Supplementary Materials of "Spectrin-Level Modeling of the Cytoskeleton and Optical Tweezers Stretching of the Erythrocyte"

J. Li, M. Dao, C.T. Lim and S. Suresh

Icosahedron Network Generator

An initial spectrin network (see Fig. 1(a)) is generated to cover a spherical surface using a public-domain software (Weber et al., 2002). It employs a recursive division and projection algorithm. The first approximation of a sphere is an icosahedron with 12 vertices and 20 (initially equilateral) triangular faces. Every level of refinement subdivides each triangular face by a factor of 4. After each refinement, the vertices are projected to the sphere surface. A total of six refinements are made. The final network has 20×4^6 = 81,920 triangular faces, 40,962 junction complexes, and 122,880 spectrin links. Almost all the vertices on the icosahedron network are degree-6. However, a topological minimum of twelve degree-5 vertices (defects) must be present when mapping any triangular network onto a sphere, which is achieved exactly by the icosahedron network here. Fig. 2(b) shows a closer look at one of twelve such defects. The structure is rendered using AtomEye, a public-domain molecular visualization software (Li, 2003).

In the ensuing CGMD simulations using this structure, we have found shape artifacts associated with these degree-5 vertices, as for instance a greater propensity to form extruded corners near these defects; see e.g. Figs. 1(c) and 1(d). These are obtained using the volume deflation procedure detailed in the main text. After some analysis, it is found that these artifacts are caused by a global residual stress distribution which is long-ranged, and therefore are rather difficult to be removed. The icosahedron cytoskeleton structure generator used here leads to a residual stress field (in-plane) for the following reason. Basically, 20 flat triangular pieces of membrane (if the spontaneous curvature angle θ_0 is zero) are deformed to "patch up" a spherical surface. The stress-free states of these membranes are equilateral triangles. But in order to patch up a spherical surface without leaving gaps, these pieces should first be stretched on their circumferences. This is done implicitly in the recursive division and projection procedure.

As evidence of such geometrically necessary in-plane stretching, consider the angle at the tip of a piece of membrane (see Fig. 1(b)). It is 60° in the stress-free state, but must become 360°/5=72° if it is to cover a spherical surface. As such, spectrin links a, b of originally equal lengths must now become unequal: $b \neq a$, creating hoop stresses around the defect. This is not merely a local condition: one may alternatively draw a much larger loop around the defect, count the numbers of circumferential versus radial spectrin links, and realize that the circumferential links must be stretched compared to the radial ones, which leads to residual stress field deep within each "crystalline" membrane piece. This is a fundamental geometrical consequence of our ordered network structure. It turns out to be highly nontrivial to come up with a network generation algorithm to create an ordered cytoskeleton structure (meaning a small number of defects arranged in symmetric fashion) that covers a spherical surface with equilateral triangles as tiling units, which also gives small residual stresses.

Another problem with the icosahedron network generator is that there are limited choices for the size of the network. If the icosahedron is refined 5 times, 10,242 junction complexes are obtained; for a six-fold refinement, there are 40,962 junction complexes. The real RBC has about 30,000 junction complexes if tessellated by triangles. In order to use the icosahedron network with L_0 = 75 nm, the spherical RBC model needs to be significantly larger than the real RBC, with total area $A_{\text{total}}^{\text{sphere}} = 200\ \mu\text{m}^2$, total volume $\Omega_{\text{total}}^{\text{sphere}} = 265\ \mu\text{m}^3$ and diameter (before deflation procedure) $D_0 = 7.97\ \mu\text{m}$. In contrast, a spherical RBC which has the same surface area as the actual biconcave RBC average (Evans and Skalak, 1980) should have $A_{\text{total}}^{\text{sphere}} = 134.1\ \mu\text{m}^2$, $\Omega_{\text{total}}^{\text{sphere}} = 146\ \mu\text{m}^3$ and $D_0 = 6.53\ \mu\text{m}$. A random network generator is developed in the main text which satisfactorily addresses both the residual stress and size problems. This random network generator is also not limited to creating just triangular networks.

