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Type-I Collagen Modeled as a Liquid Crystal

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Introduction

Type-I collagen is found in numerous parts of the human body. It initially came under study in order to better understand the structure of bone and how this structure changes over time. From this information, we now have a much better understanding of a multitude of bone diseases such as osteoporosis. Consequently, this research has been focused on the macrostructures of type-I collagen and its implications on the physical properties of certain tissues. Furthermore, the self-assembly of the three α chains into the collagen triple helix has been studied in order to understand the biochemical pathway in which type-I collagen is produced. However, the self-assembly of triple helices in solution which enables the formation of the initial structure, collagen microfibrils, is not fully understood.

The scope of this research is to focus in on the ordering of type-I collagen in solution after the helices have been formed but prior to the formation of any kind of macrostructure. Specifically, a model has been constructed to predict the most stable ordering of type-I collagen molecules in aqueous solution as a

Figure 1. *The chiral nematic phase.*

function of concentration and temperature. The basis for this model lies in the liquid crystalline properties of type-I collagen; while it can exist in the isotropic phase with no long-range ordering similar to a regular liquid, it will adopt liquid crystalline properties after reaching a critical concentration and shift into the chiral nematic phase[2]. This phase depicted is by Figure 1, the molecules form layers that are ordered in two dimensions.

This model revolves around the structure of the type-I collagen triple helix and approximating it in a suitable fashion. Moreover, probability distributions are analyzed in order to establish the foundation of the model.

Background

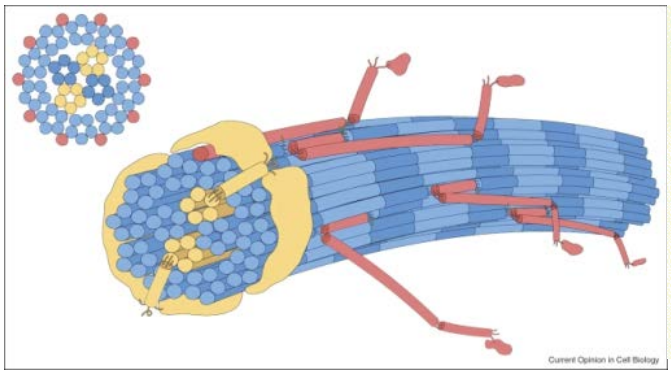

Figure 2. *Microfibril structure.*

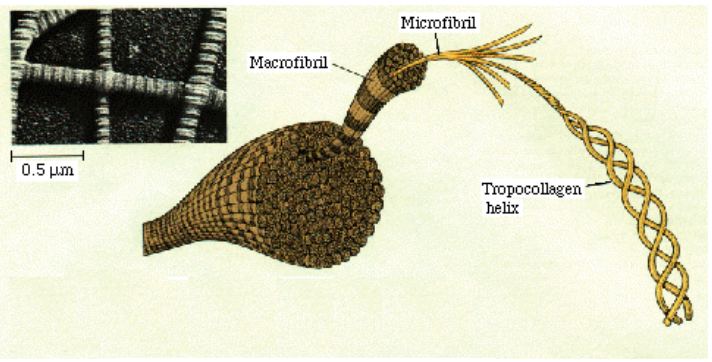

Figure 3. *Macomolecular structures of collagen.*

The structure of focus is the micrfibril made of type-I collagen. It forms a D-periodic, D=67nm, fibril that can be 4-stranded, 5-stranded, or 8-stranded with most of the collagen triple helices aligning with the orientation of the fibril as a whole. These microfibrils then are assembled into macrofibrils and then into tissues. Thus, the ordering of the macrostructure would not be possible without the initial self-assembly of the triple helices into the structure corresponding to the chiral nematic phase. The ability to synthetically reproduce these structures from an engineered substitute for collagen while maintaining the same biochemical properties is

of the upmost importance. As a result, this model is targeted at gaining insight into the role that liquid crystalline properties play during the formation of macrostructures such as the ones presented in Figures 2 and 3.

As previously stated, there is a critical concentration at which type-I collagen will undergo a phase transition from the isotropic phase to the chiral nematic phase. This can also be interpreted as the point in which type-I collagen triple helices go from molecules in solution to a lyotropic liquid crystal. The definition of a liquid crystal according to Merriam Webster is "an organic liquid whose physical properties resemble those of a crystal in the formation of loosely ordered molecular arrays similar to a regular crystalline lattice and the anisotropic refraction of light." The key elements of this are that it is a "loosely ordered molecular array," it has long – range ordering in one or two dimensions while a crystal has long-range ordering in three dimensions and a liquid has long-range ordering in zero dimensions; this is portrayed in Figure 4. Therefore, the orientation of one type-I collage helix can have an influence on another type-I collagen helix. This is the essence of the term lytropic; it indicates that a compound will only adopt liquid crystalline properties within a certain concentration range. This implies that in order for collagen to self-assemble into these useful orderings, it must first be locally concentrated past a critical concentration in that part of the human body. Therefore, the comparison of the observed structures and the ones predicted by that of the model can shed light on the early stages of development and how certain tissues arise form microfibrils and other macro structures.

