Electronic Supplementary Material (ESI) for ChemComm.

Electronic supplementary information

Double-Site Recognition of Pathogenic Bacterium Whole Cells Based on Antibiotic-Affinity Strategy

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Preparation of bacteria sample

Materials

Strains of *S. aureus*, *E. coli*, *Salmonella*, *P. aeruginosa*, *M. luteus* and *B. subtilis* were provided by the Chongqing Center for Disease Control and Prevention (China).

Bacteria culture and counting

The pure culture of bacteria was grown in Luria Broth medium with shaking at 37 ^{o}C for 12 h. Subsequently, the culture medium was diluted with sterile phosphate buffer saline (PBS) at 0.10 M to different concentrations. The diluted culture was centrifuged at 12000 rpm for 10 min, and the supernatant was discarded. After washing twice with sterile water, the resultant pellets were re-dispersed in 5.0 mL of PBS. The washed bacteria were enumerated by plating 10-fold serial dilutions on Luria Broth agar plates and incubating at 37 ^{o}C overnight. The medium colonies on the plates were calculated to appraise the concentrations of bacteria in CFU mL^{-1}.

Confocal microscopy imaging of the dual fluorescence-stained bacteria

Materials and apparatus

FITC-tagged pig IgG was provided by Beijing Biosynthesis Biotechnology Co., Ltd. (China). Vancocin, *N*-hydroxysuccinimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and PE were purchased from Sigma-Aldrich (USA). Ultrapure water (18.2 MΩ) purified by an ELGA PURELAB Classic system (France) was used to prepare all aqueous solutions. All other reagents were of analytical grade and used as receiving.

The purification of PE-tagged vancocin was accomplished by using an AKTAprimeTM protein purification system (GE healthcare Co., Ltd., USA) equipped with a Sephadex G-25 column. The confocal microscopy images were obtained from a LSM710 confocal laser scanning microscopy (Carl Zeiss AG, Germany).

Preparation and characterization of PE-tagged vancocin

Five hundred microliter of vancocin (20 mg mL^{-1}) was mixed with 300 μL of freshly-prepared *N*-hydroxysuccinimide (10 mg mL^{-1}) and 300 μL of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (20 mg mL^{-1}). The obtained mixture was allowed to react for 5 min at room temperature to activate the carboxylate groups in vancocin. Subsequently, 250 μL of PE (1.0 mg mL^{-1}) dissolved in PBS at pH 7.0 was mixed with the activated vancocin. After an overnight reaction at 4 ^{o}C, the reaction was stopped by adding 40 μL of glycine solution at 2.0 M. PE worked as a limited substrate in

the conjugation reaction of PE and vancocin. Thus the product was purified with a flow rate of 0.5 mL/min and a detection wavelength of 280 nm, by using a Sephadex G-25 column.

As seen in Fig. S5, PE-tagged vancocin (the first peak) was absolutely separated from excessive vancocin (the second peak). The purified PE-tagged vancocin showed fluorescence emission of PE. Furthermore, bactericidal halo test indicated that the product remained antibiotic activity to *S. aureus* (Fig. S6). The above results demonstrated that the collected product is conjugate of PE and vancocin.

Microscopy imaging of stained S. aureus

The cultured *S. aureus* was grown in Luria Broth medium at 37 ^{o}C for 12 h under constant shaking. Then the culture was centrifuged at 5000 rpm for 5 min to discard the supernatant, followed by re-dispersing in 1.0 mL of PBS. Subsequently, 75 μL of the obtained bacteria solution was incubated with same volume of PE-tagged vancocin and FITC-tagged pig IgG (100 μg mL^{-1}) for 30 min. After that, the stained *S. aureus* cells were collected by centrifugation at 4000 rpm for 3.5 min and washed twice with PBS at pH 7.0. Finally, 15 μL of the resultant stained bacteria solution was mounted onto a microscopic slide, and observed under the confocal laser scanning microscopy with a magnification of 1000. The excitation wavelengths for PE and FITC were 542 nm and 488 nm, respectively, while the emission wavelengths for PE and FITC were 575 nm and 525 nm, respectively.

