

**An assay for *Staphylococcus aureus* based on self-catalytic ampicillin-metal
(Fe³⁺)-organic gels-H₂O₂ chemiluminescence system with near-zero background
noise**

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S1 Experimental section

S1.1 Regent and materials

1,10-phenanthroline-2,9-dicarboxylic acid (PDA, $C_{14}H_8N_2O_4 \cdot xH_2O$), sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO, $(CH_3)_2SO$), thiourea dioxide (TD), hydroxylamine-O-sulfonic acid (HOSA) were provided by Macklin Biochemical Technology Co., Ltd (Shanghai, China). Iron (III) chloride ($FeCl_3$), ampicillin (AMP, $C_{16}H_{19}N_3O_4S$), Hydrogen peroxide (H_2O_2), ethanol (C_2H_6O) were all got from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) were both obtained from J&K Chemical Co., Ltd (Beijing, China). 2-(N-morpholino) ethanesulfonic acid (MES) and thiourea (CH_4N_2S) were purchased from Sigma-Aldrich Co., Ltd (Los Angeles, USA). $1 \text{ mg}\cdot\text{mL}^{-1}$ Rabbit anti-*S. aureus* antibody was purchased from Bioss Biotechnology Co., Ltd (Beijing, China). $5 \text{ mg}\cdot\text{mL}^{-1}$ carboxyl modified magnetic beads (MBs) with 500 nm diameter was purchased from BaseLine Chromtech Research Center (Tianjin, China).

Phosphate buffer saline (PBS) (pH 7.4) was prepared through dissolving 2.37 g NaH_2PO_4 , 23.20 g Na_2HPO_4 and 3.6 g NaCl in 400 mL ultrapure water. MES buffer at 10 mM was made by dissolving 19.5 mg MES in 100 mL ultrapure water.

Sea water sample was got from Qingdao first bathing beach (N $36^\circ 03' 24.55''$, E $120^\circ 20' 13.85''$). River water sample was obtained from Licun river located in Qingdao. Apple juice sample was purchased from supermarket. All the three samples were pre-treatment free.

S1.2 Instruments

The as-prepared MOGs (Fe) was characterized by transmission electron microscopy (SEM, JEM-2100 scanning electron microscope) (JEOL, Tokyo, Japan). FI-CL instrument (Xi'an Remax analyse instrument Co., Ltd. China) was adopted as the CL signal receiver. Ultrapure water was distilled and purified by purification system (Shanghai Yarong Biochemistry Instrument Factory Co., Ltd. China). The micro morphologies of AMP modified MB captured bacteria were observed by Leica TCS SP5 II confocal laser scanning microscope (Leica, Wetzlar, Germany).

S1.3 Synthesis of Fe(III)-based MOGs (MOGs (Fe))

Fe(III)-based MOGs (MOGs (Fe)) was synthesized by a facile and convenient method which had been reported previously^{1,2}. 26.8 mg PDA was added into 500 μL DMSO and dispersed by ultrasound. Then, $0.1 \text{ mol}\cdot\text{L}^{-1}$ $FeCl_3$ was dropped into the PDA solution under stirring. Light yellow gel was appeared only after about ten seconds. The result gel was washed three times by ultrapure water and centrifuged at 12,000 rpm for 30 min. After that, MOGs (Fe) was got though freeze-drying treatment to remove the residual solvent.

S1.4 CL catalytical performance of MOGs (Fe)

Experiments were carried out to investigate the catalysis performances of MOGs (Fe) in AMP CL system. 50 μL $1 \text{ mg}\cdot\text{mL}^{-1}$ AMP and 50 μL $1 \text{ mg}\cdot\text{mL}^{-1}$ MOGs (Fe) were placed in the 96-well-plate and 100 μL $0.1 \text{ mol}\cdot\text{L}^{-1}$ H_2O_2 was injected. CL signal was collected by FI-CL instrument.

S1.5 Preparation of AMP conjugated MB (MB@AMP)

200 μL carboxyl modified MB was purified by MES buffer for three times through magnetic separation. The washed MB was dispersed by 200 μL MES buffer containing 20 mg EDC and 5 mg NHS. After 40 min, the activated carboxyl modified MB was purified thrice by PBS through magnetic separation and dispersed in PBS with same volume. Then, 2 mg AMP was added into the activated MB solution and reacted for 12 h under 4 $^{\circ}\text{C}$. The resulted MB@AMP was washed and dispersed into 200 μL PBS containing 0.1% BSA and put under 4 $^{\circ}\text{C}$ for further use.

