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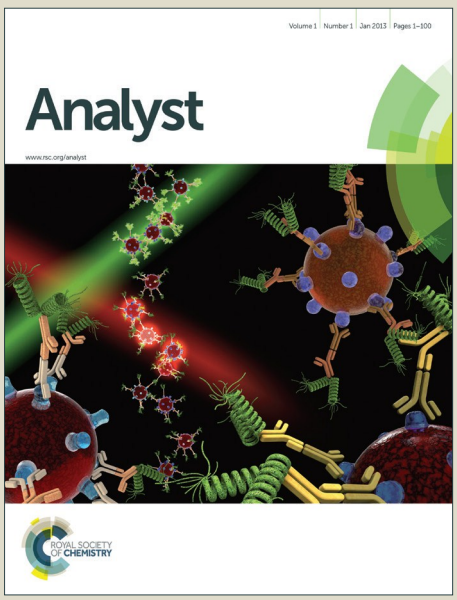





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ARTICLE

# Light Up $ClO^-$ in Live Cells Using an Aza-coumarin Based Fluorescent Probe with Fast Response and High Sensitivity

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Hypochlorous acid (HClO)/hypochlorite ($ClO^-$), one of reactive oxygen species (ROS), is a key microbicidal agent used for natural defense; however, HClO is also responsible for some human diseases. Although much effort has been made to develop HClO-selective fluorescent probes, many of them display a delayed response time and nanomole-sensitivie probes are rare. In this study, we designed and synthesized an aza-coumarin based fluorescent probe **AC-ClO** for the $ClO^-$ determination with fast response (completed within 2 min) and high sensitivity (detection limit is 25 nM). **AC-ClO** displayed a color change from pink to light yellow and a remarkable "turn-on" fluorescence response towards $ClO^-$. Confocal fluorescence microscopy experiments demonstrated that the probe could be applied for the live-cell imaging of exogenous and endogenous $ClO^-$.

## Introduction

Reactive oxygen species are emerging as critical signaling molecules[1-2] and play a crucial role in various physiological and pathological processes. Among the various ROS, hypochlorous acid (HClO)/hypochlorite ($ClO^-$), generated from $H_2O_2$ and $Cl^-$ by myeloperoxidase (MPO)[3-4], is a potent antimicrobial agent for the immune system[5]. However, as a result of the highly reactive and diffusible nature of HClO/$ClO^-$[6], its uncontrolled production within phagocytes is involved in a variety of human diseases such as cardiovascular disease and inflammatory disease[7-10]. Therefore, monitoring cellular HClO/$ClO^-$ concentration is significant for biological research and clinical diagnoses.

Because of its high spatial and temporal resolutions, fluorescence imaging technology is regarded as a promising method for monitoring biological species in living cells[11-22]. The designed strategies for HClO/$ClO^-$ fluorescent probes are based on specific reactions between recognition groups and HClO/$ClO^-$, affording highly fluorescent products[1, 23-45]. These HClO-reactive groups include *p*-methoxyphenol[34], dibenzoyl hydrazide[24], rhodamine-hydroxamic acid[35], selenide[30], and so on. These reactions can efficiently differentiate HClO/$ClO^-$ from other ROS. Under the physiological condition, HClO/$ClO^-$ is highly reactive, short-lived, and at low level (nanomole)[6, 46]. Thus, its detection with fast response and high sensitivity is desirable for real-time monitoring of the fluctuation of HClO/$ClO^-$ in its cellular site of action[43]. Unfortunately, many of the reported HClO/$ClO^-$ probes display a delayed response time[24-32] and nanomole-sensitive probes are rare[28, ]. Recently, Goswami *et al*[47] have developed a chemodosimeter **DPNO** for $ClO^-$ with fast response by the combination of 1,8-diaminonaphthalene and 2-hydroxynaphthalene aldehyde. Nevertheless, **DPNO** showed short emission wavelength (< 4 nm) and was not suitable for the bioimaging application.