Large Deformation Elasticity of WLC Sheet

Suppose that the spectrin network is a 2-D triangular crystal (Fig. 2) with Bravais lattice vectors $\mathbf{a} \equiv (a_x, a_y)$ and $\mathbf{b} \equiv (b_x, b_y)$. The four numbers $\{a_x, a_y, b_x, b_y\}$ completely specify the geometry of the system. For a given reference lattice, $\{a_x, a_y, b_x, b_y\}$ can be made equivalent to two diagonal and one off-diagonal strain and one rotational degree of freedom. Then,

$$\mathbf{c} \equiv \mathbf{b} - \mathbf{a} = (b_x - a_x, b_y - a_y), \tag{1}$$

$$a \equiv |\mathbf{a}| = \sqrt{a_x^2 + a_y^2},\ b \equiv |\mathbf{b}| = \sqrt{b_x^2 + b_y^2},\ c \equiv |\mathbf{c}| = \sqrt{(b_x - a_x)^2 + (b_y - a_y)^2}. \tag{2}$$

All triangles in the perfect crystalline state have the same area:

$$A_0 = A_1 = A_2 = A = \frac{1}{2}|\mathbf{a} \times \mathbf{b}| = \frac{1}{2}|a_x b_y - a_y b_x|. \tag{3}$$

If a right-handed system is always chosen, that is, if $\mathbf{b}$ is always chosen to be counter-clockwise to $\mathbf{a}$, then the above symbols || for absolute values can be omitted.

The next step is to perform a nonlinear elasticity analysis for the given Bravais lattice $\{\mathbf{a}, \mathbf{b}\}$. Each vertex is connected to 6 spectrin links, but each link is shared by two vertices, such that each vertex has three links associated with it. These three links are conveniently chosen to be $\mathbf{a}$, $\mathbf{b}$, $\mathbf{c}$, respectively, with the associated WLC energy being $V_{\text{WLC}}(a) + V_{\text{WLC}}(b) + V_{\text{WLC}}(c)$. Similarly, each vertex is connected to 6 triangular plaquettes, but a triangular plaquette is shared by 3 vertices. Thus each vertex really has two affiliated plaquettes, with associated membrane energy being $2 \times C_q A^{-q}$. So for each vertex that occupies a current area $2A$ the associated strain energy is $V_{\text{WLC}}(a) + V_{\text{WLC}}(b) + V_{\text{WLC}}(c) + 2C_n A^{-n}$. Using the Virial theorem (Allen and Tildesley, 1987), the Cauchy stress is derived as:

$$\tau_{\alpha\beta} = -\frac{1}{2A}\left[\frac{f_{\text{WLC}}(a)}{a} a_\alpha a_\beta + \frac{f_{\text{WLC}}(b)}{b} b_\alpha b_\beta + \frac{f_{\text{WLC}}(c)}{c} c_\alpha c_\beta\right] - qC_q A^{-q-1} \delta_{\alpha\beta}. \tag{4}$$

with

$$C_q = \frac{3A_0}{4qL_{\max}x_0}\cdot\frac{k_B T}{p}\left\{\frac{1}{4(1-x_0)^2}-\frac{1}{4}+x_0\right\}(x_0L_{\max})^2 = \frac{3A_0L_{\max}x_0^2k_BT(6-9x_0+4x_0^2)}{16pq(1-x_0)^2}. \quad (5)$$

where $a = b = c = x_0L_{\max}$ and $A_0 = \sqrt{3}(x_0L_{\max})^2/4$ when $\tau_{\alpha\beta} = 0$. In the case of $q=1$ (Discher et al., 1998),

$$C_1 = \frac{3\sqrt{3}L_{\max}^3x_0^4k_BT(6-9x_0+4x_0^2)}{64p(1-x_0)^2}. \quad (6)$$

The pure shear stress-strain response of the homogenized WLC sheet, envisioned solely as a triangulated network of molecules with WLC potential, undergoing large elastic deformation is plotted in Fig. 3 on the basis of the foregoing analysis.

Linear Elastic Area Compression Modulus K of WLC Sheet

When $a=b=c=L_{\max}x$ and the angle between $\mathbf{a},\mathbf{b}$ is exactly 60°, the Cauchy stress is diagonal:

$$\tau_{\alpha\beta} = \frac{1}{2A}\cdot\frac{k_BT}{pL_{\max}x}\left\{\frac{1}{4(1-x)^2}-\frac{1}{4}+x\right\}\left[(xL_{\max})^2\frac{3\delta_{\alpha\beta}}{2}\right]-qC_qA^{-q-1}\delta_{\alpha\beta}. \quad (7)$$

