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Ni aptamer: DNA mimic of His-tag to recognize Ni-NTA†

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We introduced Ni Apt as the first aptamer with a characterized dissociation constant for recognizing Ni-NTA. Serving as a nucleic acid analog of the His-tag commonly employed for protein purification using Ni-NTA resin, Ni Apt displays a remarkable binding affinity (K_d = 106 nM) towards Ni-NTA. Furthermore, it can be eluted from the resin using imidazole or EDTA, similar to the removal of His-tag from Ni-NTA resin. The versatile capabilities of Ni Apt make it a valuable molecular tool in nucleic acid purification and recognition applications.

Protein tags, also known as fusion tags or affinity tags, are short peptide sequences that are genetically fused to a target protein of interest. These tags provide a variety of advantages in protein research, purification, detection, and characterization. The most commonly used protein tag is the polyhistidine-tag (His-tag). It is a short amino acid sequence, usually consisting of at least six consecutive histidine residues, and is often genetically engineered onto the terminal sequences of a protein of interest. His-tag allows for easy purification of the protein using immobilized metal affinity resins such as Ni-NTA (Nickel-nitrilotriacetic acid) resin, which contains nickel ions that have a high affinity for the imidazole side chain of several consecutive histidine residues. When a protein with a His-tag is added to the Ni-NTA resin, the histidine residues on the His-tag bind to the Ni^{2+} ions on the resin *via* coordination bonds. This interaction is highly specific and reversible, allowing for efficient purification of the His-tagged protein. After binding to the Ni-NTA resin, the His-tagged protein can be washed to remove any non-specifically bound proteins or contaminants, and then eluted from the resin using an imidazole-containing buffer that competes with the histidine residues for binding to the nickel ions. The His-tag/Ni-NTA system is widely used in protein purification and characterization,[1] and has become a very popular tool in both academic and industrial settings due to its high specificity, efficacy and ease of use.

In this paper, we reported a DNA aptamer, Ni Apt, as a nucleic acid mimic of His-tag to recognize Ni-NTA. Aptamers are single-stranded oligonucleotides that can fold into complex 3D structures, enabling them to specifically recognize unique targets, including proteins, nucleic acids, small molecules, and even cells.[2–4] Aptamers are selected from a large random pool of polynucleotides *via* an iterative selection process called systematic evolution of ligands by exponential enrichment (SELEX).[5,6] DNA aptamers are less expensive to produce than antibodies and can be manufactured using general chemical synthesis.[7–12] DNA aptamers also have low immunogenicity, in comparison to antibodies. Therefore, DNA aptamers can be a useful molecular tool in disease therapeutics and diagnostics.

Previously, adenosine-rich sequences in DNA or RNA were reported to interact with Ni-NTA.[13] However, in that report, the dissociation constant (K_d) value was not revealed. Additionally, beside PolyA, some reports imply a greater stability of Ni^{2+} complexes with guanine.[14,15] Herein, we developed Ni Apt, which exhibits high binding affinity towards Ni-NTA. The binding specificity of Ni Apt was confirmed by its loss of binding to Ni-NTA upon removal of Ni^{2+} ions, which could be restored upon recharging the Ni-NTA resin with fresh Ni^{2+} ions again. Furthermore, Ni Apt could be eluted from the Ni-NTA resin using high concentrations of imidazole solution, similar to the removal of His-tag from Ni-NTA resin. Additional, Ni Apt can also be eluted from Ni-NTA by EDTA wash. As a functional mimic of the His-tag in proteins, Ni Apt can have wide-ranging applications in DNA purification and characterization.

During our recent aptamer SELEX experiment targeting the PD-L1 protein, we identified an intriguing aptamer candidate which we named Ni Apt (5′–TCGGGACGACGACAGCACA-ACCACCAACCACCCAA CCCATTAATCGGTCGTCCCG–3′). We observed that Fluorescein-labelled Ni Apt (Fam-Ni Apt) demonstrated a strong binding affinity towards Ni-NTA resin beads, even in the absence of PD-L1, as shown in Fig. 1A. In contrast,

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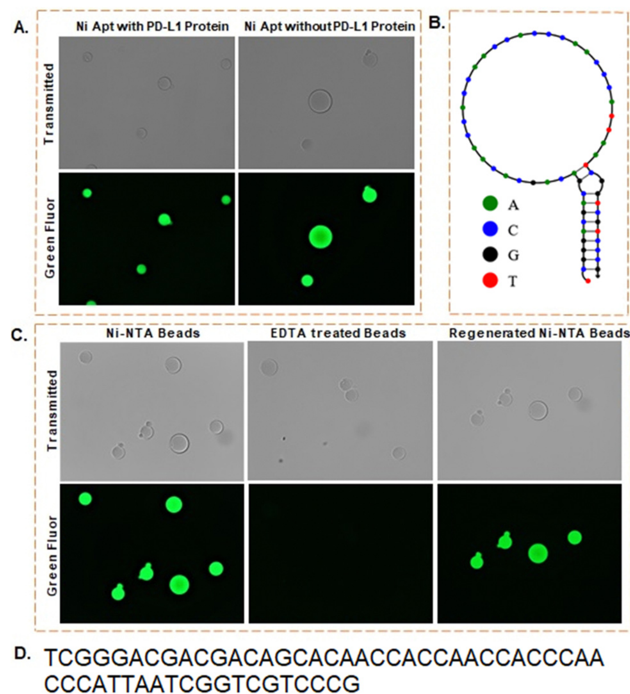

