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IMMERSIVE RESEARCH INTERNSHIP EXPERIENCE
MOLECULAR DIAGNOSTICS

Selecting DNA Aptamers Against Airway Mucin Proteins for Therapeutics and Diagnostics

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Intent & Motivation

Intent: To select and characterize **DNA aptamers against the mucin protein MUC5AC** for therapeutic and diagnostic applications.

Motivation: Selected aptamers could be used in therapeutics to deliver engineered **proteases to cleave mucin proteins** and in diagnostics and respiratory models to **tag mucin proteins**.

Background

- **Mucus** is a viscous, protective function of the body comprised of mucin proteins, inorganic salts, and water.
- **MUC5AC**, a mucin protein, creates a thickened mucus layer and provides shelter for viruses or bacteria.[1]
- Elevated MUC5AC levels are associated with **impaired mucus clearance** and indicate diseases such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease.[1,2,3]
- Current treatments that cleave mucin proteins involve **chemical treatments and enzymes** are often nonspecific, resulting in adverse side effects.[4]
- **Aptamers** are synthetic, short, single-stranded DNA or RNA molecules that fold into distinct 3D shapes, which allows them to bind with high affinity and specificity to target molecules.

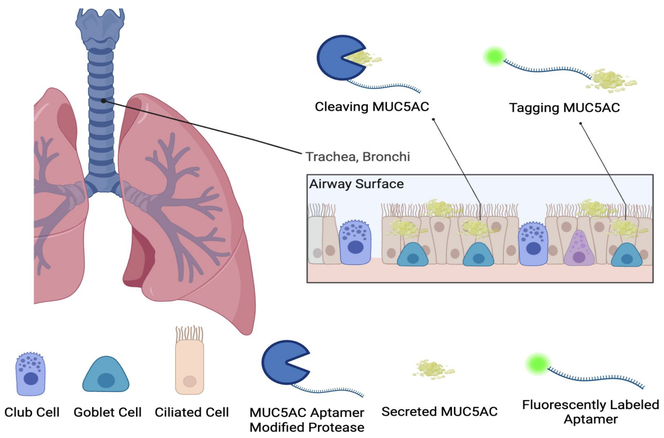


Figure 1. MUC5AC, secreted from goblet cells, makes up airway mucus. It forms hydrogels that are transported by cilia along airway surfaces. Aptamers could be used to fluorescently label MUC5AC or for targeted delivery of engineered proteases for efficient cleavage.[2]

Acknowledgments & References

QR code links to references. We would like to acknowledge for their guidance and support: our advisor, Dr. Catherine Spirito; the Whelan Lab at the University of Kansas; Dr. Jeffrey DeStefano; Dr. Quira Zeidan; the OUR Immersive Research Internship Experience (IRIE) program; and Grand Challenges collaborator faculty Dr. Louisa Wu, Dr. Gregg Duncan, and Dr. Phillip Bryan. Figures 1 to 6 were made with BioRender.

Methods

One Pot Selection of Aptamers Against MUC5AC

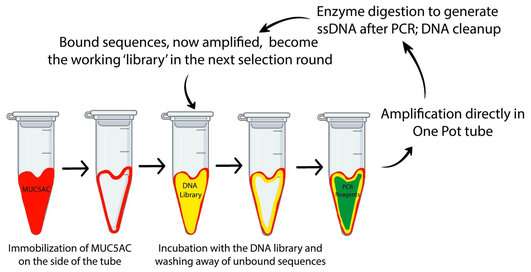


Figure 2. One Pot SELEX is a cyclical process where DNA sequences that bind to a target protein are 'evolved' from a starting library, a pool of 10^{15} randomized sequences. The in-tube process was adapted from from Scoville et. al.[5]

- Mucus enriched with **MUC5AC is immobilized on a PCR tube** using a **SpeedVac Concentrator**.
- Immobilized **protein is incubated** at 25 °C for 30 minutes with a guanine and cytosine enriched **DNA library** (63 nt long w/ 25 nt randomized region).[5]
- Unbound sequences are removed and **PCR** reagents are added. **Bound sequences are amplified** directly in the tube with a 5' phosphorylated reverse primer.
- **Lambda exonuclease digestion** generates single stranded DNA (ssDNA) for future rounds of selection and binding affinity assays.[6]