The resulting pressure is

$$P = qC_qA^{-q-1}-\frac{3k_BTxL_{\max}}{4Ap}\left\{\frac{1}{4(1-x)^2}-\frac{1}{4}+x\right\}. \quad (8)$$

The linear elastic area compression modulus K is defined as,

$$K \equiv -\left.\frac{\partial P}{\partial \log A}\right|_{A=A_0} = -\frac{1}{2}\left.\frac{\partial P}{\partial \log x}\right|_{x=x_0}. \quad (9)$$

After some derivations,

$$K = \frac{\sqrt{3}k_BT}{4pL_{\max}(1-x_0)^2}\left\{(q+\frac{1}{2})(6-9x_0+4x_0^2)+\frac{1+2(1-x_0)^3}{1-x_0}\right\}. \quad (10)$$

Fig. 4 shows the variation of pressure with the equibiaxial area strain of the effective cell wall, triangulated with the spectrin molecular network. In contrast to the shear deformation (Fig. 3), the WLC sheet area expansion response softens initially for $(A/A_0)-1 < 2$, and begins to harden above this value.

Linear Elastic Shear Modulus μ of WLC Sheet

Consider a reference lattice in equilibrium with the coordinates

$$\mathbf{a}_0 = x_0 L_{\max}\left(\frac{\sqrt{3}}{2},\ \frac{1}{2}\right),\quad \mathbf{b}_0 = x_0 L_{\max}\left(0,\ 1\right),\quad \mathbf{c}_0 = x_0 L_{\max}\left(-\frac{\sqrt{3}}{2},\ \frac{1}{2}\right), \tag{11}$$

If an incremental engineering shear strain γ is imposed on this lattice,

$$\mathbf{J} = \begin{pmatrix} 1 & \gamma/2 \\ \gamma/2 & 1 \end{pmatrix},\quad \mathbf{r}' = \mathbf{rJ},\quad \Delta|\mathbf{r}| \equiv |\mathbf{r}'| - |\mathbf{r}| = |\mathbf{r}|\,\gamma \hat{r}_x \hat{r}_y + O(\gamma^2), \tag{12}$$

then,

$$\mathbf{a} = x_0 L_{\max}\left(\frac{\sqrt{3}}{2}+\frac{\gamma}{4},\ \frac{1}{2}+\frac{\sqrt{3}\gamma}{4}\right),\ \Delta a = x_0 L_{\max}\frac{\sqrt{3}\gamma}{4} + O(\gamma^2),$$

$$\mathbf{b} = x_0 L_{\max}\left(\frac{\gamma}{2},\ 1\right),\ \Delta b = O(\gamma^2),$$

$$\mathbf{c} = x_0 L_{\max}\left(-\frac{\sqrt{3}}{2}+\frac{\gamma}{4},\ \frac{1}{2}-\frac{\sqrt{3}\gamma}{4}\right),\ \Delta c = -x_0 L_{\max}\frac{\sqrt{3}\gamma}{4} + O(\gamma^2) \tag{13}$$

and

$$\Delta A \equiv (\det \mathbf{J} - 1)A_0 = O(\gamma^2), \tag{14}$$

Using the WLC force–displacement response,

$$\frac{f_{\mathrm{WLC}}(a_0)}{a_0} = \frac{f_{\mathrm{WLC}}(b_0)}{b_0} = \frac{f_{\mathrm{WLC}}(c_0)}{c_0} = -\frac{k_{\mathrm{B}}T}{p x_0 L_{\max}}\left\{\frac{1}{4(1-x_0)^2} - \frac{1}{4} + x_0\right\}, \tag{15}$$

it can be shown (Dao, Li and Suresh, 2005) that

$$\mu \equiv \frac{d\tau_{xy}}{d\gamma} = \frac{\sqrt{3}k_{\mathrm{B}}T}{4pL_{\max}x_0}\left\{\frac{3}{4(1-x_0)^2} - \frac{3}{4} + 4x_0 + \frac{x_0}{2(1-x_0)^3}\right\}. \tag{16}$$

This equation provides an explicit expression for the shear modulus of the cell membrane which is envisioned as comprising a triangulated network of WLC spectrin molecules anchored at actin notes.

