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A computational study of blebbing in lipid membranes

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Abstract

Blebs are membrane protrusions that appear during several physiological processes in eukaryotic cells. The phase behavior and kinetics of blebbing in cellular membrane is investigated computationally using a particle-based model for self-assembled lipid vesicles apposed to an elastic meshwork, mimicking a cell's cytoskeleton. We found that blebbing is induced when the cytoskeleton is subjected to a localized ablation, detachment from the membrane, or a uniform contraction. These results are in good agreement with experiments.

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Keywords: Lipid membranes; cytoskeleton, blebbing, molecular dynamics

1. Introduction

The coupling between the cortical actomyosin cytoskeleton (CSK) and the lipid bilayer of eukaryotic cells can lead to morpholigical changes to the plasma membrane in the form of transient exoplasmic spherical protrusion, commonly called blebs (Mohandas and Evans, 1994; Lim et al., 2002). Blebs occur in several cellular processes including cytokinesis, apoptosis, and cell motility. Recent experiments have shown that blebs occur in fibroblasts when the cortical CSK is subjected to a local ablation by laser (Tinevez et al., 2009) or when the CSK is compressed (Paluch et al., 2006). Red blood cells (RBCs) also undergo blebbing during their aging. Indeed, it is well documented that RBCs shed plasma membrane during the aging in the

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form of small vesicles about 100 nm in diameter, which is about the size of a single corral of the spectrin CSK of red blood cells (Backman et al., 1998).

Up to now, very few theoretical and computational studies have been performed to investigate blebbing (Tinevez, 2009; Sens and Gov, 2007; Young and Mitran, 2010). In this article, we present recent computational results of blebbing in self-assembled lipid bilayers coupled to an explicit CSK, using an efficient coarse-grained solvent-implicit molecular model (Spangler et al., 2011).

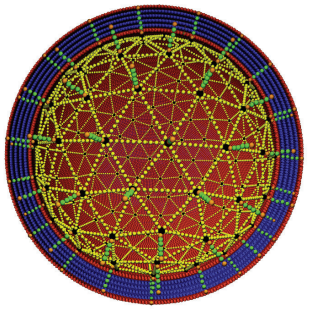

Fig. 1. A cross-section of the initial configuration of a vesicle with CSK. The head and tail beads of lipids are red and blue, respectively. CSK beads are yellow. The junctions (black) beads connect the CSK to the lipid bilayer through bola-like lipids (green and orange).

2. Model and method

To model a system composed of a self-assembled lipid bilayer apposed to a CSK meshwork, which is akin to that of an RBC, we use a model recently developed by us (Revalee et al. 2008). In this model, a lipid molecule is modeled as a semi-flexible chain composed of one hydrophilic (*h*) bead, connected to two hydrophobic tail (*t*) beads. The solvent is treated implicitly in this model. The lipid molecules spontaneously self-assemble through making the *t*-*t* interaction attractive. As shown in Fig. 1, the lipid bilayer vesicle, in this model, formed by the self-assembly of the lipids is apposed with a semi-flexible polymer meshwork, mimicking the spectrin CSK of an RBC. Here, the CSK is composed of 162 vertices and is tessellated into 320 triangular corrals. Most of the vertices (150) have six links, while 12 have 5 links, and each link is a semi-flexible chain composed of 8 to 22 beads called *c*. The CSK vertices are anchored to the bilayer through bola-like lipids, which mimics the more complex anchoring proteins.

