Electronic Supplementary Information

Affinity Capture Using Peptide-Functionalized Magnetic Nanoparticles to Target *Staphylococcus aureus*

Fang-Yin Kuo,[1] Wei-Lien Lin,[2] Yu-Chie Chen[*1,2]

[1]Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan
[2]Institute of Molecular Science, National Chiao Tung University, Hsinchu 300, Taiwan

[*]E-mail: yuchie@mail.nctu.edu.tw

Abstract

Staphylococcus aureus, a commonly found pathogen, can cause food poisoning and infections. Thus, it is necessary to develop analytical methods for rapid screening of *S. aureus* in suspicious samples. Magnetic nanoparticles (MNPs) are widely used as affinity probes to selectively enrich target species from complex samples because of their large specific surface area and magnetic property. The MNP surface should be functionalized to have the capability to target specific species. In this study, we propose a straightforward method to functionalize aluminum oxide-coated iron oxide (Fe_3O_4@Al_2O_3) MNPs with the peptide HHHHHHDEEGLFVD (*D*) through microwave heating for 30 s. The peptide *D* was comprised of three domains: polyhistidine (H_6) used as the linker, DEE added as the spacer, and GLFVD for targeting *S. aureus*. *D* was immobilized on the surface of Fe_3O_4@Al_2O_3 MNPs through H_6-Al chelation. Our results showed that the *D*-functionalized Fe_3O_4@Al_2O_3 MNPs (*D*-Fe_3O_4 MNPs) possess the capability to target *S. aureus*. The selective trapping experiments were conducted under microwave-heating for only 60 s, and sufficient bacterial cells were trapped by the MNPs to be identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). We demonstrated that the *D*-Fe_3O_4 MNPs combined with MALDI-MS can be used to rapidly characterize trace amounts of *S. aureus* in complex samples, such as juice and eggs. The sensitivity of this approach toward *S. aureus* is as low as ~ 3×10^5 cells mL^{-1}.

Additional Figures

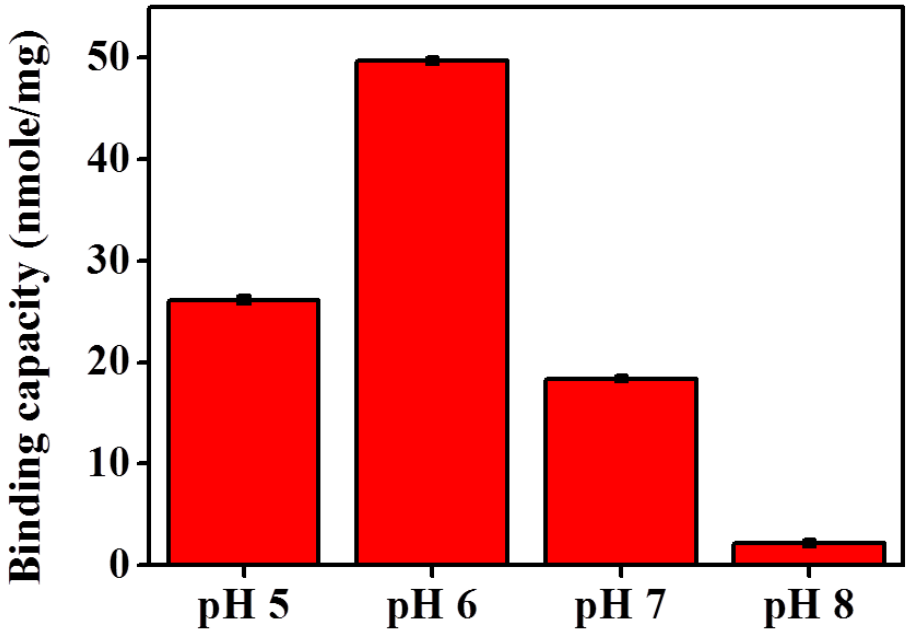

Figure S1. Examination of the binding capacity of the $Fe_3O_4@Al_2O_3$ MNPs toward *D* under different pH conditions. Bar graphs of the binding capacity of *D* (20 μL, 5×10^{-4}M) onto the $Fe_3O_4@Al_2O_3$ MPs (50 μg) at pH 5, 6, 7 and 8 under vortex-mixing for 1 h.

