Capture and Release of Protein by Reversible DNA induced Sol-gel Transition System

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1. Abstract

Biotechnology has been growing prosperously and under great research interests in recent decades because of the underlying commercial opportunities and stimuli to the change of lifestyle. In this project, we apply the latest biotechnology of designated DNA aptamer for a specific protein, together with a sol-gel system, to perform a capture and release process for the specific protein.

For our specific protein, human α-thrombin, we designed a DNA strand A with known aptamer specifically bound to it. The strand A can induce the formation of a sol-gel with a solution of thrombin, designed G1, G2 DNA stands and polyacrylamide. This sol-gel system can capture thrombin in the gel forming phase. Then, by controlling the amount of complementary strand D (to strand A) added into the sol-gel system, we can control the release of protein in gel dissolving phase. To prove our proposal, we designed and conducted an experiment using mixture of two proteins, BSA and human α-thrombin, as well as the DNA strand A (with aptamer), D, G1, G2, A' (same structure as A but without the aptamer) and D' (complementary to A'). The experiment using DNA strand A and D should show an effective separation of thrombin from the protein mixture while the control experiment using DNA strand A' and D' should show no separation of any specific protein.

This fantastic idea could be developed into various applications and bring along with enormous commercial opportunities. The controlled release of protein can be modified to be a controlled drug delivery of protein-based drugs. The capture of protein can be applied in a highly selective protein separation and purification process in the biochemical or pharmaceutical industry. Moreover, the whole protein capture and release process could be a new protein analysis technique in research application or could be incorporate to biosensors targeting at specific protein.

2. Mechanism

In the design of the experiment, there are two essential concepts: *Aptamer* and *DNA-Crosslinked Polyacrylamide Hydrogel.*

Aptamer

Aptamers are nucleic acid species that have been engineered through repeated rounds of in vitro selection or equivalently, SELEX (systematic evolution of ligands by exponential enrichment) to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Aptamers offer the utility for biotechnological and therapeutic applications as they offer molecular recognition properties that rival that of the commonly used biomolecule, antibodies. In addition to their discriminate recognition, aptamers offer advantages over antibodies as they can be engineered completely in a test tube, are readily produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications.

Compared to antibody-antigen interactions, aptamers not only have similar target binding specificities, but also exhibit good thermo stability and feasibility of automatic selection, which provides potential applications in biosensor.

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. Commercial applications of PAAm include its use in drag reduction agents, thickening agents, cutting fluids, soil stabilizers, soaps, textiles, and enhanced oil recovery. Polyacrylamide gel electrophoresis (PAGE) is a very popular method of characterizing macromolecules such as proteins and DNA. In this technique, the polyacrylamide gel functions as a porous supporting medium through which the molecules migrate in the presence of an electric field. Polyacrylamide gel is ideal in this diagnostic role because of its chemical inertness i.e., it does not react with the macromolecules. Moreover, the average pore size is easily controlled by altering the concentrations of the monomer and the crosslinker. In the medical field, the potential

of PAAm as a versatile biomaterial has been demonstrated in vascular applications in immunoabsorbents and in artery embolization reconstructive surgery, and even bone implants.

Through literature reach, DNA Oligomers were discovered as a crosslinker of the PAAm to form the hydrogel. The biopolymer DNA serves as the carrier of genetic information in living organisms. Native DNA is a polymer of the four distinct nucleotides adenine A, guanine G, cytosine C, and thymine T. Within cells, DNA molecules exist as double-stranded helical structures. This phenomenon is facilitated by the propensity to form complementary base pairs between A and T and between G and C. The highly specific nature of DNA interactions has been exploited by a number of researchers in nanotechnology e.g., the reversible selfassembly of colloidal gold nanoparticles into macroscopic aggregates.Mosaic Technologies Waltham, MA has developed a phosphoramidite trade name Acrydite for immobilizing 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 Acryditemodified 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 is then added to crosslink the polyacrylamide chains, forming a crosslinked hydrogel.

