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Ni Aptamer: DNA Mimic of His-tag to recognize Ni-NTA

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Supporting Information

Supporting Figures:

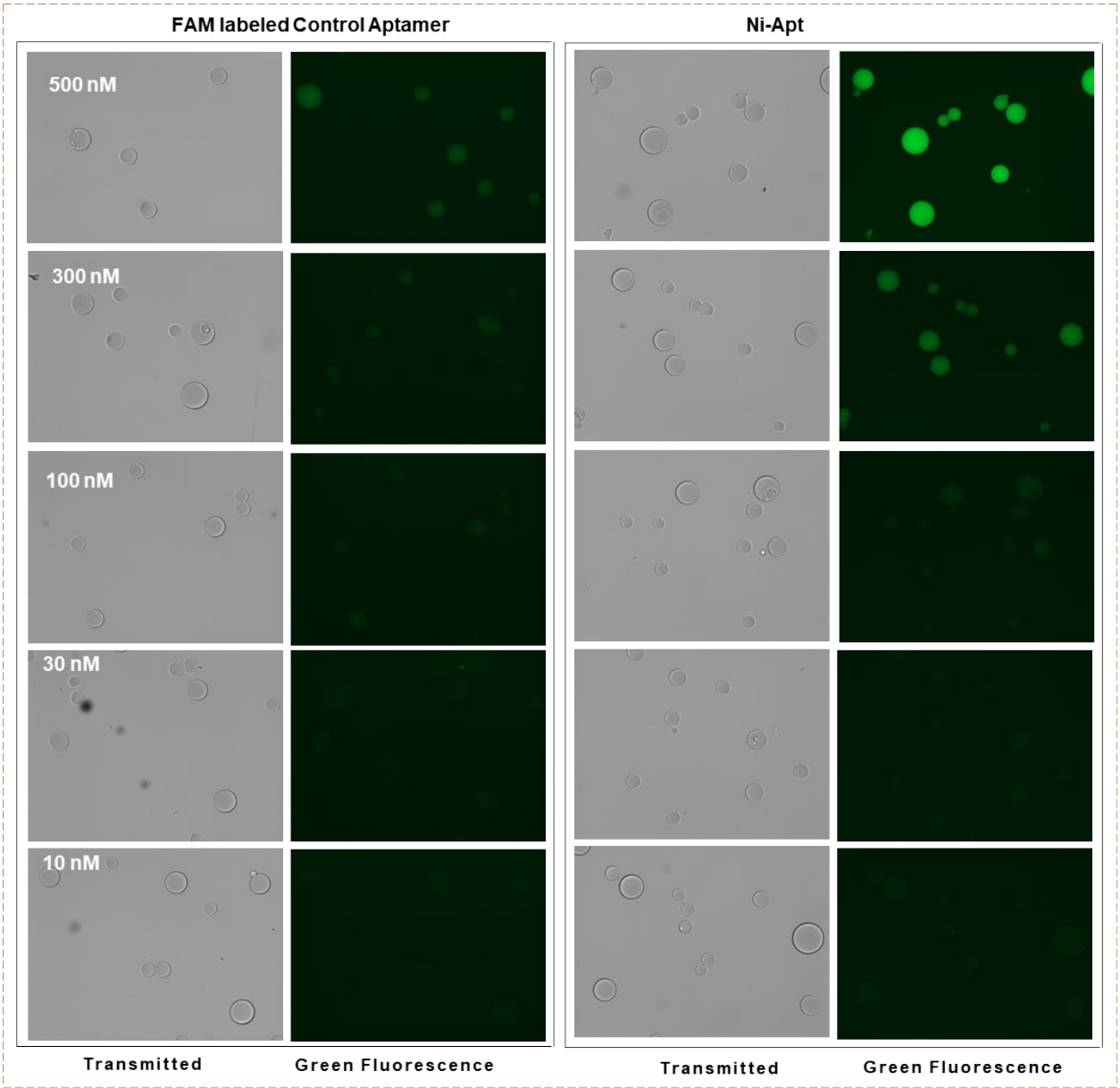

Figure S1: Determine the binding specificity of Ni Apt. The fluorescence intensity of Ni aptamer gets increased with higher concentrations of the Ni-Apt for the same amount of bead incubation. On the other hand, when Ni-NTA resin bead was incubated with a control FAM labeled aptamer, there were no visible bright fluorescence signals observed in the fluorescence microscope. The experiment was carried out with 10, 30, 100, 300, and 500 nM of the control aptamer and Ni Apt, showing the binding specificity of Ni Apt against Ni-NTA.

