Journal Name

COMMUNICATION

Double-mode detection of HClO by naked eye and concurrent fluorescence increasing in absolute PBS

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

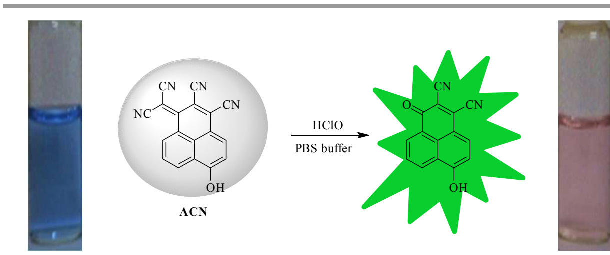
Beng Wang,‡[a] Jia Wen,‡[a] Kuo Gao,‡[b] Hui Yan, [a] Yongqian Xu,[a] Hongjuan Li,[a] Jianxin Chen,*[b] Wei Wang*[b] and Shiguo Sun*[a]

A water soluble fluorescent probe WCN was successfully designed and synthesized based on acenaphthenequinone. It can be employed for double-mode detection of HClO by naked eye and concurrent significant fluorescence increasing in absolute PBS. The detection limit is down to 77 pM. Especially, WCN works pretty well in both living cell and living mouse.

Reactive oxygen species (ROS) study is attracting more and more attention due to their essential roles in mediating a wide variety of biological events such as aging and immunity.[1] Among them, hypochlorous acid (HClO), normally produced by myeloperoxidase (MPO)-catalyzed per-oxidation of chloride ions in phagolysosome,[2] plays vital role in killing a wide range of pathogens in the innate immune system.[3] Unfortunately, uncontrolled production of HClO derived from phagocytes is involved in some diseases such as cardiovascular diseases, rheumatoid arthritis, and cancer etc.[4] Therefore, it is of great importance to develop methods for sensitive and selective detection of HClO/ClO^- for both disease diagnosis and exploration of its diverse pathophysiology.[5]

In recent years, a number of methods for detection of HClO/ClO^- have been developed.[6] Among them, synthetic fluorescent probes are generally superior in terms of high sensitivity, low cost, real-time detection and simple manipulation.[7] Typically, the design strategies are based on the specific reactions between recognition groups of the probes and HClO, which give highly fluorescent products. And the most common employed fluorophores are rhodamine,[8] fluorescein,[9] BODIPY[10] etc. Although many HClO/ClO^- fluorescent probes have been developed, most of them still have the drawbacks such as single-mode detection only, poor water solubility, relative complicated operating process, low sensitivity and selectivity etc.[11] Therefore, further study to develop double or even multi-mode detection probe together with much convenient operating for biological applications are still needed.

To fulfil this, a water soluble fluorescent probe **WCN** (Scheme 1) was successfully designed and synthesized based on acenaphthenequinone via one-pot synthetic strategy, which was fully characterized by ^{1}H NMR, HRMS (Fig. S10 - S12 in the Supporting Information). As expected, **WCN** exhibits a remarkable color change from blue to pink, together with a significant fluorescence increasing as soon as it encounters with HClO in absolute PBS, owing to the oxidative cleavage of the malononitrile in **WCN**.[12] This can be employed for double-mode detection of HClO by either naked eye or significant enhanced fluorescence.



Scheme 1 The proposed double-mode detection mechanism of HClO by **WCN**.

The water solubility of **WCN** was firstly confirmed in PBS (20 mM, pH=7.4) (Fig. S1). Next, the absorption properties of **WCN** (5 μM) were investigated in absolute PBS (Fig. S2). Next, fluorescence dynamics of **WCN** was recorded in PBS buffer (20 mM, pH 7.4) at room temperature. As shown in Fig. S3, a remarkable fluorescence increase was observed at 560 nm within 5 min and no further significant change occurred, demonstrating that **WCN** can be used to monitor HClO in real time. Thus, all of the following measurements were performed under the same conditions. To verify the sensitivity of **WCN** to HClO, it was treated with various concentrations of hypochlorite. Upon the addition of HClO, an immediate fluorescence increase was observed at 560 nm, and about 100-fold increasing was found on fluorescence intensity (Fig. 1a)

[a] College of Science, Northwest A&F University, Yangling, Shaanxi, 712100, China, E-mail: sunsg@nwsuaf.edu.cn
[b] Beijing University of Chinese Medicine, Beijing, 100029, China, E-mail: cjx@bucm.edu.cn; wangwei@bucm.edu.cn
† Electronic Supplementary Information (ESI) available: Experimental procedures and spectroscopic data for isolated compounds.
See DOI: 10.1039/x0xx00000x
‡ These authors contributed equally.

with the addition of 60 μM HClO. The fluorescence intensity at 560 nm was linearly related to the concentration of HClO added over the range of 0-60 μM (Fig. 1b), together with a lower detection limit (77 pM, LOD = 3 σ/S), indicating that **WCN** was quite suitable for accurate detection of HClO.

