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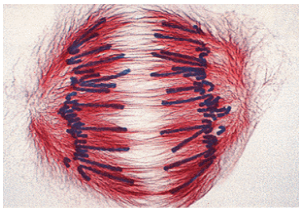
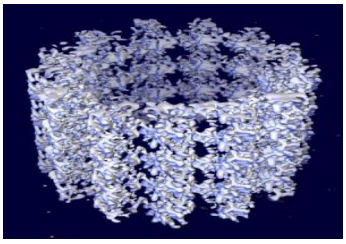
REU Summary paper

This summer my research has dealt primarily with investigating the mechanisms behind spindle assembly in mitotic cells. The spindle is a football-shaped structure that forms in all cells during cellular mitosis.



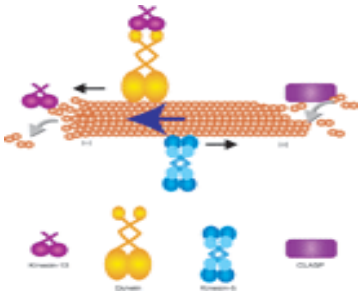
The spindle seems to emanate out of two nodes on each "pole" of the football, called *centrosomes,* and its primary function is to pull the opposing chromosomes apart during anaphase. The spindle is formed primarily of long, hollow, tube-like protein structures known as microtubules, which have been the primary focus of my research. Microtubules are an instrumental part of many different cell functions, including assisting in vesicular transport and providing the cellular superstructure. Microtubules have a diameter of approximately 26 nm and are composed of a circle of 13 chains of tubulin diamers proteins.



Microtubules have two important regimes of dynamics which are of particular interests in my studies. The first is the ability to actively polymerize and depolymerize. The microtubule growth and

shrinkage is governed by a process known as *"dynamic instability"* . That is, instead of being governed by a simple stochastic process, instead they stay in either grow or shrink phases for defined periods of time, with some probability of switching between. The process of switching from a grow phase to a shrink phase is called *"catastrophe"* and the process of switching from a shrink phase to a grow phase is called "*rescue."* A final important point about polymerization dynamics is that microtubules are polar molecules: that is, growth and shrinkage occurs on one end only.

The second important dynamic witnessed in microtubules is the presence of active motors. These motors are a special type of protein called "EG-5" which connect to two microtubules and walk along their length towards the polymerizing ends, exerting a relative force between the two.



There is some dispute between the currently accepted model and recent experimental findings. The most important aspect of the present model is that the centrosomes play an important role in "growing" the spindle structure. Purportedly the spindle fibers grow outward from the centrosomes in twin aster formations, then are eventually pulled together into the familiar football-shaped model by the EG-5 motors. The other major idea of the current model is that the spindle is formed of a few very long microtubule strands. However, discoveries dispute this model. First, detailed measurements of the length distribution of microtubules in the spindle fiber show an exponential distribution, rather than just a few very long microtubules. Second, it has been observed that if the centrosome structure is excised from asomal cells, something very much resembling the centrosome grows back. This discovery makes it hard to believe that the centrosome is the controlling structure of spindle formation, since it grows

back spontaneously. The conclusion from this is that it is very likely that the spindle undergoes some sort of spontaneous self assembly, rather than being "grown".

This is where we feel there is still some unanswered questions. In the quest to investigate the purported self assembly of the spindle structure, we are looking to map out some phase boundaries of polymerizing microtubules, and also microtubules in the presence of active motors based on parameters such as tubule concentration, temperature, pressure, motor concentration, polymerization rates and switching frequencies, etc. The hope is to eventually compare these to the quantities measured in real cells and possibly illuminate something about the ability of systems of microtubules to numatically order themselves and self assemble.

We hope to achieve this via a computational approach, taking an existing molecular simulation program (written in C) and modifying it to reflect microtubule dynamics. The microtubules themselves are simulated by two balls attached by a spring. Externally, they have a rectangle (or cylinder in 3D) that connects the widest point of the two balls, giving rise to a shape dubbed the "spherocylinder".