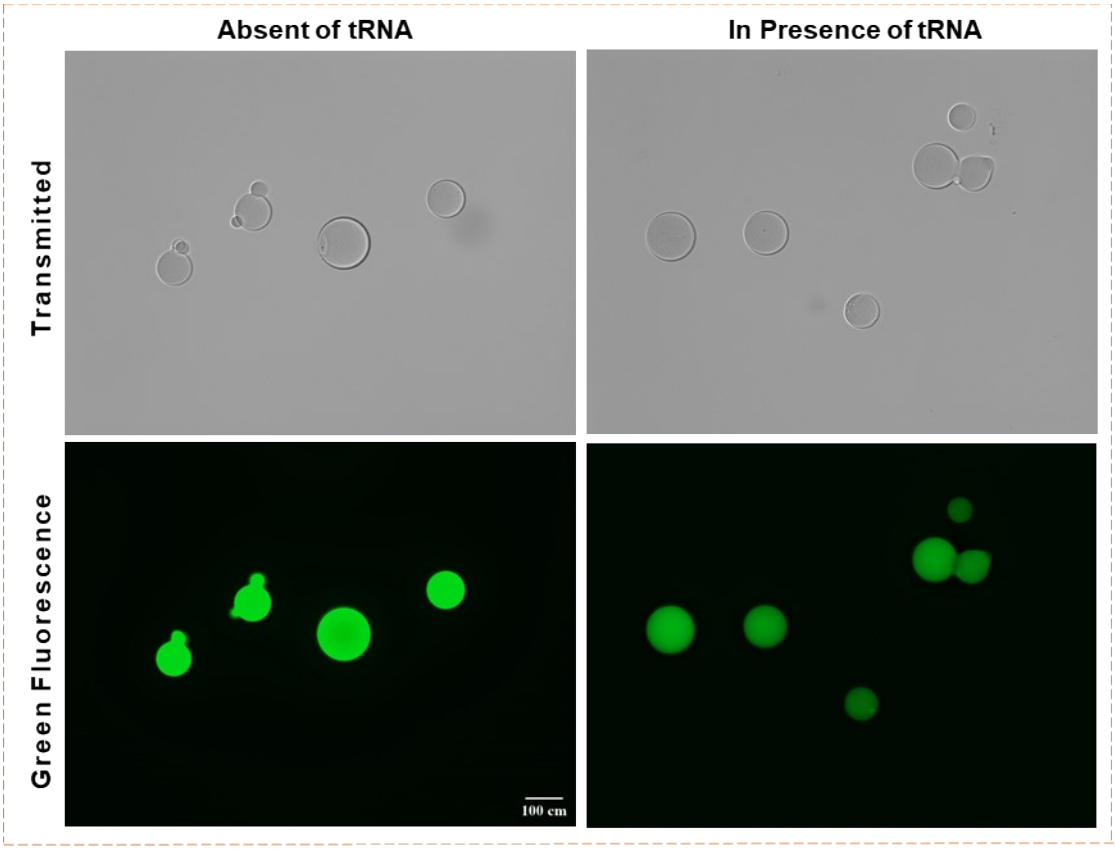

Figure S2: Determine the effect of tRNA with the binding of Ni Apt on Ni-NTA resin. This experiment data clearly exhibit that Ni Apt can still recognize the Ni-NTA resin beads with high concentration of tRNA. The scale bar is 100 μm.

