Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information

A highly water-soluble and specific BODIPY-based fluorescent probe for hypochlorite detection and cell imaging

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Contents:

1. ^{1}H NMR spectra.

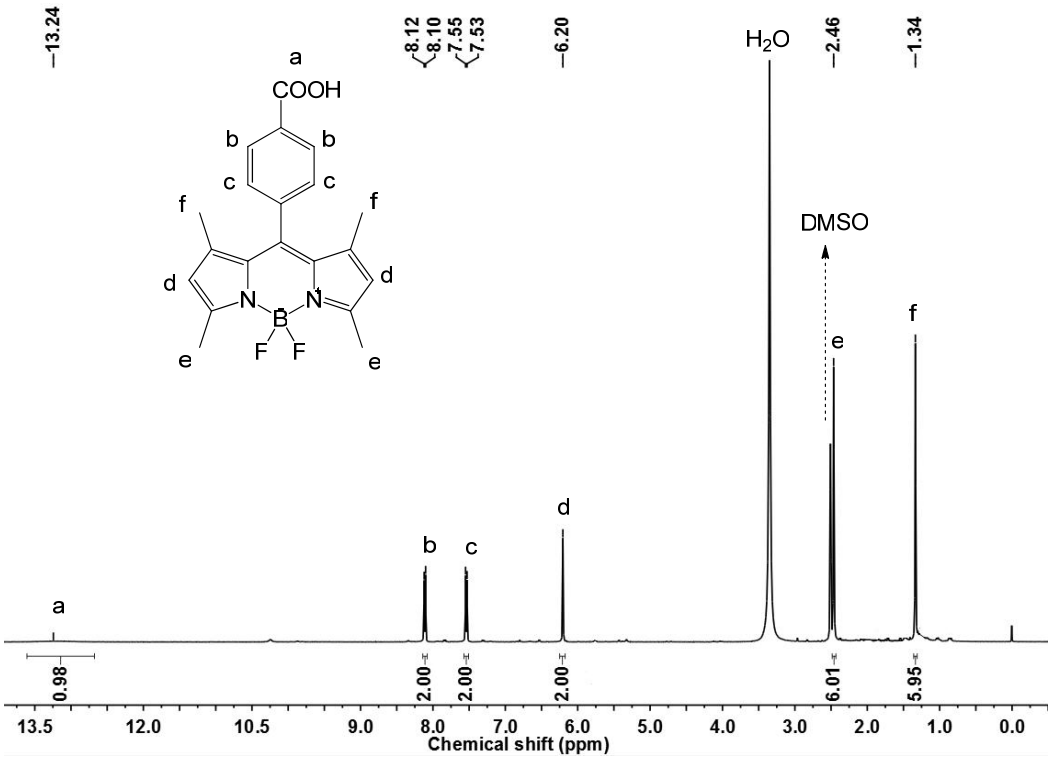


Figure S1. ^{1}H NMR spectrum of **BOD-COOH**.

