Supporting Information

Molecular Recognition Based DNA Nanoassemblies on the Surfaces of Nanosized Exosomes

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Materials and Experiments

1 Materials and Apparatus

All DNAs were synthesized on an ABI3400 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA, USA), with/without labeling with FITC or biotin, using standard phosphoramidite chemistry. A, T, G, C phosphoramidites were purchased from Glen Research (Sterling, VA). Z, P phosphoramidites were obtained from Firebird Biomolecular Sciences LLC. (Alachua, FL). The sequences were then purified by reversed-phase HPLC (ProStar, Varian Walnut Creek, CA). Binding buffer for cells and exosomes was prepared by Dulbecco's Phosphate-Buffered Saline with 5 mM/L $MgCl_2$, 2 g/mL BSA (Fisher Scientific) and 100 mg/L yeast tRNA (Sigma-Aldrich, St. Louis, MO, USA). Washing buffer contains Dulbecco's Phosphate-Buffered Saline, 5 mM/L $MgCl_2$, and 2 g/mL BSA. All chemicals were of analytical grade, and all solutions were prepared with ultrapure water obtained from an ELGA water purification system (Evoqua Water Tech., Lowell, MA, USA).

2 Cell lines

Live cancer cell lines, including HepG2, Hep3B, HuH7, PC3, HeLa, HEK293, TOV21G, CCRF-CEM and Ramos, were purchased from American Type Culture Collection (ATCC). Noncancerous liver cell line Hu1545 was a gift from Dr. Chen Liu's lab at Rutgers University, established by immortalizing primary hepatocyte with lentivirus carrying hTERT (human telomerase reverse transcriptase; the enzyme maintains telomere length at the end of chromosomes, enabling cells to grow and proliferate). The cell line retains the characteristic protein profile of normal liver cells compared to liver cancer cells. Cells were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS, Gibco®, Life Technologies, Carlsbad, CA) and 1% penicillin-streptomycin (PS, Life Technologies, Carlsbad, CA) and incubated at 37 °C with 5% CO_2.

3 Experimental Section

Preparation of LZH8-NA in solution

DNA sequences, M1, M2 and LZH8-trigger, were diluted by PBS with 5 mM Mg^{2+}. The DNA solutions were heated at 95 °C for 3 minutes and left on ice for 3 minutes. Then, the mixture was left at room temperature overnight. To prepare LZH8-NA, M1, M2 and LZH8-trigger were mixed with the ratio of 10:10:1. The formation of nanostructure was characterized using 3% agarose gel electrophoresis in TBE buffer at 100 V for 40 min by loading 1 μM M1, 1 μM M2, 0.1 μM LZH8, 1 μM M1 and M2 mixture, and LZH8-NA, respectively.

Exosome extraction

Exosomes were collected using conventional centrifugation from supernatant media of HepG2 cells. Cells were cultured in T182 cm^2 flasks in exosome-depleted FBS supplemented DMEM until they reached a confluency of 80~ 90%. Cell culture media were collected and centrifuged at 800 g for 5 min at 4 °C. Then, the supernatant was centrifuged at 2,000 g for 10 min at 4 °C to discard cellular debris, followed by filtration using a 0.22 μm filter (vacuum-driven filter, 25-229, Genesee Scientific). The filtered media were then ultracentrifuged at 27,000 rpm for 2 h at 4 °C. Pellet was pipetted and

washed with 36 mL PBS, followed by another centrifugation at 27,000 rpm for 2 h at 4 °C. Finally, the supernatant was discarded, and exosomes were resuspended in 100 μL PBS. After several collections, exosomes were characterized using Nanoparticle Tracking Analysis (NanoSight Ltd., Malvern, Worcestershire, UK). A stock solution of 10^{13} HepG2-derived exosomes/mL and 10^{13} Hu1545-derived exosomes/mL were obtained, respectively. The collected exosomes were stored at -80 °C.

Dynamic light scattering

LZH8-NA formation in buffer solution was characterized by using dynamic light scattering (DLS). 0.2 μM LZH8-trigger was incubated with exosomes at 4 ℃ for 30 minutes for aptamer binding. Then, 2 μM M1 and 2 μM M2 were added and incubated for 0.5 hour, 1 hour, 2 hours, 4 hours and 6 hours at 4 ℃. The particle size of NA was measured by DLS.