Linear Elastic Young's Modulus E and Poisson's ratio ν of WLC Sheet

For small deformations, the sheet is an isotropic elastic medium, with

$$C_{ijkl} = \lambda\delta_{ij}\delta_{kl} + \mu(\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk}),\quad i,j,k,l \in 1,2. \tag{17}$$

which complies with all symmetry requirements and tensor-transformation law:

$$Q_{ii'}Q_{jj'}Q_{kk'}Q_{ll'}C_{ijkl} = C_{i'j'k'l'} \tag{18}$$

In matrix notation,

$$\mathbf{C} = \begin{pmatrix} \lambda + 2\mu & \lambda & 0 \\ \lambda & \lambda + 2\mu & 0 \\ 0 & 0 & \mu \end{pmatrix} \tag{19}$$

Note that $K = \lambda + \mu$, or $\lambda = K - \mu$, such that

$$\begin{pmatrix} \lambda + 2\mu & \lambda \\ \lambda & \lambda + 2\mu \end{pmatrix} \begin{pmatrix} 1 \\ -\dfrac{\lambda}{\lambda + 2\mu} \end{pmatrix} = \begin{pmatrix} \lambda + 2\mu - \dfrac{\lambda^2}{\lambda + 2\mu} \\ 0 \end{pmatrix} \tag{20}$$

The Young's modulus is therefore:

$$E = \lambda + 2\mu - \frac{\lambda^2}{\lambda + 2\mu} = \frac{4\mu^2 + 4\lambda\mu}{\lambda + 2\mu} = \frac{4K\mu}{K + \mu}, \tag{21}$$

and the Poisson's ratio is:

$$\nu = \frac{\lambda}{\lambda + 2\mu} = \frac{K - \mu}{K + \mu} \tag{22}$$

Unlike pair-potential systems (Van Vliet et al., 2003), ν in general is not 1/3. However, if $q = 1$, then ν happens to be 1/3. One may directly verify that Eq. (18) is exactly twice of Eq. (31) when $q = 1$. This result was confirmed in a recent measurement, where K/μ was found to be 1.9 (Lenormand et al., 2001).

Fig. 5 shows the uniaxial stress–strain response of the spectrin-network sheet, plotted from the above analysis. It is seen here that in both tension and compression, linear elastic response occurs for an extensional strain of up to about 0.4, beyond which significant hardening is evident.

See Dao, Li and Suresh (2005) for detailed developments of the derivations in this supplementary material.

References:

Allen, M. P. and Tildesley, D. J. (1987) *Computer simulation of liquids,* Clarendon Press, New York.

Discher, D. E., Boal, D. H. and Boey, S. K. (1998) *Biophysical Journal,* **75,** 1584-1597.

Evans, E. A. and Skalak, R. (1980) *Mechanics and thermodynamics of biomembranes,* CRC Press, Inc., Boca Raton, Florida, USA.

Lenormand, G., Henon, S., Richert, A., Simeon, J. and Gallet, F. (2001) *Biophysical Journal,* **81,** 43-56.

Li, J. (2003) *Modelling and Simulation in Materials Science and Engineering,* **11,** 173-177.

Dao, M., Li, J. and Suresh, S. (2005). *Materials Science and Engineering C*, in press.

Van Vliet, K. J., Li, J., Zhu, T., Yip, S. and Suresh, S. (2003) *Physical Review B,* **67,** art. no.-104105.

Weber, D. L., Buddenhagen, J. and Leech, J. (2002) GNU General Public License.

List of Figures:

