STRUCTURE ACTIVITY RELATIONSHIP STUDIES FOR APTAMER, AFR-CM-65

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Abstract

The development of effective targeted therapies has become increasingly imperative in the treatment of cancer. Antibodies have been used for this purpose due to their precision in specificity and affinity against desired substrates. But their use can result in new toxicities and unwanted immune reactions. Recently, researchers found aptamers to be a versatile alternative.

Aptamers are single or double stranded oligonucleotides that can bind to a target molecule with high affinity and specificity. They are developed by a special method called SELEX (systematic evolution of ligands by exponential enrichment). Aptamers are analogous to antibodies in-terms of target recognition and applications. Aptamers have several key advantages. Aptamers have low molecular weight, low toxicities and no animal is involved in their production. They have fast tissue penetration and can be produced using a DNA synthesizer. Also, aptamers remain stable during long-term storage and can easily be labeled with fluorescence tags or radio-isotopes. In addition, they sustain reversible denaturation. These advantages make aptamers very appropriate for target cancer diagnostics and therapies.

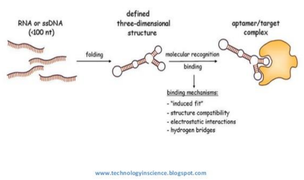
In this work, we redesigned a version, AF2 from aptamer AFR-CM-65. The aptamer, AFR-CM-65 was selected against Burkitt's lymphoma and can specifically recognize Burkitt's lymphoma cell with high affinity.

Background

What is an aptamer?

Aptamers are single or double stranded DNA or RNA molecules that can bind to a target molecule with high affinity and specificity.

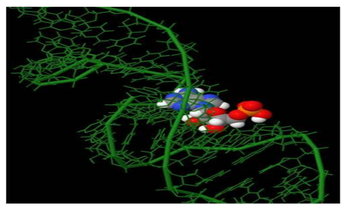
Aptamer – Target Binding Mechanism



Nature of the aptamer interaction

- ✧ van der Waals Surface contacts
- ✧ Hydrogen bonds
- ✧ Stacking interactions
- ✧ Other non covalent bonds

Advantages of an aptamer

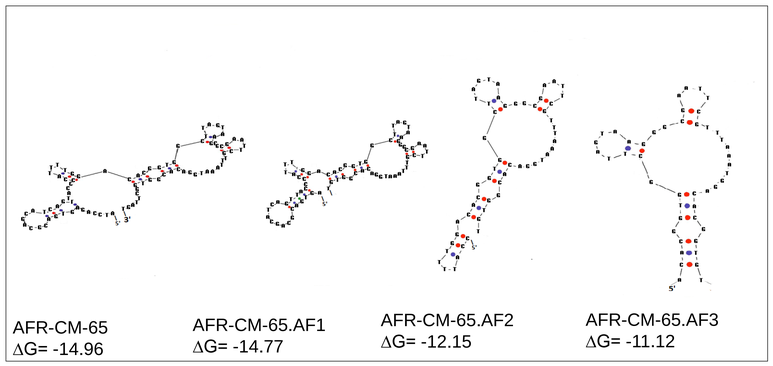


- ✧ Easy, fast and reproducible synthesis
- ✧ Easy chemical manipulation for signaling
- ✧ Fast tissue penetration
- ✧ No immune response
- ✧ Non-toxic
- ✧ Easy storage
- ✧ No animals involved in production

RNA aptamer binding to its target molecule

Goal

Systematic truncation from (3' and 5' end) to design shorter analogues of AFR-CM-65, to increase the thermodynamically most favorable fold.



Aptamer AFR-CM-65 and truncated sequences. Structures and free energies are predicted using m-Fold from www.idtdna.com

