FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

DNA Aptamer Against Alzheimer's β-Amyloid 42 Protein

Keon Abasi, Siya Kothale, Joey Munyaneza, Mariana Alcoleas

BACKGROUND

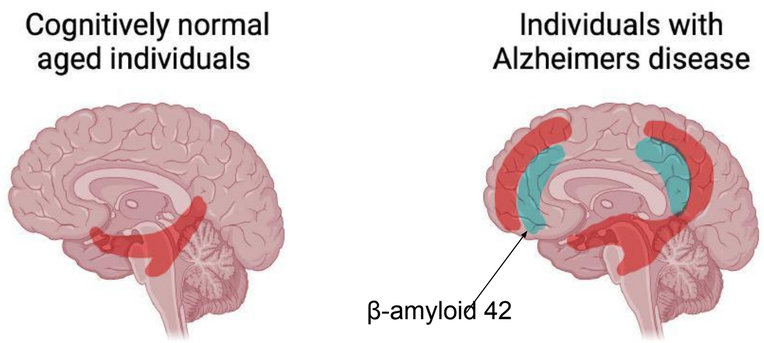

Figure 1: Increased plaque buildup in Alzheimer's patients (right) vs healthy individuals (left)

- Alzheimer's disease is a chronic neurodegenerative disease with a predicted 7% rise from the 6.7 million cases of ages 65+ by 2025.[1]
- Alzheimer's disease currently has no cure and the most common form of conclusive diagnosis is the spinal tap.[2]
- Studies have linked the presence of clusters of amyloid deposits made up of the protein β-amyloid 42 to the presence of Alzheimer's disease.[3]

INTENT & MOTIVATION

Intent: To select a DNA aptamer that binds to the β-Amyloid 42 protein for future diagnostic applications.

Motivation: An aptamer based diagnostic test would allow for early treatment of Alzheimer's disease.