S1.6 Preparation of rabbit anti-*S. aureus* antibody modified MOGs (Fe) (antibody@MOGs (Fe))

1 mg MOGs (Fe) was dispersed in 500 μL MES buffer containing 50 mg EDC and 12.5 mg NHS to active the carboxyl group of MOGs (Fe). After 3 h, activated MOGs (Fe) was collected by centrifuge and washed thrice by PBS. The purified MOGs (Fe) was dispersed in 490 μL PBS while 10 μL 1 $\text{mg}\cdot\text{mL}^{-1}$ rabbit anti-*S. aureus* antibody was dropped following. The mixture solution was incubated under 37 $^{\circ}\text{C}$ for 12 h with 200 rpm shaking. The resulted antibody@MOGs (Fe) was washed and dispersed into 200 μL PBS containing 0.1% BSA and put under 4 $^{\circ}\text{C}$ for further use.

S1.7 Bacteria culture and measurement

S. aureus strain was inoculated on the prepared tryptone soybean broth (TSB) in Erlenmeyer flask and put into incubator for 16 h under 37 $^{\circ}\text{C}$ with constantly shaking. The grown *S. aureus* was counted by plate counting method. Firstly, 1 mL of grown *S. aureus* was diluted with ultrapure water for 107 times and the diluted solution was coated on the TSB plate containing 1.5 % of agar. After further growth, the bacterial quantity can be counted by the diluted bacterial colony number.

S1.8 CL detection of *S. aureus*

1 mL of a series concentrations *S. aureus* solution was mixed with 20 μL MB@AMP and 10 μL antibody@MOGs (Fe) respectively. The mixture solution was reacted at 37 $^{\circ}\text{C}$ for 1 h under constantly shaking. Then, the formed MB@AMP-*S. aureus*-antibody@MOGs (Fe) sandwich complex was collected by magnetic separation and purified thrice by PBS. The treated sandwich complex was dispersed in 100 μL (tenth volume of initial solution) 0.1 $\text{mol}\cdot\text{L}^{-1}$ NaOH solution and added to the 96-well microplate. After that, 100 μL 0.1 $\text{mol}\cdot\text{L}^{-1}$ H_2O_2 was added immediately by static injection to trigger the CL signal. CL signal was collected by FI-CL instrument to measure the different concentration of *S. aureus*.

S1.9 Preparation of real samples

The two kinds of water samples were settling for 2 h in Clean Bench to remove the large particles. Then, the two kinds of water samples were conveyed into glass bottle at 4 $^{\circ}\text{C}$ without any other pre-treatments. As for apple juice sample, sterile was employed as the diluting solution to thinning the apple juice for 10-times. The diluted apple juice was also pre-treatments-free and put into glass bottle at 4 $^{\circ}\text{C}$ for further use.

S1.10 Real sample detection

1 mL of real sample solutions were added into the mixture solution of 20 μL MB@AMP and 10 μL antibody@MOGs (Fe). After reacted at 37 $^{\circ}\text{C}$ for 1 h with

constantly shaking, the MB@AMP-S. aureus-antibody@MOGs (Fe) sandwich complex was collected by magnet separation. The sandwich complex was washed thrice with PBS and 100 μL 0.1 $\text{mol}\cdot\text{L}^{-1}$ NaOH solution was employed as dispersant. By putting the sandwich complex into 96-well microplate, CL signal was collected after 100 μL 0.1 $\text{mol}\cdot\text{L}^{-1}$ H_2O_2 injected as trigger (tenth enriched of solution to be detected).

S1.11 CL spectra of AMP-MOGs (Fe)- H_2O_2 CL system

250 μL 1 $\text{mg}\cdot\text{mL}^{-1}$ AMP and 250 μL 1 $\text{mg}\cdot\text{mL}^{-1}$ MOGs (Fe) was added into cuvettes which was placed in the covering up the light entrance slot of AF-7000 fluorescence spectrophotometer. CL emission was collected from the range of 360 nm to 550 nm after 500 μL 0.1 $\text{mol}\cdot\text{L}^{-1}$ H_2O_2 was added quickly.

S1.12 O_2 , $\text{OH}\cdot$ and $\text{O}_2^{\bullet-}$ influence on AMP-MOGs (Fe)- H_2O_2 CL system

In order to discuss the O_2 influence on AMP-MOGs (Fe)- H_2O_2 CL system, all the reaction solutions were purged by N_2 for 30 min. The other experiment processes were same as S1.4. Meanwhile, thiourea was employed as the inhibition reagents to scavenge the $\text{OH}\cdot$. While 50 μL 1 $\text{mg}\cdot\text{mL}^{-1}$ AMP and 50 μL 1 $\text{mg}\cdot\text{mL}^{-1}$ MOGs (Fe) was placed in 96-well-plate, 10 μL 4 $\text{mmol}\cdot\text{L}^{-1}$ thiourea was added. After reacted for 0.5 h, 100 μL 0.1 $\text{mol}\cdot\text{L}^{-1}$ H_2O_2 was injected to trigger the CL reaction. As for $\text{O}_2^{\bullet-}$, inhibition experiment was similar to that of $\text{OH}\cdot$ while 10 μL 4 $\text{mmol}\cdot\text{L}^{-1}$ thiourea was replaced by 10 μL 4 $\text{mmol}\cdot\text{L}^{-1}$ ethanol.