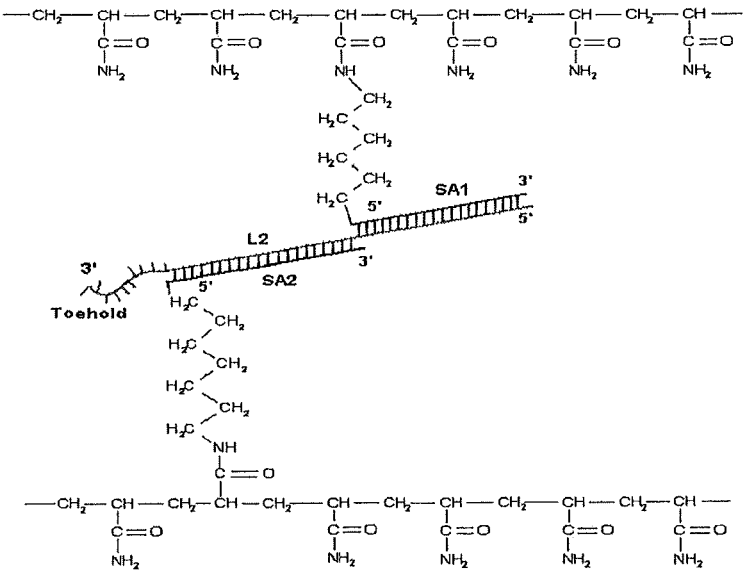
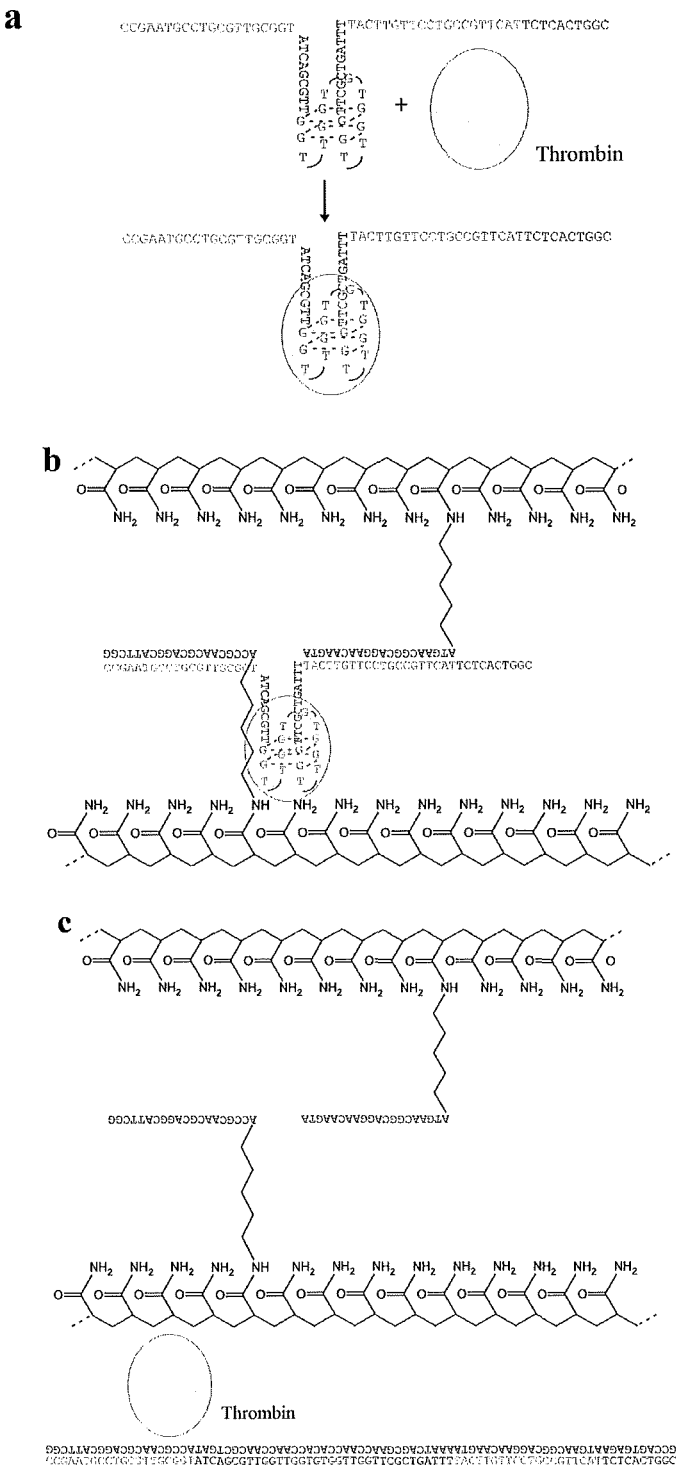