In this model, particles interact via the following potential,

$$U\left(\left\{\mathbf{r}_i\right\}\right)=\sum_{i,j}U_0\left(r_{ij}\right)+\sum_{i}U_{bond}\left(r_{i,i+1}\right)+\sum_{i}U_{bend}\left(\mathbf{r}_{i-1},\mathbf{r}_i,\mathbf{r}_{i+1}\right) \tag{1}$$

where $\mathbf{r}_i$ is the position of bead i, and $r_{ij}=\left|\mathbf{r}_i-\mathbf{r}_j\right|$. $U_0\left(r_{ij}\right)$ is the interaction potential between two beads and is given by

$$U_0(r)=\begin{cases}\left(U_{\max}^{\alpha\beta}-U_{\min}^{\alpha\beta}\right)\left(r_m-r\right)^2/r_m^2+U_{\min}^{\alpha\beta} & \text{for } r\le r_m\\ -2U_{\min}^{\alpha\beta}\left(r_c-r\right)^3/\left(r_c-r_m\right)^3+3U_{\max}^{\alpha\beta}\left(r_c-r\right)^2/\left(r_c-r_m\right)^2 & \text{for } r_m\le r\le r_m\\ 0 & \text{for } r>r_m\end{cases} \tag{2}$$

where $U_{\max}^{\alpha\beta}>0$ for any pair. The self-assembly of the lipid molecules is realized through making the interaction between the tail beads attractive, i.e. $U_{\min}^{\alpha\beta}<0$ for $\alpha=\beta=t$. $U_{\min}^{\alpha\beta}=0$ if α or $\beta\neq t$. The potential U_{bond} ensures connectivity between consecutive beads belonging to a lipid molecule or CSK and is given by

$$U_{bond}(r)=\frac{k_{bond}}{2}\left(r-a\right)^2 \tag{3}$$

where k_{bond} is a bond stiffness coefficient and a is the bond length. U_{bend} is a three-body potential to ensure bending stiffness of the chains and is given by,

$$U_{bond}\left(\mathbf{r}_{i-1},\mathbf{r}_i,\mathbf{r}_{i+1}\right)=\frac{k_{bend}}{2}\left(\cos\theta_0-\frac{\mathbf{r}_{i,i-1}\cdot\mathbf{r}_{i,i+1}}{r_{i,i-1}r_{i,i+1}}\right)^2 \tag{4}$$

where k_{bend} is the bending stiffness coefficient of a lipid chain or a cytoskeletom, and θ_0 is a preferred splay angle. Here, we chose $\theta_0=180^\circ$ for all triplets.

Specific values of the interaction parameters are $U^{hh}_{\max}=U^{cc}_{\max}=U^{ht}_{\max}=U^{hc}_{\max}=U^{tc}_{\max}=100\varepsilon$, $U^{tt}_{\max}=200\varepsilon$, $U^{hh}_{\min}=U^{cc}_{\min}=U^{ht}_{\min}=U^{hc}_{\min}=U^{tc}_{\min}=0$, $k_{bond}=100\varepsilon/r_m^2$, $k_{bond}=100\varepsilon$, $a=0.7r_m$, and $r_c=2r_m$. More details about the model can be found in (Revalee et al., 2008; Spangler et al., 2011).

Particles are moved using molecular dynamics with a Langevin thermostat (Revalee et al., 2008): $\dot{\mathbf{r}}_i(t)=\mathbf{v}_i(t)$ and $m\dot{\mathbf{v}}_i(t)=-\nabla_i U-\Gamma\mathbf{v}_i(t)+\mathbf{W}_i(t)$, where m is the mass of a bead, Γ is the friction coefficient, and $\mathbf{W}_i(t)$ is a random force, which satisfies $\langle\mathbf{W}_i(t)\rangle=0$, and $\langle\mathbf{W}_i(t)\cdot\mathbf{W}_j(t')\rangle=6k_BT\Gamma\delta_{ij}\delta(t-t')$. This ensures that the dissipation-fluctuation theorem is not violated. The equations of motion are integrated using the velocity-Verlet method with $\Gamma=\sqrt{6m\tau}$ with $\tau=(r_m^2m/\varepsilon)^{1/2}$, and r_m and ε being our time and energy scales. All simulations are performed at $k_BT=3\varepsilon$, at which the lipid bilayer is in the fluid phase (Spangler et al., 2011). The time step of the simulations, $\Delta t=0.02\tau$ and the number of lipids in a vesicle ranges between 3.5×10^4 and 2.5×10^5.

