Vibrational Spectroscopy and Normal Mode Analysis Applied to Human Prion Protein E200K:

Modeling The Effects of Targeted Microwave Radiation

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Executive Summary

The basis of our project is the excitation of normal modes of the human prion protein E200K (Protein Data Bank index 1FKC) in order to change its structure. The E200K prion has been linked to Creutzfeldt-Jakob disease, an incurable disease of the human brain. This protein is a mutated from of a protein normally found in the human body; the E200K variant transforms the normal state prion into another disease state protein and, in the process, causes neural degradation. This disease-prion relies heavily on the distribution of electric charges on its surface to latch onto and transform other prions. It was our hope to find a way to neutralize E200K prions with specific frequencies of microwave radiation by changing the molecular structure and thus surface charge distibution. Our program, in conjunction with the molecular modeling package TINKER, uses normal mode analysis to find the fundamental frequencies at which the E200K prion vibrates. We then modeled how the structure of the prion protein changed when it was bombarded with microwaves at the lower end of the molecule's fundamental frequency spectrum. We used both graphical visualizations and mathematical techniques to analyze the changes in molecular structure. We modeled the brief exposure of the prion to microwave radiation in both a vacuum and water environment. We had great success manipulating the E200K's structure in a vacuum but need to improve our techniques for modeling the prion's transformation in a more realistic water environment. The use of electromagnetic radiation to manipulate molecular structures holds great promise for revolutionizing the field of medicine; our project aims to further explore these possibilities and raise awareness about the theory and techniques involved with this novel utilization of normal mode analysis and vibrational spectroscopy.

Prion E200K

The E200K prion protein causes Creutzfeldt-Jakob disease, an incurable illness, by modifying the naturally produced PrP^{C} (prion protein) into the deadly pathogenic conformer PrP^{sc} . PrP^{C} is naturally made in the human body. The disease state variant of this protein, PrP^{sc} is created on the same gene codon but in a mutant state. Both of these prion proteins have remarkably similar structures, except in the flexible regions. The major differences are as follows: the PrP^{sc} has more Beta sheets, and the PrP^{c} has more alpha helixes, and the PrP^{sc} has a different electrostatic surface potential then the healthy form. This means that the electric charges covering the surface of the protein are arranged differently. Current research suggests that the proteins surface defects allow it to seek out and attach to the PrP^{c} variant. The E200K then acts like an enzyme, changing the structure of the substrate prion and making one chemical change at the 200^{th} codon. During the mutation process the $PrPs^{c}$ replaces a negatively charged glutamic acid with a positive charged lysine. This change results in the rapid transformation from PrP^{c} to PrP^{sc}. The loops and helixes of both of these prions are different. In one loop region this is critical because it provides a building site for Protein X, which is thought to be a crucial part in the transformation from PrP^{c} to PrP^{sc}.

The PrP^{c} utilizes these changes to covert healthy state prions to the disease state. Once the PrP^{sc} is created it transforms other normal PrP^{c} molecules into the disease state. This means that the rate of reproduction of the prion begins slow if there are few original E200K prions introduced, but accelerates rapidly after a long period of accumulation.

The disease can remain dormant for decades, making it even more deadly, for it can be passed on by the same person multiple times before that person even realizes that

they have the disease, and by then his donated blood has been given to someone else. The long incubation period is thought to be caused by the slow accumulation of PrP^{sc}. The PrP^{sc} collects in the host's brain, slowly decaying it. Eventually creating holes in the brain, and breaking down the nervous system, making Creutzfeldt-Jakob a spongiform disease. The PrP^{c}'s function is not fully understood but is believed to protect the brain from dementia and other degenerative problems associated with by old age. When the PrP^{c} is contorted into a PrP^{sc} it further onsets these symptoms in the host.

Project Goal

Our project goal was to find a way to make this dangerous prion, E200K, inert. There are currently no treatments for Creutzfeldt-Jakob disease and it is the most common prion disease. In order to render the PrP^{sc} harmless the activation sites, crucial for enzyme activity, must be rearranged in such a way that they are not functional. The way we chose to do this was to utilize specific frequencies of microwave radiation to cause the prion to vibrate and rearrange itself into a new form, where it cannot naturally return to its disease state, by activating the normal modes of the molecule. The use of electromagnetic waves to change the structure of proteins on the capsid of the Tobacco necrosis virus by Arizona State University researchers Eric C. Dykeman and Otto F. Sankey inspired our project. Their work is detailed in the essay Low Frequency Mechanical Modes of Viral Capsids: An Atomistic Approach. Their work was much more advanced and precise than ours, but utilizes many of the same basic principles. What follows is a detailed walkthrough of how the fundamental frequencies of the prion are found through normal mode analysis and then applied through vibrational spectroscopy.

One Dimensional Springs

Vibrational spectroscopy, with regard to altering the physical structure of the disease state form of the human prion protein E200K, is based on excitation of the molecule through activation of the normal modes. The normal modes are frequencies the molecule may vibrate at; they depend on both physical structure and forces acting both inside the molecular structure and interactions with the molecule's surroundings. The normal modes are derived using a combination of regular algebra, basic calculus, and linier algebra. The following section describes a method for creating and solving the matrix associated with normal mode analysis as well as an example modeling the simple three atom molecule CO_2.

To understand the derivation of normal modes it is not simplest to start on the scale of the individual atom or molecule, but in classical macro-physics. Picture two objects connected by a simple spring (one that obeys Hook's Law) with no outside forces are acting on the system. If the objects are left to rest they will stay a fixed distance away from each other—the spring will neither be extended past its rest length or contracted at all. This state is called the minimized state of the system; this is because the potential energy is at its minimal state. If an outside force pulls the two objects apart (extending the spring) or pushes them together (compressing the spring) a force acts between the two objects. The spring naturally wants to return towards its minimal state of energy and thus its natural length. The force that acts between the objects is described by Hook's law: $F = -k\Delta x$ where F is force, k is the spring constant (also called the force constant) of the spring adjoining the objects, and Δx is the change in length of the spring from its natural length (if the original length of the spring is x_0 and the new length is x_n then

$\Delta x = x_0 - x_n$). The negative sign means that the force is always the opposite the direction that the spring has been altered—i.e. force has direction. The force constant, *k*, represents the stiffness of the spring; if *k* is large the spring is hard to stretch, if *k* is small the spring is easy to stretch. If the system is mechanically excited, the spring is stretched or compressed, and there is no damping from outside forces then it will oscillate between stretching and compressing at a fixed frequency until some outside force stops it. The amount of time it takes the system to make one full cycle from the equilibrium point (minimum potential energy) to maximum extension (maximum potential energy) to the equilibrium point to maximum compression and back to the natural length is called the period of the spring and is given by $T = 2\pi\sqrt{m/k}$. At all times the center of mass ($\bar{x}$) of the system remains constant. The frequency, or number of cycles per second, in Hertz (Hz) is given as the reciprocal of the period. The potential energy of the system for any amount of stretch is described by: $\mu_e = {}^{-1}\!/_{2}\, k(\Delta x)^2$. Where μ_e is the potential energy. Note that this expression is a quadratic equation with its vertex at the minimum potential energy of the system. The first derivative of potential energy is the expression for force

$$\frac{\delta \mu_e}{\delta \Delta x} = F = -k\Delta x$$

The second derivative of potential energy is the opposite of the force constant.

$$\frac{\partial^2 \mu_e}{\partial \Delta x^2} = -k$$

This is an important relationship that makes matters simple for the time being, but will become more complicated later. The last point about the nature of the springs involves the displacement with respect to time. As the spring's length changes its motion is

described by a sine curve. The displacement of either object from its original position before excitation is given by the un-damped sine curve: $s(t) = A\sin(2\pi vt)$, where *A* is the amplitude of motion (it depends on how much the spring was originally stretched and is an arbitrary value for these calculations), *v* is the fundamental frequency of the system, and *t* is time. For this system there is only one possible value of *v* but for more complex systems there are a great many possible values—the calculation of all the possible values of *v* for our molecule is the ultimate goal of this normal mode analysis. Taking the first derivative of displacement, *s*(*t*), gives $s'(t) = (2\pi v)A\cos(2\pi vt)$. This represents the velocity of the object at any given time. The second derivative of displacement gives the acceleration with respect to time: $s''(t) = (4\pi^2 v^2)(-A)\sin(2\pi vt)$. The original expression of displacement is substituted into the equation for acceleration to yield: $s''(t) = (4\pi^2 v^2)(-s(t))$. Newton's second law of motion states that for any unbalanced force on an object force equals mass times acceleration ($F = ma$). Multiplying both sides of the expression by acceleration gives $m[s''(t)] = ma = F = -4\pi^2 v^2 m[s(t)]$. The force created by the extension/compression of the spring is given as the derivative of potential energy, $\frac{\delta \mu_e}{\delta \Delta x} = F = -k\Delta x$. Setting the forces equal to each other gives $-k\Delta x = -4\pi^2 v^2 m[s(t)]$. If the analysis of forces is not done with respect to time then the expression *s*(*t*) is equivalent to Δx and the normal mode analysis equation becomes $-k\Delta x_1 = -4\pi^2 v^2 m_2 \Delta x_2$. What this equation really represents is: the resultant displacement of object two due to the forces created by moving object one by some amount. The resultant displacement is dependent on the frequency that the object is moving at. Imagine the dual mass, single spring system again. If one mass is held still

while the other is moved and then the system is let go the second mass will be displaced by the force from the spring resulting from the movement of the first mass. In a simple one dimensional model like this one, the two displacements are equal and on the same line by virtue of Newton's third law of motion. This system becomes much more complicated when the objects are allowed to move in three dimensions.

Three Dimensional Spring Forces

Imagine, now, a cube, made up of eight masses at the points and each mass is connected to every other mass by a simple spring. Disregard that, at the center of the cube (and in the middle of each of the faces), the diagonal springs intersect each other; the important property of the springs is this. They represent a variable force between masses, their physical appearance is trivial. If any one of the masses is moved in any direction the forces created by the springs will cause all the other masses to move in various directions by various amounts. This is where the liner algebra aspect of normal mode analysis comes in to play. First: the system of masses and springs is a three-dimensional object in three-dimensional space, so all movement in space is broken down into components. The movement of a mass, m_n, from its minimized position (the minimized state of the cube is, again, when the potential energy of the system is at its lowest) is represented by $\Delta\mathbf{s}_n$. Note that **s** is boldface—this means that it represents a vector, which has both direction and magnitude; in the earlier example Δx had only one direction to represent so it was not written in boldface and only referred to magnitude. $\Delta\mathbf{s}_n$ can be broken down into unit vectors, one for each direction in x,y,z space: $\Delta\mathbf{s}_n = \Delta\mathbf{x}_n + \Delta\mathbf{y}_n + \Delta\mathbf{z}_n$. The unit vectors: $\Delta\mathbf{x}_n, \Delta\mathbf{y}_n,$ and $\Delta\mathbf{z}_n$ all represent distances along the x,y, and z axis, respectfully, from the original position of the mass m_n. Generally, at

this stage, all measurements of displacement are done so with the origin at the center of mass of the system (internal Cartesian coordinates), which is at the center of the cube in this case if all the objects are of equal mass. The center of mass with respect to any outside coordinate system is $(\bar{x},\bar{y},\bar{z})$.