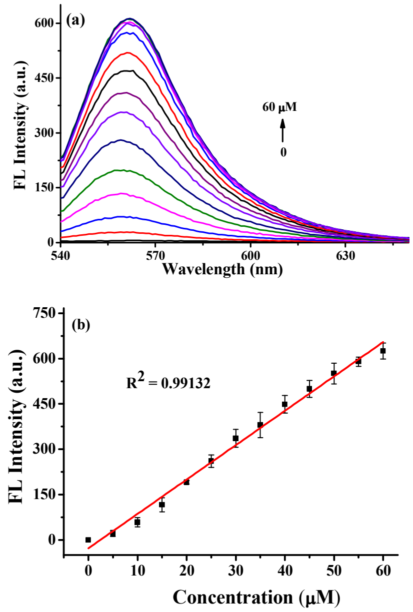


Fig. 1 Fluorescence titration studies of **WCN** upon addition of HClO. (a) Fluorescence spectra of **WCN** (5 μM) upon addition of HClO (0-70 μM) in PBS buffer (20 mM, pH 7.4) at room temperature. (b) The linear relationship between the fluorescent intensity and HClO concentration (0-60 μM). All data were collected 5 min after the addition of HClO. λ_{ex} =530 nm, λ_{em} = 560 nm. Error bars stand for the mean value of three experiments.

Meanwhile, **WCN** exhibited a remarkable color change from blue to pink (Fig. 2) along with the addition of HClO. Especially, good linear relationships were also reached between the absorbance of **WCN** at the wavelength of 595 nm, 645 nm and the concentration of HClO as shown in Fig. S4, demonstrating that **WCN** can be good candidate for double-mode detection of HClO by either naked eye or simultaneous enhanced fluorescence in absolute PBS.

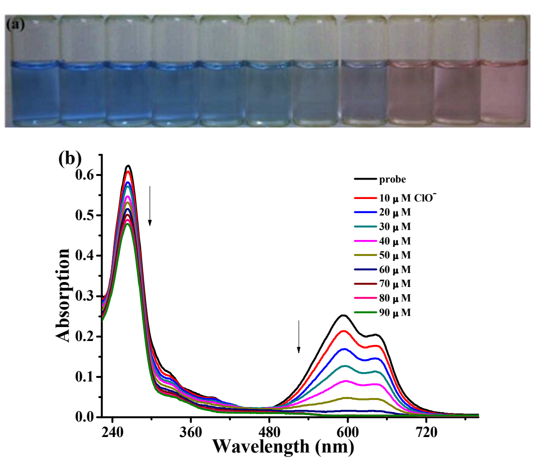


Fig. 2 (a) The color changes of **WCN** (5 μM) upon addition of various concentrations of HClO (from left to right: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 μM). (b) UV-vis spectra of **WCN** (10μM) upon the addition of increasing concentrations of sodium hypochlorite (0–90 μM) in PBS buffer (20 mM, pH 7.4).

Next, we examined the selectivity of **WCN** towards HClO over other oxidants and anions under simulated physiological conditions. **WCN** was incubated with HClO and the other relevant ROS and RNS including H_2O_2, $^1O_2$, •OH, $O_2^{\bullet-}$, TBHP (tert-butyl hydroperoxide), TBO• (tert-butoxy radical) and •NO (nitric oxide) respectively.[13] As shown in Fig. S5, almost no changes on fluorescence intensity was observed after the addition of excess ROS and RNS, demonstrating **WCN** has high selectivity towards HClO. Furthermore, the studies of some cations and anions such as Li^+, K^+, Cu^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, F^-, Cl^-, Br^-, I^-, ClO_4^-, NO_2^-, $S_2O_3^{2-}$ and SCN^- (100 μM for each) were also carried out (Fig. S6). As expected, none of these cations and anions exhibited any interference. The excellent selectivity for HClO over other analytes shows that **WCN** has potential applications for HClO detection in complex biological environments. All these can be attributed to the HClO induced specific oxidative cleavage of malononitrile in **WCN** as shown in Scheme 1, which was confirmed by ESI-MS (Fig. S11-S12).

Furthermore, the pH effect was also checked on the fluorescence of **WCN**. As shown in Fig. S7, **WCN** exhibited a stable performance with HClO over the pH value ranged from 1 to 10, suggesting that **WCN** is quite suitable for biological applications.

