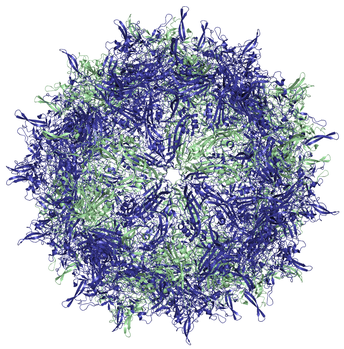

Identifying Functional Residues in Adeno-Associated Virus Through Frustration Analysis

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Motivation

- Virus infectivity remains poorly understood
- Predicting how mutations will affect Adeno-Associated Virus (AAV) infection *in silico* can save time and resources
- Better *in silico* models of AAV infection will aid the engineering of gene therapy vectors to treat diseases

Introduction

- Virus protects its cargo and unfolds at different stages in its infectious pathway
- Identifying residues that participate in these steps can elucidate more complex mechanisms
- A computational model can screen or find interesting residues before physically testing

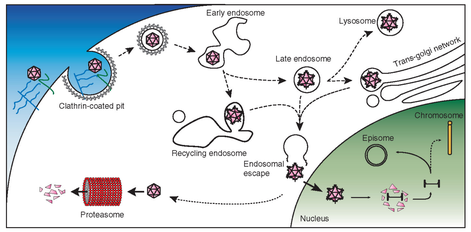

Figure 1: Cell entry and trafficking of recombinant adeno-associated virus (rAAV)[1].

The frustration model

- Frustration index is a measure of energetic stability between single residues of a protein
- Minimally frustrated residues contribute energetic stability to the native protein structure
- Highly frustrated residues appear near binding sites or sites participating in conformational changes
- Frustration can be compared between protein monomer and multimer assemblies to find residues that favor either state[2]
- Model can be used to screen for sites that may play a role in virus capsid assembly, stability, or disassembly

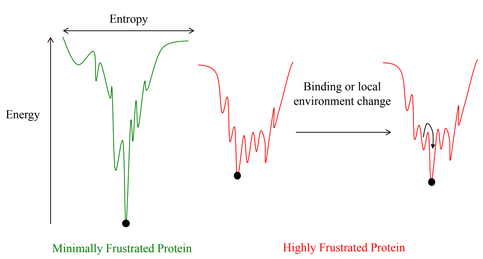

Figure 2: Frustration in proteins. The folding funnel maps all possible conformations of a protein and their energy levels. A minimally frustrated protein is unlikely to spontaneously unfold, but a highly frustrated protein is able to change conformation with less energy input through a local environment change

Frustration modeling in AAV

- Multimer assemblies of each serotype were modeled in PyMOL by selecting a central monomer and incorporating all monomers within 4.5 Å
- Monomer and multimer conformations for AAV serotypes 1 through 9 were analyzed with the Wolynes Lab Frustratometer tool[3]
- The frustration index difference (FID), defined as multimer – monomer frustration index, was computed for each residue
- Residues promoting assembly and disassembly were found by thresholding for a frustration index difference of ±1.5

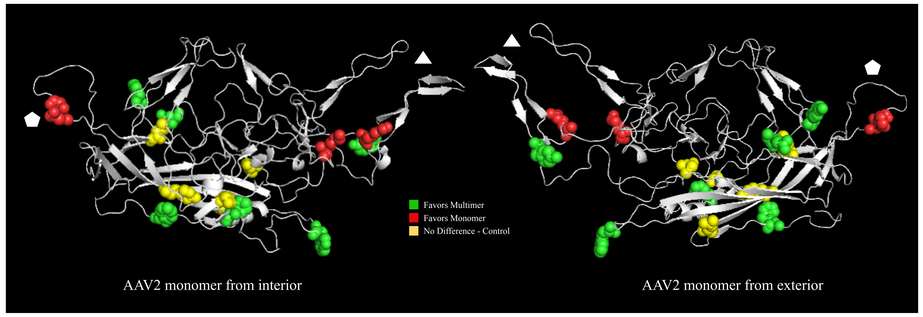

Figure 3: Selected residues for mutants in AAV2. Selected residues that will be mutated into alanines and compared against wtAAVs to test the effectiveness of the model on AAV2. Residues with a frustration index difference of +1.5 are predicted to favor a multimer conformation, while those with a frustration index difference of -1.5 are predicted to favor a monomer confirmation. Inside (left) and outside (right) capsid monomer views are shown, 5-fold loop shown with a pentagon and 3-fold axis with a triangle. Green and red residues are outside the threshold, either favoring a multimer or a monomer confirmation, respectively, of the virus capsid. Yellow residues favor neither conformation.