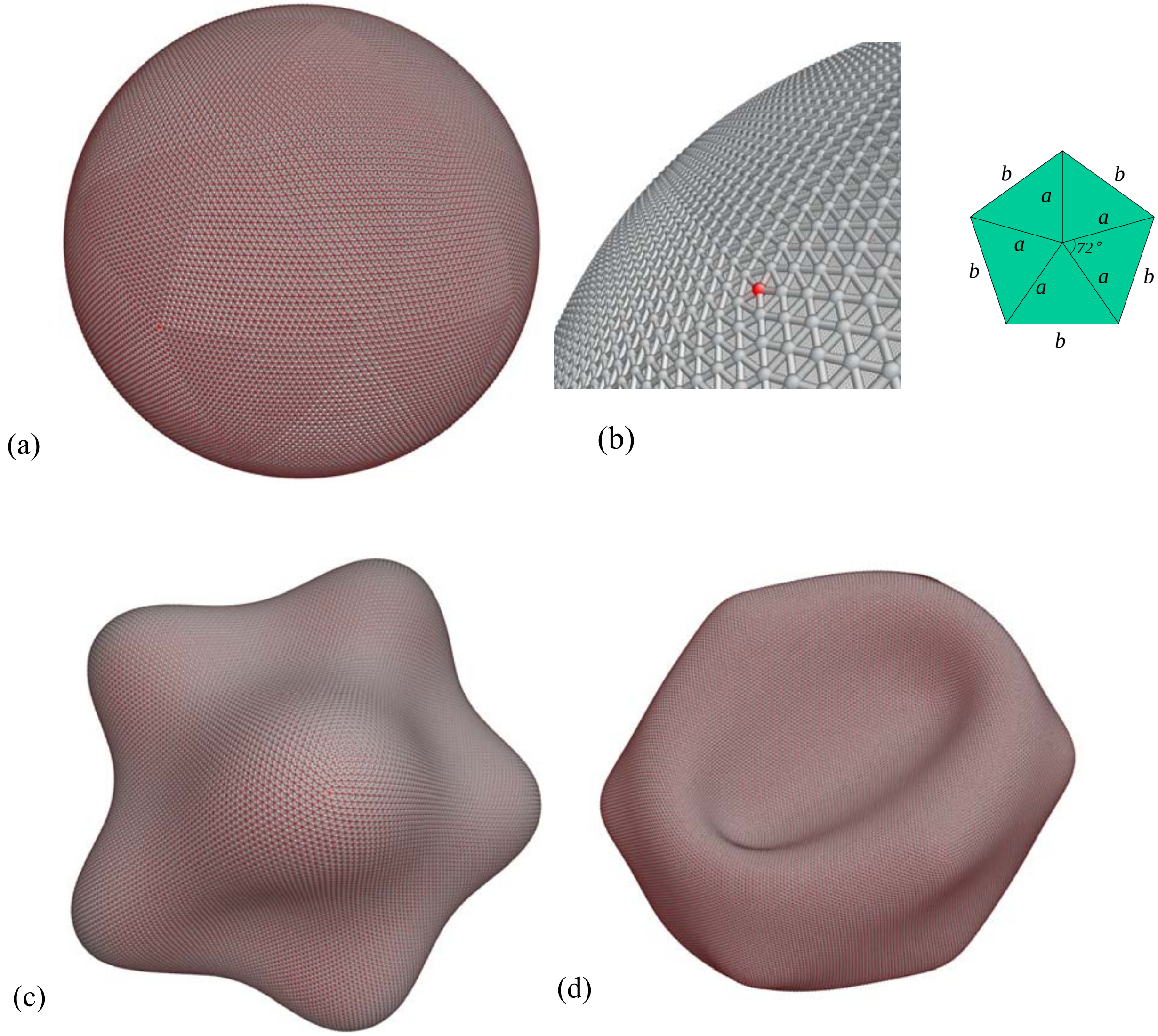

Fig. 1: Icosahedron network and shape artifacts caused by this ordered network structure. (a) Icosahedron network generated by mapping an icosahedron onto a sphere, and recursively partitioning six times. It contains 40,962 vertices, with diameter 7.97 μm. The brown particles represent degree-6 vertices, whereas the red particles represent degree-5 vertices. (b) A closer look at a degree-5 vertex (degree-6 vertices are rendered silver here to increase the contrast). There are 12 such defects among the 40,962 vertices, which are the geometrical minimum in order to cover a sphere. There is long-ranged hoop stress field around the defect because the spectrin angle is ~72º instead of the ideal 60º, so b=1.176a. (c) Global shape artifact caused by degree-5 defects. We use the Discher model and the numerical volume deflation procedure to reduce the cytosol volume to approximately 85% of the full sphere. In this parameter regime extrusions should *not* appear. Note there is a degree-5 vertex right at the tip of every extrusion. (d) Shape artifact at 65% cytosol volume.