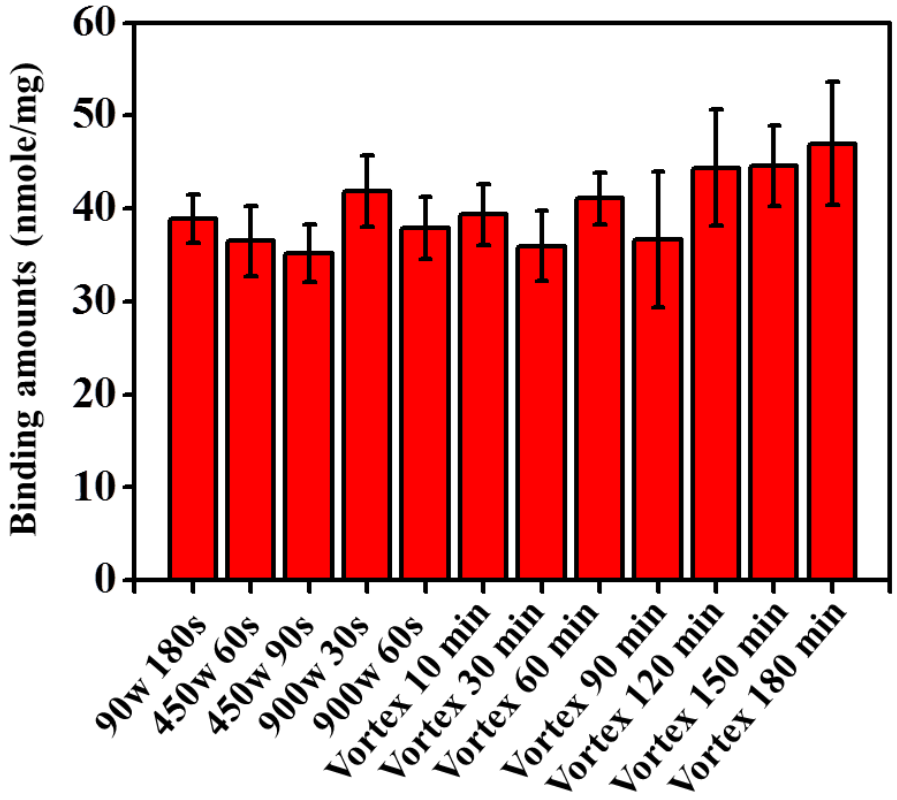

Figure S2. Examination of the binding amount under vortex-mixing and microwave-heating with different powers and time. *D* (20 μL, 5× 10^{-4} M) were incubated with the Fe_3O_4@Al_2O_3 (50 μg) at pH 6.

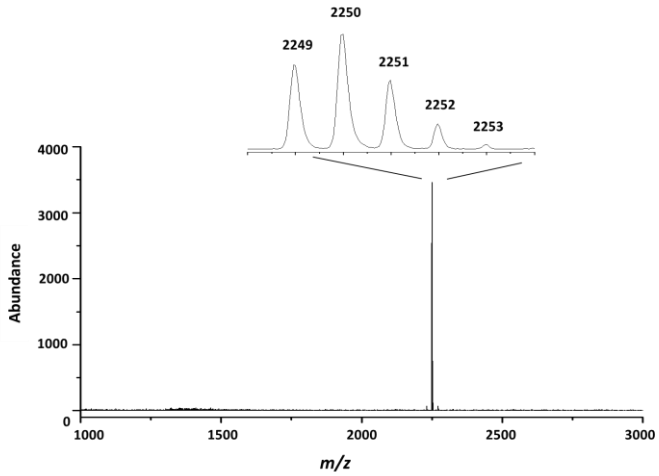

Figure S3. MALDI mass spectrum of the *D*-Fe_3O_4@Al_2O_3 MNPs. The *D*-Fe_3O_4@ Al_2O_3 MNPs MNPs (50 mg/mL, 1 μL) were mixed with CHCA (1.5 mg/mL, 2 μL) prepared in the solvent of acetonitrile/deionized water (2:1, v/v). The mixture (1 μL) was deposited on the MALDI plate. After solvent evaporation, the sample was ready for MALDI-MS analysis. The inset shows the zoom-in mass spectrum at *m/z* 2249.

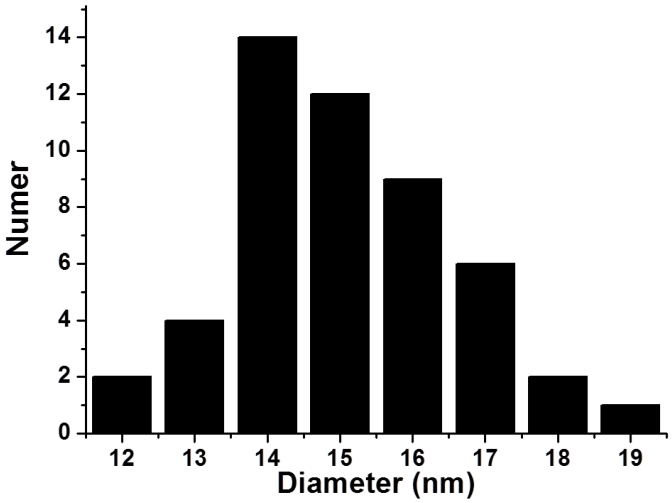

Figure S4. The size distribution of the *D*-Fe_3O_4@Al_2O_3 MNPs. The size of the MNPs were estimated to be 14.5±1.6 nm.