Transmission electron microscopy

The size of LZH8-NA was characterized by transmission electron microscopy (TEM). 0.2 μM LZH8-trigger was incubated with exosomes at 4 ℃ for 30 minutes for aptamer binding. Then, 2 μM M1 and 2 μM M2 were added and incubated with LZH8-binding exosomes overnight. Sample was loaded on TEM copper grits and dried for 2 hours. After washing with water twice, sample was negatively stained by 2% phosphotungstic acid. The dried sample was observed on a Hitachi H-7000 NAR transmission electron microscope using a working voltage of 100 kV.

Immunogold

For immunogold labeling of samples, HepG2 exosomes were dropped on grids and allowed to conjugate. The grids were blocked with 1% BSA/PBS for 1h and then placed on biotin-labeled LZH8 aptamer solution for 1 hour at 4 °C, followed by rinsing with PBS 5 times. After washing, grids were treated with drops of streptavidin-gold nanoparticles for 30 min at 4 °C. Finally, the grids were rinsed with PBS 5 times and dried at room temperature. The dried sample was observed on a Hitachi H-7000 NAR transmission electron microscope using a working voltage of 100 kV.

Immobilization of exosomes on latex beads

Exosomes were immobilized to 3.8 μm 40 mg/mL aldehyde/sulfate latex beads (Thermo Fisher Scientific, Waltham, MA USA) by mixing 10 μL exosomes and10 μL beads for 15 min at room temperature with continuous vibration. The suspension was diluted to 1 mL with PBS and continuously vibrated for 30 min at room temperature. Reaction was stopped by adding 100 μL 1 M glycine and 20 % BSA/PBS and kept under vibration for 2 hours at room temperature. Then, exosome-conjugated beads were washed with 1 mL of 2 % BSA/PBS and centrifuged for 1 min at 14,800 g twice. Afterwards, blocking solution containing 10 % BSA and 1 % salmon sperm DNA (Thermo Fisher Scientific, Waltham, MA USA) in PBS was added and vibrated at room temperature for 30 min. The beads were washed in 2 % BSA and centrifuged for 1 min at 14,800 g twice. Finally, exosome-conjugated beads were resuspended in 100 μL 2% BSA in PBS with 5 mM Mg^{2+}.

***In situ* assembly of LZH8-NA on exosome surfaces**

The ability of LZH8-NA to bind immobilized HepG2 exosomes was further characterized using flow cytometry. FITC-labeled LZH8-trigger (LZH8-trigger-FITC, 200 nM) was incubated with the immobilized exosomes (diluted in PBS with the ratio of 1: 50) in binding buffer at 4 °C for 30 min. Two FITC-labeled monomers, M1-FITC and M2-FITC (2 μM, respectively), were added to the aptamer-binding exosome solution mentioned above and incubated at 4 °C for 2 h. Then, the nonbinding aptamer was washed twice by adding 1 mL washing buffer and centrifuged at 3000 g for 1 minute. Next, the exosome-conjugated beads were suspended in binding buffer (100 μL). Scramble LZH8-trigger sequence with FITC (scrLZH8-trigger-FITC) was used as control. The fluorescence intensities of FITC on the exosomes were measured with a FACScan cytometer (Becton Dickinson Immunocytometry Systems, Sparks, MD, USA). Data were analyzed with FlowJo software.

