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RESEARCH PAPER

Breakup of spherical vesicles caused by spontaneous curvature change

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Abstract We present our theoretical analysis and coarse-grained molecular dynamics (CGMD) simulation results to describe the mechanics of breakup of spherical vesicles driven by changes in spontaneous curvature. Systematic CGMD simulations reveal the phase diagrams for the breakup and show richness in breakup morphologies. A theoretical model based on Griffith fracture mechanics is developed and used to predict the breakup condition.

Keywords Molecular dynamics · Bending modulus · Phase diagram · Fracture

1 Introduction

Intensive experimental and theoretical studies have been performed to investigate vesicle shapes and their transitions [1–7]. These studies primarily focused on the energetics of membrane-enclosed vesicles through change of curvature, surface area and volume as a result of interplay between membrane composition, temperature or physical forces that are applied to the vesicle membrane. In experiments, various shapes of cells and vesicles have been observed, for example, prolate and oblate ellipsoids, biconcave discocytes, starfish, pearling et al. [5–7]. Meanwhile, modeling and simulation studies reproduced and confirmed the existence of these various shapes [2–5].

Recently, folding, local fracture and breakup of membrane driven by change of spontaneous curvature have attracted great attention [8–11]. The change of spontaneous curvature is of great importance in affecting the transitions between different topological shapes. For example, driven by a change in spontaneous curvature, transitions of a flat membrane into a folded one through membrane cracking were observed, resembling an equilibrium first-order phase transition [8]. In addition, local fracture of a gel-phase lipid membrane was proposed as a mechanism for forming polyhedral vesicles [11]. It was also observed that partial fracture of a membrane and polyhedral morphology occurred merely through a change in experimental temperature [8]. It was speculated that a change in experimental temperature might lead to a change in spontaneous curvature [8]. Furthermore, experimental observations [12] showed that clathrin-coated membranes were precursors to coated vesicles, and a flat clathrin-coated membrane was able to transform into many small coated vesicles spontaneously. It was suggested that the transformation proceeded by a change in spontaneous curvature of the clathrin-coated membrane which is in turn controlled by a change in chemical environment of the clathrin lattice [13].

Cell membranes and membrane-enclosed organelles are all composed of lipids and proteins, and share a common general structure. Under normal circumstance, these lipid bilayer membranes are in liquid state, local fracture is unlikely when subjected to spontaneous curvature change. However, at relatively low temperatures, these membranes are in gel or even solid phase. It remains unclear under what conditions these gel or solid phase membranes may break, what are the resulting breakup structures, and whether such breakup is spontaneous and instantaneous. To answer these questions, we need to address the following issues: What is the driving force for the breakup? What are the post breakup shapes? How are the membrane defects affecting the breakup? To address these issues, we enforce the spontaneous curvature into a coarse-grained molecular dynamics (CGMD) model by introducing an energy penalty factor for any deviation from the prescribed spontaneous curvature. We then perform molecular dynamics simulations to study the breakup of vesicles and to obtain the phase diagrams of the breakup. A theoret-

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ical model based on Griffith fracture mechanics [14] is formulated to predict the breakup condition.

2 Simulation method

The membranes of cells and intracellular organelles are of a lipid bilayer structure, containing a large number of amphiphilic lipid molecules. We focus our attention on a gel phase membrane, rather than a liquid phase membrane since a gel phase membrane may crack and fracture [11, 12]. Currently it is impossible to simulate a large area of a lipid bilayer membrane by considering all the atoms. Usually coarse-grained models are used. In one extreme, a biological membrane is described as a single-layer continuum membrane, which spans in a three-dimensional space. The seminal Helfrich theory is such a model based on the single-layer membrane assumption [1]. This model has been widely used for understanding the energetics of membrane shaping of cells and vesicles, and many insightful understandings have been achieved. Hence many mechanical and physical properties of lipid bilayer membranes are independent of the detailed molecular structures of membranes. Here we employ a CGMD model to study the breakup of vesicles.