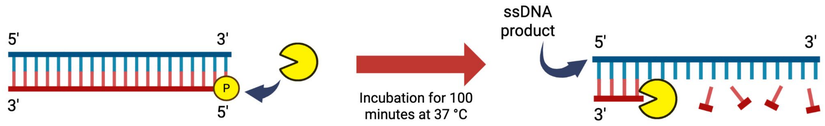


Figure 3. The lambda exonuclease enzyme is able to recognize and selectively degrade strands of dsDNA that were tagged with a 5' phosphate reverse primer during PCR.

Binding Assays to Assess Aptamer Candidates

- **Gel shift** and **nitrocellulose filter** assays can be used to qualitatively measure the binding affinity of selection products to a protein target.
- Initial testing with a known protein-aptamer pair, **thrombin and thrombin 15mer**.[7]
- A constant concentration of **5' Cy3 tagged aptamer** is incubated with **increasing concentrations of the protein**. Incubation occurs in a buffer containing 1X PBS, 3 mM Mg^{2+}, and 5 mM K^{+} at room temperature for 30 minutes.
- During a **gel shift assay**, after incubation samples are run on an unstained agarose gel at 150V for 30 minutes.
- During a **nitrocellulose filter assay**, after incubation samples are taken into a syringe and passed through a 0.45 µm nitrocellulose filter.

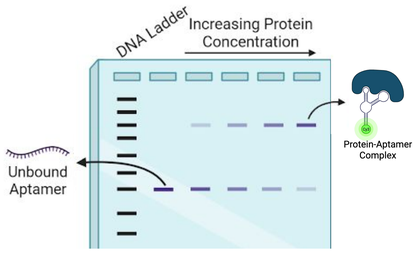


Figure 4. Gel shift assay. Binding reactions with increasing protein concentrations are run on a gel. Band intensity is used to determine qualitative binding affinity.

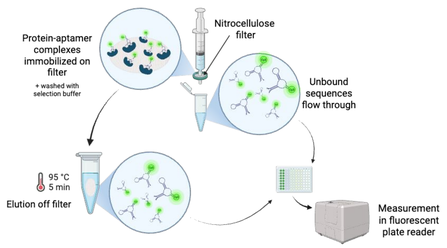


Figure 5. Nitrocellulose filter assay. Protein-aptamer complexes are caught on the nitrocellulose filter, while unbound DNA passes through. Washes remove weakly unbound DNA before quantification in a fluorescent plate reader.

Results & Discussion

One Pot Selection of Aptamers Against MUC5AC

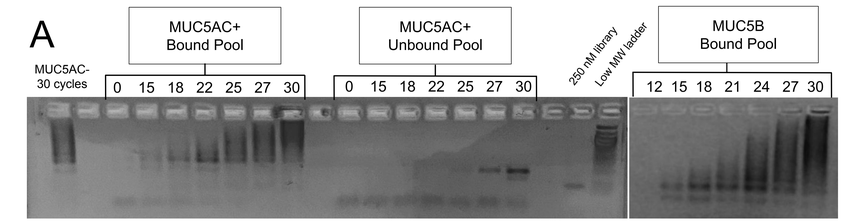


Figure 6. (**A**) Cycle course PCR of Round 7 selection products, 3% agarose gel stained with GelRed; 170V for 25 min. (**B**) 10% PAGE gel ran at 150V; Coomassie blue stain. MUC5B or MUC5AC enriched mucus sample, as indicated.

- Round 7 selection products displayed an affinity for the inside of One Pot tubes and MUC5B samples.