So, if one mass is moved it will affect the position of every other mass, according the equation derived earlier: $-k\Delta\mathbf{s}_b = -4\pi^2 v^2 m_a \Delta\mathbf{s}_a$. This equation can be expanded using knowledge of vectors.

$$\begin{aligned}
-k_{xx}^{ab}\Delta\mathbf{x}_b - k_{xy}^{ab}\Delta\mathbf{y}_b - k_{xz}^{ab}\Delta\mathbf{z}_b &= -4\pi^2 v^2 m_a \Delta\mathbf{x}_a \\
-k_{yx}^{ab}\Delta\mathbf{x}_b - k_{yy}^{ab}\Delta\mathbf{y}_b - k_{yz}^{ab}\Delta\mathbf{z}_b &= -4\pi^2 v^2 m_a \Delta\mathbf{y}_a \\
-k_{zx}^{ab}\Delta\mathbf{x}_b - k_{zy}^{ab}\Delta\mathbf{y}_b - k_{zz}^{ab}\Delta\mathbf{z}_b &= -4\pi^2 v^2 m_a \Delta\mathbf{z}_a
\end{aligned}$$

This system of equations deals with the movement of two objects—m_a and m_b in three dimensions. m_b is being moved by some outside force, this causes the spring connecting it to m_a to change length and produce a force, displacing m_a by some amount. The left hand side of this equation deals with how m_b is being moved in space—in terms of x,y, and z. The right side shows how that force will cause m_a to be displaced. Note that the force on the left side has nothing to do with the mass of the object being moved, only the distance, whereas the force on the right side involves the mass of the object and the frequency of the oscillation. Going back to the cube, to find all the possible ways that one object can be moved, in each direction, the movements of every other object, in every direction, must be considered. If the eight masses are called m_1, m_2,… m_n … m_8 where $1 \le n \le 8$ and $n \in \square$. The system of equations describing the displacement of one atom: m_1 due to the displacement of any of the other atoms is:

$$-k_{xx}^{11}\Delta\mathbf{x}_1 - k_{xy}^{11}\Delta\mathbf{y}_1 - k_{xz}^{11}\Delta\mathbf{z}_1 - k_{xx}^{12}\Delta\mathbf{x}_2 - k_{xy}^{12}\Delta\mathbf{y}_2 - k_{xz}^{12}\Delta\mathbf{z}_1 ... - k_{xx}^{1n}\Delta\mathbf{x}_n - k_{xy}^{1n}\Delta\mathbf{y}_n - k_{xz}^{1n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_1\Delta\mathbf{x}_1$$
$$-k_{yx}^{11}\Delta\mathbf{x}_1 - k_{yy}^{11}\Delta\mathbf{y}_1 - k_{yz}^{11}\Delta\mathbf{z}_1 - k_{yx}^{12}\Delta\mathbf{x}_2 - k_{yy}^{12}\Delta\mathbf{y}_2 - k_{yz}^{12}\Delta\mathbf{z}_1 ... - k_{yx}^{1n}\Delta\mathbf{x}_n - k_{yy}^{1n}\Delta\mathbf{y}_n - k_{yz}^{1n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_1\Delta\mathbf{y}_1$$
$$-k_{zx}^{11}\Delta\mathbf{x}_1 - k_{zy}^{11}\Delta\mathbf{y}_1 - k_{zz}^{11}\Delta\mathbf{z}_1 - k_{zx}^{12}\Delta\mathbf{x}_2 - k_{zy}^{12}\Delta\mathbf{y}_2 - k_{zz}^{12}\Delta\mathbf{z}_1 ... - k_{zx}^{1n}\Delta\mathbf{x}_n - k_{zy}^{1n}\Delta\mathbf{y}_n - k_{zz}^{1n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_1\Delta\mathbf{z}_1$$

Again the right sides of the equations represent the resultant displacement of one object in the system, in this case object m_1 in any direction. The left side of the equation represents all the possible movements of all the other objects in the system in all directions and the forces they induce. This system of equations can then be expanded further, instead of looking at the resultant force and displacement of one object, depending on all the objects, the system can represent the resultant force/displacement of any object as a result of any movement of any other object, in any three dimensional space. For the eight object, three-dimensional system described earlier where $1 \le n \le 8$ and $n \in \mathbb{Z}$ the system of equations is:

$$-k_{xx}^{11}\Delta\mathbf{x}_1 - k_{xy}^{11}\Delta\mathbf{y}_1 - k_{xz}^{11}\Delta\mathbf{z}_1 - k_{xx}^{12}\Delta\mathbf{x}_2 - k_{xy}^{12}\Delta\mathbf{y}_2 - k_{xz}^{12}\Delta\mathbf{z}_1 ... - k_{xx}^{1n}\Delta\mathbf{x}_n - k_{xy}^{1n}\Delta\mathbf{y}_n - k_{xz}^{1n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_1\Delta\mathbf{x}_1$$
$$-k_{yx}^{11}\Delta\mathbf{x}_1 - k_{yy}^{11}\Delta\mathbf{y}_1 - k_{yz}^{11}\Delta\mathbf{z}_1 - k_{yx}^{12}\Delta\mathbf{x}_2 - k_{yy}^{12}\Delta\mathbf{y}_2 - k_{yz}^{12}\Delta\mathbf{z}_1 ... - k_{yx}^{1n}\Delta\mathbf{x}_n - k_{yy}^{1n}\Delta\mathbf{y}_n - k_{yz}^{1n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_1\Delta\mathbf{y}_1$$
$$-k_{zx}^{11}\Delta\mathbf{x}_1 - k_{zy}^{11}\Delta\mathbf{y}_1 - k_{zz}^{11}\Delta\mathbf{z}_1 - k_{zx}^{12}\Delta\mathbf{x}_2 - k_{zy}^{12}\Delta\mathbf{y}_2 - k_{zz}^{12}\Delta\mathbf{z}_1 ... - k_{zx}^{1n}\Delta\mathbf{x}_n - k_{zy}^{1n}\Delta\mathbf{y}_n - k_{zz}^{1n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_1\Delta\mathbf{z}_1$$
$$-k_{xx}^{21}\Delta\mathbf{x}_1 - k_{xy}^{21}\Delta\mathbf{y}_1 - k_{xz}^{21}\Delta\mathbf{z}_1 - k_{xx}^{22}\Delta\mathbf{x}_2 - k_{xy}^{22}\Delta\mathbf{y}_2 - k_{xz}^{22}\Delta\mathbf{z}_1 ... - k_{xx}^{2n}\Delta\mathbf{x}_n - k_{xy}^{2n}\Delta\mathbf{y}_n - k_{xz}^{2n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_2\Delta\mathbf{x}_2$$
$$-k_{yx}^{21}\Delta\mathbf{x}_1 - k_{yy}^{21}\Delta\mathbf{y}_1 - k_{yz}^{21}\Delta\mathbf{z}_1 - k_{yx}^{22}\Delta\mathbf{x}_2 - k_{yy}^{22}\Delta\mathbf{y}_2 - k_{yz}^{22}\Delta\mathbf{z}_1 ... - k_{yx}^{2n}\Delta\mathbf{x}_n - k_{yy}^{2n}\Delta\mathbf{y}_n - k_{yz}^{2n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_2\Delta\mathbf{y}_2$$
$$-k_{zx}^{21}\Delta\mathbf{x}_1 - k_{zy}^{21}\Delta\mathbf{y}_1 - k_{zz}^{21}\Delta\mathbf{z}_1 - k_{zx}^{22}\Delta\mathbf{x}_2 - k_{zy}^{22}\Delta\mathbf{y}_2 - k_{zz}^{22}\Delta\mathbf{z}_1 ... - k_{zx}^{2n}\Delta\mathbf{x}_n - k_{zy}^{2n}\Delta\mathbf{y}_n - k_{zz}^{2n}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_2\Delta\mathbf{z}_2$$
$$\vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \ddots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots \quad \vdots$$
$$-k_{xx}^{n1}\Delta\mathbf{x}_1 - k_{xy}^{n1}\Delta\mathbf{y}_1 - k_{xz}^{n1}\Delta\mathbf{z}_1 - k_{xx}^{n2}\Delta\mathbf{x}_2 - k_{xy}^{n2}\Delta\mathbf{y}_2 - k_{xz}^{n2}\Delta\mathbf{z}_1 ... - k_{xx}^{nn}\Delta\mathbf{x}_n - k_{xy}^{nn}\Delta\mathbf{y}_n - k_{xz}^{nn}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_n\Delta\mathbf{x}_n$$
$$-k_{yx}^{n1}\Delta\mathbf{x}_1 - k_{yy}^{n1}\Delta\mathbf{y}_1 - k_{yz}^{n1}\Delta\mathbf{z}_1 - k_{yx}^{n2}\Delta\mathbf{x}_2 - k_{yy}^{n2}\Delta\mathbf{y}_2 - k_{yz}^{n2}\Delta\mathbf{z}_1 ... - k_{yx}^{nn}\Delta\mathbf{x}_n - k_{yy}^{nn}\Delta\mathbf{y}_n - k_{yz}^{nn}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_n\Delta\mathbf{y}_n$$
$$-k_{zx}^{n1}\Delta\mathbf{x}_1 - k_{zy}^{n1}\Delta\mathbf{y}_1 - k_{zz}^{n1}\Delta\mathbf{z}_1 - k_{zx}^{n2}\Delta\mathbf{x}_2 - k_{zy}^{n2}\Delta\mathbf{y}_2 - k_{zz}^{n2}\Delta\mathbf{z}_1 ... - k_{zx}^{nn}\Delta\mathbf{x}_n - k_{zy}^{nn}\Delta\mathbf{y}_n - k_{zz}^{nn}\Delta\mathbf{z}_n = -4\pi^2\nu^2 m_n\Delta\mathbf{z}_n$$

Note that this is a system of 3*n* equations in 3*n* variables ($\Delta\mathbf{x}, \Delta\mathbf{y}, \Delta\mathbf{z}$ are the components of one variable $\Delta\mathbf{s}$, or displacement). Also important is the recursive nature of the system—for each solution on the right for a displacement that is then re-substituted into the equation for the corresponding displacement on the left. This system of equations is rather unwieldy. A matrix is formed of all the force constants (i.e. the second derivatives

of potential energy). The original set of all the forces in a matrix is called the Force-matrix or F-matrix, the matrix of all the second derivatives of μ_e is called the Hessian matrix. By factoring out all of the $\Delta\mathbf{x}, \Delta\mathbf{y}, \Delta\mathbf{z}$ components the system becomes:

$$\begin{pmatrix} -k_{xx}^{11}-k_{xy}^{11}-k_{xz}^{11}-k_{xx}^{12}-k_{xy}^{12}-k_{xz}^{12}\ldots-k_{xx}^{1n}-k_{xy}^{1n}-k_{xz}^{1n} \\ -k_{yx}^{11}-k_{yy}^{11}-k_{yz}^{11}-k_{yx}^{12}-k_{yy}^{12}-k_{yz}^{12}\ldots-k_{yx}^{1n}-k_{yy}^{1n}-k_{yz}^{1n} \\ -k_{zx}^{11}-k_{zy}^{11}-k_{zz}^{11}-k_{zx}^{12}-k_{zy}^{12}-k_{zz}^{12}\ldots-k_{zx}^{1n}-k_{zy}^{1n}-k_{zz}^{1n} \\ -k_{xx}^{21}-k_{xy}^{21}-k_{xz}^{21}-k_{xx}^{22}-k_{xy}^{22}-k_{xz}^{22}\ldots-k_{xx}^{2n}-k_{xy}^{2n}-k_{xz}^{2n} \\ -k_{yx}^{21}-k_{yy}^{21}-k_{yz}^{21}-k_{yx}^{22}-k_{yy}^{22}-k_{yz}^{22}\ldots-k_{yx}^{2n}-k_{yy}^{2n}-k_{yz}^{2n} \\ -k_{zx}^{21}-k_{zy}^{21}-k_{zz}^{21}-k_{zx}^{22}-k_{zy}^{22}-k_{zz}^{22}\ldots-k_{zx}^{2n}-k_{zy}^{2n}-k_{zz}^{2n} \\ \vdots \quad \vdots \quad \vdots \quad \vdots \quad \ddots \quad \vdots \quad \vdots \\ -k_{xx}^{n1}-k_{xy}^{n1}-k_{xz}^{n1}-k_{xx}^{n2}-k_{xy}^{n2}-k_{xz}^{n2}\ldots-k_{xx}^{nn}-k_{xy}^{nn}-k_{xz}^{nn} \\ -k_{yx}^{n1}-k_{yy}^{n1}-k_{yz}^{n1}-k_{yx}^{n2}-k_{yy}^{n2}-k_{yz}^{n2}\ldots-k_{yx}^{nn}-k_{yy}^{nn}-k_{yz}^{nn} \\ -k_{zx}^{n1}-k_{zy}^{n1}-k_{zz}^{n1}-k_{zx}^{n2}-k_{zy}^{n2}-k_{zz}^{n2}\ldots-k_{zx}^{nn}-k_{zy}^{nn}-k_{zz}^{nn} \end{pmatrix} \begin{pmatrix} \Delta\mathbf{x}_1 \\ \Delta\mathbf{y}_1 \\ \Delta\mathbf{z}_1 \\ \Delta\mathbf{x}_2 \\ \Delta\mathbf{y}_2 \\ \Delta\mathbf{z}_2 \\ \vdots \\ \Delta\mathbf{x}_n \\ \Delta\mathbf{y}_n \\ \Delta\mathbf{z}_n \end{pmatrix} = -4\pi^2\nu^2 \begin{pmatrix} m_1\Delta\mathbf{x}_1 \\ m_1\Delta\mathbf{y}_1 \\ m_1\Delta\mathbf{z}_1 \\ m_2\Delta\mathbf{x}_2 \\ m_2\Delta\mathbf{y}_2 \\ m_2\Delta\mathbf{z}_2 \\ \vdots \\ m_n\Delta\mathbf{x}_n \\ m_n\Delta\mathbf{y}_n \\ m_n\Delta\mathbf{z}_n \end{pmatrix}$$

The components are 'factored' out into a separate matrix according to the rules of matrix multiplication. If the matrices are multiplied out they form the system of equations in the above step.

Working With the Hessian

Next the system is mass weighted to produce a more symmetric matrix. The process seems to complicate the equation in the short term. It ultimately makes the eigenvector/eigenvalue process simpler. The mass weighted coordinate system is based on the identity: $\Delta\tilde{\mathbf{s}}_n = \sqrt{m_n}\Delta\mathbf{s}_n$. This form is achieved by dividing all the displacement entries of the matrix on the right-hand side of the equation by the square-root of the corresponding mass term. When this is done to both sides of the equation, in order for both sides to remain equal, each *k* value is divided by two square-root mass terms—the

affecting and affected objects. So $k_{xx}^{ab} \longrightarrow \tilde{k}_{xx}^{ab} \Big/ \sqrt{m_a}\sqrt{m_b}$. This entire matrix, in the case of the cube and 3(8)x3(8) or 24x24, can be written in the form of:

$$\mathbf{H} = \left[k_{ij} \right] \text{ and } k \longrightarrow \begin{pmatrix} -\tilde{k}_{xx}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{xy}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{xz}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} \\ -\tilde{k}_{yx}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{yy}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{yz}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} \\ -\tilde{k}_{zx}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{zy}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{zz}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} \end{pmatrix}$$

$$\tilde{\mathbf{H}} = \left(\begin{pmatrix} -\tilde{k}_{xx}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{xy}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{xz}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} \\ -\tilde{k}_{yx}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{yy}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{yz}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} \\ -\tilde{k}_{zx}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{zy}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} & -\tilde{k}_{zz}^{ij} \Big/ \sqrt{m_i}\sqrt{m_j} \end{pmatrix}_{ij} \right) \quad \tilde{\mathbf{S}} = \begin{pmatrix} \Delta\tilde{\mathbf{x}}_1 \\ \Delta\tilde{\mathbf{y}}_1 \\ \Delta\tilde{\mathbf{z}}_1 \\ \vdots \\ \Delta\tilde{\mathbf{x}}_n \\ \Delta\tilde{\mathbf{y}}_n \\ \Delta\tilde{\mathbf{z}}_n \end{pmatrix}$$

$$\tilde{\mathbf{H}}\tilde{\mathbf{S}} = -4\pi^2 v^2 \tilde{\mathbf{S}} \quad 1 \le i \le n \; 1 \le j \le n$$

This then becomes the final form of the equation used for normal mode calculation. To actually solve the secular equation and get the possible values of the fundamental frequencies, or normal modes, a process called diagonalization is then used to derive the eigenvalues and eigenvectors. This system is actually more complex then it appears, further information is needed to fully comprehend it.

Simple Springs, Angular Springs, Torsion Springs

The whole basis of this system of equations and matrix relies on some assumptions about the meaning of *"k."* Earlier it was defined as the spring constant of a simple, linear spring—or the second derivative of the potential energy stored in a spring. This is fine for a very small and predominantly linear system; as the complexity of the system increases, however, some other parts of the constant *k* must be considered. Take,

for instance, the cube discussed earlier. Every point is connected to every other object with a spring that can compress and stretch in a linear fashion. Call the spring constant associated with this stretch k_α. As the masses move around there is also some bending of the angles between the springs. The angles will naturally want to return to their equilibrium points. In the case of the cube all angles equal 90 degrees. Another form of Hook's law applies to this situation. Instead of considering the 'stretching energy' of the spring, the 'bending energy' is considered. If Θ_0 is the angle of the springs in the system's minimized position and Θ is the new angle, after displacement of an object, then the bending energy involved is given by: $\mu_\Theta = -\frac{1}{2} k_\Theta (\Theta - \Theta_0)^2$. The second derivative of this expression is:

$$\frac{\delta^2 \mu_\Theta}{\delta \Theta^2} = -k_\Theta$$

This yields another constant to be factored into the Hessian matrix. The values of *k* represented in the matrix $\tilde{\mathbf{H}}$ are the sum of all the spring constants and partial derivatives of all the forces acting between the objects. As far as springs go there is only one more physical process to be explored: torsion. Not only can the lengths and angles between objects change, but they can also rotate about an axis. Take, for example, one of the faces of the cube. Call the square $abcd$. $\angle abc = \angle bcd$ and $\overline{ab} = \overline{bc} = \overline{cd}$. If the mass, *a*, is moved to the new position *a'*, but $\angle a'bc = \angle abc = \angle bcd$ ($\angle ba'd \neq bad$) and $\overline{a'b} = \overline{ab} = \overline{bc} = \overline{cd}$ then the angle $\angle a'ba = \phi$ is the angle of rotation, or the angle of twist. This idea works for any four object shape, if the angle between two planes, defined by triangles made of three points on the perimeter of the shape, changes from its minimized state then the system is considered to be a torsion spring. If the angle ϕ_0 is the original

position and ϕ is then new angle then the torsion energy stored is given as:

$\mu_\phi = -\frac{1}{2} k_\phi (\phi_0 - \phi)^2$. The second derivative of torsional potential energy is -k_ϕ. So far,

$k^{ij} = k_\alpha + k_\Theta + k_\phi$. For the most part, these are the only ways that macroscopic structures can move around: stretch, bend, and twist. The ultimate goal, however, involves analysis of forces on a microscopic scale.

Covalent and Ionic Bonds

Next, it is necessary to explore forces and force constants on the intermolecular scale. In this case the objects are no longer just floating masses, but nuclei of atoms. There are no springs attaching the nuclei to each other, instead there are electromagnetic forces acting between all of them. Atoms can be attached to one another in one of two ways; they can be bonded together due to interactions of electron clouds or they can interact through non-bonded forces.

The two main groups of chemical bonds relevant to the human prion protein, E200K, are covalent bonds and ionic bonds. Covalent bonds occur when two atoms share a valence, or outer orbital, electron between them. The electron cloud spreads between the two atoms and this holds them together. This sharing of electrons may be even (pure covalent bonding) or uneven (polar covalent bonding). If the electron is pulled so much by one atom away from another that it is considered to have 'left' its original nucleus then the bond is ionic. Covalent and ionic bonds are the strongest forces that generally exist between two atoms in a molecule. Though the equations governing the force between the two atoms are complex they are approximated with the three macroscopic phenomena listed earlier. Within very short distances from the minimized state of the molecule the bond forces are approximated very well with Hook's Law

(although the approximation breaks down over greater distances). So covalent, polar covalent, and ionic bond interactions between atoms in the protein are dealt with by summing the spring constants of stretching, bending, and twisting.

Non-Bonded Force Interactions

The nature of the atom, a positive charged nucleus surrounded by numbers of negatively charged electrons, gives rise to additional internal molecular forces that must be considered. These non-bonded interactions link every atom in the protein to every other atom, as well as to any and all atoms in the environment, by way of longer-range force fields. These forces are generally termed "van der Waals" forces after the Dutch scientist Johannes Diderik van der Waals. This set of forces describes the interactions between ions and neutral molecules (or segments of molecules). It contains both an attractive (at long distances) and repulsive part (at shorter distances). The attractive component of the van der Waals force field has three main components. The first, and strongest, involves the interactions of two permanent multipoles. When two atoms are bonded together and one has a higher electronegativity then the other the electron cloud is unevenly distributed—creating a section of the molecule with a net positive ($\delta+$) charge and a section with a net negative charge ($\delta-$). This is called a dipole moment. A molecular section that has multiple dipole moments has a multipole. The unlike charged sections of the molecule attract and the like charges repel (intramolecular force). These multiple moments in a molecule, like a prion, also interact with the molecules surrounding it (intermolecular force). The polarity of the sections of the molecule are determined by the polar-covalent or ionic bonds acting. This is called a fixed dipole-dipole van der Waals-Keesom force, sometimes referred to as a Hydrogen bond (or

sometimes a electrostatic dipole-dipole interaction). Hydrogen bonding is the strongest of the van der Waals forces, but is not considered in the same category as covalent or ionic bonding for molecular modeling. The second source of attraction in the van der Waals forces is due to induction or polarization. Because the electron cloud's position around the nucleus is variable, other surrounding charges can cause changes in the polarity of a molecule. A permanent dipole or multipole can induce another section of the molecule (or a separate molecule) to become polarized. The resulting interaction produces a force between the two charges (again this can be intermolecular or intramolecular). These forces are generally not as strong as hydrogen bonds. The final part of the van der Waals force is called the London dispersion force. It arises from interactions of two or more temporary dipole moments. These may stem from transient polar moments and/or induced moments. Neither case involves a fixed multipole moment created by a chemical bond. Quantum mechanics dictates that the distribution of charge around a nucleus is constantly in flux (due to the uncertainty in position of the electron, governed by the Heisenberg Uncertainty Principle: $\Delta x \Delta p \geq \hbar$ or uncertainty in position times uncertainty in momentum is greater then or equal to Plank's constant over two). The electron density of the cloud moves around the atom probabilistically, so at any given point in time a temporary (or transient) dipole moment can exist in a neutral molecule. Generally this dipole moment will only last for 10^{-15} to 10^{-14} seconds before dissipating. This momentary redistribution of charge in one atom can then induce polarization in other atoms. This will then repolarize the original atom after its random electron motion disrupts the force field—causing the atoms to polarize each other (oscillating off and on) and create a continuous force. This force occurs between all

molecules and atoms, but is so week in comparison to the other van der Waals and bonding forces that it is noticeable only when seen alone (generally over longer distances). The interaction between two, or more, temporarily polarized molecules is dubbed the London dispersion force.

