Anti-Interference Near-Infrared SERS Tag-Aptamer Sensor for Rapid Detection of *Staphylococcus aureus* in Complex Matrices

Rui Zhao,[a] Qishuo Wang,[a] Yunqing Wang,[a,b]* Rongchao Mei,[c] Xiaoyan Wang,[c] Lingxin Chen[b]*

[a] *Research Institute of Marine Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Qingdao, 266112, China*

[b] *Shandong Key Laboratory of Coastal Zone Environmental Processes and Ecological Security, Shandong Engineering Research Center for Coastal Zone Ecological & Environmental Monitoring Technologies and Equipment, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China*

[c] *School of Pharmacy, Binzhou Medical University, Yantai, 264003, China*

**Corresponding author.*

E-mail addresses: yqwang@yic.ac.cn (Y. Wang), lxchen@yic.ac.cn (L. Chen)

Support information

Experimental

1.1 Au nanoparticle synthesis

Au seed were synthesized according to our previously reported seed-mediated growth metho[1]. Briefly, an aqueous seed solution was prepared by reducing $HAuCl_4$ (0.5 mM, 5 mL) in CTAB (0.2 M, 5 mL) with freshly prepared ice-cold $NaBH_4$ (10 mM, 0.6 mL) at 27°C. The mixture was vigorously stirred for 30 s and then left undisturbed for 3h. 50 μL of the synthesized seed solution was mixed with CTAC (0.2 M, 2 mL) and AA (0.1 M, 1.5 mL). $HAuCl_4$ solution (0.5 mM, 2 mL) was then added, and the reaction proceeded at 27°C for 15 min. The resulting Au seed (~10 nm) were collected by centrifugation at 14000 rpm for 40 min, washed twice with deionized water, and redispersed in 1 mL water. CTAC (0.1 M, 40 mL), AA (10 mM, 3 mL), and the 10 nm seed solution (200 μL) were combined. $HAuCl_4$ (0.5 mM, 40 mL) was infused into the mixture at 40 $mL \cdot h^{-1}$ using a syringe pump. After complete addition, the reaction was maintained at 27°C for 10 min. The final Au NPs were purified by centrifugation.

1.2 Liposome encapsulation

1 mL of the synthesized Au NP solution was centrifuged at 6000 rpm for 10 min. The supernatant was discarded, and the NPs were resuspended in 1 mL of liposome solution (5 mg/mL). This mixture was incubated overnight at room temperature. Following incubation, the solution was centrifuged again at 6000 rpm for 10 min. The supernatant containing unbound lipids was discarded. The pellet was then dispersed in 300μL of deionized water to yield lipid bilayer-coated Au NPs. Finally, the Cy7 signal molecule (2.5×10^{-4} M, 10 μL) was added to the nanoparticle suspension and allowed to adsorb onto the functionalized surface.

1.3 Au@Au core-shell NP synthesis

1 mL of phospholipid-bilayer-coated Au NPs (OD_{540nm}=1.0) was mixed with AA (0.1 M, 0.1 mL) under vigorous stirring at room temperature (~25°C). $HAuCl_4$ (2.5mM, 0.1mL) was added to the reaction system, and the reaction continued for 5 minutes, synthesising Au-Au core satellite NPs. The mixture was subsequently heated to 60°C with continuous vigorous stirring. KI (10 mM, 12 μL) was introduced to initiate etching, and the reaction continued for 20 min. Finally, the solution was centrifuged at 6000 rpm for 10 min to remove excess KI reagent. The etched nanoparticles were redispersed in deionized water for further use.

1.4 Calculating the concentration of SERS tag@PS-COOH

The method for calculating the concentration of SERS tag@PS-COOH is as follows. Briefly, it is caculated from the Au seed NPs by the following formula:

$C_{Au\ seed} = (C_{Au\ element} \times M_{Au\ element})/(\rho_{Au} \times V_{single\ Au\ seed})$.

