The Hong Kong University of Science and Technology
The Department of Chemical Engineering
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Capture and Release of Protein Reversible DNA induced Sol-gel Transition System

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Executive Summary

The goal of this project is to utilize DNA aptamer to achieve a capture-release of a human protein α-thrombin by sol-gel transition. Synthetic DNA strand A can bind to thrombin forming a stable thrombin-aptamer complex, which is then mixed with strands G1- and G2-linked polyacrylamide. Hydrogel will be formed hence capturing thrombin in the matrix. Complimentary strand D dissolves thde hydrogel and thrombin is released.

The gel electrophoresis results of protein and DNA showed that thrombin was successfully captured and released by the desired mechanism. The potential applications of the capture-release sol-gel transition mechanism include drug delivery, protein separation and purification, and revolutionary protein analyzer and biosensors.

Background and Theory

Centered the Project there are two essential concepts: Aptamer and DNA-Crosslinked Polyacrylamide Hydrogel.

a)Aptamer

Aptamers are nucleic acid species that have been engineered through repeated roundsof in vitro selection or, SELEX (Systematic Evolution of Ligands by Exponential Enrichment) to bind to various molecular targets, including proteins, nucleic acids. Aptamers offer molecular recognition properties comparable to the commonly used antibodies. Additionally aptamers are engineered completely in a test tube, readily produced via chemical synthesis, hence having good thermo stability and feasibility of automatic selection.

b)DNA-Crosslinked Polyacrylamide Hydrogel

Polyacrylamide (PAAm) hydrogels are characterized by their long chain lengths and a capacity for preserving their shape and mechanical strength even while imbibing water and ions.

DNA Oligomers can be used as a crosslinker of the PAAm to form the hydrogel. Acrydite contains a phosphoramidite group which immobilizes synthesized, single- stranded DNA on solid supports for analysis. The phosphoramidite group allows attachment of the Acrydite molecule to the 58 end of an oligonucleotide. The reactive end of the Acrydite modified oligonucleotide is similar to that of acrylamide monomer. Hence, Acrydite is used in special PAGE applications such as capture assays, by co-polymerizing the oligonucleotides with crylamide and bis. The authors have devised a method whereby two Acrydite functionalized oligonucleotides are copolymerized with acrylamide. A third DNA strand can then be added to crosslink the polyacrylamide chains, forming a crosslinked hydrogel as **Figure 1**.

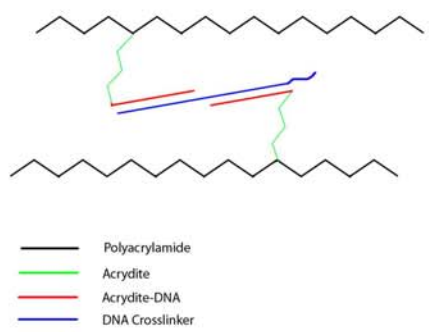


Figure 1. The schematic Diagram of Sol-Gel System

Design and Experiment

The Aptamer and DNA-Crosslinked Polyacrylamide Hydrogel are combined to create protein capture and release sol-gel system. The major components are as follows,

1)human **thrombin** was selected as the target protein

2)an **85mer crosslinking strand A was designed with a specific thrombin-binding aptamer** (GGTTGGTGTGGTTGG), which was able to form a double-stacked G quadruplex with a high affinity to thrombin, as shown in **Figure 2**,

3)two kinds of **20mer DNA strands (G1 and G2)** to be grafted on the main chains of polyacrylamide,

4)another **85mer DNA strand D**, which was fully complementary to strand A.

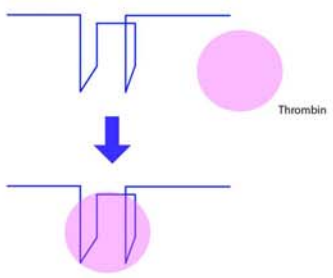


Figure 2. Strand A with aptamer binding to thrombin selectively

When strands G1 and G2 attached acrydites were copolymerized with acrylamide monomers, G1/G2 can be grafted to the main chain of polyacrylamide. The aptamer segment of strand A captures thrombin. Since the strand A has two segments complementary to G1 and G2, the hydrogel was formed by adding the thrombin-bound strand A into the system.