3. Results and discussion

3.1. Phase diagram

The equilibrium phase diagram of the system is shown in Fig. 2 in terms of the CSK's corral stress-free area $A^{(0)}_{cyto}$ and the parameter $s=A_{lip}/A^{(0)}_{cyto}$, which defines the mismatch between the area of the lipid vesicle per corral and the stress-free area of a corral. An $s=1$ corresponds to no mismatch, an $s<1$ corresponds to a CSK which is compressed within the vesicle, and an $s>1$ corresponds to a stretched CSK. Fig. 2 shows that the system exhibits two equilibrium phases corresponding to (I) a uniform phase, in which the vesicle essentially has a spherical shape with thermally induced fluctuations, and (II) a blebbed phase in which the main vesicle is underlined with the CSK, and a bud or a bleb which is devoid from CSK. The bleb and main vesicle are connected by a neck with a diameter about the linear size of a corral. The inset of Fig. 2 shows that the transition line between the uniform and blebbed phase is consistent with $s^*\sim1/l^{(0)}_{cyto}$ where $l^{(0)}_{cyto}$ is a corral's linear size. This is due to the interplay between the curvature (Helfrich's) free energy of the vesicle and the elastic energy of the CSK (Spangler et al., 2011).

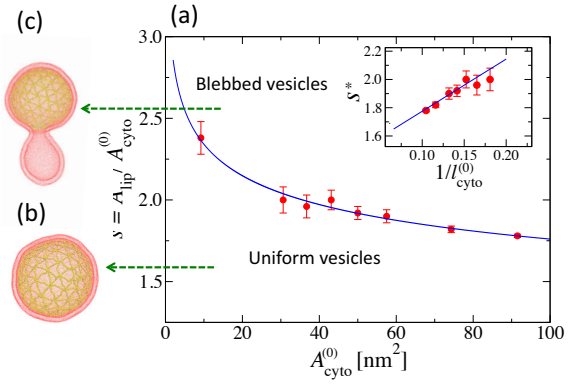

Fig. 2. (a) Phase diagram in terms of cytoskeleton corral stress-free area, $A^{(0)}_{cyto}$ and mismatch parameter s (defined in text). The inset of (a) shows the transiltion line s^* versus one over the linear size of a corral at rest. The blue line is a fit with the theory (see text above and ref. (Spangler et al., 2011)). (b) shows a uniform vesicle and (c) shows a vesicle with a single bleb.

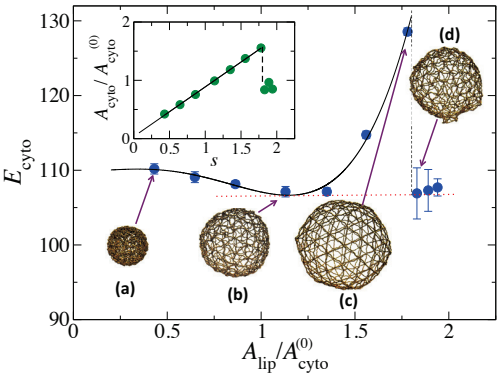

Fig. 3. The cytoskeleton elastic energy vs. the mismatch parameter for. Also shown are snapshots of the cytoskeleton for different values of s. The inset shows the actual corral area over the corral rest area vs. the mismatch parameter. The sharp decrease in the elastic energy is indicative of a first-order transition. Snapshot (a) corresponds to a compressed CSK. (b) corresponds to a stress-free CSK. (c) corresponds to a stretched CSK. (d) is a snapshot of the CSK right after the transition. The configuration of the CSK (d) is that of a stress-free CSK, similar to that of (b).

Fig. 3 shows the CSK elastic energy vs. the mismatch parameter, s, and configurations of the CSK within vesicle as a function of the mismatch parameter for the case of $A^{(0)}_{cyto} = 92r_m^2$. This figures shows that the CSK elastic energy is minimized at a value, $s_{\min}$, slightly larger than 1. The increase in the CSK's energy for $s < s_{\min}$ is due to its compression, while for $s > s_{\min}$, the high energy of the CSK is due to its stretching by the apposed lipid vesicle. We note that the sharp decrease in the CSK's elastic energy implies that the transition from the uniform to the blebbed phase is first order.

