Electronic Supplementary Information

High-Discrimination Factor Nanosensor Based on Tetrahedral DNA Nanostructures and Gold Nanoparticles for Detection of MiRNA-21 in Live Cells

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The oligonucleotide sequences used in this article are as follows:

Expected detection probe P1 (ExP1):

5’-SH-ATTTGTATAGCTTATCAGACTG-3’.

5’-Dabcyl-ATTTGTATAGCTTATCAGACTG-3’.

Expected detection probe P2 (ExP2):

5’-TCAACATCAGTCTGATAAGCTATACAA AT-3’-FAM.

5’-FAM-TCAACATCAGTCTGATAAGCTATACAA AT-3’-Cy5.

Ordinary detection probe P1 (OrP1): 5’-SH-TATAGCTTATCAGACTG-3’,

Ordinary detection probe P2 (OrP2):

5’-TCAACATCAGTCTGATAAGCTATA-3’-FAM.

Target (T): 5’-TAGCTTATCAGACTGATGTTGA-3’.

Proximal mismatch of cDNA (P): 5’-TAGCTTATCAGACTGAAGTTGA-3’.

middle mismatch of cDNA (M): 5’-TAGCTTATCTGACTGATGTTGA-3’.

distal mismatch of cDNA (D): 5’-TTGCTTATCAGACTGATGTTGA-3’.

18 insertion (18i): 5’-TAGCTT ATCAGACTGATCGTTGA-3’.

18 deletion (18d): 5’-TAGCTTATCAGACTGATGTT GA-3’.

18 mismatch (18m): 5’-TAGCTTATCAGACTGATCTTGA-3’.

MiR-200b:5’-TAATACTGCCTGGTAATGATGA-3’.

Let-7d: 5’-AGAGGTAGTAGGTTGCATAGT T-3’.

S1: CAGCACGAGTCACTCTTCCCCTCTGATAAGCTACGGTCTGATAA

GCTACTTGAC.

S2: SH-CCGTAGCTTATCAGAGGGCATTAATCAACATCACAAAAT GTACTGACGAAACTT.

S3: TTGTGATGTTGATTAATGTCTGATAACGTAACTGATGAAGAGTG ACTCGTGCTG.

S4: AAGTTTCGTCAGTACATTGTCAAGTAGCTTATCAGAATCAGTTAC GTTATCAGA.

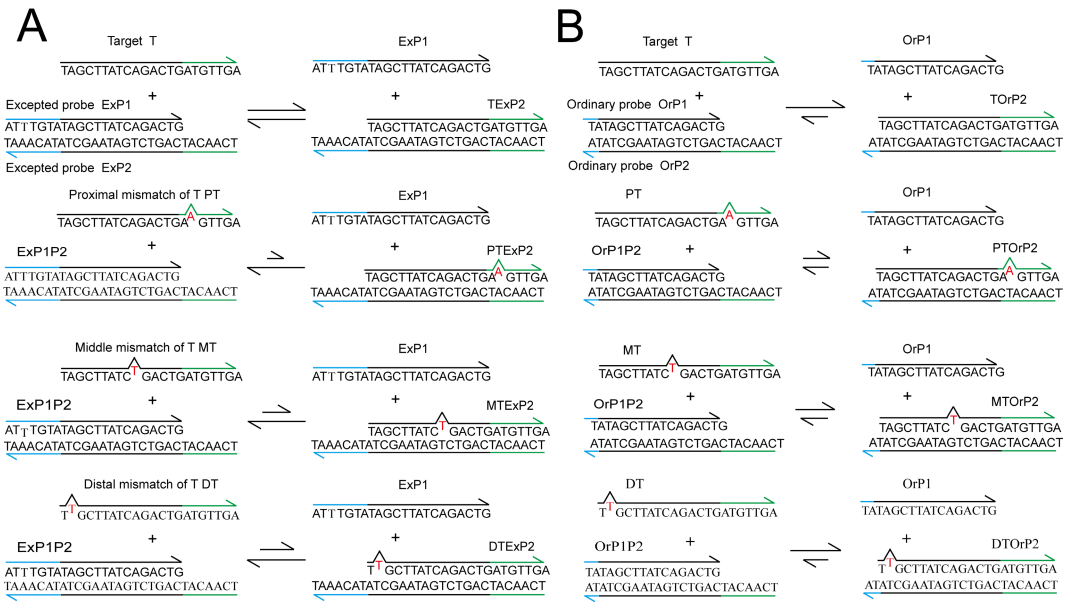

Figure S1. Working principle of ExP (A) and OrP (B) for single base mutation target and wild type target detection. Tehold exchange probe, for example, ExP1P2 consists of a pre-hybridized complement strand ExP2 and a protector strand ExP1, which can react with T to release ExP1 and the hybridized product TExP2.