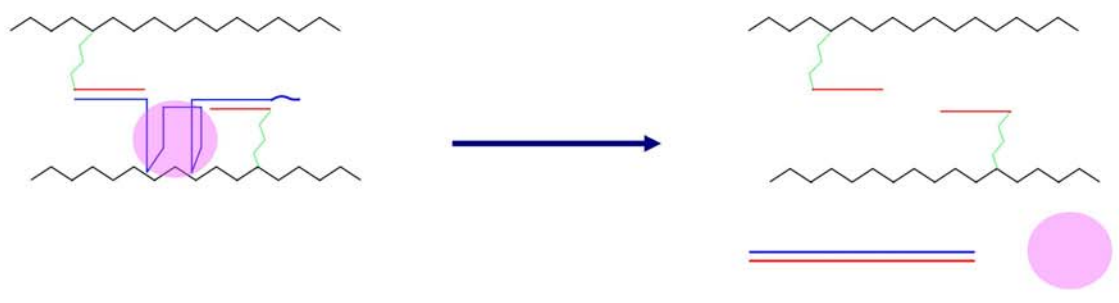


Figure 3. Introduction of D to the Sol-gel leads to dissolution

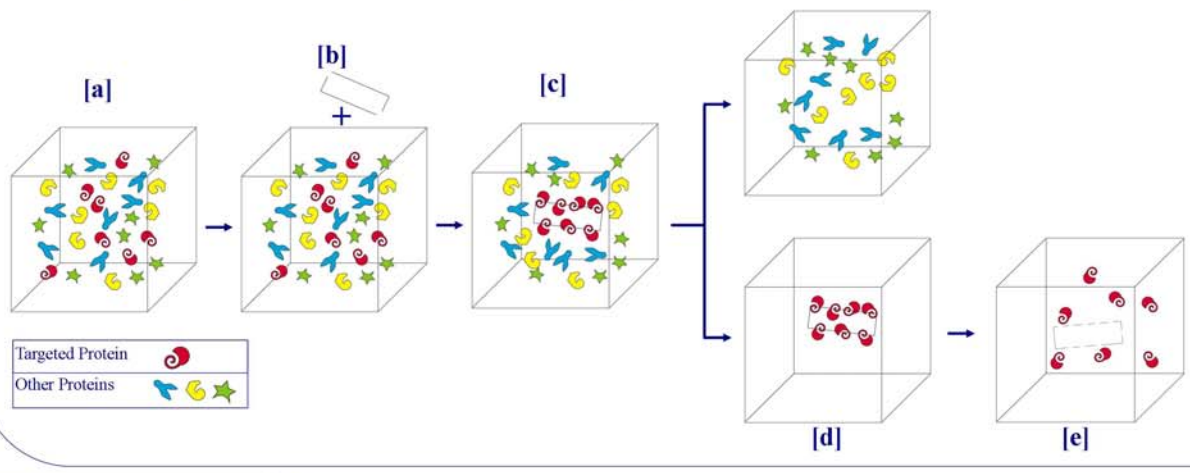
In the design, strand A has a toehold at the 3' end, which acts as a recognition tag for strand displacement. Adding the strand D that is fully complementary to strand A, the hydrogel is dissolved and thrombin is released, demonstrated in **Figure 3**.

Design and Experiment Continued

The selectivity is proven by adding other proteins into the system, where in the experiment BSA (Bovine Serum Albumin) will be particularly used. The whole mechanism is shown in **Figure 4**.

[a] There are many types of proteins in the solution. Each shape represents one particular protein. The red helical represents the target. **[b]** The PAAm with G1/G2 immobilized while the polymerization is prepared. **[c]** The aptamer embedded DNA crosslinker (Strand A) is added into the solution. After incubation, the proteins of interest are bound to the aptamer. The PAAm is added in and the hydrogel is formed while the crosslinking occurs within the PAAm.

Figure 4. Conceptual Diagram of the Sol-Gel System Design.



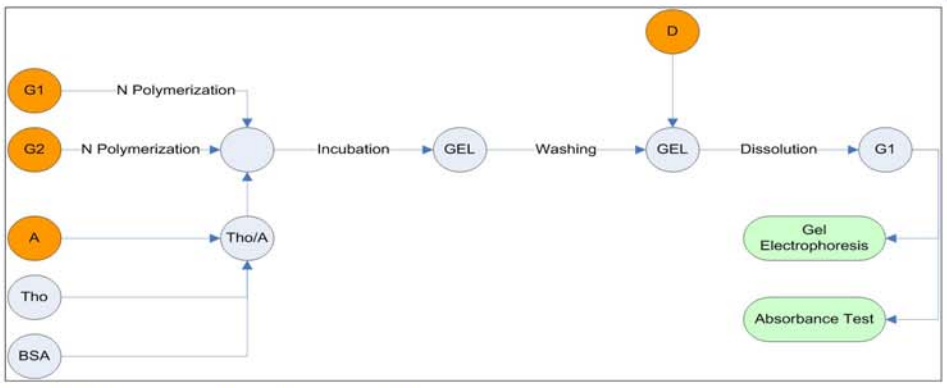


Figure 5. The process flow diagram of the experiment

[d] The hydrogel with the protein of interest is taken out and put into a buffer to leach out the free protein. **[e]** The hydrogel is dissolved by adding in the DNA strand (Strand D) which is complementary to Strand A so that the protein of interest is released. The whole separation process completes.

