Supplementary Information

SERS-based cascade amplification bioassay protocol of miRNA-21 by using sandwich structure with biotin-streptavidin system

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Part 1 Optimization of the concentration of the probe DNA

As described in Section 2.3, there are three steps in the preparation process of Ag@4MBA@DNA-biotin probe. In the second step, the concentration of the probe DNA is important to ensure the fully linking of the probe DNA with Ag@4MBA NPs. And the optimal concentration of the probe DNA can be determined by analyzing the absorption spectra of the pure probe DNA solution and the supernatants of Ag@4MBA@DNA-biotin NPs [1].

In our experiments, the three pure probe DNA solutions (0.3, 0.5 and 0.7 OD) were added to the prepared Ag@4MBA NPs solutions (3 ml) and incubated at 37 ℃ for 3 h, and then washed by centrifugation to obtain the supernatants of Ag@4MBA@DNA-biotin NPs, respectively. Here, the absorption spectra of the three pure probe DNA solutions (0.3, 0.5 and 0.7 OD, dissolved by 3ml of deionized water) and their supernatant of Ag@4MBA@DNA-biotin NPs are measured and shown in Fig. S1(a), (b) and (c), respectively. It is easy to find that all the supernatants of Ag@4MBA@DNA-biotin NPs display weak absorptions compared to the corresponded pure probe DNA solutions, which means a mass of probe DNAs were linked with the Ag@4MBA NPs. Especially, in Fig. S1(a), the absorption peak of the supernatants of Ag@4MBA@DNA-biotin NPs obviously decline, which means few of

probe DNA is remained in the supernatants. However, comparing with Fig. S1(a), the supernatants of Ag@4MBA@DNA-biotin NPs in Fig. S1(b) and (c) exhibit stronger absorptions, which implies more probe DNAs are remained in the supernatants. Base on Lambert-Beer law, the linkage amount of the probe DNA in Fig. S1(a), (b) and (c) are calculated to be 0.242, 0.273 and 0.268 OD, respectively. It presents that 0.3 OD of probe DNA is not enough to fully link with Ag@4MBA NPs, while 0.5 OD of probe DNA satisfies the requirement of fully linking with Ag@4MBA NP. Therefore, as described in Section 2.3, 0.5 OD of probe DNA was added in 3 ml of the prepared Ag@4MBA NPs solutions in our experiments. On the other word, 0.17 $OD \cdot ml^{-1}$ could be selected as an optimal concentration of probe DNA.

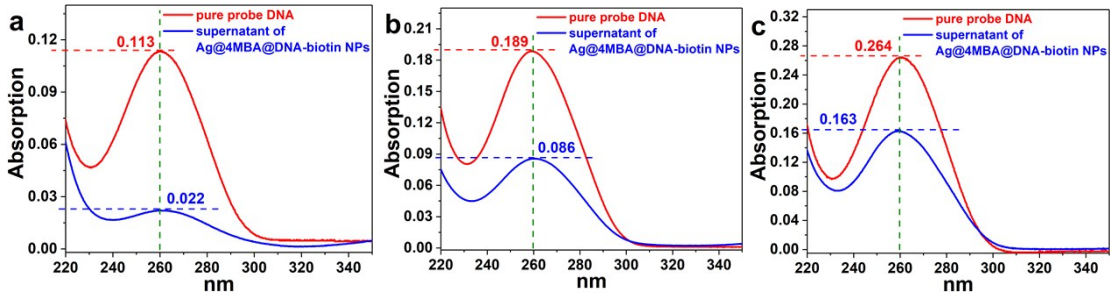

Fig. S1 UV-vis absorption spectra of the pure probe DNA solutions (red curves) and the supernatants of Ag@4MBA@DNA NPs (blue curve) corresponding to (a) 0.3, (b) 0.5 and (c) 0.7 OD, respectively.

Reference

[1] Z. Rong, C. Wang, J. Wang, D. Wang, R. Xiao, S. Wang, Magnetic immunoassay for cancer biomarker detection based on surface-enhanced resonance Raman scattering from coupled plasmonic nanostructures, Biosens. Bioelectron. 84 (2016) 15-21.

Part 2 Optimization of the concentration of the capture DNA

In this experiment, the optimal concentration of the capture DNA will be determined by the saturation concentration of anti-digoxigen immobilized on the Ag-coated wafer due to the corresponding relation between antigen and antibody.

Firstly, according to the steps described in Section 2.4, six Si@Ag@anti-digoxin/digoxin-DNA substrates were prepared by using the different concentrations of anti-digoxigen solution (0, 0.25, 0.5, 0.75, 1.0, 1.5 $\mu g \cdot ml^{-1}$), the capture DNA (2 μM) and the Ag-coated wafers. Secondly, the target RNA (2 μM of miRNA-21) and the

prepared Ag@4MBA@DNA-biotin probes solution (~ 0.3 mg·ml^{-1},) were added on the six Si@Ag@anti-digoxin/digoxin-DNA substrates, respectively. Here, the concentration of target-RNA is enough to ensure the hybridization chain reaction of capture DNA and miRNA-21. Thirdly, as described in Section 2.5, the sandwich complex composed of the Ag@4MBA@DNA-biotin probes, target RNA and the Si@Ag@anti-digoxin/digoxin-DNA substrate was used for the detection of SERS signal and the experimental results are shown in Fig. S2(a) and (b). Obviously, Fig. S2(b) exhibits that the intensities of SERS signal increase with the increase of the concentrations of anti-digoxigen solution and arrives saturation at 1.0 μg ml-1 and more. In addition, by further increasing the concentrations of the Ag@4MBA@DNA-biotin probes, the SERS signal of the formed sandwich complexes are almost unchanged, as shown in Fig. S2(c) and (d). It presents that saturation concentrations of anti-digoxigen solution is 1.0 μg·ml^{-1} (~ 1.28 μM), that is, the optimal concentration of the capture DNA can be determined as 1.28 μM. However, in our experiments, the concentration of the capture DNA is set as 1.5 μM, which is slightly greater than the optimal concentration of the capture DNA for fully linking of the capture DNA with anti-digoxigen.

