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Systematic coarse-graining of spectrin-level red blood cell models

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Abstract

We present a rigorous procedure to derive coarse-grained red blood cell (RBC) models, which yield accurate mechanical response. Based on a semi-analytic theory the linear and nonlinear elastic properties of healthy and infected RBCs in malaria can be matched with those obtained in optical tweezers stretching experiments. The present analysis predicts correctly the membrane Young's modulus in contrast to about 50% error in predictions by previous models. In addition, we develop a stress-free model which avoids a number of pitfalls of existing RBC models, such as non-smooth or poorly controlled equilibrium shape and dependence of the mechanical properties on the initial triangulation quality. Here we employ dissipative particle dynamics for the implementation but the proposed model is general and suitable for use in many existing continuum and particle-based numerical methods.

Keywords

Atomistic modeling; Dissipative particle dynamics; Spectrin model

1. Introduction

Recent experiments to probe the mechanical properties of a red blood cell (RBC) include micropipette aspiration [1,2], RBC deformation by optical tweezers [3,4], RBC edge flicker microscopy [5] and tracking of fluorescent nanometer beads attached to the RBC [6]. The first two experimental techniques subject the RBC directly to mechanical deformation, while the two latter attempt to extract the mechanical properties from passive observations of thermal fluctuations. The direct deformation techniques report overlapping results for the shear modulus of healthy cells in the range of 4–9 $\mu\text{N/m}$ for micropipette aspiration and 5–12 $\mu\text{N/m}$ in optical tweezers experiments. In contrast, the thermal fluctuations techniques predict the shear modulus to be one to two orders of magnitude smaller than those from the RBC deformation experiments. Recent theoretical developments offer explanations for the discrepancies in experimental results. Li et al. [7] suggest that the erythrocyte cytoskeleton may be subject to a continuous rearrangement due to metabolic activity or large strains. Their numerical model shows that under certain conditions, the RBC membrane consisting of a lipid bilayer with an attached cytoskeleton formed by a spectrin protein network and linked by short actin filaments may experience strain hardening and softening. In addition, the actin cytoskeleton attachments are subject to diffusion within the lipid bilayer, however

it is a slow process and hence negligible at short time scales. Gov [8] proposes an active elastic network model, where the metabolic activity controls the stiffness of the cell through the consumption of ATP. The ATP activity could also greatly affect membrane thermal undulations [9] resulting in fluctuations comparable to an effective temperature increase by a factor of three, which would result in a substantial underprediction of the RBC membrane elastic properties. However, recent experiments [10] did not find a strong dependence of RBC elastic properties and fluctuations on ATP.

The experimental findings provide clear evidence that RBCs subject to large deformations are characterized by a complex nonlinear mechanical response. However, it is plausible to assume that a nonlinear elastic model can provide an adequate description of moderate RBC deformations at small strain rates. Thus, the main focus of this paper is to derive consistent coarse-grained nonlinear elastic models, which are able to successfully describe the mechanical deformations of RBCs. Possible membrane strain hardening or softening as well as the effects of metabolic activity can be incorporated into the model, however this is beyond the scope of the present paper.

The healthy human RBC assumes a biconcave shape with an average diameter of $7.8\ \mu\text{m}$. The lipid bilayer can be considered nearly viscous and area-incompressible [11], while the attached spectrin network is mainly responsible for the membrane elastic response providing RBC integrity as it undergoes severe deformations in narrow capillaries as small as $3\ \mu\text{m}$ in diameter. An RBC model is constructed by a network of springs in combination with a bending energy and constraints for surface-area and volume conservation. Fig. 1 illustrates the difference between network and continuum based models, which are characterized by different parameters.

Atomic force microscopy experiments [12,13] have shown that the spectrin network of RBCs is highly irregular compared to the regular hexagonal network and has varying lengths of interconnections. The spectrin-level model in this paper corresponds to an *effective* spectrin network where each spring represents a single spectrin tetramer; the network is regular, i.e. nearly hexagonal. Theoretical analysis of the hexagonal network yields its linear mechanical properties, however the current theoretical results for the spectrin-level model [14] underestimate the effective membrane Young's modulus by about 50%. In this paper, we present the *corrected* analysis of elastic membrane properties for different spring models and arbitrary levels of coarse-graining. In addition, we propose a stress-free model, which eliminates non-vanishing local artifacts, such as the dependence of mechanical properties on triangulation quality and equilibrium shape stability for realistic membrane bending rigidity; the latter is often compensated with *artificially* high bending stiffness. In addition, comparison of the spring response at spectrin-level of modeling with the response of a coarse-grained single spectrin tetramer [15] is shown to yield good agreement. This provides additional model validation.

A number of numerical models have recently been developed, which include continuum descriptions [11,16,17], and discrete approximations at the spectrin molecular level [18,19] as well as at the mesoscopic scale [20–22]. Fully continuum (fluid and solid) modeling often suffers from difficulties in coupling nonlinear solid motions and fluid flow without excessive computational expense. Therefore, “semi-continuum” modeling [16,17] of deformable particles is developing rapidly and typically employs immersed boundary or front-tracking techniques. Here a membrane is represented by a set of points which move in Lagrangian fashion and are coupled to an Eulerian discretization of the fluid domain. In this work, we focus on the accurate mesoscopic modeling of RBCs. Specifically, we develop a generalized elastic model with major improvements to its mechanical properties.

The paper is organized as follows. In the next section we present the detailed RBC model. Section 3 provides a semi-analytical theory of the RBC membrane elastic properties, and Section 4 compares calculations of the stretching-deformation of healthy and parasitized RBCs in malaria with experimental data. We conclude in Section 5 with suggestions for model development.

2. Red blood cell model

The model membrane structure is analogous to the models presented in [20–22]. It is defined as a set of points with Cartesian coordinates $\{\mathbf{x}_i\}$, $i=1\dots N_v$, which are vertices in a two-dimensional triangulated network on the RBC surface. The vertices are connected by N_s edges represented by springs, which form N_t triangles. The free energy of the system is given by

$$V(\{x_i\}) = V_{\text{in-plane}} + V_{\text{bending}} + V_{\text{area}} + V_{\text{volume}}. \quad (1)$$

