Selecting Peptides Using mRNA Display

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Introduction

Research in Professor Richard Robert's lab uses the process called **mRNA display** to select peptides that bind to target proteins. The goal is for these peptides to be used as disease diagnostics and therapeutics.

Throughout the seven weeks of SHINE, my mentor, Dr. Kaori Noridomi, my lab partner, Lillian Bailey, and I discussed the mechanisms of the complex mRNA display and performed mRNA display experiments. We also researched Covid-19 and explored mRNA display's application to COVID-19.

Objectives & Impact

Through mRNA display, selected peptides could be used in diagnostic and therapeutic applications for diseases including autoimmune diseases, cancer, and COVID-19.

Autoimmune Disease

In an autoimmune disease, antibodies, whose job is to protect one's body from viruses and foreign substances, malfunction. They instead attack one's own body and tissue. Using mRNA display, peptides are developed that bind to the malfunctioning antibodies, called autoantibodies.

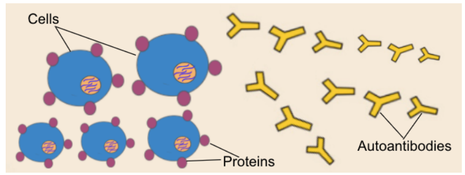

Figure 1A: Autoantibodies recognizing proteins in a body as foreign and attacking them

Diagnostic Application

The selected peptides could:

- Recognize the autoantibodies' presence → diagnosing the disease
- Measure the amount of expressed autoantibodies → identifying the disease's severity or progression

Therapeutic Application

Selected peptides could:

- Inhibit autoantibodies from binding to proteins → slowing disease progression

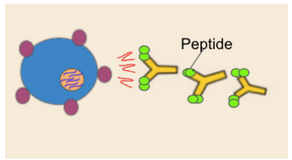

Figure 1B: Selected peptides binding to autoantibodies, inhibiting them from attacking proteins

Methodology/Skills Learned

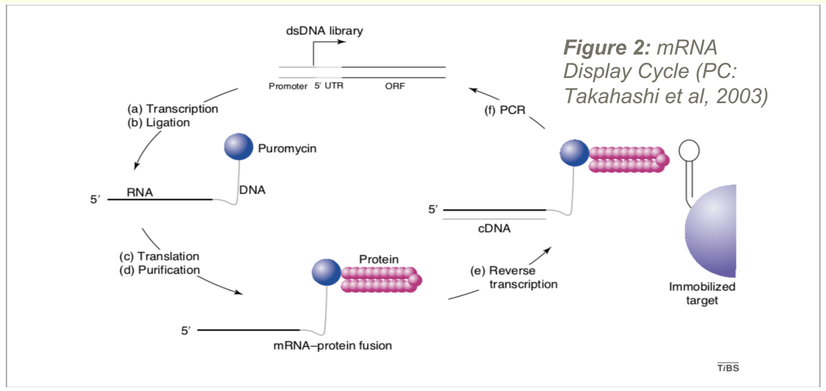

Figure 2: *mRNA Display Cycle (PC: Takahashi et al, 2003)*

Polymerase Chain Reaction (PCR) ~ technique to amplify DNA strands

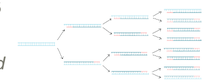

Figure 3: DNA sequences amplified through PCR

Agarose Gel Electrophoresis ~ technique to detect DNA

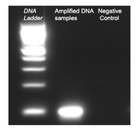

Figure 4: Agarose Gel of amplified DNA (PC: Dr. Noridomi)

Transcription ~ Reaction that codes mRNA sequences based on their DNA sequences

Ligation ~ Reaction to ligate mRNA sequences with F30P

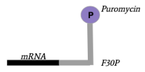

Figure 5: mRNA ligated with F30P

Urea Gel Purification ~ technique to purify ligated mRNA from unligated mRNA

Translation ~ reaction that codes peptides based on their mRNA sequences

Fusion ~ attachment of Puromycin to peptides → fuses peptides with their mRNA sequence

Figure 6: mRNA-peptide fusion

dT Purification ~ technique to purify mRNA-peptide fusions using dT beads

Reverse Transcription ~ reaction that codes complementary DNA (cDNA) sequences from mRNA sequences

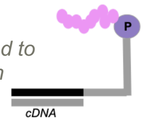

Figure 7: cDNA added to mRNA-peptide fusion

Selection ~ process of exposing peptides to immobilized target protein, selecting the peptides that bind to protein, and washing away the peptides that don't bind.

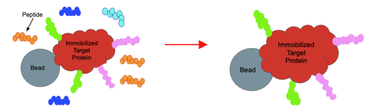

Figure 8: Selection of Peptides against immobilized target protein

Next round of Selection/ PCR ~ process of amplifying enriched DNA library and repeating all steps for the second round of selection. 6-10 rounds of selection will occur.

Characterize and Sequence ~ selected peptide and their properties are distinguished

COVID-19 Application

We researched mRNA display's application to COVID-19. In a body, a spike protein on a SARS-CoV-2 virus binds to a ACE 2 human receptor. This binding allows the virus to enter human cells.

Using mRNA display, selected peptides could bind to the spike protein on the SARS-CoV-2 virus. These peptides could block the spike protein from binding to the ACE 2 human receptor, inhibiting the SARS-CoV-2 virus from entering human cells and slowing the viral progression

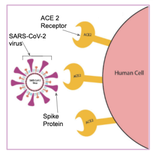

Figure 9A: Spike protein binds to human ACE2 Receptor

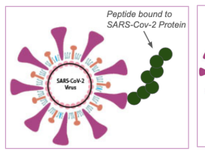

Figure 9B: Selected peptide binds to spike protein

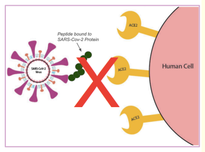

Figure 9C: Bound peptides prohibit spike protein and ACE 2 receptor binding

We used PyMOL, a visualization software, to analyze the binding interaction between a spike protein and an ACE 2 receptor.

Figure 10: Crystal structure of spike protein bound to ACE 2 (PC: Lan et al, 2020)

Understanding this interaction is useful to design a DNA library for mRNA display selection against a spike protein.

Next Steps

I have been incredibly inspired throughout my seven weeks at SHINE. Wherever I go, I will carry a new level of knowledge, expertise, and confidence. I am especially eager to begin my Honors Research in Science II class next month. From SHINE, I have a whole new perspective on the challenges of research; I now feel confident in deepening the complexity and impact of my own research project. I am also very excited to start Honors Computational Chemistry. From this program, I have observed real-world applications of chemistry, and I have developed a love for it. I can definitely see chemical engineering and research being a part of my future.

The most important next step for me is staying in contact with and hopefully involved with Dr. Noridomi and Professor Roberts. I hope that I can continue to observe and be a part of their lab in the near future.

Acknowledgements

I would like to give a sincere thank you to Professor Roberts for granting me such an amazing opportunity to be a part of his lab. I would also like to thank Dr. Noridomi for everything she has taught me, her support, and her patience. She has made my seven weeks at SHINE an exceptional and memorable experience. I would also like to acknowledge my lab partner, Lillian Bailey, for her support and friendship. Lastly, I would like to thank Dr. Mills and the whole SHINE team for their ongoing support, guidance, and effort towards making the SHINE program phenomenal.