In our previous study on a novel type of squarylium NIR absorbing dye, we found that part of the dye, 7-diethylamino-1,4-benzoxazin-2-one (aza-coumarin) showed good spectral properties[48-49]. This small molecule is not symmetrical, and the dipole moment in the excited state is much larger than in the ground state. The slight structure differences between coumarin and aza-coumarin make the latter have long absorption and emission wavelengths. In addition, it is known that the alkylation of the amino group in the coumarin dyes can shift their emission towards longer wavelength side. Keeping these in mind, we condensed 1,8-diaminonaphthalene with a 3-aldehyde-aza-coumarin dye in which diethylaniline group was replaced by julolidine, to construct a new $ClO^-$-selective fluorescent probe **AC-ClO**. It showed an obvious color change and a large fluorescence enhancement ($\lambda_{em}$ > 550 nm) in the presence of $ClO^-$. Importantly, **AC-ClO** can respond to $ClO^-$ rapidly (completed within 2 min) with high sensitivity (DL = 25 nM). Finally, **AC-ClO** was applied to detect $ClO^-$ changes in the presence of exogenous or endogenous $ClO^-$ in live MCF-7 and Raw 264.7 cells via confocal fluorescence imaging.

## Experimental

### Materials and methods

All materials used are without further purification and obtained from commercial suppliers. The purity and

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manufacture information are listed in Table S1. Lipopolysaccharides (LPS) and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich (USA). $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a VARIAN INOVA-400 spectrometer, using TMS as an internal standard. Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer. UV-visible spectra were collected on a Perkin Elmer Lambda 35 UV-Vis spectrophotometer. Fluorescence measurements were performed on a VAEIAN CARY Eclipse Fluorescence Spectrophotometer (Serial No. FL1109-M018). Excitation and emission slit widths were modified to adjust the fluorescence intensity to a suitable range. Slight pH variations in the solutions were achieved by adding the minimum volumes of NaOH or HCl (1 M). All pH measurements were made with a Model PHS-3C meter. The fluorescence imaging was performed with OLYMPUS FV-1000 inverted fluorescence microscope. All the data were repeated three times.

**Cell incubation**

MCF-7 cells and Raw 264.7 macrophages were cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen). Cells were seeded in 24-well flat-bottomed plates and incubated for 24 h at 37 °C under 5% $CO_2$. **AC-ClO** (5 μM) was added and cells were further incubated for 30 min, followed by washing thrice with phosphate-buffered saline (PBS). The fluorescence imaging was performed with OLYMPUS FV-1000 inverted fluorescence microscope. Under the confocal fluorescence microscope, **AC-ClO** was excited at 488 nm and emission was collected at 540-600 nm. For the detection of exogenous $ClO^-$, MCF-7 and Raw 264.7 cells were incubated with **AC-ClO** (5 μM, 30 min) and then NaClO (100 μM) was added. For the detection of endogenously produced $ClO^-$, Raw 264.7 macrophages were treated with different amounts of LPS (0-1000 ng/mL) for 12 h and PMA (1 μg/mL) for 15 min, followed by the addition of **AC-ClO** (5 μM) for 30 min.

**Synthetic procedures**

**Synthesis of compound 1.** To a DMF (20-30 mL) solution of 3-aminophenol (4.3 g, 40 mmol), 1-bromo-3-chloropropane (25.2 g, 160 mmol) and sodium bicarbonate (13.4 g, 160 mmol) were added. The reaction mixture was stirred at 70 °C for 12 hours. After the removal of ethanol under vacuum, the reaction mixture was extracted with EtOAc (150 mL), washed with water (3×150 mL) and dried over $MgSO_4$. The afforded syrup after evaporation was subjected to flash chromatography purification to give the product as a white solid **1** (2.7 g, yield: 45.3%). $^1$H NMR (400 MHz, MeOD) δ: 6.53 (d, *J* = 4Hz, 1H, CH), 6.05 (d, *J* = 4Hz, 1H, CH), 5.47 (s, 1H, OH), 3.03 (m, *J* = 4Hz, 4H, $CH_2$), 2.64 (m, *J* = 4Hz, 4H, $CH_2$), 1.95 (m, *J* = 4Hz, 4H, $CH_2$). TOF MS: m/z calcd for $C_{12}H_{16}NO^+$ $[M+H]^+$ 190.1154, found:190.1228.