The microtubules interact with each other via a short-rage Lennard-Jones repulsion potential given by:

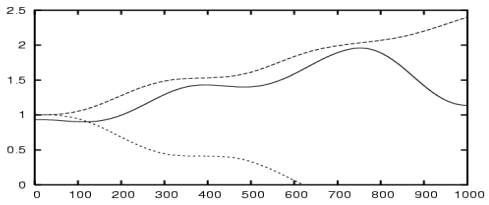
$$U_{LJ} = \frac{\varepsilon}{4} + \varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right]$$

This makes the particles bounce off of each other. Each end of the spherocylinder carries its own forces, velocities, and position, which gets updated every time step taken with a simplectic numerical integration method.

Polymerization dynamics are simulated by increasing or decreasing the equilibrium lengths of the springs connecting the two spherocylinder, making them grow or shrink as the springs readjust themselves to their lowest potential. The process of dynamics instability is recreated by specifying probabilities of switching between states. A random number is chosen every time step to compare to these probabilities. If the particles shrink to zero length, they are removed from the system entirely (like they dissolved back into the tubulin diamer, which are much smaller than an assembled microtubule). Similarly, particles may spontaneously start growing if there is room. This is determined by an "energy of insertion" test, which scales as:

$$P(insertion) = e^{-\frac{\varepsilon}{\tau}}$$

A random number is generated and if it is lower than this function, where ε is the energy of insertion and τ is the temperature of the system, then the insertion of a new particle is energetically allowable. The lifetime of a few microtubules is graphed here:



Length (y) is graphed against time step (x). Each time step interval on the graph is actually 500 true time steps, or about _____ seconds. The irregularities in the curves are due to the fact that the spring lengths of the particles are increased and the actual equilibration lags behind a bit. Also notice that the rate of polymerization is slightly lower than the rate of depolymerization. This is an important quality that any system following dynamic instability must adhere to in order to have bounded behavior.

Active crosslinking motor dynamics are also simulated by a spring force connecting two microtubules at a specific point. They are inserted with a certain probability, and once attached, the

crosslink walks towards the polymerizing ends on both, disappearing if it happens to walk off the end of one of the molecules. This part of the project is not as developed and there are many more aspects yet to be implemented. Eventually, we would like that each crosslink has a probability of falling off that scales so that each crosslink has an average of eight steps taken, consistent with real observations. Crosslinks eventually will also be able to be attached to one microtubule only and "floating free" on the other end, thus increasing the probability of this microtubule becoming reattached to another. Finally, the crosslinks should also detach if too much strain is put on them.

After the initial code development, the next important aspect of simulating microtubules is parameter matching. Taking actual data of polymerization rates, switching frequencies, temperature, etc, these parameters have to be matched to the unitless quantities specified in the simulation. In the type of molecular modeling we are doing, if a fundamental mass (m), length (σ), and energy (ε) can be specified, then all other quantities can be expressed in terms of these. In particular, we needed:

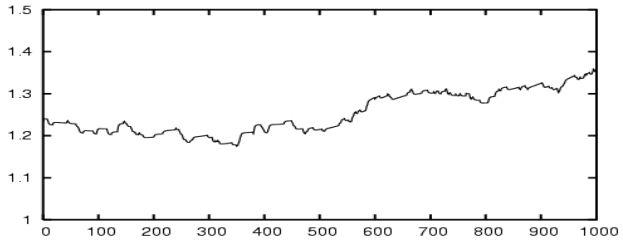
$$pressure = \frac{\varepsilon}{\sigma^3} \quad temperature = \frac{\varepsilon}{k_B} \quad time = \sigma\sqrt{\frac{m}{\varepsilon}} \quad velocity = \sqrt{\frac{\varepsilon}{m}} \quad frequency = \frac{1}{\sigma}\sqrt{\frac{\varepsilon}{m}}$$

The fundamental mass of a microtubule is well known. The mass of a tubulin monomer is approximately 50 KDa, or 8.3×10^{-20} kg. To determine the fundamental mass and energy, we started with our Lennard-Jones repulsion potential and matched it to a force/unit length vs distance curve of actual the actual repulsion force between microtubules. This yielded a length of 28.8 nm and an energy of .2 electron volts. As a check, the fundamental length we would expect would be the diameter of a microtubule (26nm) so it would appear as though the matching process yielded reasonable results.