Supplementary Tables and Figures

Table S1 Sequences used in this work

LZH8-biotin	5’-ATC CAG AGT GAC GCA GCA TAT TAG TAC GGC

	TTA ACC CPC ATG GTG GAC ACG GTG GCT TAG T-biotin-3’
scrLZH8-biotin	5’-CAG GTA CGG PCA GTA AGA ACA TCC GTG ACG CGA CTG ATA CAT TTG GCT TAG TGC CAG TGC T-biotin-3’
scrLZH8-trigger	5’-GAC CCT AAG CAT ACA TCG TCC TTC ATT TTC AGG TAC GGP CAG TAA GAA CAT CCG TGA CGC GAC TGA TAC ATT TGG CTT AGT GCC AGT GCT-3’
LZH8-trigger	5’-GAC CCT AAG CAT ACA TCG TCC TTC ATT TTA TCC AGA GTG ACG CAG CAT ATT AGT ACG GCT TAA CCC PCA TGG TGG ACA CGG TGG CTT AGT-3’
M1	5’-ATG AAG GAC GAT GTA TGC TTA GGG TCG ACT TCC ATA GAC CCT AAG CAT ACA T-3’
M2	5’-GAC CCT AAG CAT ACA TCG TCC TTC ATA TGT ATG CTT AGG GTC TAT GGA AGT C-3’
scrLZH8-trigger-FITC	5’-GAC CCT AAG CAT ACA TCG TCC TTC ATT TTC AGG TAC GGP CAG TAA GAA CAT CCG TGA CGC GAC TGA TAC ATT TGG CTT AGT GCC AGT GCT-FITC-3’
LZH8-trigger-FITC	5’-GAC CCT AAG CAT ACA TCG TCC TTC ATT TTA TCC AGA GTG ACG CAG CAT ATT AGT ACG GCT TAA CCC PCA TGG TGG ACA CGG TGG CTT AGT-FITC-3’
M1-FITC	5’-ATG AAG GAC GAT GTA TGC TTA GGG TCG ACT TCC ATA GAC CCT AAG CAT ACA T-FITC-3’
M2-FITC	5’-FITC-GAC CCT AAG CAT ACA TCG TCC TTC ATA TGT ATG CTT AGG GTC TAT GGA AGT C-3’

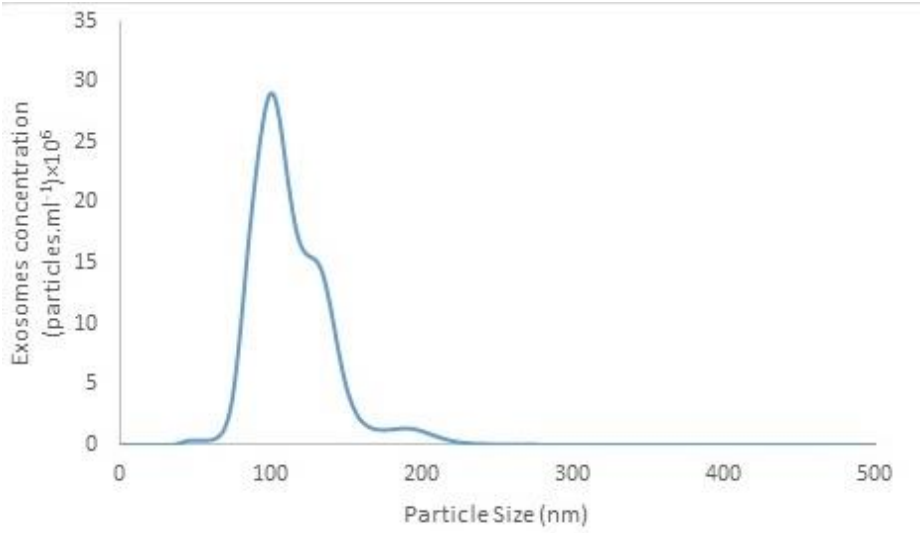

Figure S1 Exosome concentration and size distribution by nanoparticle tracking analysis of culture supernatant from HepG2 cells. Size mode: 100 nm (3 technical replicates)

