Constitutive Modeling of the Stress-Stretch Behavior of Two-Dimensional Triangulated Macromolecular Networks Containing Folded Domains

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Abstract

The mechanical behavior of the red blood cell membrane is governed by the lipid bilayer which resists changes in surface area and the underlying spectrin network which resists changes in shape. The constituent spectrin chains of the network consist of a series of domains along the chain, which exhibit noncovalent interactions. Upon sufficient extension of a chain, each folded domain undergoes mechanically-induced unfolding after reaching a chain force between 10 to 35pN. Individual spectrin chains within the network experience their first unfolding event at different levels of macroscopic strain depending on the macroscopic loading conditions and the orientation of each constituent chain with respect to the macroscopic loading. A microstructurally-informed continuum level constitutive model is developed which tracks individual chain deformation behavior as well as the overall macroscopic network stress-strain behavior. Using the introduced continuum approach and statistical mechanics based models of the chain force-extension behavior together with a transition state model of domain unfolding; a constitutive model for the membrane stress-stretch behavior is constructed. Uniaxial tension and simple shear behaviors of the membrane are simulated incorporating the unfolding of the individual chains. A Taylor averaging approach is used as a first approximation to account for the irregularities in the spectrin network which result in a near plateau-like force behavior with increasing stretch.

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1 Introduction

Many protein molecules have multidomain structures which result in a force-extension behavior with a characteristic saw-tooth pattern due to stretch-induced unfolding of domains along the molecular chain (Figure 1). During a displacement controlled extension test, the force increases in a nonlinear manner with stretch until reaching a peak, whereupon there is a drop in the force, followed by a nonlinear rise to a peak, followed by a drop, and so on [1-5]. Upon reaching a rate-dependent peak force, a domain will unfold, releasing additional chain length which increases the configurational space available to accommodate the overall extended chain length. Due to the entropic nature of the single molecule extension behavior, the increase in configurational space results in the observed drop in force and the increased compliance after the force drop.

Fig. 1. Schematics of the stages of unfolding for a single chain under mechanical loading. (1) corresponds to the undeformed chain, with a non-zero end-to-end distance, (2) corresponds to the nearly fully extended chain when the force reaches a value that will unfold a domain; a domain then begins to unfold as the force drops, and (3) corresponds to the chain after one domain has unfolded and the chain force starts increasing nonlinearly with monotonic stretching again. The dashed lines showing another level of force drop correspond to a lower extension rate. The inset of the plot shows how the peak force of unfolding changes with extension rate (Schematic adapted from [1]).
Single molecule force-spectroscopy has been used to quantify the force-extension behavior and corresponding mechanically-induced unfolding of domains along the spectrin molecule [4, 5]. Rief et al. [4] noted that the peak unfolding force is approximately $30 \text{ pN}$ when a long chain spectrin molecule is stretched at a rate of $0.3 \mu \text{m/s}$ and also identified rate dependence to the peak unfolding force. Law et al. observed spectrin unfolding to occur at forces ranging between $\sim 10 \text{ pN}$ to $\sim 37 \text{ pN}$ during extension of short (3-4 repeat units) strands of spectrin at extension rates of $1 \mu \text{m/s}$ [4, 5].

In this paper, a microstructurally-informed continuum level constitutive model which accounts for the triangulated molecular network structure is developed which tracks individual chain deformation behavior as well as the overall macroscopic network stress-strain behavior [6]. The force-extension behavior of individual chains is modeled using a statistical mechanics representation of the long chain molecule together with an Eyring type transition state model [7] to capture the rate dependence of domain unfolding. The chain constitutive model taken together with the network representation determines the effect of individual chain unfolding events on the overall network stress-strain behavior.

