Supporting Information

Dual-signal lateral flow assay using vancomycin-modified nanotags for rapid and sensitive detection of *Staphylococcus aureus*

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S1. Experimental section

S1.1 Materials and chemicals

Branched PEI (MW ~25 kDa), vancomycin, bovine serum albumin (BSA), 2-(N-morpholino)ethanesulfonic (MES), tetraethoxysylane (TEOS), Tween-20, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), and sodium azide (NaN_3) were obtained from Sigma-Aldrich (USA). Mouse monoclonal antibody to *S. aureus* were obtained from ThermoFisher (USA). Chloroauric acid tetrahydrate ($HAuCl_4 \cdot H_2O$) were purchased from Sangon Biotech Co., Ltd. (China). LFA materials and accessories were obtained from Jieyi Biotechnology Co., Ltd. (Shanghai, China). Nitrocellulose membranes were obtained from Sartorius (Spain). Carboxyl-functionalized CdSe/ZnS QDs (Catalog #CdSe-MPA-625) were obtained from Mesolight Inc. (Suzhou, China).

S1.2 Fabrication of dual-signal SiO_2-Au-QD nanocomposite

First, 200 nm SiO_2 NPs were synthesized according to a previously described Stöber method with modification.[1] Then, 1 mL of as-prepared SiO_2 spheres was mixed with 40 mL of aqueous PEI solution (0.5%, *v*/*v*), and the mixture was sonicated for 30 min. During the sonication, the PEI rapidly self-assembled onto the surface of the negatively charged SiO_2 spheres to form SiO_2@PEI NP spheres. Then, the SiO_2@PEI was completely separated from the solution and then washed twice with deionized water to remove excess PEI. Afterward, the prepared SiO_2@PEI spheres were added in 100 mL of 3 nm Au seed, and the mixture was kept sonicating for another 30 min.

The resulting SiO_2-Au NPs were separated by centrifugation (5500 rpm, 6 min), and dispersed in 5 mL of deionized water. Third, the prepared SiO_2-Au NPs were added into 40 mL of PEI aqueous solution (0.5 mg/mL), and the mixture was sonicated for 60 min to coat the second PEI layer on the surface of SiO_2-Au NPs. After washing twice by centrifugation, SiO_2-Au-PEI were mixed with 20 mL of carboxyl-functionalized CdSe/ZnS QDs (1 nM) under sonication for 30 min to form dual-signal SiO_2-Au-QD. Finally, the synthesized SiO_2-Au-QD NPs were separated by centrifugation (5000 rpm, 6 min) and stored in 10 mL of ethanol for future use.

S1.3 Fabrication of vancomycin modified-SiO_2-Au-QD tags

Vancomycin molecules were conjugated to the surface of SiO_2-Au-QD NPs via the EDC-based coupling,[2] as illustrated in Scheme 1a. In brief, 1 mL of SiO_2-Au-QD NPs was separated from ethanol by centrifugation (5000 rpm, 6 min) and resuspended in 0.5 mL of MES buffer (0.1 M, pH 5.5) containing 1 mg of EDC and 0.5 mg of vancomycin. The mixture was then sonicated for 2 h, and the resulting SiO_2-Au-QD-Van tags were washed with water and redispersed in PBS buffer (10 mM, pH 7.4). The concentration of SiO_2-Au-QD-Van tags solution was determined by weight. The freeze-dried SiO_2-Au-QD-Van tags were weighed, dissolved in PBS solution and prepared as a standard solution (2 mg/mL) for future use.

S1.4 Fabrication of LFA strip for *S. aureus* detection

A one-channel LFA strip was designed with a sample pad, NC membrane, a test line, and an absorbent pad for the detection of *S. aureus*. The detection antibody (30 μL, 0.8 mg/mL) to *S. aureus* was sprayed onto the NC membrane to build the test line by using the XYZ spraying platform (Biodot, USA) at an application volume of 0.1 μL/mm. The antibody modified NC membrane was dried overnight at 37 °C in the drying oven, and then assembled with the sample and absorbents pad onto a plastic backing card. The prepared LFA was then cut into individual 3-mm strips and stored in vacuum desiccator until use.