**Synthesis of compound 2.** 5 g compound **1** (26 mmol) was dissolved in 15 mL hydrochloric acid and 10 mL water, then sodium nitrite 2.2 g (32 mmol) in 15 mL water was added dropwise at -5 °C. Then the mixture was stirred for 1 h at room temperature. The red brown solid was washed with 100 mL saturated sodium acetate solution and water (50 mL×2), then crude product **2** (red acicular crystal) was obtained without further purification (6.3 g, yield: 91.2%). TOF MS: m/z calcd for $C_{12}H_{15}N_2O_2^+$ $[M+H]^+$ 219.1154, found: 219.1233.

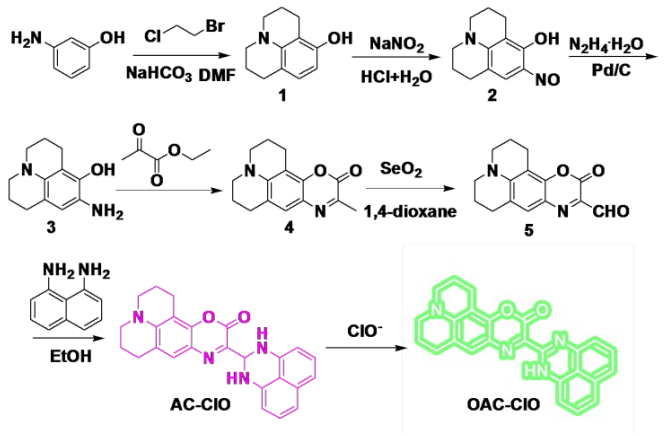
**Synthesis of compound 4.** Compound **3** was easily oxidized, and its fast degradation leads to highly-blue-colored product. To avoid the oxidative degradation, **3** was used readily for subsequent cyclization into compound **4**. 5 g (22.9 mmol) compound **2** was dissolved in 30 mL absolute ethanol, then 20 mL 85% hydrazine hydrate and 1 g palladium-carbon catalyst were added under nitrogen atmosphere at 35 °C. The mixture was refluxed for 3 h after the red color of solution vanished. 40 mL ethyl pyruvate was added and the mixture was refluxed for another 3 h. After the removal of ethanol under vacuum, the residue was purified by flash chromatography with petroleum ether/ethyl acetate = 3/1 as eluent to give the yellow powder **4** ( 4.3 g, yield: 72.9%). $^1$H NMR (400 MHz, $CDCl_3$), *δ*: 7.06 (s, 1H, CH), 3.26 (m, 4H, $CH_2$), 2.81 (t, *J* = 4Hz, 2H, $CH_2$), 2.78 (t, *J* = 4Hz, 2H, $CH_2$), 2.84 (t, *J* = 8Hz, 2H, $CH_2$), 2.45 (s, 3H, $CH_3$), 1.98 (m, *J* = 4Hz, 4H, $CH_2$). $^{13}$C NMR (400 MHz, $CDCl_3$), *δ*: 154.96, 145.54, 144.73, 144.23, 125.56, 122.37, 119.01, 106.05, 49.96, 59.57, 29.70, 27.47, 21.48, 20.59, 19.93. TOF MS: m/z calcd for $C_{15}H_{17}N_2O_2^+$ $[M+H]^+$ 257.1212, found: 257.1289.

**Synthesis of compound 5.** 300 mg (1.2 mmol) compound **4** and 200 mg $SeO_2$ were dissolved in 10 mL 1,4-dioxane, the mixture was stirred at 75 °C for 7 h. The solution turned from light yellow to deep red. After the removal of ethanol under vacuum, the residue was purified by flash chromatography with n-hexane/ethyl acetate = 1/1 as eluent to give the brownish-red powder **5** (210 mg, yield: 66.4%). $^1$H NMR (400 MHz, $CDCl_3$), *δ*: 10.07(s, 1H, CHO), 7.27 (s, 1H, CH), 3.44 (m, *J* = 4Hz, 4H, $CH_2$), 2.84 (m, *J* = 4Hz, 4H, $CH_2$), 2.02 (m, *J* = 4Hz, 4H, $CH_2$). $^{13}$C NMR (400 MHz, $CDCl_3$), *δ*: 186.81, 173.45, 156.86, 146.23, 135.01, 130.66, 128.98, 125.85, 119.68, 59.17, 27.20, 7.18. TOF MS: m/z calcd for $C_{15}H_{15}N_2O_3^+$ $[M+H]^+$ 257.1001, found: 257.1076.

