Supporting Information for

Rolling circle amplification-assisted DNA biosensors for sensitive and specific detection of hypochlorous acid and myeloperoxidase

Bo Liu [1,†], Jia-Yi Ma [1,†], Jing Wang [2], Dong-Xia Wang [1], An-Na Tang [1] and De-Ming Kong [1,*]

[1] State Key Laboratory of Medicinal Chemical Biology, Tianjin Key Laboratory of Biosensing and Molecular Recognition, Research Centre for Analytical Sciences, College of Chemistry, Nankai University, Tianjin 300071, China; nankai_lb@163.com (B.L.); 2120170751@mail.nankai.edu.cn (D.-X.W.); tanganna@nankai.edu.cn (A.-N.T.)

[2] School of Medical Laboratory, College of Medical Technology, Tianjin Medical University, Guangdong Road, Tianjin 300203, China; wj822@mail.nankai.edu.cn

* Correspondence: kongdem@nankai.edu.cn

† These authors contributed equally to this work.

Table of contents:

S1. Experimental Section

S1.1 Materials and reagents

Phi29 DNA polymerase, T4 DNA ligase, 10 × phi29 DNA polymerase reaction buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM $(NH_4)_2SO_4$, 40 mM DTT, pH 7.5), and 10 × T4 DNA ligase reaction buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP, pH 7.5) were purchased from New England Biolabs (Beijing, China). NaClO were purchased from Alfa Aesar (China). H_2O_2, L-cysteine, glucose and dopamine were purchased from Aladdin (Shanghai, China). Myeloperoxidase (MPO, human neutrophil) was purchased from Athens Research & Technology. Deoxyribonucleoside 5′-triphosphate mixture (dNTPs) was obtained from Tiangen Biotech (Beijing) Co., Ltd. Thioflavin T (ThT) was obtained from Sigma. 4-aminobenzoic acid hydrazide (4-ABAH), lipopolysaccharide (LPS), glutathione reductase (GSR) and diethyl pyrocarbonate water (DEPC) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Salicyl hydroxamic acid (SHA) and horseradish peroxidase (HRP) were purchased from Macklin. Phorbol 12-myristate 13-acetate (PMA) was purchased from MultiSciences (Lianke) Biotech Co., Ltd. Hypoxanthine, catalase (from bovine liver) and healthy human serum were purchased from Solarbio. Xanthione oxidase (XOD) was purchased from Nanjing Duly Biotech Co., Ltd. Tert-butyl hydroperoxide (TBHP) was purchased from Energy Chemical. MPO commercial kit was purchased from Shanghai Beibo Biotechnology Co., Ltd. Ultrapure water (resistance $\geq$ 18.2 MΩ·cm) was used throughout the experiments. All chemical reagents were of analytical grade and used without further purification.

S1.2 Apparatus

UV-Vis spectral measurements were performed on an Agilent Technologies Cary 60 UV/Vis spectrometer. pH values of corresponding solutions were measured by a Mettler-Toledo Delta 320 pH meter. All fluorescence measurements were carried out

on a Shimadzu RF-5301 PC fluorescence spectrometer (Shimadzu Ltd., Japan). Excitation and emission slits were set at 3.0 and 3.0 nm, respectively. Gel electrophoresis result was observed using a Gel Documentation System (Huifuxingye, Beijing, China).

S1.3 Preparation of ROS solutions

The concentrations of NaClO and H_2O_2 were measured by UV-Vis spectroscopy, based on their molar extinction coefficients at 290 nm (350 $M^{-1} \cdot cm^{-1}$) and 230 nm (81 $M^{-1} \cdot cm^{-1}$), respectively. Superoxide (O_2^-) was prepared with XOD, catalase and hypoxanthine according to the literature methods.[1] TBHP, LPS and PMA were prepared by diluting the stock solutions.

1. Srikun, D.; Miller, E. W.; Domaille, D. W.; Chang, C. J., An ICT-based approach to ratiometric fluorescence imaging of hydrogen peroxide produced in living cells. J. Am. Chem. Soc. 2008, 130 (14), 4596-4597.

S1.4 Electrophoresis characterization

S1.4.1 Site-specific cleavage of Primer-S

For HClO assay, 20 μL of samples containing 1 × hybridization buffer, 2.5 μM DNA (FAM-modified Primer-S, prepared in advance through incubation at 95 °C for 5 min and then at 25 °C for 30 min) and 5 μM HClO were prepared and incubated at 37 °C for 1 h. The samples were analyzed by 24% denatured PAGE in 0.5 × TBE buffer (44.5 mM Tris-boric acid, 1 mM EDTA, pH 8.3) at 180 V for 60 min, following by photographing with gel recording system based on the fluorescence of FAM under UV irradiation.