2 Membrane Structure of a Red Blood Cell

The mechanical properties of the red blood cell membrane are governed by the lipid bilayer, which resists changes in the membrane surface area of the red blood cell, and the spectrin network, which resists surface shearing of the membrane. The surface area stiffness ($\sim 500 \text{ dyn/cm}$) is much greater than the surface shear stiffness ($\sim 0.01 \text{ dyn/cm}$). Hence this membrane is a two-dimensional analogue to a rubbery solid where a rubber exhibits a high bulk modulus ($\sim 1 \text{ GPa}$) and a low shear modulus ($\sim 1 \text{ MPa}$). The distinct physical origins of the mechanical behavior of the surface area and that of the surface shear together with the high contrast in these properties enables separate modeling of the shear behavior from that of the surface area behavior. Indeed, in most instances, it is sufficient to approximate the surface area as remaining constant. Phenomenological neo-Hookean hyperelastic type constitutive models have been proposed and used in early work by Skalak et al. [8] and Evans [9], and higher-order hyperelastic models in recent work by Suresh and colleagues [10,11]. A microstructurally-informed continuum level
model of the large deformation behavior of the spectrin network has recently been developed by Arslan and Boyce in [6]. The Arslan and Boyce model predicts the membrane stress-strain behavior based on the force-extension behavior of the constituent chains and the triangulated network geometry. Arslan and Boyce also identified the roles of both chain rotation and chain stretching to the nonlinear stress-strain behavior of the network by examining networks with linear chain behavior in comparison to networks with nonlinear chain behavior. In this paper, the Arslan and Boyce constitutive model for the spectrin network is enhanced to take into account the unfolding behavior of the constituent chains.

3 Constitutive Model

In recent years, several investigations have adopted and extended the framework of statistical mechanics based models for rubber elasticity (e.g. Flory [12], Treloar [13], Boyce and Arruda [14]) to develop microstructurally-motivated models of biological materials (e.g., Bischoff, et al. [15,16], Bergstrom and Boyce [17], Holzapfel [18], Qi, et al. [19,20], Arslan and Boyce [6,21], Kuhl, et al. [22]). Our constitutive model for the general membrane stress-stretch behavior of the spectrin network follows this successful methodology [6]. Indeed, a recent review paper [23] highlights the need and the trend for models of the behavior of the various molecular networks of biological cells to properly account for the translation of the macroscopic strain to the constituent elements of these networks. The Arslan and Boyce model for the finite deformation of triangulated networks is reviewed and extended below in terms of (1) identification of the networked microstructure and the mapping of macroscopic deformation onto the corresponding network representative volume element (RVE); (2) description of the force-extension behavior of the RVE constituent chains together with a transition-state unfolding criterion; and (3) construction of a strain energy density function for the network and its corresponding macroscopic stress-stretch behavior.

3.1 Network Idealization, Representative Volume Element and Deformation

The spectrin network of the red blood cell is found to possess a triangulated network as shown in micrographs of spread cell membranes [e.g. 24, 25]. We note that the “spread”
state of the membrane is a highly biaxially stretched state; the triangulated network structure is in tact under these large deformations showing the robustness of the crosslink sites to mechanical deformation. Hence, as in [6], we idealize the microstructure to be perfectly triangulated as shown in Figure 2. A unit equilateral triangle is identified as the RVE (Figure 2C). Voronoi tessellation is used to assess the area affiliated with the RVE (Figure 2A). Therefore the chain density of the network is found to be \( \nu = \frac{3}{2A_{\text{triangle}}} \). A schematic of the undeformed RVE is given in Figure 2C, where \( r_o \) is the initial chain end-to-end distance and \( \phi_A, \phi_B, \text{ and } \phi_C \) represent the orientation of the constituent chains with respect to the 1-direction. In the proposed microstructurally-informed model, the change in length (the stretching of the chains) and orientation (the rotation of the chains) of the chains can be tracked with macroscopic deformation as demonstrated and discussed in Arslan and Boyce [6].