The in-plane free energy term includes the spring energy, U_s , and may also contain other elastic energy stored in the membrane as follows,

$$V_{\text{in-plane}} = \sum_{j \in 1\dots N_s} U_s(l_j) + \sum_{k \in 1\dots N_t} \frac{C_q}{A_k^q}, \quad (2)$$

where l_j is the length of the spring j , A_k is the area of the k -th triangle, and the constant C_q and exponent q need to be properly selected. Different spring models can be used here, and their performance will be discussed in Section 3. However, we highlight two *nonlinear* spring models: the wormlike chain (WLC) and the finitely extensible nonlinear elastic (FENE) spring, whose attractive potentials are given, respectively, by

$$U_{\text{WLC}} = \frac{k_B T l_{\text{max}}}{4p} \frac{3x^2 - 2x^3}{1-x}, \quad U_{\text{FENE}} = -\frac{k_s}{2} l_{\text{max}}^2 \log[1-x^2], \quad (3)$$

where $x = l/l_{\text{max}} \in (0,1)$; l_{max} is the maximum spring extension, p is the persistence length and k_s is the FENE spring constant. Note that when the distance between two connected points approaches l_{max} , the corresponding spring force goes to infinity, and therefore it limits the maximum extension to l_{max} . It is important to point out that both WLC and FENE springs exert purely attractive forces, thus they produce a triangular area compression, while the second term in Eq. (2) provides triangular area expansion. The minimum energy state of single triangle corresponds to an equilibrium spring length l_0 , which depends on the spring parameters and C_q . The relationship between these parameters and the equilibrium length can be derived by energy minimization [18] or by setting the Cauchy stress obtained from the virial theorem to zero [14]. We obtained the following expressions for WLC and FENE springs, respectively,

$$C_q^{\text{WLC}} = \frac{\sqrt{3} A_0^{q+1} k_B T (4x_0^2 - 9x_0 + 6)}{4pq l_{\text{max}} (1-x_0)^2}, \quad C_q^{\text{FENE}} = \frac{\sqrt{3} A_0^{q+1} k_s}{q(1-x_0^2)}, \quad (4)$$

where $x_0 = l_0/l_{\text{max}}$ and $A_0 = \sqrt{3} l_0^2/4$. These formulas allow us to calculate the strength of the second term in Eq. (2) for the given equilibrium length and spring parameters. Another choice is to select a spring with a specific equilibrium length (e.g., harmonic spring, WLC or

FENE in combination with a repulsive potential), and then set C_q to zero. We now introduce a repulsive force defined as a power function (POW) of the separation distance l as follows

$$f_{\text{POW}}(l) = \frac{k_p}{l^m}, \quad m > 0, \quad (5)$$

where k_p is the force coefficient and m is the exponent. The combination of WLC or FENE with the POW force defines a spring with nonzero equilibrium length, and will be called WLC-POW and FENE-POW, respectively. The strength k_p can be expressed in terms of the equilibrium length l_0 and the WLC or FENE parameters by equating the corresponding forces. The combination of WLC or FENE with the in-plane energy in Eq. (2) will be denoted as WLC-C and FENE-C throughout the paper.

The bending energy is defined as,

$$V_{\text{bending}} = \sum_{j \in 1 \dots N_s} k_b [1 - \cos(\theta_j - \theta_0)], \quad (6)$$

where k_b is the bending constant, θ_j is the instantaneous angle between two adjacent triangles having the common edge j , and θ_0 is the spontaneous angle.

The area and volume conservation constraints are

$$V_{\text{area}} = \frac{k_a (A - A_0^{\text{tot}})^2}{2A_0^{\text{tot}}} + \sum_{j \in 1 \dots N_t} \frac{k_d (A_j - A_0)^2}{2A_0}, \quad (7a)$$

$$V_{\text{volume}} = \frac{k_v (V - V_0^{\text{tot}})^2}{2V_0^{\text{tot}}}, \quad (7b)$$

where k_a , k_d and k_v are the global area, local area and volume constraint constants, respectively. The terms A and V are the total area and volume of the RBC, while A_0^{tot} and V_0^{tot} are the *desired* total area and volume, respectively. Note, that the above expressions define the global area and volume constraints while the second term in Eq. (7a) corresponds to local area dilatation.

The nodal forces corresponding to the above energies are derived from the usual formula

$$f_i = -\partial V(\{x_i\}) / \partial x_i, \quad i \in 1 \dots N_v. \quad (8)$$

Exact force expressions can be derived analytically from the above energies, however for brevity we do not present them in this paper.

3. Mechanical properties

The elastic shear modulus μ_0 measured experimentally lies between 4 and 12 $\mu\text{N/m}$ and the bending modulus k lies between 1×10^{-19} and 7×10^{-19} J, which corresponds to the range of 25–171 $k_B T$ based on the room temperature of $T = 23^\circ \text{C}$. Since the precise geometry is often not known, the discrepancies in the measurements arise, in part, from overly simplified

geometrical models used to extract values from the measured forces. In such cases, accurate numerical modeling can provide a valuable aid in experimental parameter quantification.

In recent optical tweezers stretching experiments [4] the RBC behavior was modeled as a hyperelastic material using the finite element method (FEM). From the FEM simulations the membrane shear modulus μ_0 was obtained in the range 5–12 $\mu\text{N/m}$. This corresponds to the Young's modulus of $Y=3 \mu_0=15\text{--}36 \mu\text{N/m}$ due to the use of a three-dimensional membrane model. Dao et al. [14] performed coarse-grained molecular dynamics (CGMD) simulations of the spectrin-level cytoskeleton which yielded a worse comparison to the experimental stretching response than FEM. They derived the first-order approximation of the shear modulus μ_0 and the area-compression modulus K for a two-dimensional regular hexagonal network of springs expressed through spring parameters. Even though the shear modulus in the FEM and CGMD simulations was matched, it is clear from Fig. 8 of [14] that FEM and CGMD systems have different Young's moduli as the slopes in the linear elastic regime are different. In addition, their estimated area-compressibility modulus was $K=2\mu$ which yields the Poisson's ratio of $\nu=1/3$, while the membrane was assumed to be nearly incompressible. However, for an incompressible material $\nu=1$ for a two-dimensional model and $K \rightarrow \infty$. We have confirmed that their analytical results are correct but they appear to be incomplete because not all model contributions are considered for the membrane elastic properties estimation, resulting in a Young's modulus underprediction by about 50%, which can explain the inconsistency found.

3.1. Macroscopic elastic properties

Our starting point is the linear analysis of a two-dimensional sheet of springs built with equilateral triangles [14]. Fig. 2 (left) shows an element of the equilateral triangulation with vertex $\mathbf{v}$ placed at the origin.

