Host Range Mutations Affect the Stability of RNA Virus Attachment Proteins

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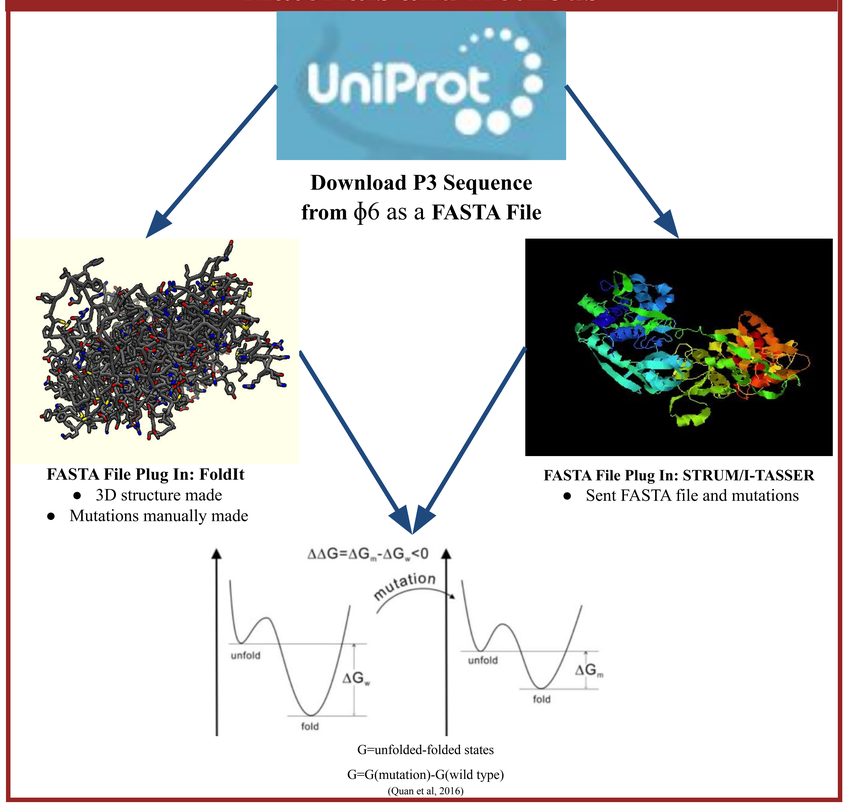
Abstract

RNA viruses generally have much higher mutation rates than other types of biological entities. Many of the deadliest and well known eukaryotic viruses such as Ebola, SARS, and influenza are RNA viruses. ϕ6, a double stranded RNA virus that infects *Pseudomonas* bacteria, is a part of the family *Cystoviridae*. Since *Cystoviridae* viruses are the only lipid-coated, segmented, and dsRNA bacterial viruses, the group in itself stands in contrast to other bacterial viruses and more closely resembles some viruses of higher eukaryotes. Studying ϕ6 opens doors to better understanding the evolution of viruses, especially because results obtained in this bacterial virus may directly apply to eukaryotic viruses that share similar genomic and physical properties.This study focused on protein P3, which is ϕ6's host attachment protein: the main protein controlling host range and its expansion for this virus. Using predictive structure programs, the 3-dimensional layout of the P3 protein was modeled. Then the effects of host range expansion mutations on the structure were determined by differences in free energy. This study begins to explain why certain host range mutations are more favored than others over evolutionary time (i.e., more stable P3), and provides insight into how host range mutations affect the accumulation of future mutations in P3. This biophysical study aims to understand the barriers to sequential host shifting in a model RNA virus.