Antibiotic-affinity assay of *S. aureus* using CL detection

Materials and apparatus

The high-affinity 96-well polystyrene microplate was obtained from Greiner Bio-One Biochemical Co., Ltd. (Germany). Pig IgG were purchased from Sigma-Aldrich (USA). SuperBlock® T20 used as the blocking buffer was provided by Thermo Fisher Scientific Inc. (USA). Lightning-LinkTM HRP conjugation kit was purchased from Innova Biosciences Ltd. (UK). The home-prepared HRP substrate for CL detection consisted of two solutions dissolved in 0.10 M PBS at pH 8.5. Solution 1 contained 5.0×10^{-5} M luminol (Sigma-Aldrich, USA) and 5.0×10^{-5} M *p*-iodophenol (Aladdin Industrial Inc., China), while Solution 2 contained 1.0×10^{-2} M H_2O_2 (Chengdu Kelong Chemical Reagent Co., Ltd., China). Washing buffer was PBS (pH 7.0) containing 0.05% Tween 20. The CL measurement was performed using an Infinite 200 PRO multifunctional microplate reader (Tecan, Switzerland). All other reagents and apparatus were same as those described in the section of "Confocal microscopy imaging of the dual fluorescence-stained bacteria".

Preparation and characterization of Vanc-HRP

Vanc-HRP was prepared by tagging vancocin using Lightning-Link^{TM} HRP conjugation kit according to the protocol provided in the manual. The obtained conjugate was purified with a protocol similar to that for PE-tagged vancocin (described in the section of "Confocal microscopy imaging of the stained bacteria").

As seen in Fig. S7, Vanc-HRP (the first peak) was absolutely separated from excessive vancocin (the second peak). Besides TMB color reaction activity, the purified product also showed similar antibiotic activity as PE-tagged vancocin, demonstrating conjugating of HRP and vancocin.

Assay procedure

In a typical experiment, a polystyrene microplate was coated with 5.0 μg mL^{-1} pig IgG dissolved in 0.10 M Tris-HCl buffer at pH 8.0 (100 μL/well) overnight at 4 ^{o}C. Thereafter, the microplate was washed thrice and blocked with the blocking buffer (150 μL/well) for 1.5 h at 37 ^{o}C. After thrice washing, the microplate was filled with *S. aureus* sample (100 μL/well) to perform an incubation of 60 min at 37 ^{o}C. Then it was washed thrice, and filled with 1.0 μg mL^{-1} Vanc-HRP (100 μL/well) to perform the second incubation for 60 min. Afterward the microplate was washed thrice, 80 μL of Solution 1 and 20 μL of Solution 2 were sequently injected into the well, and the peak value of the CL emission was recorded for quantitation.

Antibiotic-affinity assay of *S. aureus* using colorimetric detection

Materials and apparatus

The TMB substrate for colorimetric detection was composed of Solution A and Solution B (Beijing Dingguo Biotechnology Co., Ltd., China). All other reagents and apparatus were same as those described in the section of "Antibiotic-affinity assay of *S. aureus* using CL detection".

Assay procedure

Microplate coating, blocking, washing and incubation were all performed with protocols same as those described in the section of "Antibiotic-affinity assay of *S. aureus* using CL detection". Thus the sandwich complex of IgG/*S. aureus*/Vanc-HRP was formed on the microplate. Fifty microliter of Solution A was mixed with the same amount of Solution B, and then this mixture was pipetted into the well. After 10 min of color development at room temperature, the reaction was stopped by pipetting 50 μL of 2.0 M H_2SO_4. The absorbance value was recorded at 450 nm for quantitation.

Pseudo ELISA using Vanc-HRP as the signal tracer

Materials and apparatus

Mouse monoclonal antibody for *S. aureus* was provided by Beijing Biosynthesis Biotechnology Co., Ltd. (China). All other reagents and apparatus were same as those described in the section of "Antibiotic-affinity assay of *S. aureus* using colorimetric detection".

