Protein Forced Unfolding and Its Effects to the Finite Deformation Stress-Strain Behavior of Biomacromolecular Membrane and Solids

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ABSTRACT

Many mechanical load-carrying proteins have been experimentally observed to exhibit a characteristic repeating pattern of a nonlinear rise in force with imposed displacement to a peak, followed by a significant force drop upon reaching the peak (a saw-tooth pattern) in their force-extension behavior due to successive unfolding of modules during extension. This behavior is speculated to play a governing role in the some biological and mechanical functions of materials constituted of such protein networks. In this paper, models for the networks of such macromolecules are developed. A constitutive model for the force-extension behavior of a single modular macromolecule is presented using the Freely Jointed Chain (FJC) and Worm-Like Chain (WLC) models of statistical mechanics together with two-state theory as an unfolding criterion. The single molecule behavior is then used with a formal continuum mechanics approach to construct constitutive models of the finite deformation stress-strain behavior of two- and three-dimensional macromolecular membranes and solids containing folded modules. The model for two-dimensional networks has applicability to biological membrane skeletons, and the model for three-dimensional networks provides a constitutive model for cytoskeletal networks and solid biological tissues containing modular macromolecules. Simulations of these networks under different loading conditions illustrate features of the stress-strain behavior of these materials and the stretching conditions which activate unfolding in these microstructures. These constitutive models for the membrane and the solid thus are a starting point for understanding the role of modular protein mechanical behavior in issues of cell mechanics as well as in issues of protein-rich biological materials which may act as load transfer agents in biological composite structures such as spider silk and the adhesive layers of the nacre of abalone shell.

INTRODUCTION

It is well known that biomacromolecules, such as protein macromolecules, have a multi-domain architecture composed of folded modules along a macromolecular chain (Fig. 1). Unfolding of such domains can occur due to different sources including temperature, chemical denaturants, nonphysiological solvent and pH conditions. Force-induced unfolding of single modular macromolecular chains has been observed in recent atomic force microscopy (AFM) protein pulling tests\cite{1-4}. The force-extension behavior in these experiments generally exhibits a “saw-tooth” profile, a characteristic repeating pattern of a nonlinear rise in force to a peak, followed by a force drop after each peak\cite{1-4}. This “saw-tooth” pattern is due to the increase of the contour length of polymer chain after unfolding, thus the increase of configurational entropy and a distinct drop in force (e.g.\cite{5}).

Understanding how the mechanical responses of single modular biomacromolecules are
translated into material response is particularly important when they are the primary structural element of a biological system. For example, in red blood cells (RBC), the spectrin network is responsible for shear stiffness and the resilient elasticity of the cell membrane[6]. It has been observed that spectrin macromolecules are in a state of pre-tension[7]; the role of the pretension together with potential unfolding during physiological loading conditions is not well understood. A theoretical foundation that describes the behavior of modular polymer membranes and solids enables a fundamental understanding of structure-mechanical property relationships of soft tissues and cells. This research is to develop constitutive models that account for the finite deformation stress-strain behaviors of biological systems composed by modular macromolecules. In this paper, the FJC ([8-9]) and the WLC (e.g., [10-13]) models, together with the two-state theory[14-16] serving as an unfolding criterion[6,18], were employed to capture the single molecule unfolding behavior. The single macromolecular behavior was then integrated into one, two, and three dimensional macromolecular assemblies using established approaches of continuum mechanics based on macromolecular network theories of rubber elasticity. Constitutive models for the large stretch macroscopic stress versus strain behavior are formulated and used to predict general multiaxial loading conditions.

![Figure 1](image)

**Figure 1** (A) Schematic of a single modular polymer chain where the black rectangles represent folded modules. (B) A representative folded module; here, structure of an individual *Drosophila* α-spectrin segment 14 repeat domain which exhibits a three-helix bundle[19].

**CONSTITUTIVE MODELS**

**Single Macromolecule Force-Extension Model**

The FJC representation of macromolecular chain behavior is used to illustrate the formula of constitutive models; parallel model formulations utilizing the WLC model are detailed in Appendix. The polymer statistical mechanics states that as the molecule is stretched, the number of possible configurations decreases, thus giving a decrease in configurational entropy, \( \Delta S_c \).