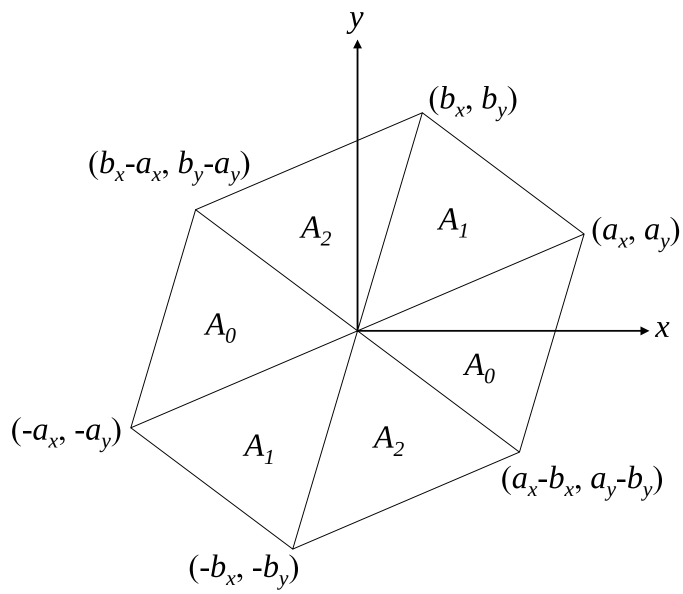

Fig. 2: Coarse-grained WLC sheet (crystal) model. The geometry is established fully by the two local Bravais lattice vectors **a** = (a_x, a_y) and **b** = (b_x, b_y), from which the Cauchy stress τ can be derived as shown in the Appendix.

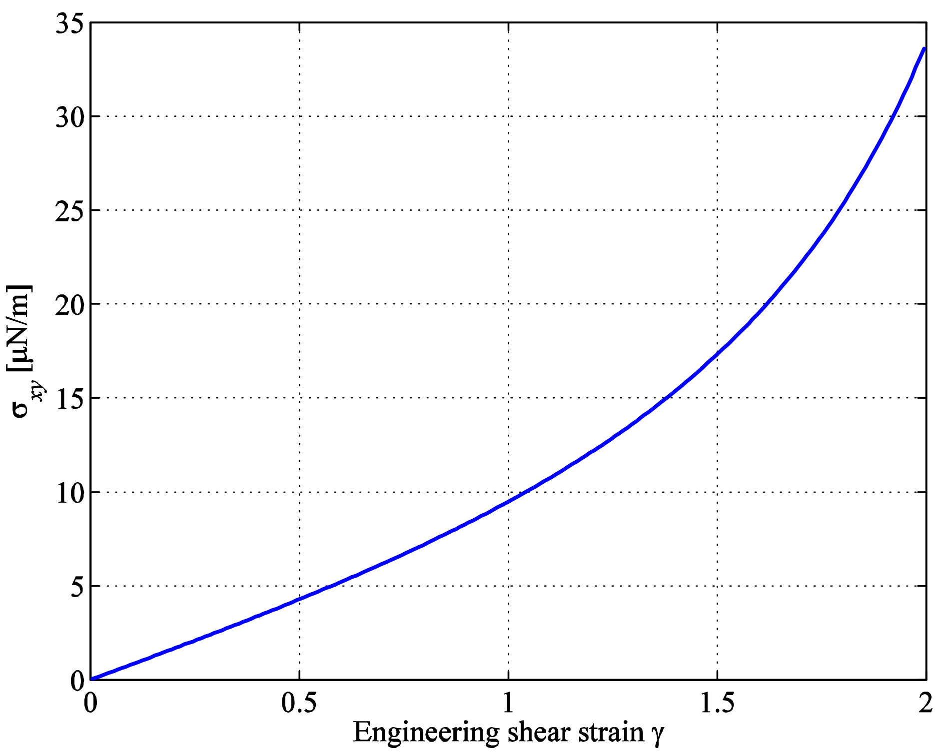

Fig. 3: Stress versus strain response of the triangulated cell sheet comprising the spectrin molecular network under pure shear. $\mathbf{a}_0 = (\sqrt{3}/2, 1/2)L_0$ and $\mathbf{b}_0 = (0, 1)\, L_0$.