Figure.2.1 Two polyacrylamide chains crosslinked by three DNA strands(SA1, SA2 and L2)

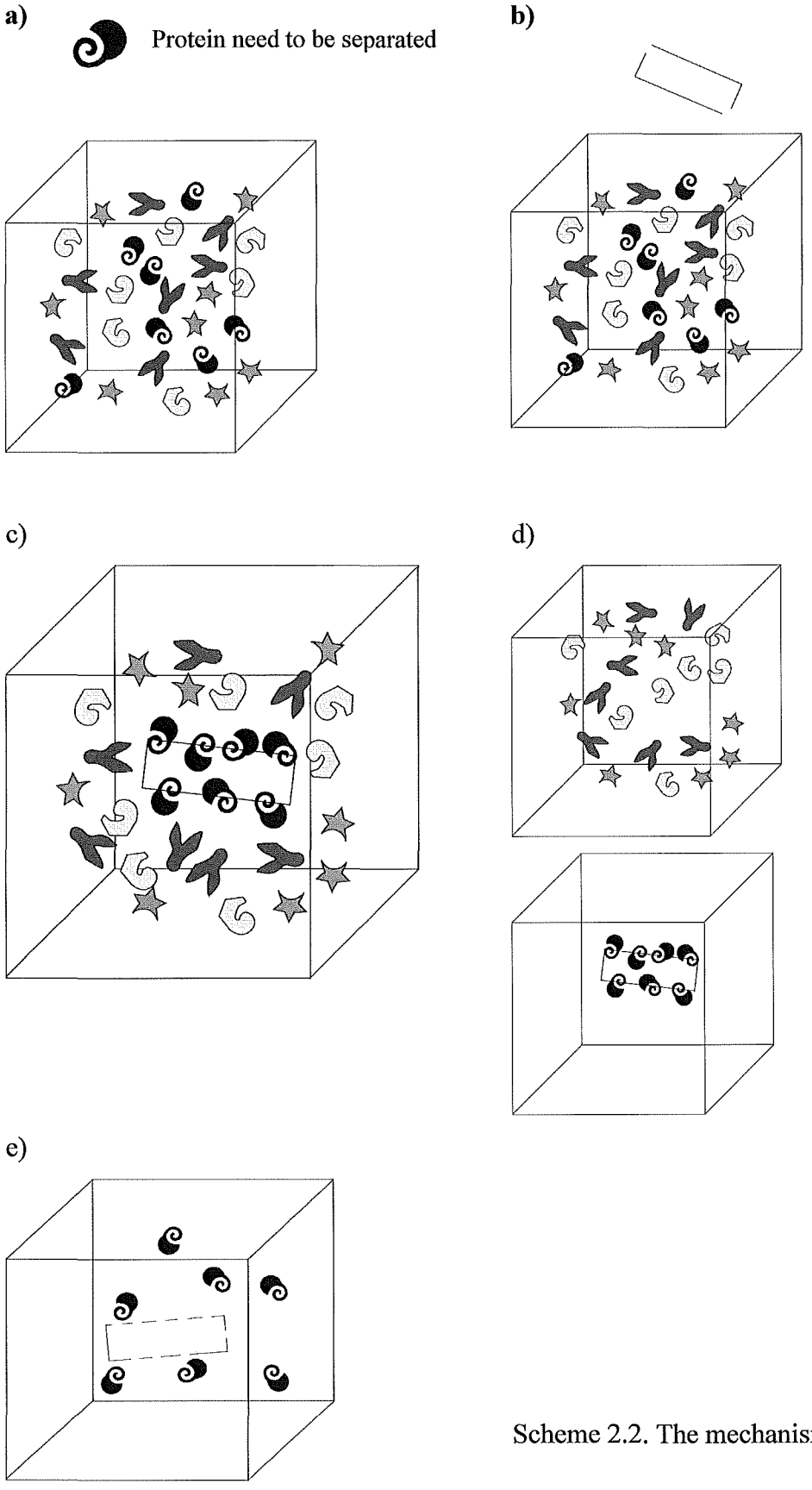
In this project, the previous two technologies are combined to create protein capture and release sol-gel system. Human thrombin was selected as the target protein and a 85mer crosslinking strand A was designed with a specific thrombin-binding aptamer (GGTTGGTGTGGTTGG), which was able to form a double-stacked G quadruplex with a high affinity to thrombin. The polyacrylamide hydrogel was prepared in two steps: (1) two kinds of 20mer DNA strands (G1 and G2) were grafted on the main chains of polyacrylamide; (2) polyacrylamide main chains were crosslinked into hydrogel by a 85mer DNA strand A with the two ends being complementary to strand G1 and strand G2, respectively. Upon adding another 85mer DNA strand D, which was fully complementary to strand A, the hydrogel was dissolved and the thrombin was released.

