FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

DNA Aptamers against Parkinson's Disease Biomarker Alpha-Synuclein

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BACKGROUND

Parkinson's Disease (PD):

➔ A progressive, neurodegenerative disorder causing uncontrollable motor movements.[1]

Alpha Synuclein and Aptamer Selection:

➔ There is a higher concentration of oligomeric alpha synuclein, a protein, in the saliva of patients with PD than in healthy patients.[2]

➔ Targeting this protein with aptamers enables efficient point-of-care diagnostics for PD

➔ Aptamers are short DNA or RNA strands that fold to bind specific molecules, like proteins, via non-covalent interactions.

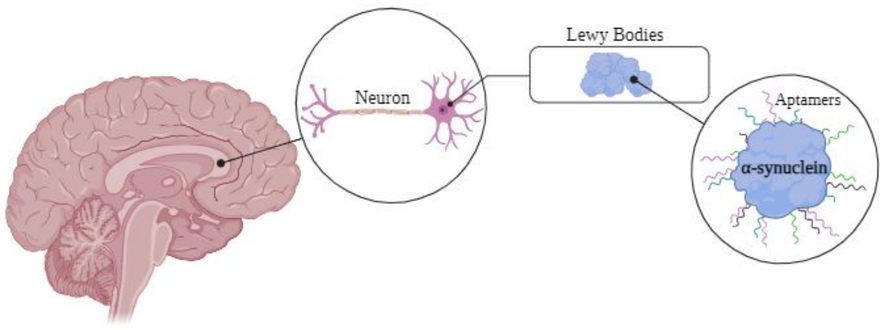


Figure 1:
The location and aptamer attachment of the alpha synuclein protein in the brain. In Parkinson's disease, this protein abnormally aggregates within neurons, forming Lewy bodies, which contribute to neurodegeneration and disease progression.[3]

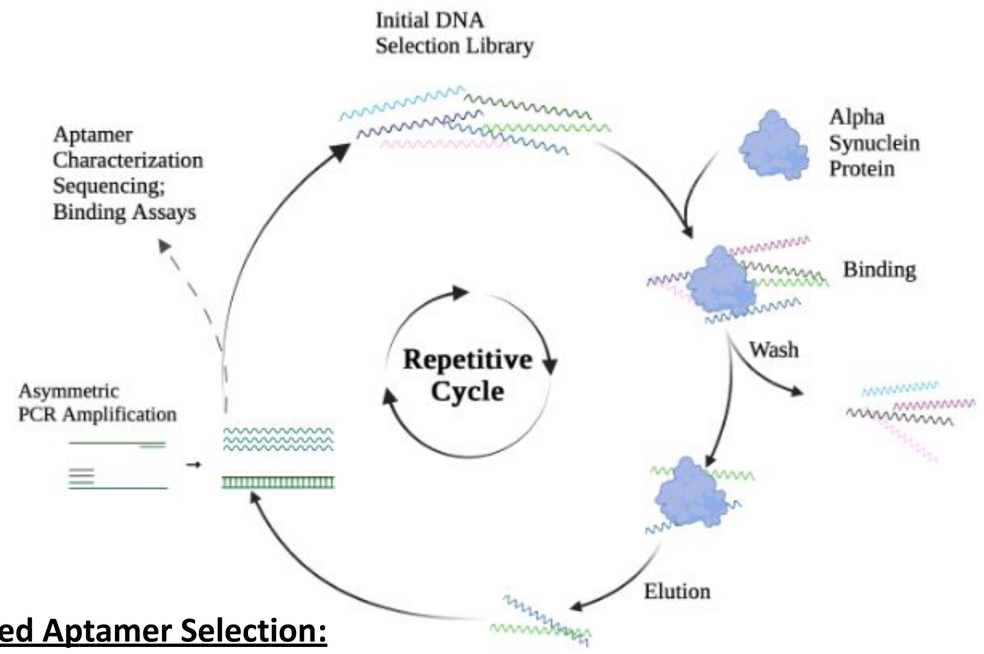
INTENT & MOTIVATION

Objective: Select an aptamer that can be used to develop a quick, non-invasive, and reliable early PD detection tool.

Significance: Current detection for Parkinson's disease involves monitoring symptoms and imaging scans, both of which are only reliable after substantial degeneration has occurred.

METHODS

Figure 2:
Histidine-tagged Alpha-synuclein protein was immobilized on magnetic beads and used in multiple rounds of SELEX aptamer selection to select a DNA aptamer.



In-vitro Bead-Based Aptamer Selection:

1. Bound the DNA sequences to alpha synuclein in PBS buffer with 5 mM KCl and 3 mM $MgCl^{2+}$ at 25ºC for 30 minutes
2. Washed non-specific/unbound sequences using PBT buffer
3. Eluted bound sequences using Zymo RNA Clean Up Kit binding buffer with guanidinium
4. Amplified high-affinity sequences and analyzed binding with gel shift assays

➔ Selection Pool: Bowser 2013 DNA Library, 65 nucleotides (nt) long, 25 nt random region[4]

➔ Repeated steps and added additional washes in subsequent rounds to enrich for aptamers with high specificity toward Alpha-synuclein

➔ Conducted negative selection against the magnetic beads and tube to control for binding aptamer to equipment

RESULTS

Cycle Course PCR:

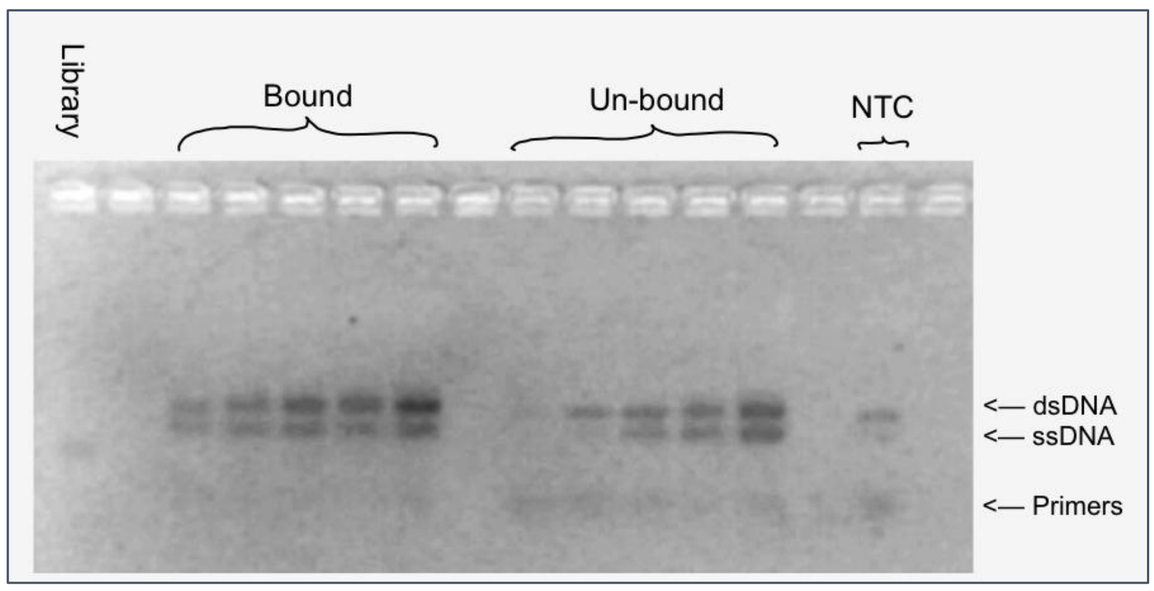


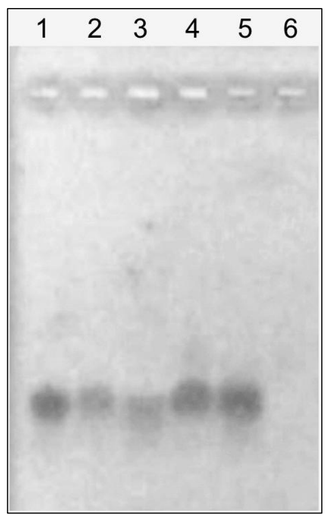
Figure 3 (left):
Cycle course PCR with symmetric PCR products run on 3% agarose gel stained with GelRed for round 4 of selection. 10, 14, 18, 22 and 25 cycles were run for the bound and unbound pool PCR products respectively with an ideal cycle number without over - amplification at 25 cycles.

Optimal Number of Cycles and DNA Concentrations:

Table 1 (below):
Optimal number of cycles without over-amplification as identified by cycle course PCR for each round of selection.

	Optimal Cycles	DNA Concentration	Protein Concentration
Round 1 Positive Selection	23 Cycles	5 µM	0.5 µM
Round 2 Positive Selection	26 Cycles	0.078 µM	0.5 µM
Round 3 Negative Selection & Positive Selection	25 Cycles	0.001 µM	0.5 µM

Modified Gel Shift Assay:



Key:
1. Library
2. 0 uM Protein & Library
3. 5.5 uM Protein & Library
4. 0 uM Protein & Aptamer
5. 5.5 uM Protein & Aptamer
6. No DNA (control)

Figure 4 (left):
Modified Gel Shift Assay performed on a 1.5% agarose gel (unstained) for Round 4 of aptamer selection. Reaction mixtures included varying volumes of selection buffer, DNA library, and aptamer, denatured at 95°C for 3 minutes, cooled to room temperature for 10 minutes, and subsequently incubated with protein for 1 hour. Loading dye (6×) was added before running the gel.

FUTURE WORK

Short Terms Goals

➔ Troubleshoot DNA concentration of selection pool from Round 3 to improve yield

➔ Conduct further rounds of selection to enrich pool

➔ Illumina sequencing of selected aptamers

➔ Binding affinity assays to quantify stringency

Long Term Goals

➔ Develop a rapid diagnostic tool using selected aptamers to detect elevated levels of Alpha-synuclein in saliva

REFERENCES & ACKNOWLEDGEMENTS

1. Parkinson's Disease: Causes, Symptoms, and Treatments. *NIH* https://www.nia.nih.gov/health/parkinsons-disease/parkinsons-disease-causes-symptoms-and-treatments#:~:text=Parkinson's%20disease%20is%20a%20brain,have%20difficulty%20walking%20and%20talking.
2. Vivacqua, G. et al. Salivary alpha-synuclein in the diagnosis of Parkinson's disease and Progressive Supranuclear Palsy. Parkinsonism & Related Disorders 63, 143–148 (2019).
3. Lücking, C. B. & Brice*, A. Alpha-synuclein and Parkinson's disease: CMLS, Cell. Mol. Life Sci. 57, 1894–1908 (2000).
4. Yang, J. & Bowser, M. T. Capillary Electrophoresis–SELEX Selection of Catalytic DNA Aptamers for a Small-Molecule Porphyrin Target. Anal. Chem. 85, 1525–1530 (2013).

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