The stress for the area element S (from the virial theorem) is given by

$$\tau_{\alpha\beta} = -\frac{1}{2A} \left[\frac{f(a)}{a} a_\alpha a_\beta + \frac{f(b)}{b} b_\alpha b_\beta + \frac{f(c)}{c} (b_\alpha - a_\alpha)(b_\beta - a_\beta) \right] - \left(q \frac{C_q}{A^{q+1}} + \frac{k_a (A_0^{\text{tot}} - N_t A)}{A_0^{\text{tot}}} + \frac{k_d (A_0 - A)}{A_0} \right) \delta_{\alpha\beta}, \quad (9)$$

where $f(\cdot)$ is the spring force, α, β can be x or y , N_t is the total number of triangles and $A_0^{\text{tot}} = N_t A_0$. In general, N_t cancels out and the global and local area contributions to the stress can be combined as $-(k_a + k_d)(A_0 - A)/A_0 \delta_{\alpha\beta}$. *Note, that the linear analysis in [14] did not take into account the global and local area contributions to the stress which significantly affect the final results.* The linear shear modulus can be derived by applying a small engineering shear strain γ to the configuration in Fig. 2 (left), followed by the first derivative of shear stress $\mu_0 = \frac{\partial \tau_{xy}}{\partial \gamma} \big|_{\gamma=0}$. The shear deformation is area-preserving, and therefore only spring forces contribute to the membrane shear modulus. For different spring models, we obtained the following expressions for μ_0 :

$$\mu_0^{\text{WLC-C}} = \frac{\sqrt{3} k_B T}{4 p l_{\text{max}} x_0} \left(\frac{3}{4(1-x_0)^2} - \frac{3}{4} + 4x_0 + \frac{x_0}{2(1-x_0)^3} \right), \quad (10a)$$

$$\mu_0^{\text{FENE-C}} = \frac{\sqrt{3} k_s}{2} \left(\frac{x_0^2}{(1-x_0^2)^2} + \frac{2}{1-x_0^2} \right), \quad (10b)$$

$$\mu_0^{\text{WLC-POW}} = \frac{\sqrt{3}k_B T}{4pl_{\max}x_0} \left(\frac{x_0}{2(1-x_0)^3} - \frac{1}{4(1-x_0)^2} + \frac{1}{4} \right) + \frac{\sqrt{3}k_p(m+1)}{4l_0^{m+1}}, \quad (10c)$$

$$\mu_0^{\text{FENE-POW}} = \frac{\sqrt{3}}{4} \left(\frac{2k_s x_0^2}{(1-x_0^2)^2} + \frac{k_p(m+1)}{l_0^{m+1}} \right). \quad (10d)$$

The linear elastic area-compression modulus K can be calculated from the area expansion with the resulting in-plane pressure given by

$$P = -\frac{1}{2}(\tau_{xx} + \tau_{yy}) = \frac{3lf(l)}{4A} + q \frac{C_q}{A^{q+1}} + \frac{(k_a + k_d)(A_0 - A)}{A_0}. \quad (11)$$

With the compression modulus K defined as

$$K = -\frac{\partial P}{\partial \log(A)} \Big|_{A=A_0} = -\frac{1}{2} \frac{\partial P}{\partial \log(l)} \Big|_{l=l_0} = -\frac{1}{2} \frac{\partial P}{\partial \log(x)} \Big|_{x=x_0}, \quad (12)$$

we use Eqs. (15) and (16) to derive the linear area-compression modulus for different spring models as follows

$$K^{\text{WLC-C}} = \frac{\sqrt{3}k_B T}{4pl_{\max}(1-x_0)^2} \left[\left(q + \frac{1}{2} \right) (4x_0^2 - 9x_0 + 6) + \frac{1+2(1-x_0)^3}{1-x_0} \right] + k_a + k_d, \quad (13a)$$

$$K^{\text{FENE-C}} = \frac{\sqrt{3}k_s}{1-x_0^2} \left[q + 1 + \frac{x_0^2}{1-x_0^2} \right] + k_a + k_d, \quad (13b)$$

$$K^{\text{WLC-POW}} = 2\mu_0^{\text{WLC-POW}} + k_a + k_d, \quad (13c)$$

$$K^{\text{FENE-POW}} = 2\mu_0^{\text{FENE-POW}} + k_a + k_d. \quad (13d)$$

Note, that if $q=1$ we obtain the expressions $K^{\text{WLC-C}} = 2\mu_0^{\text{WLC-C}} + k_a + k_d$ and $K^{\text{FENE-C}} = 2\mu_0^{\text{FENE-C}} + k_a + k_d$. Generally, for a nearly incompressible sheet of springs the area constraint coefficients have to be large such that $k_a + k_d \gg 1$, and thus $K \gg \mu_0$.

Young's modulus Y for the two-dimensional sheet can be expressed through the shear and area-compression moduli as follows

$$Y = \frac{4K\mu_0}{K + \mu_0}, \quad Y \rightarrow 4\mu_0, \text{ if } K \rightarrow \infty, \quad (14)$$

with Poisson's ratio ν given by

$$\nu = \frac{K - \mu_0}{K + \mu_0}, \quad \nu \rightarrow 1, \text{ if } K \rightarrow \infty. \quad (15)$$

The above expressions are consistent with the incompressibility assumption enforced through the condition $k_a + k_d \gg 1$. In practice, we use the value of $k_a + k_d = 5000$, which provides a nearly incompressible membrane with Young's modulus about 2% smaller than its asymptotic value of $4\mu_0$ ($\mu_0 = 100$). All the analytical expressions for μ_0 , K and Y were numerically verified by shearing, area expanding and stretching experiments of the regular two-dimensional sheet of springs. In addition, it is important to note that the modeled sheet appears to be *isotropic* for small shear and stretch deformations, however it is *anisotropic* at large deformations.

3.2. Membrane bending properties

In this section we discuss the correspondence of our bending model to the macroscopic model of Helfrich [23] given by

$$E = \frac{k_c}{2} \int_A (C_1 + C_2 - 2C_0)^2 dA + k_g \int_A C_1 C_2 dA, \quad (16)$$

where C_1 and C_2 are the local principal curvatures, C_0 is the spontaneous curvature, and k_c and k_g are the bending rigidities.

We base the derivation on the spherical shell. Fig. 2 (right) shows two equilateral triangles with sides a , whose vertices rest on the surface of a sphere of radius R . The angle between their normals $\mathbf{n}_1$ and $\mathbf{n}_2$ is equal to θ . For the spherical shell we can derive from Eq. (16) $E = 8\pi k_c (1 - C_0/C_1)^2 + 4\pi k_g = 8\pi k_c (1 - R/R_0)^2 + 4\pi k_g$, where $C_1 = C_2 = 1/R$ and $C_0 = 1/R_0$. For the triangulated sphere we have $E_t = N_s k_b [1 - \cos(\theta - \theta_0)]$ in the defined notations. We expand $\cos(\theta - \theta_0)$ in Taylor series around $(\theta - \theta_0)$ to obtain $E_t = N_s k_b (\theta - \theta_0)^2 / 2 + O((\theta - \theta_0)^4)$. From

Fig. 2 (right) we find that $2r \approx \theta R$ or $\theta = \frac{a}{\sqrt{3}R}$, and analogously $\theta_0 = \frac{a}{\sqrt{3}R_0}$. Furthermore,

$A_{\text{sphere}} = 4\pi R^2 \approx N_t A_0 = \frac{\sqrt{3}N_t a^2}{4} = \frac{N_s a^2}{2\sqrt{3}}$, and thus $a^2/R^2 = 8\pi\sqrt{3}/N_s$. Finally, we obtain

