

**Selection of DNA aptamers to anti-VSV antibodies:
a solution to extend oncolytic virus half life in
blood via reversible protection for controllable
treatment**

By

Siu-Yan Lee

Honours Thesis submitted to the Faculty of Science, Department of Chemistry
In partial fulfilment of the requirements of the B. Sc. Degree

Supervisor: Dr. Maxim Berezovski

Department of Chemistry
University of Ottawa
Ottawa, Canada

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Abstract

Aptamer-Facilitated Virus Protection (AptaVIP), a project aimed at increasing the circulation time of an oncolytic virus therapeutic, vesicular stomatitis virus (VSV), using aptamer technology, is a collaboration between Jennerex Biotherapeutics, the Bell Lab, and the Berezovski Lab. A method was developed for the selection of aptamers via SELEX against anti-VSV neutralizing antibodies (a-VSV) using protein-G magnetic beads and negative selection was performed using rabbit serum. A total of 6 pools were completed and amplified; binding affinity was analyzed via fluorescence of Alexa488 labelled primers. No significant binding affinity was observed for aptamers to a-VSV. More pools of aptamers need to be developed until significant binding affinity is observed against a-VSV before further steps can be taken such as competitive binding assays and modification of aptamers.

Acknowledgements

Thanks to Darija Muharemagic and Dr. Berezovski for their help and guidance.

Table of Contents

| | |
|------------------------------------|-----|
| Abstract | ii |
| Acknowledgements | iii |
| List of Figures | v |
| Statement of Contribution | vi |
| | |
| Introduction | 1 |
| Materials and Methods | 8 |
| <i>Aptamer Selection via SELEX</i> | 8 |
| <i>DNA Amplification</i> | 11 |
| <i>Fluorescence Analysis</i> | 12 |
| Results and Discussion | 13 |
| <i>Method Development</i> | 13 |
| <i>Fluorescence Analysis</i> | 19 |
| Works Cited | 26 |
| Appendix | 28 |
| <i>Acronyms used</i> | 28 |

List of Figures

- Figure 1: Varying methods of virus neutralization by antibodies in vertebrates.** **Left:** Virus in the absence of neutralizing antibodies is successful in infecting a host cell. **Right:** Viral neutralization prevents host infection and causes virus particle degradation and removal from circulation through multiple pathways. (Source: <http://www.virology.ws/2009/07/24/virus-neutralization-by-antibodies>) **3**
- Figure 2: Overview of aptamer development for anti-VSV neutralizing antibodies.** Left: SELEX enrichment of a naïve DNA pool against a-VSV. Right: Possible future steps for the analysis and development of a proficient oncolytic virus therapy. **7**
- Figure 3: Rounds of aptamer selection against non-target antibodies and anti-VSV using magnetic beads.** **10**
- Figure 4: Gel Electrophoresis analysis of naive and enriched DNA library at via fluorescence.** Fluorochromes were excited at 485nm and emission was measured at 535nm. DNA Lib* show the amplification of the DNA library used for fluorescence analysis. The DNA Lib is purified and 100nM in concentration. **15**
- Figure 5: Gel Electrophoresis for R5.3 purification via 30KDa cut off filters.** R5.3(pur) is concentrated after purification giving a brighter band. **17**
- Figure 6: Relative fluorescence showing affinity of naive and enriched libraries to a-VSV, Serum, and magnetic beads.** Relative Fluorescence is calculated using equation (a). No standard deviations are shown because only single readings were taken for each sample prepared. **20**
- Figure 7: Relative Fluorescence polarization demonstrating the affinity of naive and enriched libraries to a-VSV, serum, and magnetic beads.** Relative fluorescence polarization is calculated using equation (a). No standard deviations are shown because only single readings were taken for each sample prepared. **22**
- Figure 8: Fluorescence of unwashed serum-beads incubation reaction.** The PBS sample shows the fluorescence emitted from just magnetic beads. No standard deviations are shown because only single readings were taken for each sample prepared. **24**

Statement of Contribution

Darija Muharemagic helped by optimizing the fluorescence plate reader protocol (exposure times etc.) and helping with preliminary analysis of pools on capillary electrophoresis (no significant results, not included). All of the work included is of my own.

Introduction

An aptamer based technology to enhance the efficiency of an anticancer treatment by increasing the *in vivo* survival of an oncolytic virus is currently being developed; aptly named *Aptamer-Facilitated Virus Protection* (AptaVIP), this project is a collaboration between Jennerex Biotherapeutics, the Bell Lab at the OHRI, and the Berezovski Lab at University of Ottawa.