Binding Assays to Assess Aptamer Candidates

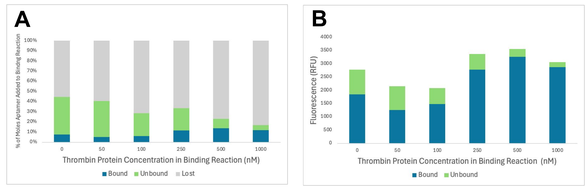


Figure 7. (**A**) Percent recovery of thrombin 15mer after nitrocellulose filter assay. Sample molarity was determined from interpolation with a standard curve and used to calculate moles. (**B**) Fluorescence of 5' Cy3 thrombin 15mer from nitrocellulose binding assay samples. Measured in a fluorescent plate reader (excitation 550 nm; emission 563 nm)

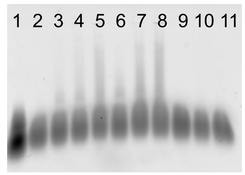


Figure 8. Gel binding assay with 400 nM R3 selection product and varying concentrations of MUC5AC, MUC5B, and human serum albumin. 1% agarose gel ran in 0.5X TAE, 150V for 20 min. Visualized with Amersham Typhoon laser scanner. **1.** 400 nM R3 sample. **2.** Protein negative. **3-5:** 2.5, 5, 7 µL MUC5AC sample. **6-8:** 2.5, 5, 7 µL MUC5B sample. **9-11:** 0.5, 1, 1.5 µM HSA.

- Mucin proteins remain in wells during electrophoresis, resulting in smearing.
- Round 3 selection products displayed no affinity for non-mucin proteins.
- Fluorescence of bound vs. unbound samples from binding reactions with increased concentration of protein qualitatively demonstrates binding affinity.

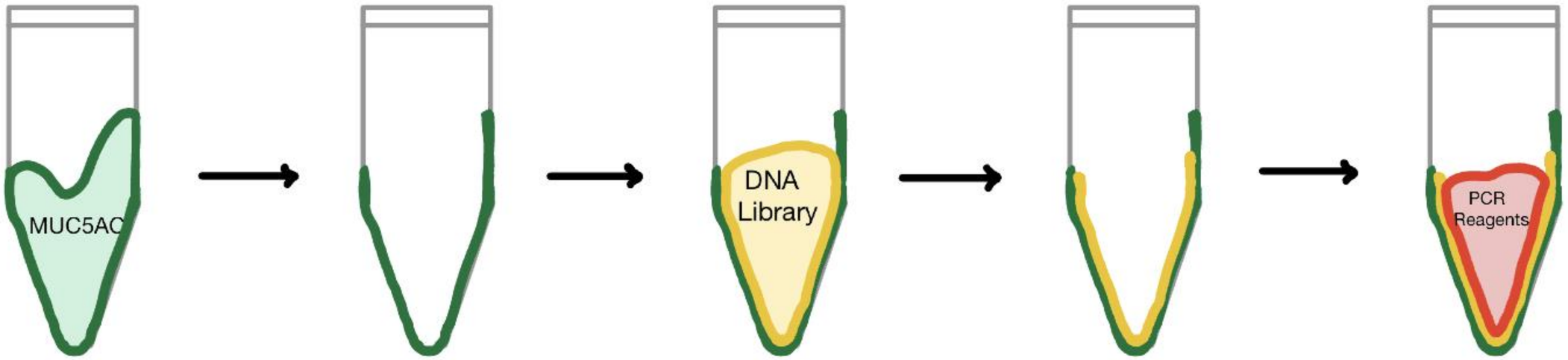
Future Work

- Verify the presence of MUC5AC in mucin culture samples used in One Pot.
- Continue One Pot aptamer selection with increased rounds of negative selection.
- Optimize the nitrocellulose filter assay to minimize the loss of aptamer sequences and nonspecific binding.
- Compare the binding affinity of MUC5AC and selection products against MUC5AC and the initial library using a nitrocellulose gel shift assay.
- Characterize potential aptamer candidates through sequencing once enrichment of binding sequences plateaus.