**Synthesis of compound AC-ClO.** Compound **5** (180 mg, 0.7 mmol) and 1,8-diaminonaphthalene (200 mg, 1.3 mmol) were dissolved in absolute ethanol (15 mL) and refluxed overnight under nitrogen. Then, the mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol =10/1 as eluent to afford the product as a deep red solid (150.5 mg, 55%). $^1$H NMR (400 MHz, $CDCl_3$), *δ*: 7.44 (s, 1H, CH), 7.14 (t, *J* = 8Hz, 2H, CH), 7.08 (d, *J* = 8Hz, 2H, CH), 6.72(d, *J* = 8Hz, 2H, CH), 3.39(s, 4H, $CH_2$), 2.82(m, *J* = 4Hz, 4H, $CH_2$), 2.00(t, *J* = 4Hz, 4H, $CH_2$), . $^{13}$C NMR (400 MHz, $CDCl_3$), *δ*: 154.97, 149.77, 149.11, 145.89, 135.54, 129.22, 128.46, 124.59, 122.43, 121.83, 119.97, 105.35, 50.83, 50.38, 29.87, 29.49, 27.52, 21.10, 20.12, 19.68, 0.16. TOF MS: m/z calcd for $C_{25}H_{12}N_4O_2^+$ $[M]^+$ 409.1659, found: 409.1656.

## Results and discussion

The synthetic route of **AC-ClO** is shown in Scheme 1. Alkylation of 3-aminophenol with 1-bromo-3-chloropropane yielded intermediate **1**. After nitrosation, compound **2** was reduced by hydrazine hydrate, followed by the cyclization with ethyl pyruvate to form azo-coumarin fluorophore **3**. Then **3** was oxidized to the corresponding aldehyde **4** which was oxidized




Scheme 1 The synthesis and the proposed $ClO^-$ sensing mechanism of **AC-ClO**.

to the corresponding aldehyde **4** which was condensed with 1,8-diaminonaphthalene to probe **AC-ClO**. The structure of **AC-ClO** was confirmed by nuclear magnetic resonance (NMR) ($^1H$, $^{13}C$) spectroscopy and mass spectrometry.

We first investigated the sensing ability of **AC-ClO** towards $ClO^-$ in aqueous solution. Free **AC-ClO** showed an absorption peak at 505 nm. Upon addition of NaClO, the absorption at 505 nm decreased, whereas a new absorption peak appeared at 298 nm (Figure S1) which changed the color of the solution from pink to light yellow (Figure S2), allowing colorimetric detection of $ClO^-$ by the naked eyes. In the corresponding fluorescence spectra, free **AC-ClO** exhibited extremely weak fluorescence ($\Phi$ = 4.3×10$^{-4}$) due to the effective photoinduced electron transfer (PET) process from the N atoms of 1,8-diaminonaphthalene to the aza-coumarin fluorophore. While the emission intensities of **AC-ClO** at 576 nm increased remarkably with the addition of NaClO upon excitation at 480 nm (Figure 1a). After 36 μM of NaClO was added, these changes were found to reach a plateau where a 372-fold fluorescence intensity enhancement accompanied with an increase in the fluorescence quantum yield to 0.030 were obtained. In the high resolving mass spectrum of **AC-ClO**+$ClO^-$, one peak appeared at m/z 408.9558 which could be assigned as the corresponding dehydrogenated product [**OAC-ClO**+H]$^+$ (Figure S3). Furthermore, density functional theory (DFT) calculation demonstrated **OAC-ClO** bearing much higher $S_1 \leftrightarrow S_0$ transition oscillator strength (f) of 0.676 than that of **AC-ClO** (0.022), which verified the "turn-on" response of **AC-ClO** towards $ClO^-$ (Figure S4). A linear relationship was observed between the fluorescence intensity of **AC-ClO** and NaClO concentration in the range 0–5 μM (Figure 1b), and the detection limit was calculated as low as 25 nM ($3\sigma/k$). The time dependent fluorescent changes (at 576 nm) of **AC-ClO** with NaClO can be achieved within 2 minutes (Figure 1c) which is very important for real-time detection.