$E_t = N_s k_b \left(\left(\frac{a}{\sqrt{3}R} - \frac{a}{\sqrt{3}R_0} \right)^2 / 2 = \frac{N_s k_b a^2}{6R^2} (1 - R/R_0)^2 = \frac{8\pi k_b}{2\sqrt{3}} (1 - R/R_0)^2$. Equating the macroscopic bending energy E for $k_g = -4k_c/3$, $C_0 = 0$ [24] and E_t gives us the relation

$k_b = 2k_c/\sqrt{3}$ in agreement with the continuum limit in [24]. The spontaneous angle θ_0 is set according to the total number of vertices N_v on the sphere. It can be shown that

$\cos(\theta) = 1 - \frac{1}{6(R^2/a^2 - 1/4)} = \left(\sqrt{3}N_s - 10\pi \right) / \left(\sqrt{3}N_s - 6\pi \right)$, while $N_s = 2N_v - 4$. The corresponding bending stiffness k_b and the spontaneous angle θ_0 are then given by

$$k_b = \frac{2}{\sqrt{3}} k_c, \quad \theta_0 = \cos^{-1} \left(\frac{\sqrt{3}(N_v - 2) - 5\pi}{\sqrt{3}(N_v - 2) - 3\pi} \right). \quad (17)$$

3.3. RBC triangulation

The average unstressed shape of a single RBC measured in the experiments in [25] is biconcave and is described by

$$z = \pm D_0 \sqrt{1 - \frac{4(x^2 + y^2)}{D_0^2}} \left[a_0 + a_1 \frac{x^2 + y^2}{D_0^2} + a_2 \frac{(x^2 + y^2)^2}{D_0^4} \right], \quad (18)$$

where $D_0 = 7.82 \mu\text{m}$ is the cell diameter, $a_0 = 0.0518$, $a_1 = 2.0026$, and $a_2 = -4.491$.

The area and volume of this RBC is equal to $135 \mu\text{m}^2$ and $94 \mu\text{m}^3$, respectively. We have investigated three types of triangulation strategies:

- *Point charges*: N_v points are randomly distributed on a sphere surface, and the electrostatic problem of point charges is solved while the point movements are constrained on the sphere. After equilibrium is reached, the sphere surface is triangulated, and conformed to the RBC shape according to Eq. (18).
- *Advancing front*: The RBC shape is imported into commercially available grid generation software Gridgen [26] which performs the advancing front method for the RBC surface triangulation.
- *Energy relaxation*: First, the RBC shape is triangulated following the point charges or advancing front methods. Subsequently, the relaxation of the free energy of the RBC model is performed while the vertices are restricted to move on the biconcave shape in Eq. (18). The relaxation procedure includes only in-plane and bending energy components and is done by flipping between the two diagonals of two adjacent triangles.

The triangulation quality can be characterized by two distributions: (i) distribution of the link (edge) length, (ii) distribution of the vertex degrees (number of links in the vertex junction). The former is characterized by the value $d(l) = \sigma(l)/l$, where l is the average length of all edges, and $\sigma(l)$ is the standard deviation. The latter defines the regularity of triangulation by providing the relative percentage of degree- n vertices $n = 1 \dots n_{\max}$. Note that the regular network, from which the mechanical properties were derived, has only degree-6 vertices. Table 1 presents the *average* mesh quality data for different triangulation methods.

The better mesh quality corresponds to a combination of smaller $d(l)$, higher percentage of degree-6, and smaller percentage of any other degree vertices, and is achieved for larger number of points N_v . It seems that the best quality is reached with the energy relaxation method while the worst is the advancing front triangulation, which will be discussed further below.

3.4. Coarse-graining

For systematic coarse-graining the parameters of the fine or spectrin-level model have to be defined. Atomic force microscopy results [12,13] show that each actin junction complex exists every $3000\text{--}5000 \text{ nm}^2$. Taking into account that the average RBC area is equal to $A = 135 \mu\text{m}^2$ [25] we obtain that the RBC spectrin network has about $27,000\text{--}45,000$ junction complexes which represent the total number of vertices N_v in the spectrin-level model. The spectrin-level model in this paper is built by $N_v = 27,344$ junction complexes. The effective equilibrium spectrin length l_0 is estimated as follow

$$A = N_t \cdot A_0 = (2N_v - 4) \cdot A_0 = (2N_v - 4) \cdot \frac{\sqrt{3}l_0^2}{4}, \quad (19)$$

and is equal to 75.5 nm . Note that l_0 lies in the range $59\text{--}76 \text{ nm}$ based on the number of junction complexes $27,000\text{--}45,000$. In order to define the maximum spectrin extension it is

more convenient to set the value of the ratio $x_0 = l_0/l_{\max}$, which is equal to 2.2 for the WLC models and 2.05 for FENE, and it governs the nonlinear spectrin response. This yields to $l_{\max} = 166.1$ nm for WLC and 154.8 nm for FENE models. Using the defined lengths and Eqs. (10a)–(10d) with $\mu_0 = 6.3$ $\mu\text{N/m}$ we obtain the persistence length $p = 18.7$ nm for the WLC-C model at the room temperature $T = 23$ °C and the spring constant $k_s = 2.4$ $\mu\text{N/m}$ in case of the FENE-C model. The persistence length estimated here is about 2.5 times longer than $p = 7.5$ nm chosen in [14,22], however both values are within the range obtained from experiments [27]. In part this difference can be reconciled by a choice of the effective spectrin-level model. From Eq. (10a) we find that in order to have the same macroscopic shear modulus for a fixed x_0 but for a different number of the actin junction complexes N_v in the spectrin-level representation, the product pl_0 has to be kept constant. This implies that for a smaller number of vertices ($N_v = 27,344$ here) the equilibrium spectrin length would increase while the persistence length becomes smaller. In addition, the estimated parameters depend on the spring model such that for the cases of WLC-POW and FENE-POW models we obtain $p = 14.68$ nm and $k_s = 3.06$ $\mu\text{N/m}$, respectively while the power force parameter k_p found by equating the corresponding spring forces for $l_0 = 75.5$ nm is equal to 1.66×10^{-27} Nm^2 and 1.73×10^{-27} Nm^2 for the POW parameter $m = 2$.

