PEBL Long Assignment 1: Hypothesis

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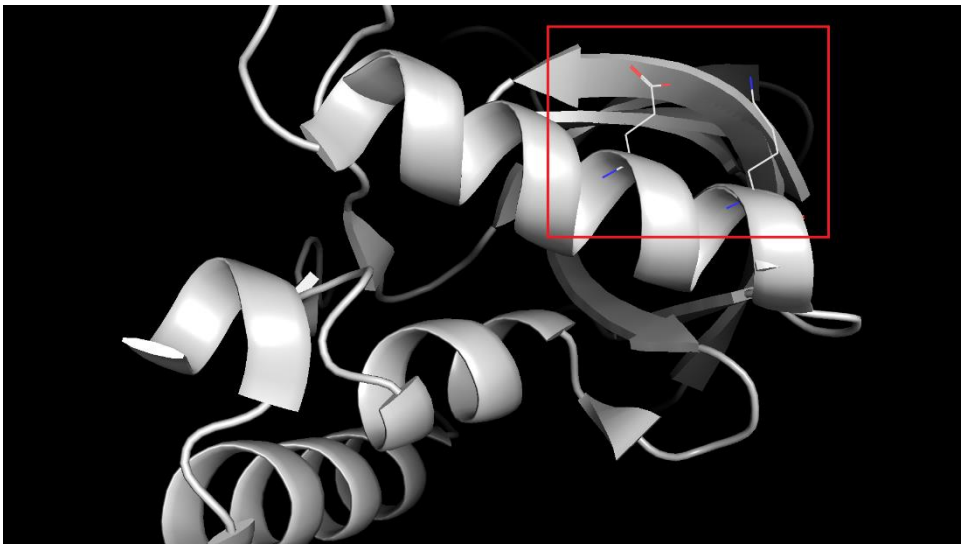
Proteins are integral to the existence of life; they are the movers and shakers of the biological world. From mechanical motion to metabolism, the actions of cells are governed by the interactions of proteins. By adopting extremely specific structures, proteins perform the multitude of tasks necessary to sustain the life of a single cell, breaking down each task into thousands of individual steps and relying on differently shaped proteins to execute each step. Before attaining their structure, proteins are simply a linear chain of amino acid building blocks. Somehow, this chemical sequence spontaneously folds, achieving the proper conformation to create extremely specifically structured proteins. How does the chemical composition of a protein relate to its structure and properties? —how does the environment of a protein change its structure and properties? —and how do the structure and properties of a protein relate to its function? These are the questions we tackle in PEBL. To study these overarching questions, we adopt a particular question regarding a model protein, SNase. We will investigate how the stability of the structure of SNase changes following a mutation to produce an ion pair on its surface. We aim to show that substituting two amino acids on the surface of SNase with glutamic acid (negative) and lysine (positive) further stabilizes its structure through the favorable, electrostatic interaction between these residues. With the advent of protein engineering, for drug development and personalized medicine, addressing these types of questions regarding the basic science of protein structure and function is increasingly important, for the difference between a life-saving drug and potent poison may be a difference of just one amino acid out of hundreds.

The specific mutant variant of SNase we will investigate is K63E/E67K. This mutation is the inversion of an existing ion pair in SNase between lysine (K, or Lys) at position 63 and glutamic acid (E, or Glu) at position 67, thereby producing a new ion pair by reversing the charges. Though on the surface of the protein, these two residue positions are distant from the active site of SNase, and thus will likely not directly produce a change of the structures actively involved in SNase's function (cleaving DNA). Furthermore, since an ion pair exists at the mutation location in wild-type (unmutated) SNase, it has a structural role (since non-covalent interactions dictate structure). Hence, this mutation and mutation site are suitable for answering the particular question of the experiment. In determining the changes in stability, certain standards must be defined to test the robustness of the protein's structure. Relating to the overarching questions about environmental factors and structure/function, we can investigate the stability of the protein by testing its functioning efficacy: measuring its ability to act on its substrate and subjecting it to different environmental conditions to see how the efficacy changes with a changing environment. By comparing the mutant protein's performance to that of the wild-type, we can determine the changes to its structural stability. The environment is one of the main factors affecting the outcome of the experiment, as the structure of proteins is heavily dependent on the chemical and physical conditions to which they are subjugated. Furthermore, the formation and disruption of noncovalent forces by the mutation will largely determine the outcome of the experiment, as these are the determinants of a protein's folded form and hence of its stability.