Fig. 1 Ni Apt recognizes the Ni-NTA resin. (A) The fluorescence intensity of Fam-Ni Apt did not change in the presence or absence of the PD-L1 protein when it was bound to Ni-NTA resin beads. The lack of change in the brightness of the Ni-NTA resin bead images indicated Ni Apt can recognize Ni-NTA. (B) The secondary structure of Ni Apt obtained using NUPACK. (C) Fluorescence images of Fam-Ni Apt incorporated with resin beads under different conditions. The original Ni-NTA resin beads exhibit strong fluorescence when incubated with Ni Apt. However, after the treatment by EDTA, the nickel ions were removed from the resin and Ni Apt lost its ability to recognize the EDTA-treated beads, resulting in no fluorescence intensity in the beads. Later, when the NTA beads were regenerated with nickel ions from a freshly prepared 250 mM $NiSO_4$ solution, Ni Apt binds regenerated resins again, as depicted by the fluorescence imaging. (D) The nucleic acid sequence of Ni Apt.

the control Fam-aptamer (5′–TTCGGGACGACAGATTTGGGTT-GTTGTTGGTGGGGGTGGGGGGAGGGGTCGTCCCG–3′) showed only weak signals for recognizing Ni-NTA beads (Fig. S1, ESI†). Based on these findings, we concluded that the strong affinity and specificity of Ni Apt for recognizing Ni-NTA may have contributed to the high fluorescence intensity on beads (Fig. 1A). Given that Ni Apt is highly negatively charged, we speculated that one of the major interactions between Ni Apt and Ni-NTA is likely to be an electrostatic interaction between the positively charged Ni^{2+} ions and the negatively charged aptamer. However, this cannot explain why the control DNA sequence only showed weak binding to Ni-NTA resin beads. Therefore, further experiments were required to fully understand the nature of the interaction between Ni Apt and Ni-NTA.

To further investigate this possibility, we tested the binding ability of Ni Apt against Ni-NTA resin beads that had been treated with EDTA, a chelating reagent known to remove Ni^{2+} ions. The fluorescence intensity of the beads was completely abolished after EDTA treatment, as shown in the middle panel of Fig. 1C, suggesting that Ni Apt does indeed recognize Ni^{2+} ions rather than the naked NTA. To confirm this again, we recharged the EDTA-treated NTA resin beads with fresh $NiSO_4$. We incubated the recharged beads again with Fam-Ni Apt, and the fluorescence was restored as we expected (the right panel of Fig. 1C). Taken together, these results strongly suggest that Ni Apt recognizes Ni-NTA resin through its interaction with Ni^{2+} ions.

The wide range of applications for the His-tag is not only due to its ability to bind to Ni-NTA resin, but also because His-tag can be specifically and easily removed by elution reagents such as imidazole.[1] Ni^{2+} ions can interact with imidazole to replace their interactions with multiple histidine residues (each contains an imidazole ring), allowing for the efficient elution of the His-tag from the Ni-NTA resin. To determine if our Ni Apt could also be eluted from the Ni-NTA resin using imidazole, we conducted similar experiments as that for purifying His-tagged proteins (Fig. 2A), and found that the fluorescence of Fam-Ni Apt was largely diminished by imidazole elution (Fig. 2B), indicating that Ni Apt can be specifically purified using a similar approach as that of His-tag. Next, we also tested the efficacy of EDTA to elute Ni Apt from the resin and found that it