S1.5 Preparation of bacterial sample

The tested bacterial concentrations were verified by classic plate counting.[3] Briefly, *S. aureus* was inoculated onto 5% sheep blood agar plates at 37 °C in an atmosphere containing 5% CO_2 for 16 h. Dozens of colonies were obtained from the plates and transferred into 1 mL of PBS solution (10 mm, pH 7.4) as the initial bacterial solution. The original bacterial solution was then diluted 1×10^5 to 1×10^7 times into 0.1 mL of sterile water and applied to a blood agar plate at 37 °C. After 12 h of incubation, the colony forming units (CFUs) on the plate was counted. Based on the CFU count

results, the initial bacterial solution can be diluted to the testing concentration. The experiments with the bacterial subculture, maintenance, and treatments were conducted in a level II biosafety cabinet. Considering biological safety, the bacteria were inactive by absolute methanol for further use.

S1.6 Characterization

Transmission electron microscopy (TEM) images of fabricated nanomaterials (including SiO_2, SiO_2-Au, and SiO_2-Au-QD) were taken on a Tecnai G2 F20 microscope (Philips, Holland) at an accelerating voltage of 200 kV. Zeta potentials and dynamic light scattering (DLS) results were investigated using a Mastersizer 2000 (Malvern, UK). Fluorescence signal of SiO_2-Au-QD-Van-based LFA strip was acquired on a portable FIC-S1 fluorescent strip reader (Suzhou Hemai, China).

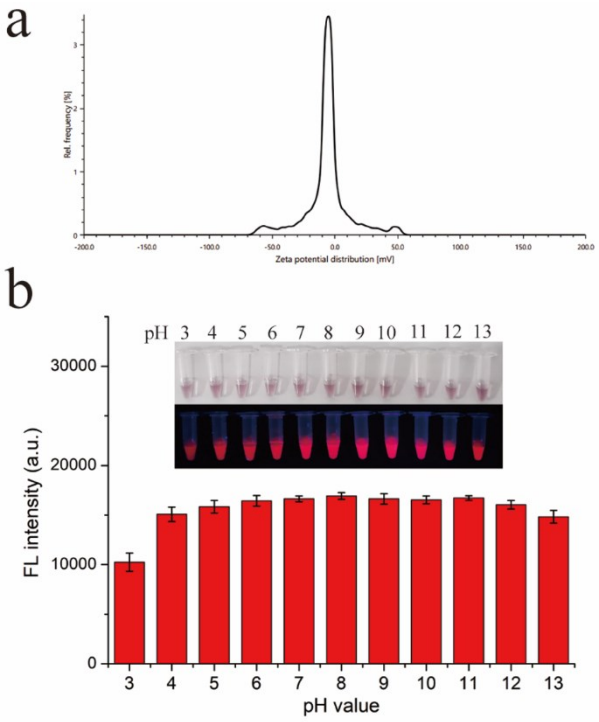

Fig. S1 (a) Zeta potential of SiO_2-Au-QD-Van NPs. (b) Fluorescence images and intensities of SiO_2-Au-QD-Van at different pH values.

Fig. S2 Agarose gel electrophoresis results of amplified PCR products by using *S. aureus* as DNA template.