Systematic RBC coarse-graining yields a model represented by a smaller number of vertices compared to the spectrin-level model, which is called the “fine” model further in text. Equating the areas of the coarse-grained and fine models, we obtain the lengths (l_0 and $l_{\max}$) for the coarse-grained RBC as follows

$$l_0^c = l_0^f \sqrt{\frac{N_v^f - 2}{N_v^c - 2}}, \quad l_{\max}^c = l_{\max}^f \sqrt{\frac{N_v^f - 2}{N_v^c - 2}}, \quad (20)$$

where the superscripts c and f correspond to coarse-grained and fine models, respectively. For a fixed x_0 the shear and area-compression moduli remain unchanged for the coarse-grained model if the parameters are adjusted as follows

$$p^c = p^f \frac{l_0^f}{l_0^c} \text{ (WLC)}, \quad k_s^c = k_s^f \text{ (FENE)}, \quad k_p^c = k_p^f \left(\frac{l_0^c}{l_0^f} \right)^{m+1} \text{ (POW)}. \quad (21)$$

The Eqs. (20) and (21) define a complete set of parameters required for the model at an arbitrary coarse-graining level derived from the fine model; they are generalizations of the formulas first presented in [22].

3.5. Model and physical units scaling

We now outline the scaling procedure, which relates the model's non-dimensional units to physical units. First, we choose the equilibrium spring length $l_0 = l_0^M$ in our model units, where the superscript M denotes “model” and $[l_0^M] = r^M$ defines model length scale. Another parameter we are free to select is the imposed shear modulus $\mu_0 = \mu_0^M$ with

$[\mu_0^M] = \frac{N^M}{r^M} = \frac{(k_B T)^M}{(r^M)^2}$, which will provide a scaling base. Use of WLC and FENE springs requires the maximum-extension length $l_{\max}^M$ to be set, however it is more convenient to set the ratio $x_0 = l_0^M / l_{\max}^M$. Further we will show that the choice of x_0 does not affect the linear elastic deformation, but it governs the RBC nonlinear response at large deformations. For given l_0^M , μ_0^M and x_0 we can calculate the required spring parameters for a chosen model

using Eqs. (10a)–(10d). Then, the area-compression modulus K^M and the Young's modulus Y^M are found for the calculated spring parameters and given area constraint parameters (k_a and k_d) using Eqs. (13a)–(13d) and (14). We then define the length scale based on the cell diameter $D_0^M = (L_x^M + L_y^M)/2$, where $[D_0^M] = r^M$ and L_x, L_y are the cell diameters in the x and y directions found from the equilibrium simulation of a single cell using the previously obtained model parameters. The length scale based on l_0^M appears to be inappropriate, because, in general, the cell dimensions will depend on the relative volume-to-area ratio and to some extent on the current triangulation artifacts (discussed below). As an example, we can define an RBC and a spherical capsule with the same l_0^M , while the cell sizes would greatly differ. However, in general, D_0^M is proportional to l_0^M for fixed volume-to-area ratio. The real RBC has an average diameter $D_0^P = 7.82 \mu m$ (superscript P denotes “physical”), and therefore the following length scale is adapted

$$r^M = \frac{D_0^P}{D_0^M} [m]. \quad (22)$$

Since we will perform simulations of RBC stretching, it is natural to involve Young's modulus as the main scale parameter. Matching the model and physical Young's modulus

$Y^M \frac{(k_B T)^M}{(r^M)^2} = Y^P \frac{(k_B T)^P}{m^2}$ provides us with the energy unit scale as follows

$$(k_B T)^M = \frac{Y^P}{Y^M} \frac{(r^M)^2}{m^2} (k_B T)^P = \frac{Y^P}{Y^M} \left(\frac{D_0^P}{D_0^M} \right)^2 (k_B T)^P. \quad (23)$$

After we determined the model energy unit (as an example for room temperature of $T=296$ K), we calculate the bending rigidity in model energy units using Eq. (17). In addition, we define the force scale, N^M , by

$$N^M = \frac{(k_B T)^M}{r^M} = \frac{Y^P}{Y^M} \frac{D_0^P}{D_0^M} \frac{(k_B T)^P}{m} = \frac{Y^P}{Y^M} \frac{D_0^P}{D_0^M} N^P. \quad (24)$$

Note that for the stretching simulations presented below mass and time scales need not be defined explicitly since we are not interested here in stretching dynamics.

4. Simulation results and discussion

4.1. RBC stretching: success and problems

Next, we perform RBC stretching simulations and compare the results with the experimental data of RBC deformation by optical tweezers [4]. Here, we use the average RBC diameter of $D_0^P = 7.82 \mu m$. The aforementioned FEM simulations of RBC membrane [4] showed an agreement with the experimental data for $\mu_0^P = 5.3 \mu N/m$, however we find that a slightly better correspondence of the results is achieved for $\mu_0^P = 6.3 \mu N/m$ and $Y^P = 18.9 \mu N/m$ (two-dimensional properties of the three-dimensional elastic model), which we select to be the targeted properties. Table 2 shows a set of the model and physical RBC parameters using the coarse-graining procedure described in Section 3.4. In all cases $\mu_0^M = 100$, while x_0 is equal to 2.2 for the WLC models and 2.05 for the FENE models, and the exponents $q=1$ (Eq. (2)) and $m=2$ (Eq. (5)). The area and volume constraints coefficients were set to $k_a=5000$, $k_d=0$,

and $k_v = 5000$ for WLC-C and FENE-C models, while $k_a = 4900$, $k_d = 100$, and $k_v = 5000$ for the WLC-POW model. The triangulation for all N_v was performed using the energy relaxation method. The imposed Young's modulus for all cases is $Y^M = 392.453$, which is about 2% lower than that in the incompressible limit $Y^M = 4\mu_0^M = 400$. Using Eq. (23) we find the energy unit $(k_B T)^M$ based on $(k_B T)^P$ at room temperature of $T = 296$ K. The bending rigidity k_c is set to 2.4×10^{-19} J, which seems to be a widely accepted value and is equal to approximately $58(k_B T)^P$. The total RBC area A_0^{tot} is equal to $N_t \frac{\sqrt{3}}{4} (l_0^M)^2$, where N_t is the total number of triangle plaquettes with the area $A_0 = \frac{\sqrt{3}}{4} (l_0^M)^2$. Note that for all triangulations used in this paper $N_t = 2N_v - 4$. The total RBC volume V_0^{tot} is found according to the following scaling $V_0^{\text{tot}} / (A_0^{\text{tot}})^{3/2} = V^P / (A^P)^{3/2}$, where $V^P = 94 \mu\text{m}^3$ and $A^P = 135 \mu\text{m}^2$ according to the average RBC shape described by Eq. (18).

The RBC is modeled by Dissipative Particle Dynamics (DPD), a mesoscale method, see reference [28] for details. The RBC is suspended in a solvent which consists of free DPD particles with number density $n = 3$. Note that the macroscopic solvent properties (e.g., viscosity) are not important here, because we are interested in the final cell deformation for every constant stretching force. Thus, we allow enough time for the RBC to reach its final deformation state without close monitoring of the stretching dynamics. Meanwhile, the solvent maintains the temperature at the constant value of $(k_B T)^M$.