Considering the nature of the specific mutation in the context of the factors discussed above, the K63E/E67K mutation will likely reduce the structural stability of SNase. Conservation information from the Lab 2 WebLogo supports this hypothesis, as the Lys at

position 63 is largely conserved across SNase-like genes from many organisms, suggesting it is important to the structure of SNase and that the mutation will disrupt SNase function. Investigations of the interaction between the wild-type ion pair and mutant ion pair further strengthens this prediction. In the wild-type, the length of the interaction is 2.7 Å, while it is 3.6 Å in the mutant (using least strain rotamers), suggesting that the structure will more easily be disrupted due to this weakened (longer) ionic interaction. Though the hypothesis predicts detrimental changes to SNase, the experimental process may produce beneficial results in increasing our understanding of the nature of proteins. The process highlights the relationship between structure and function, as the measurement of impaired SNase function allows inference of altered SNase structure. The relationship between environment and structure is highlighted by the consideration of the strength of the Coulombic interaction between Glu and Lys, as the weaker interaction of the mutant will likely break more easily than the wild-type in adverse conditions. Investigating the specific question of this experiment provides a gateway into answering the overarching questions of the course, and thus understanding the basic nature of proteins, the molecular machines of life.

Figure 1: The entire SNase protein shown as a cartoon, with the mutation site boxed in red, and the individual mutated residues (K63E and E67K) shown as sticks.



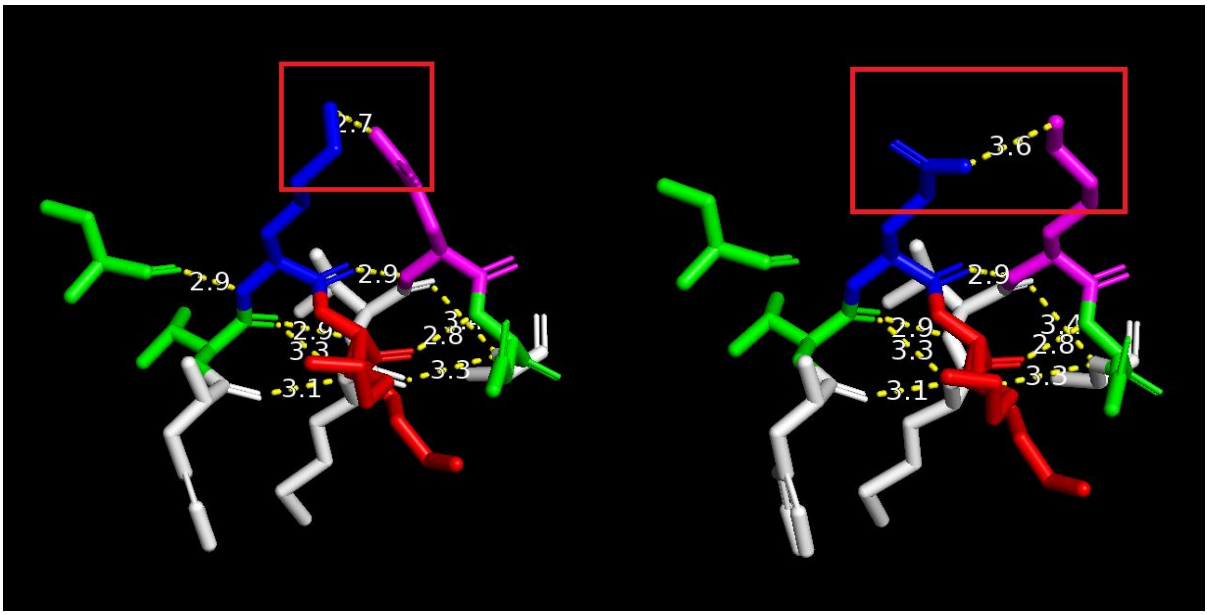


Figure 2: The mutation site in detail.

Left: Wild-Type SNase, with Lys63 in blue and Glu67 in magenta, also showing resiudes within H-bonding distance of both positions, with H-bonds shown as yellow dashes and distances indicated by number labels. The red box contains the Coulombic interaction. Polar residues are shown in green, nonpolar in white, and basic in red.

Right: Mutant SNase, with Glu63 in blue and Lys67 in magenta. Same coloring as wild-type.