Selectivity is one of the most important requirements for all kinds of detection methods. The present probe was treated with a wide variety of ions and ROS to examine its selectivity. For the representative ions including $Na^+$, $K^+$, $Mg^{2+}$, $Zn^{2+}$, $Hg^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$, $HPO_4^{2-}$, $Cl^-$, $I^-$, $Br^-$, $OH^-$, $SO_4^{2-}$, $SO_3^{2-}$, $NO_3^-$, $CO_3^{2-}$, and $HCO_3^-$, **AC-ClO** showed nearly no change in the fluorescence spectra(Figure S5). After addition of various ROS

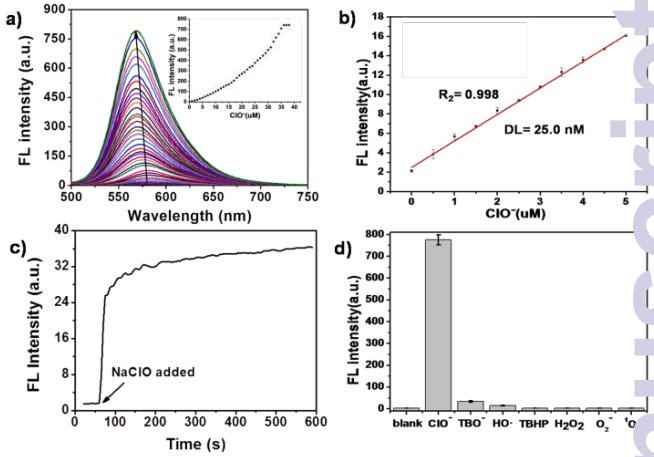



**Figure 1** a) The fluorescence spectra of probe **AC-ClO** (5 μM) upon the addition of NaClO (0–38 μM). Inset: the changes in the emission intensities at 576 nm with the increasing NaClO concentration. b) f **AC-ClO** (1 μM) change as a function of NaClO (0–5 μM). c) The time course of fluorescence intensity of **AC-ClO** (5 μM) at 576 nm after adding 3 equiv. NaClO, time range: 0–600 s. d) Fluorescence responses of **AC-ClO** (5 μM) towards NaClO (10 equiv.) and other ROS (100 equiv.). Conditions: PBS buffer (pH 10.0, 20 mM) and DMF (1/4, v/v). $\lambda_{ex}$ = 480 nm. Error bar = RSD (n =3).

including $ClO^-$, hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($HO^·$), singlet oxygen ($^1O_2$), superoxide ($O_2^{·-}$), tertbutyl hydroperoxide (TBHP) and tert-butoxy radical($TBO^·$), only $ClO^-$ caused a robust fluorescence enhancement (Figure 1d). The excellent selectivity of **AC-ClO** for over other analytes show that the present probe has potential applications for $Cl^-$ detection in complex biological environments. In addition, the effect of pH on the fluorescence response of probe **AC-ClO** to $ClO^-$ was also investigated. As shown in Figure S6, the fluorescence intensities of **AC-ClO** kept an almost constant and minimal value at a pH range of 2-14. After the addition of NaClO, the fluorescence intensity becomes significantly higher at a pH range of 7.6-11.9, indicating that the probe could be used under neutral or basic conditions. Given the fact that the pKa of HClO is around 7.6[50], probe **AC-ClO** detects $ClO^-$ rather than HClO.

Next, we evaluated **AC-ClO** in live-cell imaging assays utilizing an Olympus FV 1000-IX81 confocal microscope. After incubation of the MCF-7 and Raw 264.7 cells with 5 μM **AC-ClO** for 30 min at 37 °C, negligible intracellular green fluorescence at 540-600 nm was observed upon excitation at 488 nm (Figure S7b and Figure 2b). However, when NaClO was added, the intense fluorescence emerged in the green channel, indicating that **AC-ClO** can detect $ClO^-$ in live cells (Figure S7c and Figure 2c). The promising results obtained from the above fluorescence imaging study of externally added $ClO^-$ in the live cells encouraged us to evaluate the feasibility of **AC-ClO** for detecting endogenous $ClO^-$. Previous study suggested thatlipopolysaccharide (LPS) and phorbol 12-myristate 13-acetate (PMA) can stimulate Raw 264.7 cells to produce ClO$^-$. As discussed above, Raw 264.7 cells loaded with only **AC-ClO**