Oncolytic viruses are a promising treatment against tumours for cancer patients aiming to improve outcomes through their tumour selective mode of action and multimodality attack against cancers. Although this virus based technology has shown efficacy in animal tumour models, it has seen less success so far in clinical settings. The safety profile of oncolytic viruses already shown in human trials are much better than the typical forms of cancer treatment; being much less toxic than chemotherapy or radiation therapy (1). The large variation in the population as well as the genetic variations between cancers and patients makes it difficult to provide a new effective clinical treatment. In recent times, some viral platforms are approaching the status of an approved therapeutic in North America. Under this scope, our project is working with the vesicular stomatitis virus (VSV) and its use as an oncolytic virus.

VSV is a small enveloped rhabdovirus containing a genome consisting of a single strand of negative sense RNA. It is able to replicate and spread between infected cells very quickly. With a broad cancer cell tropism, it is shown to be effective when administered intravenously in several murine models (2). The genome of VSV encodes of 5 major proteins, more importantly, the matrix protein. A mutation in the M-protein is found to increase oncolytic properties; VSV has been shown to reduce the tumour size as well as reduce the spread in melanoma, lung cancer, colon cancer, and certain brain tumours in laboratory models of cancer (2).

Oncolytic viruses are an effective delivery vector for the transfer of foreign genetic material into other cells for cancer therapy. These viruses can carry large amounts of foreign DNA, infect cells, and can be easily produced in large amounts making viruses an attractive route of development for cancer therapy. Oncolytic viruses are capable of targeting metastatic cancer cells via intravenous injection (3). Intravenous injection requires a therapeutic in which the virus can gain access to disseminated tumour cells and have a long enough half life in circulation. One major issue with the development of an intravenous oncolytic viral therapy is the short half life in blood. Due to the viral nature of the therapy, the innate immune system in vertebrates will produce neutralizing antibodies which inactivate the oncolytic virus and clear the body of it quickly.

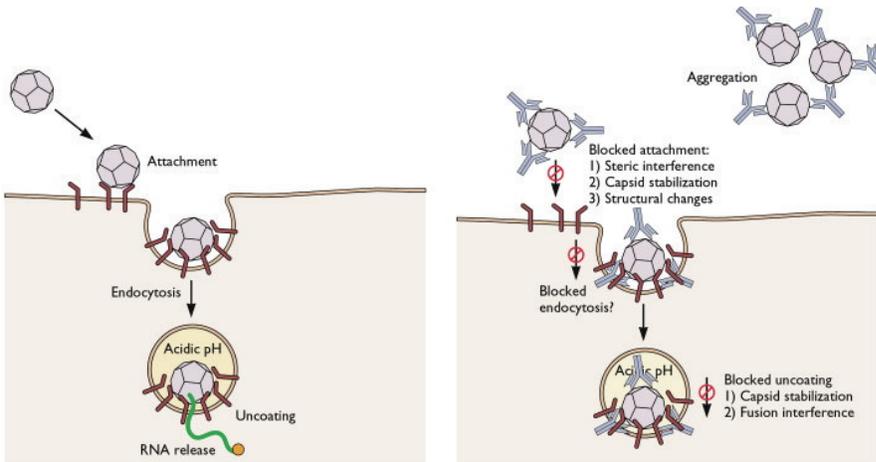


Figure 1: Varying methods of virus neutralization by antibodies in vertebrates. Left: Virus in the absence of neutralizing antibodies is successful in infecting a host cell. **Right:** Viral neutralization prevents host infection and causes virus particle degradation and removal from circulation through multiple pathways. (Source: <http://www.virology.ws/2009/07/24/virus-neutralization-by-antibodies>)

In vertebrates, a virus infection will elicit an immune reaction that will produce antibodies against virus surface proteins. Neutralization is the process of which antibodies block virus infection by interfering with the virus binding to receptors, blocking uptake into the cell, preventing uncoating of the viral genome in endosomes, or by causing the aggregation of virus particles. In addition to all these processes (depicted in Fig. 1 above, right), many enveloped viruses are lysed when the antiviral antibodies and immuno-proteins in serum bind, disrupting membranes. All these factors contribute to the inhibition of oncolytic viral infection thus decreasing the efficacy of this developing cancer therapy. The durability of the oncolytic virus in blood needs to be elongated, but not affect the uptake of the oncolytic virus by tumour cells. Modifications to extend the circulation times in blood include conjugation to polymers such as N-(2-hydroxypropyl) methacrylamide (HPMA) (4) or poly (ethylene glycol) (PEG) (5). Conjugation of this therapeutic to polymers increase life time in blood, but also decrease virus infection of the tumour cells due to blockage of essential virus-protein interactions needed for viral uncoating.

Aptamers are a growing new field of technology that could extend the half time of oncolytic viruses in circulation without affecting the potency of the oncolytic virus. Using aptamer technology, a shielding methodology can be developed against anti-VSV antibodies (a-VSV) by either surface masking of the virus using aptamers or causing neutralization of the a-VSV via binding of aptamers to elongate the virus survival time in whole blood. Both of these two projects are being explored in the Berezovski lab currently; however, herein discussion will continue on the neutralization of the anti-VSV antibodies using ssDNA aptamers.

