Supplementary Information

Sensitive and enzyme-free fluorescence polarization detection for miRNA-21 based on decahedral sliver nanoparticles and strand displacement reaction

Qingyue Zhu, Hui Li, Danke Xu*

State Key Laboratory of Analytical Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, No 163, Xianlin Avenue, Nanjing 210023, China.

1. Synthesis and characterization of decahedral silver nanoparticles ($Ag_{10}NPs$) and the nucleic acids modified $Ag_{10}NPs$:

The synthesis method of the decahedral silver nanoparticles ($Ag_{10}NPs$) referred to the previous literature. In a word, 15ml trisodium citrate (50mM), 15 ml polyvinylpyrrolidone (PVP, 6%), 2.5 ml L-arginine (10 mM), 10 ml $AgNO_3$ (10 mM) were added into 175 ml ultra-pure water. After mixing evenly, 0.8 ml fresh $NaBH_4$ (0.5M) was added into the solution, and the next step was irradiating by the blue light for 24 hours, then the nanoparticles were obtained. The fluorescence and absorption spectrums of the nucleic acids modified $Ag_{10}NPs$ were also listed in Fig S1.

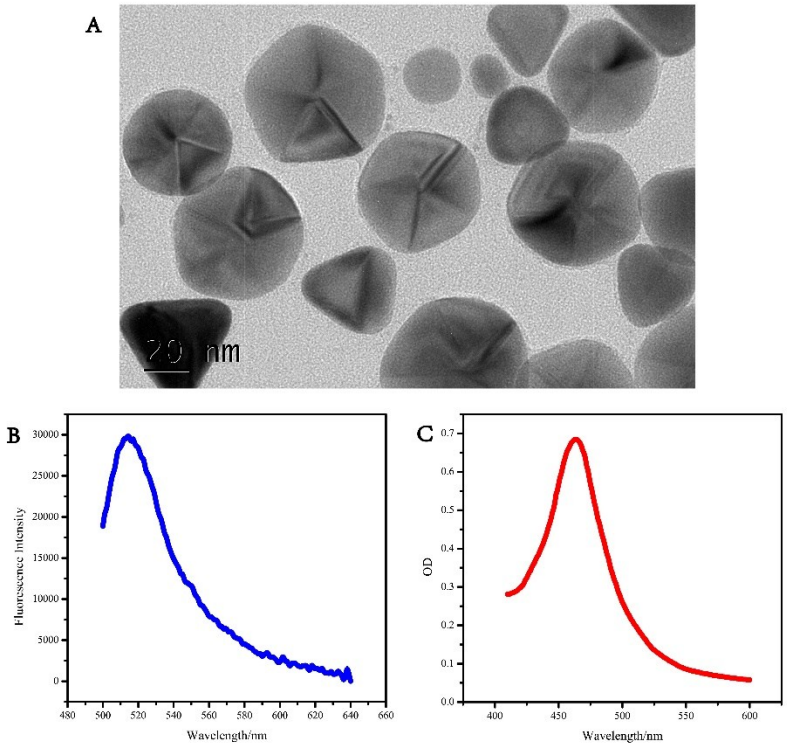

Fig. S1. (A)TEM images of Ag_{10}NPs. (B) The fluoresce spectrum of the nucleic acids modified Ag_{10}NPs (C) The absorption spectrum of the nucleic acids modified Ag_{10}NPs

2. Principles of strand displacement reaction without cyclic amplification.

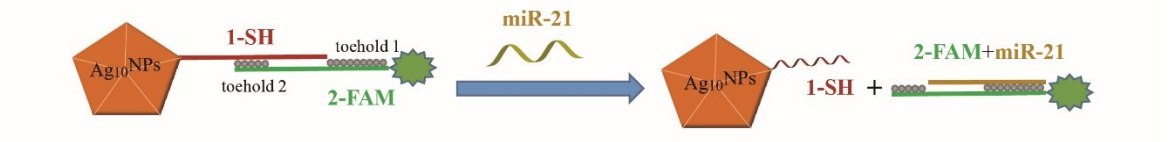

Fig. S2. Detection principle of miRNA-21 without cyclic strand displacement reaction (SDR).

3. Optimization of nucleic acid concentration conditions:

Followed the previous steps to synthesize probes with nucleic acid concentrations of 100,200,300,400,500,600,700nM (1-SH: 2-FAM = 1: 1). When the probes were synthesized, it was necessary to be kept at room temperature for 24 hours before

being used. After centrifugation at 15000 rpm twice, the probes were resuspended in 1xPBS. Probes were mixed with 5nM miRNA-21 and reacted for a period of time, and then detected with a microplate reader.

4. Optimization of reaction time:

Probes with a concentration of 300 nM (1-SH: 2-FAM = 1: 1) were synthesized and kept at room temperature for 24 h. The probes were centrifuged twice at 15,000 rpm and resuspended in 1 x PBS. The probes were mixed with 10 nM miRNA-21 and kept in a 37 ° C and 220 r • min^{-1} constant temperature shaker, the signals were detected by a microplate reader at 10, 20, 30, 40, 50, 60, 70 min.

5. Signals comparison of HEK-293 and MCF-7 total RNA extracts:

After mixing the optimized probe with 10nM miRNA-21(diluted by 1×PBS , 350 ng total RNA·μl^{-1} HEK-293 and MCF-7 extracts), the FP value generated by the probe at different times were continuously recorded with the kinetic mode of the microplate reader, and the fluorescence polarization change value ΔFP caused by different reaction times was also calculated.

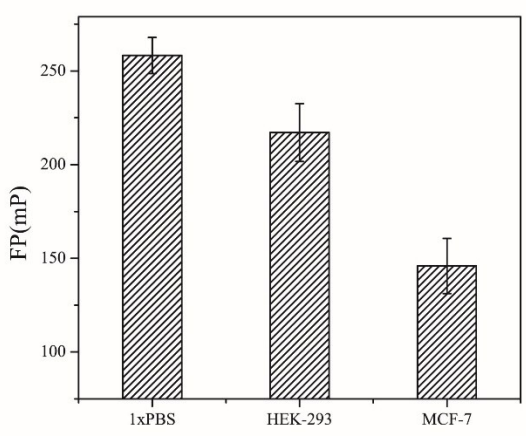

Fig. S3. Response signals of detection methods to different reactants.

6. The calculation method of miRNA-21 concentration in MCF-7 total RNA extracts.

Standard addition methods to measure the miRNA-21 concentration in MCF-7 total RNA extracts[15], miRNA-21 with different concentration gradients was added and mixed, then the fitting curve of dots was described as y=Ax+B, and the concentration of total RNA extracts(ng • L^{-1}) was named C, so the miRNA-21 concentration N was calculated:

$$N=B/AC \qquad \text{Eq 1}$$