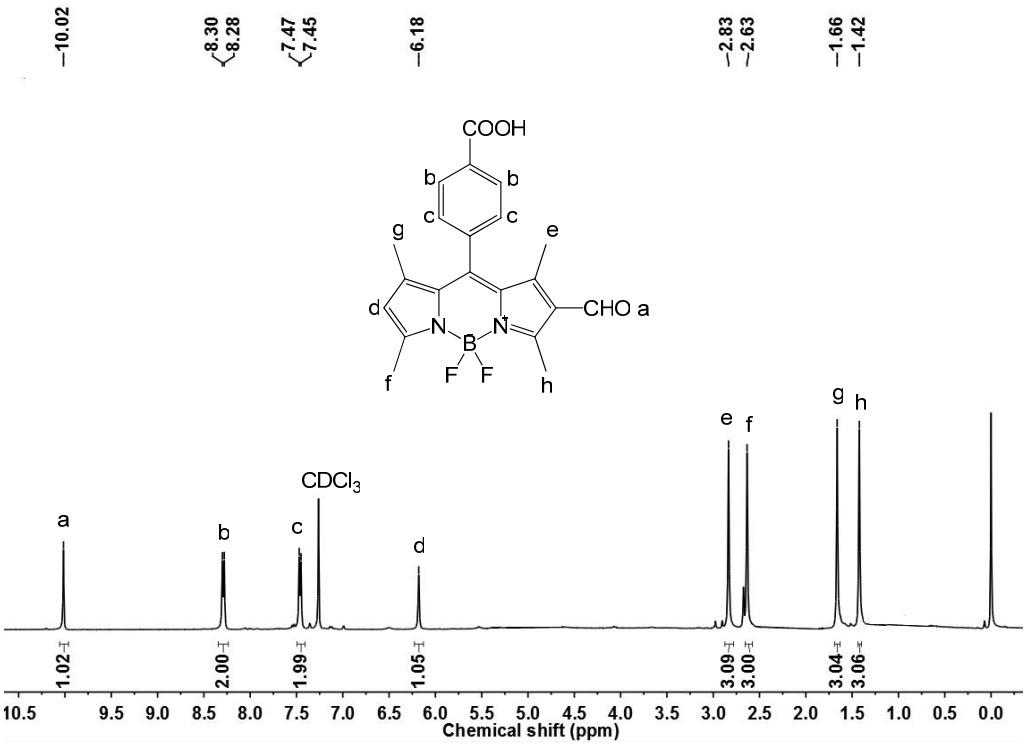


Figure S2. ^{1}H NMR spectrum of **BOD-CHO**.