Table S1. Thermodynamic parameters evaluated by software

Name	Complementary bases	T (K)	ΔH^0 (Kcal mol^{-1})	ΔS^0 (Cal $mol^{-1}K^{-1}$)	ΔG^{0*} (Kcal mol^{-1})
ExP1P2	22	310	-168.2	-467.7	-23.1
TExP1	22	310	-165.1	-451.8	-25.0
OrP1P2	17	310	-128.7	-357.0	-18.0
TOrP1	22	310	-165.1	-451.8	-25.0

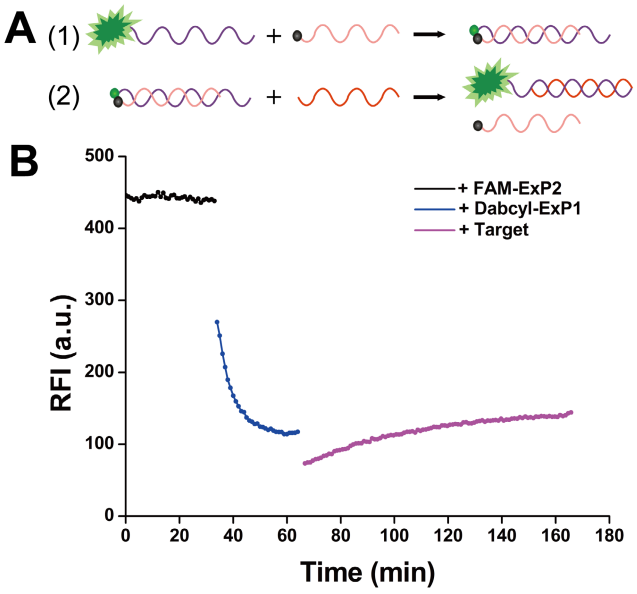

Figure S2. (A) Kinetics experiments of the hybridization reaction and strand displacement reaction of ExP1P2. (B) 100 μL FAM-ExP2 (black curve); 100 μL Dabcyl-ExP1 and 100 μL FAM-ExP2 at 30 min (blue curve); 50 μL target was added to the above solution (purple curve) at 60 min. DNA concentrations were 200 nM.

Preparation of Au-NPs:

AuNPs (20 ± 3 nm) were synthesized using the sodium citrate reduction method [1,2]. Typically, 1.8 mL of 1% aqueous trisodium citrate solution (freshly prepared) was quickly added to a boiling aqueous solution of HAuCl4 (100 mL, 0.25 mM) with vigorous stirring. The color of the solution changed from blue to brilliant red in several minutes. After boiling for 30 min, the heat source was removed to allow the reaction solution reached room temperature. The solution was stored at 4 °C before use.

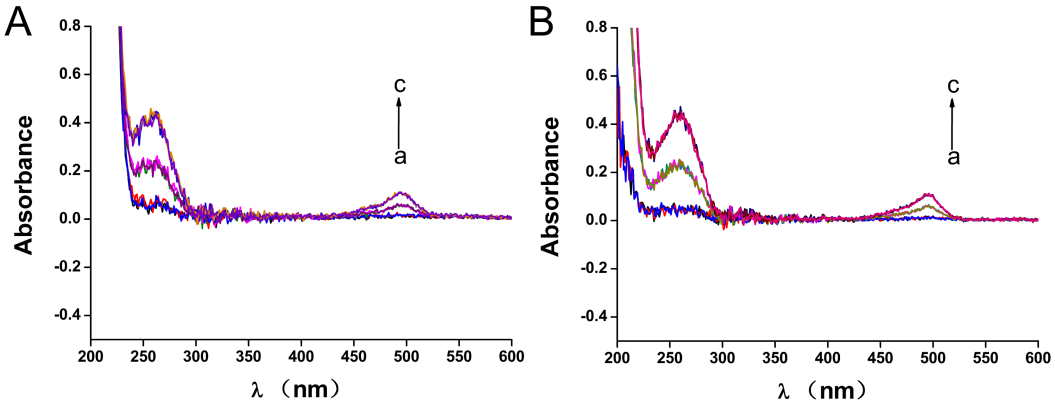

Figure S3. (A) UV-vis detection of supernatant of Au-NPs@ExP1 after incubation with FAM-ExP2 for 2 h at room temperature in the dark. a-c represents 10 μL, 50 μL, and 100 μL 10 μM FAM-ExP2. (B) a-c represents 10 μL, 50 μL, and 100 μL 10 μM of FAM-ExP2 without Au-NPs@ExP1, other components of the solution were the same with experiments of (A). Nucleic acid absorption peak was 260 nm. All these experiments were repeated thrice.