Materials and Methods

All DNA molecules were purchased from Integrated DNA Technologies, Inc. (www.idtdna.com). All DNA samples received as lyophilized powder with instruction to dissolve in nanopore water to make 100 μM. PD-L1 proteins were obtained from Sino Biological as HPLC purified form without further purification. Ni-NTA agarose resin (HisPur™ Ni-NTA Resin, Catalog # 88221) was obtained from Thermo Fischer Scientific. Ni-NTA magnetic beads (Ref# 062N-A) were purchased from G-Biosciences and used as required dilutions suitable for the experiments. All other chemicals were purchased from Sigma-Aldrich.

SELEX procedure.

We conducted a DNA-SELEX experiment targeting the PD-L1 protein. To initiate the general SELEX process, we employed an oligonucleotide library composed of 40 random nucleotides flanked by constant primer sequences. In the first round, 100 pmole of the

PD-L1 protein and 1 μL of nickel nitrilotriacetic acid (Ni-NTA) beads (G-bioscience) were diluted in 100 μL of SELEX buffer (PBST-Mg buffer, PBS with 1 mM $MgCl_2$, pH 7.4, 0.01% Tween) and incubated at room temperature while rotating for 1 h. Meanwhile, 3 nmole of the DNA library was diluted in 100 μL of PBST-Mg and treated at different temperatures (5 min at 95°C, 5 min at 0°C, and 5 min at room temperature, before finally being placed at 0°C). After the 1h incubation period, the protein-bead (P-B) complex was washed twice with 200 μL of SELEX buffer and then combined with the DNA library and tRNA, followed by incubation for 1 h at room temperature. Subsequently, the protein-bead-library (PBL) complex was washed twice with 200 μL of SELEX buffer to remove the nonspecific library. After washing, the bound library was eluted twice with 30 μL of hot water. Finally, the selected library was amplified via the polymerase chain reaction (PCR).

The PCR mixture contains 60 μL of the library, 39 μL of nuclease-free water, 100 μL of 2 × PCR solution, and 1 μL of Easy Taq polymerase (TransGen Biotech). The 50 μL of the PCR mixture is loaded into each PCR tube and amplified in the conditions of 2 min at 95°C; 9 cycles of 45 s at 95°C; 30 s at 54°C; 30 s at 72°C, and 2 min at 72°C. After completing PCR, all PCR product was collected in a tube. To optimize the PCR cycle number for bulk amplification, 5 μL of the PCR product, 119 μL water, 125 μL of 2×PCR solution, and 1.25 μL Easy Taq polymerase were mixed in a tube and then distributed equally (50 μL) into 5 PCR tubes. Amplification conditions were: 2 min at 95°C; 3-11 cycles of 45 s at 95°C; 30 s at 54°C; 30 s at 72°C; and extra 2 min at 72°C. The PCR tubes were taken out in 3, 5, 7, 9, and 11 cycles, respectively, and kept in ice. Then PCR products were assessed with 2 % agarose gel electrophoresis to determine the suitable number of PCR cycles (X). The suitable number of the PCR cycles provides the right PCR product which was confirmed by DNA agarose gel analysis. Once the number of suitable PCR cycles (X) was determined, the bulk PCR reaction was run for that particular PCR cycles (X) to generate 1 (or 2) mL of PCR mixture (20 μL of the 1st round PCR solution, 475 μL water, 500 μL of 2 x PCR solution, and 5 μL of Easy Taq Polymerase). The PCR amplification conditions were set to be 2 min at 95°C; X cycles of 45 s at 95°C; 30 s at 54°C; 30 s at 72°C; and extra 2 min at 72°C.

After a bulk PCR, 20 μL of Neutravidin Agarose (Thermo Scientific) were washed two times by 400 μL of SELEX buffer and incubated with 1 mL of PCR products for 15 minutes rotating at RT. Then the beads were washed two times with 400 μL of the SELEX buffer. The sense strand was separated from the beads by denaturing in 200 μL of 100 mM NaOH solution for 1 min; the solution was immediately neutralized by 0.2 M HCl. Then the beads were eluted again by 212 μL of SELEX buffer, combined with the previous solution, and centrifuged using a desalting column (3K) at 12000 g for 10 min. The remaining solution in the desalting column was washed two times using 400 μL of the SELEX buffer. The eluted library was quantified by Nanodrop, then it was treated at 95°C for 5 min, 4°C for 5 min, and RT (room temperature) for 5 min and stored at -20°C.