$C_{Au\ seed}$: quantity concentration of Au seeds; $C_{Au\ element}$: molar concentration of $HAuCl_4$ used in synthesis of Au seeds (assuming the reaction is complete); ρ_{Au}=19.32 g/cm^3; $V_{single\ Au\ seed}$: volume of single Au seed ($4\pi R^3/3$); R: radius of single Au cluster (assuming the average R=1 nm). $C_{Au\ element}$ = (0.5 mM×5 mL)/10.6 mL = 2.36×10^{-4} M ; $M_{Au\ element}$ = 196.97g/mol; $V_{single\ Au\ core} = 4\pi R^3/3 = 4.19\times10^{-27}$ m^3; C_{Auseed} = $(2.36\times10^{-4}\text{M}\times 196.97\text{g/mol})/(19.32\text{g/cm}^3\times4.19\times10^{-27}\ \text{m}^3) = 5.75\times10^{17}$ particles/L. Since the concentration of Au seed is calculated to be 5.75×10^{17} particles/L, the concentration of SERS tag@PS-COOH is 2.30 $\times10^{13}$ particles/L (Au seed is first diluted 416 times, then concentrated 3 times, and finally diluted 1.2 times, then concentrated 5 times, based on the synthesis process of 45 nm of Au NPs).

Figures

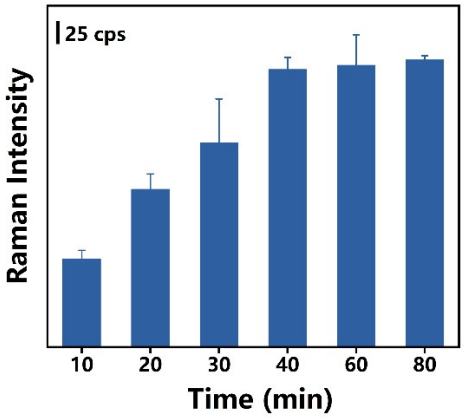

Figure S1. Raman signal intensity of magnetic bead-SERS tag aptamer sensor reacting with bacteria at different detection times.

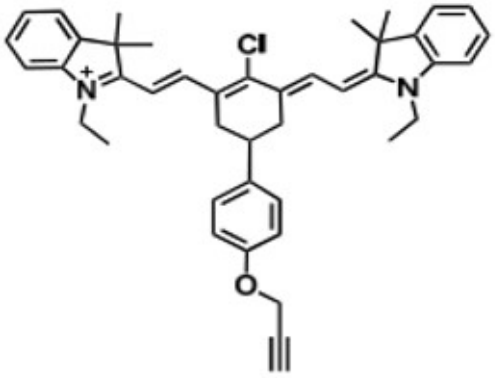

Figure S2. Chemical structure of Raman reporters Cy7.

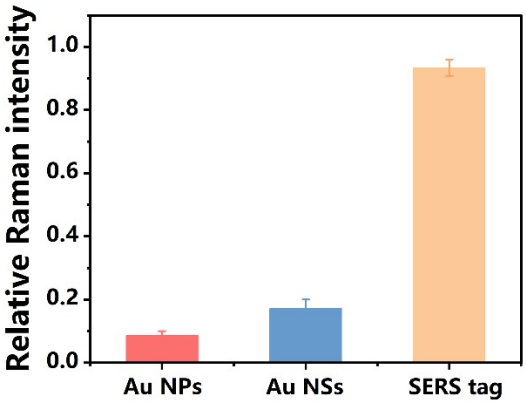

Figure S3. Signal retention levels of different particles before and after PS carboxylation.