When we synthesized Au-NPs@ExP1P2, an interesting phenomenon was found the stability of Au-NPs increased with the total volume increasing of 10 μM ExP1. We observed that Au-NPs were totally gathered in the bottom of the centrifuge tube in 10 μL 10 μM ExP1 and were partly gathered in 50 μL 10 μM of ExP1. However, they kept very stable in 100 μL 10 μM of ExP1. Figure S2A showed supernatant of Au-NPs@ExP1 after incubation with FAM-ExP2 for 2 h at room temperature in the dark. We supposed that the Au-NPs need enough negatively charged nucleic acid to maintain their stability. Meanwhile, we did not observe the aggregation phenomenon in the nucleic acid hybridization step on Au-NPs@ExP1. Nucleic acid absorption peak was at 260 nm and the Au-NPs absorption peak was at 520 nm. We initially thought the absorption peak at 520 nm in Figure S2A was caused by Au-NPs, however, we again observed the absorption peak at 520 nm in Figure S2B in the absence of Au-NPs. We supposed that the absorption peak at 520 nm was mainly caused by FAM in Figure S2A, because the FAM fluorescence emission peak was also 520 nm.

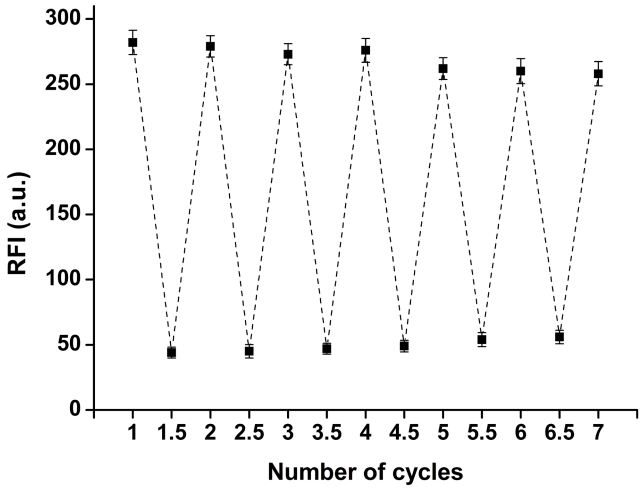

Figure S4. Repeatability of recycled Au-TDNNs through six times detection of target.

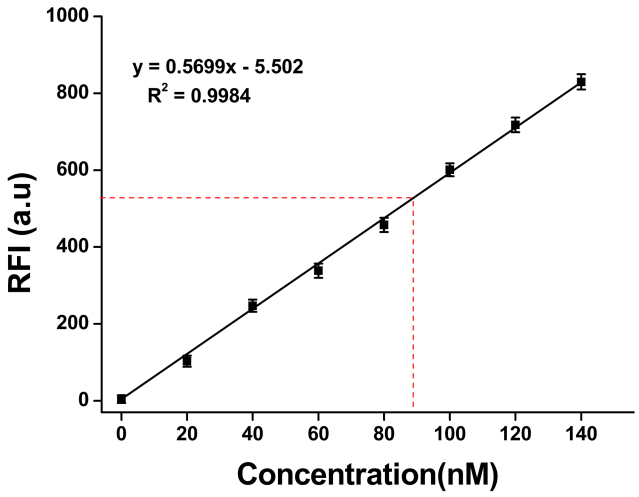

Figure S5. Calibration curve of pure FAM-ExP2. The red dotted line represents the relative fluorescence intensity of the nanosensor when detecting target. The final concentration of Au-TDNNs was 1 nmol/L.

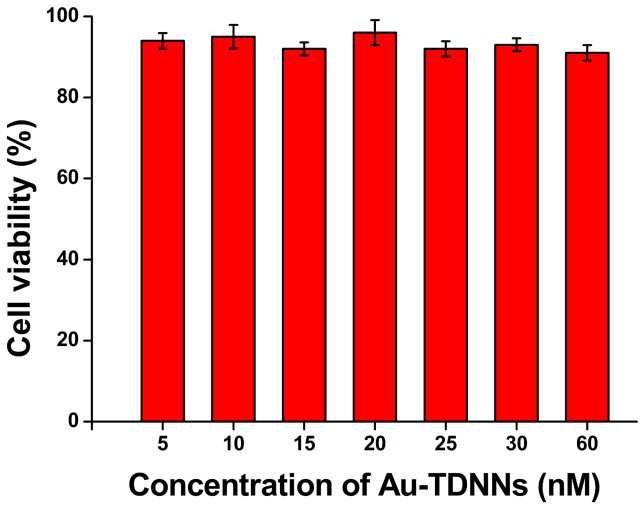

Figure S6. MCF-7 cell viability analysis with addition of different concentrations of Au-TDNNs.

1. Slot JW, Geuze HJ. A new method of preparing gold probes for multiple-labeling cytochemistry. Eur J Cell Biol. 1985; 38: 87-93.

2. Li S, Xu L, Ma W, Wu X, Sun M, Kuang H, Wang L, Kotov NA, Xu C. Dual-mode ultrasensitive quantification of microRNA in live cells by chiroplasmonic nanopyramids self-assembled from gold and upconversion nanoparticles. J Am Chem Soc. 2016; 138: 306-312.