Fig. 2. Schematic of an area of the triangulated network in (A) the undeformed state, also depicting Voronoi tessellation (the superposed hexagon) to identify the area of the RVE, and (B) an arbitrary deformed state. Schematic of the isolated (C) undeformed representative volume element and (D) the RVE when subjected to an arbitrary loading condition.

An arbitrary macroscopic deformation is mapped onto the unit cell equilateral triangle RVE. The membrane deformation gradient \( \mathbf{F}_{2D} \) is defined in the 1-2 frame as:
\[ \mathbf{F}_{2D} = \nabla \mathbf{x} = \begin{bmatrix} F_{11} & F_{12} \\ F_{21} & F_{22} \end{bmatrix} \] where \( \mathbf{x} \) is the deformed position of a material point and \( \mathbf{X} \) is the reference position. The RVE is subjected to an arbitrary deformation gradient giving the stretch of constituent network chains A, B, C in terms of the macroscopic deformation gradient. The simplicity of the unit cell triangle RVE provides a unique, kinematically-determined mapping of the macroscopic deformation gradient to the microscopic network deformation. Denoting the current end-to-end distance of each chain as \( r_i (i = A, B, C) \), the axial stretch of each chain in the network is, \( \lambda_i = \frac{r_i}{r_o} (i = A, B, C) \) and can be expressed in terms of an arbitrary deformation gradient as demonstrated by Arslan and Boyce [6]:

\[
\lambda_A = \left( F_{11}^2 + F_{21}^2 \right)^{\frac{1}{2}} \\
\lambda_B = \frac{1}{2} \left( (F_{11} - F_{12} \sqrt{3})^2 + (F_{21} - F_{22} \sqrt{3})^2 \right)^{\frac{1}{2}} \\
\lambda_C = \frac{1}{2} \left( (F_{11} + F_{12} \sqrt{3})^2 + (F_{21} + F_{22} \sqrt{3})^2 \right)^{\frac{1}{2}}
\]

### 3.2 Force-extension Behavior of Constituent Chains

The mechanical behavior of the constituent chains (the spectrin molecules of the triangulated skeletal network of the red blood cell are long chain molecules containing many repeat units along the length of chain between crosslink sites) of the RVE (A, B, C) is modeled using the non-Gaussian Freely Jointed Chain (FJC) model (e.g., Treloar [13])\(^1\). The chain force-extension expression is given by:

\[
P_{ch} = \frac{N k_b \theta \beta}{L_c}
\]

\(^1\) We note here that these flexible molecular chains can be alternatively represented using the worm-like chain (WLC) model. Qi et al. (20) have found WLC and FJC models to give quite similar results. Therefore, we only present FJC here.
with corresponding chain strain energy:

\[
\mathcal{U}_c = k_b \theta N \left( \frac{r}{L_c} \beta + \ln \left( \frac{\beta}{\sinh \beta} \right) \right),
\]

where \( N \) is the number of Kuhn segments along the chain, \( k_b \) is Boltzmann’s constant, \( \theta \) is the absolute temperature, \( L_c = Nl \) is the contour length of the chain, \( l \) is the Kuhn segment length, \( r \) is the chain end-to-end distance and \( \beta \) is the inverse Langevin function. The Langevin function is defined as \( \mathcal{L}(\beta) = \coth(\beta) - \frac{1}{\beta} \), with the inverse, \( \beta = \mathcal{L}^{-1} \left( \frac{r}{L_c} \right) \).

To incorporate unfolding of a folded domain in the microstructurally-informed model [6], we utilize a transition state model.