With these results in hand, W**CN** was applied to image HClO in Hela cells using a confocal laser microscope with the widely employed 488 nm laser excitation. Hela cells were incubated with **WCN** (5 μM) for 30 min at 37 ˚C in DMEM and washed three times with PBS to remove the excess **WCN**. Upon excitation at 488 nm, there was negligible intracellular green fluorescence at 540-620 nm (Fig. 3a-c). When NaClO (40 μM) was added and then incubated for another 30 min, intense fluorescence emerged in the green channel showing that **WCN** can detect HClO rapidly in living cells (Fig. 3d-f). MTT assay show that 5 μM **WCN** has no obvious cytotoxicity to the Hela cells (Fig. S9).

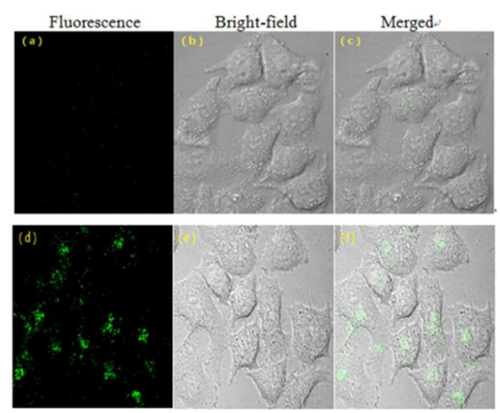


Fig. 3 Fluorescence, bright-field, and merged images of the **WCN**-loaded HeLa cells in the absence (a, b, c) and presence (d, e, f) of HClO (50 μM).

We next assessed the ability of our probe to visualize HClO in living mouse. The nude mouse was first subcutaneously injected with **WCN (**20 μM, 50 μL, PBS buffer**)**, and 5 min later, 10 equiv. HClO (in PBS buffer) was injected into the same region, and strong fluorescence was observed after 30 min (Figure 4b). In contrast, control nude mouse that was injected with saline only followed by the probe showed no significant fluorescence (Figure 4a). Thus, **WCN** is applicable for not only in vitro but also in vivo imaging of HClO.

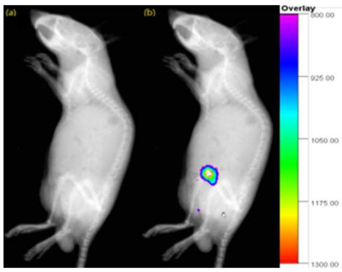


Fig. 4 Fluorescence images of living nude mice. Subcutaneous injection of the solution of **WCN** and then a solution of HClO (a) and PBS (b) were injected separately.

In summary, a water soluble fluorescent probe **WCN** was designed and synthesized based on acenaphthenequinone via one-pot synthetic strategy. **WCN** can not only detect HClO in absolute PBS by naked eye and enhanced fluorescence simultaneously but also work well for imaging living cell and living mouse. For more practical applications, the probe can be made into portable tool like test paper etc. Thus, this probe is expected to provide valuable reference for double and multi-mode investigation of HClO in the future.

All of the experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by Northwest A&F University.

This work was financially supported by the Scientific Research Foundation of Northwest A&F University (Z111021103 and Z111021107), the National Natural Science Foundation of China (No. 21472016, 21272030 and 21476185), State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University (No. 2013005).

Notes and references

1 (a) R. Noubade, K. Wong, N. Ota, S. Rutz, C. Eidenschenk, P.A. Valdez, J. Ding, I. Peng, A. Sebrell and P. Caplazi, *Nature*, 2014, **509**, 235; (b) M. Yu, X. Wu, B. Lin, J. Han, L. Yang and S. Han, *Anal. Chem*., 2015, **87**, 6688.

2 C.C. Winterbourn, *Toxicology*, 2002, **181–182**, 223.

3 (a) D. Lapenna and F. Cuccurullo, Gen. *Pharmacol.*, 1996, **27**, 1145; (b) Z. Prokopowicz, F. Arce, R. Biedron, C. Chiang, M. Ciszek, D. Katz, M. Nowakowska, S. apotoczny, J. Marcinkiewicz and B. Chain, *J. Immunol*., 2010, **184**, 824.