Based on this description, the FJC model determines the elastic strain energy, \( u_c \), to be:

\[
u_c = k_B T N \left( \frac{r}{L} \beta + \ln \frac{\beta}{\sinh \beta} \right) ,
\]

where \( k_B \) is Boltzmann’s constant, \( T \) is absolute temperature, \( L \) is the contour length of the chain, \( L = NL \) where \( l \) is the Kuhn segment statistical length, \( N \) is the number of Kuhn links in the chain, \( r \) is the chain end-to-end distance, and \( \beta = L^{-1} (r/L) \) is the inverse Langevin function,
which is defined as $L(\beta) = \coth \beta - 1/\beta$. The axial force versus extension relationship is obtained via $f_c = du_c/dr$, giving:

$$f_c = \frac{Nk_BT}{L} \beta.$$  \hspace{1cm} (2)

The axial force is then employed to determine the occurrence of unfolding using the unfolding criterion described below. Upon unfolding, the molecule structural parameters including the number of folds and the contour length are updated, i.e., the number of folds decreases by one, and the contour length increase by an amount of $\Delta L$.

**Unfolding Criterion**

Unfolding and its rate-dependence have been successfully modeled by the two-state transition theory [1,6,14-16,18]. The two-state theory assumes that a domain can exist in one of two states: folded and unfolded. A transition from folded state to unfolded state requires an energy barrier, $\Delta G$, to be overcome, which determines the frequency of unfolding. When an extension force, $f_c$, is applied to the chain ends, $\Delta G$ is reduced by $f_c x_u$, where $x_u$ is the width of the activation barrier. The frequency of unfolding, $\omega_u$, is thus given as:

$$\omega_u = \omega_0 \exp\left(-\frac{\Delta G - f_c x_u}{k_BT}\right) = \alpha \exp\left(\frac{f_c x_u}{k_BT}\right),$$ \hspace{1cm} (3)

where $\omega_0$ is an attempt frequency parameter, and $\alpha = \omega_0 \exp(-\Delta G/k_BT)$ is a lumped parameter. In practice, $\alpha$ and $x_u$ are parameters which are fit to data obtained at different extension rates. Noting that the unfolding force at a given extension rate has been experimentally observed to possess a distribution [4], we extend Eq.3 to sample such a distribution by randomly sampling over a range in $\alpha$ when assessing unfolding.

**Constitutive Models of Modular Macromolecular Assemblies**

Models for assemblies of modular polymers are presented below, including one-dimensional, two-dimensional, and three-dimensional structures.

**Multi-Molecule Strands**

Here, a multi-molecule "strand" is defined as an assembly of macromolecules. Two forms of molecular strands are simulated: (A) A strand consisting of $k$ molecules arranged in parallel with different initial contour lengths, but otherwise identical nanomechanical behavior; (B) A strand consisting of $k$ identical molecules having a slight distribution in initial tethered positions of chain ends. The total force, $F$, required to stretch the strand is simply the summation of the axial force contribution from each molecule. For the FJC model, the total force is:

$$F = \sum_{i=1}^{k} f_{ci} = k_B T \sum_{i=1}^{k} N_i/L_i \beta_i \cos \theta_i,$$ \hspace{1cm} (4)

where the subscript $i$ denotes the $i$th molecule, $\beta_i = L^{-1}(r_i/L_i)$, and $\theta_i = 90^\circ$ for strands of type A and $\beta_i = L^{-1}(r_i/L)$ and $\theta$ evolves with stretch for strands of type B. Unfolding is continually
monitored on each constituent macromolecule during stretching. When the unfolding criterion is satisfied, the number of folded modules and the contour length of the corresponding molecule are updated.

\[
\begin{align*}
\lambda_c = \left( \frac{I_{\text{planar}}}{2} \right)^{1/2} & \quad \text{is the chain stretch and} \quad I_{\text{planar}} = \lambda_1^2 + \lambda_2^2. \\
\end{align*}
\]

Furthermore, in cell membranes, the membrane area is typically preserved during stretching due to the constraint of the fluid-like lipid bilayer which is connected to the membrane skeleton [6]. The planar area constraint is expressed as \( \lambda_1 \lambda_2 = 1 \). The strain energy of the RVE subjected to an in-plane stretch is simply the sum of the strain energy in the four constituent chains due to their change in entropy resulting from membrane stretch. Therefore, the stored energy per unit initial planar area is:

\[
\widetilde{U} = \nabla k_N T \left[ \frac{\lambda_c r_0}{L} \beta_c + \ln \left( \frac{\beta_c}{\sinh \beta_c} \right) - c \right]
\]

where \( \beta_c = L^{-1}(r/L) = L^{-1}(\lambda_c r_0 / L) \), \( c \) is the contribution from the initial entropy of the macromolecule prior to application of external loading, and \( c \) is a constant.