Following transition state theory, a domain can be modeled as being in one of two states: the folded state, or the unfolded state. To transfer from the folded state to the unfolded state, an energy barrier of \( \Delta G \) has to be overcome. Following Rief et al. [26] and Qi et al. [20], we use the Bell [27] adaptation of the Eyring model [7]: the energy barrier to translate from one state to the other is reduced by the applied chain force, \( P_{ch} \), multiplied by the width of the activation barrier, \( x_u \), giving a frequency of unfolding, \( \alpha \)

\[
\alpha(P_{ch}) = \omega \exp \left( \frac{-(\Delta G - P_{ch} x_u)}{k_b \theta} \right) = \alpha \exp \left( \frac{P_{ch} x_u}{k_b \theta} \right)
\]

where \( k_b \) is Boltzmann’s constant, \( \theta \) is absolute temperature, and \( \alpha \) is a lumped parameter: \( \alpha = \omega \exp \left( -\Delta G / k_b \theta \right) \). \( \alpha \) and \( x_u \) are then obtained from the data of peak unfolding force as a function of the strain rate. For completeness, we note that the energy
barrier to unfolding depends on the particular folded domain through its molecular geometry and the nature of intermolecular interactions; the axial chain force will map to a combination of shear and normal intermolecular stresses within the domain. Hence, the unfolding of a domain will be due to some combination of normal and shear separation and will depend on how the domain is being loaded. In a long chain molecule with many domains in series, the domain most favorably oriented to unfold (i.e. the domain which will unfold at the lowest axial chain force) will be the domain to unfold; therefore, the experimentally observed unfolding force at a given rate of extension is repeatable and the effect of the chain force on lowering the energy barrier is effectively captured by equation (4).

After a folded domain unfolds, additional chain length is released from the fold and the contour length increases leading to an increase in the available configurational space (implying higher entropy), and a corresponding drop in the chain force. This behavior of a repeating sequence of a force rise with extension to a peak followed by a load drop, gives rise to a “saw-tooth pattern” (Figure 1). Rief, et al. [4] have tested the force-extension behavior of long chain spectrin molecules at different extension rates, observing unfolding to occur at an average of ~27pN at 0.08 µm/s and ~32pN at 0.80 µm/s.

Prior to unfolding, the number of the effective rigid links along the chain is:

\[ N_{(t=0)} = n - m_{(t=0)} (q - l), \]

where \( n \) is the total number of rigid Kuhn links, \( m \) is the number of folded domains and \( q \) is the effective number of links of length \( l \) in a folded domain [19]. When a domain unfolds, the number of folded domains decreases by 1, giving

\[ m_{(t_{i+1})} = m_{(t_i)} - 1. \]

After one domain unfolds, the effective number of rigid links at \( t = t_i \), \( N_{(t=t_i)} \) is updated according to:

\[ N_{(t=t_i)} = n - m_{(t_i)} (q - l). \]

The contour length, \( L_c \), of the molecule increases, giving a new contour length of

\[ L_{(t=t_i)} = N_{(t_{i+1})} l, \]

where \( l \) is the length of the rigid links. The summary of the formulation of the procedure for force-extension with unfolding is given in Appendix A.
3.3 Strain Energy Density of the RVE

To determine the behavior of the network, the strain energy of the RVE, $U$, is calculated by the summation of the strain energy in each chain. The strain energy density, $U^*$, is given by:

$$U^* = \frac{1}{2 A_{\text{triangle}}} \left( U_A + U_B + U_C \right), \quad (5)$$

where $A_{\text{triangle}}$ is the area of the RVE. Thus, the strain energy density is given by:

$$U^*_\text{NGC} = \frac{\nu}{3} \left\{ k_o \theta N \sum_{i=A,B,C} \left[ \frac{\lambda_i r_i}{L_c} \beta_i + \ln \left( \frac{\beta_i}{\sinh \beta_i} \right) \right] - \left( \frac{r_o}{L_c} \beta_o + \ln \left( \frac{\beta_o}{\sinh \beta_o} \right) \right) \right\} \quad (6)$$

where $\nu$ is the chain density ($= \frac{3}{2 A_{\text{triangle}}}$) given earlier, $r_o$ is the initial end-to-end distance of a chain (i.e. the initial chain length or distance between cross-links), $\lambda_i (i = A, B, C)$ are the constituent chain stretches defined earlier as a function of the macroscopic deformation gradient, and $\beta_i = \lambda_i r_i \left( \frac{\lambda_i r_o}{L_c} \right)$.