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Comment [1]: Darija:
give full names

add a sentence or two describing the mechanism of action or how they work.

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Comment [2]: daraija : rather say "or coating a-VSV neutralizing by ..."

Aptamers are single stranded DNA or RNA sequences that are usually 15-40 nucleotides long; they can bind with both organic and inorganic molecules with selectable affinity. Easily and inexpensively synthesized, aptamers are an attractive alternative as 'synthetic DNA antibodies' due to their binding and inhibitory properties (6). As a new developing technology, aptamers have found applications in multiple fields, including diagnostics, biotechnology, imaging, and therapeutics. Currently, only one aptamer-based pharmaceutical is available on the market, Macugen; it is a pegylated anti-VEGF (vascular endothelial growth factor) aptamer for the treatment of neovascular age-related macular degeneration. It is administered once every six weeks in an intravitreal injection that helps prevent angiogenesis and leakage from blood vessels in the eyes that are responsible for this type of vision loss (7).

Systemic Evolution of Ligands by Exponential Enrichment (SELEX), the classical method for aptamer selection, involves three critical steps: 1, Pool generation, development of a combinatorial library containing random nucleotide sequences that are flanked by conserved primer binding regions; 2, Selection, separation of binding sequences from non-binding sequences to target molecules; and 3, Amplification, polymerase chain reaction (PCR) amplification of the binding sequences (8). One repetition of these steps comprises a single cycle of SELEX. Typically, the starting naïve DNA library becomes an enriched library after as few as 5 or as many as 40 rounds of SELEX. The enriched library will contain nucleic sequences with higher affinity and specificity to the target molecule. Once sufficient pools have been obtained and their binding affinity analyzed, further analysis may include cloning, competitive binding analysis, and sequencing of the most successful aptamers. Post selection modification of the

aptamers is an important step in the elongation residency time of the oligonucleotide in the circulatory system.

Partly based on the Aptamer-Facilitated Biomarker Discovery (AptaBiD) first developed by Berezovski et al (9), where negative selection is performed before positive selection (Fig. 2) due to the efficiency shown. Working with the 80 nucleotide DNA library, SELEX is used to achieve a pool containing nucleotide sequences with higher affinity to a-VSV than non-target proteins in serum. Aptamers are to be selected for a-VSV with the intention of halting the neutralizing ability of the antibody to elongate the life time of a VSV based oncolytic therapy in general circulation. The scope of this term project intends to detail the development of an efficacious methodology for the selection of aptamers for a-VSV using magnetic beads and the results achieved thus far using the developing method.

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Comment [3]: I think that you have to say a-VSV antibody, not just a-VSV.

You could also insert a paragraph explaining different method of selection (capillary electrophoresis, magnetic beads, cells, ...) and explain your selection.. magnetic beads, protein G-a-VSV coupling...

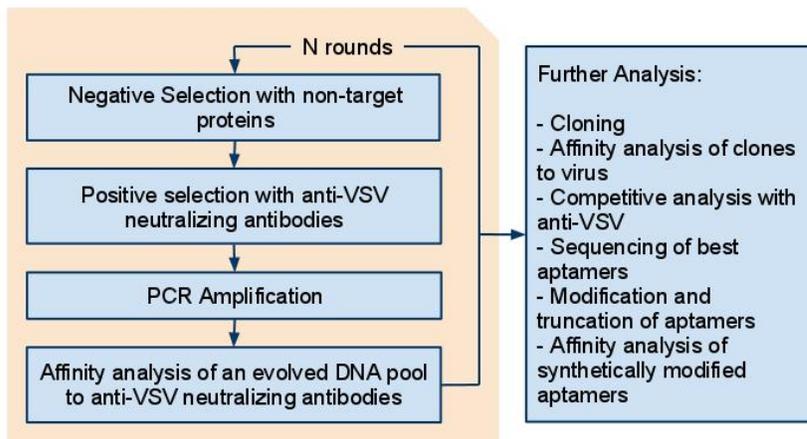


Figure 2: Overview of aptamer development for anti-VSV neutralizing antibodies. Left: SELEX enrichment of a naïve DNA pool against a-VSV. Right: Possible future steps for the analysis and development of a proficient oncolytic virus therapy.