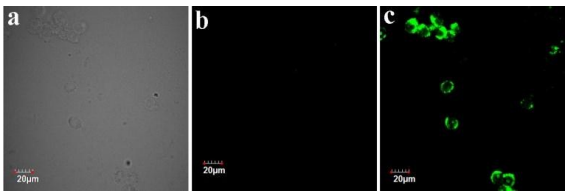



**Figure 2** Confocal fluorescence imaging of exogenous $ClO^-$ in RAW264.7 cells using probe **AC-ClO**. (a) Bright-field images of cells in panel. (b) Fluorescence images of RAW 264.7 cells loaded with **AC-ClO** (5 μM) at 37 ℃ for 30 min. (c) Fluorescence images of cells after treatment with probe **AC-ClO** (5 μM) for 30 min and subsequent treatment of the cells with 100 μM NaClO for 15 min at 37 $^{o}$C. Emission intensity were collected in an optical window 540-600 nm, $\lambda_{ex}$ = 488 nm. Scale bar = 20 μm.

displayed almost no fluorescence. After the cells were incubated with different amounts of LPS (0-1000 ng $mL^{-1}$) for 12 h and PMA (1 μg $mL^{-1}$) for 15 min, followed by the addition of probe **AC-ClO** (5 μM) for 30 min, fluorescence images were recorded. As shown in Figure 3, the emission intensities of **AC-ClO** enhanced as the increasing concentration of LPS. Taken together, these results indicate that **AC-ClO** was capable of detecting the changes in $ClO^-$ level in the presence of exogenous or endogenous $ClO^-$ in live cells via confocal fluorescence imaging.

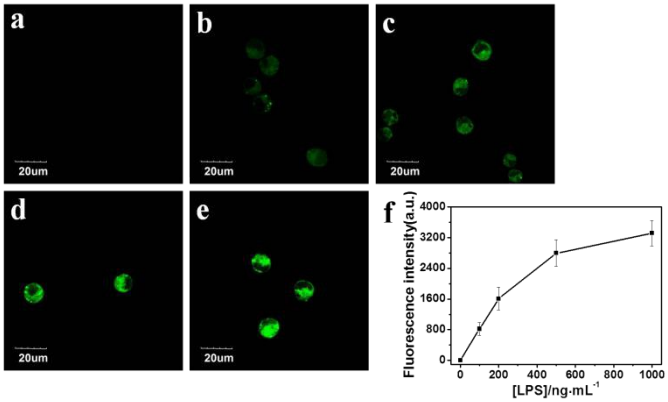



**Figure 3** Confocal fluorescence images in live Raw 264.7 cells of probe **AC-ClO** (5 μM) to different LPS concentration and and PMA (1 μg/mL) induced endogenous $ClO^-$. Insert: a) blank; b) 100 ng/mL; c) 200 ng/mL; d) 500 ng/mL; e) 1000 ng/mL; f) the relationship between the relative fluorescence intensity and the LPS concentrations. Emission intensity were collected in an optical window 540-600 nm, $\lambda_{ex}$ = 488 nm. Scale bar = 20 μm. Error bar = RSD (n = 3).

To evaluate cytotoxicity of probe **AC-ClO**, we performed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays in MCF-7 and RAW264.7 cells with 1, 5 and 10 μM probe **AC-ClO** for 24 h, respectively. The result clearly showed that our proposed probe was almost of no toxicity to cultured cells under the experimental conditions at the concentration of 10 μM for 24 h (Figure S8).

## Conclusions

In summary, by linking the 1,8-diaminonaphthalene moiety to the aza-coumarin fluorophore, we developed a new fluorescent probe **AC-ClO** for the selective detection of $ClO^-$ over other ROS and ions. **AC-ClO** showed a large fluorescence enhancement at 576 nm accompanied by a ratiometric color change from pink to light yellow in the presence of $ClO^-$ with fast response (completed within 2 min) and high sensitivity ($K_d$ = 25 nM). The sensing mechanism was verified by HRMS and DFT calculations. Living cell imaging experiments established the utility of this probe for the detection of exogenous and endogenous $ClO^-$. It is therefore believed that **AC-ClO** provides a promising chemical tool for the study of hypochlorite science in the biological systems.