The Cauchy stress is determined by proper differentiation of the strain energy density function (Appendix B) and is given by:

$$T = \left[ \frac{\partial U^*_A}{\partial \lambda_A} \frac{\partial \lambda_A}{\partial F_{2D}} + \frac{\partial U^*_B}{\partial \lambda_B} \frac{\partial \lambda_B}{\partial F_{2D}} + \frac{\partial U^*_C}{\partial \lambda_C} \frac{\partial \lambda_C}{\partial F_{2D}} \right] F_{2D}^T + h I. \quad (7)$$

Here $h$ is the additional equibiaxial membrane stress (due to the preservation of area constraint) required to satisfy equilibrium. The $\left( \frac{\partial \lambda}{\partial F} \right)$ terms are independent of chain
constitutive behavior and obtained by direct differentiation of the kinematically specified relationships (Equation (1)) connecting the stretch of each chain to the macroscopic deformation.

3.4 Material Properties

Table 1. Spectrin properties

<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>Spectrin Network</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial end-to-end distance, ( r_o ) (nm)</td>
<td>75</td>
</tr>
<tr>
<td>Persistence length, ( \ell ) (nm)</td>
<td>10.25</td>
</tr>
<tr>
<td>Initial contour length (nm), ( L_o )</td>
<td>180</td>
</tr>
<tr>
<td>Increase in contour length due to unfolding, ( \Delta L ) (nm)</td>
<td>28.8</td>
</tr>
<tr>
<td>Activation barrier width, ( x_u ) (nm)</td>
<td>1.7</td>
</tr>
<tr>
<td>( \alpha ) (x10^6 s^-1)</td>
<td>6.0</td>
</tr>
</tbody>
</table>

The network and chain properties for \( r_o \), \( \ell \), and \( L_o \) are found as discussed in Arslan and Boyce [6] and are given in Table 1. As discussed in [6], the initial elastic stiffness of the model with these properties captures the experimentally reported modulus for the skeletal spectrin network (as measured, for example, using cell aspiration tests). The unfolding properties consist of \( x_u \), \( \alpha \), and \( \Delta L \). These properties are determined from fitting the model to the AFM data of Rief et al. [4, 26] as given in [19] and therefore capture the experimentally observed dependence of unfolding force on extension rate.

4 Results

4.1 Uniaxial Tension
Fig. 3. (A) Stress-stretch behavior of the membrane under uniaxial tension in the 2-direction at a strain rate of 0.1/s where Cauchy and nominal stress are compared, (B) constituent chain force-extension behaviors in the RVE during uniaxial tension in the 2-direction.

Figure 3A shows the Cauchy (true) and nominal stress versus stretch behavior of the network when subjected to uniaxial tension in the 2-direction. The nominal stress curve has a plateau-like peak stress region where successive unfolding events occur at a nominal peak unfolding stress of 0.5 dyn/cm. The first unfolding event happens at a stretch of $\lambda = 2.75$. In terms of the Cauchy (true) stress, the unfolding stress increases with each unfolding event due to the decrease in load-bearing area as the membrane is stretched. Once an unfolding event occurs in a constituent chain, the macroscopic stress drops due to the force drop in the chain(s). After the stress drop, the stress increases in a nonlinear manner with further extension, but now with a lower stiffness because of the increase in the compliance of the constituent chains which have experienced an unfolding event.

Figure 3B shows the constituent chain behavior during tensile stretching. Chains B and C are observed to extend and exhibit a sawtooth force-extension behavior. Chain A does not contribute to the unfolding events since it is only compressed under this applied macroscopic stretch.
Fig. 4. Stress-stretch behavior of the membrane under uniaxial tension (A) in a range from $0^\circ$ to $30^\circ$ loading, depicting the mean stress; (B) in the 11 and the 22-directions, the “average” curve depicting the macroscopic composite stress behavior.