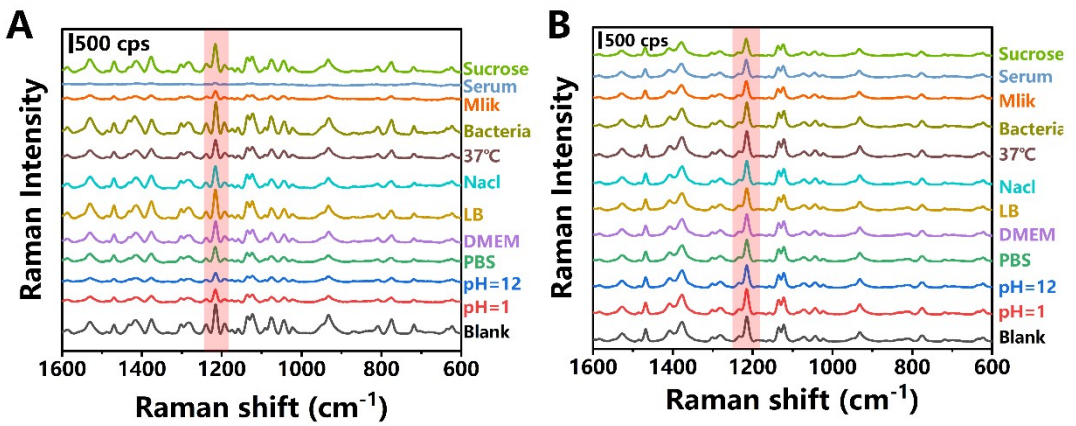

Figure S4. Stability Raman spectra: (A) bare Au NP-based SERS tags exhibited drastic signal fluctuations attributed to nanoparticle aggregation or reporter molecule detachment; (B) performance of PS shell-coated SERS tags under interference conditions.

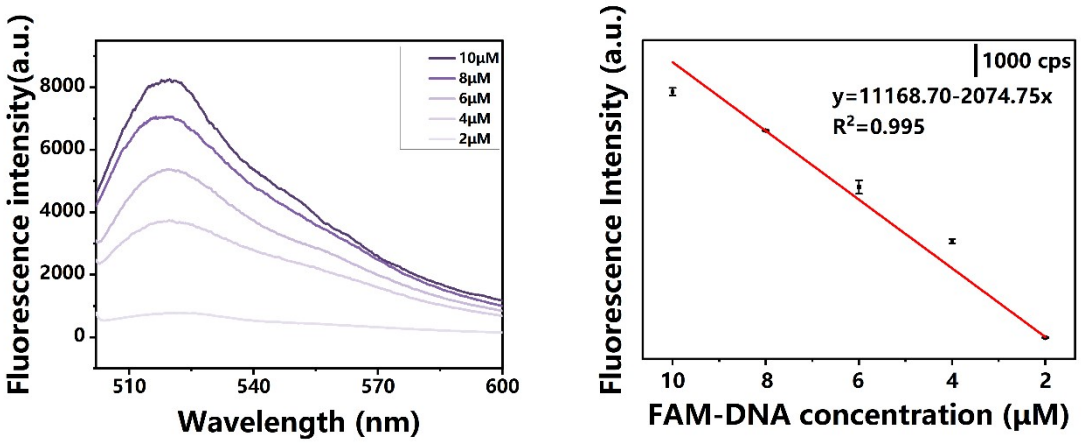

Figure S5. Fluorescence calibration curve for FAM-DNA concentration.

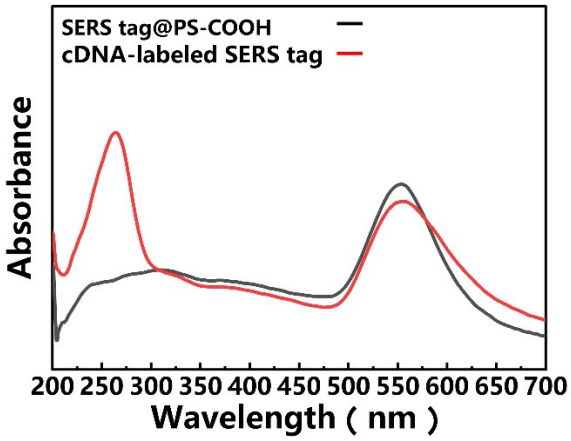

Figure S6. UV spectra of SERS tag@PS-COOH and cDNA-labeled SERS tag.