Fig. 3 shows a sketch of the red blood cell before and after deformation. The total stretching force F_s^P is in the range $0 \dots 200$ pN, which can be scaled into model units F_s^M according to Eq. (24). The total force F_s^M is applied to $N_+ = \varepsilon N_v$ vertices (drawn as small black spheres in Fig. 3) of the membrane with the largest x -coordinates in the positive x -direction, and correspondingly $-F_s^M$ is exerted on $N_- = N_v - N_+$ vertices with the smallest x -coordinates in the negative x -direction. Therefore, a vertex in N_+ or N_- is subject to the force

$f_s^M = \pm F_s^M / (\varepsilon N_v)$. The vertex fraction ε is equal to 0.02 corresponding to a contact diameter of the attached silica bead $d_c = 2 \mu\text{m}$ used in experiments. The contact diameter was measured as $(\max_{ij} |y_i^+ - y_j^+| + \max_{ij} |y_i^- - y_j^-|) / 2$, where y_i^+ , y_j^+ and y_i^- , y_j^- are the y -coordinates of vertices in N_+ and N_- , respectively. The simulations for the given force range were performed as follows: (i) $B = 16$ is chosen, which defines the force increment

$\Delta F_s^P = 200 \text{ pN} / B$ with corresponding ΔF_s^M . (ii) The loop $i = 1 \dots B$ is run with the stretching force ΔF_s^M during time 2τ each. The time τ is long enough in order for the RBC to converge to the equilibrium stretched state for the given force. Thus, the time $[0, \tau]$ is the transient time for convergence, and during time $[\tau, 2\tau]$ the deformation response is calculated. The axial diameter D_A is computed over time τ as $|x_{\max} - x_{\min}|$, where $x_{\max}$ is the maximum x position among the N_+ vertices, while $x_{\min}$ is the minimum among N_- . The

transverse diameter D_T is calculated as $2 \times \max_{i=1 \dots N_v} \sqrt{(y_i - c_y)^2 + (z_i - c_z)^2}$, where c_y , c_z are the y and z center of mass coordinates.

Fig. 4 presents the RBC stretching response for different number of vertices N_v (left) and spring models (right) with RBC parameters from Table 2; also included are experimental results [4] and the spectrin-level RBC model results of [14]. Independent of the number of vertices or spring model we find excellent agreement of the simulation results with the experiment. A noticeable disagreement in the transverse diameter may be partially due to experimental errors arising from the fact that the optical measurements were performed from a single observation angle. RBCs subjected to stretching may rotate in y - z plane as observed in our numerical simulations, and therefore measurements from a single observation angle

may result in under-prediction of the maximum transverse diameter. However, the simulation results remain within the experimental error bars.

The solid line in Fig. 4 corresponds to the spectrin-level RBC [14] of similar type employing the WLC-C model. In [14] the derivation of linear elastic properties did not include a contribution of the area constraint, which results in Young's modulus being underpredicted by about 50%. From the region of small near-linear deformation (0–50 pN) it is clear that the solid line corresponds to a membrane with a larger Young's modulus compared to the experiment. In addition, the ratio α_0 was set to 3.17, which results in near-linear elastic deformation, and ignores the nonlinear RBC response at large deformations. Finally, we note that the FENE-C model appears to be less stable (requires a smaller time step) at large deformations due to a more rapid spring hardening compared to WLC-C. The WLC-POW model performs similarly to WLC-C, however a weak local area constraint ($k_d > 0$) may be required for stability at large deformations as it mimics the second in-plane force term in Eq. (2) for the WLC-C model.

Fig. 5 demonstrates typical RBC shape evolution from equilibrium (0 pN force) to 100 pN total stretching force for different N_v using the WLC-C model. Note that the RBCs show local anomalous surface features (hills) in equilibrium which are due to local membrane stresses since it not possible to have regular hexagonal triangulation of the RBC surface with equal edges. The strength of the local buckling depends on the relative interplay of the in-plane elasticity and bending rigidity. Increase of the membrane bending stiffness results in smoother RBC surface, while a decrease would result in a more buckling. However, this feature seems to be less pronounced for higher N_v . Other membrane models yield similar shapes.

Despite the demonstrated success of the RBC models, several problems remain due to the fact that the membrane is not stress-free. Fig. 6 shows the RBC response of the WLC-C ($N_v = 500$) model for different stretching directions (left) with energy relaxation triangulation and the RBC response for models with different triangulations (right). While the RBC triangulated through the energy relaxation method gives satisfactory results with differences in the stretching response on the order of 5–8%, RBCs triangulated by other methods show a much greater discrepancy with the experiment.

Fig. 7 shows the RBC shapes at equilibrium and at the stretching force of 100 pN for point charges, advancing front triangulations (WLC-C model), and for a “stress-free” model introduced in the next section. The RBCs triangulated by point charges and advancing front methods show pronounced buckling and a non-biconcave shape for realistic bending and elastic RBC properties due to stronger local stresses arising from more irregular triangulation when compared to the energy relaxation mesh. In order to obtain a smooth biconcave shape the membrane bending rigidity has to be set to about $500(k_B T)^P$ and $300(k_B T)^P$ for point charges and advancing front methods, respectively, which is much higher than the bending rigidity of the real RBC of about $56(k_B T)^P$. Local buckling features are less pronounced for stretched cells since the membrane is subject to strong stretching stresses. Moreover, Fig. 6 shows that these models have higher effective elastic moduli than those measured as they are subject to a higher membrane stress at equilibrium due to triangulation artifacts. Also, they appear to give a stronger stretching anisotropy (10–15%) compared to the free energy relaxation method. The effect of local stresses on the membrane equilibrium shape appears to be a drawback for existing models [19], which is often compensated by setting artificially high values for the bending rigidity. Fig. 7 also shows the corresponding RBC shapes (advancing front triangulation) with a “stress-free” model which proves to be independent of triangulation and will be proposed next.

4.2. Stress-free membrane model

To eliminate the aforementioned membrane stress anomalies we propose a simple “annealing” procedure. For each spring we define $l_0^i, i=1 \dots N_s$ which are set to the edge lengths after the RBC shape triangulation, since we assume it to be the equilibrium state. Accordingly we define $l_{\max}^i = l_0^i \times x_0$ and $A_0^j, j=1 \dots N_t$ for each triangular plaquette. The total RBC area $A_0^{\text{tot}} = \sum_{j=1 \dots N_t} A_0^j$ and the total volume V_0^{tot} is calculated from the RBC triangulation. Then, we define the average spring length as, $\bar{l}_0 = \frac{1}{N_s} \sum_{i=1 \dots N_s} l_0^i$, and the average-maximum spring extension as $l_{\max} = l_0 \times x_0$; these are then used in the linear elastic properties estimation using Eqs. (14c,d) and (17c,d). Here, we omit the WLC-C and FENE-C models because it may not be possible to define a single in-plane area expansion potential (the second force term in Eq. (2)) which would define different individual equilibrium spring lengths for a triangle with distinct sides. However, for the WLC-POW and FENE-POW models the individual equilibrium spring length can be simply defined. Based on given $l_0, l_{\max}$ and μ_0^M the WLC or FENE spring parameters (p or k_s) can be calculated analogously to the previous model and then set to the same value for all springs. Then, the individual power force coefficients $k_p^i, i=1 \dots N_s$ (Eq. (5)) are defined for each spring in order to set the given equilibrium spring lengths l_0^i . An additional generalization of the model is to define individual spring parameters (p^i or k_s^i) and the power force coefficients k_p^i for all springs. Here, a system of two constraints (equilibrium length l_0^i and imposition of μ_0^M) needs to be solved for every spring. However, computational results did not differ for both stress-free approaches for the studied membranes.