Background should include:

- Define mucin and MUC5AC
- Introduce aptamers more smoothly than in original poster
- Current methods of treatment with antibodies and why they can be problematic
- Diseases & disorders related to MUC5AC
- Possible applications of aptamers

Any new figures for the poster?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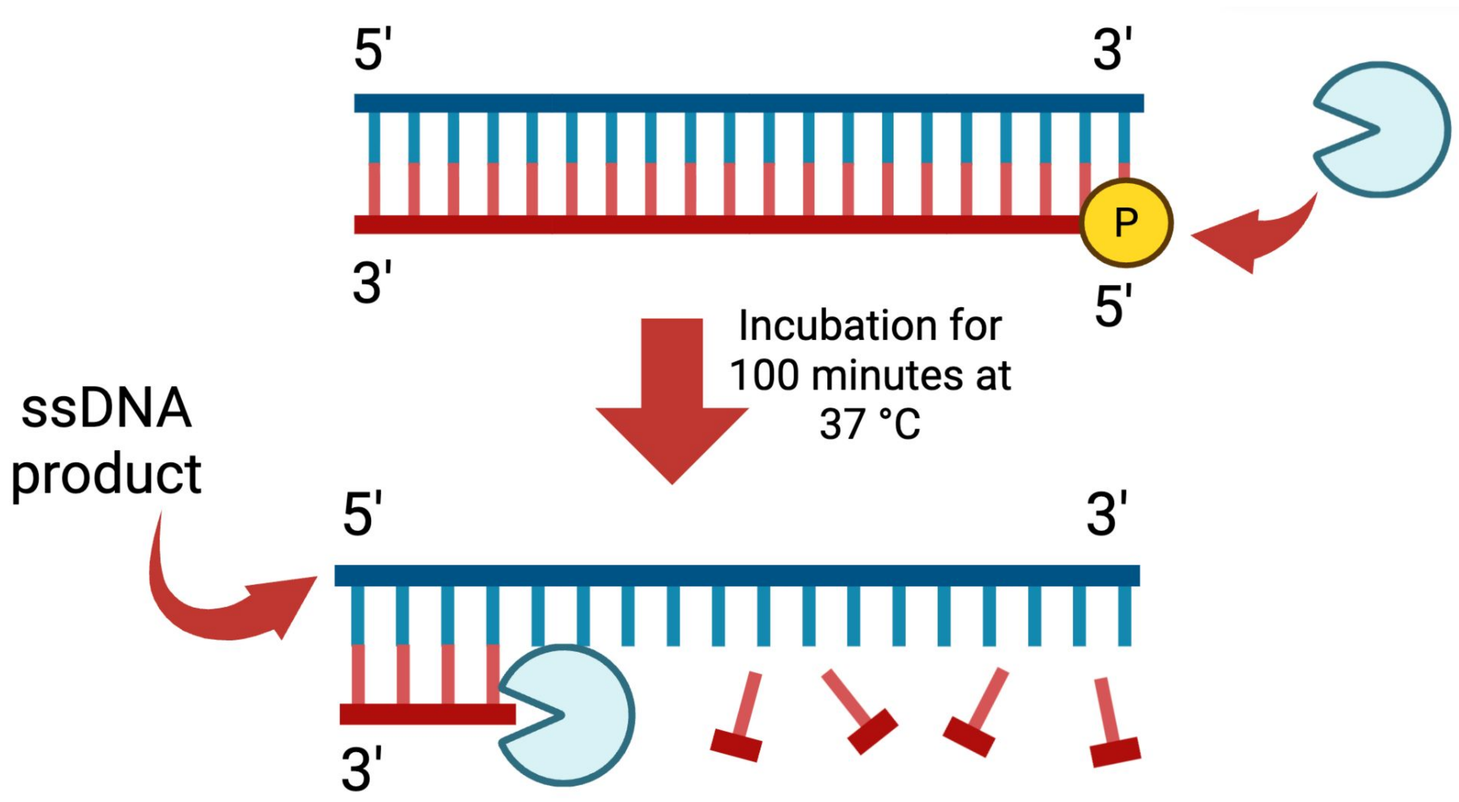
Reagents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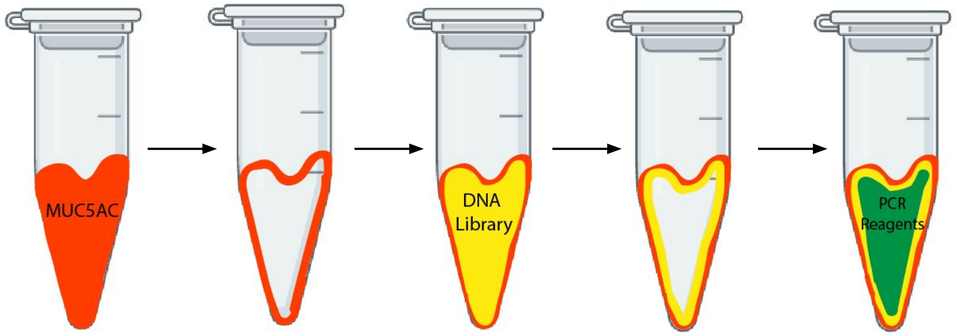
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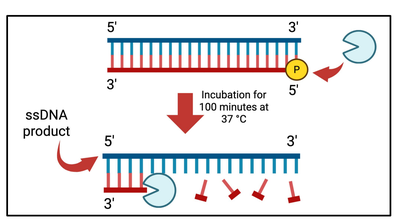
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Figure 2. In-tube selection process from Scoville et. al. against semi pure MUC5AC to generate aptamer candidates after multiple rounds.[6]

