Selecting Peptide Binders through mRNA Display

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USC Viterbi School of Engineering

SHINE Summer High School Intensive in Next-Generation Engineering

Introduction

The researchers of Professor Richard Roberts' chemical engineering lab are using mRNA display to develop protein-specific peptide binders. Invented by Dr. Roberts, mRNA display is an *in vitro* technique used to develop and select peptides that target specific proteins. mRNA display has therapeutic and diagnostic purposes for various conditions and diseases.

While we were unable to be physically present in the lab, my partner and I, researching under Dr. Kaori Noridomi, gained much exposure to the technique and purpose of mRNA display, and learned the skills to apply it to COVID-19 experimental design, as well as to think deeply about its application to medicine.

Objective & Impact

Through the development of protein-specific peptide binders, the diagnosis and treatment of many diseases (including cancer and various autoimmune diseases) could be simplified and improved.

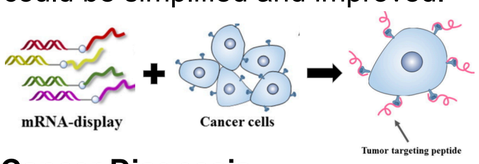

Figure 1: *using mRNA display to develop a tumor-targeting peptide (PC: Liu et al., 2017)*

Cancer Diagnosis

mRNA display can be used to develop and select peptides that bind to specific proteins unique to a certain type of cancer, increasing the efficiency and accuracy of the diagnosis.

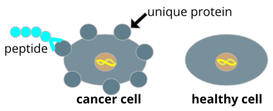

Figure 2: *peptide binds to unique protein expressed by cancer cell*

Cancer Treatment

Peptides can be used as a drug delivery method to target a protein unique to a cancer type, or can be used to inhibit protein function of overexpressed proteins. This would reduce negative side effects of treatment, in contrast to a treatment like chemotherapy which attacks both healthy and cancerous cells.

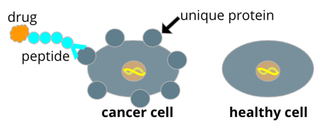

Figure 3: *drug delivered only to cancer cell by fusing to selected peptide*

Methods & Skills Learned

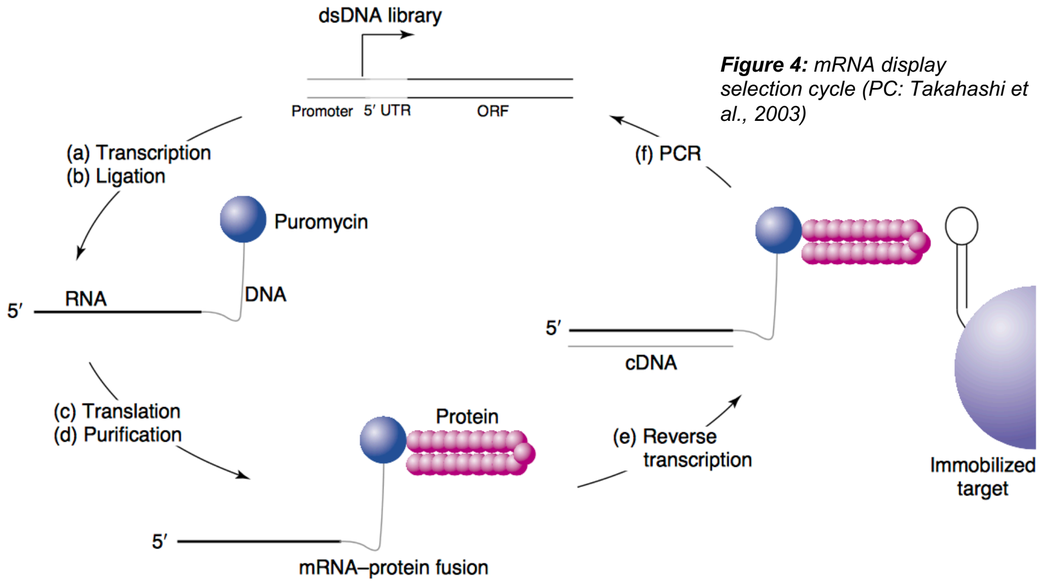

Figure 4: *mRNA display selection cycle (PC: Takahashi et al., 2003)*

1. **DNA library / Polymerase Chain Reaction (PCR):** amplifies DNA library (many kinds of DNA sequences)