Figure 4A shows the uniaxial stress versus macroscopic stretch for tension applied in different directions ranging from $\theta = 0^\circ$ to $30^\circ$. Because of the symmetry, $60^\circ$ loading gives the same stress response as the $0^\circ$ loading, likewise, $90^\circ$ loading gives the same response as $30^\circ$ loading. $0^\circ$ loading reflects uniaxial tension in the 1-direction and $90^\circ$ loading reflects uniaxial tension in the 2-direction. The six-fold symmetry of the undeformed microstructure results in isotropy of the very initial modulus. A nearly direction-independent behavior is shown to be retained up to rather large stretches ($\lambda=2.2$), showing the isotropic robustness of the triangulated microstructure. However, at very large stretch values the developing anisotropy begins to manifest itself. The first unfolding event occurs at a smaller macroscopic stretch when uniaxial tension is applied at $0^\circ$ than in the $30^\circ$ case. This shows that the number of unfolding events and the degree of deformation is dependent on the loading direction as the six-fold symmetry condition does not hold at larger stretches due to the developing microstructural anisotropy as captured naturally in the microstructurally-informed constitutive model. In actual networks, there exist some irregularities in the network structure which may lead to ongoing load and deformation redistribution within the network (and isotropy to larger
stretches) as macroscopic stretching increases, especially when unfolding begins to occur in some chains.

In order to achieve a stress-stretch curve that represents the red blood cell membrane behavior more accurately, a Taylor averaging approach [30] is used. As a first approximation, to account for a slightly irregular triangulated network, we “average” the behavior of constituent unit triangles inclined at $0^\circ$, $10^\circ$, $20^\circ$, and $30^\circ$ with the 1-direction. It is assumed that each constituent triangle experiences the same deformation gradient, $F^2$. Based on this assumption, the individual Cauchy Stress, $T$, of each constituent triangle is calculated as described earlier and the macroscopic composite stress is calculated by taking a volume average of the stress from all constituent triangles. The “average” curve in Figure 4A depicts the mean of the stress response for directions: $0^\circ$, $10^\circ$, $20^\circ$, and $30^\circ$. The dramatic saw-tooth response obtained for a perfectly triangulated structure is observed to smooth out to a behavior with lower peaks and lower drops. In order to achieve an even more precise and smoothened stress-stretch response, the macroscopic composite stress was ascertained by calculating the average response for a network comprised of unit triangles whose orientation varied in 1 degree intervals; see the “average” curve shown in Figure 4B. The numerous distributed orientations of the chains in the network result in a plateau-like force behavior in contrast to the “saw-tooth” pattern of the perfectly triangulated network. This plateau corresponds to simultaneous chain stretching and domain unfolding events taking place in different chains in the rather irregular network. The unfolding peaks and drops are contained within a stress band of mild-fluctuations. This plateau-like behavior gives compliance to the network that enables large scale deformations at nearly constant stresses.

\[ We recognize that the Taylor approach does not capture the ongoing interplay of local load and deformation redistributions on the network chains that an actual irregular structure experiences, but feel this first approach to averaging illustrates the basic influence of a more complex triangulated network structure on the overall macroscopic stress-strain behavior.\]
Figure 5 shows the strain-rate dependence of the stress-stretch behavior under large stretches for strain rates from $1/s$ to as low as $0.001/s$. When the strain-rate decreases, the stretch at which the unfolding occurs drops, the peak stress decreases and the stress drop decreases. The decrease in the peak stresses and the stress drops also leads to a plateau-like stress level at very low strain rates which limits and controls the level of force required for large deformations.