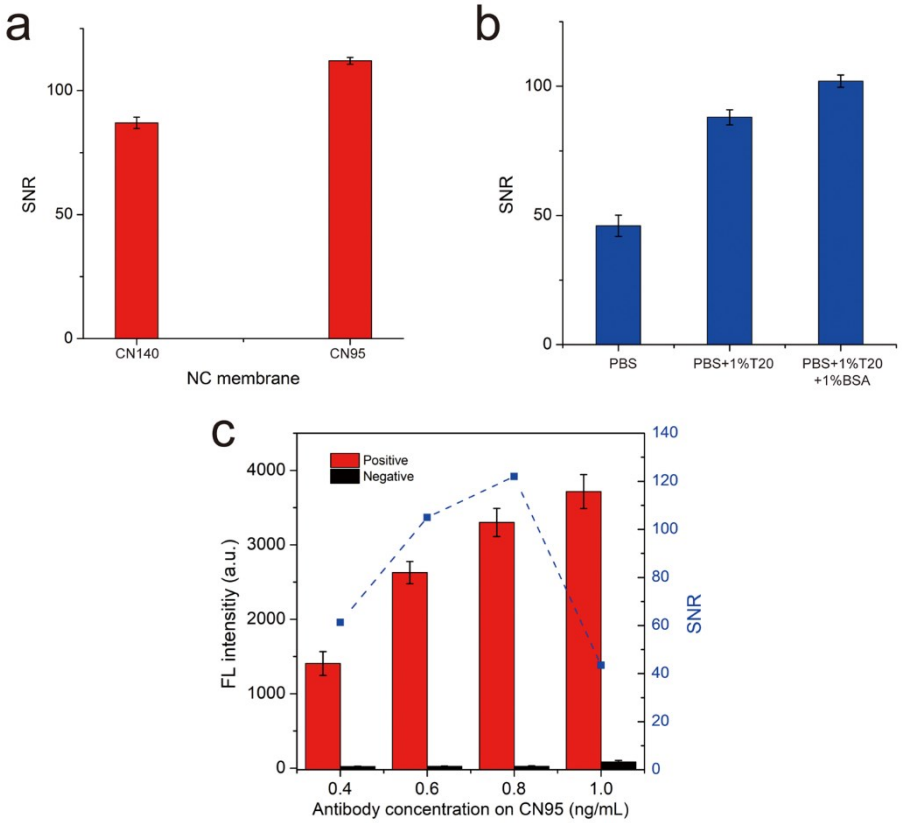

Fig. S3 Optimization of NC membrane (a), running buffer (b), and detection antibody concentration on the test line (c) for SiO_2-Au-QD-Van-based LFA strip.

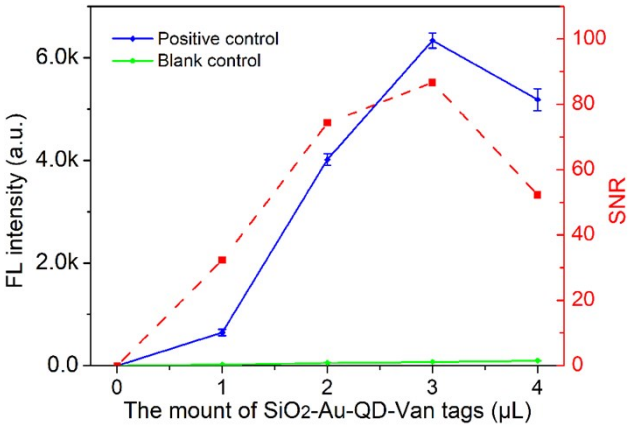

Fig. S4 Effects of the SiO_2-Au-QD-Van (2 mg/mL) amount on the test line intensity of the LFA strip.