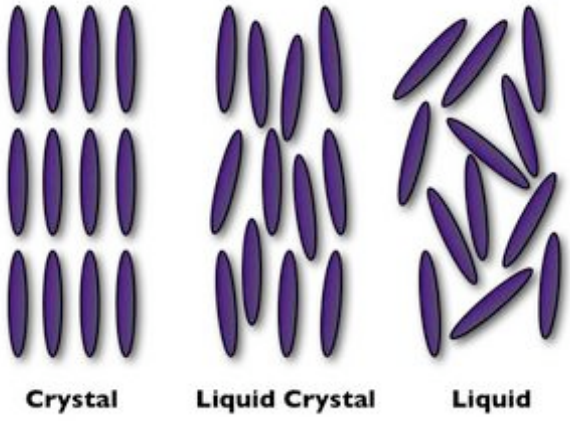

Figure 4. *Ordering of different compounds.*

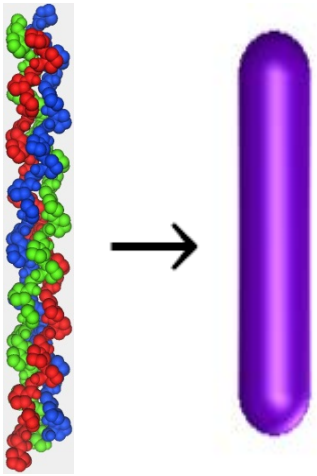

Figure 5. *Approximation of the triple helix.*

Previous theoretical models have assumed that the collagen triple helix can be approximated by a lyotropic mesophase of a charged semi-flexible spherocylinder that is approximately 300nm long and has an aspect ratio of about 250[1]. This can be seen pictorially in Figure 5. The term mesophase indicates a phase between solid and liquid, which is very appropriate for a liquid crystal. The next term, charged, reflects that the triple helices contain charged amino acids and the model takes those charges into account. The next term, semi-flexible, is one of many possible classifications that can be applied to a molecule in order to categorize the rigidity of the system in solution. For the purposes of this research, the type-I collagen triple helix was assumed to be a rigid-rod molecule with no ability to flex in solution. This change was implemented in order to include the effects of excluded volume while not increasing computation time by a drastic amount. The next term, spherocylinder, is accurately depicted by the shape on the right hand side of Figure 5; a cylinder with half a sphere on top and half a sphere on bottom. The last term, aspect ratio, refers to the ratio of length divided by diameter. Since the length is 300nm, the diameter is very small compared to the length of the molecule and this is reflected in the large aspect ratio of 250. Moreover, it should be noted that the diameter is relatively constant at any point along the triple helix so the aspect ratio is treated as a constant. This approximation is used to make all of the rods identical and therefore much easier to manipulate.

Once the critical concentration of type-I collagen is reach and the helices take on liquid crystal properties, it is possible to demonstrate that all packing schemes are just an effort to minimize excluded volume[2]. Excluded volume is the idea that in long chain polymer, such as

collagen, one part of the chain cannot occupy a space that is already occupied by another part of the chain. While it may seem intuitive that two things cannot exist in the same space, implementing this in modeling has posed challenges for Monte Carlo simulations. The model takes excluded volume into account and ensures that no rods overlap in order to maximize accuracy of the predicted structure of rods. Moreover, the phase transition from isotropic to chiral nematic also depends on the charge density of the molecules and thus pH of the solution under study[2]. Thus, the phase transition is merely an allocation of space to each molecule such that it experiences minimal effects from excluded volume and minimizes the increase in potential energy of solution when more molecules are placed into solution.