Materials and Methods

Aptamer Selection via SELEX

Prior to each selection and binding experiment, the naïve or enriched library and the aptamer pools were denatured by heating them at 95°C for 5 minutes in PBS+Mg buffer (1x PBS with 5mM MgCl) and renatured on ice for 10 minutes. Protein G Magnetic beads (PureProteome, Millipore) were incubated with rabbit serum or a-VSV at 37.1ug protein per 1 million beads (10.6uL of bead suspension) for 15 minutes at 37°C in 100uL of PBS+Mg buffer. The rabbit serum and a-VSV were both provided from the Bell Lab and stored at 4°C prior to use. The incubations were all done in a thermomixer (Eppendorf) set at 37°C and 550rpm. The beads were then washed once (100uL PBS+Mg buffer) and resuspended with 100uL PBS+Mg buffer. Negative selection was performed by incubating the rabbit serum with the magnetic beads prior to incubation with the naïve or enriched ssDNA library at an incubation concentration of 250nM ssDNA for 1 hour at 37°C with shaking (incubation volume of 100uL made up with PBS+Mg buffer). For the positive selection, the supernatant from negative selection was incubated with a-VSV bound to protein G magnetic beads for 1 hour at 37°C with shaking. Masking DNA, Salmon Sperm DNA, was used for more stringent selection conditions in rounds 2 (0.1ug), 3 (0.2ug), and 4.2 (1.0ug). After washing the beads three times with 100uL PBS+Mg buffer, two methods of elution were used, using NaOH and with heat. A soft elution was conducted by adding 20uL NaOH (0.1M) to the magnetic beads, mixing and separating the supernatant; it was then neutralized by adding 2uL HCl (1.0M) to give a final elution volume of 22uL. The alternative heat elution was done by suspending the beads in 30uL PBS+Mg buffer and heating to 85°C for 10 minutes and then immediately separating the supernatant from the magnetic beads to give a final elution volume of 30uL. The eluted solutions were then used as

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Comment [4]: Is this a discussion sentence???

I would put it in discussion But you did use masking for a few rounds, so you could say "in round x and y, z ug/ul of masking dna was used."

template in tandem PCR amplification for further rounds of selection. Rounds of selection and amplification were repeated for a total of 6 times to give 6 ssDNA pools that were then analyzed for affinity to a-VSV, rabbit serum, and the magnetic beads (Fig. 3).

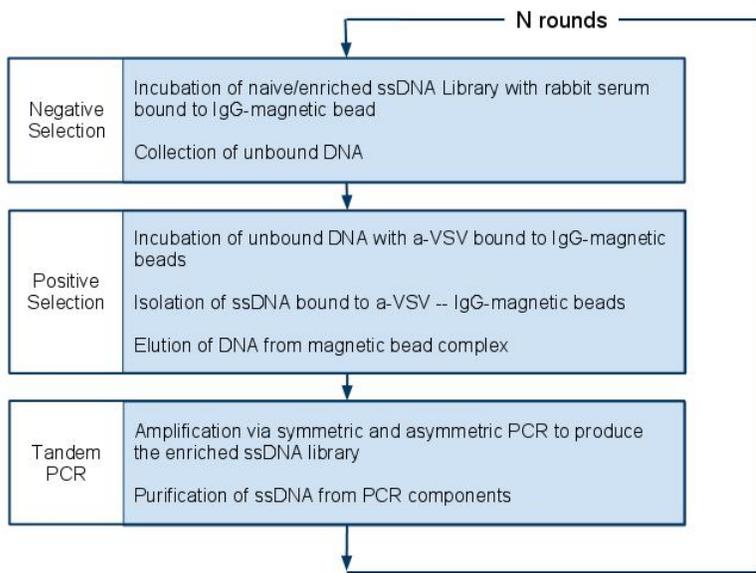


Figure 3: Flowchart detailing rounds of aptamer selection against non-target antibodies and anti-VSV using magnetic beads.

DNA Amplification

The naïve ssDNA library(Integrated DNA Technologies, Inc.) contained a central randomized sequence of 40 nucleotides that was flanked by 20 nucleotide long primer hybridization sequences (5'- CTC CTC TGA CTG TAA CCA CG-(N40)-GC ATA GGT AGT CCA GAA GCC -3'). The forward primer was labelled with Alexa-532 (5'-Alexa532- CTC CTC TGA CTG TAA CCA CG -3') whereas the reverse primer used was unlabeled (5'- GGC TTC TGG ACT ACC TAT GC -3'). The labelled forward primer and unlabelled primer were used for both symmetric and asymmetric steps of tandem PCR. An equal amount of primer was used for symmetric PCR while asymmetric PCR used 20 times more forward primer to give the naïve or enriched ssDNA library. All of the DNA pools were amplified using the GoTaq® PCR kit (Promega). The symmetric master mix consisted of 1x Green flexi buffer, 2.5mM MgCl₂, 0.2mM dNTP mixture, 0.3uM forward primer, 0.3uM reverse primer, and 0.025uM hot start Taq polymerase. Similarly, the asymmetric master mix contained the same concentrations of buffer, MgCl₂, dNTPs, and Taq polymerase but instead contained 1uM forward primer and 0.05uM reverse primer. Nuclease free water (Integrated DNA Technologies, Inc.) was used for the master mix as well as a negative control for PCR. DNA eluted from each round of aptamer selection was amplified via tandem PCR which consisted of 15 rounds of symmetric PCR and 11-16 rounds of asymmetric PCR. Temperature programs were the same for both symmetric and asymmetric PCR (preheating at 94°C for 2 minutes; cycles of 94°C for 30 s, 56°C for 15 s, and 72°C for 15 s; finishing with 72°C for 2 minutes). PCR optimization was only performed for rounds 4 and 5 to determine the optimal number of asymmetrical amplification rounds gave the highest concentration of ssDNA. For PCR optimization, the temperature program was modified to shorten the last 2 minutes at 72°C to 1 minute. The amplified ssDNA pools were purified using

