KINARI-Mutagen Case Study: 1CRN
Crambin

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Crambin (Figure 1(a)) is a 46 amino acid plant protein, whose crystals diffract to ultra-high resolution. The quick-start option was used to invoke default curation, modeling and rigidity analysis options. From start to finish, the total computation time of the experiment was approximately 30 seconds.

![Cartoon Rendering of Crambin, PDB file 1CRN](image)

![Rigidity Results of the non-mutated Crambin](image)

![Rigidity Results when Residue 3 was mutated by KINARI-Mutagen](image)

Figure 1: Protein 1CRN, Crambin, is a 46 amino acid protein composed of two alpha helices and a short anti-parallel beta sheet (1(a)). When rigidity analysis is performed on the non-mutated form of Crambin, the majority of the atoms in the proteins core are part of a single large rigid cluster (shown in purple)(1(b)). KINARI-Mutagen was used to generate several *in-silico* mutants of crambin. When excision was performed on residue 3, the size of the largest rigid cluster decreased(1(c)).

When no mutation was performed, the majority of the core residues of Crambin were calculated to be part of a single large rigid cluster (Figure 1(b)). When excision was performed on residue 3, the size of the largest cluster decreased, and the number of clusters increased, when compared to the non-mutated protein(Figure 1(c)).

We wanted to know if KINARI-Mutagen could identify critical residues. KINARI-Mutagen uses the *SurfRace* program[1] to calculate the Solvent Accessible Surface Area (SASA)[2] of each residue. A residue that is not exposed to the solvent has a low SASA value, measured in Å². Residues closer to the surface of a protein have higher SASA values, and completely buried residues have a SASA value of 0.

Several residues in the core of Crambin had a pronounced effect on the protein’s predicted rigidity when they were mutated (residue 3 for example). Similarly, many residues (7, 15, and
that are solvent accessible, when mutated, had little effect on the largest rigid cluster. These findings were not surprising, because residues on the surface of a protein are not expected to help maintain a protein’s stability[3]. However, the software was able to identify critical residues on the surface of the protein that affected the protein’s rigidity when mutated to a glycine.

The Distribution of Rigid Bodies, By Residue plot (Figure 3) can be used to determine which excision(s) caused the large rigid bodies of a protein to break down into smaller rigid bodies. The left axis of the plot lists mutants that were generated by the user. For example, the row labeled 1crn.A.0026 indicates that a mutant was generated by excising residue 26 of chain A of protein 1crn. The vertical color legend on the right-hand side assigns colors to the rigid body sizes found among the mutants. Residues on which excision was performed are on the x-axis. The color at each x-y position in the plot indicates the size of the cluster that residue x belongs to for the mutant specified on row y. If a computational mutation had no effect on the largest rigid body of a protein, and all residues were contained in one large rigid cluster, then the color for that row will have a uniform color (for example 1crn.A.18, 1crn.A.24, and 1crn.A.30). If performing excision on a residue caused each of the residues to be part of small rigid bodies, then the row for such an excision would be colored dark. For example, when a mutation was performed on residues 2 or 3, or on residue 40, then the largest rigid body in the protein was far smaller than about 500, which is the size of the largest rigid body of the protein when no mutants were generated.

We inspected the Largest Rigid Cluster and SASA vs Excised Residue plot (Figure 2), to identify critical residues that could not be located by using the SASA calculations alone. Of the 11 mutants that had largest rigid clusters below the critical residue threshold, eight of them (residue 2, 10, 17, 35, 36, 40, 41, and 44) had SASA values in the wild-type protein that were well above zero. Of these eight, 4 are known to be identical among viscotoxin A3 and α1-purothionin[4], while another 3 of them were conserved among two of these three homologous proteins. Only residue 44, with a SASA value of 70, was identified as critical, but which is not conserved among the three homologues.

The set of critical residues identified by our method is different that the set of amino acids that are ranked by just the strength of hydrogen bonds or number of stabilizing hydrophobic interactions. Moreover, KINARI-Mutagen can identify conserved surface exposed residues that could not be detected using Solvent Accessible Surface Area measurements alone.
Figure 3: The Distribution of Rigid Bodies, By Residue plot can be used to determine which excision(s) caused the large rigid bodies of a protein to break down into smaller rigid bodies.

References