Assay procedure

A polystyrene microplate was coated with 5.0 μg mL^{-1} mouse monoclonal antibody for *S. aureus* dissolved in 0.10 M Tris-HCl buffer at pH 8.0 (100 μL/well) overnight at 4 ^{o}C. All other processes including blocking, washing, incubation, color development and signal detection were all performed with protocols same as those described in the section of "Antibiotic-affinity assay of *S. aureus* using colorimetric detection". The dose-response relation for *S. aureus* detection is shown as Fig. S8.

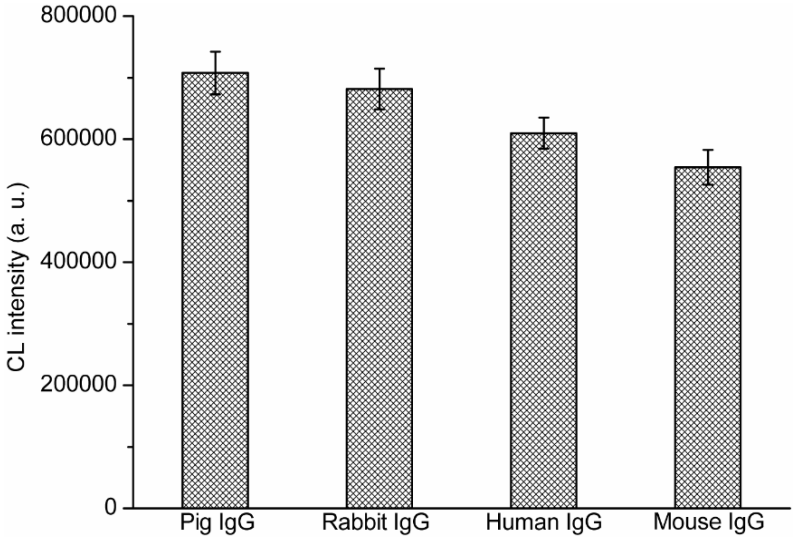

Fig. S1 CL responses from *S. aureus* at 10^5 CFU mL^{-1} by using IgG from different animals. All other conditions were the optimal conditions (n = 5).

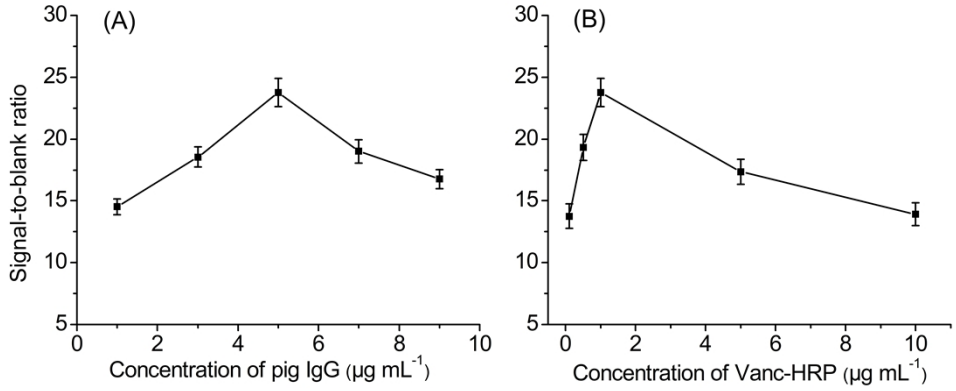

Fig. S2 Effects of the concentrations of (A) pig IgG and (B) Vanc-HRP on the signal-to-blank ratios. The signal value and the blank value were obtained from *S. aureus* at 10^5 CFU mL^{-1} and PBS, respectively. All other conditions were the optimal conditions (n = 5).