From the theory of continuum mechanics, for a general membrane deformation described by the planar deformation gradient \( \mathbf{F}_{2D} = \partial \mathbf{x} / \partial \mathbf{X} \) and the planar left Cauchy Green tensor \( \mathbf{B}_{2D} = \mathbf{F}_{2D} \mathbf{F}_{2D}^T \), the Cauchy (true) membrane stress is given by:
\[ \mathbf{s} = k_B T \frac{r_0 N}{2 \lambda_c L} \mathbf{B} + \tilde{\mathbf{h}} \mathbf{I}_{2D}, \]

where \( \mathbf{I}_{2D} \) is the 2-D identity tensor, \( \lambda_c = \sqrt{tr(\mathbf{B})/2} \), and \( \tilde{\mathbf{h}} \) is the additional equibiaxial stress (due to the constant area constraint \( \lambda_1 \lambda_2 = 1 \)) obtained by satisfying boundary conditions. The stress considering a distribution in the initial contour length of chains is obtained by differentiating a volume averaged strain energy density function where the distributed strain energy density is approximated using a simple mixtures rule.

Utilizing Eq. 6 together with the unfolding criterion of Eq. 3 gives a constitutive model for the multiaxial finite deformation stress-stretch behavior of membranes composed of a modular macromolecule network. Furthermore, this expression can be extended to emulate a more random network containing chains of different initial contour lengths by volume averaging with RVEs of different initial contour lengths.

**Three-Dimensional Networks**

In order to study biological solids constituted of modular macromolecules, a three-dimensional network model that incorporates the single molecule behavior is necessary. Here, the eight-chain network model in rubber elasticity[21] is utilized where the three-dimensional randomly oriented macromolecular network is homogenized as a network consisting of crosslinks or entanglements arranged in a perfectly staggered array (Fig. 3). Isolating an RVE from this network, one identifies eight chains, each chain emanating from the center to a corner of the cube where the cube is aligned with the principal axes of stretch.

![Figure 3](https://example.com/figure3.png)

**Figure 3** Schematic of the eight-chain network: (A) An idealized macromolecule network before deformation; (B) An isolated eight-chain network representative volume element (RVE) highlighted in gray in (A); (C) The 8-chain network RVE after deformation.

When the material is subjected to deformation, the macromolecular chains stretch and rotate towards the maximum principal stretch axis(es). From Fig. 3C, the chain stretch ratio \( \lambda_c \) is

\[ \lambda_c = \frac{r}{r_0} = \sqrt{\frac{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}{3}} = \sqrt{\frac{I_1}{3}}, \]

where \( \lambda_1, \lambda_2, \) and \( \lambda_3 \) are the three principal stretches, and \( I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \). Following rubber elasticity, the deformation is taken to be incompressible (note this can be extended to cover the case of compressible materials by inclusion of pressure-volume contributions to the strain energy (see, for example, [22,23]). The strain energy per unit reference volume is given by:
\[ 
\bar{U} = v k_B T N \left[ \frac{\lambda_c r_0}{L} \beta_c + \ln \frac{\beta_c}{\sinh \beta_c} - c \right],
\]

where \( v \) is the number of macromolecular chains per unit reference volume, or chain density, \( \beta_c = L^{-1} (r/L) = L^{-1} (\lambda_c r_0 / L) \), \( c \) is the contribution of initial entropy of a macromolecule and is a constant. This expression is extended to account for a distribution in initial chain contour lengths using a simple mixtures volume averaging approach as done for the membrane.

