. Commun.*, 2016, **52**, 12350–12352.
16 J. S. Wu, W. M. Liu, J. C. Ge, H. Y. Zhang and P. F. Wang, *Chem. Soc. Rev.*, 2011, **40**, 3483–3495.
17 B. C. Zhu, P. Li, W. Shu, X. Wang, C. Y. Liu, Y. Wang, Z. K. Wang, Y. W. Wang and B. Tang, *Anal. Chem.*, 2016, **88**, 12532–12538.
18 B. C. Zhu, L. Wu, M. Zhang, Y. W. Wang, Z. Y. Zhao, Z. K. Wang, Q. X. Duan, P. Jia and C. Y. Liu, *Sens. Actuators, B*, 2018, **263**, 103–108.
19 N. Sarkar, K. Das, S. Das, A. Datta, D. Nath and K. Bhattacharyya, *J. Phys. Chem.*, 1995, **99**, 17711–17714.
20 K. Das, N. Sarkar, A. K. Ghosh, D. Majumdar, D. N. Nath and K. Bhattacharyya, *J. Phys. Chem.*, 1994, **98**, 9126–9132.
21 D. P. Murale, H. Kim, W. S. Choi and D. G. Churchill, *Org. Lett.*, 2013, **15**, 3946–3949.
22 H. R. Zheng, L. Y. Niu, Y. Z. Chen, L. Z. Wu, C. H. Tung and Q. Z. Yang, *Chin. Chem. Lett.*, 2016, **27**, 1793–1796.
23 R. Hu, J. A. Feng, D. H. Hu, S. Q. Wang, S. Y. Li, Y. Li and G. Q. Yang, *Angew. Chem., Int. Ed.*, 2010, **49**, 4915–4918.
24 S. Erbas-Cakmak, S. Kolemen, A. C. Sedgwick, T. Gunnlaugsson, T. D. James, J. Yoon and E. U. Akkaya, *Chem. Soc. Rev.*, 2018, **47**, 2228–2248.
25 D. P. Murale, H. Liew, Y. H. Suh and D. G. Churchill, *Anal. Methods*, 2013, **5**, 2650–2652.
26 W. Shu, L. G. Yan, J. Liu, Z. K. Wang, S. Zhang, C. C. Tang, C. Y. Liu, B. C. Zhu and B. Du, *Ind. Eng. Chem. Res.*, 2015, **54**, 8056–8062.