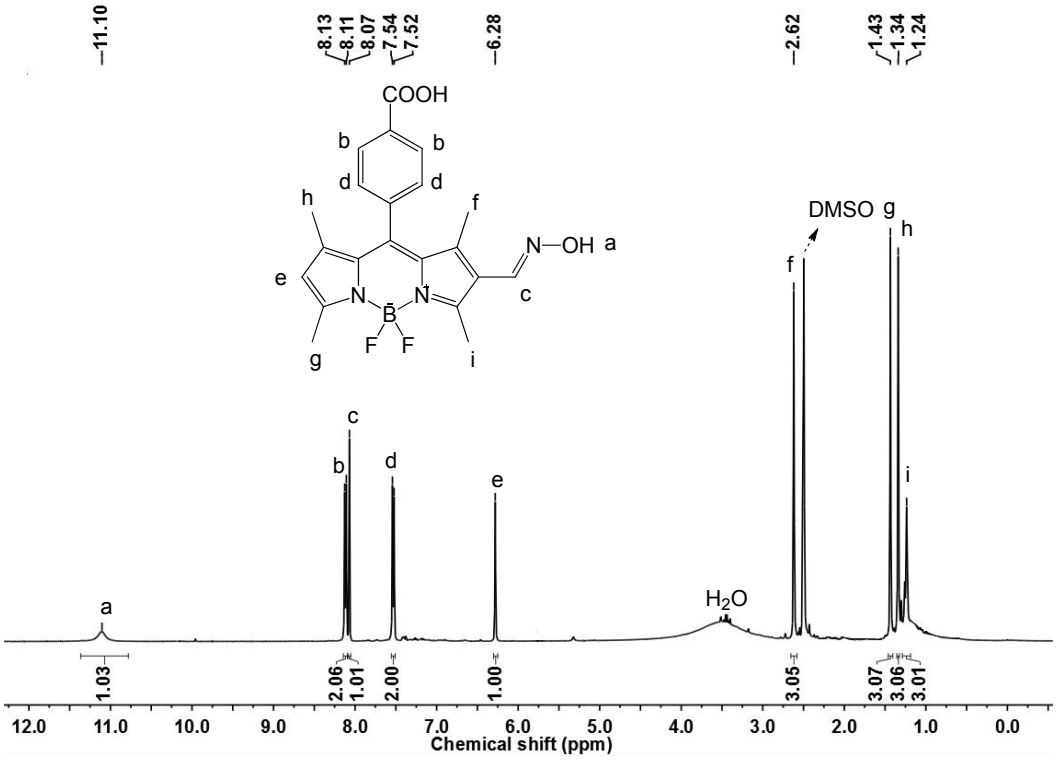


Figure S3. ^{1}H NMR spectrum of **BOD-OXIME.**

2. Mass spectra (ESI).

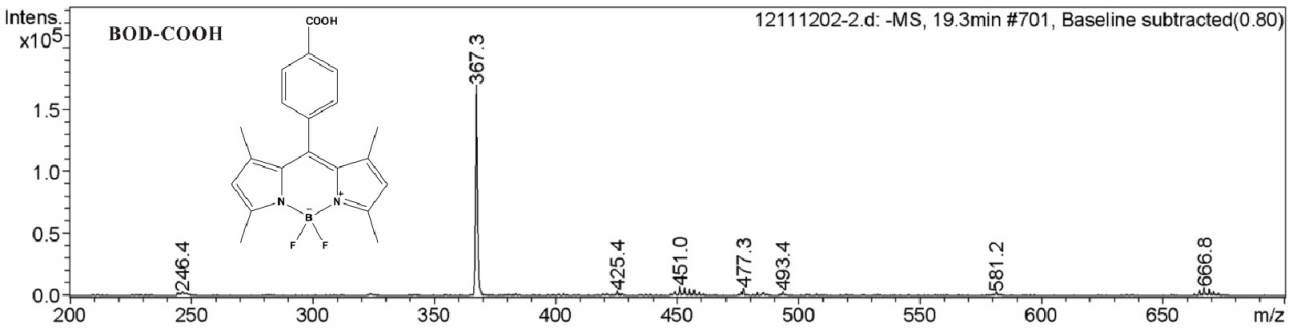


Figure S4. MS (ESI) spectrum of **BOD-COOH**.

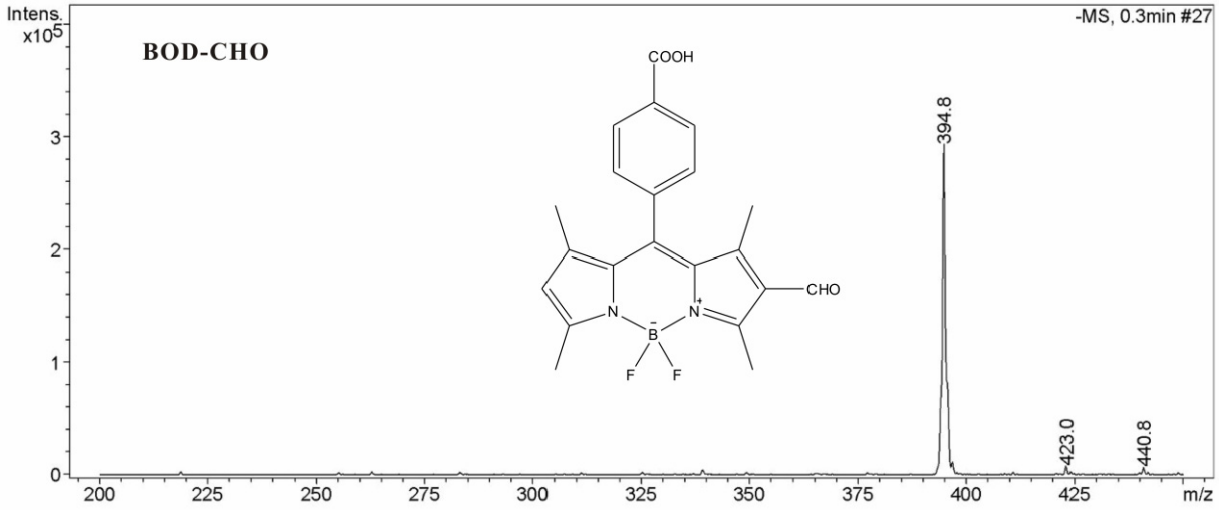


Figure S5. MS (ESI) spectrum of **BOD-CHO**.