A general multiaxial deformation can be described by the deformation gradient \( \mathbf{F} = \partial \mathbf{x} / \partial \mathbf{X} \), where \( \mathbf{x} \) represents the current position and \( \mathbf{X} \) represents the reference position, and the left Cauchy Green tensor is given by \( \mathbf{B} = \mathbf{F} \mathbf{F}^T \). The Cauchy stress tensor is then obtained by proper differentiation of Eq. 8 and is given by:

\[ 
\mathbf{s} = v k_B T \frac{r_0 N}{3 \lambda_c L} \beta_c \mathbf{B} - p^* \mathbf{I},
\]

where \( \mathbf{I} \) is the 3D diagonal identity tensor, \( p^* \) is the additional pressure (due to volume conservation constraint), and \( \lambda_c = \sqrt{\text{tr}(\mathbf{B})/3} \). Furthermore, each chain will unfold when the unfolding criterion is satisfied.

\[ \text{Rate: 0.3 m/s} \]

\[ \text{Rate: 0.3 m/s} \]

\[ \text{Figure 4} \]

\[ \text{FJC model compared to experiment: (A) Spectrin force versus extension behavior; experimental data from Rief et al. (1999)[6]; the inset shows the dependence of unfolding force on extension rate; (B) Predicted force versus extension behavior for spectrin (1 µm/s) using a distribution in unfolding barrier based on Law et al. (2003) data[4].} \]

**RESULTS AND DISCUSSION**

**Single Modular Macromolecule**

Fig. 4 compares the FJC model predictions of force versus extension to experimental data reported in the literature for single molecule native spectrin[6]. The model parameters were obtained by fitting to the experimental data and are given in Table 1. The model captures the overall force versus extension behavior as well as the force at which unfolding occurs for this spectrin. Similar agreement was obtained using the WLC model (results not shown). The Fig. 4A
inset shows the dependence of the spectrin unfolding force on extension rate. Fig. 4B shows the model prediction for spectrin force versus extension using the distribution in unfolding force data [4] with \( x_u=1.7 \text{ nm} \) and \( a = .000125-.0079 \text{ /s} \). The inset shows the distribution in unfolding force at an extension rate of 1 \( \mu \text{m/s} \). The distribution in unfolding barrier is randomly sampled in the model: here, three curves are shown to illustrate the ability of the model to capture this aspect of unfolding, where unfolding events are observed to occur at slightly different forces and extensions.

Table 1. Modular Chain Structure and Properties

<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>Spectrin, Single Molecule</th>
<th>Spectrin, Networked Heterodimer Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WLC</td>
<td>FJC</td>
</tr>
<tr>
<td>Persistence length, s, (WLC) or statistical segment length (FJC), ( l ) (nm)</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Initial contour length (nm), ( L_0 )</td>
<td>197.</td>
<td>174.</td>
</tr>
<tr>
<td>Increase in contour length due to unfolding, ( \Delta L ) (nm)</td>
<td>32.0</td>
<td>28.8</td>
</tr>
<tr>
<td>Activation barrier width, ( x_u ) (nm)</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>( \alpha ) (( \times 10^{-5} \text{s}^{-1} )) (for Rief, et al. (1999) data)</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>100.</td>
<td>100.</td>
</tr>
</tbody>
</table>

**Multi-Molecule Strands**

Accurate single macromolecule stretching results can be achieved where the macromolecular surface density is sufficiently low and the chains are long enough to extend beyond the nonspecific range of surface forces. However, in some systems [18], under the physiological conditions, the macromolecular surface density is quite high and nanomechanical testing results in pulling multiple macromolecules of varying lengths and/or from different tethering locations along the polymer chain simultaneously. To emulate such experiments, simulations are presented for the strands of 5 and 20 macromolecules with different initial contour lengths arranged in parallel (A) and for the strands with slight variation in tether locations amongst the inherent molecules (B). The parameters in Table 1 were used in these simulations.

Fig. 5 shows the simulation results for the normalized force versus extension behavior for molecular strands composed of 5 and 20 molecules compared to single molecule behavior. The total force values were normalized by the number of molecules in the strand. For strands (A), the initial contour lengths of constituent chains were taken to have a small distribution of 174. ± 26. nm. For strands (B), tether locations were distributed such that the initial end-to-end distance ranged from 0 - 40 nm. The force-extension behavior of strands containing multiple molecules show numerous peaks with smaller peak values and smaller force drops after each peak than the single molecule behavior. This indicates that small variations in bundles of modular molecules can provide a plateau region in force-extension behavior.
Figure 5 Force vs. extension curves from simulations of stretching multi-molecule strands for (A) type A strands with a distribution in initial contour lengths; (B) type B strands with a distribution in tether locations. The force is reported as force per unit molecule.