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Darija: yes

cut off filters (Pall Life Sciences) to remove PCR components between each round of purification as well as to separate the 80nt library from primers. Quantification of ssDNA was done via Nanodrop2000 (Thermo Scientific). Success of DNA amplification and purification was verified by using gel electrophoresis (3% agar gel (VWR) in 1x TAE buffer) and imaged via fluorescence (Alpha Innotech) for the Alexa488 labelled DNA and primers.

Fluorescence Analysis

After each selection, the pool of DNA was amplified and purified to give ssDNA in PBS+MgCl buffer. Analysis of each pool (1, 2, 3, 4.2, 5.3, and 6.2), the DNA library and PBS as a control was done via fluorescence on a micro plate reader (Molecular Devices). For each pool, 250nM of ssDNA was incubated with a-VSV bound to protein G magnetic beads (as in aptamer selection), rabbit serum bound to magnetic beads, or just magnetic beads for one hour using 50uL incubation volume and half a million beads. Two replicates were prepared for the rabbit serum; one of the replicates will not be washed after incubation to give a total fluorescence reading for the samples. The magnetic beads were washed once with 50uL and then resuspended in 50uL PBS+Mg buffer before plate analysis. The plate was analyzed for fluorescence intensity from the top as well as fluorescence polarization at both with excitation at 485nm and emission at 532nm.

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Comment [6]: Excitation at 485nm and emission at 535nm

Results and Discussion

Method Development

Although machine automated procedures have been developed for aptamer selection (10), often times it is unfeasible due to space limitations, complex equipment, and require long set-up and optimization time. Based on various other protocols (11-13), the developed procedure aims to use magnetic beads for their ease of separation. Although streptavidin-coated beads (10) and tosyl-activated beads (11-13) have been used with measurable success, these procedures require extra steps for biotinylation (10) or long incubation times for hydrophobic binding of analytes to beads (11-12). In this aspect, utilizing protein G –magnetic beads to quickly and effectively bind to a-VSV and non-target immuno-proteins for positive and negative selection lead to a much quicker preparation by taking advantage of the natural affinity of antibodies to protein G. Magnetic beads upon which protein A and G have bound have already been shown to be effective binders of immunoglobulins, and are popularly used in bench top purification of antibodies from serum.

Protein G is a cell surface protein and there is binding to antibodies. But this would then indicate that only antibodies are bound excluding other important serum proteins including albumin and other immune-proteins. Despite this, the convenience of using magnetic beads to select for aptamers allows for much faster selection at much larger amounts. And in reality, a small molecule selected specifically for an anti-viral neutralizing antibody is very unlikely to have any significant binding to additional serum proteins due the vast difference in tertiary and quaternary structure.

Tandem PCR asymmetric amplification requires an excess of forward primer to give a single stranded DNA library. Tandem PCR works first by rounds of symmetric PCR, containing equal

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Comment [7]: protocols

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Comment [8]: I don't quite understand what you are trying to say here.. do you mean "additional serum proteins"?

amounts of both forward and reverse primer which will amplify the template normally. This symmetric PCR mixture is then used as template for the asymmetric PCR mix which contains 20 times more forward primer than reverse. The excess amount of forward primer amplifies the forward strand specifically building the enriched ssDNA libraries which are then used for selection against target molecules.

Prior to each selection, the naive and enriched libraries were denatured at 95°C and then renatured on ice to ensure that all of the ssDNA was set to its single stranded 3d conformation. The primers used for amplification are labelled with Alexa488 allowing for their detection quickly and easily via fluorescence, excitation at 485nm and emission at 535nm. Salmon sperm DNA was used to mask a-VSV to provide more stringent selection conditions; although, masking DNA was excluded in later rounds, R5-6, due to lack of DNA binding detected.