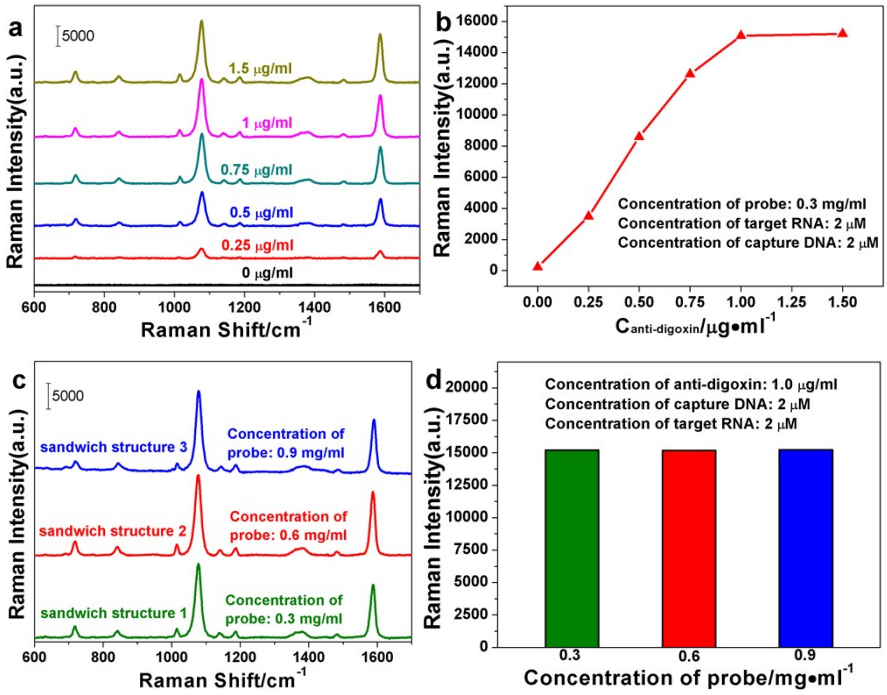

Fig. S2 (a) SERS spectra of Ag@4MBA@DNA-biotin probe at the different concentrations of anti-digoxigenin; (b) the intensities of peak at 1078 cm^{-1} corresponding to (a); (c) SERS spectra of Ag@4MBA@DNA-biotin probe at the different sandwich complexes; (d) the intensities of peak at 1078 cm^{-1} corresponding to (c).

Part 3 Optimization of the amplification order

According to the SERS-based cascade amplification bioassay protocol described in Section 2.5 of main text, the miRNA-21 was detected by measuring of SERS signal of 4MBA in the sandwich structure with fourth-order amplification, the experimental results are shown in Fig. S3(a). As shown in Fig. S3(b), the SERS intensities of peaks located at 1078 cm^{-1} in Fig. S3(a) are chosen to obtain the dose-response curves of the fourth order amplification. From Fig. S3(b), the LOD of SERS-based bioassay with fourth-order amplification is calculated to be 42.95 fM. Comparing with the results in Section 3.3, the LOD (42.95 fM) of SERS-based bioassay with fourth-order amplification is higher than that of SERS-based bioassay with third-order amplification (38.02 fM). Therefore, in terms of the LOD, the third order amplification was selected as an optimal amplification order in our detection scheme.

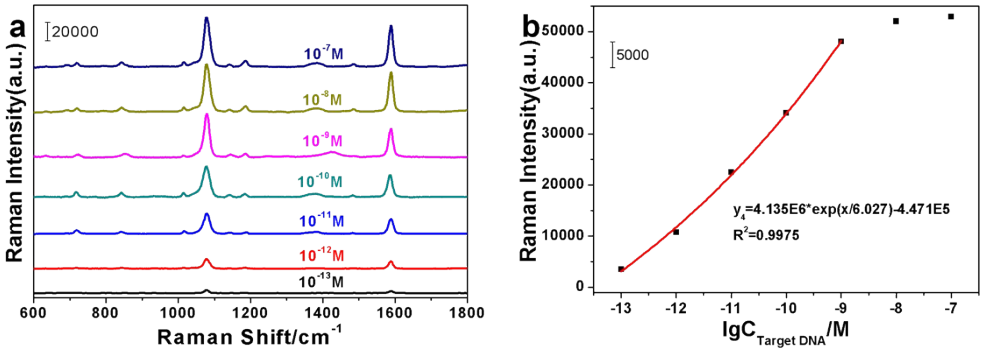

Fig. S3 (a) SERS spectra corresponding to the different concentrations of miRNA-21 in the fourth order amplification detection; (b) the dose-response curves of the fourth order amplification.

Part 4 Contrast data table of miRNA-21 detection

Table S1 Results of miRNA-21 detection with different methods

Methods	Detection range	LOD	Sources
SERS-based sandwich detection	7.40 nM-1.48 μM	0.85 nM	Ref. 18
SERS-based cascade amplification detection	100 fM - 100 nM	38.02 fM	This work
RT-qPCR	1 pM-1 μM	>200 fM	Table 3 of this work