The process of experiment is determined according to our design, as illustrated by **Figure 5**. A Polyacrylamide Gel Electrophoresis with SDS as de-naturant (SDS- PAGE) analysis is conducted to test the practicability of the DNA sol-gel system.

Results and Discussions

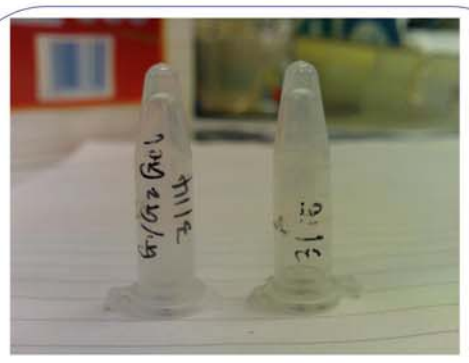


Figure 6. The Sol-Gel System was formed successfully on the tip of 2 Tubes, using Strand A (Left) and Strand A' (Right).

1) Formation of Polyacrylamide Hydrogel

The polyacrylamide hydrogel was prepared in two steps.G1 and G2 were grafted on the main chains of polyacrylamide by nitrogen bubbling. Polyacrylamide main chains were crosslinked into hydrogel by A with the two ends complementary to G1 and G2. Upon adding another D the hydrogel was dissolved.

Figure 6 shows that the system began to gel after adding A to the mixture of G1 and G2 with incubation. This is because strand A formed complementary base pairing with G1 and G2 upon incubation, the crosslinkings between the DNA strands made the system become hydrogel, hence proving the proposed gelatification mechanism. Compared to the figure, before adding A and after adding D, the content demonstrated liquid property in which the drops could not stick on the tips of inverted tubes.

2) The Selective Binding between the aptamer and thrombin

To investigate the selective binding, samples of A, A', A+Thrombin, A'+Thrombin, A+BSA, A+D+Thrombin, A'+D'+Thrombin, and A+D were analyzed by electrophoresis with a DNA Ladder, as displayed in **Figure 7**. The observation can be summarized in the following,

- The affinity of the thrombin to the aptamer is proved by lane 2 and 4. The band in lane 3 did not move down as the molecular weight increased dramatically due to the binding of thrombin to strand A.
- The strand A' without aptamer will not have the affinity to thrombin. This was shown in lane 3 and 5.
- The selectivity of the aptamer to thrombin over BSA is shown in lane 1 and 6. The addition of BSA did not influence the position of the bands.
- Lane 7 and 9 show that strand A has the greater affinity to strand D than thrombin, since the position of the bands appeared in the same position.
- Lane 8 shows that strand A' and D' formed the double-helix.

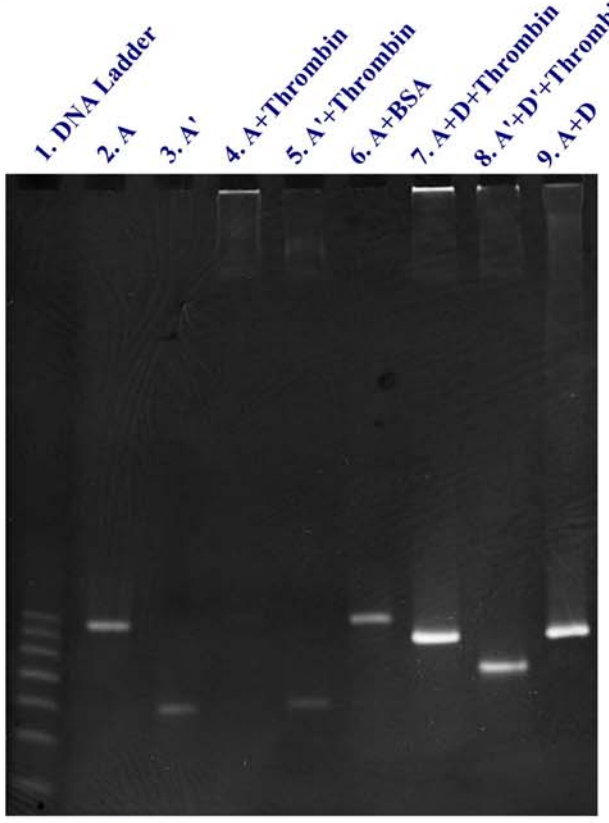


Figure 7. The DNA Electrophoresis Analysis of the capture and Release Mechanism

Applications and Commercialization

1) Drug Delivery: Controlled Release of Protein-based Drugs

Protein-based drugs could be controlled to release conveniently.

2) Biochemical/Pharmaceutical Process: Protein Separation and Purification

The process could be tailor-made for a specific protein in separation and purification process.

3) New Research Technique for Protein Analysis and Protein Biosensors

The process could be applied to determine the presence of protein with accuracy in protein analysis and biosensors experiments.