Supplementary material

Construction of two-dimensional DNA-RNA hybridized membrane for collecting tumor-derived exosomes

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Experimental Section

Materials and Reagents DNA oligonucleotides were purchased from Integrated DNA Technologies (IDT). T4 DNA ligase was purchased from Promega (Madison, USA). Nxgen Phi 29 DNA polymerase including 10X Phi 29 polymerase buffer was purchased from Lucigen (Madison, USA). dNTP mix was purchased from Thermo Fisher Scientific. T7 RNA polymerase, 10X RNA polymerase reaction buffer, and ribonucleotide solution mix were purchased from New England Biolabs (NEB). GelRed was purchased from Biotium. Cy3-dCTP and 5-propargylamino-dCTP-cy5 were purchased from Jena Bioscience. Cy3-CTP and

cy5-UTP were purchased from Enzo. RNase III, DNase I, and RNase H were purchased from NEB and RNase I was purchased from Epicentre.

Synthesis of the DRMs Before dual enzyme polymerization reaction, two partially complemented sequences of circularized DNAs were prepared (Table S1). For rolling circle DNA replication, the first circularized template (from linear ssDNA and primer for DNA) was incubated with Phi29 DNA polymerase (10 U μL^{-1}), dNTP mix (8 mM) and reaction buffer (80 mM Tris-HCl, 100 mM KCl, 20 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 8 mM DTT) for 10 min at 30℃. To perform RCT, the second circularized template (from linear ssDNA and primer for RNA) was mixed in T7 RNA polymerase (10 U μL^{-1}), rNTP mix (8 mM) and reaction buffer (80 mM Tris-HCl, 12 mM $MgCl_2$, 2 mM DTT, 4 mM spermidine) for 10 min at 37℃. After 10 min, the two separated reactants (DNA polymerase reaction and RNA polymerase reaction) were mixed together. The reaction temperature was then changed periodically between the optimal activity temperatures of DNA or RNA polymerase for 20 h. After the polymerization process, the cap of the tube was opened to initiate the EISA process. The tube was incubated overnight at 37℃. For labeling, 5-propargylamino-dCTP-cy5 (0.02 mM) and Cy3-CTP (0.02 mM) were added to the reaction mixture. The fabricated DRM was characterized with SEM and fluorescent microscopy.

Degradation of DRMs For the degradation experiment, cy3-dCTP (0.02 mM) and cy5-UTP (0.02 mM) stained DRMs were prepared with RNases (0.2 U μL^{-1} of RNase III and 0.2 U μL^{-1} of RNase I), DNase I (0.4 U μL^{-1}), and RNase H (0.25 U μL^{-1} of RNase H) in nuclease-free water (final volume of 1 ml). After the incubation for 24 h at 37℃ with a gentle rotation, DRM samples were analyzed with fluorescent microscopy and SEM for investigating the structure of the membrane.

Exosome capturing assay and separation Exosomes were isolated via serial centrifuge and filtration steps. Briefly, MDA-MB-231 cells were seeded at a density of 220,000 cells per dish in a 100 pi culture dish (SPL Lifesciences) and cultured for 24 h. Then, cells were washed with phosphate-buffered saline (PBS) and media were replaced with serum-reduced MEM for 24 h to collect conditioned media (CM). Collected CM were centrifuged at 300×g for 10 min to remove cell debris. Exosomes were isolated by tangential flow filtration (TFF) using a 300-kDa MWCO ultrafiltration membrane filter capsule (Pall Corporation, Port Washington, NY, USA). Isolated exosomes were analyzed with nanoparticle tracking analysis (NTA) to estimate the size distribution and concentration. For exosome capturing assay, DRMs were treated with exosomes at the concentration of 1×10^{9} exosomes mL^{-1}. After an hour incubation at 37℃ with a gentle rotation, the existing solution was exchanged three times with fresh nuclease-free water to remove the unbound exosomes. The capturing efficiency of DRM was calculated from the concentration of exosomes in supernatant measured with NTA. For SEM analysis, DRM samples were lyophilized after treatment of 10% of DMSO to preserve the biological structures while freezing. Moreover, based on previous research, isolating of collected exosomes from the DNA-RNA membrane could be achieved. Due to the controllability of the nucleic acid-based materials, techniques to elute the captured target from the aptamer such as adjusting temperature gradient[1] or buffer condition[2–4] have been introduced. Various elution strategies could be applied depending on the character of targeted molecules encapsulated in exosomes.