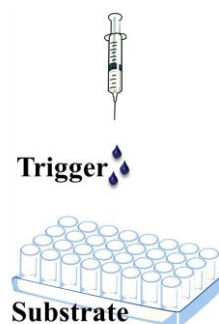
S1.13 Anti ions-interference property test

NiCl_2 , BaCl_2 , CaCl_2 , KCl , NaCl , MgCl_2 , CoCl_2 , CuSO_4 , NaF , FeCl_3 , FeCl_2 , Na_2SO_3 , Na_2CO_3 and KNO_3 were employed as interference ions reagents with a concentration at 2.5×10^{-5} $\text{mol}\cdot\text{L}^{-1}$. Experiments were carried out similarly. 50 μL 1 $\text{mg}\cdot\text{mL}^{-1}$ AMP and 50 μL 1 $\text{mg}\cdot\text{mL}^{-1}$ MOGs (Fe) and 10 μL interference ions were placed in the 96-well-plate and 100 μL 0.1 $\text{mol}\cdot\text{L}^{-1}$ H_2O_2 was injected followed.

S2. Results and discussions

S2.1 Static injection

Static injection is a traditional injection method for CL behavior detection while substrate is put in the container and the reaction trigger is added by injector. In this study, AMP and MOGs (Fe) are put in the 96-well plate as substrate and H_2O_2 is injected as trigger (Scheme S1).



Scheme S1. Diagram of static injection model. AMP and MOGs (Fe) are put in the 96-well plate as substrate and H_2O_2 is injected as trigger.

S2.2 Oxidant selection

The performances of the two types of new oxidants, thiourea dioxide (TD) and hydroxylamine-O-sulfonicacid (HOSA), were investigated in the AMP-MOGs (Fe) system by replacing H_2O_2 at the same concentration respectively. Results suggested that HOSA and TD were not suitable oxidant for AMP-MOGs (Fe) system because the CL signals of either were much lower than the CL signal triggered by H_2O_2 (Fig. S1). Thus, H_2O_2 was chosen as the most suitable oxidant for AMP-MOGs (Fe) system.

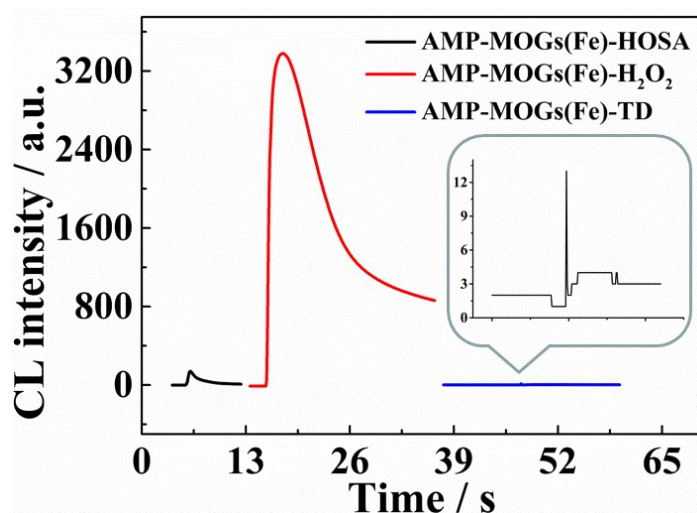


Fig. S1. CL intensity of AMP-MOGs (Fe) system triggered by HOSA, H_2O_2 and TD respectively.

S2.3 Comparison of catalytic performances

To further prove the catalysis ability of MOGs (Fe) in AMP- H_2O_2 CL system, experiments were carried out which employed PDA, Fe^{3+} , without-freeze-drying MOGs (Fe) as comparative catalysis and the results were displayed in Fig. S2. Under the alkaline condition, AMP was mixed with different materials respectively including MOGs (Fe) (curve a), PDA (curve b), Fe^{3+} (curve c), without freeze-drying MOGs (Fe) (curve d) and ultrapure water (blank signal, curve e) while H_2O_2 was injected. CL signal of MOGs (Fe) catalyzed AMP- H_2O_2 system was about 2387.8 a.u.. It was about 200-fold, 150-folds and 240-fold higher compared with the CL signal of AMP- H_2O_2 system catalyzed by PDA (12.4 a.u.), Fe^{3+} (15.9 a.u.) and ultrapure water (background, 10.4 a.u.), which revealed the excellent catalysis ability of MOGs (Fe) in the AMP- H_2O_2 CL system. Meanwhile, the catalysis performance of without-freeze-drying MOGs (Fe) was also lower than MOGs (Fe) apparently. It suggested that the freeze-drying is beneficial for the CL catalysis.