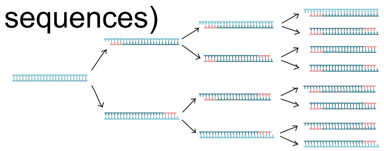

Figure 5: *amplification of DNA after 3 PCR cycles (PC: Khan Academy)*

2. **Agarose Gel Electrophoresis:** detects DNA to ensure PCR works
3. **Transcription:** DNA library → mRNA library
4. **Ligation:** ligate mRNA to F30P (DNA with puromycin)

Figure 6: *ligated mRNA/F30P*

5. **Urea Gel Purification:** purifies ligated mRNA from unligated mRNA

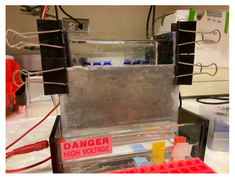

Figure 7: *running urea gel in lab (PC: Kaori Noridomi)*

5. **Translation:** mRNA translated to amino acids (form peptides)

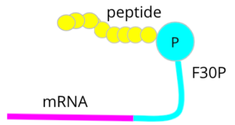

Figure 8: *ligated mRNA with peptide*

6. **Reverse Transcription:** complementary DNA is encoded from mRNA

Figure 9: *reverse transcription – opposite central dogma*

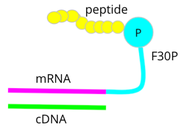

Figure 10: *cDNA strand (colored in green) encoded from mRNA*

7. **Selection:** select peptides that bind to target protein

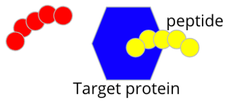

Figure 11: *find peptide (red, yellow, green) to bind to target protein (blue)*

8. **PCR and Re-selection:** perform PCR to create enriched library

Repeat selection cycle 6-10 times

9. **Characterization of peptide:** perform further experiments to characterize the peptide and determine its sequence

Application to COVID-19

We applied mRNA display to SARS-CoV-2 (COVID-19) research and experimental design. One of the ways in which the SARS-CoV-2 virus infects human cells is through its spike protein's interaction with the human ACE2 receptor protein. Our aim is to develop peptides to target the SARS-CoV-2 spike protein to inhibit its binding with the human ACE2 receptor for usage in potential therapy or in a vaccine.

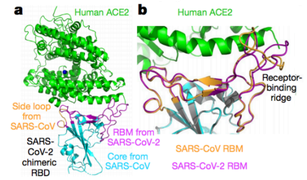

Figure 12:
a) overview of spike protein/ACE2 interaction
b) zoomed in view of interface
(PC: Shang et al., 2019)

We used PyMOL software to study the interface between the spike protein and ACE2. Understanding this interaction will allow us to design a doped DNA library to be used in mRNA display selection against the spike protein.

My STEM Journey

From my experience in SHINE, I have come to understand what a career or further studies in a lab might include, and I am excited to see where my STEM fascination and growing passion for biochemistry will lead me beyond high school.

Acknowledgements

Sincere thanks to Professor Roberts for welcoming me to his lab and encouraging my learning amongst such an inspiring group of researchers. Thank you to Dr. Kaori Noridomi for her time, support, and knowledge, and to Stella Grynberg for sharing this experience with me and leading me to ask more questions and think more deeply. This experience would not have been possible without the ongoing organization, leadership, and encouragement of Dr. Katie Mills, Dr. Megan Herrold, my center mentors, and the SHINE team. Finally, I would like to thank my parents and family for supporting me to pursue my interest in STEM this summer.