For MPO assay, 20 μL of samples containing 1 × NEBuffer2.1, 2.5 μM DNA (FAM-modified Primer-S, prepared in advance through incubation at 95 °C for 5 min and then at 25 °C for 30 min), 20 μM H_2O_2 and 1 μg/mL MPO were prepared and incubated at 37 °C for 15 min. The samples were analyzed by 24% denatured PAGE in

0.5 × TBE buffer, same as above.

S1.4.2 Hybridization between Primer-S and Padlock

20 μL of samples containing 1 × hybridization buffer, 5 μL 10 μM Primer-S (prepared in advance through incubation at 95 °C for 5 min and then at 25 °C for 30 min) and 5 μM HClO (or 10 μM H_2O_2 and 300 ng/mL MPO) were prepared and incubated at 37 °C for 30 min. After that, 5 μL Padlock (10 μM) and 1 × T4 DNA ligase reaction buffer were added, and the resultant reaction mixture were incubated at 16 °C for 2 h for the cyclization of Padlock. Then, 20 μL above solution was mixed with 2 μL 6 × Super GelRed Prestain loading buffer and analyzed by 10% non-denaturing PAGE in 0.5 × TBE buffer at 120 V for 50 min, following by photographing with gel recording system.

S1.4.3 RCA reaction

20 μL of samples, obtained from 100 μL RCA reaction systems in HClO or MPO detection, were mixed with 2 μL 6 × Super GelRed Prestain loading buffer and then analyzed by 10% non-denaturing PAGE in 0.5 × TBE buffer at 120 V for 50 min, following by photographing with gel recording system.

S1.5 MPO inhibitor assay

The experiment was the same as that for MPO detection, except that the mixture of 300 ng/mL MPO and different concentrations of 4-ABAH, which was pre-incubated at 37 °C for 10 min, was added in the sensing system instead of MPO.
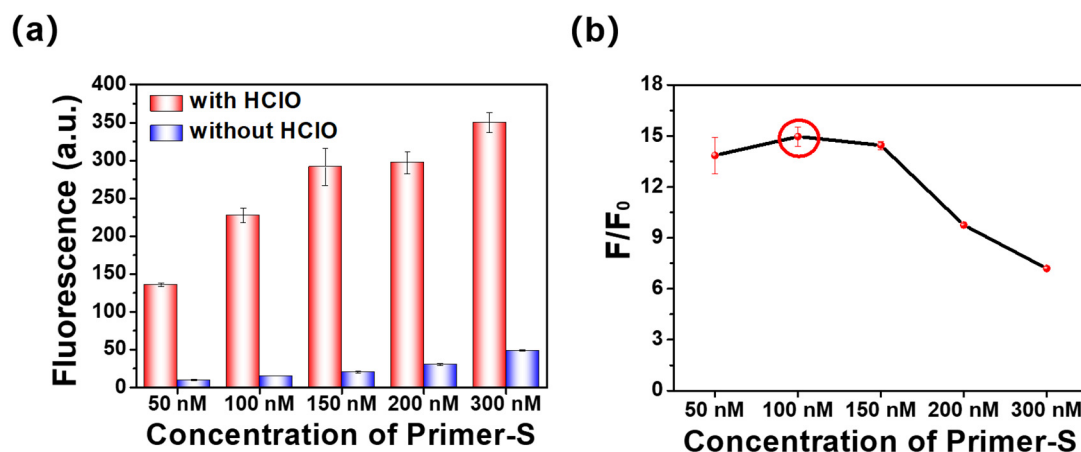
The relative activity (RA) of MPO was calculated according to the following equation:

RA = $(F_i - F_0)/(F_t - F_0) \times 100\%$

Where F_0, F_t, and F_i represent the fluorescence intensity without MPO, with 300 ng/mL MPO, with 300 ng/mL MPO and different concentrations of 4-ABAH.
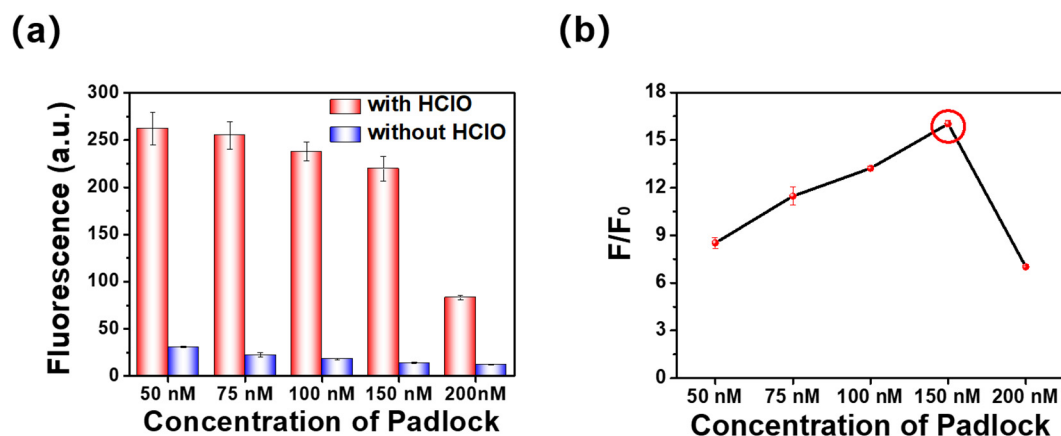
S2. HClO-sensing system
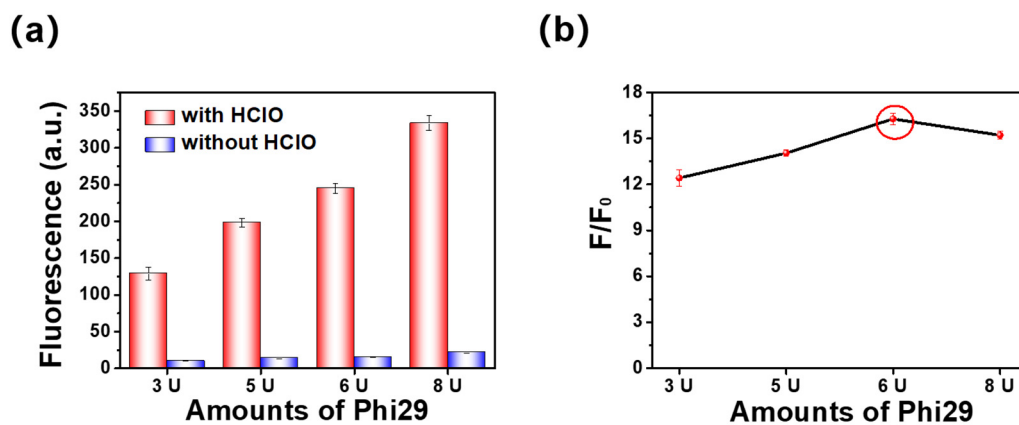
S2.1 Optimization of Primer-S concentration

(a)

(b)

Figure S1 Variance of (a) fluorescence intensity and (b) signal-to-noise ratio (F/F_0) with the Primer-S concentration. F and F_0 are the fluorescence intensities at 485 nm in the presence and absence of HClO, respectively. [HClO] = 3 μM.

S2.2 Optimization of Padlock concentration

(a)

(b)

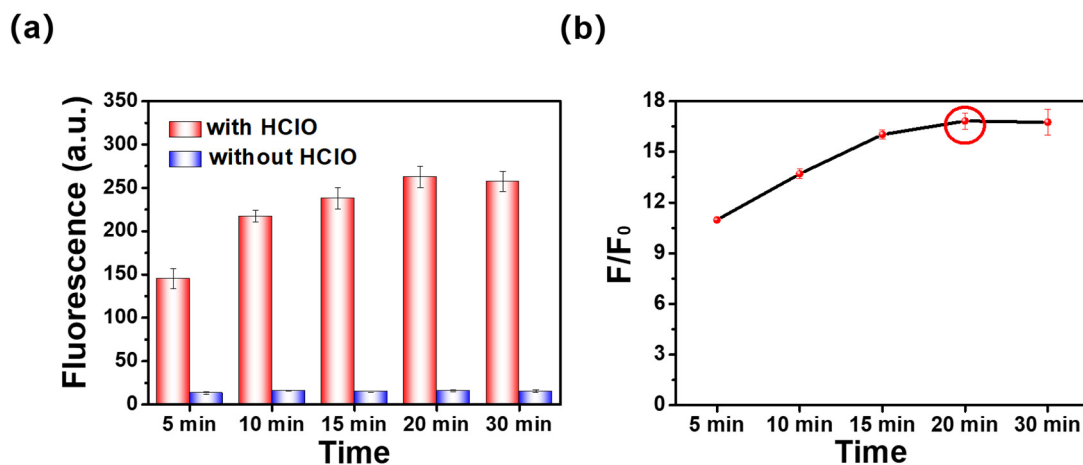**Figure S2** Variance of (a) fluorescence intensity and (b) signal-to-noise ratio (F/F_0) with the Padlock concentration. F and F_0 are the fluorescence intensities at 485 nm in the presence and absence of HClO, respectively. [HClO] = 3 μM.

