Mechanics of membrane bulging during cell-wall disruption in Gram-negative bacteria

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The mechanics of membrane bulging during cell-wall disruption in Gram-negative bacteria

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The bacterial cell wall is a network of sugar strands crosslinked by peptides that serves as the primary structure for bearing osmotic stress. Despite its importance in cellular survival, the robustness of the cell wall to network defects has been relatively unexplored. Treatment of the Gram-negative bacterium *Escherichia coli* with the antibiotic vancomycin, which disrupts the crosslinking of new material during growth, leads to the development of pronounced bulges and eventually of cell lysis. Here, we model the mechanics of bulging of the cytoplasmic membrane through pores in the cell wall. We find that the membrane undergoes a transition between a nearly flat state and a spherical bulge at a critical pore radius of ~ 20nm. This critical pore size is large compared to the typical distance between neighboring peptides and glycan strands, and hence pore size acts as a constraint on network integrity. We also discuss the general implications of our model to membrane deformations in eukaryotic blebbing and vesiculation in red blood cells.

I. INTRODUCTION

In most bacteria the peptidoglycan cell wall, a composite of long sugar strands (glycans) crosslinked by stretchable peptides, is the primary stress-bearing and shape-maintaining structure [1]. In *E. coli*, the osmotic-pressure differential across the cell membrane ranges from ~ 0.5 to 3 atm depending on the osmolality of growth [2], and the cell wall plays an essential role in maintaining the integrity of the cell. Importantly, the cell wall is a growing structure and bonds must be broken to permit growth and division, potentially leading to defects in the peptidoglycan network. Despite the potential vulnerability of the cell, a quantitative understanding of the sensitivity of the cell to such defects is still lacking. Such an understanding would be particularly valuable given that many antibiotics target the growing cell wall. In this Letter, we analyze the mechanical deformations of the membrane resulting from pores in the cell wall.

In a recent paper, Huang *et al.* [3] investigated the effect of the antibiotic vancomycin on a vancomycin-sensitive strain of the Gram-negative bacterium *E. coli* [4]. In the presence of vancomycin, cells often developed a pronounced bulge (inset to Fig. 1), which grew with time and led eventually to cell lysis. Such bulge formation is a typical response to perturbations of the peptidoglycan synthesis pathway [5], and vancomycin disrupts the formation of new peptide crosslinks [6]. We hypothesize that the accumulation of crosslink defects in a small region creates a pore in the cell wall. Above a critical pore size, our model predicts that the osmotic pressure differential drives bulging of the plasma membrane out through the pore. Bulging is irreversible, hence once the membrane bulges out, the cell has no mechanism for repair and ultimately loses its viability. Our model and analysis is also relevant for a broad spectrum of cellular phenomena, including blebbing in eukaryotic cells [7, 8], vesiculation in red blood cells [9, 11–13], and budding in multicomponent membranes [14].

II. MODEL AND RESULTS

To study the observed bulge formation in Gram-negative bacteria, we model the energetics of the plasma membrane using the functional

$$\begin{aligned} E &= E_{\rm bend} + E_{\rm surf} + E_{\rm press} \\ &= \frac{\kappa}{2}\left[\int dA\ (2\bar{C} - C_0)^2\right] + \sigma A - PV. \end{aligned} \tag{1}$$

The surface integral in the first term corresponds to the Helfrich bending energy [15], where $\bar{C}$ is the local mean curvature of the membrane, C_0 is its spontaneous curvature, and κ is the bending modulus. In the second term, A is the area of the bulge and σ is the surface tension. In the third term, P is the osmotic pressure differential across the membrane and V is the volume contained in the bulge. We consider the lowest energy conformation of a membrane constrained by a flat external cell wall with a circular pore of radius r. For small r, bulging is disfavored due to the cost of bending, while for large r turgor pressure favors the formation of a membrane bulge, which we model as an axisymmetric truncated sphere of radius R [16] as shown in Fig. 1. For brevity, we ignore the additional resistance to bulging produced by the bending energy of the neck, which depends on the details of the morphology of the neck region.

In order to estimate the critical pore radius for large bulge formation, we calculate the spherical portion defined by the bulge angle θ in Fig. 1. When the surface is essentially flat and the membrane does not protrude out of the pore, $\theta \approx 0$, while for a large protrusion where the bulge tends towards a complete sphere, $\theta \approx \pi$. R,

r, and θ are not independent, but rather satisfy the relation $\sin\theta = r/R$. The solid angle subtended by the bulge surface is given by $2\pi(1-\cos\theta)$.