The initial end-to-end distance $r_o=75nm$ implies a pretension in the network [6, 20]. Figure 6 compares the effect of two different initial end-to-end distances ($75nm$, $125nm$) on the uniaxial tensile response of the network. When the initial end-to-end distance is taken to be $125nm$ (an initial chain end-to-end distance close to the initial contour length of the chain), the areal chain density decreases which would normally be associated with a decrease in modulus. However, the fact that $r_o=125nm$ is close to the contour length gives an increase in the initial modulus and a decrease in the extensibility of the network; the unfolding events are found to initiate at much lower stretch levels.
Hence we can see that network pretension strongly influences the overall mechanical behavior.

4.2 Simple Shear

Fig. 7. (A) Stress-stretch behavior of the membrane under simple shear in the 12-direction and in the 21-direction, the “average” curve depicting the macroscopic composite stress behavior; (B) chain force-extension behavior in the RVE under simple shear in the 12-direction.

Figure 7A shows the shear stress vs. nominal shear strain, tan γ, behavior of the network at a shear rate of 0.1 rad/s. We note that the curves of shear stress in the 12- and the 21-direction are nearly coincident up to a shear strain of tan γ=1.5, demonstrating the robustness of this triangulated microstructure in providing isotropic mechanical behavior to relatively large strains. At a shear rate of 0.1 rad/s, when shear is applied in the 12-direction, the first unfolding happens at tan γ=1.97, while for shear in the 21-direction, the first unfolding occurs at tan γ=2.2. The shear strains at which the network experiences initial unfolding is very close for shearing in different directions. Figure 7A also shows the macroscopic composite shear stress versus stretch behavior calculated following the Taylor averaging approach as discussed in the case of uniaxial tension where we average over 1° increments between 0° and 30°. The averaging is seen to result in the plateau-like behavior once a critical unfolding stress is reached.
Figure 7B shows the chain force versus macroscopic stretch for shearing in the 12-direction. When shear is applied in the 12-direction, only chains B and C deform, stretching and rotating with the deformation. Chain B compresses until it makes an angle of $\theta_B = 90^\circ$ with the 1-direction, it then extends. This delayed extension results in chain C initiating the unfolding in the overall RVE. Therefore, the shear stress-shear strain plot shows an initial unfolding behavior which is identical to chain C’s unfolding behavior.

5 Summary

The mechanical behavior of the red blood cell membrane is governed by the lipid bilayer which resists changes in surface area and the underlying spectrin network which resists changes in shape. The constituent chains of the spectrin network consist of a series of domains along the chain, which exhibit noncovalent interactions. Unfolding of these folded domains can be triggered by the application of large deformation to the macromolecular network, depending on the extension rate and also the statistical distribution of the strength of the internal bonds of the module. The force-extension behavior of a single modular macromolecule exhibits a “saw-tooth” pattern due to unfolding giving a sequence of force rise to a peak followed by a load drop, rise to a peak and drop etc. A microstructurally informed continuum level constitutive model which tracks individual chain deformation behavior as well as the overall macroscopic network stress-strain behavior is developed by Arslan and Boyce [6]. In this paper, using the introduced continuum approach together with single molecule force-extension behavior and a transition state model of unfolding, large deformation behavior of two-dimensional triangulated networks of biomacromolecules is studied. Uniaxial tension and simple shear behaviors of the membrane are simulated incorporating the unfolding of the individual chains. The constituent chains of the representative volume element (RVE) of the idealized network do not unfold at the same time since the stretch (and force) in each chain is different depending on the macroscopic deformation. The triangulated network provides a more realistic approach to modeling the spectrin network than the “four chain” network representation [20] of macromolecular membranes since it directly accounts for the network geometry. The triangulated structure is found to be rather robustly isotropic in mechanical behavior to very large stretches. However, the individual chain
deformations evolve much differently and each molecule unfolds at different stretch levels during a macroscopic deformation which amplifies the effect of developing anisotropy on the mechanical response of the network. In actual networks, there exist some irregularities in the network structure which may provide additional robustness to isotropy as macroscopic stretching increases since the load and deformation will redistribute amongst the network molecules. In order to account for the irregularities in the spectrin network structure, a Taylor model approach is used as a first approximation for averaging, whereby the same deformation gradient is applied to RVEs with different initial orientations and the resulting stress is the volume average of the stress on each RVE. It is found that the distribution in the orientations of RVE’s result in a rather plateau-like stress-stretch response after reaching a critical stress level that initiates the first unfolding event; the plateau is due to the multitude of unfolding events occurring at different stretch levels balanced by ongoing force increases in other chains. The plateau controls the level of stress required for the deformation of the red blood cell membrane. Therefore, the averaging approach gives a first approximation of the stress-strain response of the red blood cell membrane accounting for aspects of distributions in the network structure. The effect of the strain rate on the mechanical response is also investigated in this paper. When the strain rate is as low as $0.001/s$, the stress peaks and drops become less apparent due to the decrease in the critical stress level needed to initiate unfolding as the rate is reduced; the predicted network rate dependence is strongly dependent on the rate-dependence of single molecule unfolding for which there is currently limited data [4,5].