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## Notes and references

1 S. Kenmoku, Y. Urano, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 7313-7318.
2 A. Manke, S. Luanpitpong, C. Dong, L. Wang, X. He, L. Batts, R. Derk, T. Stueckle, D. Porter, T. Sager, H. Gou, C. Dinu, Y. Wu, R. Mercer and Y. Rojanasakul, *Int. J. Mol. Sci.*, 2014, **15**, 7444-7461.
3 J. E. Harrison and J. Schultz, *J. Biol. Chem.*, 1976, **251**, 1371-1374.
4 C. C. Winterbourn, *J. Clin. Invest.*, 1986, **78**, 545-550.
5 D. Lapenna and F. Cuccurullo, *Gen. Pharmacol. Vasc. Syst.*, 1996, **27**, 1145-1147.
6 S. J. Klebanoff, *J. Leukocyte Biol.*, 2005, **77**, 598-625.
7 S. Hammerschmidt, N. Büchler and H. Wahn, *CHEST J.*, 2002, **121**, 573-581.
8 S. M. Wu and S. V. Pizzo, *Arch. Biochem. Biophys.*, 2001, **391**, 119-126.
9 T. Hasegawa, E. Malle, A. Farhood and H. Jaeschke, *Am. J. Physiol. Gastrointest. Liver. Physiol.*, 2005, **289**, G760-767.
10 S. Sugiyama, Y. Okada, G. K. Sukhova, R. Virmani, J. V. Heinecke and P. Libby, *Am. J. Pathol.*, 2001, **158**, 879-891.
11 H. Zhu, J. Fan, B. Wang and X. Peng, *Chem. Soc. Rev.*, 2015, doi: 10.1039/C4CS00285G.
12 J. Fan, M. Hu, P. Zhan and X. Peng, *Chem. Soc. Rev.*, 2013, **42**, 29-43.
13 Z. Xu, J. Yoon and D. R. Spring, *Chem. Soc. Rev.*, 2010, **39**, 1996-2006.
14 J. Du, M. Hu, J. Fan and X. Peng, *Chem. Soc. Rev.*, 2012, **41**, 4511-4535.
15 Q. Xu, P. Pu, J. Zhao, C. Dong, C. Gao, Y. Chen, J. Chen, Y. Liu and H. Zhou, *J. Mater. Chem. A*, 2015, **3**, 542-546.



 