Lennard-Jones Potential and the Electrostatic Term

All three of the above mentioned forces are more complicated then the bond forces (where the force equations can be approximated by a direct relationship of force to distance). The van der Waals forces are all relate force to distance via elaborate exponential functions that are too complex to be useful in this normal mode analysis. Very commonly the potential energy created by the van der Waals forces is approximated by a mathematical model called the Lennard-Jones potential. Lennard-Jones (L-J) potential approximates the combinations of potential energy equations for the attractive forces of: Hydrogen bonding (van der Waals-Keesom interaction), dipole-induced dipole interactions, and London Dispersion forces; as well as the short range repulsive part of the force due to Pauli repulsion (the orbits of negatively charged electrons resisting being in proximity to one another). The equation looks like:

$$\mu_v = 4\varepsilon\left[\left(\frac{\sigma}{d}\right)^{12} - \left(\frac{\sigma}{d}\right)^{6}\right]$$

Where μ_v is potential energy, ε is the minimum potential energy of the system (called the van der Waals minimum, the distance at which the attractive and repulsive forces cancel each other out), σ is the distance between the two atoms when the potential energy is at a minimum, and d is the distance between the two atoms. μ_v is a function of d where ε and σ are constants. Notice that this function's second derivative does not resolve to a

constant, *k*, like the various spring approximations did. At distances very close to the minimized state the equation for L-J potential is sometimes approximated by a quadratic (that does resolve to a constant) equation—In our program the entire second derivative is used in the Hessian matrix.

The final component in the term, k^{ij}, is derived by treating each atom as a simple point charge. If the whole atom is regarded as one ball that has a net charge of *q*, then the force between two atoms is given by Coulomb's Law:

$$F = \frac{q_1 q_2}{4\pi\varepsilon_0 d^2}$$

where q_1 and q_2 are the net charges, ε_0 is the electric permittivity of free space, and *d* is the distance between the two atoms. The potential energy utilized by the two atoms in proximity to one another is the integral of the force equation with respect to *d*. The potential energy is called Coulomb potential and the equation describing it, "the electrostatic term."

$$\mu_c = \frac{q_1 q_2}{4\pi\varepsilon_0 d}$$

The Final Form of "*k*"

Now the final meaning of the entries, k^{ij}, in the Hessian matrix is apparent. The values for $\tilde{k}^{ij}$ are the sum, in component form, of all the second derivatives of potential energy equations. Though the true physical processes are even more complex, this equation is what the program we are using, parameters set AMBER99, uses to compute the Hessian Matrix. The net potential energy between a set of two atoms is the sum of all

the sources of potential energy: stretching bonds, bending angles, twisting structures, L-J potential, and Coulomb potential (electrostatic term):

$$\mu_{net} = -\frac{1}{2}k_{\alpha}(d-d_0)^2 - k_{\Theta}(\Theta-\Theta_0)^2 - \frac{1}{2}k_{\phi}(\phi-\phi_0)^2 - 4\varepsilon\left[\left(\frac{\sigma}{d}\right)^{12} - \left(\frac{\sigma}{d}\right)^{6}\right] - \frac{q_i q_j}{4\pi\varepsilon_0 d}$$

Then the angles are rewritten using trigonometric functions to put them in terms of *d*. The *k* function is then the second derivative of potential energy with respect to distance (or position).

$$k^{ij} = \frac{\partial^2 \mu_{net}}{\partial d^2}$$

Then the distances are written in terms of vector displacements ($\Delta\mathbf{s}$), and broken down into component form ($\Delta\mathbf{x}, \Delta\mathbf{y}, \Delta\mathbf{z}$). Then they are substituted into the $\tilde{\mathbf{H}}$ matrix. The result is the Hessian matrix and the normal mode analysis equation. The parameters set AMBER99 (or Assisted Model Building and Energy Refinement, 1999) provides all necessary values for constants for any chemical bond or other force acting.

When this system of equations is solved to produce the eigenvalues and eigenvectors we get the fundamental frequencies of the molecule. There are a total of $3n-6$ frequencies for any non-linear molecule (six of the frequencies produced cause the center of mass to move and are thus not valid for our purpose). In the case of the prion E200K, 1734 atoms, this produces 5196 frequencies. Of these frequencies we then pick one of the lowest frequencies for use in normal mode for excitation. Generally the lower frequencies allow for greater displacements of large sections of the molecule while smaller scale structures remain mostly intact and individual atoms do not move around as much. There is a program in the molecular dynamics package, TINKER, that selects the

most efficient frequency based on desired displacements. Once the frequency is chosen we use it to produce movement in the molecule through the property of resonance.

Resonance and Problems With the Model

Resonance is quite well known in its mechanical sense; a sound wave from one tuning fork will cause a second tuning fork of the same fundamental frequency to vibrate. Resonance, the idea that an object vibrating at its natural frequency will force another object of the same natural frequency to vibrate, works not only with sound waves, but with all kinds of waves—including electromagnetic waves. If the E200K protein is bombarded with electromagnetic waves that are very close to one of its normal modes it will be forced to vibrate at that frequency and thus its atoms will move in accordance with the displacement vectors associated with that frequency. The frequency of the EM wave we are hitting the modeled protein with is in the range of microwaves. If we introduce enough energy into the prion quickly enough the amplitude of vibration will be great; actually causing the molecule to refold in a different structure with a different minimized state. If enough movement of large structures in the prion is achieved then the imaginary springs connecting the atoms are stretched out enough so that they will reform in a different configuration. Then the excess energy will be re-radiated or lost to heat—allowing the molecule to stay in this new arrangement. This could potentially change the structure of a damaging protein into something harmless by rearranging the positions of the activation sites.

The main problems with this process are due to absorption, anharmonicity, and instantaneous modes. If the calculated frequency is not close enough to the true frequency, the molecule will not absorb the energy very well, and a much higher

amplitude (higher intensity) wave is required to excite the molecule. Any solvent surrounding the molecule will also likely absorb a great deal of the energy before it reaches the prion. Anharmonicity is a problem created by overtones. If you imagine a guitar string, the string does not only vibrate at one frequency; its rich sound quality is made up of multiple notes (frequencies) emanating from one vibration. The majority of the vibration is at the fundamental frequency with decreasing amounts of vibrational energy being transferred into integer multiples of that frequency. If this happens in the molecular vibration it can disrupt the movement of the atoms. We get around this problem by not allowing the molecule to vibrate for very long, we use one burst of radiation for an extremely short period of time—so anharmonicity does not develop. The final problem comes down to flaws in approximation. The normal mode frequencies are only valid fundamental frequencies when the molecule is in, or very close to, its minimized state; as soon as the molecule starts to move the frequencies change to something called an instantaneous mode. The instantaneous mode is the fundamental frequency in a non-minimized state, using more accurate models and factoring in the kinetic energy of the moving atoms. This requires use of a G-Matrix rather the F-Matrix, a much more complicated problem. We, again, are working around this problem by applying all the energy to the prion very quickly, before it can move enough to significantly change the fundamental frequency and reduce the absorption of the EM waves.

The Carbon Dioxide Example

The last part of this section, devoted to background research and theoretical understanding, gives an example of the process involved in finding normal modes with a

much-simplified model of a carbon dioxide atom. If we focus only on the stretch of the bonds in one direction (the **x** component) and use only the simple spring approximation (ignoring Lennard-Jones Potential and Coulomb forces) it greatly reduces the complexity of the problem. The carbon dioxide molecule is linear, an oxygen double bonded to a carbon in the center with another oxygen on the opposite side. Atom 1 is the left hand oxygen, atom 2 is the center carbon, and atom 3 is the right hand oxygen. The mass weighted Hessian matrix for this problem is:

$$-\begin{pmatrix} k_{xx}^{11}/\sqrt{m_1}\sqrt{m_1} & k_{xx}^{12}/\sqrt{m_1}\sqrt{m_2} & k_{xx}^{13}/\sqrt{m_1}\sqrt{m_3} \\ k_{xx}^{21}/\sqrt{m_2}\sqrt{m_1} & k_{xx}^{22}/\sqrt{m_2}\sqrt{m_2} & k_{xx}^{23}/\sqrt{m_2}\sqrt{m_3} \\ k_{xx}^{31}/\sqrt{m_3}\sqrt{m_2} & k_{xx}^{32}/\sqrt{m_3}\sqrt{m_2} & k_{xx}^{33}/\sqrt{m_3}\sqrt{m_3} \end{pmatrix} \begin{pmatrix} \tilde{\mathbf{x}}_1 \\ \tilde{\mathbf{x}}_2 \\ \tilde{\mathbf{x}}_3 \end{pmatrix} = -4\pi^2 v^2 \begin{pmatrix} \tilde{\mathbf{x}}_1 \\ \tilde{\mathbf{x}}_2 \\ \tilde{\mathbf{x}}_3 \end{pmatrix}$$

Next we use Newton's third law of motion to equate some of the terms; this is not often possible due to the complexity of the L-J potential and electrostatic term. $k_{xx}^{12} = k_{xx}^{21}$ and $k_{xx}^{32} = k_{xx}^{23}$ because of Newton's third law of motion. We assume $k_{xx}^{13} = k_{xx}^{31} = 0$ because they are not directly bonded together and the non-bonded interaction forces are not considered. The masses of each atom are inserted, in atomic mass units (carbon is 12amu and oxygen is 16amu). Finally the values of *k* are inserted. Because k_{xx}^{12} is attached to k_{xx}^{11} by a bond, the spring constant between the two is the same, but the direction opposite so $k_{xx}^{11} = -k_{xx}^{12}$. The same logic can be applied to k_{xx}^{11} and k_{xx}^{21} as well as $k_{xx}^{33} : k_{xx}^{23}$ and k_{xx}^{33} k_{xx}^{32}. The approximate force constant for k_{xx}^{11} is picked to be 1600Nm^{-1}, which, because of symmetry is assumed to be the same as k_{xx}^{33} (they are both double bonds between oxygen and carbon). k_{xx}^{22} is approximately 3200Nm^{-1}. Applying the above identities produces:

$$-\begin{pmatrix} {}^{1600}\!/_{\sqrt{16}\sqrt{16}} & {}^{-1600}\!/_{\sqrt{16}\sqrt{12}} & 0 \\ {}^{-1600}\!/_{\sqrt{12}\sqrt{16}} & {}^{3200}\!/_{\sqrt{12}\sqrt{12}} & {}^{-1600}\!/_{\sqrt{12}\sqrt{16}} \\ 0 & {}^{-1600}\!/_{\sqrt{16}\sqrt{12}} & {}^{1600}\!/_{\sqrt{16}\sqrt{16}} \end{pmatrix}\begin{pmatrix} \tilde{\mathbf{x}}_1 \\ \tilde{\mathbf{x}}_2 \\ \tilde{\mathbf{x}}_3 \end{pmatrix} = -4\pi^2 v^2 \begin{pmatrix} \tilde{\mathbf{x}}_1 \\ \tilde{\mathbf{x}}_2 \\ \tilde{\mathbf{x}}_3 \end{pmatrix}$$

This reduces to:

$$\begin{pmatrix} -100 & 115.47 & 0 \\ 115.47 & -266.67 & 115.47 \\ 0 & 115.47 & -100 \end{pmatrix}\begin{pmatrix} \tilde{\mathbf{x}}_1 \\ \tilde{\mathbf{x}}_2 \\ \tilde{\mathbf{x}}_3 \end{pmatrix} = -4\pi^2 v^2 \begin{pmatrix} \tilde{\mathbf{x}}_1 \\ \tilde{\mathbf{x}}_2 \\ \tilde{\mathbf{x}}_3 \end{pmatrix}$$

Taking the eigenvalues and eigenvectors produces only two valid solutions. Eigenvalue$_1$=-100, Eigenvectors$_1$=-0.707107, 0, 0.707107 and Eignevalues$_2$=-366.669, Eigenvectors$_2$=-0.369272, 0.852805, -0.369272. Converting the units on the eigenvalues and then turning them into wavenumbers (a wavenumber is the reciprocal of wavelength, and $v = f\lambda$ so wavenumber equals frequency divided by velocity) produces E_1=1303cm^{-1} and E_2=2495cm^{-1}. The actual wavenumbers for the corresponding frequencies are E_1=340cm^{-1} and E_2=2349cm^{-1}. There is a lot of error, but a lot of assumptions and simplifications were made. The first eigenvalue is quite inaccurate, and the second set of eigenvectors show the center mass moving directly through the rightmost mass—which cannot happen in a real world situation. Looking at the eigenvectors, the first set shows symmetric stretch (both masses moving the same amount in opposite directions) and the second set represents asymmetric stretch (the two outer masses moving in the same direction and the center mass opposite—the center of mass remains constant, however, because the single light mass moves much farther then the two heavier ones).