For a subsequent round of selection, the 100 pmole DNA library (~2 μg) was incubated with 100 pmole protein (PBL). The amount of protein and incubation time were consistently decreased in the following selection rounds to increase the selection pressure. While the number of washes to the PBL complex was consistently increased to ensure the removal of the unspecific libraries. The bound libraries were eluted two times

by 30 µL of hot water. 20 µL of elution was used for the PCR amplification, and the remaining was stored at -20° C. The PCR amplification, purification, desalting, and quantification were similar as before. After the second selection round, we introduced the counter selection (CS) in each alternative round. For that, we incubated the ssDNA with Ni-NTA magnetic beads (without protein); in this step, the unspecific library bound to the magnetic bead was discarded while the specific library present in the supernatant was used to start the next round of selection.

The label-free dsDNA obtained from the appropriate selection rounds were purified using NucleoSpin Gel and PCR Clean-up kit (Ref. # 740609-250) and used as an insert. We used the TOPO TA cloning kit (Ref. # 45-0071) for ligation and transformed *E. coli* component cells using recombinant DNA. The ampicillin-resistant bacterial colonies were cultured on the ampicillin (100 µg/mL) containing Luria broth (LB) agar plate (follow the standard protocol). The bacterial culture was subjected to PCR to assess the correct insert using gel electrophoresis. We extracted the plasmids from the bacterial solution containing a desirable insert using E.Z.N.A.® Plasmid DNA Mini Kit (Ref. # D6942-01) and sequenced by the Human Genetics Comprehensive Cancer Center DNA Sequencing Facility at the University of Chicago.

Fluorescence microscope-based binding assay.

Magnetic bead binding assays were conducted by EVOS® FL Imaging Systems by Thermo fisher scientific. The bead binding assays determine the fluorescence intensity of Ni-Apt binding with Ni-NTA resin bead complex. In this experiment, 10 µL of Ni-NTA agarose resin (HisPur™ Ni-NTA Resin, Thermo Fischer, Catalog # 88221) beads were incubated with different concentrations up to 500 nM of Ni-Apt for 1 hour. After incubation, the beads were washed with 500 µL of PBST-Mg buffer (0.01% Tween-20 and 1mM $MgCl_2$) for three times to remove the unspecific aptamer to the Ni ions. Then the Ni-NTA resin beads were resuspended with 50 µL of fresh PBST-Mg buffer for consistent sample preparation. Use 10 µL of one sample at a time to record the fluorescence intensity of the binding affinity through green fluorescent and transmitted light channels.

Measuring binding affinity by flow cytometer

Flow cytometer, Guava easyCyte 5HT system from Luminex Corporation (Catalog # 0500-4005) was used to determine the binding affinity of Ni Apt. In this experiment, 2 µL of Ni-NTA magnetic beads (Ref# 062N-A; G-Biosciences) was diluted in 100 µL of PBST-Mg buffer. This bead solution was incorporated with 500 nM of Ni Apt and incubated for 2 hours with a rotating mixer. After incubation, the magnetic beads were washed with 200 µL of PBST-Mg buffer for three times to remove any unbound aptamers. After the final washing, we centrifuged the beads and resuspended them in fresh PBST-Mg buffer. For the determination of binding affinity of Ni Apt, we kept the bead quantity same for all the samples and the bead solution was incubated with 100 µL of 1, 3, 9, 30, 90, 300 nM of Ni

Apt. This complex was incubated for 2 hours and was washed before determining the binding affinity (K_d) of Ni Apt against Ni-NTA magnetic beads using flow cytometer. All the samples of each concentration were measured for three trials, counting approximately 5000 events each time to calculate mean fluorescence intensity values (*Xc* value). The data analysis of the acquired data was done by the Origin software through plotting all the data points in intensity Vs concentration plot.