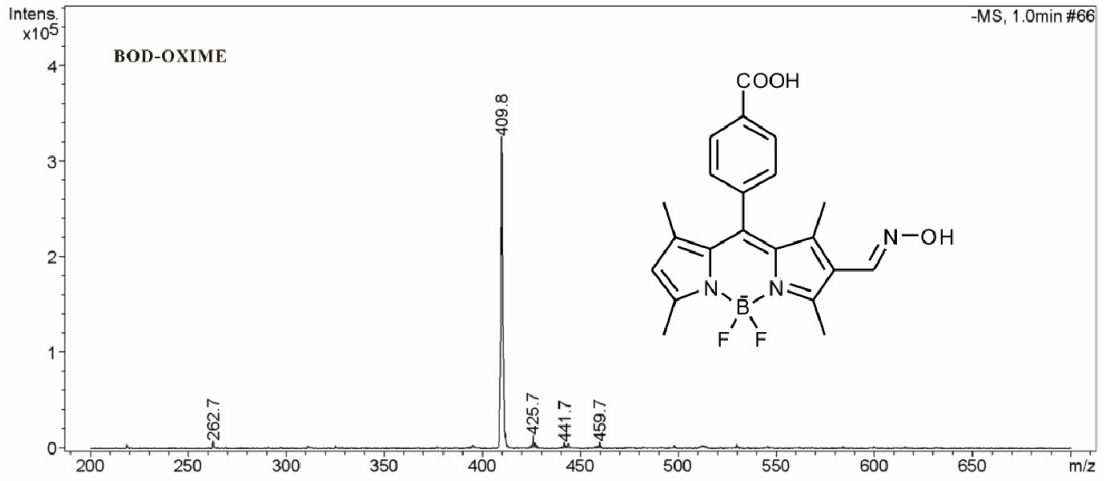


Figure S6. MS (ESI) spectrum of **BOD-OXIME**.

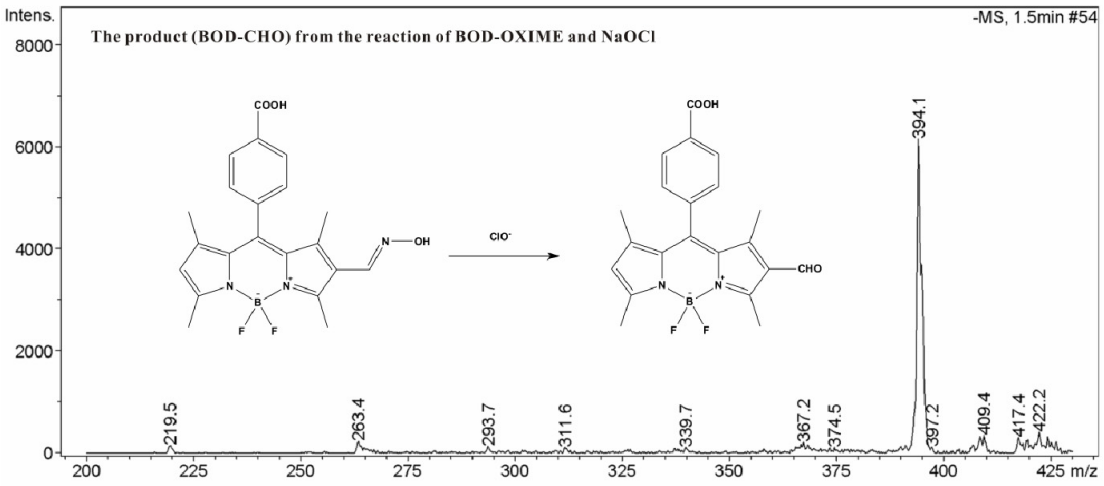


Figure S7. MS (ESI) spectrum for the product from the reaction of **BOD-OXIME** and NaOCl

3. Quantum Yields.

Quantum yields were determined using Rhodamine B in ethanol as a standard (Φ_{ST} = 0.49 in ethanol[1]). Non-degassed, spectroscopic grade ethanol and a 10 mm quartz cuvette were used. In order to minimize reabsorption effects, only dilute solutions with an absorbance below 0.1 at the excitation wavelength λ_{ex} were used in the measurements. All spectra were recorded at room temperature on non-degassed samples. The quantum yield was calculated according to the equation: $\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta^2_X}{\eta^2_{ST}}\right)$; Where the subscripts ST and X denote standard and test respectively, Φ is the fluorescence quantum yield, ***Grad*** the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and η the refractive index of the solvent. Both **BOD-OXIME** and **BOD-CHO** were dissolved in 0.1M PBS buffer (pH=7.4).
Quantum yield of **BOD-OXIME**: Φ = 0.96. Quantum yield of **BOD-CHO**: Φ = 0.04

[1] Kelly G. Casey, Edward L. Quitevis, *J. Phys. Chem.*, 1988, **92**, 6590.