16 K. Huang, C. Jiang and A. A. Martí, *J. Phys. Chem. A*, 2014, **118**, 10353-10358.
17 L. Zhu, Z. Yuan, J. T. Simmons and K. Sreenath, *RSC Adv.*, 2014, **4**, 20398-20440.
18 Z. Yu, R. M. Schmaltz, T. C. Bozeman, R. Paul, M. J. Rishel, K. S. Tsosie and S. M. Hecht, *J. Am. Chem. Soc.*, 2013, **135**, 2883-2886.
19 Z. Yu, T. Kabashima, C. Tang, T. Shibata, K. Kitazato, N. Kobayashi, M. K. Lee and M. Kai, *Anal. Biochem.*, 2010, **397**, 197-201.
20 T. Kabashima, Z. Yu, C. Tang, Y. Nakagawa, K. Okumura, T. Shibata, J. Lu and M. Kai, *Peptides*, 2008, **29**, 356-363.
21 J. Hu and C. J. Easley, *Analyst*, 2011, **136**, 3461-3468.
22 K. Sreenath, Z. Yuan, J. R. Allen, M. W. Davidson and L. Zhu, *Chem. Eur. J.*, 2015, **21**, 867-874.
23 X. Chen, X. Tian, I. Shin and J. Yoon, *Chem. Soc. Rev.*, 2011, **40**, 4783-4804.
24 X. Chen, X. Wang, S. Wang, W. Shi, K. Wang and H. Ma, *Chem. Eur. J.*, 2008, **14**, 4719-4724.
25 P. Panizzi, M. Nahrendorf, M. Wildgruber, P. Waterman, J.-L. Figueiredo, E. Aikawa, J. McCarthy, R. Weissleder and S. A. Hilderbrand, *J. Am. Chem. Soc.*, 2009, **131**, 15739-15744.
26 L. Yuan, W. Lin, J. Song and Y. Yang, *Chem. Commun.*, 2011, **47**, 12691-12693.
27 T.-I. Kim, S. Park, Y. Choi and Y. Kim, *Chem. Asian J.*, 2011, **6**, 1358-1361.
28 Y. Zhou, J.-Y. Li, K.-H. Chu, K. Liu, C. Yao and J.-Y. Li, *Chem. Commun.*, 2012, **48**, 4677-4679.
29 L. Yuan, W. Lin, Y. Xie, B. Chen and J. Song, *Chem. Eur. J.*, 2012, **18**, 2700-2706.
30 Z. Lou, P. Li, Q. Pan and K. Han, *Chem. Commun.*, 2013, **49**, 2445-2447.
31 Z. Lou, P. Li, P. Song and K. Han, *Analyst*, 2013, **138**, 6291-6295.
32 F. Liu, T. Wu, J. Cao, H. Zhang, M. Hu, S. Sun, F. Song, J. Fan, J. Wang and X. Peng, *Analyst*, 2013, **138**, 775-778.
33 J. Shepherd, S. A. Hilderbrand, P. Waterman, J. W. Heinecke, R. Weissleder and P. Libby, *Chem. Biol.*, 2007, **14**, 1221-1231.
34 Z.-N. Sun, F.-Q. Liu, Y. Chen, P. K. H. Tam and D. Yang, *Org. Lett.*, 2008, **10**, 2171-2174.
35 Y.-K. Yang, H. J. Cho, J. Lee, I. Shin and J. Tae, *Org. Lett.*, 2009, **11**, 859-861.
36 Y. Koide, Y. Urano, K. Hanaoka, T. Terai and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 5680-5682.
37 G. Chen, F. Song, J. Wang, Z. Yang, S. Sun, J. Fan, X. Qiang, X. Wang, B. Dou and X. Peng, *Chem. Commun.*, 2012, **48**, 2949-2951.
38 Q. Xu, K.-A. Lee, S. Lee, K. M. Lee, W.-J. Lee and J. Yoon, *J. Am. Chem. Soc.*, 2013, **135**, 9944-9949.
39 G. Cheng, J. Fan, W. Sun, K. Sui, X. Jin, J. Wang and X. Peng, *Analyst*, 2013, **138**, 6091-6096.
40 X. Wu, Z. Li, L. Yang, J. Han and S. Han, *Chem. Sci.*, 2013, **4**, 460-467.
41 S. T. Manjare, J. Kim, Y. Lee and D. G. Churchill, *Org. Lett.*, 2013, **16**, 520-523.
42 S.-R. Liu and S.-P. Wu, *Org. Lett.*, 2013, **15**, 878-881.
43 H. Zhu, J. Fan, J. Wang, H. Mu and X. Peng, *J. Am. Chem. Soc.*, 2014, **136**, 12820-12823.
44 X. Wang, X. Wang, Y. Feng, M. Zhu, H. Yin, Q. Guo and X. Meng, *Dalton Trans.*, 2015, **44**, 6613-6619.
45 J. Li, F. Huo and C. Yin, *RSC Adv.*, 2014, **4**, 44610-44613.
46 Y. Aratani, H. Koyama, S.-i. Nyui, K. Suzuki, F. Kura and [illegible] Maeda, *Infect. Immun.*, 1999, **67**, 1828-1836.
47 S. Goswami, A. Manna, S. Paul, C. Kheng Quah and H.-K. Fun, *Chem. Commun.*, 2013, **49**, 11656-11658.
48 M. Hu, J. Fan, H. Li, K. Song, S. Wang, G. Cheng and X. Peng, *Org. Biomol. Chem.*, 2011, **9**, 980-983.
49 J. Fan, W. Sun, M. Hu, J. Cao, G. Cheng, H. Dong, K. Song, [illegible] Liu, S. Sun and X. Peng, *Chem. Commun.*, 2012, **48**, 811[illegible] 8119.
50 J. C. Morris, *J. Phys. Chem.*, 1966, **70**, 3798-3805.
51 S. E. Gomez-Mejiba, Z. Zhai, M. S. Gimenez, M. T. Ashby, J. Chilakapati, K. Kitchin, R. P. Mason and D. C. Ramirez, *J. Biol. Chem.*, 2010, **285**, 20062-20071.