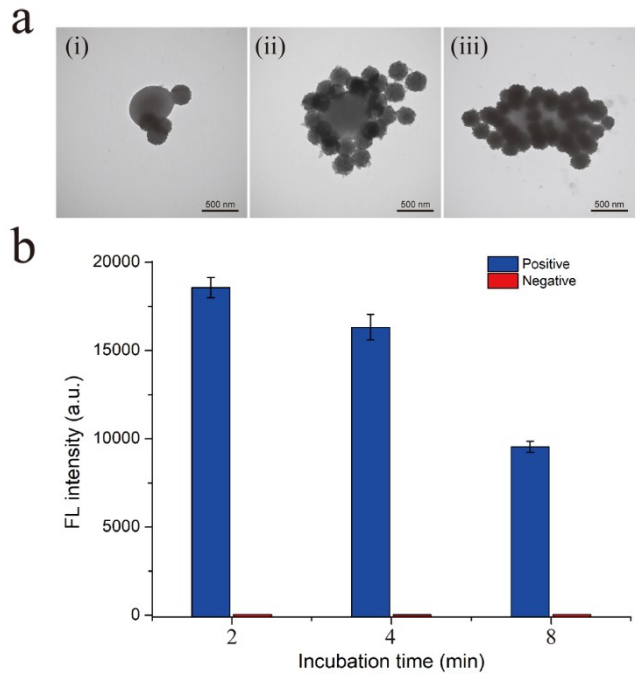

Fig. S5 Optimization of incubation time of SiO_2-Au-QD-Van-based LFA strip. 10^4 cells/mL of *S. aureus* was spiked into PBS solution as the bacteria sample. (a) TEM images of the SiO_2-Au-QD-Van-*S. aureus* complexes from different incubation time: (i) 2 min, (ii) 4 min and (iii) 8 min. (b) Effects of different incubation time for SiO_2-Au-QD-Van-based LFA strip.

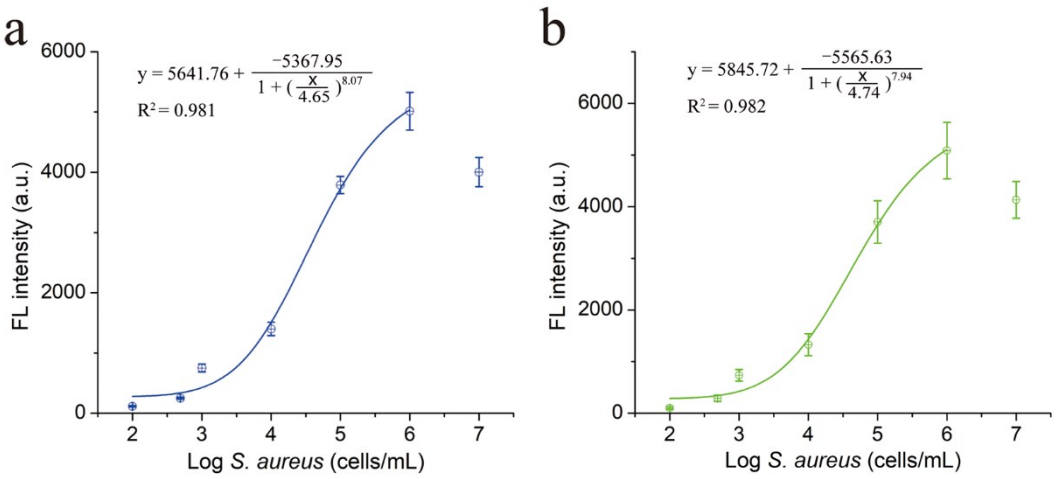

Fig. S6 Corresponding calibration curves for *S. aureus* detection in (a) PBS buffer (10 mM, pH7.4) and (b) vegetable juice. Error bars are standard deviation of three repetitive tests.

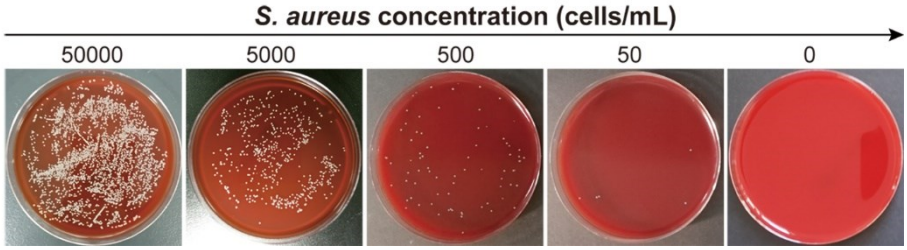

Fig. S7 Control experiments using the plate counting method for *S. aureus* detection. 100 μL of the bacterial samples with different concentrations (50000–0 cells/mL) was coated on the blood agar plates.