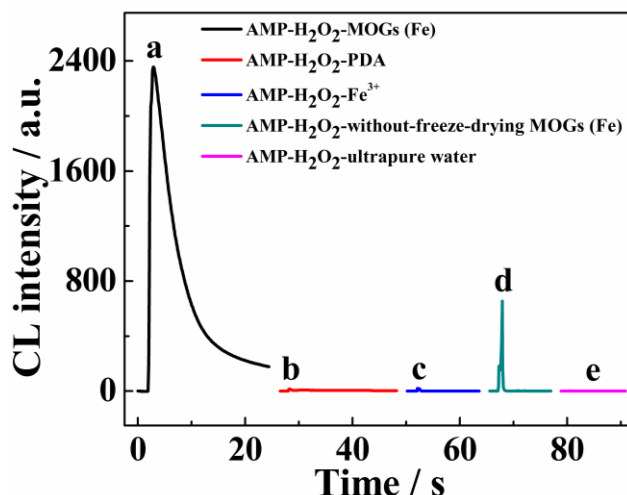


Fig. S2. AMP-H₂O₂ CL catalyzed by MOGs (Fe) (a), PDA (b), Fe³⁺ (c), without freeze-drying MOGs (Fe) (d), ultrapure water (e). The concentrations of AMP and H₂O₂ were 1 mg·mL⁻¹ and 0.1 mol·L⁻¹ respectively.

S2.4 Optimization of experiment conditions

As is known to us, the signal of traditional CL system such as luminol-H₂O₂ system usually had a closer relationship to pH. As for AMP-H₂O₂ CL system, experiment was carried out firstly to investigate the influence of pH and the result was illustrated in **Fig. S3 (A, B)**. We had tried the influence of pH gradient from 8.0 to 14.0 (curve a to g) on the AMP-H₂O₂ CL system and it is obvious that the CL intensity increased with the increasing of pH until 13.0. When pH was 14.0, CL intensity was decreased distinctly. Therefore, the most suitable pH was 13.0.

Another important factor of AMP-H₂O₂ CL system was the concentration of H₂O₂ due to the capability of providing the oxygen radical (See the possible CL mechanism of AMP-MOGs (Fe)-H₂O₂ CL system in main text). Concentration of H₂O₂ greatly affects the CL intensity so the optimized concentration of H₂O₂ was discussed. As shown in **Fig. S3 (C, D)**, CL intensity increased gradually with the increasing concentration of H₂O₂ and the largest CL intensity was appeared until H₂O₂ concentration reached to 0.1 mol·L⁻¹. After that, CL intensity began to fall. So, 0.1 mol·L⁻¹ was chosen as the optimal concentration for AMP-H₂O₂ CL system.

As AMP was modified on the surface of MB to accomplish capture bacteria and CL emission at the same time, the consumption of AMP was particularly important. Optimization experiment was carried out followed the experiment processes above while the amounts of AMP was controlled as 1 mg·mL⁻¹, 5 mg·mL⁻¹, 10 mg·mL⁻¹, 20 mg·mL⁻¹ and 30 mg·mL⁻¹, respectively. The results indicated that 10 mg·mL⁻¹ of AMP to modify MB can get the biggest CL response of the strategy (**Fig. S3 (E, F)**). Similarly, the quantity of MOGs (Fe) which labeled with rabbit anti-*S. aureus* antibody was investigated at the same time. The results shown in **Fig. S3 (G, H)** indicated that the best CL response was appeared when 2 mg·mL⁻¹ MOGs reacted with sufficient rabbit anti-*S. aureus* antibody.