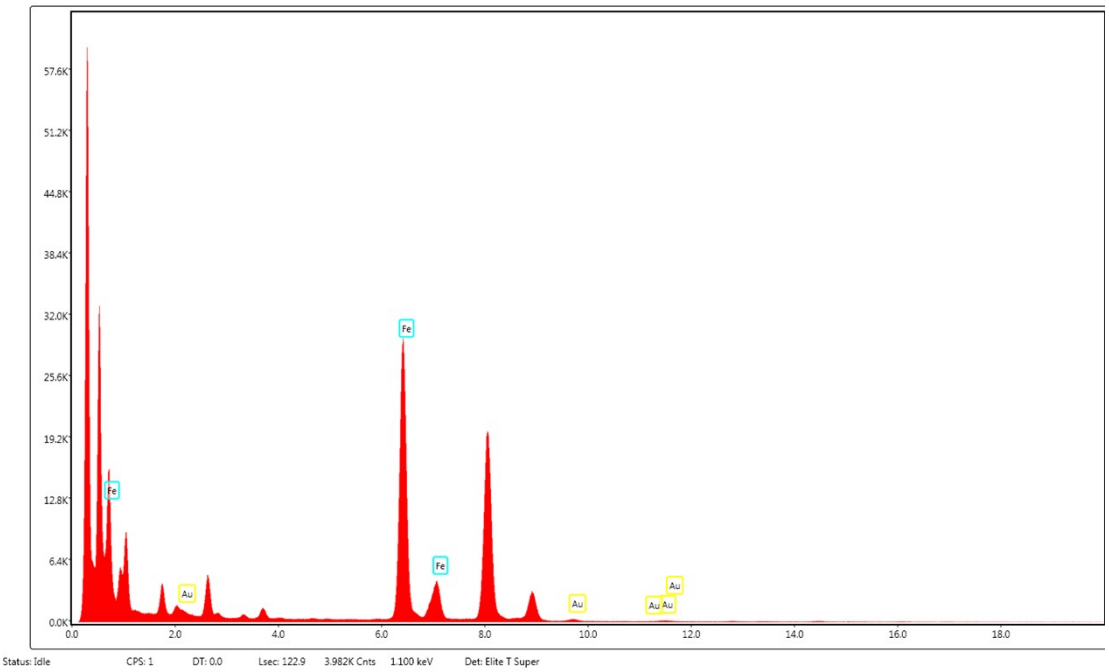

Figure S7. EDS spectrum of the magnetic bead-SERS tag aptamer sensor.

Table

Table S1. DNA Sequence Used in This Work

Name	Sequence (5' to 3')
Aptamer DNA for *S. aureus*	Biotin- GCA ATG GTA CGG TAC TTC CTC GGC ACG TTC TCA GTA GCG CTC GCT GGT CAT CCC ACA GCT ACG TCA AAA GTG CAC GCT ACT TTG CTA A
cDNA for *S. aureus*	TGC GAT GAA ACG ATT -$(CH_2)_6$-NH_2
FAM-DNA	FAM-$(CH_2)_6$- ACG CTA CTT TGC TAA
DNA for amide bonds	TGC GAT GAA ACG ATT-$(CH_2)_6$-NH_2
DNA for Au-S bonds	TGC GAT GAA ACG ATT-$(CH_2)_6$-SH

Table S2. Characteristics of the SERS biosensor developed in this work compared to reported methods

Method/ capture element	Pathogenic bacteria	Capture substrate	LOD (CFU/ mL)	Irradiation wavelength	Applicable matrices	Ref
SERS/antibody	*S. aureus*	$MnFe_2O_4$@Au	10	——	——	[2]
	E. coli	Fe_3O_4	10	785nm	Beef	[3]
	MRSA, E. coli,S. typhimurium	Fe_3O_4@Ag	10	532nm	——	[4]
SERS/boric acid	*E. coli, S. aureus*	AgNPs	10^2	632.8 nm	Blood	[5]
SERS/antibiotic	*E. coli, S. aureus,* MRSA	Fe_3O_4@Ag-Van MNPs	10^2	785nm	Milk, blood	[6]
SERS/aptamer	*S. aureus*	$MnFe_2O_4$@Au	10	780nm	——	[7]
	S. typhimurium	Fe_3O_4@AuNPs	15	——	Pork	[8]
	V. parahaemolyticus	Au-PDMS film	18	632.8nm	Seafood	[9]
	S. typhimurium		27			
	E. coli O157:H7	Au/Fe^{3+} nanoclusters	2	——	Water, lettuce, chicken	[10]
This work	*S. aureus*	Au-Au core shell@PS	10	785nm	Seawater, Serum, Milk, TCM	

Reference

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