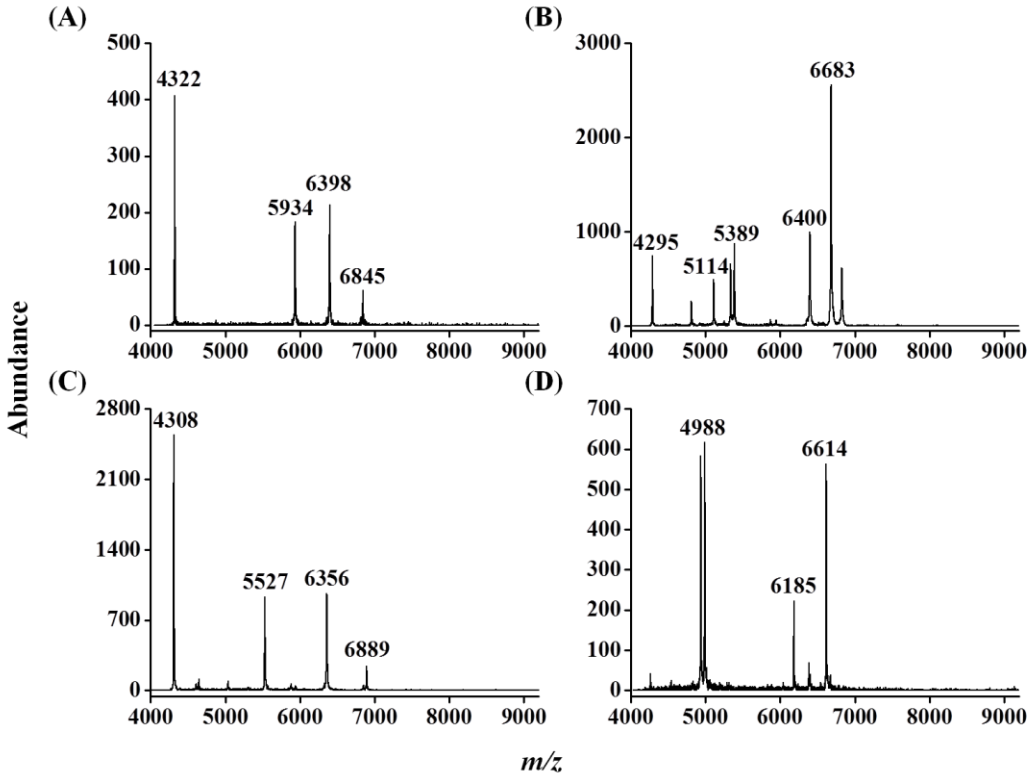

Figure S5. MALDI mass spectra of (A) *S. simulans,* (B) *S. epidermidis,* (C) *S. aureus,* and (D) *S. saprophyticus*. α-Cyano-4-hydroxycinnamic acid (15 mg mL^{-1}) prepared in the solvent of acetonitrile and deionized water (2:1, v/v) containing 1% trifluoroacetic acid. The mixture (2 μL) was spotted onto a MALDI sample plate for MALDI-MS analysis.

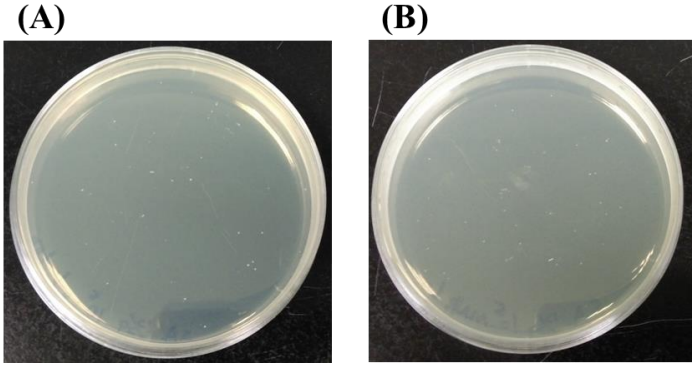

Figure S6. (A) Representative photograph obtained by inoculating a *S. aureus* sample (pH 6, 20 μL) directly on an agar plate for overnight. The culture experiment was conducted three times. The colony number was 34±19. (B) Representative Photograph obtained after using the *D*-Fe_3O_4@Al_2O_3 MNPs (50 μg) to trap *S. aureus* from the same sample (20 μL) as used to obtain Panel (A) followed by rinse, re-suspension in acetate buffer (pH 6, 20 μL), and culturing the suspension (20 μL) on an agar plate for overnight. Three replicated experiments were conducted. The colony number was 34±19.

Additional Table

Table S1. The cell concentration of different bacteria at OD_{600}= 1.

Bacteria	cells/mL
S. aureus	1.56×10^9
S. saprophyticus	1.25×10^9
S. simulans	6.73×10^8
P. aeruginosa	4.66×10^8
E. coli J96	2.80×10^8
B. cereus	1.47×10^7