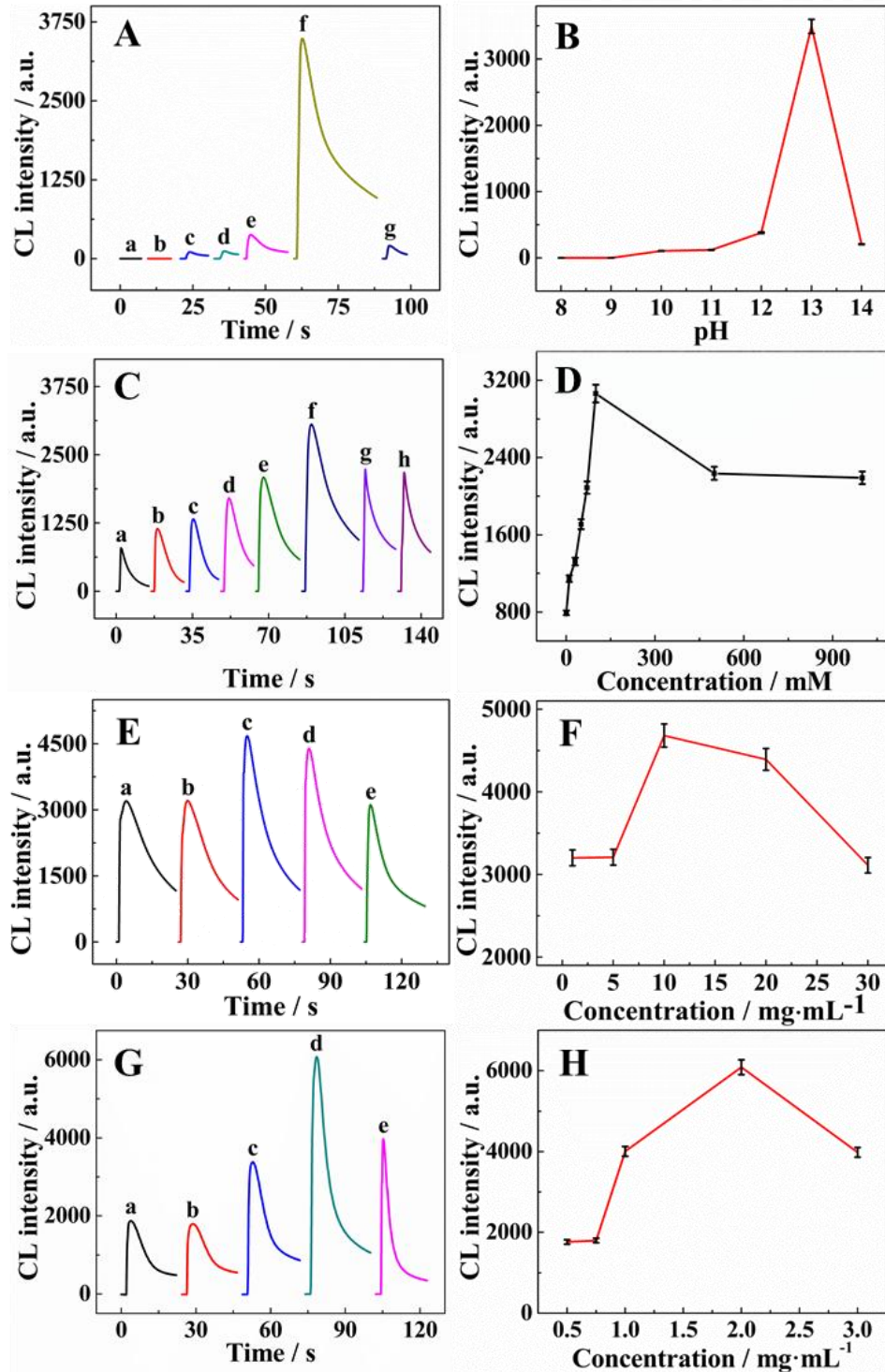


Fig. S3 (A), (B) CL response of AMP-MOGs (Fe)- H_2O_2 system under different pH. 8.0 (a), 9.0 (b), 10.0 (c), 11.0 (d), 12.0 (e), 13.0 (f), 14.0 (g). (C), (D) Effect of H_2O_2 concentration on CL intensity. 1 $mmol \cdot L^{-1}$ (a), 10 $mmol \cdot L^{-1}$ (b), 30 $mmol \cdot L^{-1}$ (c), 50 $mmol \cdot L^{-1}$ (d), 70 $mmol \cdot L^{-1}$ (e), 100 $mmol \cdot L^{-1}$ (f), 500 $mmol \cdot L^{-1}$ (g), 1,000 $mmol \cdot L^{-1}$ (h). (E), (F) Effect of amounts of AMP on CL intensity. 1 $mg \cdot mL^{-1}$ (a), 5 $mg \cdot mL^{-1}$ (b), 10 $mg \cdot mL^{-1}$ (c), 20 $mg \cdot mL^{-1}$ (d) and 30 $mg \cdot mL^{-1}$ (e). (G), (H) Effect of the dose of MOGs (Fe) labeled with rabbit anti-*S. aureus* antibody on CL intensity. 0.1 $mg \cdot mL^{-1}$ (a), 0.5 $mg \cdot mL^{-1}$ (b), 1 $mg \cdot mL^{-1}$ (c), 2 $mg \cdot mL^{-1}$ (d) and 3 $mg \cdot mL^{-1}$ (e).

