A new probe based on rhodamine B and benzothiazole hydrazine for sensing hypochlorite in living cells and real water samples

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Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

Various ROS and RNS including NaClO, H_2O_2, *t*-BuOOH, ·OH, *t*-BuO·, NO, $ONOO^-$, $^-O_2$, $^1O_2$ were prepared according to the following methods. Sodium hypochlorite (NaOCl), H_2O_2, Nitric oxide (NO) and *tert*-butylhydroperoxide (*t*-BuOOH) were diluted from the commercially available solution to 0.1 M in water.

Preparation of ·OH

Hydroxyl radical (·OH) was generated by Fenton reactions by mixing $FeSO_4 \cdot 7H_2O$ with 1 equivalent of H_2O_2, the concentration of ·OH was estimated from the concentration of Fe^{2+}.

Preparation of *t*-BuO·

Tert-butoxy radical (*t*-BuO·) was prepared according to Fenton reactions by mixing $FeSO_4 \cdot 7H_2O$ with 1 equivalent *tert*-butylhydroperoxide (*t*-BuOOH), the concentration of *t*-BuO· was estimated from the concentration of Fe^{2+}.

Preparation of $ONOO^-$

Peroxynitrite ($ONOO^-$) was prepared according to the reported method.[1] The mixing of 10 mL 0.6 M HCl and 10 mL 0.7 M H_2O_2 was stirring at 0 °C for 3 h. Then 10 mL 0.6 M $NaNO_2$ was quickly added to the above solution and stirred. The mixture was quickly added to 10 mL 3 M NaOH at 0 °C to get peroxynitrite ($ONOO^-$).

Preparation of $^-O_2$

Superoxide ($^-O_2$) was generated from KO_2 according to the literature.[2]

Preparation of $^1O_2$

Singlet oxygen $^1O_2$ was generated on mixing of NaOCl with 2 equivalent of H_2O_2 according to the literature.[3]

1. J.W. Reed, H.H. Ho, W.L. Jolly, Chemical synthesis with a quenched flow reactor. Hydroxytrihydroborate and peroxynitrite, *J. Am. Chem. Soc*. 1974, **96**, 1248–1249.

2. Z. Ma, W. Sun, L.Z. Chen, J. Li, Z.Z. Liu, H.X. Bai, M.Y. Zhu, L.P. Du, X.D. Shi, M.Y. Li, A novel hydrazino-substituted naphthalimide-based fluorogenic probe for *tert*-butoxy radicals, *Chem. Commun*., 2013, **49**, 6295-6297.

3. X. Li, G. Zhang, H. Ma, D. Zhang, J. Li, D. Zhu, 4,5-Dimethylthio-4-[2-(9-anthryloxy)ethylthio]tetrathiafulvalene, a highly selective and sensitive chemiluminescence probe for singlet oxygen, *J. Am. Chem. Soc*. 2004, **126**, 11543–11548.

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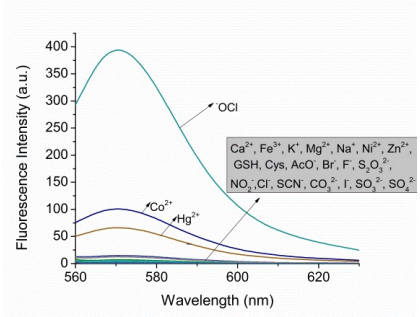


Fig. S1 Fluorescence spectra of probe RBT (5 μM) to OCl^- and Co^{2+}, Hg^{2+}, Ca^{2+}, Fe^{3+}, K^+, Mg^{2+}, Na^+, Ni^{2+}, Zn^{2+}, GSH, Cys, AcO^-, Br^-, F^-, $S_2O_3^{2-}$, NO^{2-},Cl^-, SCN^-, CO_3^{2-}, I^-, SO_3^{2-}, SO_4^{2-} (10 equiv.). Condition: MeCN – PBS (v/v = 3:7, pH = 7.4; λ_{ex}: 550 nm (slit widths: 12 nm/3 nm).