We perform tests using the WLC-POW model for different triangulation methods and number of vertices. Table 3 shows a set of the model and physical RBC parameters. Other parameters are $\mu_0^M=100, x_0=2.2, m=2$ (Eq. (5)), $k_a=4900, k_d=100$, and $k_v=5000$. Fig. 8 presents simulation results for $N_v=500$ with different triangulations (left) and a range of the number of vertices N_v from 100 to 27,344 (right). A substantial improvement is observed when compared with the results in Fig. 6 (right). Note that the stress-free model, when probed along different stretching directions results in deviation in the stretching response on the order of 1% for the free energy triangulation method and about 3–5% for the other triangulation techniques. In addition, the stress-free model eliminates equilibrium shape artifacts for different triangulations shown in Fig. 7, and can be used even in cases of much lower bending rigidity. In addition, the stretching response for different number of vertices gives excellent agreement with the results of experiment. Here, $N_v=27,344$ corresponds to a spectrin-level of RBC modeling as in [19], while $N_v=100-500$ is highly coarse-grained RBC.

Fig. 9 presents RBC shapes for the cases of high coarse-graining and spectrin-level models. Even though the coarse-grained model of $N_v=100$ yields correct mechanical deformation results, it does not provide an accurate or smooth RBC shape description, which can be of importance in RBC dynamics. We suggest the minimum N_v to be used for the RBC model should be about 250–300.

The dependence of the RBC deformation response on the ratio x_0 and on the number of vertices N_+, N_- (Fig. 3) is shown in Fig. 10. As mentioned above, small RBC deformations are independent of the ratio x_0 , however at large deformations this parameter plays a significant role and governs the nonlinear RBC response. In addition, Fig. 10 (right) shows that the RBC response is sensitive to the fraction of vertices (shown in percent) to which the

stretching force is applied. It is equivalent to changing d_c in Fig. 3, which characterizes the attachment area of silica bead in the experiments.

4.3. Comparison with a single spectrin tetramer

It is rather remarkable that RBCs can be accurately modeled with just a few hundred points, which is about one hundred times computationally cheaper than the spectrin-level RBC model, where $N_v \sim 27,000$. At the spectrin-level of RBC modeling, each spring represents a single spectrin tetramer, and therefore the spring force WLC-POW should mimic the spectrin tetramer deformation response. We are not aware of any experimental single spectrin stretching results, however in [15] this has been done by means of numerical simulation using coarse-grained molecular dynamics (CGMD).

Fig. 11 compares the single spectrin tetramer stress–strain response to the spring force of the spectrin-level RBC model. The “WLC-POW fit” curve assumes that the maximum-extension spring length is 200 nm as in the CGMD simulations of [15], which corresponds to $l_0 = 91$ nm with $x_0 = 2.2$. This equilibrium length corresponds to an effective spectrin-level model represented by $N_v = 18,826$ (Eq. (19)) actin junction complexes, which is lower than that found in atomic force microscopy experiments [12,13]. The dashed line in Fig. 11 corresponds to the spring force of the spectrin-level model in [14] with parameters $l_0 = 75$ nm, $x_0 = 3.17$, and $l_{\max} = 237.75$ nm which results in about 50% underprediction of the macroscopic Young’s modulus. Finally, the dash-dotted line corresponds to our stress-free spectrin-level model with $N_v = 27,344$. The discrepancy between the CGMD and the spectrin-level models arises from great variability in the spectrin structure characterized by variable spectrin lengths and numbers of actin junction complexes. As discussed in the coarse-graining Section 3.4 for the effective spectrin-level model, the equilibrium spectrin length is directly related to the number of junction complexes. However, the spectrin-level model spans a wide range in terms of the number of junction complexes, i.e. 27,000–45,000, as documented in [12,13].

4.4. Infected RBCs in malaria

One of the main characteristics of the malaria disease is progressive changes in RBC mechanical properties and geometry. Infected RBCs in malaria become considerably stiffer compared to healthy ones [4,29]. Infected RBCs are characterized by three stages from the earliest to the latest: ring $\rightarrow$ trophozoite $\rightarrow$ schizont. After invasion of RBCs malaria parasites grow and take up more of the inner space of RBCs, such that the final stage (schizont) is often characterized by “near spherical” shape, while the preceding stages maintain their biconcavity. The progression through the stages of malaria is also characterized by considerable stiffening of the RBC membrane as found in optical tweezers stretching experiments [4] and in diffraction phase microscopy by monitoring thermal fluctuations [29].

Fig. 12 shows a comparison of simulation results of healthy and infected RBCs at different stages compared with the experiments [4]. The simulation results were obtained with the stress-free model ($N_v = 500$) having $\mu_0 = 6.3$ $\mu\text{N/m}$ for the healthy RBC, 14.5 for the ring stage, 29 for the trophozoite, and 60 $\mu\text{N/m}$ for the schizont, which is consistent with the experiments [4,29]. The ratio x_0 is equal to 1.8 for the infected RBCs in malaria. The bending rigidity is set to 2.4×10^{-19} J for all cases, as the dependence of the membrane bending stiffness for different stages is not known. The additional simulation curve for the schizont stage marked “near spherical” corresponds to stretching a membrane of ellipsoidal shape with the axes $a_x = a_y = 1.2a_z$. Here, the membrane shear modulus is found to be 40 $\mu\text{N/m}$ in order to match the stress–strain response with the experiment, which is smaller than that for the biconcave-shape simulation. For the near-spherical cell geometry a membrane is

subject to a stronger local stretching for the same uniaxial deformation compared to the biconcave shape. In the case of the deflated biconcave shape the inner fluid volume can be deformed in response to stretching, while in the near-spherical shape the fluid volume applies an additional resistance onto the stretched membrane. Experiments show that for the schizont stage the RBC has a near-spherical shape, and therefore the ellipsoidal geometry should be more accurate. As a conclusion, the cell geometry plays an important role and has to be closely modeled for accurate extraction of parameters from experiments.