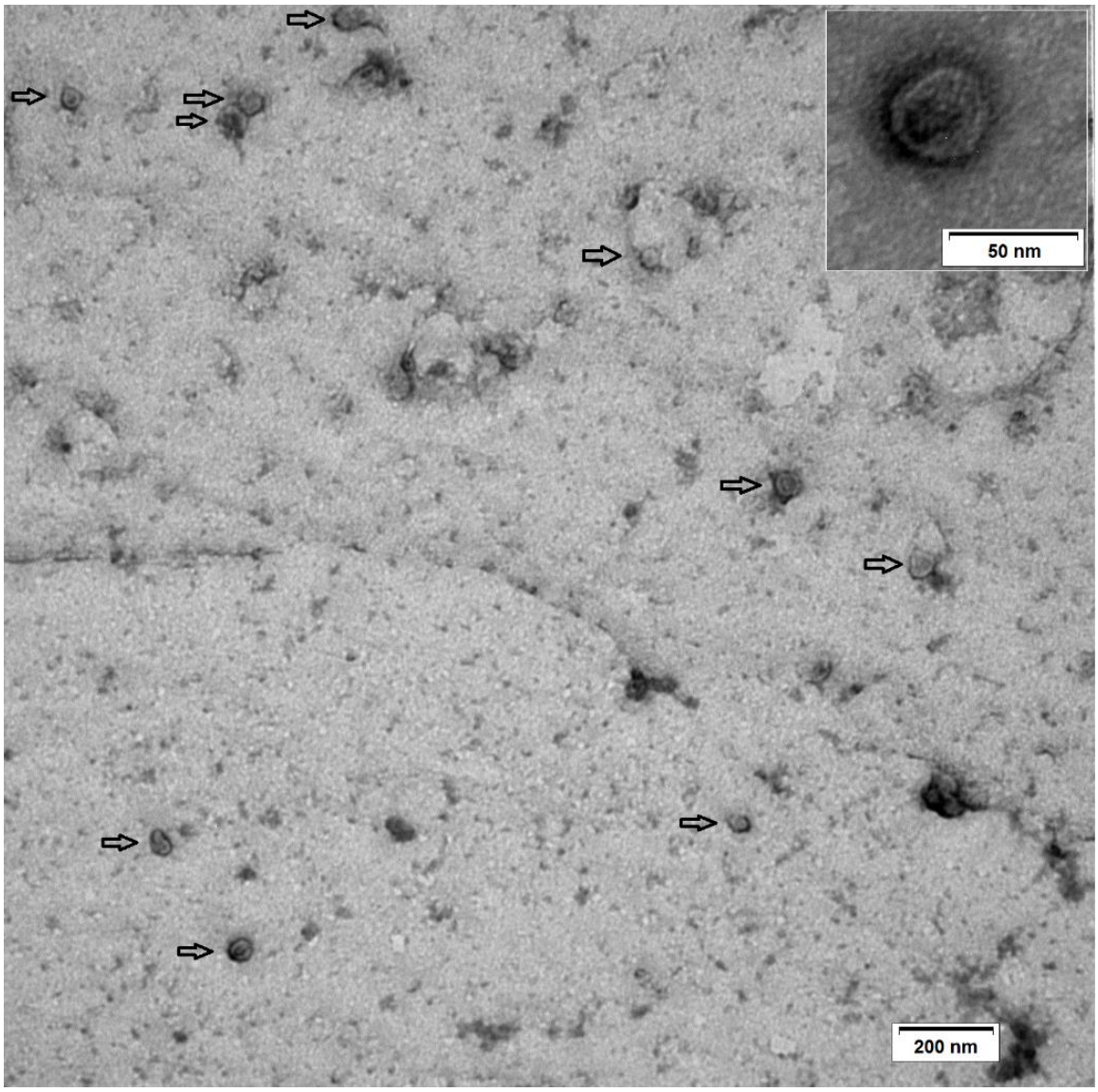

Figure S2 Transmission electron microscopy images showing the extracellular vesicles. The arrows point to the extracellular vesicles. Insert is a single extracellular vesicle with size around 50 nm.