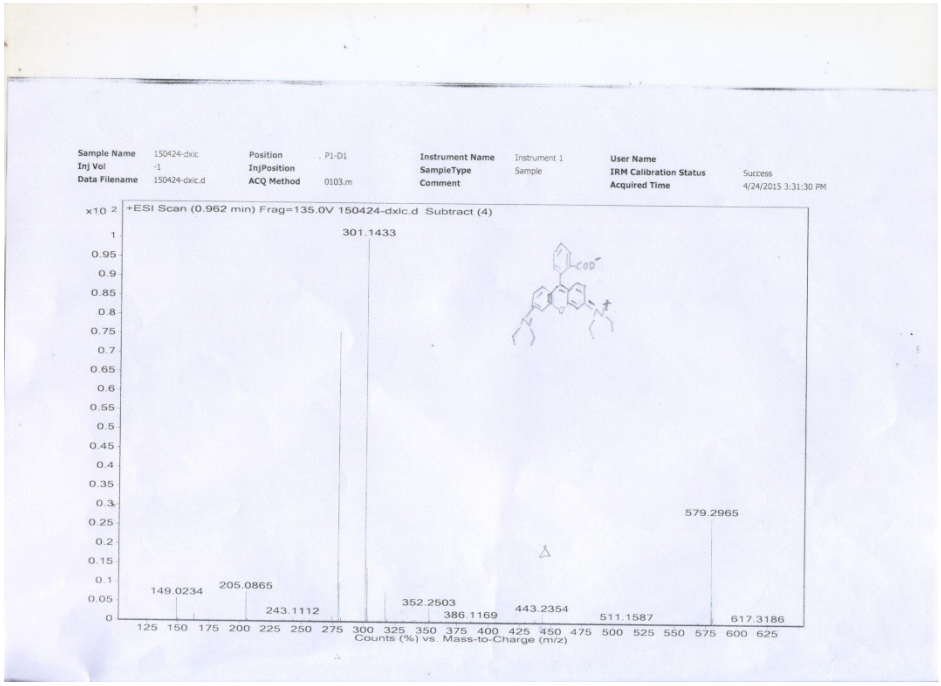


Fig. S2 HRMS of the reaction product of probe RBT with HOCl

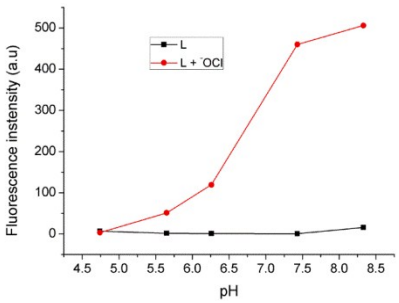


Fig. S3 Fluorescence intensity of probe RBT (5 μM) in the absence and the presence of ClO^- (50 μM) in MeCN – PBS (v/v = 3:7, pH = 7.4; λ_{ex}: 550 nm (slit widths: 12 nm/3 nm).

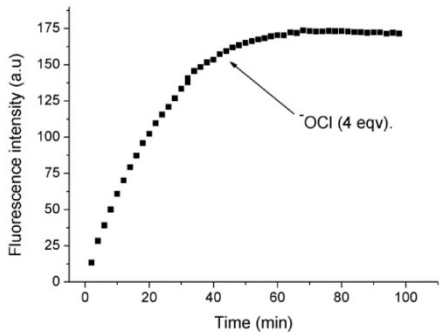


Fig. S4 Time-dependent fluorescence intensity changes of probe RBT (5 μM) in the presence of ClO^- (20 μM). Condition: MeCN – PBS (v/v = 3:7, pH = 7.4; λ_{ex}: 550 nm (slit widths: 12 nm/3 nm).

