Simulating the Aggregation of Globular Proteins at Various Salt Concentrations

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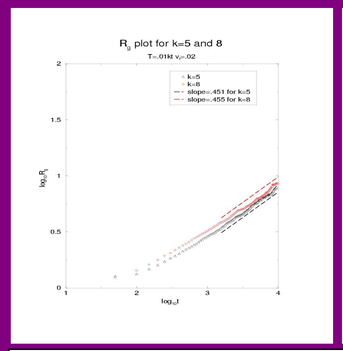
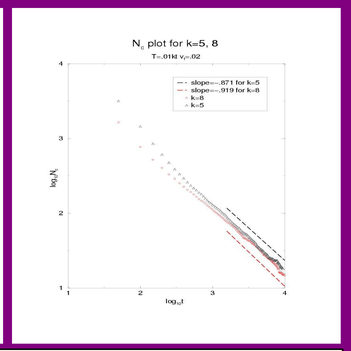
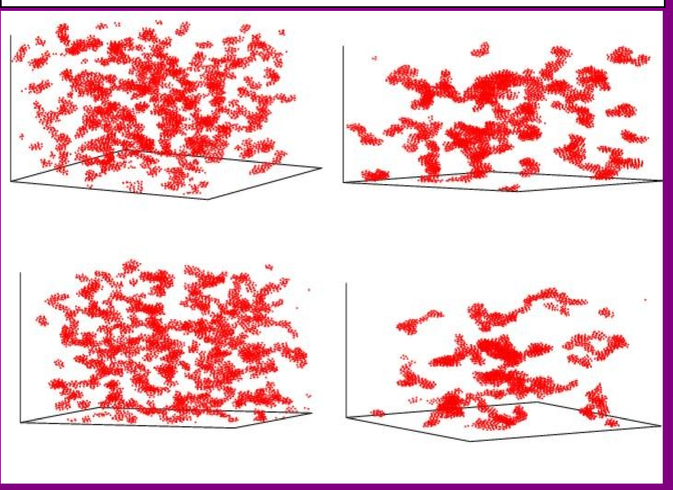




Introduction:
Aggregation can be defined as the act of individual pieces of matter coming together as a whole. It has become important in the medical world to control the aggregation of proteins such as insulin in order to obtain aggregates that make the protein deliverable to patients in need. My simulations of globular proteins attempts to model such aggregation. The conditions of constant temperature and volume were chosen due to similar conditions seen in the human body. The monomers I used are spherical, have a diameter of 3-4 nanometers, have a uniform charge distribution, and are in solution. Therefore, there is a short range attractive Van der Waals force between any two particles along with a long range Coulombic repulsion. This Coulombic repulsion can be screened by effectively adding a certain amount of salt to the solution containing the proteins.

Data:
A graph of the potential as a function of radial distance (r being separation distance and sigma being monomer diameter) between the monomers in the system at various salt concentrations can be seen to the right. Notice that at high concentrations there is no potential barrier. This means that as the particles have enough neutral screening due to the high number of negative ions in solution to aggregate fairly well due to the weak Coulombic repulsion. This should yield stringy, fractal like aggregates with a fractal dimension of 1.8, indicative of Diffusion-limited Cluster Aggregation (DLCA). Upon analyzing the graphs represented above, one can see that if you take the negative ratio of the slope of the number of clusters(Nc) and the radius of gyration(Rg) plots above for k=5 and 8, you get 1.93 and 2.02 respectively. This is not quite the DLCA limit, but the slope of the Nc plot should yield a kinetic exponent of z=-1 for DLCA, and k=8 has a slope closer to the DLCA limit then does k=5. The morphology of clusters under the two different salt concentrations is shown below, and k=8 yields more fractal like aggregates, which agrees with the data presented above.
Below:From left to right, the top row shows the morphology of a system with 10000 monomers and a box size of 64 (volume fraction of .02) for k=8 and T=.1kt after 500 time steps (left) and 5000 (right). The bottom row contains the same conditions except the salt concentration has been changed to k=5. The graphs relate to the Nc and Rg plots above.



