Biophysical Journal, Volume *114*

Supplemental Information

Cytoskeleton Remodeling Induces Membrane Stiffness and Stability Changes of Maturing Reticulocytes

He Li, Jun Yang, Trang T. Chu, Renugah Naidu, Lu Lu, Rajesh Chandramohanadas, Ming Dao, and George Em Karniadakis

The RBC membrane model and validation

The applied coarse-grained molecular dynamics (CGMD) membrane model describes the RBC membrane as a two-component system, including the cytoskeleton and the lipid bilayer. The lipid bilayer is represented by three types of CG particles, including lipid particles, band-3 particles and glycophrin particles. The cytoskeleton consists of two types of CG particles, representing action junction complex and spectrin proteins. The cytoskeleton consists of the hexagonal spectrin network and actin junctions. The actin junctions are connected to the lipid bilayer via glycophorin. As shown in Fig. 2(A-B), the spectrin filament is represented by 39 spectrin particles (grey particles) connected by unbreakable springs. The spring potential, $u_{\mathbf{cy}}^{\mathbf{s\text{-}s}}(r) = k_0(r - r_{\mathbf{eq}}^{\mathbf{s\text{-}s}})^2/2$, with equilibrium distance $r_{\mathbf{eq}}^{\mathbf{s\text{-}s}} = L_{\max}/39$, where $L_{\max}$ is the contour length of the spectrin (~200 nm) and $r_{\mathbf{eq}}^{\mathbf{s\text{-}s}} \cong 5\,\text{nm}$. The spectrin chain is connected to the band-3 particles (white particles). The two ends of the spectrin chains are connected to the actin junction via a spring potential, $u_{\mathbf{cy}}^{\mathbf{a\text{-}s}}(r) = k_0(r - r_{\mathbf{eq}}^{\mathbf{a\text{-}s}})^2/2$ where the equilibrium distance $r_{\mathbf{eq}}^{\mathbf{a\text{-}s}} = 10\,\text{nm}$. The spring constant is determined subsequently. The spectrin particles, which are not connected by the spring potential, interact with each other via the repulsive part of the L-J potential

$$u_{rep}(r_{ij}) = \begin{cases} 4\varepsilon\left[\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^{6}\right] & r_{ij} < R_{cut,LJ} \\ 0 & r_{ij} \geq R_{cut,LJ} \end{cases} \quad (1)$$

where ε is the energy unit and σ is the length unit. r_{ij} is the distance between the spectrin particles. The cutoff distance of the potential $R_{\text{cut,LJ}}$ is chosen to the equilibrium distances $r_{\mathbf{eq}}^{\mathbf{s\text{-}s}}$ between the spectrin particles. The spring constant k_0 is selected to be $1000\varepsilon/\sigma^2$.

The CG particles, which form the lipid bilayer and transmembrane proteins, carry both translational and rotational degrees of freedom ($\mathbf{x}_i$, $\mathbf{n}_i$), where $\mathbf{x}_i$ and $\mathbf{n}_i$ are the position and the orientation (direction vector) of particle *i*, respectively. The rotational degrees of freedom obey the normality condition $|\mathbf{n}_i| = 1$. Thus, each CG particle effectively carries 5 degrees of freedom. $\mathbf{x}_{ij} = \mathbf{x}_j - \mathbf{x}_i$ is defined as the distance vector between particles *i* and *j*. Correspondingly, $r_{ij} \equiv |\mathbf{x}_{ij}|$ is the distance, and $\hat{\mathbf{x}}_{\mathbf{ij}} = \mathbf{x}_{\mathbf{ij}}/r_{\mathbf{ij}}$ is a unit vector. The CG particles, forming the lipid membrane and membrane proteins, interact with one another via a pair-wise additive potential

$$u_{\text{mem}}(\mathrm{n}_i, \mathrm{n}_j, \mathrm{x}_{ij}) = u_{\mathrm{R}}(r_{ij}) + A\left(\alpha, a(\mathrm{n}_i, \mathrm{n}_j, \mathrm{x}_{ij})\right) u_{\mathrm{A}}(r_{ij}), \quad (2)$$

$$\begin{cases} u_R(r_{ij}) = k\varepsilon\left(\left(R_{cut,mem} - r_{ij}\right)/\left(R_{cut,mem} - r_{eq}\right)\right)^8 - k\varepsilon & \text{for} \quad r_{ij} < R_{cut,mem} \\ u_A(r_{ij}) = -2k\varepsilon\left(\left(R_{cut,mem} - r_{ij}\right)/\left(R_{cut,mem} - r_{eq}\right)\right)^4 - k\varepsilon & \text{for} \quad r_{ij} < R_{cut,mem} \\ u_R(r_{ij}) = u_A(r_{ij}) = 0, & \text{for} \quad r_{ij} \geq R_{cut,mem} \end{cases} \quad (3)$$

where $u_R(r_{ij})$ and $u_A(r_{ij})$ are the repulsive and attractive components of the pair potential, respectively. α is a tunable linear amplification factor. The function $A(\alpha,a(\mathbf{n}_i,\mathbf{n}_j,\mathbf{x}_{ij}))= 1+\alpha(a(\mathbf{n}_i,\mathbf{n}_j,\mathbf{x}_{ij}) -1)$ tunes the energy well of the potential. In the simulations, α is chosen to be 1.55 and cutoff distance of the potential $R_{cut,mem}$ is chosen to be 2.6σ. The detailed information about applied potentials and the selection of the potential parameters can be found from author's previous work (1). k is selected to be 1.2 for the interactions among the lipid particles and k = 2.8 for interactions between the lipid and the protein particles, such as glycophorin and band-3. Given the diameter of the lipid particles $r_{lipid} = 2^{1/6}\sigma$ = 5 nm, the length unit is calculated to be σ = 4.45 nm. The temperature of the system is maintained at k_BT/ε = 0.22 by employing the Nose-Hoover thermostat. At equilibrium, the average end-to-end spectrin length is ~65 nm.

The key mechanical property of erythrocyte membrane is the shear modulus. In order to compute the shear modulus of the RBC membrane, we shear the membrane to a shear strain (γ) of 1, as shown in Fig. 2D. Based on the shear response of the RBC membrane, we found the shear modulus of the membrane is ~7.7 μN/m at small deformation while it is increased to ~12.1 μN/m at large deformation, which is consistent with the experimentally measured values of 4–12 μN/m (2).

References

1. Li, H., and G. Lykotrafitis. 2014. Erythrocyte membrane model with explicit description of the lipid bilayer and the spectrin network. *Biophysical Journal* 107:642-653.
2. Hochmuth, R., and R. Waugh. 1987. Erythrocyte membrane elasticity and viscosity. *Annual Review of Physiology* 49:209-219.