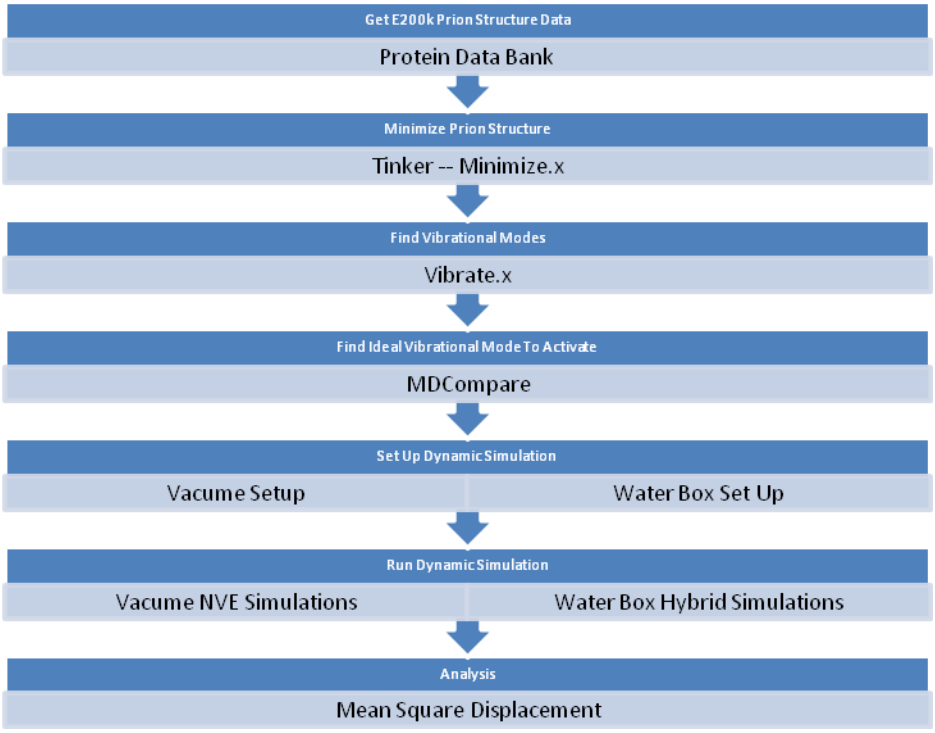
The Computer Model: Tinker

Tinker is both a molecular dynamics and molecular mechanics modeling packages. This package uses the 1999 version of amber force constants to model the interactions between atoms.

We felt it to be an ideal choice because it is open source and written in Fortran, a language well suited for large mathematical models that we are capable of understanding and modifying. Most importantly, however, it contained necessary programs that could run that could run the necessary functions required for our project.

Program Flow

The project may be broken down into many components. First we obtained the structural data for the E200K variant Prion through the protein data bank which contains data, obtained through x-ray crystallography in addition to other methods, on may different proteins. After obtaining the structural data we minimized the protein for reason described early. Next we ran the tinker program vibrate.x, which we slightly modified so it would automatically print all the vibrational modes of the molecule. Next we ran our program MDCompare which evaluated the modes and ranked them in order of interest to our goal of deforming the Prion. Next, we ran another of our programs to set up a file to run a Microcanonical Ensemble simulation that modeled the trajectories of the atoms assuming that the Prion was activated with a specific frequency of electromagnetic radiation and absorbed a certain amount of energy.



Minimizing the Protein

Tinker's program to find the vibrational modes of a molecule is limited to molecules in a minimized state where the molecule's potential energy exists in a local minima meaning that if the molecule was slightly perturbed in any fashion it would gain potential energy. Although the theory behind calculating fundamental frequencies or normal modes of molecules that are not minimized and that have kinetic energy, an implementation of this algorithm is unknown to our team and the complexity of the theory and time needed to incorporate such a model made this expansion unfeasible for our team.

For the simulations run inside of a vacuum this is a good approximation for what the structure would look like were it inside of a vacuum. In addition, the original protein structure we received from the protein data bank had been minimized according to their force field. Since the exact nature of intermolecular force fields remains unknown to the

scientific community, there are many different programs that approximate intermolecular forces and they all are slightly different.

However, for the simulations inside of a water box, which more closely resembles a blood sample, this method fails to take into account the change in structure induced by the water as well as the increase in kinetic energy due to the ambient heat of the blood. Thus, if this method was to be considered for application, a more accurate method of finding the vibrational modes is suggested.

Finding Ideal Vibrational Modes

In choosing vibrational modes to activate we felt that it would be most effective to find the ones that appeared they would most effectively change the conditions we consider in the analysis of the final, modified, Prion. Thus, we searched for Modes that most effectively change the electrostatic surface potential and that would specifically target our two identified activation sites where it is believed the E200K Prion attaches onto other in order to get the host to conform to its structure and become a pathogenic Prion.

In considering which modes would change the electrostatic surface potential the most we determined that a change in atomic structure of the Prion would directly equate to a change in the surface potential. In addition we found that water, which is a major component of blood, absorbs less energy near the visible spectrum. We believe that the more energy the Prion absorbs the more it will deform; thus a high frequency mode that is close to the visible region is the ideal choice for this criterion.

In addition we specifically observed the activation sites of the Prion which were located at atoms 697-769 and 1138-1207. We found modes where all or most of the

absorbed energy translated into the movement of these groups of atoms. In quantifying this displacement we used the composite of the Mean Square Displacement Theorem one each site.

$$MSD = \left\langle \left| r(t) - r(0) \right|^2 \right\rangle$$

We found these two selection criterion to be complementary to each other. Absorption in the form of molecular vibrations generally occurs with wavelength smaller than that of Infra-red radiation. In addition normal modes that are localized to particular regions tend to be higher frequency where as the lower frequency modes create more global movements of the atoms in the protein. Thus the criteria we observed lead us to choose a higher frequency vibration mode.

Prion Vacuum Simulation

The fist series of trials deforming the E200k variant of the human Prion are conducted in a vacuum. These simulations involved the activation of normal modes of the Prion assuming 0 to ____ J of energy is absorbed from an electromagnetic wave. This instantaneous mode activation is translated into velocities of each atom in the Prion.

This data is inserted into the molecular dynamics Microcanonical(NVE) ensemble simulation included with the Tinker molecular modeling package. This computer model determines the trajectories of each atom while conserving the amount of atoms, volume in which they are contained, and the energy of the system. They are run for a period 20 picoseconds with a time step of 1 femtosecond, which is the default for tinker. It was observed that after 10ps many of the proteins in the NVE simulation

stopped deforming thus it was decided that a run length of 20 ps would allow sufficient time for the Prion to reach a different local minima.

The scope of the project is to render inert the E200k mutant Prion in blood donations, which makes this simulation inside of a vacuum unrealistic. By failing to take into account a solvent, the ability to deform is greatly increased because as the atoms of the Prion move, they do not interact with other molecules in the environment.

Prion Water Box Simulation

Water is chosen as a solvent to submerse the Prion for the molecular dynamic simulations because it accounts for about 83% of human blood. In addition, the polarization of water is related to the protein folding in such a way that the hydrophilic parts align themselves towered the exterior while the hydrophobic portions dwell in the interior of the Prion protein.

This simulation consisted of a hybrid of both the Microcanonical ensemble and canonical ensemble. We recognized that blood samples are not stored at absolute zero, so we assumed a temperature 298k for the blood samples to be stored at. After heating up the water along with the protein a vibrational mode for each simulation was activated in a Microcanonical ensemble, which conserves energy as compared to temperature. We believe that during the activation of the normal mode, energy conservation is more important because the electromagnetic wave is providing energy that will heat up a sample of blood making temperature no longer a conserved quantity.

The tinker molecular modeling package did not come well suited for the creation and use of a water solvent. Initially the water type hydrogen of the 1999 version of amber parameters lacked a Van-Der-Waals radius causing any simulation involving water

molecules to become unstable and crash due to the occurrence of infinite energies created by the hydrogen atoms overlapping other atoms. Thus, we decided to create insert our own parameters into the 1999 amber parameter set. We used structural data of the water molecule from London South Bank University. Additionally, we used force constants from the CHARMM force field parameter set. This information allowed for the formulation of a fairly stable water box.

The water box is created using the molecular mass and specific gravity. It is found that the specific gravity of water is 995.65 Kg/m^3 at around 300 degrees Kelvin. Thus:

$$Specific\ gravity\ of\ water = \frac{9.9565 * 10^{3} Kg}{m^3} * \frac{10^3 g}{Kg} * \frac{m^3}{(10^{10})^3 A^3} = \frac{9.9565 * 10^{-25} g}{A^3}$$

Then by using the molecular mass of water, 18.01508, the number of molecules per gram is found:

$$Molecules\ Per\ Gram = \frac{6.0221417930 * 10^{23} molecules}{mol} * \frac{1\ mol}{18.01508 g} = \frac{3.342833778 * 10^{22}\ molecules}{g}$$

Knowing these two values it is then possible to determine the number of molecules per Angstrom cubed in a sample of water:

$$Atoms\ per\ Angstrum\ cubed = \frac{9.9565 * 10^{-25} g}{A^3} * \frac{3.342833778 * 10^{22} molecules}{g} = \frac{3.328292451 * 10^{-2}\ molecules}{A^3}$$

This information may then be used in a computer model to create a box of water to submerse the Prion protein.

The water box used in the simulations has sides of 40 angstroms. Using the xyzedit tool from tinker the E200K mutant Prion is submersed into the water. Initially it was believed that the 40 angstrom cube would be small enough to keep a relatively short runtime while large enough to completely submerse the protein. However, later observations proved that the cube was not large enough to house the protein which

caused an increase of the box size and the dispersion of the water molecules during the simulation as shown in the visual representations below.