Two-Dimensional Macromolecular Membrane Using the 4-Chain Network Model

The stress-stretch behavior of a two-dimensional membrane is simulated under conditions of uniaxial tension and simple shear to understand and demonstrate how single molecule behavior translates and contributes to membrane deformation. The material properties used in the simulation were those provided earlier for spectrin in Table 1, with the exception of the initial end-to-end distance \( r_0 \), the Kuhn length \( l \), and the initial contour length \( L_0 \). The initial chain end-to-end distance between crosslink sites was taken to be \( r_0 = 75 \text{ nm} \) [25], which also determines the chain density \( v \) to be \( 6 \times 10^{14} \text{ m}^{-2} \). The initial contour length is taken to be nominally 180 nm based on micrographs of spread membranes which depict fully extended chains in the spectrin network [20]; \( L_0 \) is observed to be distributed over a range from 150 nm-210 nm and we take the distributed network to have contour lengths of 150 nm, 170 nm, 190 nm, and 210 nm. The Kuhn length was chosen to be 10.25 nm to best fit the observed shear modulus of the spectrin network. It is noted that the value of the Kuhn length for the networked spectrin differs substantially from that used in the single molecule fits; we speculate this difference to be due to the more complicated heterodimer structure of the networked spectrin [19] and also due to the networked spectrin interaction with other molecules. Unfolding is triggered using Eq. 3 together with either a uniform unfolding barrier or a distribution in unfolding barrier based on the unfolding force distribution observed [4].

Fig. 6A shows the uniaxial stress-stretch behavior for the uniform network (\( L_0 = 180 \text{ nm} \)) and the distributed network (\( L_0 \) ranging from 150-210 nm). At a nominal strain rate of 1.0/s, unfolding first occurs at an axial stretch ratio of 3.1 with an axial membrane stress of about 18(10^-4) N/m. In the distributed network, unfolding first occurs at a stretch ratio of 2.65 and a membrane stress of \( \sim 5(10^-4) \) N/m. A decrease in strain rate decreases the stretch ratio and the initial stress of unfolding: at the strain rate of 0.1/s, the distributed network first unfolds at an axial stretch ratio of 2.3 and peak membrane stress of \( \sim 1.5(10^-4) \) N/m. Once unfolding first occurs, there is a significant decrease in the overall tangent modulus and a nearly plateau-like region to
the stress-stretch curve. These predictions of the stress-stretch behavior are consistent with the experimental data of Lee and Discher [26], where an increase in thermal fluctuations was observed in a highly axially stretched cell membrane. The increase in fluctuations was speculated to be related to unfolding, consistent with the predicted unfolding of Fig. 6A.

**Figure 6** (A) Uniaxial tensile stress vs stretch behavior of a uniform and distributed network membrane, where the distributed network behavior is also shown at different strain rates; (B) Membrane shear stress-tanγ behavior;

Simple shear of the membrane is also simulated, where γ is the shear angle. Results are presented in Fig. 6B in terms of the membrane Cauchy shear stress $\bar{\sigma}_{12}$ vs tangent shear angle tanγ. At a shear rate of 0.1/s, the distributed network experiences initial unfolding at tanγ = 2.1 whereas the uniform network experiences initial unfolding at tanγ = 2.7. At the shear rate of .01/s, initial unfolding occurs at a much smaller shear strain, tanγ = 0.75.