The DNA attached acryditeTM (Integrated DNA Technologies, Inc, IA, USA) is reported to have the similar activity of acrylamide monomers. When strands G1 and G2 attached acrydites were copolymerized with acrylamide monomers, G1/G2 can be grafted to the main chain of polyacrylamide. The average length between the grafted DNA strand (G1 or G2) is 30 acrylamide monomers. Scheme 1a shows the capture of thrombin by the thrombin-binding aptamer segment of strand A. Since the strand A has two segments complementary to G1 and G2, respectively, the hydrogel was formed by adding the thrombin-bound strand A into the system as shown in scheme 1b. One should note that, in scheme 1b, strand A has a toehold at the 3' end, which acts as a recognition tag for strand displacement. The process of gel dissolving and thrombin release can be operated by adding strand D that is fully complementary to strand A, as shown in scheme 1c.



The scheme 2.1. The scheme of capture and release of thrombin by sol-gel transition. (a) The 15mer segment of strand A, highlighted in red, forms a G-quadruplex, which can bind strongly to thrombin in solution to form a thrombin-aptamer complex. (b) When the thrombin-aptamer complex is mixed with strands G1- and G2-linked polyacrylamide, the G1 and G2 complementary segments of strand A bind to strands G1 and G2 to form the hydrogel and thrombin is captured in the hydrogel matrix. (c) When strand D, which is fully complementary to strand A, is added to the hydrogel, the hydrogel is dissolved and thrombin is released.

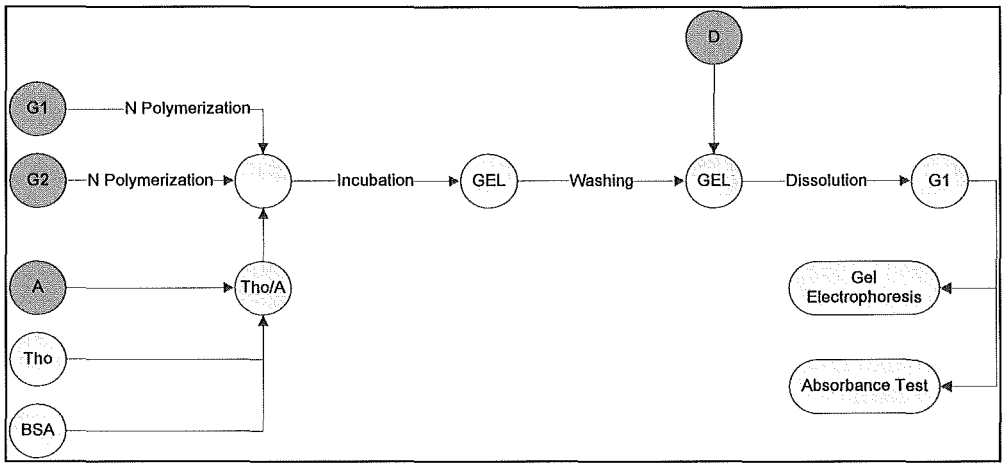
The selectivity was proved by adding another protein Bovine Serum Albumin (BSA) in to the system. The whole mechanism is shown in the following scheme.



Scheme 2.2. The mechanism flow chat

Scheme 2.2. a) There are many types of proteins in the solution. Each shape represents one particular protein. The red helical shape represents the protein which is of interest and need to be separated out. b) The PAAm with G1/G2 immobilized while the polymerization is prepared. c) The DNA 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 happens within the PAAm. 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 which in complementary to Strand A (Strand D) so that the protein of interest is released. The whole separation process completes.

The designed process flow of the experiment is shown as follow.