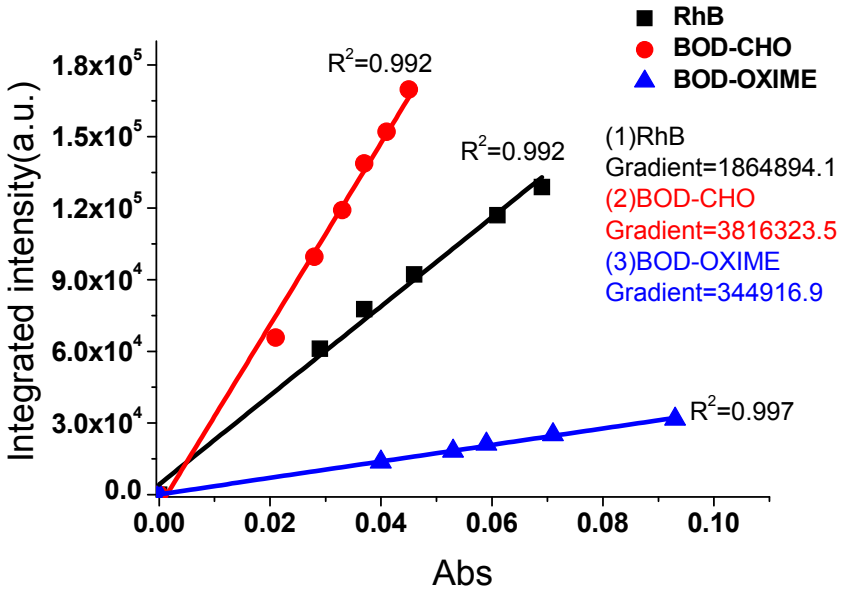


Figure S8. Integrated fluorescence intensity and absorbance of **RhB**, **BOD-CHO**, **BOD-OXIME**.

4. Detection limit.

The detection limit was calculated based on the fluorescence titration. The Probe **BOD-OXIME** concentration was 2 μM and the slit was adjusted to 5 nm/5 nm. The emission intensity of the probe **BD-OXIME** without ClO^- was measured by 5 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the ClO^- concentration could be obtained in the ClO^- concentration range of 0 - 6 μM (R=0.997).

The detection limit of **BOD-OXIME** was determined from the following equation: **Detection Limit = K × SD/S**, where K = 3; SD is the standard deviation of the blank solution; S is the slope of the calibration curve. Detection Limit = K × SD/S = 3 × 0.3099/1.7464 × 10^7 = 1.77× 10^{-8} M, namely, the detection limit is determined to be 17.7 nM at S/N = 3 (signal-to-noise ratio of 3).