Primer DNA for DNA polymerization (22 nt)	TTA GAG GCA TAT CCC TAT AGT G
Linear ssDNA for AS1411 aptamer DNA (92 nt)	Phosphate - AGG GAT ATG CCT CTA ATA AAT ATT AAC CAC CAC CAC CAC CAC AAC CAC CAC CAC CAA TAA TAA GAA GTT TCG ACG TTA ATA CGA CTC ACT AT
T7 promotor for RNA polymerization (22 nt)	TAA TAC GAC TCA CTA TAG GGA T
Linear ssDNA for RNA (92 nt)	Phosphate -ATA GTG AGT CGT ATT AAC GTC GAA ACA AAA CTT CAG GGT CAG CTT GCT TAC TTG AAG CAA GCT GAC CCT GAA GTT TTT AGA GGC ATA TCC CT

Table S1. Sequences of linear DNA and primers for AS1411 aptamer and RNA template circular DNA. DNA sequences for hybridization with primer to form circular DNA are shown green and complement DNA sequences of AS1411 aptamer region are shown red.

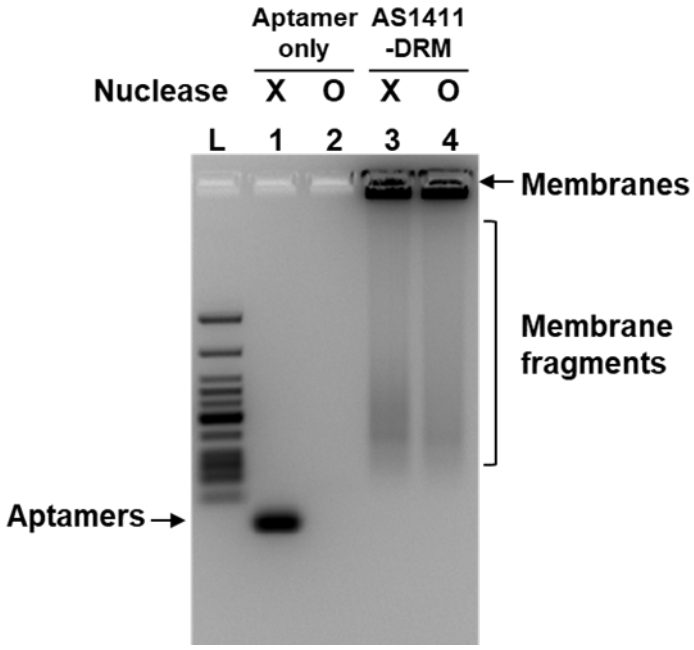

Figure S1. Gel electrophoresis result of naked aptamer and aptamer-DRM left untreated (lane 1, 3) or 2.0 U/μl of exonuclease I/III (lane 2, 4) for 30 min at 37℃. Naked aptamers were destroyed within 30 min when exposed to exonuclease. The aptamer-DRM observed within the loading wells and showed nuclease stability.

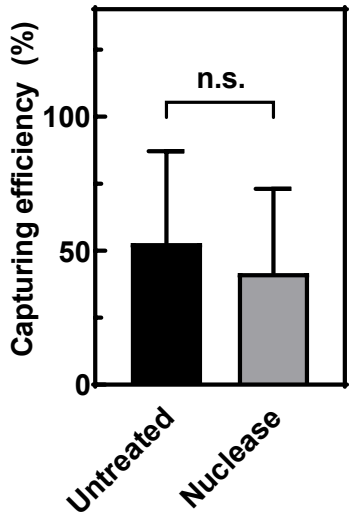

Figure S2. Capturing efficiency change of the AS1411-DRM after the treatment of 2.0 U/μl of nuclease. Statistical significance was determined by unpaired t-test, compared with untreated AS1411-DRM (n.s.; not significant).

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