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**Appendix A**

**Procedure for Force-Extension with Unfolding**

The Monte-Carlo algorithm used to model the random unfolding events can be summarized as follows:

1. A chain is subject to a stretch and its force at that stretch is determined using the FJC model of equation (2),
2. The unfolding frequency is calculated using equation (4),

3. The probability of unfolding a domain is calculated according to: 
   \[ dp = m_t \Delta \theta \] , where \( m_t \) is the number of folded domains in the chain at time \( t \),

4. The domains are sampled to determine their unfolding status,

5. If unfolding occurs, the contour length \( L_0 \) of the chain is updated and the force on that chain is re-computed using its new, updated structural parameters,

6. The steps are repeated where the macroscopic stretch is incrementally increased in each step.

Rief et al. [26] and Qi et al. [20] applied the Monte Carlo Simulation algorithm to various molecules including dextran and spectrin to model the single molecule unfolding.

**Appendix B**

**Formulation of Macroscopic Membrane Stress-Strain Relationship**

\( \mathcal{U}^* \), the strain energy density function, is defined to be a function of \( F \), the membrane deformation gradient and the number of effective rigid links along the chain, \( N \):

\[ \mathcal{U}^* = \mathcal{U}^*(F, N). \]

(B1)

Following the approach taken by Holzapfel [28], Qi and Boyce [20, 29], from the 2nd law of thermodynamics, the Clausius-Planck inequality for an isothermal process is written as:

\[ T_o \cdot \dot{F} - \mathcal{U}^* \geq 0, \]

(B2)

where, \( T_o \) is the 1st Piola Kirchhoff stress.

Following (B1), the derivative of the strain energy density function gives:

\[ \mathcal{U}^{*'} = \frac{\partial \mathcal{U}^*}{\partial F} \cdot \dot{F} + \frac{\partial \mathcal{U}^*}{\partial N} \dot{N}. \]

(B3)
Using (B3) and rearranging (B2):

\[
\left( T^* - \frac{\partial \mathcal{W}^*}{\partial F} \right) \cdot \dot{F} - \frac{\partial \mathcal{W}^*}{\partial N} \dot{N} \geq 0 .
\] (B4)

For an arbitrary deformation,

\[
T^* = \frac{\partial \mathcal{W}^*}{\partial F},
\] (B5)

and

\[- \frac{\partial \mathcal{W}^*}{\partial N} \dot{N} \geq 0 .
\] (B6)

For an incompressible material, equation (B5) gives the 1st Piola Kirchhoff stress and the Cauchy stress is then found to be:

\[
T = \frac{\partial \mathcal{W}^*}{\partial F} F^T - pI .
\]

Inequality (B6) shows that unfolding a domain is a dissipative process.

References