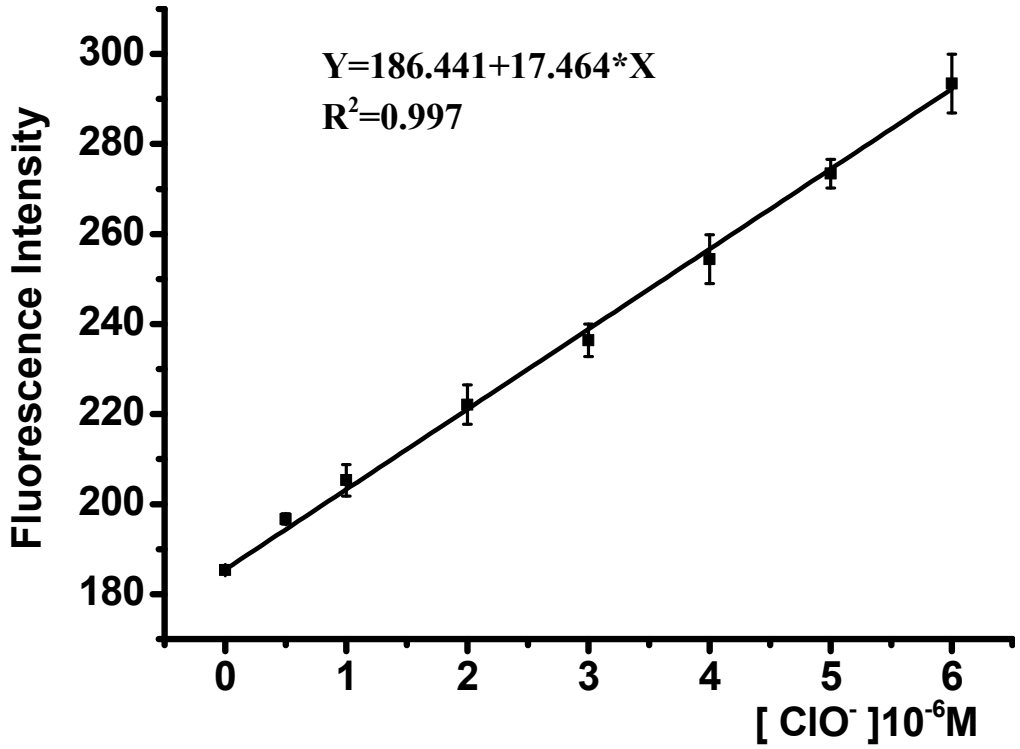


Figure S9. Fluorescence intensity of **BD-OXIME** against the hypochlorite concentration from 0 to 6μM in 0.1 M pH 7.4 PBS buffer. $\lambda_{ex}/\lambda_{em}$ = 500/525 nm.

5. Photostability

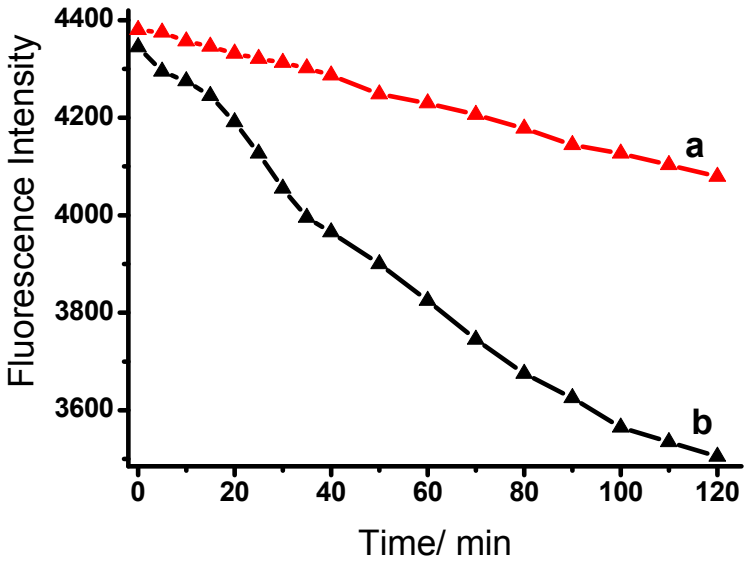


Fig. S10 Photostablility results for **BOD-OXIME** (10 μM) after addition of 100 μM NaClO (a) and 0.1 μM rhodamine B (b) in 0.1 M pH 7.4 PBS buffer by fluorescence spectrophotometer (λ_{ex}=500 nm, λ_{em}=525 nm for **BOD-OXIME** after addition of 100 μM NaClO; λ_{ex}=540 nm, λ_{em}=575 nm for rhodamine B).

As shown in this Fig. S10, for **BOD-OXIME**, small changes (<7%) of the fluorescence intensity was observed up to 2 h under continuous excitation on a fluorescence spectrophotometer, In contrast, emission of rhodamine B decreased by about 18% after 2 h of continuous excitation. These results indicate that, **BOD-OXIME** upon addition of ClO^- shows excellent photostability.