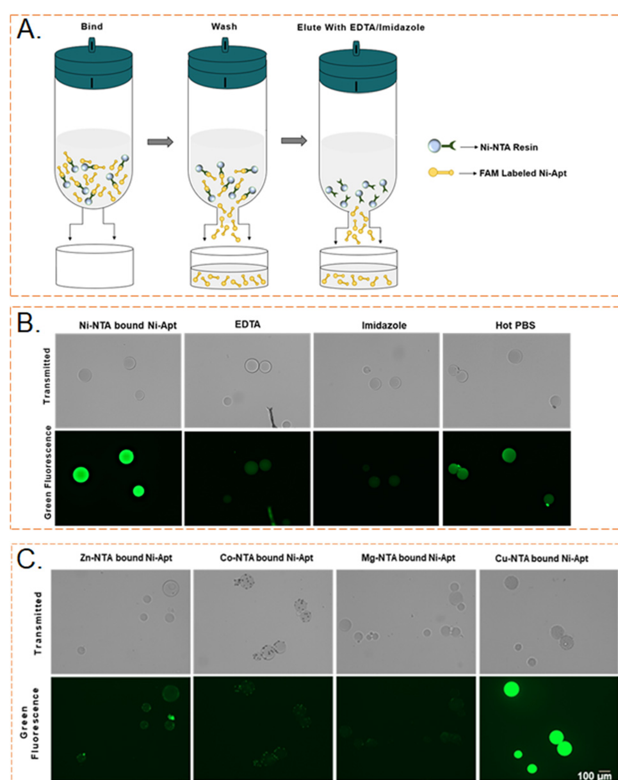

Fig. 2 Elution of Ni Apt from the Ni-NTA resin through different methods. (A) The workflow involved the binding of the aptamer to Ni-NTA resin, followed by washing and elution. (B) The complex of Fam-Ni Apt/Ni-NTA was treated by 500 mM EDTA, 500 mM imidazole, and hot PBS for 10 minutes, respectively. The fluorescence images of the Ni-NTA resin were recorded, indicating EDTA and imidazole are effective elution reagent to remove Ni Apt from the Ni-NTA resin. (C) Prior to the binding of Fam-Ni Apt, Ni-NTA was subjected to treatment with 500 mM EDTA and subsequently recharged with various divalent ions. The fluorescence images of the Ni-NTA resin were recorded.

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was similarly effective. Additionally, since most aptamers rely on their 3D structure to recognize their targets, we also investigated whether disrupting the 3D structure of Ni Apt could elute it from the resin. We treated the aptamer/resin complex with hot PBS (95 °C) and observed that hot buffer was not as effective in removing the aptamer from the resin compared to elution using EDTA or imidazole (Fig. 2B).

Based on the obtained results, it appears that the binding of Ni Apt to Ni-NTA resin is not solely reliant on its 3D structure; instead, it is influenced by additional, currently unknown interactions. To further investigate this phenomenon, we conducted binding specificity tests involving several other divalent ions. The Ni-NTA resin was subjected to EDTA treatment and subsequently recharged with 250 mM of Cu^{2+}, Co^{2+}, Zn^{2+}, and Mg^{2+}, respectively. We then incubated Fam-Ni Apt and conducted bead-based fluorescence imaging. As depicted in Fig. 2C, only the NTA resin recharged with Cu^{2+} was recognized by Ni Apt. In contrast, beads recharged with Co^{2+}, Zn^{2+}, or Mg^{2+} were not recognized. This observation suggests that the binding between Ni Apt and Ni-NTA or Cu-NTA is not exclusively governed by electrostatic interactions between Ni Apt and the positive charges of divalent ions.

As such an interesting nucleic acid mimic of His-tag, we measured the dissociation constant (K_d) of Ni Apt against Ni-NTA, by employing the flow cytometry assay using the Ni-NTA magnetic beads. The beads were incubated with various concentrations (1, 3, 9, 30, 90, and 300 nM) of Fam-Ni Apt, and samples were subjected to the flow cytometry measurement after washing. As shown in Fig. 3, Ni Apt has a low nanomolar binding affinity toward Ni-NTA. The K_d value was determined to be 106 nM.

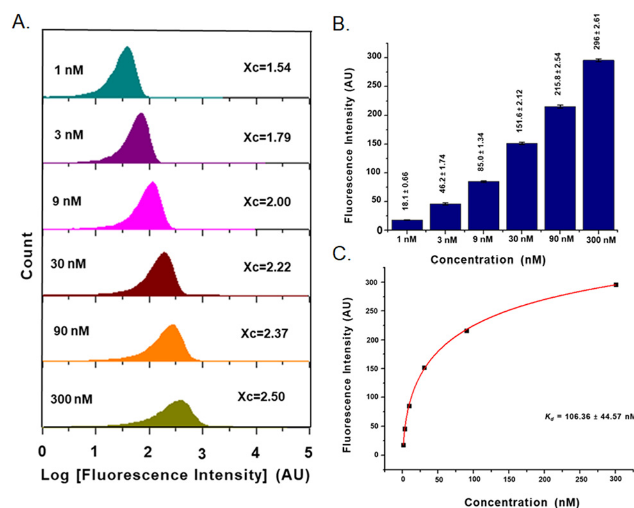

Fig. 3 The binding affinity of Ni Apt with Ni-NTA magnetic beads. (A) The flow cytometry used to measure the fluorescence intensity value (X_c) denoted for the mean green fluorescence intensity calculated from Ni Apt bound with Ni-NTA magnetic beads and form a stable covalent complex. (B) The increment of X_c values as a function of Ni Apt concentration bound to the same quantity of Ni-NTA beads. The error bars indicate the standard deviation of X_c values from the mean X_c value of three trials. (C) The binding affinity for Ni Apt immobilized with Ni-NTA magnetic bead complex was derived by the equilibrium dissociation constant (K_d) analysed by the Origin Pro software.