S2.3 Optimization of Phi29 DNA polymerase amount

Figure S3 Variance of (a) fluorescence intensity and (b) signal-to-noise ratio (F/F_0) with the Phi29 DNA polymerase amount. F and F_0 are the fluorescence intensities at 485 nm in the presence and absence of HClO, respectively. [HClO] = 3 μM.

S2.4 Optimization of the reaction time for HClO-induced Primer-S cleavage

Figure S4 Variance of (a) fluorescence intensity and (b) signal-to-noise ratio (F/F_0) with the reaction time for HClO-induced Primer-S cleavage. F and F_0 are the fluorescence intensities at 485 nm in the presence and absence of HClO, respectively. [HClO] = 3 μM.

S2.5 Optimization of RCA reaction time

(a)

(b)
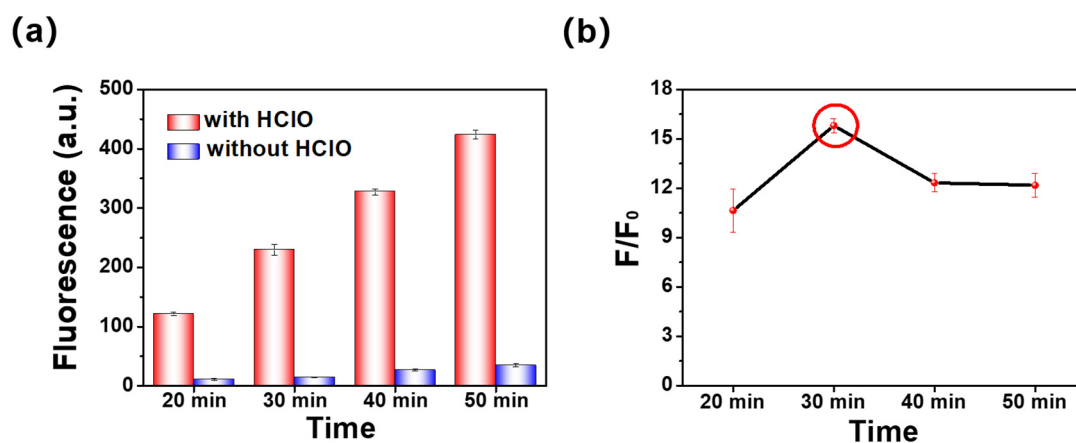
Figure S5 Variance of (a) fluorescence intensity and (b) signal-to-noise ratio (F/F_0) with the RCA reaction time. F and F_0 are the fluorescence intensities at 485 nm in the presence and absence of HClO, respectively. [HClO] = 3 μM.

S2.6 Comparison of our HClO biosensor with reported ones

Table S1. Comparison of several HClO detection methods.

Method	Limit of detection	Liner range	Reference
CD/CCM@ZIF-8	67 nM	0.1-50 μM	29
PC-UCNPs sensor	66.88 nM	0.1-5.0 μM	19
MOF-based nanoprobe	6.7 μM	15-180 μM	11
NIR fluorescent probe Cy7-NphS	0.62 μM	0-3.84 μM	30
TDN-HCR-based HClO sensor	0.8 nM	2.5-200 nM	24
CRISPR/Cas12a-based biosensor	0.33 μM	1-5 μM	31
RCA-assisted HClO biosensor	1.67 nM	5-5000 nM	This work

S2.7 HClO detection in real sample

Table S2. Recovery of HClO from human serum sample.

