

Supplementary Materials

Gold Nanoparticle-based Enzyme-assisted Cyclic Amplification for the Highly-sensitive Detection of miRNA-21

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1. Materials

Table S1 Synthesized Oligonucleotides sequences employed in this work

Oligonucleotide	Sequence
DNA-a	5'-Texas Red-ATGTTGATACGATTCCGGCGCAATCAACATCAGTCTGATAAGCTA-3'
DNA-b	5'-CGGGCCGAATCGTATAACCATTTT- C6 SH-3'
DNA-c	5'-CCCGGCCCGTTTTTTTTTTTTTTT -Biotin-3'
DNA-d	5'-TTGCGCCGGAATCGTATCAACAT-3'
MiRNA-21	UAG CUU AUC AGA CUG AUG UUG A
MiRNA-16	UAG CAG CAC GUA AAU AUU GGC G
MiRNA-141	UAA CAC UGU CUG GUA AAG AUG G
MiRNA-205	UCC UUC AUU CCA CCG GAG UCU GU
Let-7a	UGA GGU AGU AGG UUG UAU AGU U
MiRNA-221	AGC UAC AUU GUC UGC UGG GUU UC
MiRNA-210	CUG UGC GUG UGA CAG CGG CUG A

2 Characterization of AuNPs

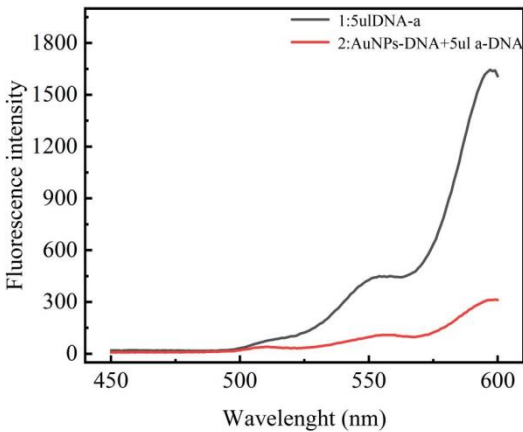


Figure S1. Excitation spectra at 620 nm before and after the reaction of AuNPs-DNA and Texas Red.

3. Feasibility study of the miRNA assay

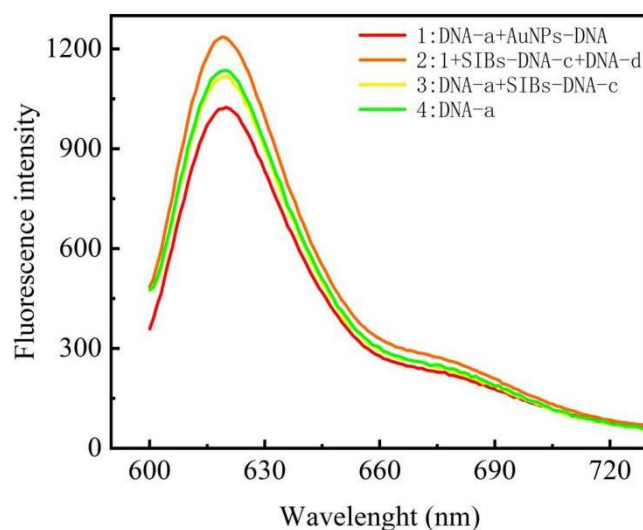


Figure S2. Effects of AuNPs-DNA and SIBs-DNA on fluorescence in the absence of miRNA-21.

4. Repeatability and longtime stability of biosensor

Table 2 is a t-test analysis table, which is analyzed by standard t-test results with a confidence level of 95% ($\alpha=0.05$) on the data measured within one day and within a few days. The 10 groups of fluorescence data tested within one day are 946, 1008, 1008, 959, 996, 1023, 1046, 1001, 980, 1021; the 10 groups of data tested within a few days are 948, 985, 934, 1033, 1031, 1027, 1034, 958, 961, 931. The selected linear regression equation was substituted into the linear equation $F_0 - F = 680.4C + 192.7$ ($R^2 = 0.993$) with 1.2 nM as the test concentration, and the theoretical average value was 1009.18, the target number was 10, and the t value, P value and confidence interval were calculated. The results are shown in Table 2, and the P values are all greater than 0.05. The results show that the experiment is repeatable, and the two confidence intervals are not much different, indicating that the experimental results are within a certain range and have certain stability.

Table S2 T-test analysis table. On the left is the analysis of 10 groups of experimental data in one day. On the right are 10 sets of experimental data analysis within a few days.

In one day (Flourescence intensity) t-test	In a few days (Flourescence intensity) t-test
$\alpha=0.05$	$\alpha=0.05$
sample average=998.8	sample average=984.2
null average=1009.18	null average=1009.18
standard delviation=30.2096379	standard delviation=43.16583011
N=10	N=10

$$t_1=1.086555298$$

$$t_2=1.830005255$$

$$P_1=0.305476152$$

$$P_2=0.100494771$$

$$\text{confidence interval}=[980.07, 1017.52]$$

$$\text{confidence interval}=[957.45, 1010.96]$$

5. Specificity analysis

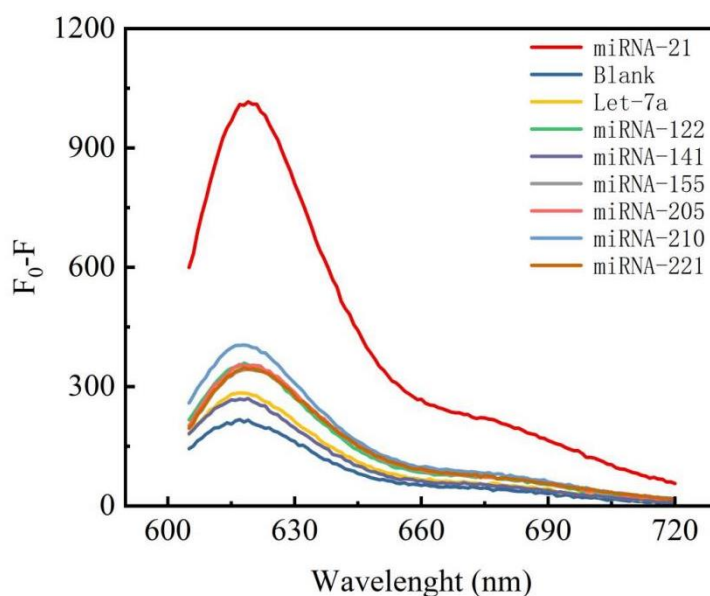


Figure S3. Experimental results Fluorescence values corresponding to various miRNAs.

6. Determination of target DNA in human serum samples

Table S3 The t-test in the practical application of human serum. The p-values of the experimental results are all greater than 0.05, indicating that the 95% confidence interval is within the 95% confidence interval, indicating that it also has good feasibility in the practical application of human serum.

Sample	Added (nM)	Found (nM)	t	P
1	0.24	0.218	1.502	0.272
		0.221		
		0.244		
		0.538		
2	0.54	0.525	2.462	0.133
		0.525		
		0.747		
		0.753		
3	0.75	0.750	0	1
		0.750		