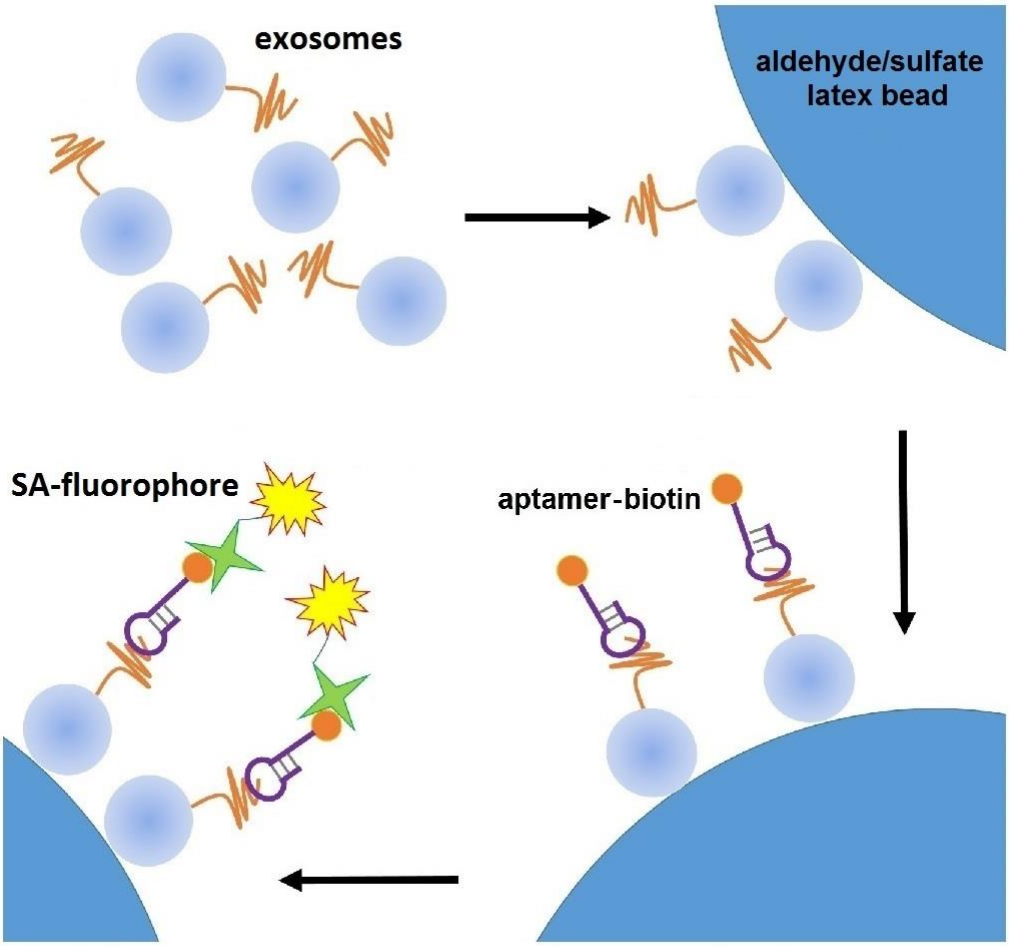

Figure S3 Schematic of fluorescent labeling on immobilized exosomes

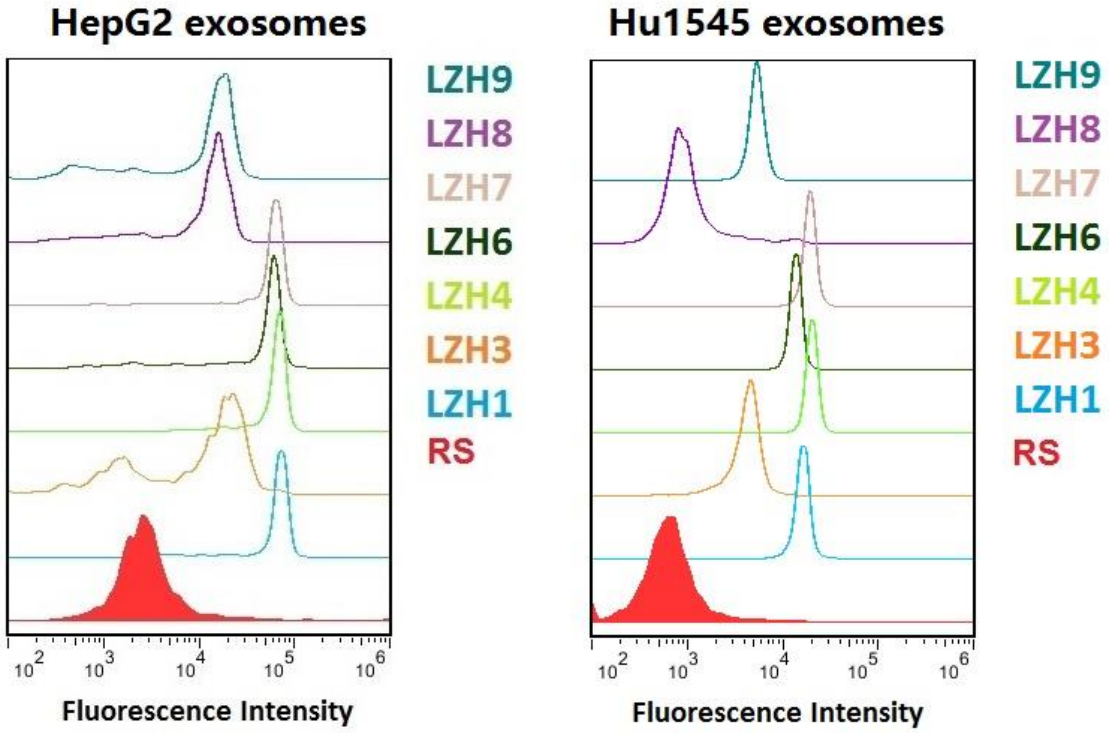

Figure S4 Flow cytometry assay results of binding extracellular vesicles using six-nucleotide aptamers. (Left) Histogram showing binding results of six-nucleotide aptamers on HepG2 extracellular vesicles-conjugated latex beads. (Right) Histogram showing binding results of six-nucleotide aptamers on Hu1545 extracellular vesicles-conjugated latex beads.