For the current model, a membrane is assumed to consist of single-layered particle with a diameter of r_0, which is also the membrane thickness [15]. For particle i, 5 degrees of freedom are considered, that is, $(\boldsymbol{x}_i, \boldsymbol{n}_i)$, where $\boldsymbol{x}_i$ is the position of particle i and $\boldsymbol{n}_i$ is the surface normal vector at particle i, subject to the constraint that $\boldsymbol{n}_i \cdot \boldsymbol{n}_i = 1$. Between particles i and j, $\boldsymbol{x}_{ij} = \boldsymbol{x}_j - \boldsymbol{x}_i$, its distance is $r_{ij} = \left|\boldsymbol{x}_{ij}\right|$ and unit vector $\bar{\boldsymbol{x}}_{ij} = \boldsymbol{x}_{ij}/r_{ij}$. The potential can be written as [15]

$$V_{ij} = \begin{cases} \varepsilon\left(\dfrac{R_{\text{cut}} - r_{ij}}{R_{\text{cut}} - R_{\min}}\right)^8 - 2\varepsilon\left(\dfrac{R_{\text{cut}} - r_{ij}}{R_{\text{cut}} - R_{\min}}\right)^4 \\ \quad \times A(\boldsymbol{n}_i,\ \boldsymbol{n}_j,\ \bar{\boldsymbol{x}}_{ij}), \quad r_{ij} < R_{\text{cut}}, \\ 0, \quad r_{ij} \geqslant R_{\text{cut}}, \end{cases} \tag{1}$$

where, ε is the energy unit, a_0 is the length unit and R_{cut} is the cutoff distance of the interaction, which is taken as $2.5a_0$. $R_{\min}$ is the diameter of the particles and is taken as $2^{1/6}a_0 = 5\,\text{nm}$. $A(\boldsymbol{n}_i,\ \boldsymbol{n}_j,\ \bar{\boldsymbol{x}}_{ij})$ is the penalty function related to the spontaneous curvature and the energy penalty incurred due to the deviation from the spontaneous curvature, and is taken as $1 + \alpha(B - 1)$, where B is taken as

$$B = \boldsymbol{n}_i \cdot \boldsymbol{n}_j - \left(\boldsymbol{n}_j \cdot \bar{\boldsymbol{x}}_{ij}\right)\left(\boldsymbol{n}_i \cdot \bar{\boldsymbol{x}}_{ij}\right) + \beta(\boldsymbol{n}_j - \boldsymbol{n}_i) \cdot \bar{\boldsymbol{x}}_{ij} - \beta^2, \tag{2}$$

where $\beta = r_{ij}/2R_0$, and R_0 is the radius of spontaneous curvature. B is maximized and equal to 1 when $\boldsymbol{n}_i$ is rotated from $\bar{\boldsymbol{x}}_{ij}$ by $\pi/2 + \theta_0/2$ and also symmetrically for $\boldsymbol{n}_j$. α is an energy penalty factor for the deviation of the membrane curvature from its spontaneous curvature.

The governing equation of motion for particle i can be written as [15]

$$m_i \ddot{\boldsymbol{x}}_i = -\frac{\partial V}{\partial \boldsymbol{x}_i}, \tag{3}$$

$$\tilde{m}_i \ddot{\boldsymbol{n}}_i = -\frac{\partial V}{\partial \boldsymbol{n}_i} + \left(\frac{\partial V}{\partial \boldsymbol{n}_i} \cdot \boldsymbol{n}_i\right)\boldsymbol{n}_i - \tilde{m}_i\left(\dot{\boldsymbol{n}}_i \cdot \dot{\boldsymbol{n}}_i\right)\boldsymbol{n}_i, \tag{4}$$

where $\tilde{m}_i$ is a pseudomass and the right-hand side conforms to the constraint of $\boldsymbol{n}_i \cdot \boldsymbol{n}_i = 1$. The integration method which was proposed by Beeman [16] is used to solve the equiations. The particle number of the vesicle is 5 072 and the initial radius of the vesicle is $20a_0$.

3 Simulation results

Vesicle may change its shape, volume or surface area arising from the properties of the membrane itself and/or the presence of external loadings. For example, by adjusting the ambient temperature, vesicles undergo shape change as the surface area is changed while holding the volume roughly constant; by changing spontaneous curvature, vesicles may change their shapes, for example, from prolate to oblate and to stomatocyte shapes. Phase diagram for vesicle topologies at different membrane properties and external loading conditions reveals important thermodynamic behavior of vesicle membranes. So far the phase diagram for vesicles has been only partially explored. For example, the phase diagram at different spontaneous curvatures and vesicle temperatures was studied in Refs. [17, 18]. Phase diagram at different area difference and different volumes was studied in Ref. [19]. Different phases ranging from stomatocytes, pears, prolates and oblates were obtained [19]. This study was based on continuum mechanics models [1], and thus it is difficult to consider the breakup of vesicles. Here we use the CGMD simulations to obtain phase diagrams for different temperatures, spontaneous curvatures and energy penalty factors.