S2.5 Functional testing of MB@AMP

As is known to us, the most important structure for antibacterial ability of AMP is β -lactams compounds which can combine with bacteria and inhibit the synthesis of cell wall. In order to capture and enrich bacteria, MB@AMP was formed by amidation reaction in the front part. But chemical reaction is often accompanied by unknown side reaction that may damage to the antibacterial ability of AMP. So, confocal laser scanning microscope was employed to observe the micromorphology while *S. aureus* and MB@AMP was mixed together. It is illustrated clearly that the formed MB@AMP was combine with bacteria perfectly (**Fig. S4**) and further proved the feasibility of the detection strategy.

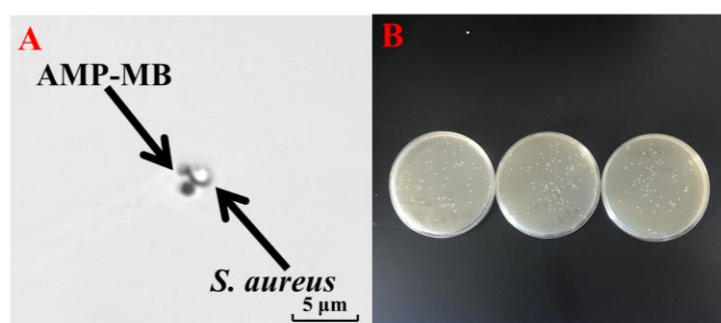


Fig. S4. (A) Micromorphology of MB@AMP conjugated *S. aureus* observed by confocal laser scanning microscope. (B) Photograph of bacteria cultural.

S2.6 Analytical performance of developed strategy

The CL response signal of this method under optimal condition showed a logarithmic relation with the linear concentration of *S. aureus* from 1.0×10^2 CFU·mL⁻¹ to 1.0×10^7 CFU·mL⁻¹ (**Fig. 3A**). The regression equation between CL intensity and concentration of *S. aureus* is $I = -1404.88 + 375.64 \ln(c + 56.99)$ (c , CFU·mL⁻¹) with the limitation of 31 CFU·mL⁻¹ and the correlation coefficient was 0.9984. The LOD was compared with the reported methods. They are provided in **Table S1**.

Table S1. LOD comparison of different CL analysis methods.

LOD	1 ^a	290 ^a	5 ^a	3.3 ^a	1 U·mL ⁻¹	0.5×10^{-18} mol·mL ⁻¹	1.3×10^{-16} mol·mL ⁻¹	31 ^a
Ref	[26]	[27]	[28]	[29]	[30]	[31]	[32]	This work

^a CFU·mL⁻¹.

Note: the number of references is inconsistent with that in the main text.

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S2.7 Selectivity of the strategy

Selectivity is a crucial factor which determines whether the developed strategy can recognize *S. aureus* specifically and accomplish to detection. The developed protocol utilized AMP as capture molecule to enrich the *S. aureus*. AMP is a kind of broad-spectrum antibiotic so that can recognize many pathogenic bacteria in the sample, which lead to the low selectivity. Therefore, rabbit anti-*S. aureus* antibody was introduced as the second recognition molecule to overcome the shortcoming. In the selectivity experiment, 1.0×10^6 CFU·mL⁻¹ three types of bacteria, *Salmonella typhimurium* (*S. typhimurium*), *Escherichia coli* (*E. coli*) and *Listeria monocytogenes* (*L. monocytogenes*) were employed as interference while concentration of *S. aureus* was 5.0×10^3 CFU·mL⁻¹ and the CL responses shown in **Fig. S5**. It can be seen that CL response of *S. typhimurium* (curve b, 196.0 a.u.), *E. coli* (curve c, 196.0 a.u.) and *L. monocytogenes* (curve d, 202.0 a.u.) were quite low and close to the CL response of blank (curve a, 206.0 a.u.). Meanwhile, mixture 1 (mixed *S. typhimurium*, *E. coli*, *L. monocytogenes* and *P. aeruginosa* together at the concentration of 5.0×10^4 CFU·mL⁻¹) and mixture 2 (*S. aureus* added into mixture 1 with the final concentration of 1.0×10^4 CFU·mL⁻¹) were further investigated. CL response of mixture 1 (curve f, 244.0 a.u.) was still close to blank while CL response of mixture 2 (curve g, 1954.0 a.u.) was close to the CL response of *S. aureus* at a concentration of 5.0×10^3 CFU·mL⁻¹ (curve e, 1922.0 a.u.). The satisfying result revealed that the selectivity of the developed protocol was acceptable and potential for application.