Running simulations to collect data typically consists of two phases. First the simulation is run with subroutines that keep the system at a constant pressure (called the barostat) to let the particle

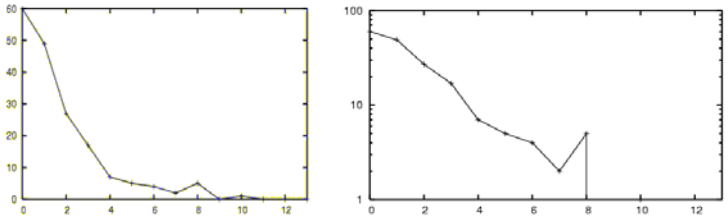
concentrations, lengths, and particle orientations reach some sort of steady state distribution. Then, the barostat is turned off and data collection begins. This is done because the barostat controls pressure by adjusting the size of the box, and it is much harder to analyze data with the box changing size.

Here is a plot of average length over a 500,000 step run after equilibration:



While we expect the average length of an equilibrated system to hold steady, It looks like there might be a slight upward trend. This could either be a normal waver in length and indicates that this sample time is too small, or it could indicate that the equilibration phase of this run was run for long enough.

Here is a graph of length distribution. To accomplish this, a histogram approach was used, counting up the particles by bin.



On the left is a linear graph, and on the right is a logarithmic graph. Notice that the logarithmic graph is fairly straight. This indicates that the length distribution is exponential, which is exactly what has been observed in experiments and is therefore shows that the model might be on the right track. The one glaring anomaly in the data is the spike in the distribution at bin eight. This is especially interesting

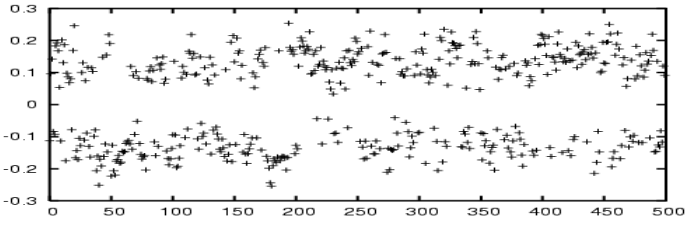
because this peak doesn't seem to average out as we continue to graph length distribution over time. This could be another indication that a longer equilibration time period is needed to smooth this out.

The main analysis we would like to do on these systems is an order analysis. To do this, a dxd order matrix is usually used, where d is the number of dimensions of the system. The order matrix is defined by:

$$Q_{ij} = 1/N \ \Sigma \ [(v_{ij}^2 - 1/d \ \delta_{ij}]$$

Where v_{ij} is the (i,j)th component of the vector along each tubule, N is the number of particles in the system, d is the number of dimensions of the system and Σ is the sum over all i and j. The scalar order parameter is calculated by diagonalizing this matrix, then taking 3/2 multiplied by the largest eigenvalue (diagonal element). However, since our system is composed of polydisperse particles and the order parameter calculation becomes useless when comparing long rods and short rods, again a histogram approach was used, binning up the particles according to length and calculating a separate order parameter for each. Eventually the goal is to combine these separate order parameters, weighting them somehow by length to derive an overall order parameter for the system.

Here is a preliminary graph of the order of the same system shown above:



This is the order parameter graphed against time of the smallest (and therefore most populated) bin of particles. We are unsure as to why the order is clustered around +0.15 and -.015.

In the future, we hope to continue this work to eventually map out the phase boundaries of systems of microtubules at various pressures and temperatures. One idea we have is to “lock in” the orientations of the microtubules while letting them equilibrate their lengths, then “unlock” them and watch the resulting motion.