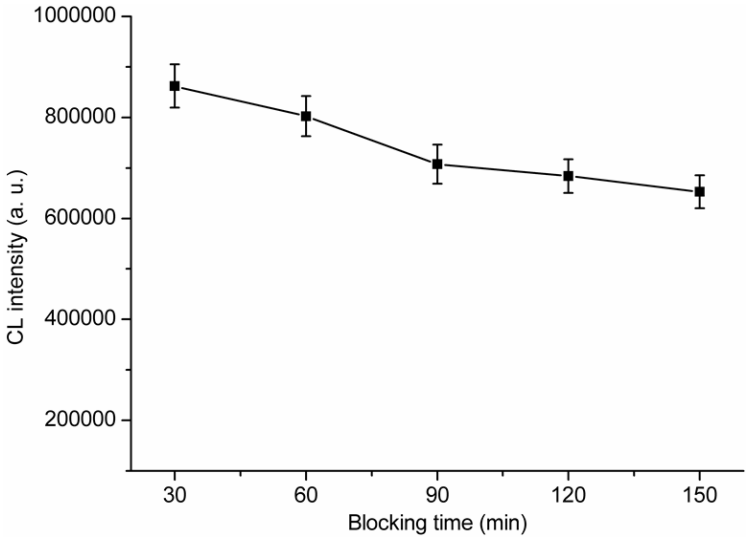

Fig. S3 Effect of the blocking time on the CL response for *S. aureus* at 10^5 CFU mL^{-1}. All other conditions were the optimal conditions (n = 5).

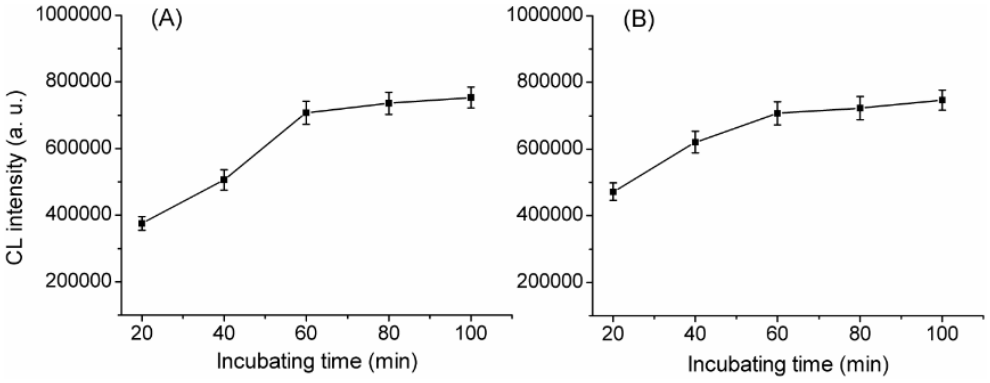

Fig. S4 Effects of the incubation time of (A) *S. aureus* and (B) Vanc-HRP on the CL responses for *S. aureus* at 10^5 CFU mL^{-1}. All other conditions were the optimal conditions (n = 5).

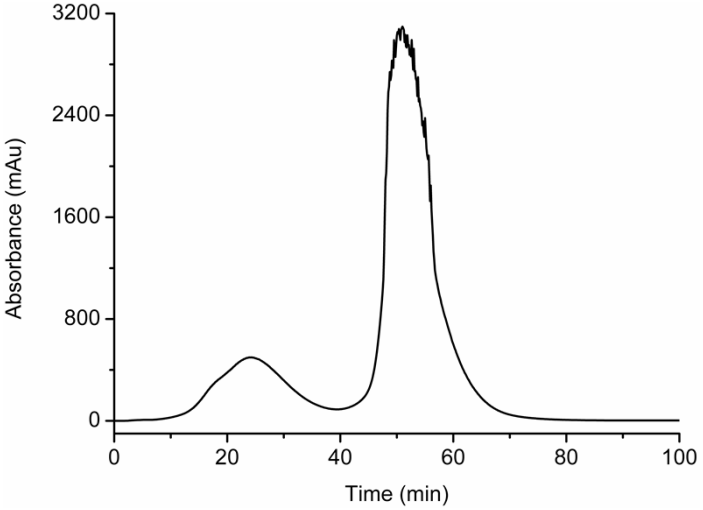

Fig. S5 The purification chromatogram of PE-tagged vancocin.