Materials and Methods



Results

P3 Mutations	FoldIt	STRUM Trial 1	STRUM Trial 2
Amino Acid Mutations	ΔΔG (Kcal/mol)		
G5S	4.984	-2.45	-2.59
E8K	-20.65	-0.99	-2.3
E8G	-23.939	-2.2	-3.36
D145G	-24.144	-3.13	-4.47
N146S	-2.01	-1.69	-2.88
E178D	-6.444	-0.49	-0.93
P339H	-5.019	-1.21	-2.08
T516A	-149.446	-0.23	-2.16
D533A	-13.792	-1.14	-2.31
D535N	-1.611	-1.61	-3.36
D554V	-6.954	-1.21	-2.94
D554N	-2.751	-1.6	-3.41
D554A	-2.086	-2.94	-3.74
D554G	-0.887	-3.26	-4.47
E8A	-21.095	-0.78	-2.11
L555F	-3.701	-0.02	-0.68
A133V	180.657	-0.71	-1.5
Q140R	115.626	-2.56	-3.36
K144R	-2.797	-1.14	-3.56
N146K	-0.651	-1.03	-1.82
S299W	1.836	-0.25	-1.72
G515S	375.202	-2.49	-3.02
D35A	34.076	-2.05	-3.72
S246T	0.147	-2.18	-2.88
K311T	-2.576	-1.91	-3.65
E8D	-17.776	N/A	-1.68
Q130R	-2.258	N/A	-2.4
G515S + A133V	486.55	N/A	N/A
E8G + A133V	89.529	N/A	N/A
E8G + S299W	-22.067	N/A	N/A
E8K + S299W	-20.982	N/A	N/A
E8K + A133V	112.866	N/A	N/A
D554G + F260Y	-4.0821	N/A	N/A
D554G + L106L	-0.895	N/A	N/A

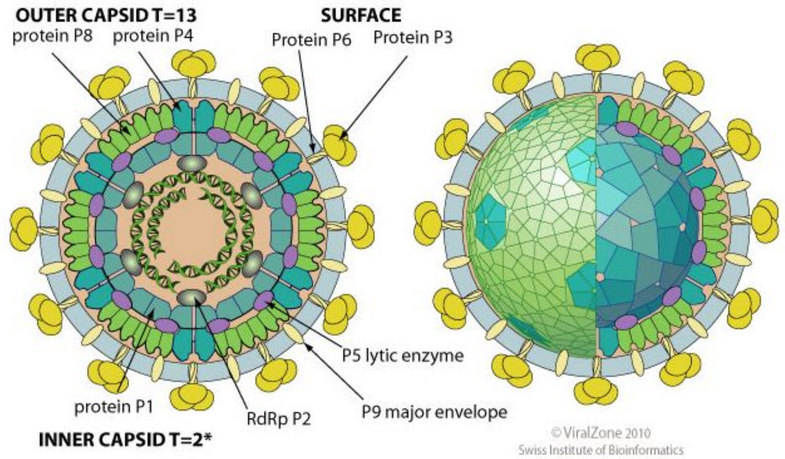


Figure 1: An illustration of ϕ6. At left, a cross section virion, at right the outer surface of the virion. The P3 host attachment protein is visible on the surface in both.

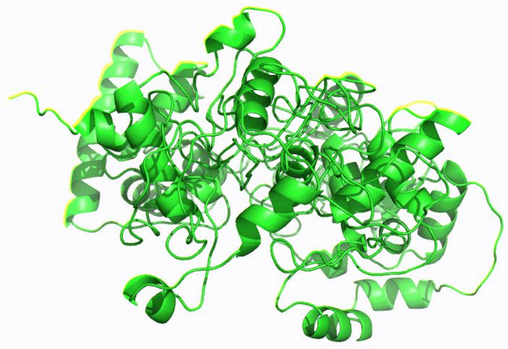


Figure 2: A 3-dimensional rendering of a predicted model of P3.

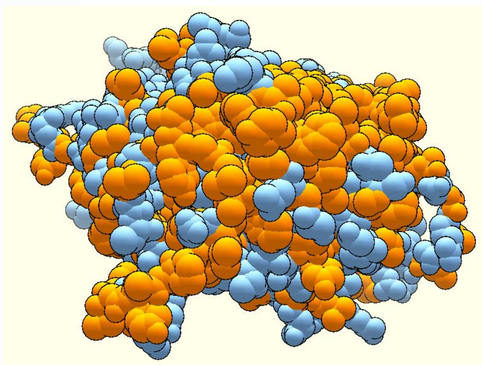


Figure 3: A 3-dimensional rendering of the P3 protein with a focus on the hydropathy of the residual regions. Orange showing more hydrophobic surfaces and blue showing hydrophilic.

Discussion

- Foldit and STRUM each have differing trends of changes in free energy.
  - Large differences can be seen in several mutations such as A133V, G515S,T516A, E8D, E8K, E8G, and D145G.
- The overall trend, with the few exceptions, is that many of the mutations lead to a smaller free energy, thus making the protein less stable.
- There is very little consistency among the data collected with large differences between the STRUM trials and the Foldit values. Since the exact 3-dimensional structure of the protein has yet to be made, each of the values should be taken with a grain of salt; foldit even more so as the process was more manual than STRUM.

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