The electrophoresis below (Fig. 4) shows presence of the 80nt band in each of the 6 amplified pools with a less intense band visible for round 5 and 6. The negative control shows the brightest band representative of primers remaining after tandem PCR, the amplified pools (DNA Lib*-R6.2) visibly had less intense bands for the primers because a portion of the primers had been incorporated into the amplified DNA library. Some smearing observed first above the 80nt band becomes less visible in later rounds, R5-6, however another band appeared between the DNA lib and the primers. A small band may be observed at a size larger than the 80nt band, this due to the fact that the majority of the library is single stranded; double stranded DNA could separate and form a separate visible band.

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Comment [9]: You should add that this is probably the doubles-stranded DNA.

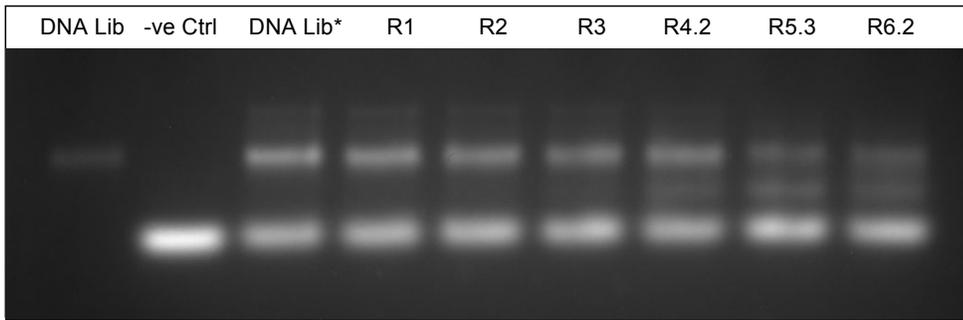


Figure 4: Gel Electrophoresis analysis of naive and enriched DNA library at via fluorescence. Fluorochromes were excited at 485nm and emission was measured at 535nm. DNA Lib* show the amplification of the DNA library used for fluorescence analysis. The DNA Library (Integrated DNA Technologies, Inc.) is diluted to 100nM in Green Flexi buffer and run against samples as a reference (80nt).

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Comment [10]: It hasn't been purified, we ordered it from IDT. The concentration is right.

30kDa cut off filters are used for DNA purification from PCR components as well as isolating the amplified ssDNA library from primers (Fig. 5). The side product visible faintly in R3, and more strongly in R5-6, between the 80nt band and the primers, remains even after purification. Smearing was observed in the purified sample but this is due to the high concentration loaded. Two possible reasons could explain the repeated appearances of this band; the side product could be binding to a-VSV and thus be selected for and repeatedly amplified. An alternate reason could be the side products observed are due to over activity from Taq polymerase. It should be noted that the same master mix and the same stock of Taq polymerase was used implying that the side products should be consistent if it were caused by the PCR components. Primary non specific products of PCR are known to be primer dimers, which are gradually accumulated with increasing cycle number; yield of the by-product was found to depend on the initial template concentration (14). The inconsistencies between studies in literature may be related to the randomness of the library and it is suggested that PCR optimization should be done for optimal amplification and the minimization of side products (14) (15). However, due to the nature of the SELEX, the contents, and thus randomness, of the ssDNA library are changing after each round and thus the number of asymmetric cycles should be optimized after each SELEX round.

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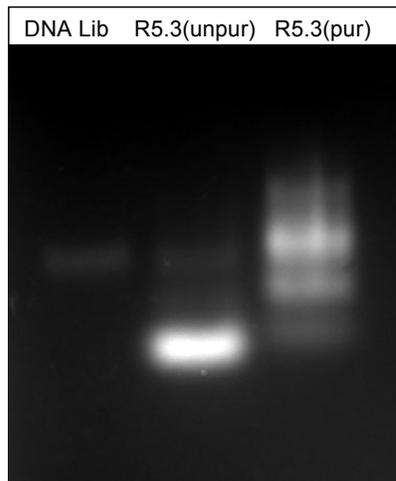


Figure 5: Gel Electrophoresis for R5.3 purification via 30KDa cut off filters. R5.3(pur) is concentrated after purification giving a brighter band.

Initial iterations of the selection protocol called for elution of bound DNA from the protein G magnetic beads by using 0.1M NaOH; this gave a soft elution that eluted only the bound DNA. This method worked for rounds 1-4 but its efficiency decreased and altogether stopped working for rounds 5-6. This could possibly be due to a decrease in DNA binders or more strongly binding aptamers that could not be eluted as efficiently as previously. Denaturing elution was employed with success once the soft elution stopped working. Denaturing with heat will cause some protein G to be unbound from the polymer coated magnetic beads, however this is not an issue because the primers used will specifically amplify the 80nt library and any remaining protein will be degraded at the high temperatures needed for PCR.