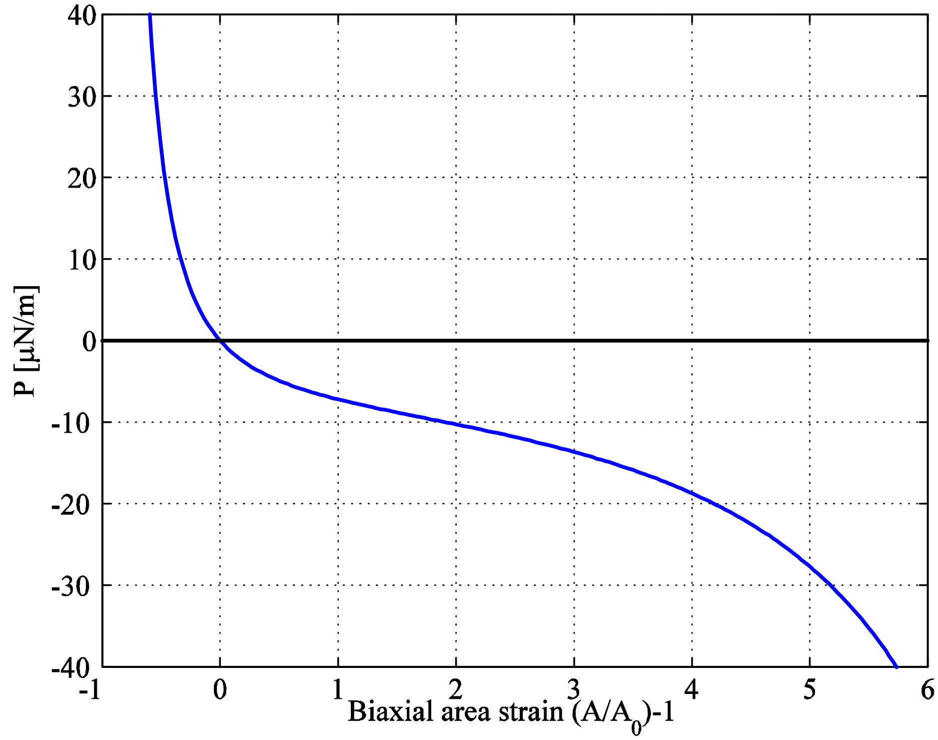

Fig. 4: A plot of the pressure versus biaxial area strain for the triangulated spectrin molecular network.

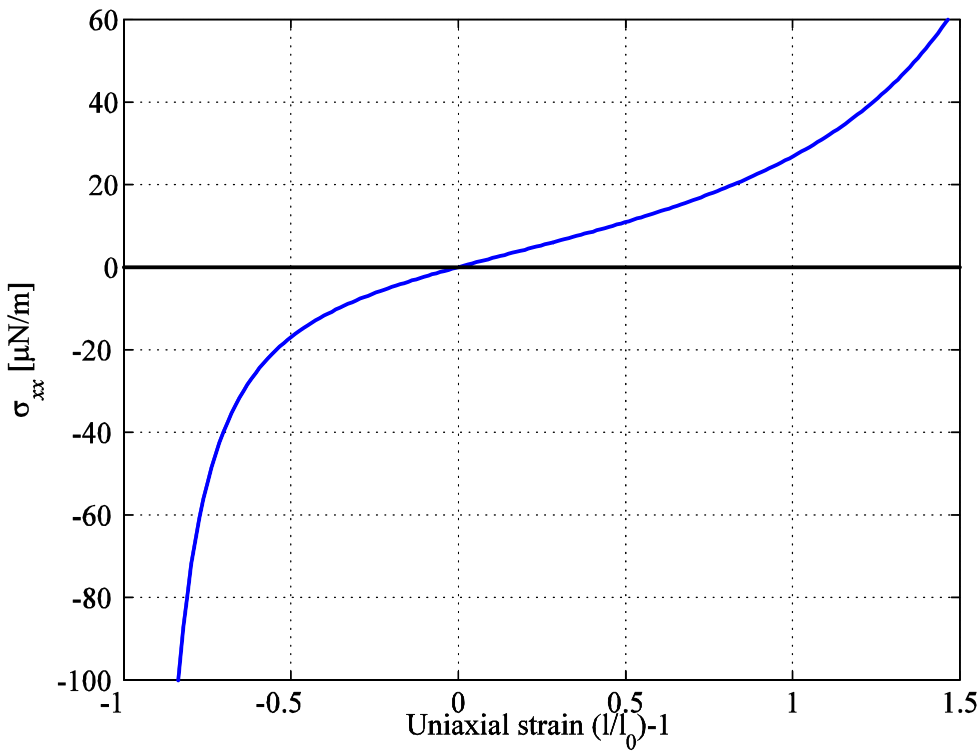

Fig. 5: Uniaxial stress versus strain response with $\mathbf{a}_0 = (\sqrt{3}/2\,,\ 1/2)L_0$ and $\mathbf{b}_0 = (0, 1)\ L_0$.