6. Effect of pH Values

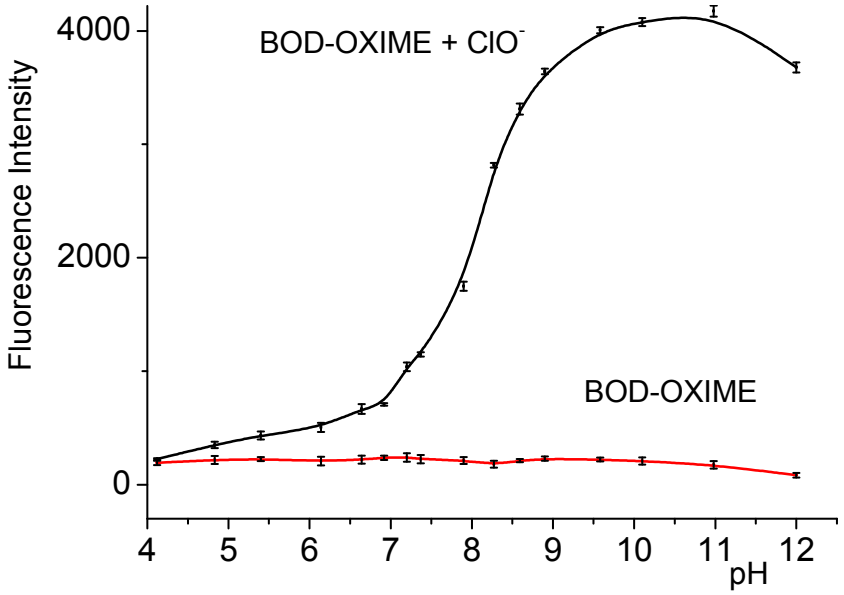


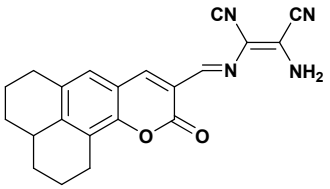
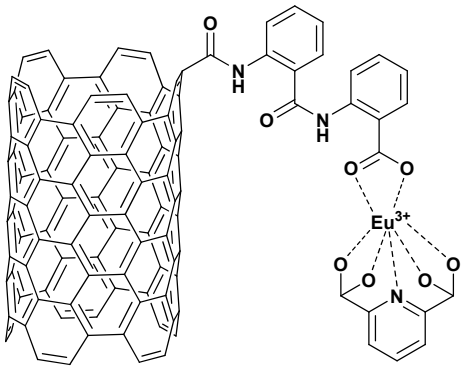
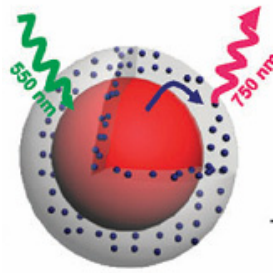
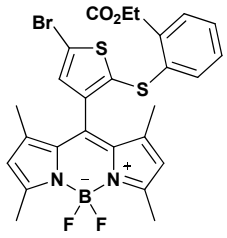
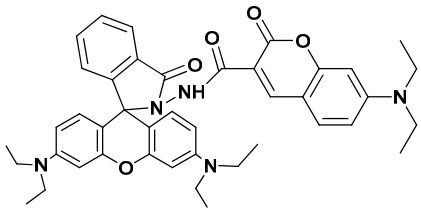
Fig. S11 Fluorescence response of free probe BOD-OXIME (2 μM) and after addition of ClO^- (50 μM) under different pH values (in Britton–Robinson buffer). λex/λem = 500/525 nm.

