Controlling the Immune System with FOXP3 Inhibitors

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INTRODUCTION

The immune system is responsible for protecting the body against any invading organisms. After eliminating the invaders, the immune system uses regulatory T cells, or Tregs, to turn down the immune response. However, certain diseases, such as cancer, recruit Tregs at tumors to "hide" the tumor[5]. In order to prevent this, inhibitors have been created to suppress regulatory T cells. Using the programs AutoDock Tools and Vina, this project explores possibilities to improve these inhibitors through computational biology.

BACKGROUND AND SIGNIFICANCE

A key obstacle is that the human immune system is unable to detect the areas of abnormal cell growth, called tumors, because of the presence of Tregs. They influence the immune system by maintaining self-tolerance and preventing autoimmune disease by turning down the immune response[4]. Thus, an excess of Tregs in tumor cells can prevent the immune system from recognizing and destroying cancerous cells, while a deficiency of Tregs causes the immune system to attack the body's own tissues.

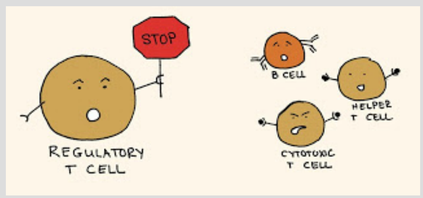

Figure 1: A visual representation of the role of Tregs

The master regulator of Tregs is the FOXP3 transcription factor. Using inhibitors derived from FOXP3 could be key to harnessing NFAT1, which starts immune responses[1]. Further development could possibly lead to treatments for cancer. There have already been experiments done to show that vaccine efficacy can be improved in mice through FOXP3 inhibitors[2], so further experimentation and improvement to the inhibitor could yield one usable in humans. Finding and trying to improve the inhibitor is the goal of this project.

RESEARCH METHODOLOGIES

1. Creating the models

Three models were created using information on their 3D structures from the Protein Data Bank (PDB): the NFAT1 RH domain (392-574) and two peptides, 393-403 and 393(E399A)[3]. The number ranges indicate the base pairs and E399A means that amino acid E at base 399 is replaced with A. However, PDB files don't come with hydrogens as they are assumed to be present. Hydrogens are vital for docking because they act as the basis for hydrogen bonds. Thus, using AutoDock Tools, hydrogens were added and the file was converted to the PDBQT extension so that it is later compatible with Vina.

2. Setting the testing zone

Next, a testing zone, or grid box, was created so that Vina would know where to test dock the various inhibitors. This is an important step because it improves efficiency and lowers the risk of errors.

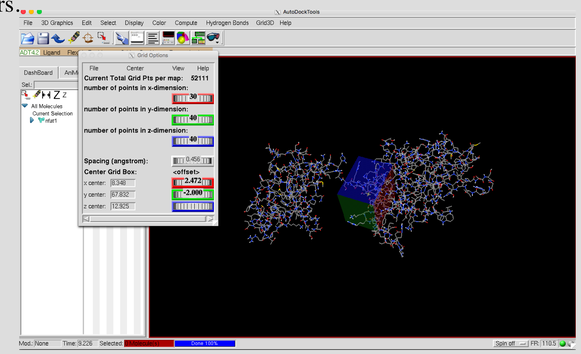

Figure 2: The grid box and NFAT1 RH domain

3. Testing the Protein Complex and Inhibitor

Finally, running Vina through Terminal, the inhibitors and protein docking complex were simulated. A report log was generated afterward.

```
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Output will be peptide1_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 28220914
Performing search ... done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1       -5.8      0.000      0.000
   2       -5.6      5.315     10.920
   3       -5.5      4.199      8.604
   4       -5.4      3.780     13.504
   5       -5.4      5.578     10.720
   6       -5.3     20.635     25.847
   7       -5.3      4.712     13.982
   8       -5.3      4.074      9.965
   9       -5.3      5.473     10.599
Writing output ... done.
```

Figure 3: Report log for peptide 393-403

DATA ANALYSIS AND RESULTS

Both peptides inhibit the NFAT1 domain. However, between the mutated 393(E399A) and 393-403 peptide, the latter had a stronger affinity to the NFAT1 domain. The affinity is the Gibbs free energy of the bond, which describes the amount of energy required to create a bond. Logically, the more negative the number, less energy needs to be used to create a bond. All trials of 393-403 have a lower Gibbs free energy value, so the chance that a bond is created is more likely because less energy is required. The mutated sequence replaced E, which is glutamic acid, with A, which is alanine. The weaker affinity could have been caused by size differences, since E is larger than A.

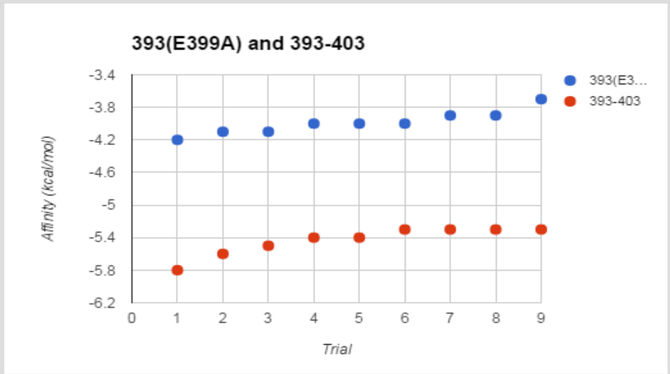

Figure 4: Graph of affinities of two peptides to NFAT1

ACKNOWLEDGEMENTS / REFERENCES

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Works Cited:

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