When $c/c_0 = 0.25$, where c is the curvature of the initial vesicle and c_0 is the spontaneous curvature, the phase diagram of the temperature T, the energy penalty factor α is showed in Fig. 1. There are four phase zones being observed. At a small value of α, the vesicle remains roughly spherical (phase 1) and no break-up is observed. In high temperature regime, with increasing α, the vesicle breaks up, and the final

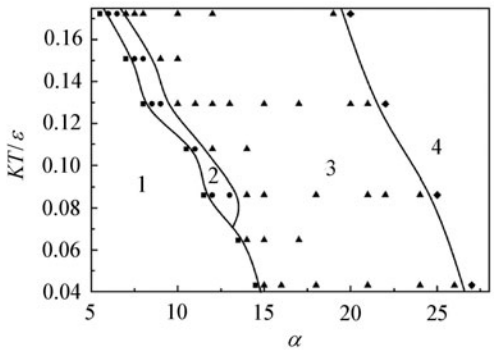

Fig. 1 Phase diagram for vesicle topologies as a function of energy penalty factor α and temperature at $c/c_0 = 0.25$. In total, four phases are determined. The dots represent the simulation results

phase (phase 2) is manifested by the co-existence of small double-layer vesicles and single-layer vesicles. Figure 2 shows the evolution pathway to reach phase 2. It can be seen from Fig. 2a that a crack appears first. Subsequently, the vesicle breaks up and each fragment changes their curvature as shown in Figs. 2b and 2c. Eventually, the vesicle breaks up into a mixture of closed double-layer spheres and single-layer spheres as shown in Fig. 2d. The fragment indicated by "1" in Fig. 2 shows how a double-layer sphere forms. The radius of spheres in Fig. 2d is in the range of $5a_0$ to $8.5a_0$, which is slightly above the prescribed radius of spontaneous curvature, $5a_0$. This deviation from the prescribed value is due to the relatively small value of the energy penalty factor used in the simulations.

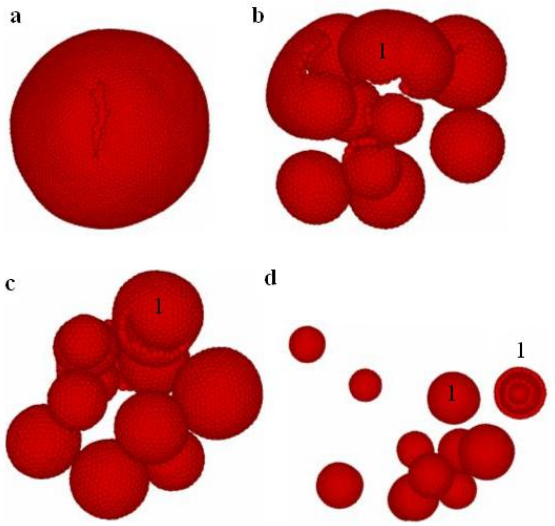

Fig. 2 Snapshots of the evolution pathway for a vesicle to reach phase 2 with double-layer spheres and single-layer spheres

With further increasing α, a combination of single-layer vesicles and arc-like pieces (phase 3) is observed after the breakup of the vesicle. Figure 3 shows the snapshots of the breakup process. It is seen that again cracks are formed first (Fig. 3a), and the vesicle breaks up into single-layer vesicles and arc-like pieces as shown in Fig. 3b. During the evolution, there are some pieces in helical shape as shown in Figs. 3c and 3d. The helical shape is unstable for it ends up either with a closed single-layer sphere as shown in Figs. 3c and 3d indicated by "1" or with a closed single layer sphere and an open arc-like piece as shown in Figs. 3d and 3e indicated by "2". At the final stage, only single-layer spheres and arc-like pieces are observed as shown in Fig. 3e.

When α is very large, a phase characterized by all arc-like pieces (phase 4) is shown in Fig. 1 after the breakup. Figure 4 shows the evolution pathway for the breakup. It is observed that many cracks appear at the early stage of the breakup as shown in Fig. 4a. The crack propagations lead to the vesicle break-up as shown in Figs. 4b and 4c. Finally, each fragment adopts an arc shape as shown in Fig. 4d and their curvatures are closely around the spontaneous curvature due to the relatively large energy penalty factor.