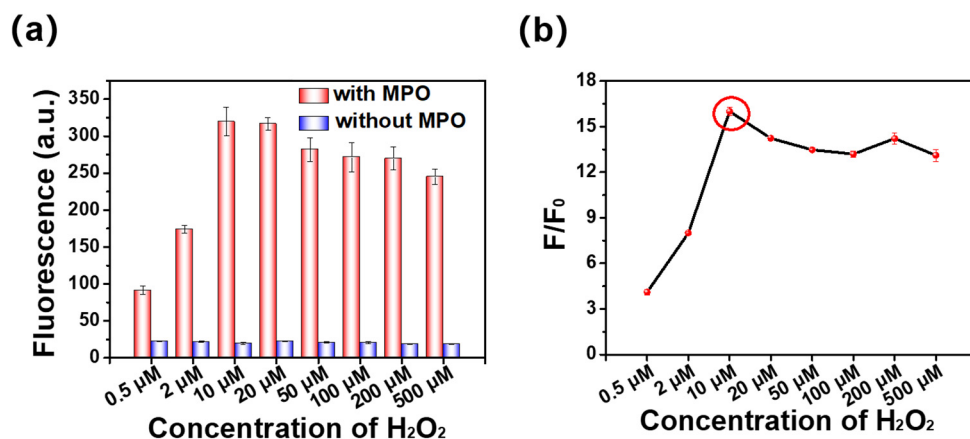| Spiked HClO (μM)	Detected HClO (μM)	Recovery (%), n=3
0.00	ND[a]	-
1.00	1.02 ± 0.03	102.17 ± 2.79
3.00	2.88 ± 0.08	96.11 ± 2.81
5.00	4.67 ± 0.07	93.43 ± 1.49

[a]ND, not detected.

Serum was subjected to a 100-fold dilution before analysis.

S3. MPO-sensing system

S3.1 Optimization of H₂O₂ concentration

(a)

(b)

Figure S6 Variance of (a) fluorescence intensity and (b) signal-to-noise ratio (F/F_0) with the H₂O₂ concentration. F and F_0 are the fluorescence intensities at 485 nm in the presence and absence of MPO, respectively. [MPO] = 300 ng/mL.

S3.2 Optimization of the reaction time for MPO-dependent Primer-S cleavage

(a)

(b)
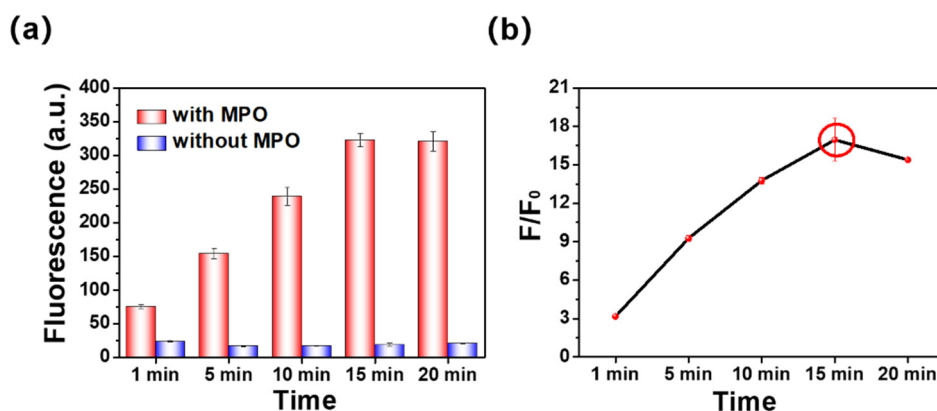
Figure S7 Variance of (a) fluorescence intensity and (b) signal-to-noise ratio (F/F_0) with the reaction time for MPO-dependent Primer-S cleavage. F and F_0 are the fluorescence intensities at 485 nm in the presence and absence of MPO, respectively. [MPO] = 300 ng/mL.

S3.3 Comparison of our MPO biosensor with reported ones

Table S3. Comparison of several MPO detection methods.

Method	Limit of detection (ng/mL)	Liner range (ng/mL)	Reference
PC-UCNPs sensor	2.44	10-200	19
TDN-HCR-based HClO sensor	0.6	2-25	24
Chronoamperometric magneto immunosensor	0.4	0.9-60	34
Chemosensor 7-HCCO	5	0-120	35
Near-infrared probe FD-301	0.22	50-250	20
MHC-HCR amplification	8.35	25-2500	23
CRISPR/Cas12a-based biosensor	0.67	2-600	31
RCA-assisted HClO biosensor	0.33	1-400	This work

S3.4 MPO detection in real sample

Table S4. Recovery of MPO from human serum sample.

Spiked MPO (ng/mL)	Detected MPO (ng/mL)	Recovery (%), n=3	Kit assay (ng/mL)
0.00	ND[a]	-	-
60.00	61.43 ± 0.79	107.39 ± 1.31	54.57
150.00	141.88 ± 2.16	94.59 ± 1.44	144.54
300.00	290.37 ± 11.02	96.79 ± 3.67	308.26

[a]ND, not detected.

Serum was subjected to a 100-fold dilution before analysis.