Characterization of Target Proteins Utilized in mRNA Display

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Introduction

mRNA display is a technique invented by Professor Richard Roberts to develop specific peptide binders to a target molecule (protein, RNA, etc.) from an initial library of high complexity. In this research, we sought to understand the specific characteristics about target proteins used in past mRNA display experiments in order to gain a better understanding of prominent selection factors, which could potentially improve future mRNA display experiments. We also understood the medical applications of mRNA display.

Benefits of mRNA Display

- Totally *in vitro*
- Can isolate high affinity binding peptides from libraries up to 10^{15} independent sequences
- Helps us understand complex protein-protein interactions
- Useful in therapeutic applications

Application

Tumor Cell Targeting with mRNA Display

mRNA display selections could be performed against tumor cell markers/receptors to develop high binding affinity **tumor-targeting peptides** (TTPs). These selected peptides could be useful tools for diagnosis/treatment of diseases such as cancer.

Diagnosis Applications

Peptides could recognize and bind to specific tumor markers found in a blood sample, potentially providing an early diagnosis of a certain type of cancer.

Treatment Applications

TTPs can be fused with cancer drugs, and only tumor cells would be treated *(figure 1).* This could potentially be more efficient than chemotherapy treatments, which often attacks both cancer and healthy cells.

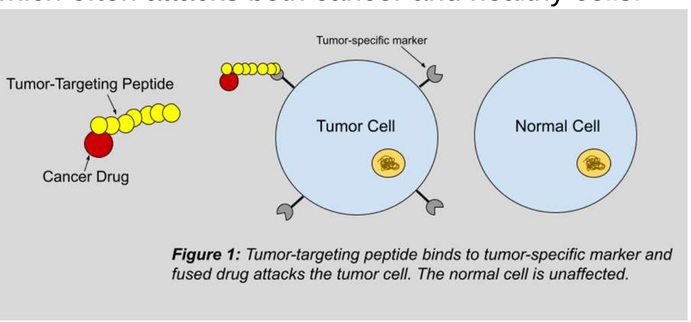
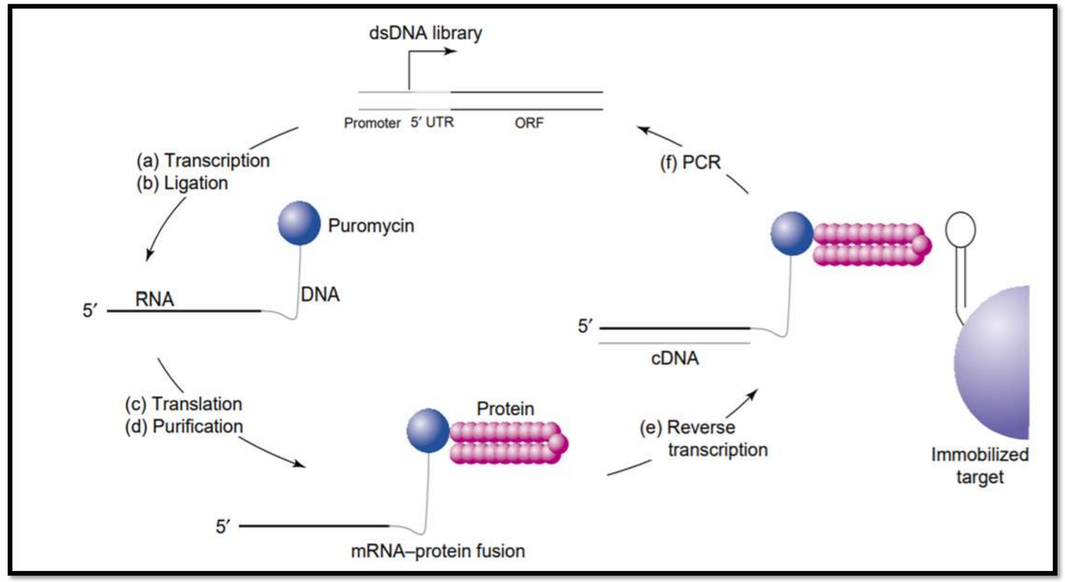


Figure 1: *Tumor-targeting peptide binds to tumor-specific marker and fused drug attacks the tumor cell. The normal cell is unaffected.*