At the same energy penalty factor, the breakup of vesicle is also dependent on the specified spontaneous curvature.

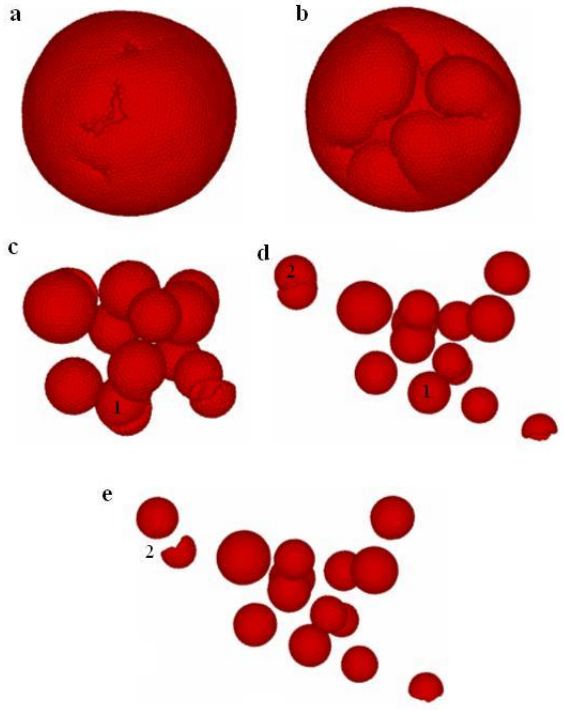

Fig. 3 Snapshots of the evolution pathway for a vesicle to reach phase 3 with single-layer spheres and arc-like pieces

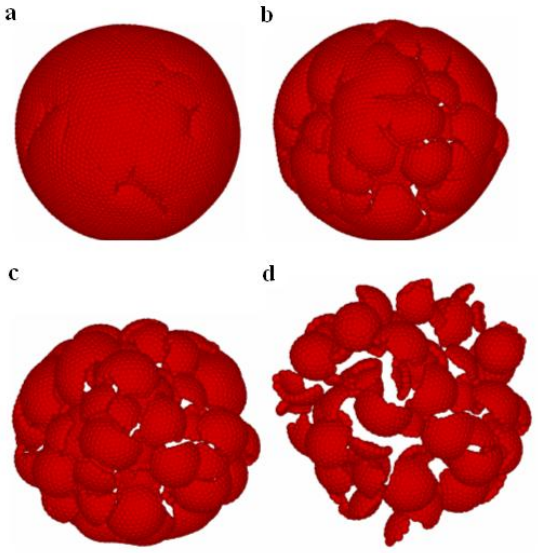

Fig. 4 Snapshots of the evolution pathway for a vesicle to reach phase 4 with arc-like pieces

For example, Fig. 5 shows the phase diagram of $T - C_0$ at $\alpha = 22$. Within the parametric regime, it is seen that three phases are observed: Phase 1 with pure arc-like pieces, phase 2 with a mixture of single-layer vesicles and arc-like pieces, and phase 3 with the spherical shape roughly maintained and no breakup is observed.

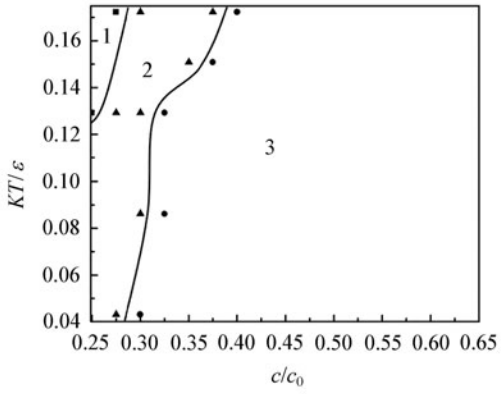

Fig. 5 Phase diagram for vesicle topologies as a function of spontaneous curvature and temperature at $\alpha = 22$. In total, three phases are determined. The dots represent the simulation results

4 Formulation of breakup condition

It can be seen from Fig. 1 that a vesicle breaks up only when the energy penalty factor α is larger than a critical value at a fixed temperature. In the following, we would like to develop a theoretical model based on Griffith fracture mechanics to predict the propagation condition of a crack on a vesicle membrane in gel-like state, that is, at relatively low temperature.