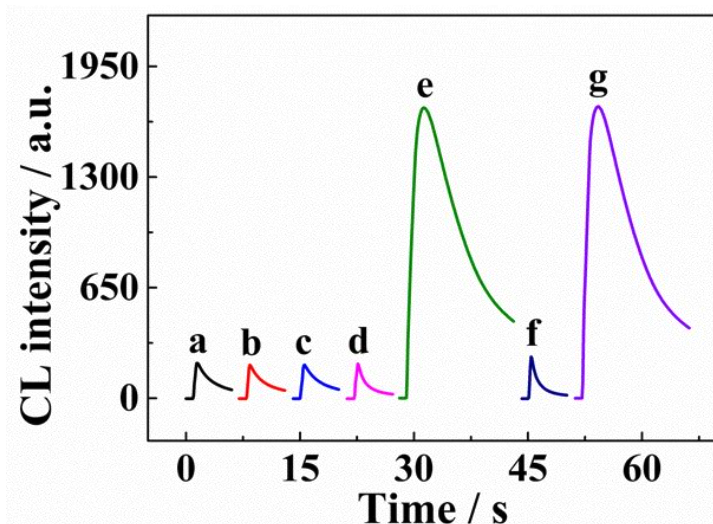


Fig. S5. CL response of different bacteria. (a) Blank, (b) *S. typhimurium*, (c) *E. coli*, (d) *L. monocytogenes*, (e) *S. aureus*, (f) Mixture 1 (mixture of *S. typhimurium*, *E. coli* and *L. monocytogenes*), (g) Mixture 2 (mixture of *S. typhimurium*, *E. coli*, *L. monocytogenes* and *S. aureus*). All the interference bacteria concentrations were $5.0 \times$

$10^4 \text{ CFU}\cdot\text{mL}^{-1}$.

S2.8 Specificity at LOD concentration

In order to further discuss the property of the developed strategy, specificity at LOD concentration of our sensor was discussed while *S. aureus* was at $31 \text{ CFU}\cdot\text{mL}^{-1}$ and other interfering bacteria were at $310 \text{ CFU}\cdot\text{mL}^{-1}$. Meanwhile, mixture of *S. aureus*, *S. typhimurium*, *E. coli* and *L. monocytogenes* when the final concentration of each bacteria was same as above was also investigated. Result indicated that CL response of interfering bacteria was similar to blank signal. Besides, there was little different between CL response of *S. aureus* and mixture of all the bacteria. All the result displayed in **Fig. S6** verified that the developed strategy has outstanding specificity in at LOD concentration.

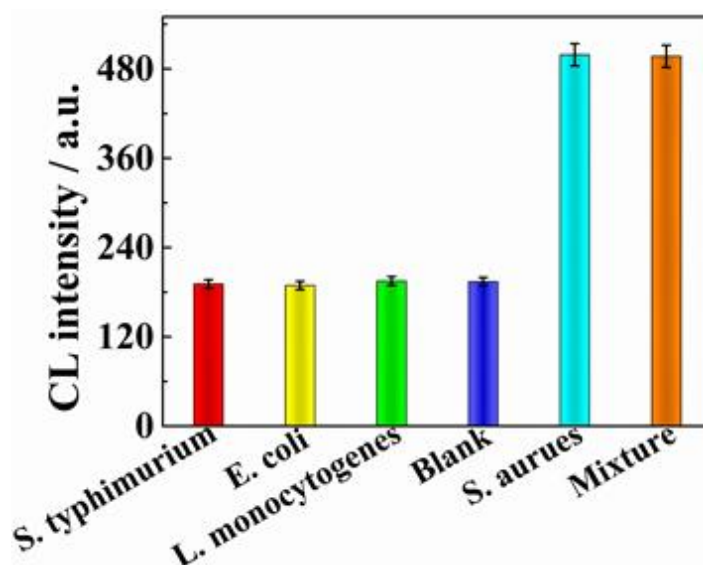


Fig. S6. CL response of different bacteria at LOD concentration.

S2.9 Real sample detection

To further investigate the potential application of the developed strategy, real sample detection experiment was carried out. Three samples, river water, sea water and apple juice, were detected by official method³ and our strategy firstly. Then, standard addition method was utilized to verify the accuracy of our strategy (**Table. S2**). Comparing with the result of official method, our strategy showed perfect accuracy in the detectable range. Meanwhile, range from 99.08 % to 102.36 % of recovery radio indicated the reliable and potential for application.