Furthermore, We conducted a test to determine the binding efficiency of Ni Apt to Ni-NTA resin in the presence of random nucleic acids, such as yeast tRNA. This was important as the presence of such nucleic acids, which can potentially compete for binding sites on the resin, may interfere with the binding of Ni Apt to the resin. To conduct the experiment, we introduced a five molar excess of yeast tRNA into the binding reaction consisting of Ni Apt and Ni-NTA resin in PBS-Mg buffer. The mixture was then incubated under identical conditions as previously established. Despite the presence of tRNA, our Ni Apt maintained its high binding ability targeting Ni-NTA, as supported by the strong fluorescence intensity observed from the Ni-NTA beads incubated with both Ni Apt and yeast tRNA (Fig. S2, ESI†).

Finally, we conducted an experiment to explore the potential applications of utilizing Ni Apt for nucleic acid purification. For this experiment, we acquired Ni Apt with a 3′ T20 tail (5′–TCGGGACGACGACAGCACAACCACCAACCACCCAACCCATTAATCGGTC GTCCCG TTTTTTTTTTTTTTTTTTTT–3′). We then combined this Ni Apt-T20 with an A20 single-stranded DNA (5′–AAAAAAAAAAAAAAAAAA AA–3′) to form 500 nM of Ni Apt-T20/A20 complex. Subsequently, 20 μl of Ni-NTA resin was introduced into the solution to capture the complex. After a 1 hour incubation period, we quantified the DNA concentration by measuring the UV absorbance of the supernatant of the incubation solution at 260 nm. As depicted in Fig. 4A, almost half of the Ni Apt-T20/A20 complex was captured by the Ni-NTA resin. In contrast, a control aptamer-T20 (5′–GCTATGCCCTGGCATCC TTCAGCTTTTTTTTTTTTTTTTTTTT–3′) also formed a complex with the A20. However, in this control capture assay, Ni-NTA exhibited a capture rate of only 4.3%. (Fig. 4B). This data demonstrates the capacity of Ni Apt to purify nucleic acid through DNA complementary interactions.

In summary, His-tag is widely used in many applications due to its ability to bind to Ni-NTA resin and be specifically eluted with imidazole. Interestingly, in our recent aptamer SELEX experiment targeting PD-L1, an aptamer named Ni Apt was identified and found to have a strong affinity for Ni-NTA resin. Ni Apt also can bind to Cu-NTA resin, while showing minimal affinity for other divalent ion-charged NTA beads, such as Co^{2+}, Zn^{2+}, and Mg^{2+}. Ni Apt was also shown to be

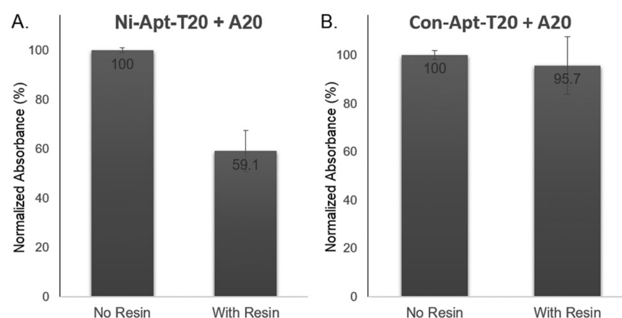

Fig. 4 Utilizing Ni-NTA and Ni Apt for capturing complementary DNA. (A) Ni Apt-T20 was hybridized with an A20 ssDNA, followed by capturing the complex using Ni-NTA resins. (B) A control aptamer-T20 was employed as a control.

specifically eluted with imidazole or EDTA. The dissociation constant of Ni Apt against Ni NTA was found to be in the low nanomolar range (K_d = 106 nM). The binding efficiency of Ni Apt in the presence of random nucleic acids such as tRNA was tested and found to be effective, suggesting that Ni Apt may be useful in complex biological samples. Finally, we demonstrated that Ni Apt and Ni-NTA can be used to capture nucleic acid through DNA complementary interactions. All data suggest the wide applications of Ni Apt for nucleic acid based purification and characterization.

X.T., and R.J. designed research; R.J., A.S., S.T., S.A., R.M. A.R., and X.T. performed research and analyzed data; and X.T., and R.J. wrote the paper with contributions from all authors.

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Conflicts of interest

There are no conflicts of interest to declare.

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