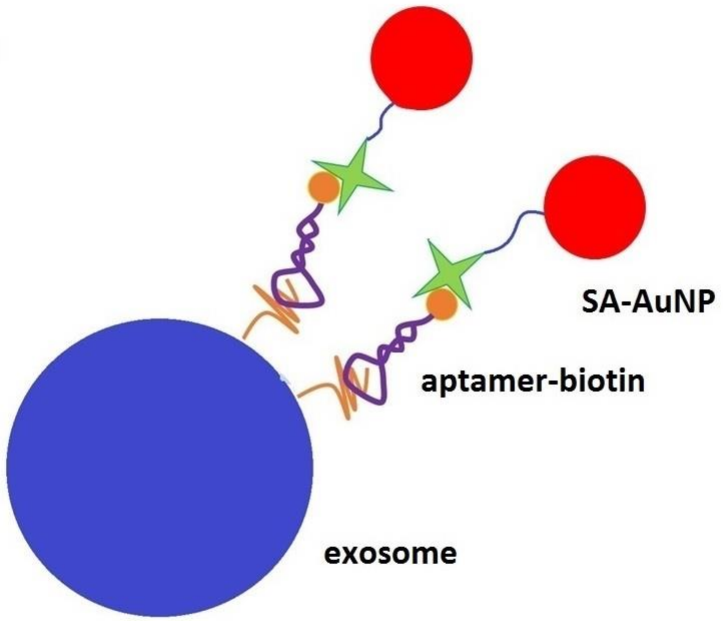

Figure S5 Schematic of immunogold labeling of exosomes using aptamer.