Assume that a through-thickness crack with an initial crack length of $2a$ exists on the vesicle membrane. During crack propagation, the curvature of a particle ahead of the crack tip will be different from that in the wake of the crack tip. The energy release associated with this curvature change provides driving force for crack propagation. On the other hand, crack propagation increases the membrane edge energy. According to Griffith fracture criterion, the crack will propagate if the energy release rate k_y^2/E is larger than the energy creating the membrane edges, $2\gamma/r_0$, where, k_y is the stress intensity factor, E is the Young's modulus of the membrane, and γ is the edge energy.

We observed that along the crack direction, the curvature is more or less the same before and after the crack propagation. However, the curvature perpendicular to the crack direction changes according to the spontaneous curvature. Assume that the initial radius of the vesicle is R_1, the radius of spontaneous curvature being R_0, and the x-direction is along the crack propagation, the y-direction is perpendicular to the crack length and the z-direction is normal to the vesicle surface (see Fig. 6). Further assume that the membrane curvature perpendicular to the crack length changes to the spontaneous curvature. Then the curvature change before and after the crack propagation is $\chi_x = 1/R_1 - 1/R_1 = 0$ and $\chi_y = 1/R_1 - 1/R_0$. The corresponding bending moments are: $M_x = -D\left(\chi_x + \nu\chi_y\right)$ and $M_y = -D\left(\chi_y + \nu\chi_x\right)$, where, D is the bending modulus of the membrane, which is defined as $D = \dfrac{Eh^3}{12\left(1-\nu^2\right)}$. Since M_x does not cause any singularity at the crack tip, we only consider M_y. The stress intensity factor can thus be written as [20]

$$k_y = \frac{6M_y\sqrt{a}}{h^2} = \frac{Eh\left(R_1 - R_0\right)\sqrt{a}}{2\left(1-\nu^2\right)R_1R_0}. \tag{5}$$

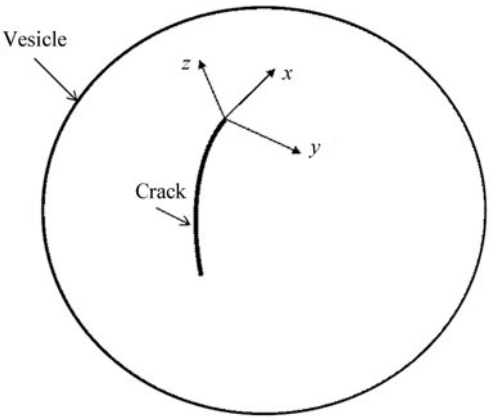

Fig. 6 Schematic of a through-thickness crack in the vesicle membrane. x, y and z form an orthogonal coordinate system with the x- and the y- directions along and perpendicular to the crack propagation direction, respectively, and the z-direction normal to the vesicle surface

The Young's modulus can be obtained by using the potential (Eq. (1)). The membrane bending energy can be written as [1, 19]

$$V = \frac{1}{2}D\int_S (C_1 + C_2 - C_0)^2 \mathrm{d}A, \tag{6}$$

where, C_1 and C_2 are the two principal curvatures, and C_0 is the spontaneous curvature. In the present scenario, $C_1 = C_2 = 1/R_1$, and $C_0 = 2/R_0$. Since the average area per particle is $\Delta A = \sqrt{3}r_0^2/2$, the bending energy per particle is

$$V_a = \sqrt{3}D\left(\frac{1}{R_1} - \frac{1}{R_0}\right)^2 r_0^2. \tag{7}$$

The bending energy per particle can also be calculated by the change in potential energy when the curvature of the vesicle changes from $1/R_1$ to $1/R_0$. Hence, V_a can also be written as

$$V_a = \frac{3c\alpha\varepsilon}{2}\left(\frac{1}{R_1} - \frac{1}{R_0}\right)^2 r_0^2, \tag{8}$$

where, $c = [(R_{\mathrm{cut}} - r_0)/(R_{\mathrm{cut}} - R_{\mathrm{min}})]^4$. From formulas (7)

and (8), we can obtain $D = \sqrt{3}c\alpha\varepsilon/2$. Hence the Young's modulus of the membrane can be obtained (assuming that Poisson's ratio and the membrane thickness are known). The edge energy can also be calculated by using the potential (Eq. (1)). Along the edges, there are broken bonds, causing excessive energy at the edges. The energy which is associated with the broken bonds can be calculated using Eq. (1), which forms the basis to calculate the edge energy. The edge energy can be written as

$$\gamma = \frac{c\varepsilon}{r_0}\left[1 - \frac{c}{2} - \frac{\alpha r_0^2}{4}\left(\frac{1}{R_1} - \frac{1}{R_0}\right)^2\right]. \tag{9}$$