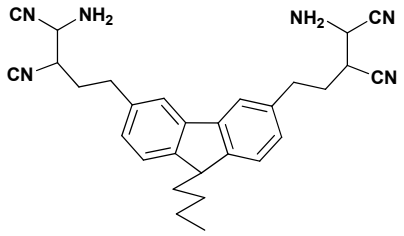
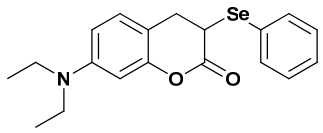
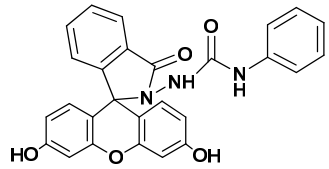
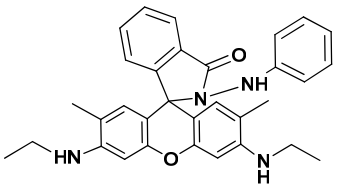
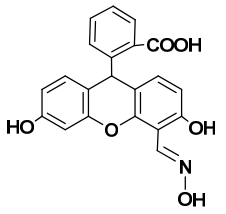
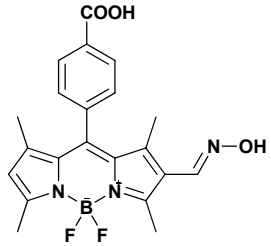
Britton-Robinson buffers were prepared by mixing 0.04 mol L^{-1} of H_3PO_4–CH_3COOH–H_3BO_3 solution and 0.2 mol L^{-1} of NaOH solution to the required pH value[2].

[2]H. T. S. Britton, R. A. Robinson, *J. Chem. Soc.*, 1931, **458**, 1456.

7. Comparison of the recently reported ClO^- fluorescent probes

Table S1. Comparison of the recently reported ClO^- fluorescent probes.

Probe	Detection limit	Response time	Comments
 *Chem. Commun.*, 2011, **47**, 12691	**0.2 μM**	**10 min**	**PBS/DMF (v/v, 2:8)** **I_{505}/I_{585}=235-fold** **Ratiometric fluorescent probe** **Living cell imaging(only exogenous ClO^-)**
 Analyst, 2012, **137**, 1872	**0.09 μM**	**1s**	**Pure water** **Fluorescent turn-off response**
 *Chem. Commun.*, 2012, **48**, 2949	**No data available**	**5 min**	**H_2O/EtOH (v/v, 4:1)** **I_{575}/I_{750}** **Ratiometric fluorescent sensor** **Living cell imaging(only exogenous ClO^-)**
 Analyst, 2013, **138**, 2829	**No data available**	**~4 min**	**Pure water** **Fluorescent turn-on response** **(I_{560})= 400-fold** **Living cell imaging(only exogenous ClO^-)**
 Anal. Chim. Acta., 2013, **775** ,100	**0.024 μM**	**1 min**	**0.1 M potassium phosphate buffer, pH 8.5, containing 40% DMF** **I_{578}/I_{501}= 3955-fold** **Ratiometric fluorescent probe**

Probe	Detection limit	Response time	Comments
 *Dalton Trans.*, 2013, **42**, 10097.	**1.07 μM**	**1 min**	**CH_3CN/H_2O (v/v, 4:6)** **I_{283}/I_{316}= 82-fold** **Ratiometric fluorescent probe**
 *Org. Lett.*, 2013, **15**, 2002.	**10 nM**	**within seconds**	**PBS buffer** **Fluorescent turn-on response** **Imaing both exogenous and endogenous ClO^- in living cells.**
 *Anal. Methods*, 2012, **4**, 4334	**0.33 μM**	**2 min**	**PBS buffer with less than 1% EtOH** **Fluorescent turn-on response** **(I_{528})=156 fold** **(Hg^{2+} induces ~80 fold enhancement at 530 nm)**
 *Anal. Methods*, 2012, **4**, 616	**5.0 nM**	**More than 60min**	**water** **Fluorescent turn-on response** **(I_{548})= 82 fold**
 *Chem. Commun.*, 2011, **47**, 11978	**No data available**	**Very fast**	**HEPES buffer (pH 7.05) containing 10% (v/v) DMSO** **Fluorescent turn-on response** **Living cell imaging(only exogenous ClO^-)**
 *This work* **BOD-OXIME**	**17.7 nM**	**Very fast (<1s)**	**PBS buffer** **Fluorescent turn-on response** **Imaing both exogenous and endogenous ClO^- in living cells.**