**Three-Dimensional Macromolecular Solid Using the 8-Chain Network Model**

The three-dimensional behavior of a solid composed of a modular macromolecular network is illustrated for two loading conditions: uniaxial tension and equibaxial tension. The same single molecule material properties used for the spectrin planar network were adopted to simulate the stress-stretch behavior of the solid. Fig. 7A shows the uniaxial tension stress-stretch behavior when the network chains are assigned identical initial contour length (dotted line) and when a distribution in initial contour length is considered. The uniform network stress-stretch behavior is found to exhibit a similar saw-tooth pattern as the single molecule and membrane behavior with a stress at initial unfolding of about 0.75 MPa and stretch ratio of 3.6. For the distributed network, the stress and stretch at first unfolding are significantly lower, 0.25 MPa and 3.0, respectively; the stress-stretch curve exhibits a smoother character. In equibaxial tension (Fig. 7B), due to extension in two directions, the chain stretch is $\lambda_c = \sqrt{(2\lambda^2 + \lambda^{-4})/3}$, which is larger than the chain stretch in uniaxial tension at any given specimen stretch. Therefore, unfolding occurs at a smaller stretch ratio of 2.7 at a stress of 0.40 MPa for the uniform network and a stress of 1.7 MPa and stretch of 2.2 for the distributed network.
Figure 7. 8-chain network model results for: (A) Uniaxial tension stress vs stretch behavior; (B) Equibiaxial tension stress vs stretch behavior.

CONCLUSION

Constitutive models for the force-extension behavior of multi-molecule strands of modular macromolecules and the finite deformation multiaxial stress-stretch behavior of membranes and solids containing modular macromolecules have been presented. Modular macromolecules take on a network structure in many biological materials and structures, and it is important to capture the behavior of the networked structure when attempting to understand the role of the single molecule saw-tooth force extension behavior in governing mechanical aspects of biological materials and structures. This paper presented a formal continuum mechanics approach which directly incorporates measured single molecule force-extension behavior into constitutive models of the large deformation stress-stretch behavior of protein-rich membranes and solids. The model specifically accounts for the single molecule force-extension behavior, the initial end-to-end distance of chain segments (the pre-tension), and the crosslink density; these structural features are all directly measurable quantities. While the model provides the multiaxial stress-stretch behavior of the membrane, it also retains intimate details of the underlying network structure and its evolution with loading including the stretch and force on the constituent molecules, the forces acting on the crosslink sites, as well as the number of folded and unfolded modules along the chain. These constitutive models for the membrane and the solid thus are a starting point for understanding the role of modular protein mechanical behavior in issues of cell mechanics as well as in issues of protein-rich biological materials which may act as load transfer and/or dissipative agents in biological composite structures such as the adhesive layers of the nacre of abalone shell [27].

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APPENDIX: A. Worm-Like Chain Equations

The mechanical behavior of biomolecules has also been successfully modeled using the worm-like chain (WLC) model. The WLC model originates from the Porod-Kratky chain model [28-31], and has been successfully to numerous biomolecules (e.g., [10-12]). The WLC model, as approximated by [10], gives the stored energy during axial stretch to be:

\[ u = \frac{k_B T}{A} L \left[ \frac{1}{2} \left( \frac{r}{L} \right)^2 + \frac{1}{4} \left( \frac{1}{1-r/L} - \frac{1}{4} \frac{r}{L} \right) \right] \tag{A1} \]

where \( r \) is the end-to-end distance, \( A \) is the persistence length. It is noted that the FJC Kuhn statistics length \( l = 2A \) [29]. Below we provide the constitutive model equations using WLC as the molecule behavior for the single molecule, multi-molecule strand, planar network and three-dimensional network models.

**Single Molecule Force-Extension:**

\[ f_c = k_B T \frac{A}{4} \left( \frac{r}{L} + \frac{1}{4(1-r/L)^2} - \frac{1}{4} \right) \tag{A2} \]

**Multi-Molecule Strand Force-Extension:**

\[ F = k_B T A \sum \left( \frac{r}{L_i} + \frac{1}{4(1-r/L_i)^2} - \frac{1}{4} \right) \tag{A3} \]

**Planar Membrane Cauchy Stress Tensor:**

\[ \tilde{\mathbf{s}} = \nu k_B T \frac{r_0 N}{2 \lambda c L} \left[ \frac{\lambda_c r_0}{L} + \frac{1}{4(1-\lambda_c r_0/L)^2} - \frac{1}{4} \right] \mathbf{B} + \tilde{\mathbf{h}} \mathbf{I}_2 \tag{A4} \]

**8-Chain Network Model Cauchy Stress Tensor:**

\[ \mathbf{s} = \nu k_B T \frac{r_0 N}{3 \lambda c L} \left[ \frac{\lambda_c r_0}{L} + \frac{1}{4(1-\lambda_c r_0/L)^2} - \frac{1}{4} \right] \mathbf{B} - p^i \mathbf{I} \tag{A5} \]

REFERENCES