Assume the plane-stress condition, the energy release rate is $g = k_y^2/E$. The crack will propagate when

$$\frac{k_y^2}{E} \geqslant \frac{2\gamma}{r_0}. \tag{10}$$

Substitute all known relevant quantities into formula Eq. (10) and finally we obtain the condition for crack propagation on the vesicle membrane

$$\alpha \geqslant \frac{2 - c}{\left[\dfrac{3\sqrt{3}a}{2(1-v^2)r_0} + \dfrac{1}{2}\right]\left(\dfrac{1}{R_1} - \dfrac{1}{R_0}\right)^2 r_0^2}. \tag{11}$$

If the initial crack is taken as a vacancy, the crack length $2a$ can be approximated as $1.2a_0$, which is roughly the membrane thickness. Such vacancies were indeed observed in our simulations. For the present consideration, $r_0 = 2^{1/6}a_0$, $R_1 = 20a_0$, $R_0 = 5a_0$, $c = 1$ and $v = 0.25$, according to Eq. (11), we can obtain $\alpha \geqslant 16$. In our molecular dynamics simulation at relatively low temperature, it is observed that when $\alpha \geqslant 15$, a crack on a vesicle membrane start to propagate from a vacancy, causing the breakup of the vesicle, which agrees well with the calculated α value. For $r_0 = 2^{1/6}a_0$, $R_1 = 20a_0$, $R_0 = 7a_0$, $c = 1$ and $v = 0.25$, according to Eq. (11), we can obtain $\alpha \geqslant 42$. From our simulation cases, we observed that the critical α is 46 in relatively low temperature regime. This also agrees well with the calculated α value.

5 Discussion

From Fig. 1, it can be seen that with an increase in temperature, a smaller value of α is needed for the shape transition since the thermal energy at higher temperatures reduces the energy barrier for crack nucleation and propagation by assisting the break of atomic bond. With increasing spontaneous curvature, the stress intensity factor increases, thus, the breakup of vesicle can be achieved at a smaller energy penalty factor. For the same energy penalty factor, the larger the spontaneous curvature, the easier the breakup of the vesicle. Hence, spontaneous curvature, temperature and the energy penalty factor all play important roles in determining the phase diagrams.

Under normal condition, that is, in liquid phase, lipid bilayer membranes are a stable and fracture-resistance structure. Hence it may be difficult to break up these large membranes into individual amphiphilic molecules or vesicles, and such breakup requires substantial and persistent energy input. The present analysis suggests that the gel-state membranes can be broken up into smaller molecular structures, such as smaller vesicles or arc-like pieces, merely by changing their spontaneous curvatures.

We have shown that a change in spontaneous curvature can cause the breakup and fragmentation of a large vesicle with a gel phase membrane into smaller vesicles and/or arc-like pieces. These predictions are consistent with the previous experimental results which showed that a flat clathrin-coated membrane is able to transform into smaller clathrin-coated vesicles by changing the spontaneous curvature [12]. It should be pointed out that many factors can induce the change of spontaneous curvature of membranes. For example, the spontaneous curvature can be produced by chemical asymmetry via lowering the extracellular pH, or via changing the lipid composition, or via protein insertion into membranes, or via pulling force generated by molecular motors, or via protein binding to the membrane surface et al. [21, 22].

6 Conclusion

Through coarse-grained molecular dynamics simulations and theoretical analysis, we have shown that a large vesicle enclosed by a gel-state membrane may break up into smaller vesicles and/or small-membrane pieces by changing its spontaneous curvature. In the coarse-grained molecular dynamics model, we developed a method to prescribe spontaneous curvature, and an energy penalty factor was introduced to penalize the deviation from the spontaneous curvature. It was found that a crack on the vesicle membrane starts from a defect and then propagates, causing the vesicle to break up. The breakup was found to depend on the spontaneous curvature change, temperature and the energy penalty factor. In particular, it was found that for at a given temperature and given spontaneous curvature, when the energy penalty factor is larger than a critical value, the breakup of a vesicle was observed. A theoretical model based on the Griffith fracture criterion was proposed and used to predict the vesicle breakup condition satisfactorily.

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