Description of mRNA Display

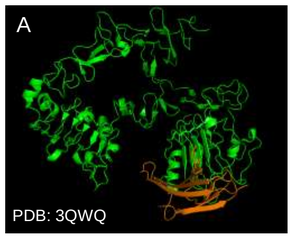
Figure 2**:** *mRNA Display Cycle (1)*



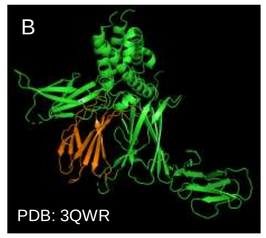
1. **PCR:** starting DNA library is amplified
2. **Transcription:** DNA library → mRNA library
3. **Ligation:** puromycin is ligated to mRNA (connects *"genotype to phenotype"*)
4. **Translation:** mRNA library → peptide library
5. **dT Purification:** mRNA-peptide fusions are purified with dT beads
6. **Reverse Transcription:** cDNA is generated from mRNA
7. **Selection:** peptides are exposed to an immobilized target on bead. High binding affinity peptides will stick on bead after several washes
8. **PCR and Re-selection**: cDNA of high binding peptides is amplified via PCR to create enriched DNA library for next cycle

Peptide Binders Interaction with Target Proteins

Figure 3: *X-Ray crystal structures of two target proteins bound by Adnectins selected via mRNA Display (2)*



Epidermal Growth Factor Receptor (EGFR) protein (green) bound with mRNA display generated Adnectin (orange)



Interleukin-23 (IL-23) protein (green) bound with mRNA display generated Adnectin (orange)

References

1. Takahashi, T. T., Austin, R. J., & Roberts, R. W. (2003). mRNA display: ligand discovery, interaction analysis and beyond. *Trends in biochemical sciences*, *28*(3), 159-165.
2. Ramamurthy, V., Krystek Jr, S. R., Bush, A., Wei, A., Emanuel, S. L., Gupta, R. D., ... & Sheriff, S. (2012). Structures of adnectin/protein complexes reveal an expanded binding footprint. *Structure*, *20*(2), 259-269.

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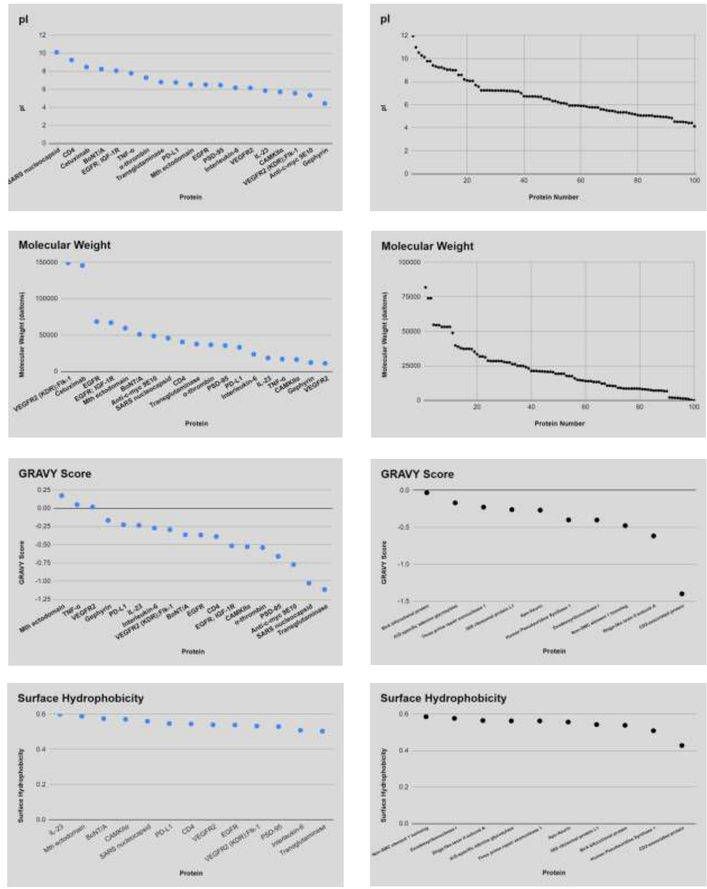
Data Analysis of Target Proteins

Figure 5: *Characterization of target proteins utilized in mRNA display experiments*



- From the graphs, we were not able to find unique characteristics for the targets
- Future work (ex. larger sample size of target proteins) is needed to identify prominent selection factors of mRNA display target proteins

My STEM Journey

Throughout this 7 weeks of SHINE, I have learned so much about mRNA display and have gained insight on how a college lab functions. I would love to continue working in this lab after SHINE and make more progress on my project. Furthermore, I am planning to major in biochemistry in college and am looking forward to performing undergraduate research in the near future!

Advice to Future SHINE Students

- Connect and get to know your mentor well! Your mentor will always be there to help you, so make sure to ask all the questions you need!
- Talk to others and make friends with people from different labs!
- Be observant and put time and effort into your research!