Scheme 2.3. The process flow diagram for capture and release of Thrombin

A Polyacrylamide Gel Electrophoresis with SDS as de-naturant (SDS- PAGE) analysis is conducted to test the efficiency of the DNA sol-gel system. The concentrations of the proteins are represented by the intensity of the band in the PAGE gel.

3. Results:

1. The polyacrylamide hydrogel:

Polyacrylamide can form thermo-sensitive hydrogel, which makes it a desirable material for therapeutic applications such as regenerative medicine and drug release. In this project, a DNA-induced sol-gel transition system was combined with a protein-binding aptamer in order to capture and release proteins.

The polyacrylamide hydrogel was prepared in two steps: (1) two kinds of 20mer DNA strands (G1 and G2) were grafted on the main chains of polyacrylamide; (2) polyacrylamide main chains were crosslinked into hydrogel by a 85mer DNA strand A with the two ends being complementary to strand G1 and strand G2, respectively. Upon adding another 85mer DNA strand D, which was fully complementary to strand A, the hydrogel was dissolved and the thrombin was released.

The polyacrylamide hydrogel formation results are illustrated as below:

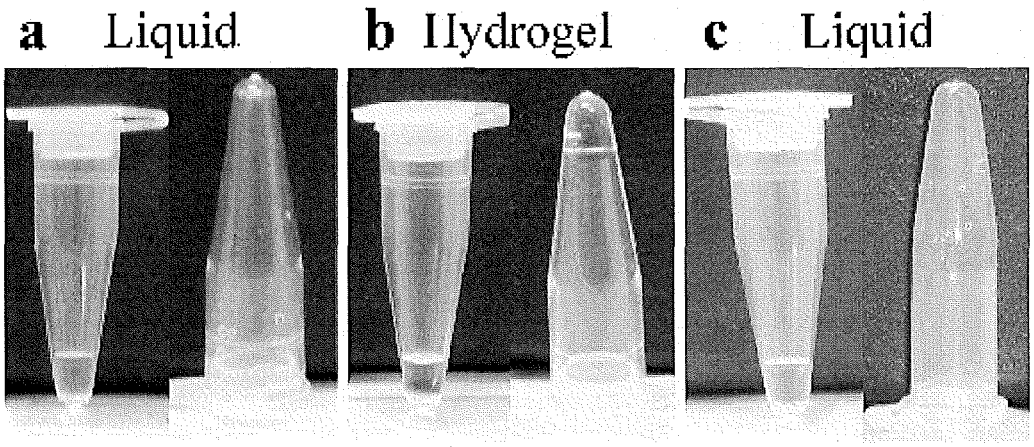


Figure 3.1 Polyacrylamide hydrogel formation results

As can be seen from the figure,

Figure 3.1 a) shows that before strand A was added to the mixture of DNA strands G1 and G2, the system was in liquid state. The reason it was called liquid is when the tube was turned upside down, the system flew downwards.

Figure 3.1 b) shows that after the strand A was added to the mixture of G1 and G2 strands, the system began to form gel. This is because strand A formed complementary base pairing with G1 and G2, the crosslinkings between the DNA

strands made the system form gel.

Figure 3.1 c) shows that after the strand D was added into the hydrogel, the system was dissolved into liquid state again. The reason is strand D formed complementary base pairs with strand A, which led to the dissolve of thrombin, strand A and strand D.

2. The polyacrylamide gel electrophoresis (PAGE) results:

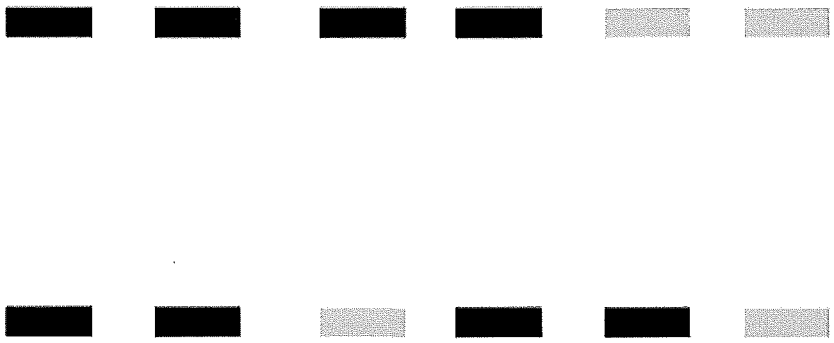


Figure 2.2 The expected PAGE result of protein

From the expected gel electrophoresis result, the upper bands are BSA, since they have larger molecular weight than that of thrombin. The lower bands are thrombin, because of their smaller molecular weight; they can run faster than BSA in the gel electrophoresis process. The black bands represent thrombin or BSA exists and the grey bands represent that neither thrombin nor BSA exists in that position.