Additionally, selection reactions were initially conducted in un-lubricated Eppendorf tubes which inadvertently lead to DNA binding to the plastic. This was initially solved by pipetting the beads to a new tube prior to base or heat elution of bound DNA. A more simple solution was to switch to using pre-lubricated Eppendorf tubes that minimized DNA binding to plastic.

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Comment [12]: And the protein will degrade when succumbed to high temperatures of the PCR reaction.

Fluorescence Analysis

Aptamer affinity to a-VSV, rabbit serum, and magnetic beads was analyzed via fluorescence.

The naive ssDNA library and the enriched ssDNA were each incubated with a-VSV bound to magnetic beads, rabbit serum bound to magnetic beads, and unbound magnetic beads.

Additional samples were prepared for the serum-magnetic beads incubation that remained unwashed to assay the total fluorescence of all DNA added. The data analysis was performed via the formula (a):

$$\text{Relative fluorescence} = \frac{(\text{Sample fluorescence} \times \text{Sum of fluorescence})_{\text{Washed}}}{(\text{Sample fluorescence} \times \text{Sum of fluorescence})_{\text{Unwashed}}} \quad (\text{a})$$

Giving a ratio that is normalized to the total amount of ssDNA added. The calculated relative fluorescence for each pool is then compared for their affinity to a-VSV, serum, or just magnetic beads (Fig. 6). There is no significant increase in fluorescence and thus affinity for a-VSV compared to serum. Additionally, there is no significant difference between the enriched DNA pools and the naive DNA library. Relatively, R2, R5.3, and R6.2 had the highest relative fluorescence compared to the naive library, whereas R1, R3, and R4.2 had relative fluorescence lower than the naive library. Out the six pools amplified, R2 appears has the best binding affinity with higher binding to a-VSV compared to rabbit serum; however the minute difference between R2 and the DNA library makes this insignificant and more rounds of SELEX selection are needed to mature more enriched ssDNA pools.

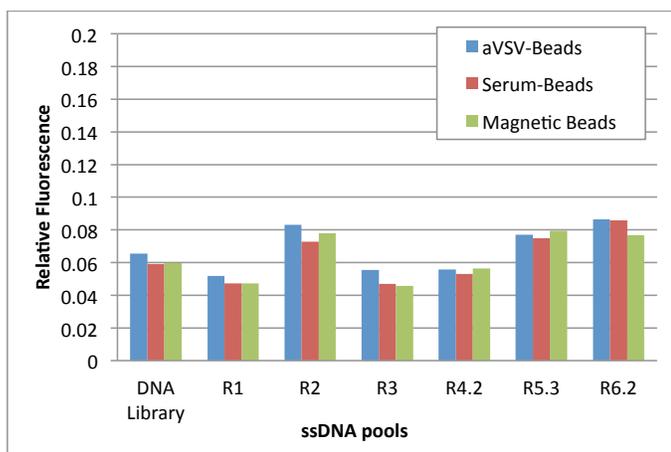


Figure 6: Relative fluorescence showing affinity of naive and enriched libraries to a-VSV, Serum, and magnetic beads. Relative Fluorescence is calculated using equation (a). No standard deviations are shown because only single readings were taken for each sample prepared.

Fluorescence anisotropy was also assayed for the plate although no significant data, and thus no significant binding interaction, was seen (Fig. 6). Anisotropy is directly related to the polarization and is the ratio of the polarized light to the total light intensity. This analysis works on the basis that polarized light striking fluorescent molecule will emit polarized fluorescence. The emitted polarized fluorescence can be transformed back into unpolarized light depending on rotational diffusion and various other factors. If the fluorophore is linked to a small molecule, which will have faster motion and rotation, the emitted light will be depolarized whereas the fluorescence emitted from a fluorophore linked to a large molecule, with slower motion, will remain polarized (16). As an alternate method for analyzing binding affinity, the data achieved through fluorescence polarization does not require additional steps and can be measured at the same time that fluorescence is measured and can ascertain the presence of binding affinities. As determined above, there was no significant difference in binding affinity between the a-VSV, serum, and beads nor was there significant binding affinity between the naive ssDNA library and the enriched DNA pools. However, the pattern observed in relative fluorescence polarization matches the pattern observed in relative fluorescence, an increased binding affinity for R2, R5.3, and R6.2.