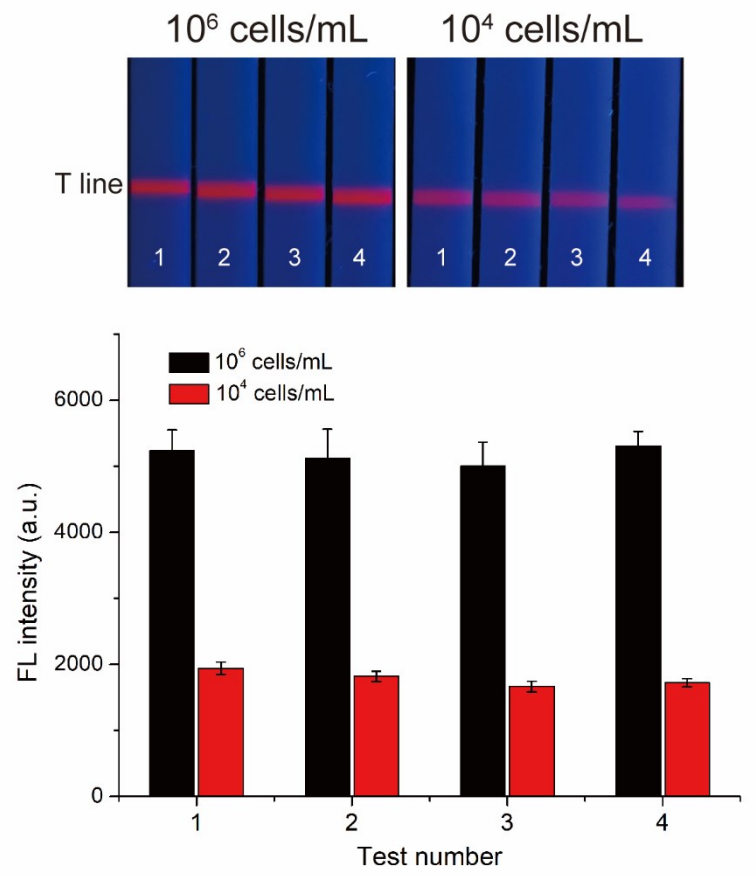

Fig. S8 Assay reproducibility of *S. aureus* at concentrations of 10^6 cells/mL and 10^4 cells/mL. The error bars represent the standard deviations from three separate experiments.

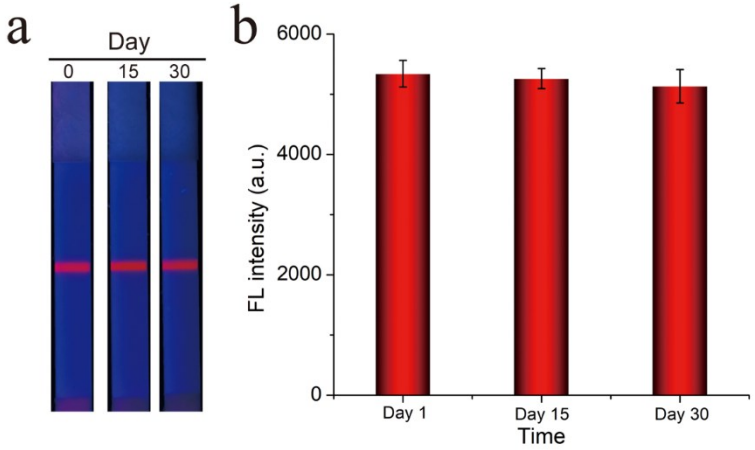

Fig. S9 Long stability of SiO_2-Au-QD-Van based-LFA strips stored for 30 days. (a) Photographs and (b) corresponding test line intensities of the test strips. Error bars are standard deviation of three repetitive tests.

References

1. C. Wang, M. Li, Q. Li, K. Zhang, C. Wang, R. Xiao and S. Wang, *RSC Adv.*, 2017, **7**, 13138-13148.
2. C. Wang, K. Zhang, Z. Zhou, Q. Li, L. Shao, R. Z. Hao, R. Xiao and S. Wang, *Int J Nanomed*, 2017, **12**, 3077-3094.
3. J. Li, C. Wang, L. Shi, L. Shao, P. Fu, K. Wang, R. Xiao, S. Wang and B. Gu, *Mikrochim Acta*, 2019, **186**, 475.