THE USE OF AI AND ADVANCED COMPUTER SYSTEMS TO DEVELOP DRUGS AGAINST NEW EMERGING THREATS

Tailoring Oligonucleotide Lengths: Optimizing The Binding Specificity And Affinity For Target Respiratory Viruses

Richelle Ntumy United States Military Academy **Stephen Hummel** United States Military Academy **Colette Muteteke** United States Military Academy **Brian Lunn** United States Military Academy **Bryant Nguyen** United States Military Academy **Tess Huchun-Walker** United States Military Academy **Jonah Landis** United States Military Academy

Oligonucleotide (aptamer) biosensors have served as useful tools for the detection and diagnosis of the presence of biological molecules, proteins, and pathogens. The length of oligonucleotides affects the efficiency of complementary binding between sequences, affinity, sensitivity concerning target detection, nuclease degradation, synthesis, and specificity. This project aims to employ an artificial intelligence (AI) algorithm in conjunction with a machine-learning model to analyze how various lengths of single-stranded oligonucleotide sequences affect the possibilities of high binding affinities and specificity. The designs will be centered around the five major respiratory viruses target/counter- target molecules: MERS-CoV, SARS-CoV-2, Hemagglutinin, Respiratory Syncytical Virus, and Rhino Virus, with the ACE2 protein serving as the control molecule.

The target molecules listed and oligonucleotides are processed through in vitro validation by attaching streptavidin magnetic beads to oligonucleotides, incubating them in concentration of molecule-flurophore complex, thoroughly mixed, and placed in a flow cytometer to determine percent positive population. This information will be used to calculate dissociation values (Kd values) which will aid in discovering the binding affinities.

Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technology has been used to select aptamers and optimize the ligand binding. Sequences will be designed to maximize length and structure. Longer oligonucleotides should have higher specificity. Within this experiment, there are varying lengths of oligonucleotides that were 20, 30, 40, 50, and 60 nucleotides long, which undergo in vitro validation to access target binding to the amino acid clusters of the target proteins. So far, our results have shown that oligonucleotides with longer lengths (more nucleotides) have resulted in higher Kd values and oligonucleotides with shorter lengths have lower Kd values. For example, the SARS-Spike Oligonucleotide with a length of 40 nucleotides had a Kd value of 18.93 nM, whereas the oligonucleotide with a length of 60 nucleotides had a Kd value of 50.12 nM. Our goal is to aim for lower Kd values for higher specificity. The results show that we may need to consider reducing oligonucleotides lengths for better specificity results.

Understanding and identifying the specificity and affinity of oligonucleotides based on oligonucleotide is essential to developing therapeutic agents and detection technology for viral infections and diseases. The production of such tools becomes more cost-effective, time-efficient, and accurate when the synthesis of oligonucleotides is based on optimal lengths for various target proteins derived from machine-learning models and algorithm templates used in our experiment. Overall, the information from this project will facilitate countering emerging viral threats through the proper identification of biological threats.

For future research, we hope to incorporate immunoassays to properly examine specificity of binding spike proteins and ligands, and even broaden our scope outside of respiratory viruses to test for how effective our machine-learning model can optimize oligonucleotide length for an increase in uniqueness and better target binding.

Project Funded by DTRA Chemical and Biological Defense (MIPR: 14463237)
Army Cyber Institute is supported by Army Development Command (DEVCOM). ACI provides the servers to house and run digitalSELEX.