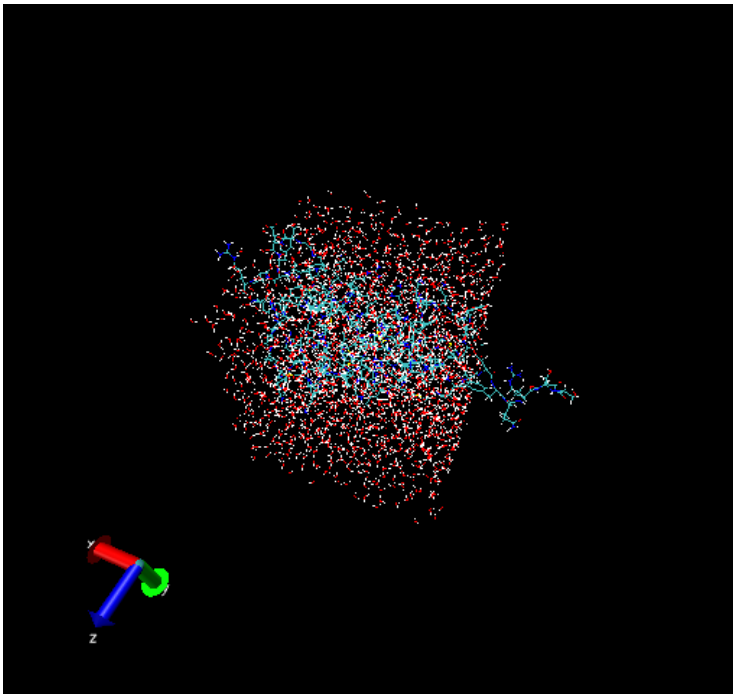


Figure 1 before Activation of Normal Mode. Notice the Prion, the blue and turquoise atoms, is not completely contained within the red and white water molecules

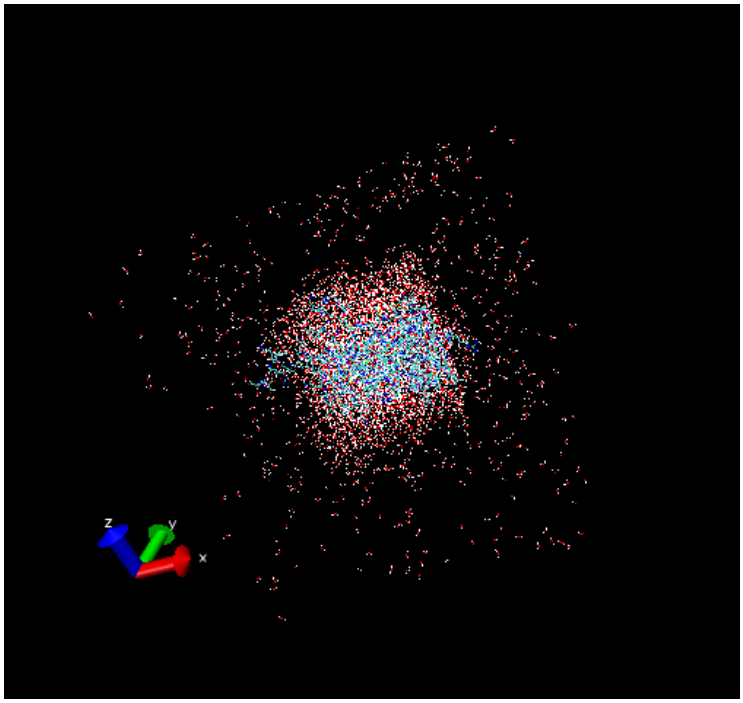


Figure 2 After a NVE simulation of a activated mode

Although the box stayed fairly together the results obtained uncountable are affected by the error. Obviously the portions outside of the smaller box are freer to move and reach different local minima than that of the initial. In addition, the ability of the water molecules to escape resulted in a decrease in density of the water molecules around the Prion thus allowing the entire Prion to deform more easily.

A larger water box that has sides 75 angstroms in length has already been initially developed. However, due to the addition of new atoms the preparation time has increased substantially, which has been compounded by the limitation that the minimization program provided by tinker is not parallelized, limiting the preparation to a single processor. In addition, the larger water box contains ten times as many atoms as the original did so the model runtime which was already at twelve hours using 32 processors is expected to dramatically increase.

Analysis Methods

Although we may only discover if a Prion has been sufficiently deformed to be rendered inert through testing, there are physical aspects that may be observed to hypothesize if it is still functional. It is believed that the loops consisting of residues 697 through 769 and 1138 through 1207 are key activation sites that enable the Prion to latch on to other health and convert them to the pathogenic form of the Prion. In addition, it is believed that the electrostatic surface potential also plays a vital role in the ability of the E200K mutant Prion to make other conform to the unhealthy state.

To evaluate the effectiveness of the deformation based on these observations we used the Mean Square Displacement Theorem:

$$MSD = \left\langle \left| r(t) - r(0) \right|^2 \right\rangle$$

We evaluated the function for three intervals: Once for each of the two loops and once over the entire protein since electrostatic surface potential is the direct result of the positioning of the atoms of the protein.

Results

From our current data it appears as if the lower frequency modes which result in more global deformations of the Prion deformed the entire Prion and the loop regions more than the mode that specifically targeted the loop region. In additon, the simulations of the Prion in the vacume seem to reflect the results of the simulations in the water box except in a much more exagerated manner.

Frequency 0.11cm^{-1} in Vacume

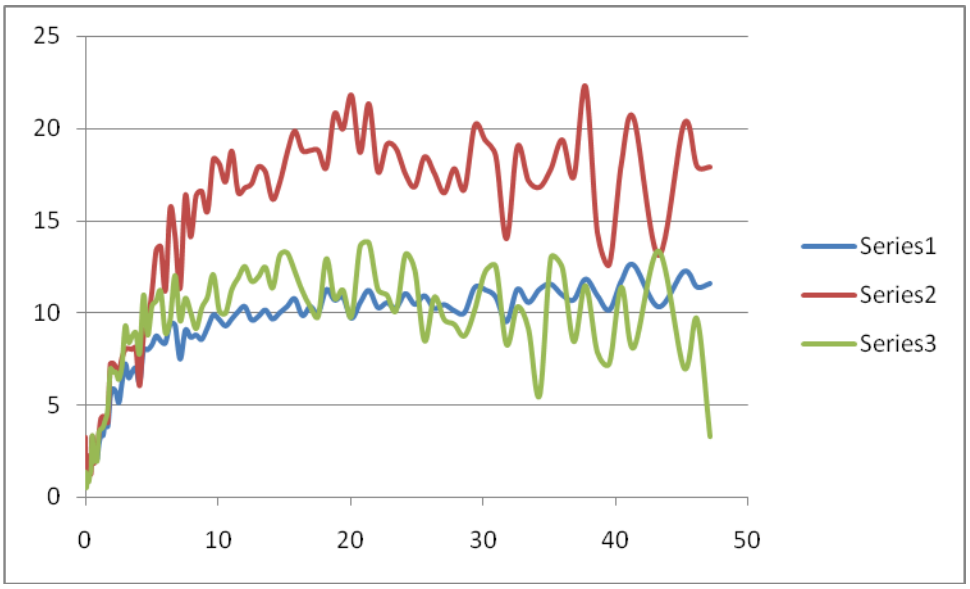


Figure 3: Series1 - Full protein Mean Square Displacement; Series 2 - First Loop Mean Square Displacement; Series3 - Second Loop Mean Square Displacement

Frequency 0.11cm^{-1} in Water Box

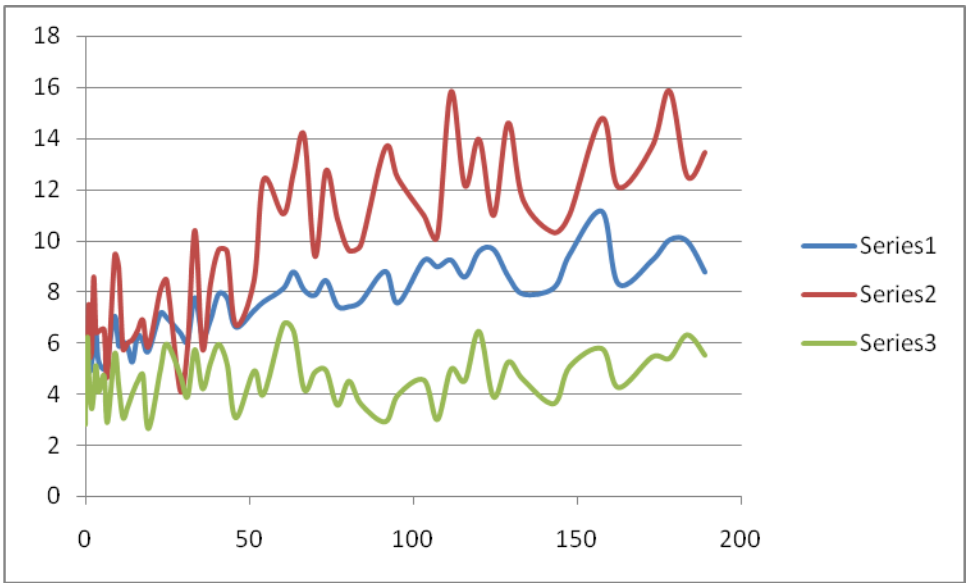


Figure 4: Series1 - Full protein Mean Square Displacement; Series 2 - First Loop Mean Square Displacement; Series3 - Second Loop Mean Square Displacement

Frequency 2999cm^{-1} in Vacume

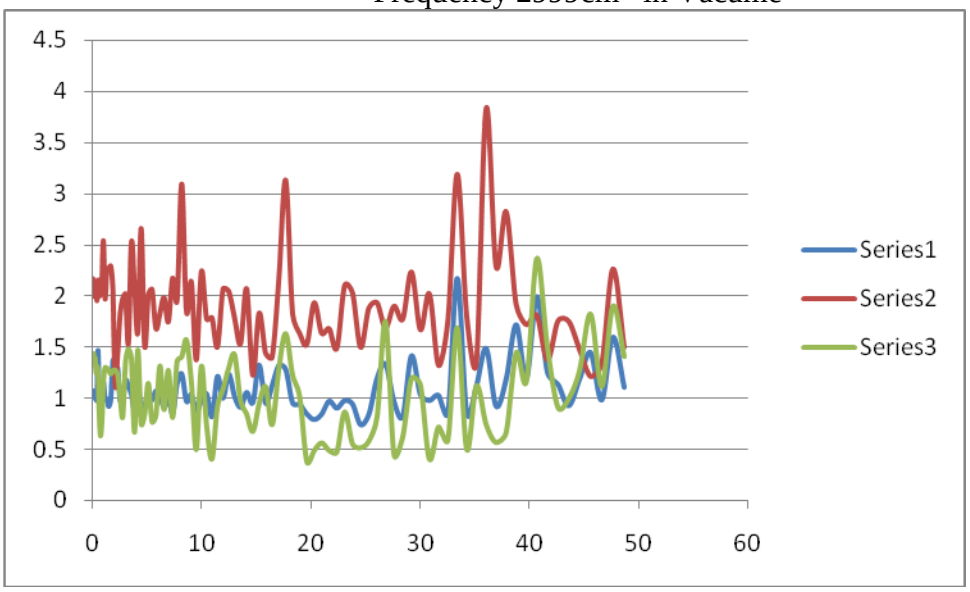


Figure 5: Series1 - Full protein Mean Square Displacement; Series 2 - First Loop Mean Square Displacement; Series3 - Second Loop Mean Square Displacement

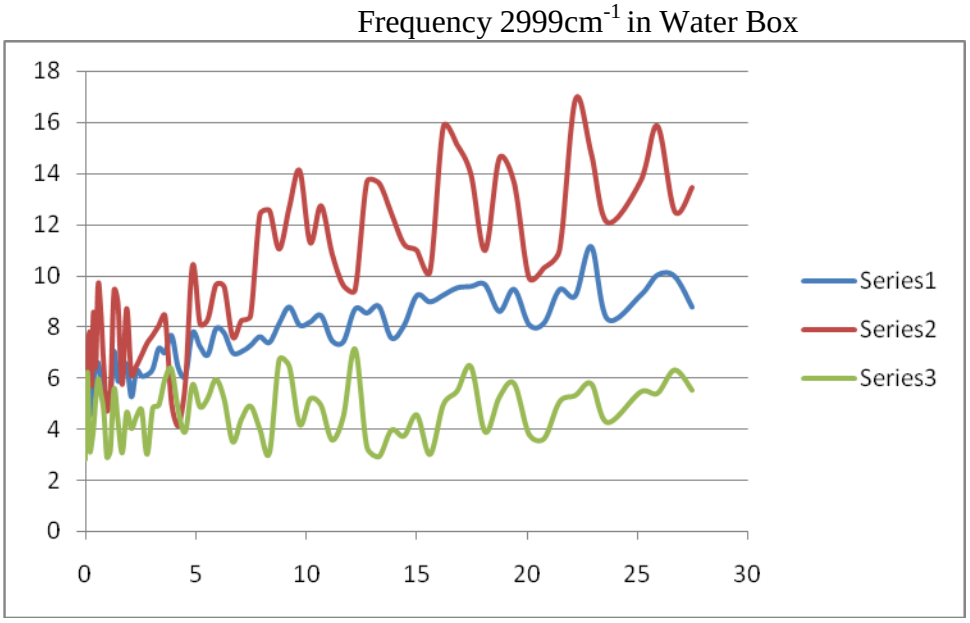


Figure 6: Series1 - Full protein Mean Square Displacement; Series 2 - First Loop Mean Square Displacement; Series3 - Second Loop Mean Square Displacement

Conclusion

We demonstrated that in a vacuum it is possible to significantly deform the Prion to the point where we can consider it probably inert. However, when applying the same method to the protein in the water box much more energy is needed to create the same deformation in the Prion. In addition, the water box we built in tinker still had issues with stability that prevented us from exploring activating these modes with the higher amounts of energy needed. Consequently, as a future expansion, it would be beneficial if we used a different molecular modeling package better suited for modeling water.