Brownian Dynamics equation of motion:

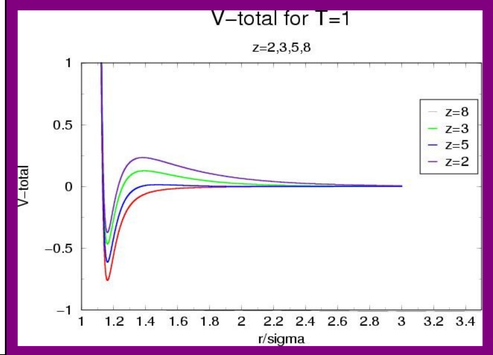
$$\ddot{\vec{r}}_i = -\vec{\nabla} U_i - \Gamma \dot{\vec{r}}_i + \vec{W}_i(t)$$

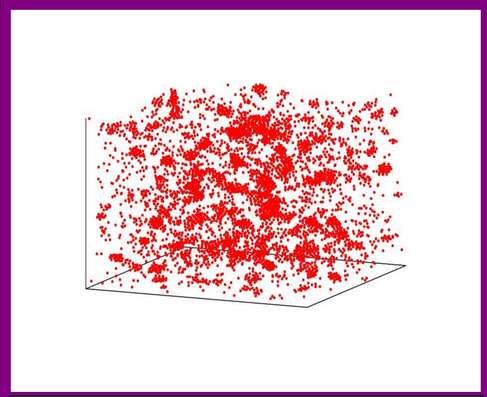
Modified Lennard-Jones potential:

$$U_1(r) = \frac{4\varepsilon}{\alpha^2}\left[\frac{1}{\left[r^2-1\right]^6} - \frac{\alpha}{\left[r^2-1\right]^3}\right]$$

Yukawa potential for hard spheres:

$$U_2(r) = A\frac{e^{[-kr]}}{r} \quad 0 < r < r_{cutoff}$$



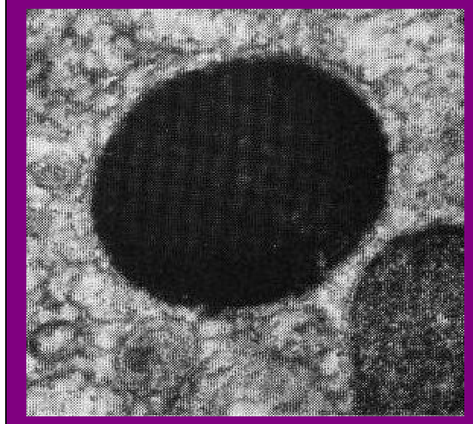


Above: Morphology of a system containing 6000 particles and box with L=64 (volume fraction =.012) for k=3 and T=.1kt after 12500 time steps.

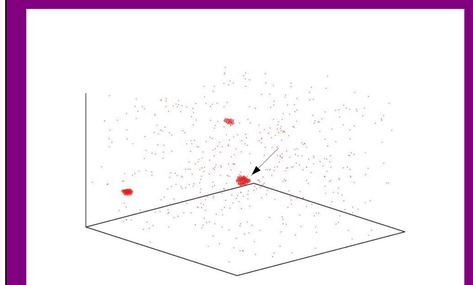
Equations:
To the left are the equations I used in my simulations. The first equation represents the Brownian Dynamics equation of motion for the i-th protein as a function of radial distance. The first term is the force present due to the short range attractive Van der Waals interaction and the screened Coulombic long range repulsion. The second term is a resistive force that exists due to the viscosity of the solvent in the system. The third term is the random Brownian force that exists due to the random collisions between the solvent and the charged proteins in solution. The second equation is a modified version of the Lennard-Jones potential which I used to represent the short ranged attractive Van der Waals force. The variable r represents radial distance. The epsilon represent the depth of the potential well, and it maintains a constant value on 1 in my simulations. Alpha represents the width of the potential well, and it maintains a constant value of 50. These values were chosen to agree with experimental results obtain on the study of Lysozyme proteins in solutions of varying NaCl concentrations. The first term in parenthesis accounts for the direct interaction between the proteins(the fact that the particles cannot overlap), while the second term accounts for the normal Van der Waals force. The third equation is the Yukawa potential for hard spheres and is used to represent the long raged repulsive screened Coulombic repulsion between the particles. The variable k represents salt concentration. The boundary condition of r-cutoff was chosen in order to simplify the calculations and it varies depending on which salt concentration is used for a given simulation. A represents the surface charge of the proteins, and is conveniently set equal to 1 for my simulations.