The core mathematical concept for this model is the quadrupolar tensor order parameter, Q. This is often used to describe the ordering of liquid crystals in the chiral nematic phase as well as other phases. Technically, it is defined to be the kernel of the second moment of the orientation distribution function. A better framework for the quadrupolar tensor order parameter is that it is a 3x3 matrix that places weights on the deviation from the isotropic ordering. In other words, it places a probability weight on the long-range orientation of the molecules with a reference of no long-range order. While it does not have a direct physical interpretation without the use of a multitude of other equations, the matrix itself can be seen below.

$$Q = \begin{bmatrix} Q_{XX} & Q_{XY} & Q_{XZ} \\ Q_{YX} & Q_{YY} & Q_{YZ} \\ Q_{ZX} & Q_{ZY} & Q_{ZZ} \end{bmatrix}$$

Additionally, this matrix can be generalized into specific parameters for uniaxial and biaxial ordering; uniaxial order refers to long-range order in one dimension and biaxial order refers to long-range order in two dimensions. The Landau-de Gennes theory of liquid crystals applies the

assumption of uniaxial ordering in order to simplify the system to a manageable point. For instance, according to the Landau-de Gennes theory, liquid crystals in the nematic phase can be approximated by uniaxial ordering due to rapid molecular tumbling .This reduces the orientation distribution function to a function of a single angle in three space, the director angle, n. The director angle can be interpreted as the angle relative to some defined zero that the molecules will most likely be aligned with in solution. This can be seen in Figure 6; the molecules are mostly pointed towards the director angle which would be 0º from the vertical in this particular case. In conjunction with this assumption, it must be noted that the chiral nematic phase is a lamellar phase, it has layers, and thus the periodicity of the system can be related to the director angle[2]. This is depicted in Figure 7, each layer has a varying director angle. However, it is commonly accepted that Landau-de Gennes merely provides a basis for the Onsager model that is more commonly used to describe three dimensional ordering in lyotropic liquid crystals and then biaxial ordering and the chirality of the molecules must be taken into account[2]. Hence, correcting terms are often added to the model in order to incorporate deviations from the rigid-rod assumption due to rod flexibility and electric charges[2]. Thus, for this research, the Onsager model is used as a basis since it incorporates the

Figure 6. *The nematic phase.*

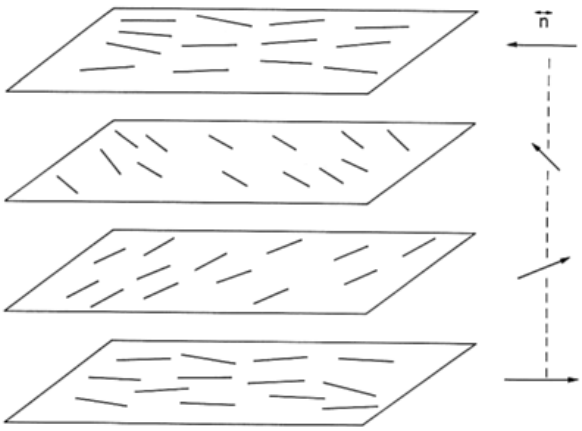

Figure 7. *The chiral nematic phase with director angles n.*

chirality of the triple helices, the biaxial ordering in the chiral nematic phase, and the charge density of the helices.

Methods

All programming was done in MATLAB R2010a. The initial model was following the structure as outlined in "A lattice model of the translational dynamics of nonrotating rigid rods." The model was expanded to incorporate orientations of rods that were not strictly along the x, y, and z axes. Initially, rigid rods that approximate the type-1 collagen triple helices are generated. These rods are described by a midpoint with x y z coordinates, an angle off of the z-axis, Φ, an angle off of the x-axis, Θ, and a length to diameter ratio. This is depicted by Figured 8. Each pixel in the three dimensional space is defined to be a cube with a volume of the triple helix diameter cubed, $1.728nm^3$. The rods are randomly generated and then placed into the space only if they do not overlap with any pre-existing rods. This is checked by calculating the minimum distance between all pairs of lines defined by the midpoints and orientation vectors. This is calculated by:

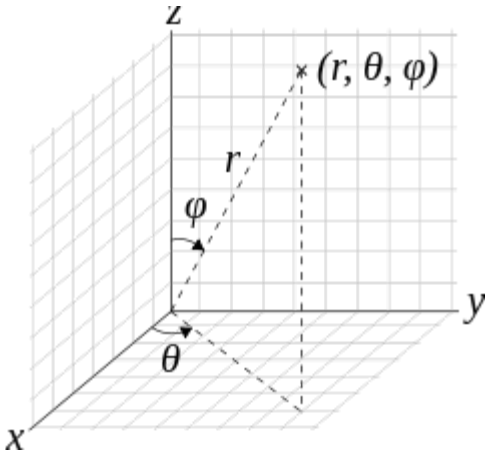

Figure 8. *The coordinate system.*

$minimum\ distance = \left|\frac{(u \times u')\cdot(m_0 - m_1)}{|u \times u'|}\right|$. Where u is the director vector for a given rod with midpoint m. If the minimum distance is less than one, the distance from the midpoints to the location where the minimum distance occurs is calculated. A rod is accepted if it has no minimum distance less than one or the distances that are less than one are farther than half the length to diameter ratio away from the midpoints of the rods involved. The core assumption for

these calculations is that no two rods are created exactly parallel. Since uniformly distributed random number generators are used to for the angles off of the x-axis and z-axis, this is a valid assumption. Once the desired number of rods is achieved, the total free energy of solution must be calculated and minimized.