Fig. 13 presents typical RBC shapes for the schizont stage using the original WLC-C model and the stress-free model for biconcave and near-spherical geometry. The WLC-C model shows strong local buckling due to local stress anomalies, which is not completely eliminated even for the stretching force of 100 pN, while the stress-free model yields a smooth RBC surface.

5. Summary

We developed coarse-grained RBC models represented by a network of springs in combination with bending rigidity, area, and volume conservation constraints. The modeled RBC accurately captures the elastic response at small and large deformations, and agrees very well with experiments of RBC stretching by optical tweezers. The linear elastic properties of the RBC membrane are derived analytically, and therefore no manual adjustment of the model parameters through numerical tests is required. We also proposed a stress-free RBC model which leads to triangulation-independent membrane properties, while the other RBC models suffer from stress anomalies, which result in triangulation-dependent deformation response and an anisotropic equilibrium shape. The model was tested for different levels of coarse-graining starting from the spectrin-level modeling ($N_v = 27,344$ vertices) and ending with only $N_v = 100$ vertices for the full membrane representation. However, we suggest that the minimum number of vertices to be used for the RBC membrane should be about $N_v = 250\text{--}300$ as the lower N_v may not accurately represent the RBC's smooth shape, which is of importance for RBC dynamics. In case of the spectrin-level model we compared the single spring force with the spectrin tetramer response obtained from the coarse-grained molecular dynamics simulations. The proposed model is general enough, and therefore can be easily applied in many numerical methods, such as semi-continuum methods (Immersed Boundary and Advanced Front Tracking), mesoscopic methods (Lattice Boltzmann and Brownian Dynamics), and mesoscopic particle methods (Dissipative Particle Dynamics and Multiparticle Collision Dynamics).

Here, we summarize the procedure for the RBC model. First, we obtain a triangulation of the equilibrium RBC shape defined by Eq. (18) for the given number of vertices N_v . This triangulation sets the required equilibrium lengths for the springs, triangle areas and the total RBC area and volume. Second, we choose the modeled membrane shear modulus μ_0 , and area and volume constraint coefficients (Eqs. (7a) and (7b)). This defines our RBC model parameters using Eqs. (10a)–(10d), (13a)–(13d), (14) and (17) with the average equilibrium spring length, which scales to the real units using Eqs. (20) and (21). In addition, we need to define the length scale (Eq. (19)) based on the RBC diameter. We suggest the RBC diameter to be obtained through an equilibrium simulation rather than assuming it from the analytical RBC shape (Eq. (18)) as they may be slightly different depending on the relative contributions of in-plane elasticity and membrane bending rigidity. After these two simple steps, the linear elastic properties of the model will match those of the real RBC. In addition, we mention that for large RBC deformations we may need to adjust the spring maximum-extension length which governs the nonlinear RBC response. However, it is convenient to set the ratio $x_0 = l_0/l_{\max} = 2.2$ for the WLC springs and $x_0 = 2.05$ for the FENE springs. We

emphasize that our procedure does not involve parameter adjustments through numerical testing.

The spectrin stretching comparison provides additional justification of using the spring model for accurate RBC deformation response. From these results we can draw the conclusion that: *an appropriate spring model for the RBC should have the maximum allowed extension length, in the neighborhood of which the spring force rapidly hardens in order to prevent further membrane strain.* In view of this, the harmonic spring used in [20] gives an adequate response at small deformations but it will not capture the nonlinear RBC deformations. Furthermore, the neo-Hookean spring used in [21] provides good RBC stretching response but it may also fail at very large deformations. At this point, an experimental confirmation of the single spectrin tetramer stress–strain relation would be of great interest.

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Fig. 1.
A sketch of network and continuum models.

Fig. 2.

An element of the equilateral triangulation (left) and two equilateral triangles placed on the surface of a sphere of radius R (right).

Fig. 3.
RBC sketch before and after deformation.

Fig. 4.

Computational results for *different N_v* (left) and *spring models* (right) compared with the experiments in [4] and the spectrin-level RBC model in [14].

Fig. 5. RBC shape evolution at *different* N_v and *total stretching forces* for the WLC-C model.

Fig. 6. RBC stretching along lines with *different orientation angles* (left) and *triangulation methods* (right) compared with the experiments in [4].

Fig. 7. RBC shape evolution for *different triangulations* and *stress-free model* introduced in the next section.

Fig. 8. Stress-free RBC model for different triangulation methods with $N_v = 500$ (left) and number of vertices with the energy relaxation triangulation (right) compared with the experiments in [4].

Fig. 9. RBC shapes for highly coarse-grained models ($N_v = 100, 250$) and the spectrin-level model ($N_v = 27,344$).

Fig. 10.

The stretching response of the stress-free RBC model for different ratio x_0 (left) and number of vertices in percents which are subject to the stretching force (right) compared with the experiments in [4].

Fig. 11.

A single spectrin tetramer stress-strain response [15] compared to the spring force of the spectrin-level RBC model.

Fig. 12.

The stretching response of healthy and infected RBCs in malaria for different stages compared with the experiments in [4].

Fig. 13. Infected RBC shape evolution at the schizont stage for original and stress-free models, and nearly spherical shape.

Table 1

Mesh quality for different triangulation methods.

Method	$d(l)$	degree-6	degree-5 and degree-7	other degrees
point charges	[0.15,0.18]	90%–95%	5%–10%	0%
advancing front	[0.13,0.16]	45%–60%	37%–47%	3%–8%
energy relaxation	[0.05,0.08]	75%–90%	10%–25%	0%

Table 2

RBC physical (“P” in SI units) and simulation (“M” in model units) parameters.

Model	N_v	l_0^P	p^P or k_s^P	l_0^M	D_0^M
WLC-C	500	5.58×10^{-7}	2.53×10^{-9}	0.56	8.267
WLC-C	1000	3.95×10^{-7}	3.58×10^{-9}	0.4	8.285
WLC-C	3000	2.28×10^{-7}	6.19×10^{-9}	0.23	8.064
FENE-C	500	5.58×10^{-7}	2.4×10^{-6}	0.56	8.265
WLC-POW	500	5.58×10^{-7}	1.99×10^{-9}	0.56	8.25

Table 3

RBC physical (“P” in SI units) and simulation (“M” in model units) parameters. Stress-free model.

N_v	l_0^P	p^P	$\bar{l}_0^M$	D_0^M
27,344	7.55×10^{-8}	14.68×10^{-9}	0.15	15.87
9128	1.31×10^{-7}	8.48×10^{-9}	0.13	8.12
3000	2.28×10^{-7}	4.86×10^{-9}	0.23	8.07
1000	3.95×10^{-7}	2.81×10^{-9}	0.4	8.07
500	5.58×10^{-7}	1.99×10^{-9}	0.56	8.06
250	7.8×10^{-7}	1.4×10^{-9}	0.79	8.08
100	1.25×10^{-6}	8.88×10^{-10}	1.23	8.05