Thanks!

We all send our thanks to Mr. McBeth for sponsoring us and making this all happen and to Mark Fleharty, an excellent mentor who helped us through some difficult science, math, and programming. Also, to all those who work behind the scenes to make the NM Supercomputing Challenge happen—it has been a phenomenal experience!

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Parallelized Code

```
#include <cstdlib>
#include "mpi.h"
#include <iostream>
#include <sstream>
#include <string>

using namespace std;

int main(int argc, char *argv[])
{
   string baseName = "1FKCWM";
   //numprocs - number of processors
   //mynum - current processor's number
   int numprocs, mynum;

   //prepare comunication between nodes
   MPI_Init(&argc, &argv);

   //get the number of processors
   MPI_Comm_size(MPI_COMM_WORLD, &numprocs);

   //get processor number
            MPI_Comm_rank(MPI_COMM_WORLD, &mynum);

   float start = atof(argv[1]);
   float end = atof(argv[2]);
   float res = atof(argv[3]);

   int size = ( end - start ) / res;

   //number of simulations for each precessor.... +1 is to make sure program does more runs if sze/numprocs != whole #
   int blkSize = size / numprocs + 1;

   //create Dyn Files
   stringstream command;
   command << "./mode 1FKC.xyz vibrate.log " << blkSize * mynum * res  + start << " " << blkSize * ( mynum + 1 ) * res + start << " "
<< res;
   system(command.str().c_str());

   //runDynamic
   for ( int i = 0; i < blkSize; i++ )
   {
      float curAmp = start + (float)mynum * blkSize * res + (float)i * res;
```

```
    //set Files for dynamic
    stringstream command2;
    command2 << "cp " << baseName << ".xyz " << baseName << "_" << curAmp << ".xyz";
    system(command2.str().c_str());
    //run Dynamic
    stringstream command3;
    command3 << "/users/jfenc/tinker/bin/dynamic.x " << baseName << "_" << curAmp << " 20000 1 .1 1";
    system(command3.str().c_str());
    //clean up
    stringstream command4;
    command4 << "cat " << baseName << "_" << curAmp << ".[0-9][0-9][0-9] > " << baseName << "_" << curAmp << ".arc";
    system(command4.str().c_str());
    stringstream command5;
    command5 << "cp " << baseName << "_" << curAmp << ".200 ../data";
    system(command5.str().c_str());
    stringstream command6;
    command6 << "cp " << baseName << "_" << curAmp << ".arc ../data";
    system(command6.str().c_str());
            stringsream command7;
            command7 << "mkdir " << curAmp;
            system(command7.str());
            stringstream command8;
            command8 << "mv " << baseName << "_" << curAmp << "* " << curAmp;
            system(command8.str());
  }
            //close all connecntions
            MPI_Finalize();

  system("PAUSE");
  return EXIT_SUCCESS;
}
```

Dynamic File Creator

```
/*****************************************************
//Title:            DYNMAKE
//
//Description:    Create dynamic Files for a NVE simulation
//
//
//Arguments:     1) Log File Name
//            2) XYZ File
//            3) Start
//            4) End
//            5) Resolution
//            6) Mode
*****************************************************/



#include <cstdlib>
#include <iostream>
#include <string>
#include <sstream>
#include <vector>
#include <fstream>

#include "VibrateMode.h"
#include "AtomSet.h"

using namespace std;


int main(int argc, char *argv[])
{

   float start = atof(argv[3]);
   float stop = atof(argv[4]);
   float res = atof(argv[5]);
   int mode = atoi(argv[6]);

   string fName = argv[1];
   string log = argv[2];
```

```
   AtomSet set(fName);
   set.GetD_(log, mode);

   for ( float i = start; i < stop; i += res )
   {
                ofstream dyn;
                dyn.open ( "i" );
      stringstream name;
      name << "data/" << mode << "_" << i;
      set.writeDyns( name.str(), i );

   }
   system("PAUSE");
   return EXIT_SUCCESS;
}
```

Table Creator

```
/*******************************************************
//Title:            Table
//
//Description:    Reads the output of the NVE water Box
            simulations and creates a graph
//             a graph showing MSD of selected atoms
//
//Arguments:     1) XYZ Original File
            2) baseName
//             3) start
//             4) stop
*******************************************************/



#include <cstdlib>
#include <iostream>
#include <string>
#include <sstream>
#include <vector>
#include <fstream>

#include "VibrateMode.h"
#include "AtomSet.h"

using namespace std;


int main(int argc, char *argv[])
{

   float start = 0;
   float stop = 1000;
   float res = 10;
   int mode = 4881;

   string baseName = "1FKCWM";
   string original = "1FKC.xyz";
   string vibratelog = "vibrate.log";

               ofstream table;
```

```
            table.open ( "MSDTable" );
   for ( float i = start; i < stop; i += res )
   {
      stringstream file;
      file << baseName << "_" << i << ".200";
            //check to see if file is there
      ifstream tFile;
               tFile.open( file.str().c_str() );
               if ( tFile )
               {
        tFile.close();
        AtomSet set(file.str());
        AtomSet set2(file.str());
        set2.ReadParam("SAmber");
        set2.GetD_( vibratelog, mode );
        set2.RemoveWater();
        table << i << "\t" << set2.energy(i) << "\t" << set.MSD(original, 0, 1734) << "\t" << set.MSD(original,  697, 769) << "\t" <<
set.MSD(original,  1138, 1207)  << endl;
      }

   }
   table.close();
   system("PAUSE");
   return EXIT_SUCCESS;
}
```

Mode Comparison

```
/*******************************************************
//Title:            MDCompare
//
//Description:    Compares the different modes and creates
//             a graph showing MSD of selected atoms
//
//Arguments:     1) Log File Name
              2) XYZ File
//             3) Start Atom
//             4) End Atom
*******************************************************/



#include <cstdlib>
#include <iostream>
#include <string>
#include <sstream>
#include <vector>
#include <fstream>

#include "VibrateMode.h"
#include "AtomSet.h"

using namespace std;


int main(int argc, char *argv[])
{
   if ( argc != 5 ) { cerr << "INCORRECT ARGS" << endl; exit(1); }


   stringstream temp;
   temp << argv[1];
   string logFile;
   temp >> logFile;

   stringstream temp1;
   temp1 << argv[2];
   string xyzFile;
   temp1 >> xyzFile;

   stringstream temp2;
   temp2 << argv[3];
```

```
  int start;
  temp2 >> start;

  stringstream temp3;
  temp3 << argv[4];
  int end = 1207;
  temp3 >> end;

  AtomSet::AtomSet protein( xyzFile );
  int atCt = protein.count();
  int fCt = 5201;//VibrateMode::ct( logFile, atCt);

  stringstream compName;
  compName << "mdComp_" << start << "_" << end << ".txt";
  ofstream outdata;
            outdata.open( compName.str().c_str() );
            if ( !outdata ) { cerr << "FAILED" << endl; }
            vector<float> total(5201);
  for ( int i = 1; i < fCt + 1; i++ )
  {
    VibrateMode::VibrateMode mode( atCt, logFile, i );
    float sec1 = mode.MSD( 697, 769 );
    float sec2 = mode.MSD( 1138, 1207 );
    total[i - 1] = sec1 + sec2;
    outdata << i << "\t" << sec1 << "\t" << sec2 << "\t" << mode.getF() << endl;
  }
  ofstream top;
  top.open("top.txt");
  for ( int i = 0; i < 15; i++ )
  {
    int topLoc = 0;
    for ( int j = 0; j < fCt; j++ )
    {
      if ( total[j] > total[topLoc] )
        topLoc = j;

      top << topLoc + 1 << "\t" << total[topLoc] << endl;
     }
  }
  top.close();
  system("PAUSE");
  return EXIT_SUCCESS;
}
```

AtomSet.h

```
#ifndef ATOMSET_H
#define ATOMSET_H

#include <iostream>
#include <fstream>
#include <vector>
#include <string>
#include <cstdlib>
#include <cmath>
#include <sstream>
#include "DataStructures.h"
#include "VibrateMode.h"

using namespace std;

class AtomSet
{
private:
   vector<Atom> *atoms;
   vector<float> *f;
   vector<float> *m;
            int atCt;

public:
   AtomSet( string xyzFile );
   ~AtomSet();
   void GetD_( string vibFile, int mode );
   void writeArc( string arcFile, int res );
   void writeDyns( string dynFile, float mag);
   void ToPDB( string PDBFile, string name );
   float MSD( string xyzOriginal, int start, int stop );
   float energy( float Amplitude );
   void ReadParam(string SAParam );
   void RemoveWater();
   float GetF();
   int count();
private:
   void GetP_( string xyzFile );
   string fileLine( float x, float y, float z, Atom atom, int number );
   string cordIntro( int atomCt, string name );
   float getPosition( float cord, float totalChange, float t );

   float mag( Point p1, Point p2 );
};
```

```
#endif
```