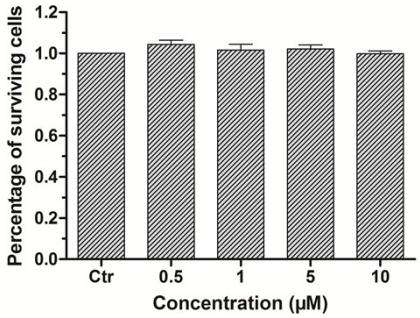
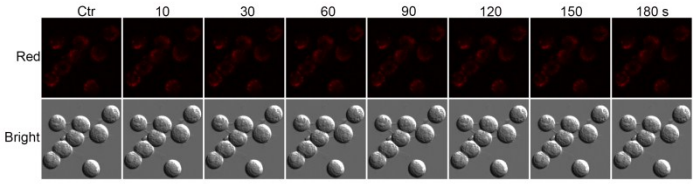


Fig. S5 Toxicity detection of probe RBT in RAW264.7 cells. The SRB assay of RAW 264.7 cells were performed with different concentration probe (0.5, 1, 5 and 10 μM) for 12 h. Data are presented as mean ± SE, n = 3.

(a)



(b)

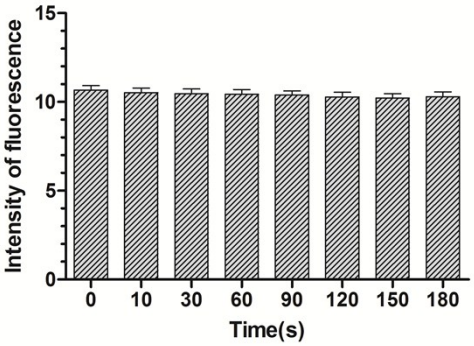


Fig. S6 Photostability of probe RBT in RAW 264.7 cells. (a) RAW 264.7 cells were incubated with 1.0 μM probe RBT for 1 h and the intensity was observed at different time (0, 10, 30, 60, 90, 120, 150 and 180 s). (b) The fluorescence statistics were obtained by ImageJ. The probe was excited by 555 nm and the emission was collected by red channel (555-700nm). Data are presented as mean ± SE, n = 3.