Computational model results

- Three residues promoting assembly (W288, Y397, W694) and one promoting disassembly (N656) were found to be conserved across all serotypes tested
- Residues promoting disassembly were found near the capsid five-fold axis, supporting the theorized mechanism of N-terminal extrusion[4]
- Residues promoting assembly are found near the capsid three-fold axis, suggesting that this region promotes structural stability

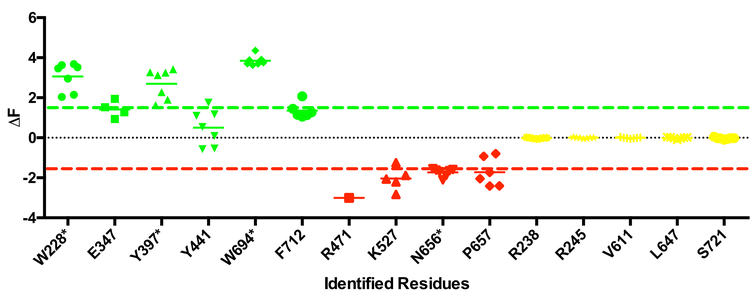

Figure 4: Frustration index difference for selected residues in AAV2. Residues outside the ±1.5 FID conserved accross all serotypes tested are shown with an asterisk; controls have an FID within ±0.1 in all serotypes tested.

Testing the model

- Alanine scanning was performed on selected residues by making a physical mutant
- Mutants are being tested against wtAAVs for capsid formation, thermal stability, and transduction efficiency
- qPCR, benzonase, and GFP output flow cytometry are used to test these parameters, respectively

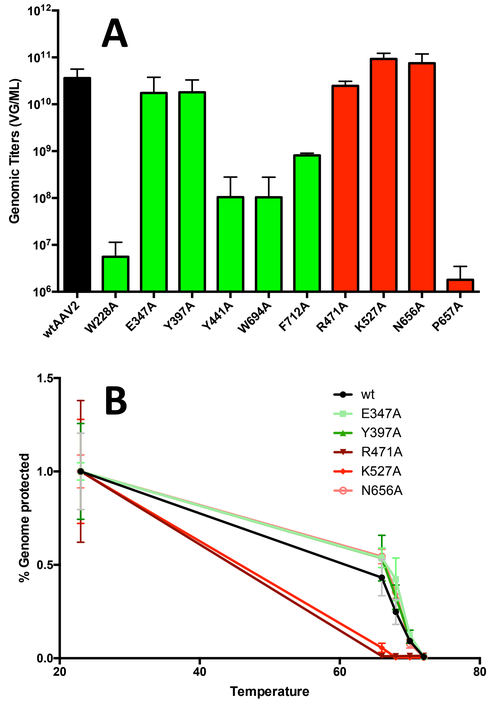

Figure 5: Capsid formation and thermal stability results of completed mutants. (Panel A) Genomic titers were measured by qPCR or virus preparations to determine whether the virus formed correctly or not. Viruses which formed at genomic titers of above 1E10 were tested for genome protection through a benzonase assay at different temperatures (Panel B). Mutants of residues favoring assembly were expected to form less stable capsids and be less thermally stable (green), while mutants of residues promoting disassembly were expected to form a more stable capsid and have higher thermal stability (red) when compared to wild type.

Future work: complete testing

- Complete screening of panel
- Further investigate mutants that defy initial hypothesis
- Expand computational analysis to incorporate bond-based frustration indices, electrostatic information, pH

Acknowledgements

This work was financially supported by the American Heart Association Summer Cardiovascular Research Internship Program.

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