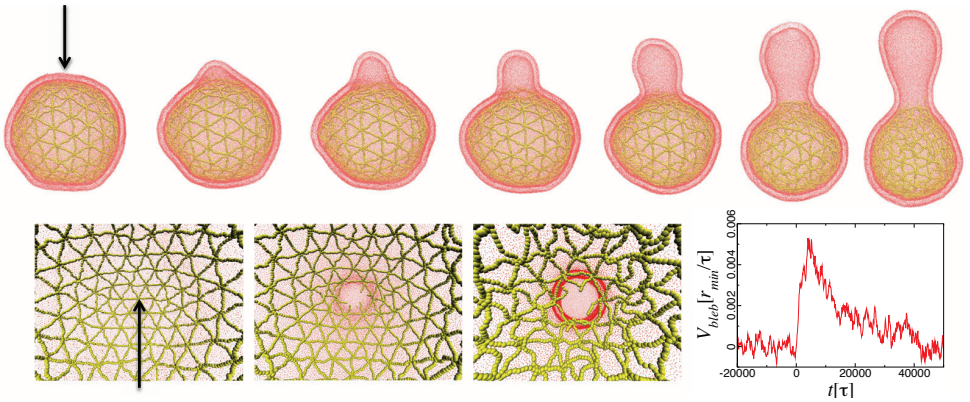

Fig. 4. Top snapshot sequence of a vesicle undergoing blebbing as a result of a localized ablation of the CSK. The localized ablation is indicated by the arrow on left. Snapshots from left to right correspond to times t = 0, 1500, 3000, 6000, 9000, 24000, 46500τ, respectively. Bottom snapshot sequence shows views of the vesicle taken from inside it and showing evolution of the bleb. The location of the ablation (inidicated by the arrow). Snapshots from left to right correspond to t = 0, 1500, 7500τ, respectively. Only lipid head beads (red) and CSK beads (yellow) are shown in snapshots. Bottom right graph shows the velocity of the tip of the bleb versus time.

3.2. Kinetics of bleb formation and growth

We also investigated the kinetics of bleb formation as a result of localized ablation of the CSK. Here, an initially equilibrated vesicle in the uniform phase with the CSK's coral size $A^{(0)}_{cyto} = 43.1r_m^2$, and with a mismatch parameter $s = 1.9$, right below the transition line in Fig. 2.

The snapshot time series of the system resulting from a sudden ablation at $t = 0$, shown in Fig. 4, demonstrates that the membrane caps right after the ablation, and exactly where the ablation is performed. The velocity of the bleb's tip defined as $v = d(\Delta h)/dt$, where Δh is the distance between the bleb's tip and its base, rapidly increases right after ablation of the CSK. The velocity reaches a maximum and then decays asymptotically to zero. The qualitative behavior of the blebs height vs. time is similar to that in the experimental study of (Charras et al, 2005). We also performed a series of computational experiments where the CSK is uniformly compressed or locally peeled from the bilayer, and found that blebbing can also occur in these situations.

4. Conclusions

We presented here a numerical study of blebbing of a model of a self-assembled lipid vesicle coupled to an explicit cytoskeleton. In this study, the solvent is accounted for implicitly, thus allowing us to perform large scale simulations and over long times. The phase behavior of the system, as expressed by the CSK corral size and by a mismatch parameter, *s* (defined by the area of the lipid bilayer over the area of a stress-free CSK), exhibits a uniform vesicle at low values of *s* and a blebbed vesicle at high values of *s*. In the uniform phase, the whole lipid membrane conforms to the CSK. However, in the blebbed phase, only part of the vesicle is apposed to the CSK, while part the other part of the lipid bilayer, i.e. bleb, is devoid of the CSK. The transition between the uniform phase and the blebbed phase is first order. From a series of computational experiments, we found that blebs occur when the lipid vesicle is subjected to a localized ablation, uniform compression or a localized peeling of the CSK.

Acknowledgements

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