The Helmholtz free energy of solution is given by:

$$A(\Psi(u)) = ck_bT\left[\ln c - 1 + \int \Psi(u)\ln\Psi(u)\,du + \frac{1}{2}c\int\int \Psi(u)\Psi(u')\beta(u,u')du'du\right].$$

Where c is the rod concentration, k_b is Boltzmann's constant, T is temperature, u is the direction of a rod, $\Psi(u)$ is the probability associated with orientation u, and $\beta(u,u') = 2dL|u \text{ x } u'|$. The probability distribution used is the form assumed by Onsager, $\Psi(u) = \frac{\alpha}{4\pi\sinh\alpha}\cosh(\alpha u \cdot n)$, where α is a parameter that is determined by minimizing A. With the rods are in solution, the total free energy is minimized by means of Newton's method. α is approximated by the formula $\alpha^{k+1} = \alpha^k - \frac{f(a^k)}{f'(\alpha^k)}$ where $f'(\alpha^k) = \frac{f(\alpha^k+\delta\alpha)-f(\alpha^k-\delta\alpha)}{2\delta\alpha}$. The convergence criteria utilized for this research is $|\alpha^{k+1} - \alpha^k| < 0.01$. Once α is calculated, the probability distribution associated with the orientation of the rods relative to the respective director angle is calculated. The director angle is calculated by the equation $\Theta_e = \frac{2\pi}{p_o}z$, where p_o is the cholesteric pitch. From that, the director vector is calculated by $n = [\cos\Theta_e \quad \sin\Theta_e \quad 0]$.

The model for movement is adapted from "Phase optimization of a kinoform by simulated annealing." First, a rod is randomly chosen and a direction of movement is randomly selected. The size of the move is randomly selected, a uniform distribution between 1 and some maximum move size, and then the move is performed. If the move causes two or more rods to overlap then it is rejected. If the move increases or maintains the alignment of the rods and does

not overlap with any other rod, then it is accepted. If it decreases the alignment of the rods and does not overlap with any rod then the probability of acceptance is $P = e^{\frac{-\Delta Tensor}{\left(\frac{1}{1+0.5*Iteration\#}\right)}}$, where the tensor is $S = \langle\frac{3\cos\theta-1}{2}\rangle$. $\langle\quad\rangle$ denotes the statistical average over all rods and θ is the angle between the orientation vector and the director vector.

Results

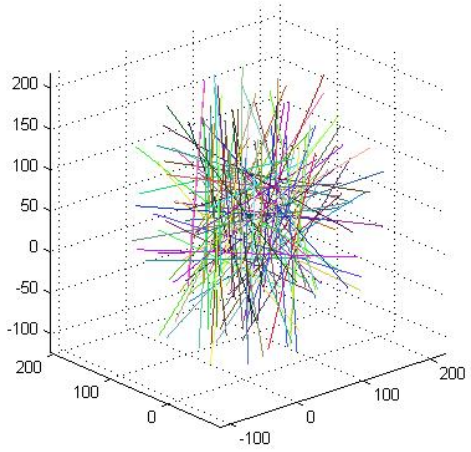

Figure 9. *Approximately 100 rods prior to any movement.*

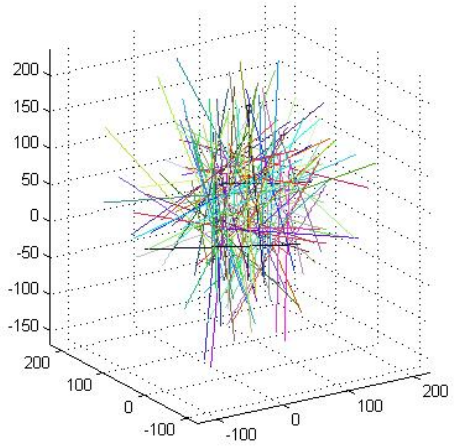

Figure 10. *Approximately 100 rods after 10,000 moves.*

Without the inclusion of electrostatics, it is difficult to pictorially judge if the rods become more aligned in solution after random movement as seen in Figures 9 and 10. Moreover, the most stable state estimated, Figure 10, has little to no resemblance to the structure of a collagen microfibril.

Further work will include the incorporation of the surface charge on the rods and thus adding the variable pH of solution and boundary conditions to ensure that the program converges for all concentrations that type-1 collagen is in the chiral nematic phase but does not converge for all concentrations associated with the isotropic phase.

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