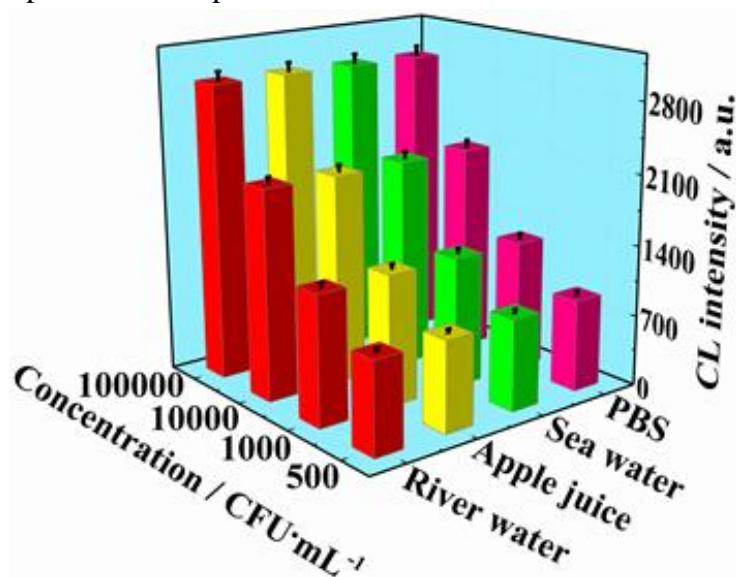
Table. S2. Results of determination of *S. aureus* in real sample.

Sample	Original		Added ^a	Detected ^a	RSD (%)	Recovery (%)
	This method ^a	Official method ^c				
River water	- ^b	9	1.00×10 ²	1.08×10 ²	6.1	99.08
	1.12×10 ²	1.14×10 ²	1.00×10 ³	1.13×10 ³	4.8	101.43
	- ^b	2	1.00×10 ⁴	9.96×10 ³	2.6.	99.58
Sea water	0.67×10 ²	0.69×10 ²	1.00×10 ²	1.73×10 ²	5.8	102.36
	- ^b	2	1.00×10 ³	9.94×10 ²	3.9	99.20
	- ^b	- ^b	1.00×10 ⁴	1.01×10 ⁴	2.3	101.00
Apple juice	- ^b	- ^b	1.00×10 ²	1.02×10 ²	7.9	102.00
	- ^b	- ^b	1.00×10 ³	1.01×10 ³	5.2	101.00
	- ^b	- ^b	1.00×10 ⁴	9.98×10 ³	3.3	99.80

^a CFU·mL⁻¹^b Concentration of *S. aureus* was lower than the limitation of this strategy.^c Official method: National Standards of the People's Republic of China GB 4789.10-2016. National food safety standard food microbiological examination: *Staphylococcus aureus*.

S2.10 Stability of method in different samples

In consideration of the different components of PBS and the real samples, stability experiment was carried out while employ sterilized samples including sea water, river water and apple juice as the detection solution. Then, *S. aureus* at different concentrations were added into different solutions and detection processed were the same as before. Result shown in **Fig. S7** revealed that our strategy has pretty stability in different compositional samples.

**Fig. S7.** CL response of *S. aureus* in different detection condition at a series concentration.

S2.11 CL spectra of AMP-MOGs (Fe)-H₂O₂ system

In order to discuss the mechanism of the developed AMP-MOGs (Fe)-H₂O₂ CL system, CL spectra was introduced and collected by AF-7000 fluorescence spectrophotometer by covering up the light entrance slot and shown in **Fig. 2 (Left)**. The CL spectra showed clearly that fluorescence emission wavelength of CL intermediate was always concentrated on 430 nm. According to the previous report, fluorescence emission of penicillamine was near 430 nm, which can be produced by the hydrolysis of AMP^{4,5}. Meanwhile, the CL intensity of AMP-MOGs (Fe)-H₂O₂ CL system after treated by N₂, thiourea and ethanol were decreased accordingly. Thus, the CL spectra proved luminophore of AMP-MOGs (Fe)-H₂O₂ CL system was penicillamine.

S2.12 Anti ions-interference property of this strategy

In order to further investigate the application potential, anti ions-interference property was discussed (**Fig. 1(C)**, **Table. S3**) while concentrations of interfering ions were $2.5 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$. It is easy to see that AMP-MOGs (Fe)-H₂O₂ CL system was influenced a little in the existence of interfering ions. The anti ions-interference property test was repeated five times and RSD was showed in **Table. S3**.

Table. S3. CL response of AMP-MOGs (Fe)-H₂O₂ CL system with different interfering ions (n=7).

Interfering ions	CL intensity	RSD (%)
None	1774.1	1.8
Ni ²⁺	1775.0	1.5
Ca ²⁺	1695.5	2.5
K ⁺	1797.3	1.9
Ba ²⁺	1789.0	2.1
Na ⁺	1725.8	1.5
Mg ²⁺	1710.4	1.8
Cu ²⁺	1785.9	2.0
Co ²⁺	1703.8	2.3
Fe ³⁺	1724.7	1.7
Fe ²⁺	1695.8	1.2
NO ₃ ²⁻	1756.2	1.9
SO ₄ ²⁻	1689.4	2.7
SO ₃ ²⁻	1741.2	2.4
CO ₃ ²⁻	1714.8	1.8
F ⁻	1746.6	2.3
Cl ⁻	1788.5	1.4

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