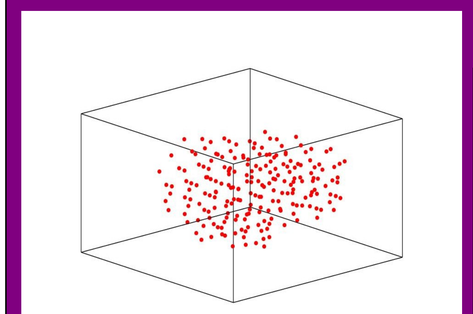
Nucleation:
At a given temperature, there is a volume fraction that is ideal for the growth of nucleating clusters in a system. These conditions appear near the boundary of the phase diagram that exists if you plot temperature as a function of concentration for a given system. To the right shows the morphology of three nucleating clusters at a concentration k=8, T=.15kt, number of monomers=1000, and box size =128 (volume fraction=.00025). Nucleating clusters grow uniformly and have a compact structure, resembling that of a sphere with a fractal dimension nearing the value of 3. To study the structure of the nucleating clusters, I plotted the radial distribution function of the largest cluster in a system as a function of radial distance (figure to the bottom left). The slightly sharp peaks are indicative of a more tightly bound structure due to the large probability that a monomer will be at a given position In contrast a liquid would have very broad distribution with dull peaks due to the particles ability to move around more freely.

Data:
Upon looking at the plot of the potential for the lower concentrations (k=2,3), one can see that there is a potential barrier that is of relative size to the potential well for the given concentrations. This barrier is indicative of a finite probability that the particles will not become part of a cluster since there is a chance they might not fall into the potential well. However, there is a probability that the monomers will make it into the well (similar to electron tunneling through a potential barrier), and some clusters do form. The type of aggregation that occurs when such a barrier exists in known as Reaction Limited Cluster Aggregation (RLCA) and usually yields more compact clusters, having a fractal dimension of 2.1. The image to the left shows some aggregates that look dense and slightly spherical. I was able to yield aggregates at k=3 due to a relatively effective neutral shielding, but k=2 does not yield aggregates due to the less effective screening of the Coulombic potential between the particles.



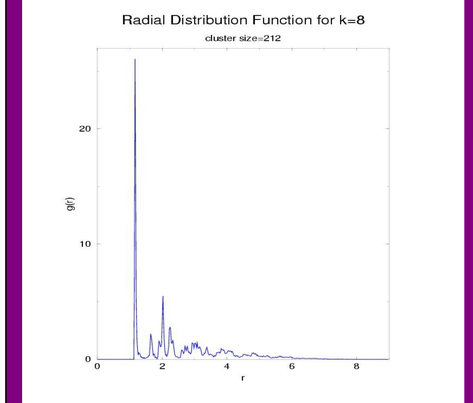
Lysozyme:
The protein Lysozyme is a globular protein commonly found in chicken egg-whites that has been studied thoroughly due to its vast abundance in nature and its radial symmetry. It is commonly used in medicine and in the food industry to fight against bacterial infections The ability to form such spherical aggregates is important in order to ensure safe delivery of Lysozyme to the human body. The image to the left is a cluster of Lysozyme proteins with a spherical structure that looks very similar to the clusters beneath it which was formed in my simulation. The arrow points to the largest cluster in the system which can be more thoroughly examined in the figure below it.





Conclusion:
Overall my simulations yield realistic examples of how these types of proteins do act in solution. At higher salt concentrations there is a more effective neutral screening allowing the particles to come together and behave under DLCA aggregation kinetics. The morphology of these cluster yields fractal like aggregates which is to be expected. Also, the lower concentration simulations did not yield too many aggregates due to the existence of the large repulsive barrier. However, there is still a lot of refining to be done on this simulation in order to make sure it agrees with the aggregation of proteins that can be seen through experimentation

Future:
After finely tuning this model to make sure it agrees with aggregation that occurs in the physical world, the next step is to include a patchy interaction between the monomers. The interactions should be patchy since the surface charge distribution of the monomers is not uniform, unlike the ideal situation in my simulations. I plan to continue working on this phenomenon in order to create the ideal most of the aggregation of these globular proteins.



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Sources:
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