From the expected gel electrophoresis result figure, the result can be explained.
In lane 1 and lane 2, thrombin and BSA exist in both of the lanes because in original sample, BSA and thrombin are mixed with A and A' respectively in order to form hydrogel in the later process.
In lane 3, only BSA exists but thrombin is disappeared. This is because thrombin is captured by DNA strand A by complementary base paring principle. However, strand A will not capture BSA since they have no complementary base pairs. Therefore, BSA resumes in the system after the leaching process.

In lane 4, both thrombin and BSA exist. This is because DNA strand A' is added to the system, however, A' will not form any complementary base pair with thrombin or BSA. Therefore, after the leaching process, BSA and thrombin are both resumed in the system.

In lane 5, the gel containing thrombin captured by strand A is dissolved by DNA strand D. DNA strand D will form complementary base pairs with DNA strand A, therefore the hydrogel is dissolved and thrombin, not captured by strand A anymore, is released from the system. Therefore, thrombin can be observed in land 5.

In lane 6, the hydrogel is also dissolved due to the forming of complementary base pairs between DNA strand A' and DNA strand D'. However, since thrombin is not captured by A' in the previous step, there is nothing exists in the hydrogel. Therefore, neither thrombin nor BSA is released from the system.

Analyzing the results:

The PAGE result obtained from the project is illustrated below:

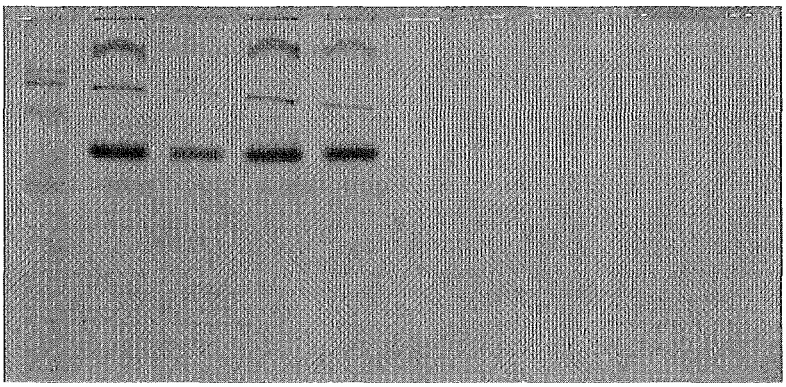


Figure 3.2 PAGE result obtained from the experiment

The materials in each lane are labeled as the following:

Lane1: Ladder

Lane2: A+Thrombin+BSA

Lane3: A'+Thrombin+BSA

Lane4: A+Thrombin+BSA

Lane5: A'+Thrombin+BSA

Lane6: The residue of Sol-gel with A

Lane7: The residue of Sol-gel with A'

Lane8: D+A+Thrombin+BSA

Lane9: D'+A'+Thrombin+BSA

The thrombin has a molecular weight of about 37K da, the BSA has a molecular weight of 60k da. In the gel electrophoresis experiment, due to the larger molecular weight, BSA will run slower than thrombin and therefore resume in a higher position in the figure 3.2. However, due to denature of thrombin during transportation, the molecular weight of thrombin somehow becomes larger than that of BSA. This can explain the strange result obtained in figure 3.2. Due to the larger molecular weight, thrombin ran slower than BSA, therefore resumed in a higher position.

As can be seen from the figure, the colors of lane 6, 7, 8 and 9 are too shallow to observe, this might due to the over de-stain of the gel sample. The expected result of this gel electrophoresis is discussed in the expected results part.

