Supporting Information for

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

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Materials information

Materials	Manufacture information	Purity
3-aminophenol	Sinopharm Chemical Reagent Co.,Ltd	≥99%
1-bromo-3-chloropropane	Tianjin Kemiou Chemical Reagent Co.	≥99%
sodium nitrite	Tianjin Kemiou Chemical Reagent Co.	99%
palladium-carbon	Aladdin Chemical Reagent Co.	85%Pd
hydrazine hydrate	Tianjin Kemiou Chemical Reagent Co.	85%
ethyl pyruvate	Energy Chemical Reagent Co.	99.5%
SeO ₂	Sinopharm Chemical Reagent Co.,Ltd	97%
1,8-diaminonaphthalene	Energy Chemical Reagent Co.	99%

Table S1 Purity and manufacture information of the materials used

All the solvents used are dehydration by dewatering equipment obtained from the Securall Safes Office.

Determination of the detection limit

The detection limit was calculated based on the fluorescence titration curve (Figure 1b) of AC-CIO in the presence of NaClO (0-5 μ M). The fluorescence intensity of AC-CIO was measured by three times and the standard deviation of blank measurement was achieved. The detection limit was calculated by using detection limit was calculated with the following equation:

Detection limit = $3\sigma/k$

Where σ is the standard deviation of the blank measurement, k is the slope between the

fluorescence ratios versus NaClO concentration.

Determination of quantum yields

The fluorescence quantum yields of **AC-ClO** and **OAC-ClO** were determined according to the method below¹.

$$\varphi_u = \frac{(\varphi_s)(FA_u)(A_s)(\lambda_{exs})(\eta_u^2)}{(FA_s)(A_u)(\lambda_{exu})(\eta_s^2)}$$

Where φ is fluorescence quantum yield; FA is integrated area under the corrected emission

spectra; A is the absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the subscripts u and s refer to the unknown and the standard, respectively. We chose rhodamine B as standard, which has a fluorescence quantum yield of 0.49 in ethanol².



Figure S1 Absorption spectra of AC-ClO (5 $\mu M)$ upon the titration of NaClO (0-38 $\mu M)$ in a

mixture of PBS buffer (pH 10.0, 20 mM) and DMF (1/4, v/v)



Figure S2 The colorimetric detection of ClO⁻ (0, 10, 20, 30, 40, 50 μ M) by naked eyes with AC-ClO (5 μ M).



Figure S3 MS spectra of OAC-ClO.



Figure S4 HOMO-LUMO energy levels and interfacial plots of the orbitals for AC-CIO and

OAC-CIO with the calculated percentage of transition.



Figure S5 Fluorescence responses of the 5 μ M AC-CIO in the presence of ClO⁻ (10 equiv.) or other ions (10 equiv.) and the corresponding photographs inset in figure(1. control 2. ClO⁻ 3. S₂O₃²⁻ 4. Cl⁻ 5. Na⁺ 6. SO₄²⁻ 7. K⁺ 8. HCO₃⁻ 9. Mg²⁺ 10. NO₃⁻ 11. Hg²⁺ 12. Fe³⁺ 13. Co²⁺ 14. HPO₄²⁻ 15. I⁻ 16. Br⁻ 17. OH⁻ 18. SO₃²⁻ 19. Zn²⁺ 20. CO₃²⁻).



Figure S6 Fluorescence intensity of **AC-ClO** (5 μ M) in the absence and presence of 3 equiv. ClO⁻ in DMF-PBS (20 mM, 4/1, v/v) solution with different pH conditions (λ_{ex} =480 nm).



Figure S7 Confocal fluorescence images of MCF-7 cells. Cells incubated with probe **AC-ClO** (5 μ M) for 30 min (top); images of cells after treatment with probe **AC-ClO** (5 μ M) for 30 min and subsequent treatment of the cells with 100 μ M NaClO for 15 min (bottom). (a and d) Bright-field images of the MCF-7 cells in samples; (b and e) green emission (540–600 nm); (c) overlay image of (a and b); (f) overlay image of (d and e). λ_{ex} =488 nm. Scale bar=20 μ m.



Figure S8 Cytotoxicity assays of probe **AC-ClO** at 1 μ M $_{\sim}$ 5 μ M and 10 μ M against MCF-7 cells and RAW264.7 cells for 24 h. Error bar = RSD (n = 3).







Figure S10 ¹HNMR of compound 4.



Figure S11 ¹³CNMR of compound 4.







Figure S13 ¹³CNMR of compound 5.



Figure S14 ¹HNMR of AC-ClO.



Figure S15¹³CNMR of AC-CIO.

Reference

- 1 T. Karstens and K. Kobs, J. Phys. Chem., 1980, 84, 1871-1872.
- 2 K. G. Casey and E. L. Quitevis, J. Phys. Chem., 1988, 92, 6590-6594.