4 Y. Tang, D. Lee, J. Wang, G. Li, J. Yu, W. Lin and J. Yoon, *Chem. Soc. Rev.* 2015, **44**, 5003.

5 P. D. Ray, B.-W. Huang and Y. Tsuji, *Cell. Signal*., 2012, **24**, 981.

6 (a) Y. Koide, Y. Urano, K. Hanaoka, T. Terai and T. Nagano, *J. Am. Chem. Soc*., 2011, **133**, 5680; (b) Y. Lin, W. Y. Lin, J. Z. Song and Y. T. Yang, *Chem. Commun*., 2011, **47**, 12691; (c) G. Chen, F. Song, J. Wang, Z. Yang, S. Sun, J. Fan, X. Qiang, X. Wang, B. Dou and X. J. Peng, *Chem. Commun*., 2012, **48**, 2949; (d) Q. Xu, K.-A. Lee, S. Lee, K. M. Lee, W. J. Lee and J. Yoon, *J. Am. Chem. Soc*., 2013, **135**, 9944; (e) M. T. Sun, H. Yu, H. J. Zhu, F. Ma, S. Zhang and D. J. Huang, *Anal. Chem.,* 2014, **86**, 671; (f) H. Zhu, J. Fan, J. Wang, H. Mu and X. Peng, *J. Am. Chem. Soc.*, 2014, **136**, 12820; (g) H. Xiao, K. Xin, H. Dou, G. Yin, Y. Quan and R. Wang, *Chem. Commun.*, 2015, **51**, 1442.

7 (a) H. D. Xiao, K. Chen, N. N. Jiang, D. D. Cui, G. Yin, J. Wang and R. Y. Wang, *Analyst*, 2014, **139**, 1980; (b) P. Puangploy, S. Smanmoo and W. Surareungchai, *Sens. Actuat. B-Chem.*, 2014, **193**, 679; (c) R. Azadbakht and J. Khanabadi, *Chem. Commun*., 2013, **30**, 21; (d) J. Liu, G. Liu, W. Liu and Y. Wang, *Biosens. Bioelectron*., 2015,**64**, 300; (e) J. Kim and Y. Kim, *Analyst*, 2014, **139**, 2986; (f) T. Cheng, J. Zhao, Z. Wang, J. An, Y. Xu, X. Qian and G. Liu, *Dyes Pigments*, 2016, **126**, 218; (g) X. Wang, F. Song and X. Peng, *Dyes Pigments*, 2016, **125**, 89; (h) H. Xiao, J. Li, J. Zhao, G. Yin, Y. Quan, J. Wang and R. Wang, *J. Mater. Chem. B*, 2015, **3**, 1633.

8 (a) Y.-K. Yang, H. J. Cho, J. Lee, I. Shin and J. Tae, *Org. Lett.*, 2009, **11**, 859; (b) G. Shyamaprosad, D. Sangita, A. Krishnendu, K. N. Prasanta, G. Kakali, K. Q. Ching, B. Maitree, H.-K. Fun and H. A. Abdel-Aziz, *RSC Adv*., 2014, **4**, 24881.

9 Y. Zhou, J.-Y. Li, K.-H. Chu, K. Liu, C. Yao and J.-Y. Li, *Chem. Commun*., 2012, **48**, 4677.

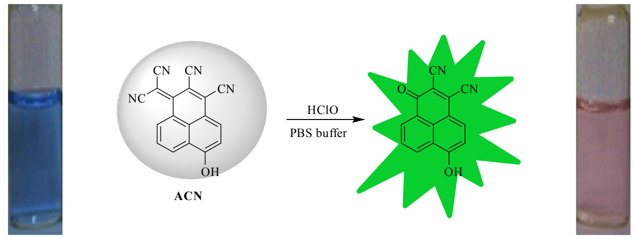
10 (a) T.-I. Kim, S. Park, Y. Choi and Y. Kim, *Chem. Asian J.* 2011, **6**, 1358; (b) S.-R. Liu and S.-P. Wu, *Org. Lett.*, 2013, **15**, 878.

11 (a) Y. R. Zhang, X. P. Chen, J. Shao, J. Y. Zhang, Q. Yuan, J. Y. Miao and B. X. Zhao, *Chem. Commun*., 2014, **50**, 14241; (b) M. Emrullahog˘lu, M. U¨ çu¨ncu¨ and E. Karakus, *Chem. Commun*., 2013, **49**, 7836; (c) F. N. Lu and T. Nabeshima, *Dalton Trans*., 2014, **43**, 9529.

12 H. D. Xiao, J. H. Li, J. Zhao, G. Yin, Y. W. Quan, J. Wang and R. Y. Wang, *J. Mater. Chem. B*, 2015, **3**, 1633.

13 (a) J. Zhao, H. J. Li, K. Yang, S. G. Sun, A. P. Lu and Y. Q. Xu, *New J. Chem*., 2014, **38**, 3371; (b) J. Y. Zha, B. Q. Fu, C. Q. Qin, L. T. Zeng and X. C. Hu, *RSC Adv*., 2014, **4**, 43110.

For TOC



A fluorescent probe **WCN** for double-mode detection of HClO by naked eye and concurrent significant fluorescence increasing in absolute PBS.