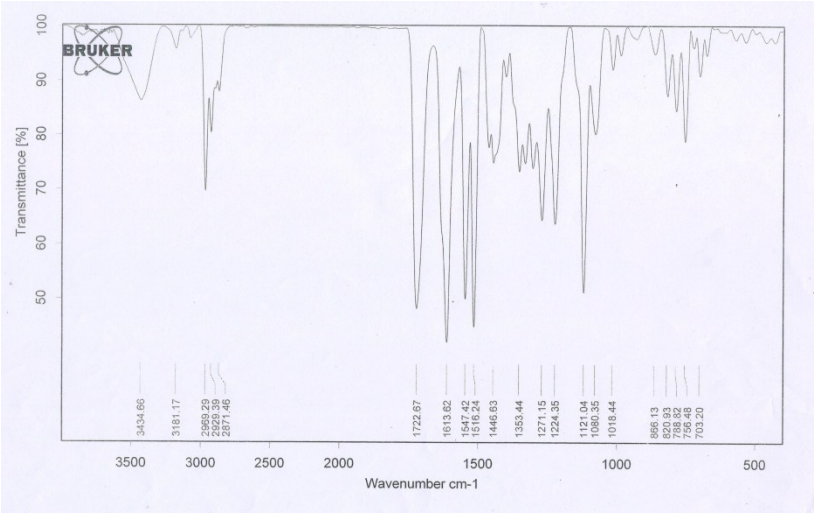


Fig. S7 IR of probe BRT

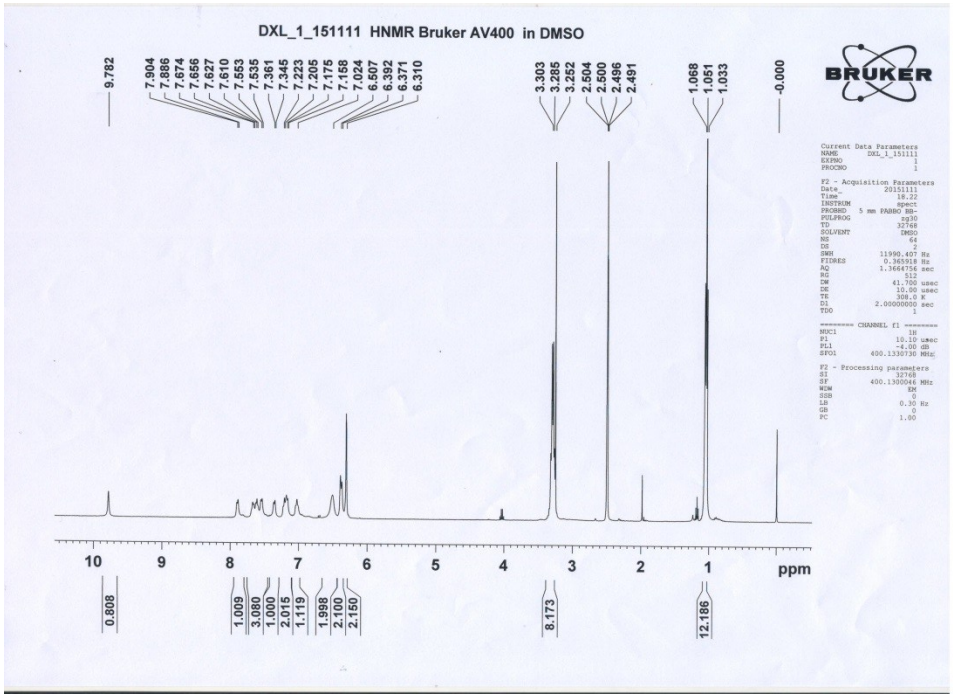


Fig. S8 ^{1}H NMR of probe BRT

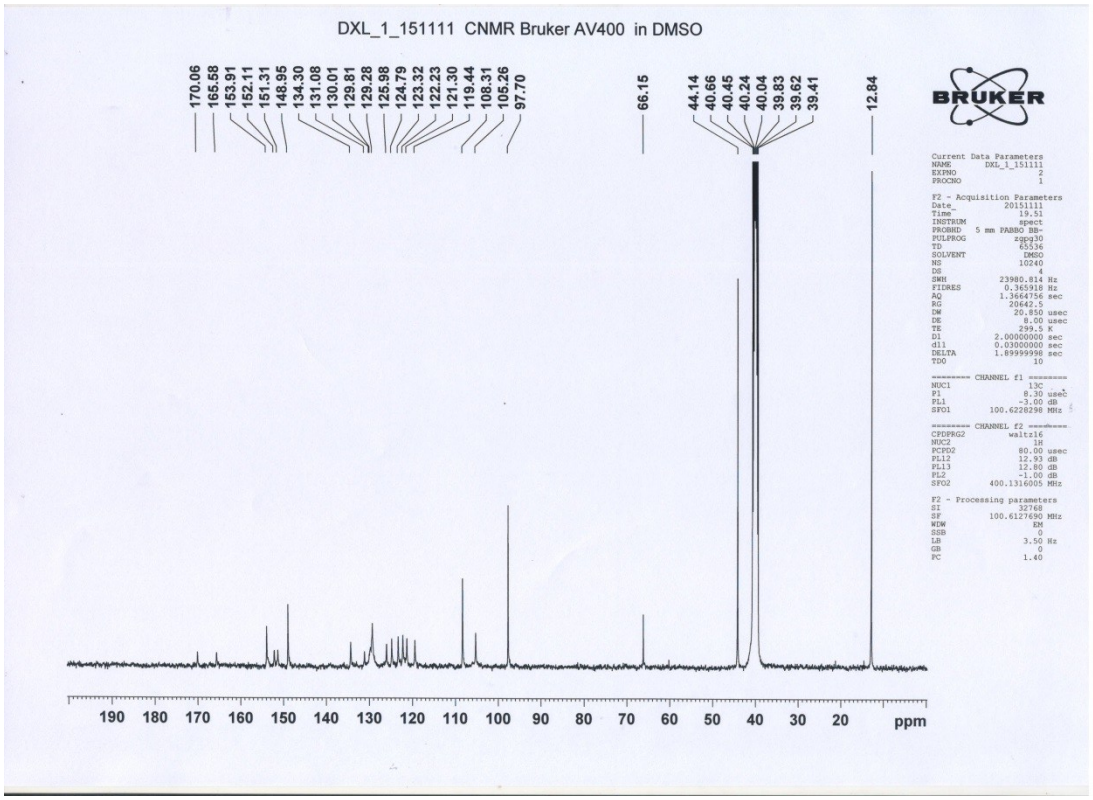


Fig. S9 ^{13}C NMR of probe BRT

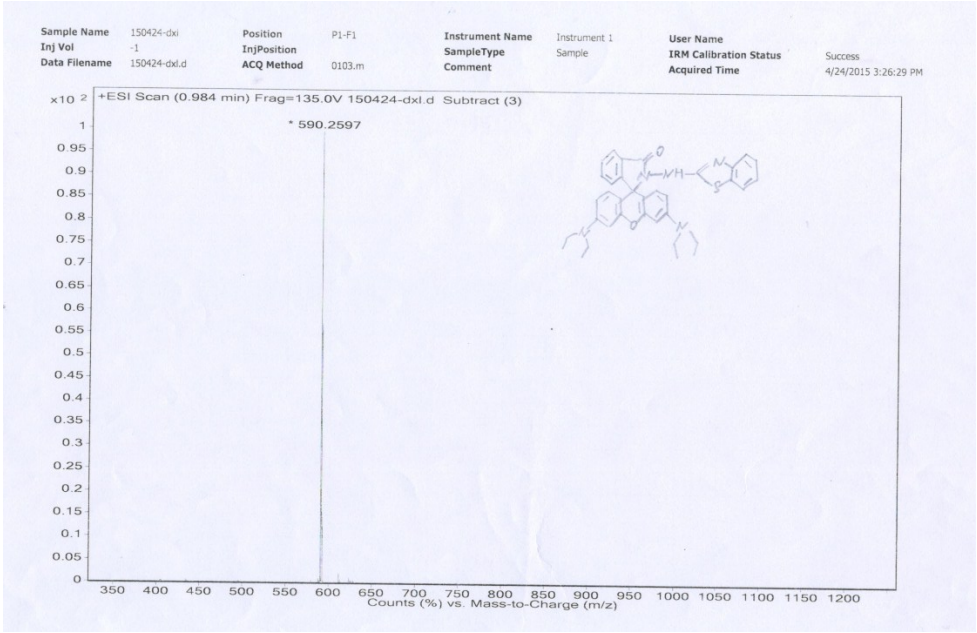


Fig. S10 HRMS of probe BRT

Table S1. A comparison about the detection limit for hypochlorite

Method [Ref.]	Fluorescence	Solvent	Detection limit
[22]	Turn on	HEPES, pH 7.0	2.0×10^{-8} M
[23]	Turn on	phosphate buffer pH 7.8	3.0×10^{-7} M
[27]	Turn on	CH_3CN/HEPES (6:4) pH 7.4	3.3×10^{-6} M
[29]	Turn on	DMSO/PBS buffer (4:6) pH 7.4	5.0×10^{-6} M
[39]	Turn on	DMF(40%)/phosphate buffer, pH 8.5	2.4×10^{-8} M
[40]	Turn on	CH_3CN/H_2O (1:1) pH 7.2	5.5×10^{-8} M

[24]	Turn off	PBS buffer pH 7.4	8.0×10^{-7} M
[26]	Turn off	CH_3CN/HEPES (1:1) pH 7.0	3.7×10^{-8} M
[30]	Turn off	MeOH/HEPES (3:1) pH 7.0	6.5×10^{-8} M
[32]	Turn off	THF/PBS (9:1) pH 7.4	3.4×10^{-7} M
This work	Turn on	MeCN/PBS (3:7) pH = 7.4	1.06×10^{-9} M

Table S2. Determination of OCl^- concentrations in natural water samples

Sample	OCl^- spiked (μM)	OCl^- recovered (μM)	Error
	17.50	17.35	0.008
Tap water	20.00	19.25	0.037
	22.50	22.15	0.015
	20.00	19.70	0.014
Daming Lake	22.50	23.55	0.048
	25.00	25.30	0.012
	20.00	20.65	0.033
Purified water	22.50	22.95	0.021
	25.00	24.55	0.018