AtomSet.cpp

```
#include "AtomSet.h"

#define	MAXBONDS			5
#define ATOMDIGITS		4
#define PI				3.14159265

using namespace std;

AtomSet::AtomSet( string xyzFile )
{
    atoms = new vector<Atom>(0);
    f = new vector<float>(0);

    GetP_( xyzFile );
};
AtomSet::~AtomSet()
{
   delete atoms;
   delete f;
};
void AtomSet::GetP_( string xyzFile )
{
		ifstream indata;
		indata.open( xyzFile.c_str() );
		if ( !indata )
		{
			cout << "FAILED" << endl;
		}
		indata >> atCt;

   //skip line
		char line[256];
		indata.getline(line, 256);

		for ( int i = 0; i < atCt; i++ )
		{
			int linenum = 0;
			indata >> linenum;
			Atom na;
			//indata.getline(line, 256);
			indata >> na.name;
			indata >> na.p_.x;
			indata >> na.p_.y;
```

```
                        indata >> na.p_.z;
    atoms->push_back(na);
                        indata.getline(line, 256);
                        stringstream ln(stringstream::in | stringstream::out);
                        ln << line;

                        for ( int j = 0; j < MAXBONDS; j++ )
      ln >> (*atoms)[ i ].bonds[ j ];
            }
};
void AtomSet::GetD_( string fileName, int modenum )
{
   VibrateMode mode(atCt, fileName, modenum);
   (*f)[ 0 ] = mode.getF();
   for ( int i = 0; i < atCt; i++ )
      (*atoms)[ i ].d_ = (*mode.d_)[ i ];

};

void AtomSet::writeArc(string arcFile, int res )
{
            int c = f->size();
            int p = atoms->size();

            ofstream arc;
            stringstream file;
            file << arcFile << "_" << (*f)[0] << ".arc";
            arc.open (file.str().c_str());
            for ( int j = 0; j < res; j++ )
            {
                        arc << cordIntro(atCt, arcFile) << endl;

                        for ( int k = 0; k < atCt; k++ )
                        {
                                    float x = getPosition( (*atoms)[ k ].p_.x, (*atoms)[ k ].d_.x, 2 * PI * (float)j / (float)res );
                                    float y = getPosition( (*atoms)[ k ].p_.y, (*atoms)[ k ].d_.y, 2 * PI * (float)j / (float)res );
                                    float z = getPosition( (*atoms)[ k ].p_.z, (*atoms)[ k ].d_.z, 2 * PI * (float)j / (float)res );
                                    arc << fileLine(x, y, z, (*atoms)[ k ], k + 1 ) << endl;
                                    if ( k > 0 )
                                                int x = 3;
                        }
            }
            arc.close();
};
void AtomSet::ToPDB ( string PDBFile, string name )
{
   ifstream indata;
            indata.open( PDBFile.c_str() );
            if ( !indata ) { cerr << "FAILED" << endl; }

   ofstream outdata;
            outdata.open( name.c_str() );
            if ( !outdata ) { cerr << "FAILED" << endl; }

            bool isText = true;
            while ( isText )
            {
      isText = false;

      char line[256];
      indata.getline(line, 256);
      stringstream lstream;
      lstream << line;
      string fword;
      lstream >> fword;
```

```
      if ( fword.length() > 0 )
        isText = true;

      int atNum = 0;

      if ( fword == "ATOM" )
      {
         stringstream nLine;
         nLine << "Atom \t";
         for ( int i = 0; i < 5; i++ )
         {
            string word;
            lstream >> word;
            nLine << word << "\t";
         }

         nLine << (*atoms)[ atNum ].p_.x << "\t";
         nLine << (*atoms)[ atNum ].p_.y << "\t";
         nLine << (*atoms)[ atNum ].p_.z << "\t";

         for ( int i = 0; i < 3; i++ )
         {
            string word;
            lstream >> word;
            nLine << word << "\t";
         }

         atNum++;
      }
      else
         outdata << line;
   }
}
float AtomSet::GetF()
{
    return (*f)[ 0 ];
}
int AtomSet::count()
{
   return atCt;
}
string AtomSet::cordIntro( int atomCt, string name )
{
            stringstream ln(stringstream::in | stringstream::out);
            ln << "    " << atomCt << "    " << name ;
            return ln.str();
};

string AtomSet::fileLine( float x, float y, float z, Atom atom, int number )
{
            stringstream ln(stringstream::in | stringstream::out);
            ln << "    " << number << "    " << atom.name << "    " << x << "    " << y \
                  << "    " << z << "    ";
            for ( int i = 0; i < MAXBONDS; i++  )
            {
                  ln << "    ";
                  ln << atom.bonds[i];
            }
            return ln.str();
};

float AtomSet::getPosition( float cord, float totalChange, float t)
{
            return 3 * totalChange * sin(t) + cord;
};
```

```
void AtomSet::writeDyns(string dynFile, float mag)
{
		cout << "writeing dns" << endl;
		ofstream dyn;
		dyn.open ( dynFile.c_str() );
		dyn << "Number of Atoms and Title :" << endl;
		dyn << "     " << atoms->size() << "     " << dynFile << endl;
		dyn << "Periodic Box Dimensions :" << endl;
		dyn << "   " << "90" << "    " <<  "90" << "    " << "90" << endl;
		dyn << "   " << "90" << "    " <<  "90" << "    " << "90" << endl;
		dyn << "Current Atomic Positions : " << endl;
		for ( int j = 0; j < atoms->size(); j++ )
		{
			dyn << "     " << (*atoms)[ j ].p_.x << "           " << (*atoms)[ j ].p_.y << "           " << (*atoms)[ j ].p_.z << endl;
		}
		dyn << "Current Atomic Velocities :" << endl;
		for ( int j = 0; j < atoms->size(); j++ )
		{
			dyn << "     " << mag * (*atoms)[j].d_.x << "   " << mag * (*atoms)[j].d_.y << "   " << mag * (*atoms)[j].d_.z <<
endl;
		}
		dyn << "Current Atomic Accelerations :" << endl;
		for ( int j = 0; j < atoms->size(); j++ )
		{
			dyn << "     " << 0 << " " << 0 << " " << 0 << endl;
		}
		dyn << "Previous Atomic Accelerations :" << endl;
		for ( int j = 0; j < atoms->size(); j++ )
		{
			dyn << "     " << 0 << " " << 0 << " " << 0 << endl;
		}
		dyn.close();
};

float AtomSet::MSD(string originalFile, int start, int stop)
{
   AtomSet orig(originalFile);

		float sum = 0;
		for  ( int i = start; i < stop; i++ )
		{

		sum += sqrt(pow((*atoms)[i].p_.x - (*orig.atoms)[i].p_.x,2)+pow((*atoms)[i].p_.y -
(*orig.atoms)[i].p_.y,2)+pow((*atoms)[i].p_.z - (*orig.atoms)[i].p_.z,2));
		}
		sum /= stop - start;
		return sum;
};
float AtomSet::energy( float Amplitude)
{
    //1/2MV^2 on each atom
    double E = 0;

    for ( int i = 0; i < atCt; i++ )
    {
       Point z;
       z.x = 0;
       z.y = 0;
       z.z = 0;
       float v = Amplitude * mag((*atoms)[i].d_, z) * 100;
       float mass = (*m)[ atoi((*atoms)[ i ].bonds[ 0 ].c_str()) - 1];
       // KE = 1/2mv^2; u -> kg; angstroms/picosecond -> meters/sec; J -> eV
       E +=  mass *  pow(v, 2) / 2 * 1.66053 * 6.2415 * 1e-9;
    }
		cout << E << endl;
    return E;
```

```
}
//gets the mass of all atoms as identified by their number which is the location in the array + 1
void AtomSet::ReadParam ( string SAParam )
{
    m = new vector<float>(1252);
   ifstream indata;
                indata.open( SAParam.c_str() );
                if ( !indata ) { cout << "FAILED" << endl; }

                for ( int i = 0; i < 1252; i++ )
                {
                indata >> (*m)[i];
   }
}
void AtomSet::RemoveWater()
{
    vector<Atom> newSet(0);
    for ( int i = 0; i < atCt; i++ )
    {
       int ID = atoi((*atoms)[ i ].bonds[ 0 ].c_str());
       if ( ID > 1254 )
       {
          atoms->erase(atoms->begin() + i);
          atCt--;
          i--;
       }
    }

}
float AtomSet::mag( Point p1, Point p2 )
{
                return sqrt(pow(p1.x - p2.x,2)+pow(p1.y - p2.y,2)+pow(p1.z - p2.x,2));
};
```

‘

VibrateMode.h

```
#ifndef VIBRATEMODE_H
#define VIBRATEMODE_H

#include <iostream>
#include <fstream>
#include <vector>
#include <string>
#include <sstream>
#include <cstdlib>
#include <cmath>
#include <sstream>
#include "DataStructures.h"

using namespace std;

class VibrateMode
{
friend class AtomSet;

private:
    vector<Point> *d_;
    float f;
    string logFile;
    int *atCt;
public:
    VibrateMode( int atCt, string logFile, int mode );
    ~VibrateMode();
    float MSD( int first, int last);
    static int ct( string log, int atomCt );
    float getF();
    void print();
private:
    void ReadMode( int mode );
    void ReadLine( float *x, float *y, float *z, ifstream& file );
    void GoToMode( ifstream& fileStrm );
    static void SkipLines( ifstream& fileStrm, int lines );
    float mag( Point p1 );
};

#endif
```

VibrateMode.cpp

```
#include "VibrateMode.h"

using namespace std;

VibrateMode::VibrateMode ( int atCt, string logFile, int mode )
{
   d_ = new vector<Point>(0);
   int temp = atCt;
   this->atCt = new int(temp); // memory stompping issues
   this->logFile = logFile;
   ReadMode ( mode );
};
VibrateMode::~VibrateMode()
{
   delete d_;
};
void VibrateMode::ReadMode ( int mode )
{
    //Open File
            ifstream indata;
            indata.open(logFile.c_str());
            if (!indata) { cout << "FAILED" << endl; }

   GoToMode(indata);

            string modeHdr = "Vibrational Normal Mode";
            bool isMode = true;
   d_->resize( *atCt );
   int fCt = 0;
            while( isMode ) //Read Displacements
            {
                        //seek down 2 lines
      char modeHeader [ 256 ];
                        indata.getline(modeHeader, 256);
                        string hdr = modeHeader;

                        if ( hdr != "" )
                        {
                  if ( 0 == modeHdr.compare( hdr.substr(1,23) ) )
                  {
                        isMode = true;
                        SkipLines(indata, 3);
```

```
                        fCt++;
                        cout << fCt << endl;
                        if (fCt == mode )
            {
                        d_->resize( *atCt );
                        stringstream head;
                        head << hdr;
                  //seek to right word of title
                  string fstr;
               for ( int i = 0; i < 7; i++ )
                           head >> fstr;
                           f = atof(fstr.c_str());


                        //save data
                        for ( int i = 0; i < *atCt; i++ )
                  ReadLine( &(*d_)[ i ].x, &(*d_)[ i ].y, &(*d_)[ i ].z, indata );
               break;
               break;
            }
            else
            {
               isMode = true;
            }
         }
                        }
   }
               indata.close();
};

float VibrateMode::MSD ( int first, int last )
{
   int temp = *atCt;
   float sum = 0;
               for  ( int i = first; i < last + 1; i++ )
               {
                        sum += pow(mag((*d_)[i]), 2);
               }
               sum /= (float)*atCt;
               return sum;
};
void VibrateMode::print()
{
    for ( int i = 0; i < 1734; i++ )
       cout << (*d_)[i].x << "     " << (*d_)[i].y << "       " << (*d_)[i].z << endl;
}

int VibrateMode::ct ( string log, int atomCt )
{
   //Open File
               ifstream indata;
               indata.open(log.c_str());
               if (!indata) { cout << "FAILED" << endl; }

   int lines = 23 + ( 3 * atomCt / 5 ) * 2 ;
   SkipLines(indata, lines);

               string modeHdr = "Vibrational Normal Mode";
               bool isMode = true;
   int fCt = 0;
               while( isMode ) //Read Displacements
               {
                        isMode = false;
                        //seek down 2 lines
      SkipLines(indata,1);
      char hdr [ 256 ];
```

```
                        indata.getline(hdr, 256);
                        string hdrs = hdr;
                        if ( 0 == modeHdr.compare( hdrs.substr(1,23) ) )
                                fCt++;
                }
                indata.close();
                return fCt;
}
float VibrateMode::getF ()
{
    return f;
}
void VibrateMode::ReadLine ( float *x, float *y, float *z, ifstream& file )
{
   char line[256];
   file.getline(line,256);
   stringstream ln;
   ln << line;
   int atom;
   ln >> atom;
   ln >> *x;
   ln >> *y;
   ln >> *z;

};

//Go to first line of atom displacements
void VibrateMode::GoToMode ( ifstream& fileStrm )
{
                int lines = 24 + ( 3 * *atCt / 5 ) * 2 ;
  SkipLines(fileStrm, lines);
};
void VibrateMode::SkipLines ( ifstream& fileStrm, int lines )
{
  char line[256];
  for ( int j = 0; j < lines; j++ )
     fileStrm.getline(line,256);
}
float VibrateMode::mag(Point p1)
{
                return sqrt(pow(p1.x, 2)+pow(p1.y, 2)+pow(p1.z, 2));
};
```

DataStructures.h

```
#ifndef DATASTRUCTURES_H
#define DATASTRUCTURES_H



#include <vector>
#include <string>

#define	MAXBONDS			5

using namespace std;

struct Point
{
   float x;
   float y;
   float z;
};

struct Atom
{
   Point p_;
		Point d_;

		string name;
		string bonds[MAXBONDS];
};
#endif
```