METHODS

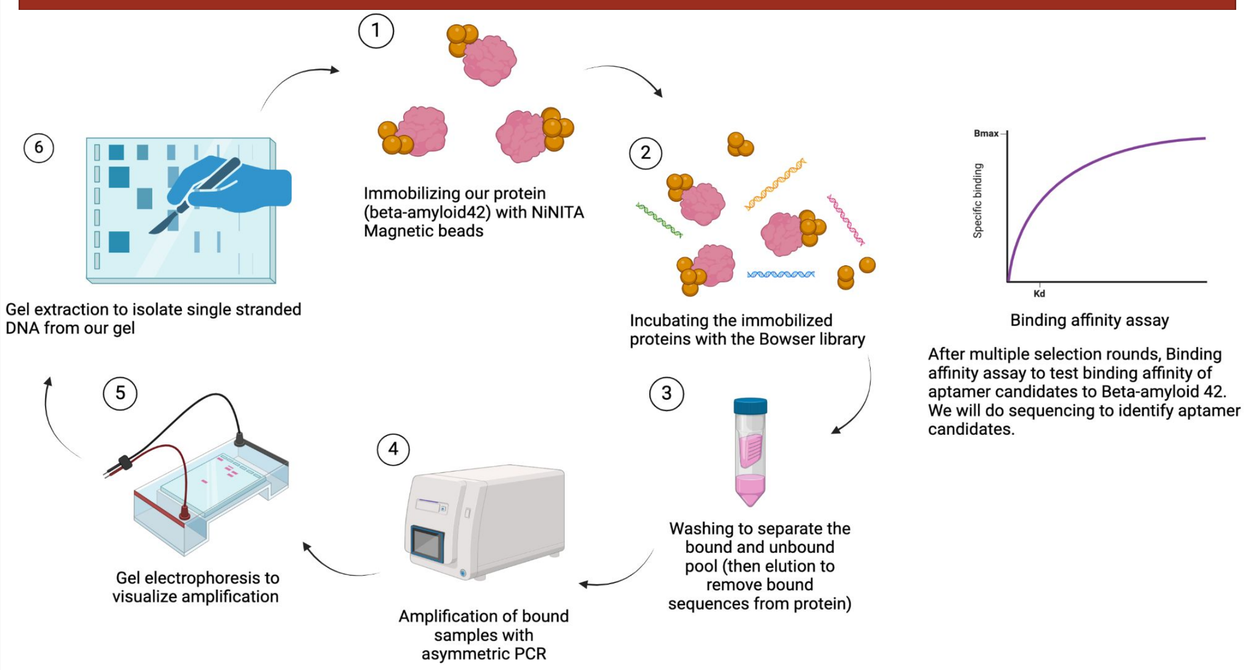

Figure 2: Steps of bead-based in vitro aptamer selection against β-amyloid 42

- Conducted positive aptamer selection process against β-amyloid 42 protein immobilized on Nit-NTA magnetic beads
- Conducted negative aptamer selection process against tube without protein to ensure binding specificity to solely the protein
- Used Qubit to quantify DNA concentrations within selection rounds
- Planned sequencing assays for aptamer candidates
- Library used (Yang and Bowser 2013):
  5'-AGCAGCACAGAGGTCAGATG (25N)
  CCTATGCGTGCTACCGTGAA-3'

RESULTS

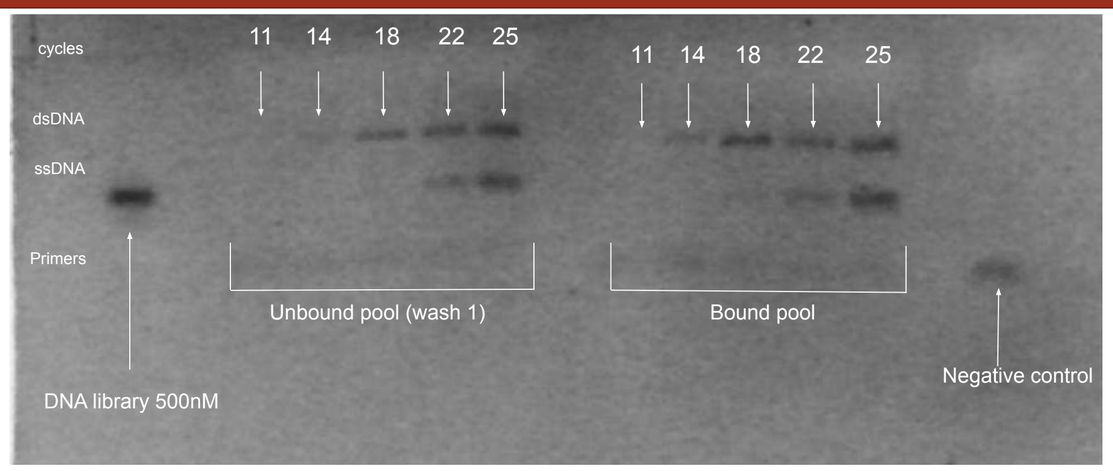

Figure 3: Cycle course asymmetric PCR was performed on the unbound pool (wash 1) and the bound (selected) pool and run on a 4% gel stained with GelRed to determine the optimal number of cycles to amplify the selected pool. 24 was optimal (not pictured)

Round	DNA Concentration (μM)	Protein Concentration (μM)	Rounds CC PCR
R1 (+)	0.458	0.5	23
R2 (+/-)	2.44	0.5	24
R3 (+)	0.0964	0.5	22

Table 1: Qubit readings from three rounds of selection. Low values of DNA concentration in R3 due to inefficient gel extraction.

Cycle Course Polymerase Chain Reaction (PCR):

- Asymmetric Cycle Course PCR and gel indicated that 24 cycles led to best amplification results of double and single stranded DNA at round 2
- As the rounds of selection progressed, a smaller than original optimal round of PCR shows an increase in the binding affinity of aptamers to the target, β-Amyloid 42
- 24 cycles was selected as optimal due to 22 cycles being under amplified and 25 cycles being over amplified

Qubit:

- DNA concentration increased from round 1 (0.458 μM) to round 2 (2.44 μM), possibly showing effective enrichment of bound sequences. However, in cycle 3, DNA concentration reduced to 0.0964 μM, most likely due to inadequate gel extraction and older clean up kits in lab

FUTURE WORK

Continue to carry out more rounds of aptamer selection against the target protein to select a sequence with a higher binding affinity.

- Conduct a binding affinity assay for the first three rounds of selection to confirm an increase in binding affinity throughout the rounds of selection
- Refine the gel extraction protocol to improve DNA recovery and increase overall yields
- Increase the stringency of selection by increasing number of washes during aptamer selection
- Continue to change our protocols to adapt to our project as we proceed in the lab

REFERENCES & ACKNOWLEDGEMENTS

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