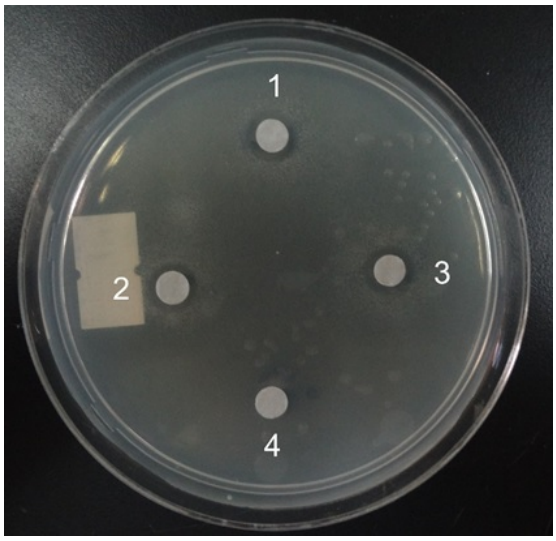

Fig. S6 Bactericidal halo test for PE-tagged vancocin. 1, 2 and 3: PE-tagged vancocin; 4: blank (PBS).

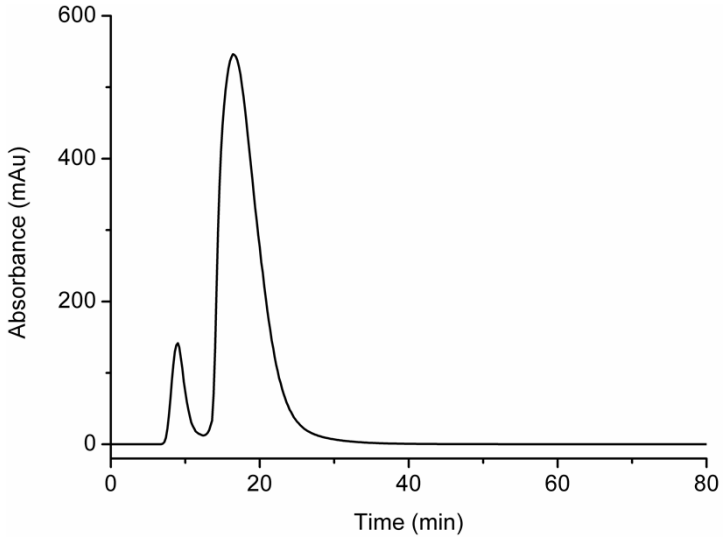

Fig. S7 The purification chromatogram of Vanc-HRP conjugate.

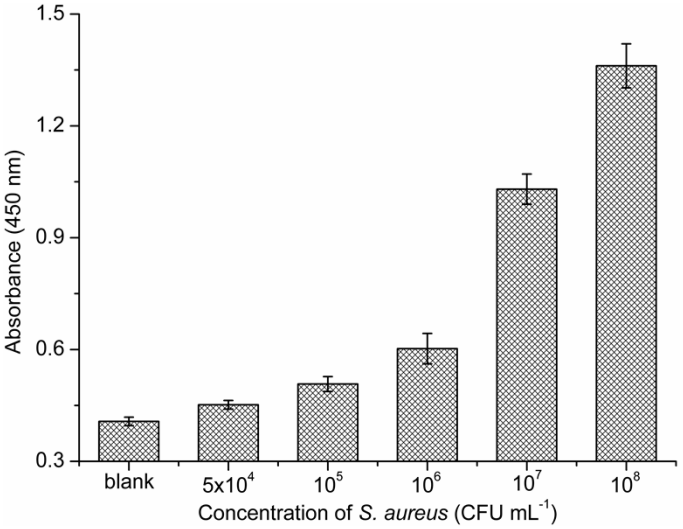

Fig. S8 Dose-response relation for *S. aureus* using pseudo ELISA, in which Vanc-HRP was used as the signal tracer.