4. Applications

The protein capture and release is indeed a high-tech bioprocess that has various potential applications with enormous commercial opportunities underlying. In this section, we would like to present some of its potential applications in drug delivery, biochemical or pharmaceutical separation process, new research technique for protein analysis and protein biosensors:

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

Drug delivery is a USD 10 billion industry. Without an effective drug delivery mechanism to the targeted applied area, even a powerful drug cannot perform well. Especially for protein-based drugs, the most common way of drug delivery, oral intake, seems to be impossible because protein cannot withstand the severe pH changes along the oesophagus, stomach and intestine. Therefore, a more sophisticated drug delivery mechanism is needed for most protein-based drugs.

We have done some research in this aspect and found that there are some good delivery mechanisms for protein-based drugs in research area. For instance, Dr. Niren Murthy and Mark R. Prausnitz et al. from Georgia Institute of Technology wrote a paper, "Minimally Invasive Protein Delivery with Rapidly Dissolving Polymer Microneedles"1, introducing their protein delivery mechanism using rapid dissolving polymer needles of micrometer dimensions they designed and synthesized. In their design, the drug is encapsulated within polymer microneedles and, after insertion into the skin, the biocompatible polymer dissolves within minutes to release the encapsulated cargo, not requiring removal, and leaving behind no biohazardous sharps. Inspired by this technology, we think that our process can be modified to deliver protein-based drugs. Firstly, the polyacrylamide sol-gel should be replaced by biocompatible polymer, like biopolymer made from polydimethylsiloxane (PDMS) monomer as used in Dr. Murthy's application. Without this modification, our sol-gel system cannot be applied in human because polyacrylamide is poisonous. With this modification, the protein release mechanism of our process can be well-applied in the delivery of protein-based drug because the presence of complementary DNA strand to the aptamer-DNA strand would initiate the release of protein drugs. And one of the advantages of our process over Dr. Murthy's is the release of protein can be well-

controlled by the concentration of the complementary DNA strand in our process. However, in Dr. Murthy's application, it cannot control the rate of release of protein.

2.Biochemical/Pharmaceutical Process: Protein Separation and Purification

In traditional practice, people separate proteins using methods like sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), capillary reversed-phase high performance liquid chromatography (HPLC). However, these methods were developed over 20 years and they cannot separate proteins with similar charge to mass ratio or molecular weight. Thus, new technologies are needed to facilitate a higher selective and effective separation process of proteins.

In this regard, our protein capture and release process can be tailor-made for a specific protein. For every protein, there is specific binding site which is complementary to specific DNA aptamer, which could be found through some research database for DNA aptamer. After indicating the aptamer, we can construct its corresponding DNA strands A, G1 and G2. With all these DNA strands available, we can then use the sol-gel system to separate our desired protein using the similar experimental procedure we did in our experiment.

Using protein capture and release process to separate protein can ensure a highly selective separation and purification process for the desired protein. However, to become a commercial large-scaled separation process, more research should be done to industrialize our process.

3. New Research Technique for Protein Analysis and Protein Biosensors

In research area, like biochemistry, biology or bioengineering, researchers always need to identify some unknown protein or biomolecules. For protein, they would use SDS-PAGE and compare it with standard SDS-PAGE, or in higher-end term, they would use biotechnology, like immune precipitation or amino acid sequencing, to identify the protein structure and sequence. For some biomoleculars, they may use biosensors corresponding to the suspected biomolecules to test the existence of the biomolecules.

Concerning with these applications, our protein capture and release process offers an alternative way to determine the presence of some suspected protein with significant accuracy. To make it as a commercialized or handy applicant to test the existence or concentration of a specific protein, we can further do research on our process and convert it into a Protein Biosensor to detect the specific protein. For some

biomolecules which have specific binding site with DNA aptamers, like RNA, we can also modify our process to capture the specific biomolecule and make a commercial biosensor for it.

Reference:

1. Bryan Wei, Immensee Cheng, Kathy Q. Luo and Yongli Mi*. "*Capture and Release of Protein by Reversible DNA-induced Sol-gel Transition System*" **Angew_Chem, 2008**

2. David C. Lin, Bernard Yurke, Noshir A. Langrana* "*Mechanical Properties of a Reversible, DNA Crosslinked Polyacrylamide Hydrogel*" **ASME, vol. 26 2004**

3. Sean P. Sullivan, Niren Murthy and Mark R. Prausnitz, *"Minimally Invasive Protein Delivery with Rapidly Dissolving Polymer Microneedles". **Adv. Mater.*** **2008**, 20, 933 - 938.