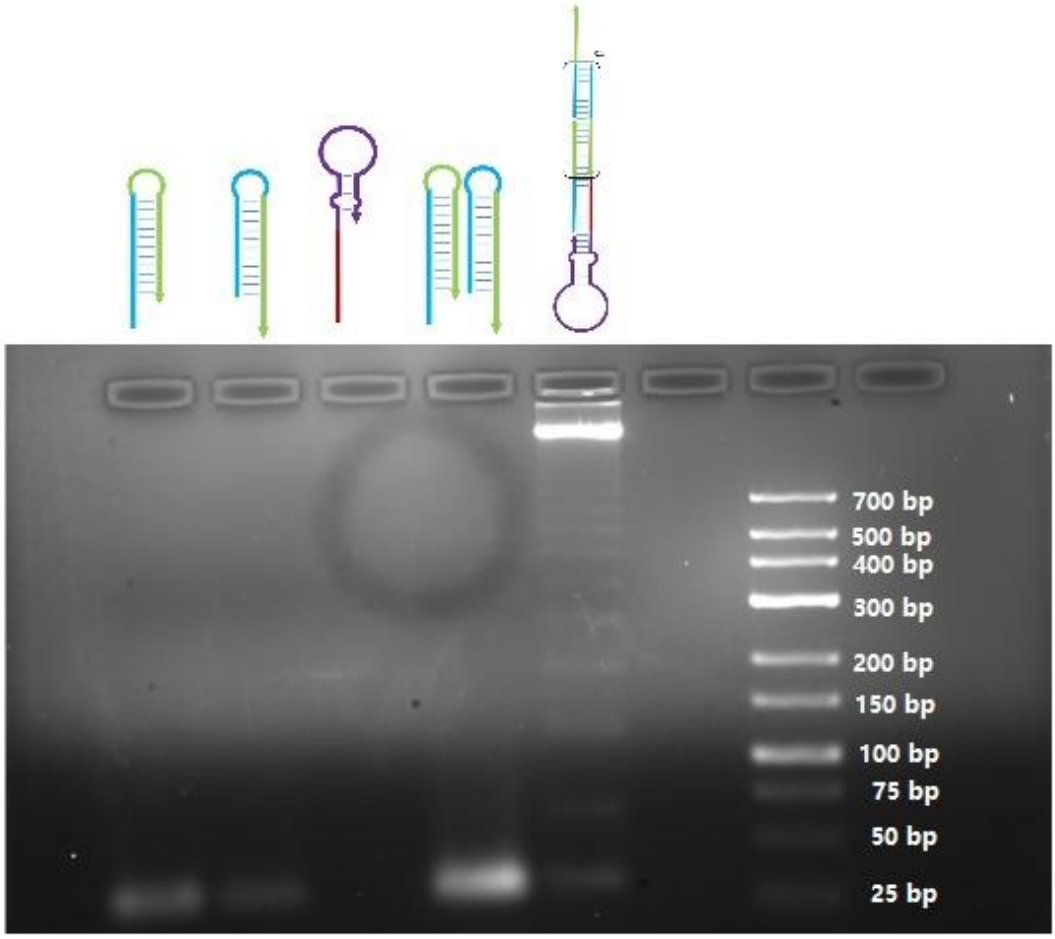

Figure S6 Agarose gel electrophoresis showing the self-assembly of LZH8-NA initiated by LZH8–trigger.

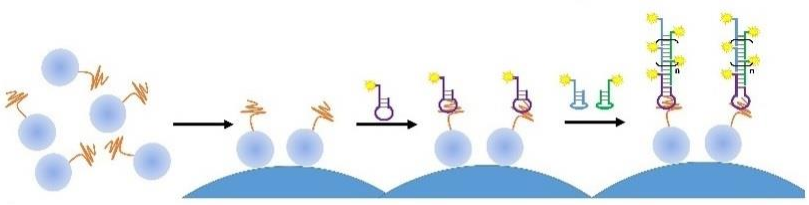

Figure S7 Schematic showing fluorescent NA on immobilized exosomes.

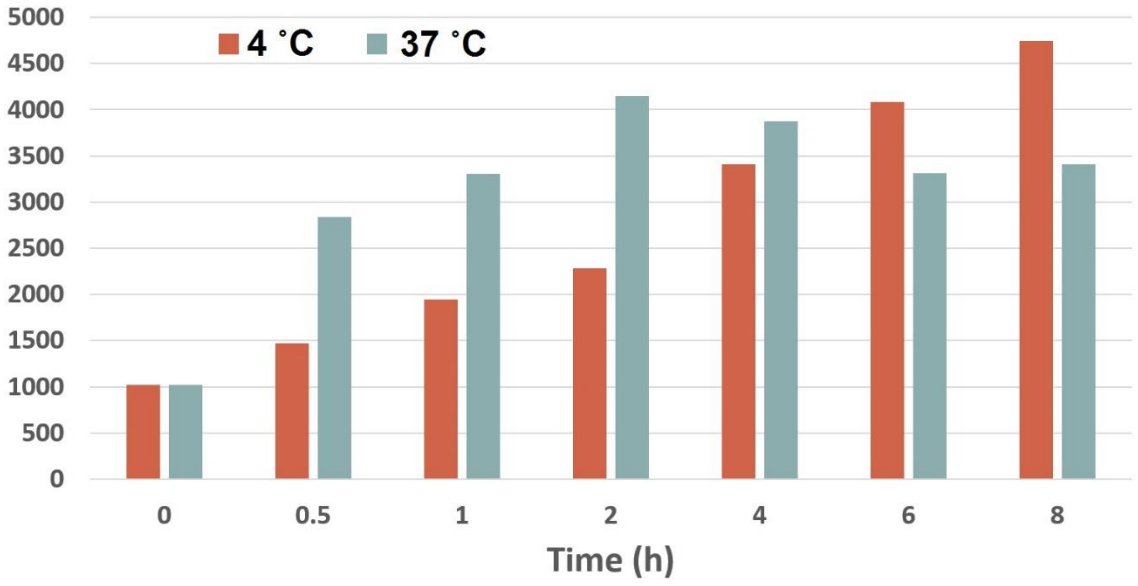

Figure S8 Time-course of *in situ* assembly of nanodevice on membrane surface of exosomes under different temperatures.

Exosomes	Aptamer	Aptamer-NAs
HepG2 (liver cancer)	+	+++
HU1545 (liver)	-	-
Hep3B (liver cancer)	+	+++
HuH7 (liver cancer)	-	-
PC3 (prostate cancer)	-	+
HeLa (cervical cancer)	-	-
HEK293 (embryonic kidney)	-	-
TOV21G (ovarian cancer)	-	-
CCRF-CEM (T lymphoblast)	-	-
Ramos (B lymphocyte)	-	-

Figure S9 Selectivity of in situ fluorescent NA on surfaces of 10 different types of cell lines.