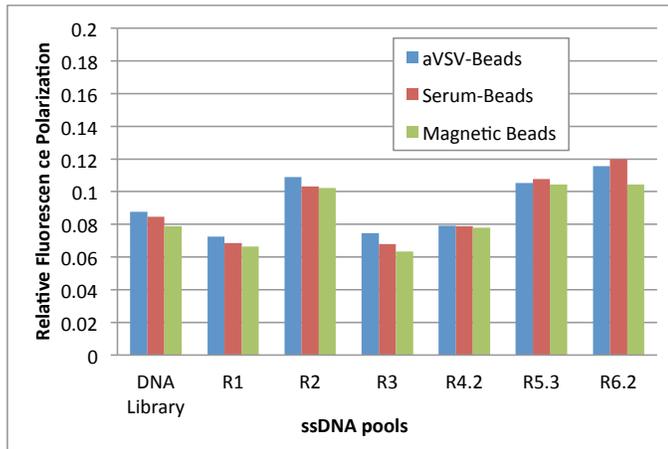


Figure 7: Relative Fluorescence polarization demonstrating the affinity of naive and enriched libraries to a-VSV, serum, and magnetic beads. Relative fluorescence polarization is calculated using equation (a). No standard deviations are shown because only single readings were taken for each sample prepared.

Purification of the PCR reaction mixture was not always successful, meaning it was necessary to check via gel electrophoresis after each round (See Fig. 5). SsDNA concentrations were determined by Nanodrop after purification, although from fluorescence analysis, it is shown to be not accurate. The concentrations of the amplified pools were diluted to give an incubation concentration of 250nM; however, the unwashed set of incubation gave inconsistent fluorescence varying from 1.5 million units, R6.2, to 2.8 million units, R3 (Fig. 8). This indicated that the ssDNA concentration in each sample was inconsistent. The likely cause of this is inaccurate readings due to contamination of dNTPs or primers inflating the ssDNA concentration. The sample containing just PBS was used as a control because due to the fluorescence from the magnetic beads, giving a baseline for fluorescence units emitted.

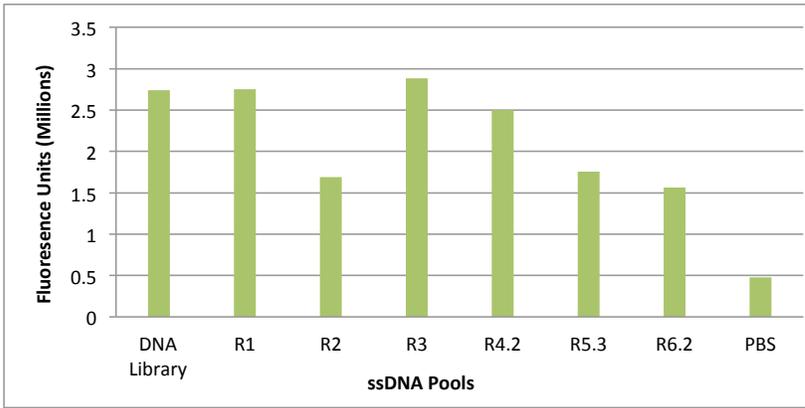


Figure 8: Fluorescence of unwashed serum-beads incubation reaction. The PBS sample shows the fluorescence emitted from just magnetic beads. No standard deviations are shown because only single readings were taken for each sample prepared.

Conclusion

Although no significant binding affinity data of aptamers to a-VSV was attained, significant portions of the selection method were developed and optimized. Future steps for project would definitely include conducting further rounds of selection and analyzing binding affinity until an adequate number of pools with preferential binding to a-VSV over normal serum are acquired. Binding affinity could be analyzed again via fluorescence and fluorescence polarization or via flow cytometry. Fluorescence measurement, via plate reader, is much more accessible compared to flow cytometry due to availability of equipment in addition to quicker preparation and analysis time. After the best pools are selected, the aptamers will undergo screening for binding affinity using both VSV and a-VSV antibodies. Further modification of the protocol may positively impact future aptamer selection experiments such as different incubation temperatures. Although current selections are performed at 37°C, lower temperatures such as room temperature or even 4°C should be considered because biomolecules are more stable at lower temperatures. A possible pathway to consider if SELEX does not provide results is Non-SELEX which differs only by the absence of amplification in each round of SELEX. The protection of oncolytic viruses without interference of the infectious properties is necessary for the elongation of its residency in blood and is possible via the use of DNA aptamers. Aptamers once selected for and properly modified, will be able to selectively bind to the a-VSV antibodies and prevent coating of the oncolytic virus particles.

Darija 2011-4-20 3:12 PM

Comment [13]: And also performing selections at different temperatures. For example, at 4C, biomolecules are more stable.

Darija 2011-4-20 3:15 PM

Comment [14]: Once selected, the aptamer pools will be screened for its binding efficacy using the VSV virus and a-VSV.

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Appendix

Acronyms used

AptaBiD: Aptamer-Facilitated Biomarker Discovery
AptaVIP: Aptamer-Facilitated Virus Protection
a-VSV: anti-VSV neutralizing antibodies
PBS: Phosphate buffered solution
PCR: Polymerase chain reaction
SELEX: Systemic Evolution of Ligands by Exponential Enrichment
ssDNA: single stranded DNA
VEGF: vascular endothelial growth factor
VSV: vesicular stomatitis virus