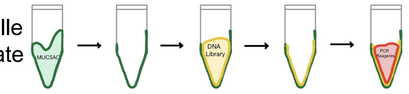


Figure 3. ssDNA is generated by lambda exonuclease digestion. Lambda exonuclease degrades dsDNA from 5' phosphorylated end.

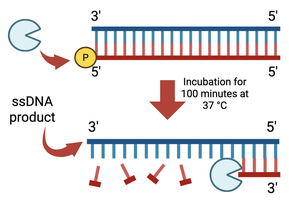
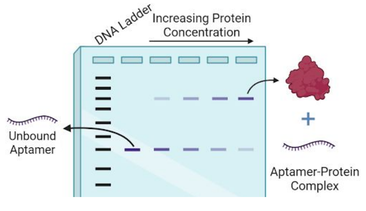


Figure 4. Gel shift assay. Selection product is run on a gel with increasing protein concentration. Band intensity shows binding strength.



In-Tube ELISA of MUC5AC

- Left tube: Positive samples containing MUC5AC protein immobilized via SpeedVac
- Right tube: Negative controls containing nuclease free water
- Blue color change indicates presence of MUC5AC protein

Figure 5. ELISA test with washes and a blocking agent to prevent nonspecific binding showed expected results

Isolating & Assessing ssDNA

- Gel of exonuclease digestion shows successful separation
- Qubit readings show consistent retention of DNA during selection and digestion

Table 1: Qubit fluorometry dsDNA & ssDNA concentrations of DNA aptamer pool during each round of aptamer selection.

Round	dsDNA Concentration	ssDNA Concentration
1	60.8 ng/μL	78.0 ng/μL
2	34.6 ng/μL	28.2 ng/μL
3	13.7 ng/μL	15 ng/μL
4	24.6 ng/μL	34.4 ng/μL
5	30.8 ng/μL	44.0 ng/μL

Lambda Exonuclease Digestion

- Amplified DNA library visualized on a GelRed pre-stained 4% agarose gel run at 90V for 45 minutes
- Incubation condition A produced the best results of ssDNA generation, outperforming conditions B & C

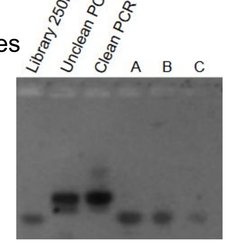


Figure 6. A) 60 minutes incubation, 0.21 U/μL enzyme. **B**) 2 hour incubation, 0.14 U/μL enzyme. **C**) 2 hour incubation, 0.21 U/μL enzyme.