Methods

DNA synthesis, purification and quantification

Synthesis of DNA using solid-phase synthesis using a DNA synthesizer

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DNA de-protection

↓

DNA purification using reversed phase column and de-tritylation

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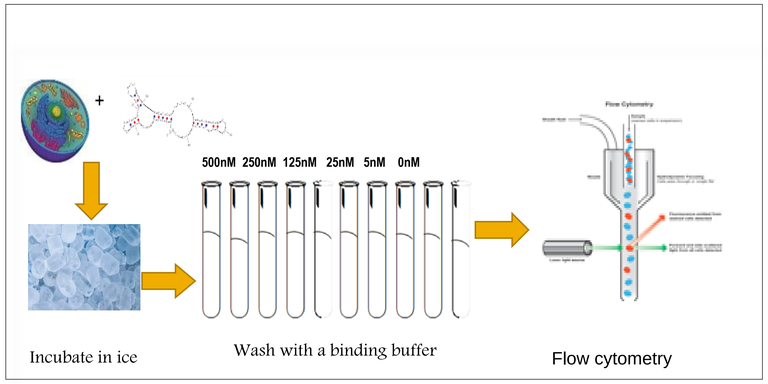
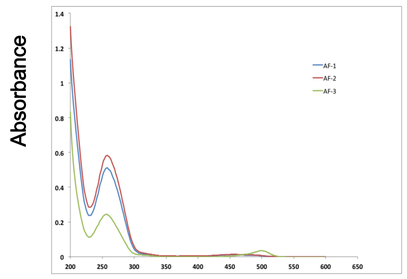
Spectrometric analysis and calculation of concentration

Cell-binding assay



Results

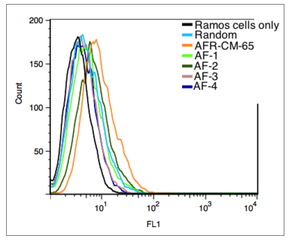
UV Visible absorption spectra of aptamer analogues



Name of the sequence	Concentration mol/L
AF-1 (76mer)	1.21×10^{-4}
AF-2 (55mer)	1.89×10^{-4}
AF-3 (46mer)	9.43×10^{-5}

UV/Visible absorbance spectra of AF1, AF2 and AF3. Absorbance maxima at 260nm was used to calculate the concentration of each molecule

Binding analysis of AFR-CM-65, its analogues against Ramos cells, a Burkitt's lymphoma cell line

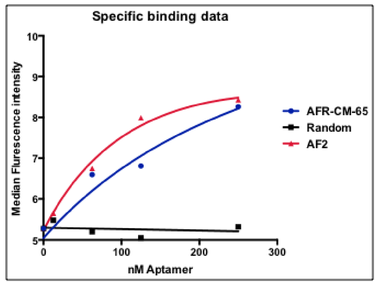


- ✧ The original aptamer AFR-CM-65 shows highest binding
- ✧ AFR-CM-65-AF2 shows significant binding
- ✧ AF1 and AF3 shows almost no binding to the cells

Binding analysis of truncated aptamers and AFR-CM-65. Each aptamer (500nM) incubated with 2×10^5 Ramos cells on ice for 40 minutes. Then the cells were washed with 3 mL of wash buffer, reconstituted in 1 mL of wash buffer. Binding of aptamers with target cells was subsequently analyzed by flowcytometry.

Results

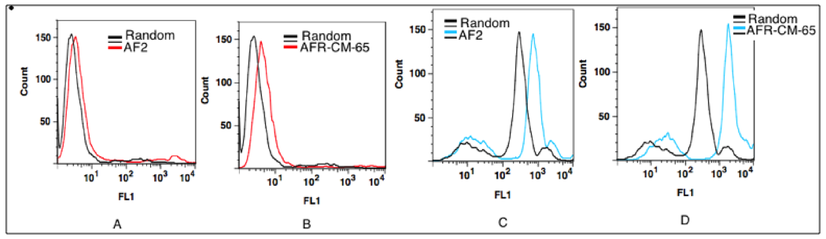
Analysis of $B_{max/2}$ for AFR-CM-65, AF2 and Random sequence



Molecule	Bmax/2
AFR-CM-65	371nM (R^2 = 00.93)
AF-2	191nM (R^2 = 00.99)

Concentrations from 500 nM to 12.5nM of Random, AFR-CM-65 and AF2 were incubated with 2×10^5 of Ramos cells 40 minute on ice. After washing with 3mL, cells were reconstituted in 0.5mL of wash buffer; binding of each molecule at each concentration was subsequently analyzed by flowcytometry. Median fluorescence of each histogram was plotted as a function of concentration

Analysis of specificity of AFR-CM-65 and AF2



(A and B) HL 60, a myeloid leukemia cells and (B and C) Daudi, a Burkitt's lymphoma cells. A 500nM of aptamer or random sequence was incubated with 2×10^5 cells for 40 minutes in ice. Then washed the unbound sequences with 3mL of wash buffer. Cells were suspended in 0.5mL of wash buffer prior to flowcytometric analysis. Higher fluorescence shift on x-axis against Daudi cells compared to non-targeting myeloid cells shows that specificity of AF2 is retained.

Conclusion

We have re-designed a novel analogue of AFR-CM-65 with high affinity and specificity using a simple systematic truncation approach. Future work will be aimed at stabilizing the secondary structure using unnatural DNA bases to increase the stability in human serum and at designing bivalent aptamer analogues to further increase the affinity.

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