

Electronic Supplementary Information

Functional Oligonucleotide Probe Encapsulated Silver Nanocluster

Assembled by Rolling Circle Amplification and Their Application in Label-

Free Sensor

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2. Experimental

Table S1 DNA oligonucleotides sequence used in this work. the underlined letters is the aptamer sequences of *E. coli* O157:H7. **P** in the padlock probe represents phosphate at the 5' end.

Oligonucleotide name	sequence (5' to 3') description
aptamer-primer probe	<u>GCAATGGTACGGTACTTCCACTTAGGTCGAGGTTAGTTTGTCTTGCTGGCGCA</u> <u>TCCACTGAGCGCAAAGTGCACGCTACTTTGCTAA</u> TTTTTTTTTTTGTACATGG CGGCATTACT
padlock probe	P- CCATGTACATTTTTTTTCTTGTTTCCTTTCCTTGTTTTTTTTTTTTTCTTGTTTCC TTTCCTTGTTTTTAGTAATGCCG
ligation probe	ATGTACATGGCGGCATTACT
FOP 1	TTCTTGTTTCCTTTCCTTGTTTTTCCCTTAATCCCC
FOP 2	TTCTTGTTTCCTTTCCTTGTTTTTCCCCCCCCCCCC
FOP 3	TTCTTGTTTCCTTTCCTTGTTTTTCCCTTAATCCCC
FOP 4	TTCTTGTTTCCTTTCCTTGTTTTTCCC TTC CTT CCT TCC AAC CAA CCC ATC CCA TTC TGC AGC
FOP5	TTCTTGTTTCCTTTCCTTGTTTTTACCCGAACCTGGGCTACCACCCTTAATCCC CAATCC GTCGAGCAGAGTT

2.2. Apparatus

Fluorescence spectra were obtained with a RF-5301PC spectrophotometer (Shimadzu, Japan) equipped with a 150 W xenon lamp (Ushio Inc., Japan). Morphology observation of AgNCs and self-assembled AgNCs in PB buffer (pH 7.0, 20 mM) was carried via transmission electron microscopy (TEM; JEM-2100 microscope) at an acceleration voltage of 200 kV. Gel electrophoresis was conducted using DYCZ-24DN electrophoresis cell (LIUYI, Beijing, China) and Bio-Rad Gel imaging system (Bio-Rad, USA). Cyclic voltammetry (CV) and differential pulse

voltammetry (DPV) were carried out on a CHI 660D electrochemical workstation (Shanghai Chen Hua Instrument Co. Ltd., China) with a conventional three-electrode system composed of a bare or functionalized Au electrode as the working electrode, a platinum wire as the auxiliary electrode, and a Ag/AgCl reference electrode with saturated KCl solution as the reference electrodes.

Results and discussion

Electrochemical characterization of the modification of the electrode.

The characterization of the electrode was also conducted by cyclic voltammetry (CV). As shown in Fig. S1, the redox peak current decreased with the modification of the electrode surface with the antibody, *E. coli* O157:H7, PAP, RCA product and AgNCs. This is due to the hindrance of the electrontransfer process of the modified biomacromolecules on the electrode, which is consistent with the observed increases of the R_{et} in the EIS measurements.

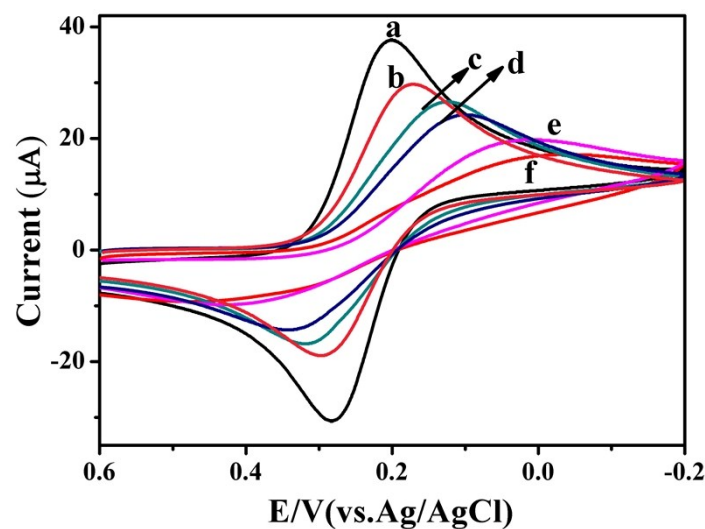


Fig. S1. CVs obtained for the bare Au electrode (a), antibody/Au electrode (b), *E. coli* O157:H7/BSA/antibody/Au electrode (c), PAP/*E. coli* O157:H7/BSA/antibody/Au (d), RCA products-linked PAP/*E. coli* O157:H7/BSA/antibody/Au electrode (e), AgNCs/RCA products-linked PAP/*E. coli* O157:H7/BSA/antibody/Au

electrode (f). Measurements were performed in 10 mM PBS (pH 7.4) containing of 0.25 mM KCl and 5.0 mM $K_3[Fe(CN)_6]$.

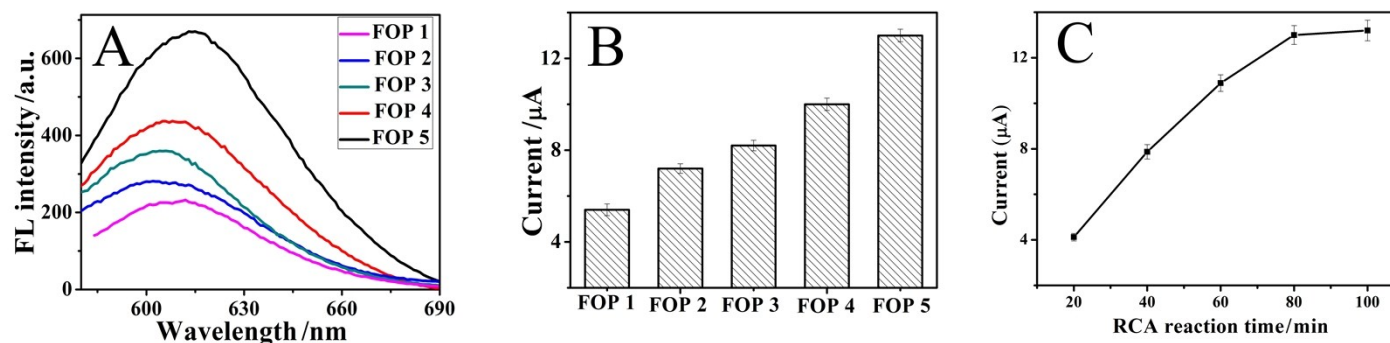


Fig. S2. (A) Effect of the FOP sequences on fluorescence intensity of AgNCs. (B) Effect of the FOP sequences on the DPV peak current of biosensor. (C) Effect of reaction time of RCA on the DPV peak current of biosensor.

Table S3 *E. coli* O157:H7 analysis in synthetic samples.

Samples	Spiked amount (cfu mL ⁻¹)	Our method (cfu mL ⁻¹)	Recovery (%)	plate count method (cfu mL ⁻¹)
milk samples	3.7×10^2	$(3.46 \pm 0.15) \times 10^2$	93.4	3.83×10^2
	3.7×10^3	$(3.77 \pm 0.21) \times 10^3$	101.9	3.54×10^3
	3.7×10^4	$(3.58 \pm 0.17) \times 10^4$	96.8	3.61×10^4
	3.7×10^5	$(3.80 \pm 0.13) \times 10^4$	102.7	3.52×10^4