In its unbulged state, the plasma membrane typically has excess area relative to the cell surface, including excess area contained in membrane fluctuations. The surface tension of a thermally fluctuating membrane is given by [17]

$$\sigma \approx \frac{\pi^2\kappa}{a_0}\exp\left(-\frac{8\pi\kappa}{k_BT}\left(\frac{A-A_p}{A_p}\right)\right), \qquad (2)$$

where $a_0 \approx 0.7$ nm^2 is the area per lipid, A is the total membrane area, and A_p is the projected area. Based on the observation that osmotic shock causes a noticeable elongation of *E. coli* cells, a lower-bound estimate estimate of $(A-A_p)/A_p \approx 0.05$ yields an overall factor of $\sim 10^{-11}$ due to the exponent in Eq. 2, suggesting that unbulged membranes may have very low surface tension. Moreover, in the analysis below, we will demonstrate that there is typically an energy barrier to large bulge formation that occurs for small bulges, thus the membrane can be stabilized against bulging simply by its resistance to bending, without the stabilizing effect of surface tension. Therefore, we initially set the surface tension to zero.

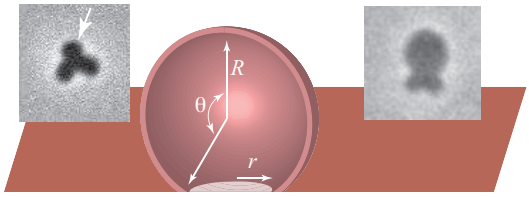

FIG. 1. (Color online) Schematic of a spherical membrane bulge with radius R protruding from a circular pore in the cell wall with radius r. The angle θ defines the portion of the sphere that has been pushed through the pore. Inset: a vancomycin-treated *E. coli* cell in late stages after bulge formation (left, arrow indicates bulge), and a cell wall shell after lysis (right).

The surface area of the bulge is

$$A(\theta) = \int_0^{\theta}\int_0^{2\pi} R^2\sin\theta' d\theta' d\phi = 2\pi R^2(1-\cos\theta), \qquad (3)$$

and the volume of the bulge is

$$V(\theta) = \frac{1}{3}\pi R^3\left(2-3\cos\theta+\cos^3\theta\right). \qquad (4)$$

Neglecting the bending energy of the neck, where the surface of the bulge connects with the flat membrane, the energy of the bulge for $\sigma = 0$ and $C_0 = 0$ is

$$E = 4\pi\kappa(1-\cos\theta) - \frac{\pi P}{3}\left(\frac{r}{\sin\theta}\right)^3(2-3\cos\theta+\cos^3\theta). \qquad (5)$$

To determine the general dependence of the membrane energy on pore radius, we define the dimensionless pore radius $\tilde{r} = r/l_p$, where $l_p = (\kappa/P)^{1/3}$ is the length scale associated with the transition between the unbulged and bulged states. The dimensionless rescaled membrane energy is then

$$\tilde{E} = \frac{E}{\pi\kappa} = 4(1-\cos\theta) - \frac{1}{3}\left(\frac{\tilde{r}}{\sin\theta}\right)^3\left(2-3\cos\theta+\cos^3\theta\right), \qquad (6)$$

Notice that $\tilde{E}$ depends only on the dimensionless pore radius $\tilde{r}$ and on the extent of bulging represented by θ.

In Fig. 2, we plot $\tilde{E}$ versus θ for several values of $\tilde{r}$. We find a critical value of $\tilde{r}$ given by $\tilde{r}_c \approx 2$, such that if $\tilde{r} < \tilde{r_c}$, there is a local minimum energy state at a small (nonzero) value of θ corresponding to an almost flat membrane, separated by an energy barrier from the bulged shapes represented by large values of θ. As $\tilde{r}$ approaches $\tilde{r_c}$, the energy barrier shrinks, and disappears for $\tilde{r} > \tilde{r_c}$. Thus, for $\tilde{r} > \tilde{r_c}$ there is no metastable state corresponding to an almost flat membrane and no energy barrier to prevent a membrane bulge from growing. In this sense, $\tilde{r}_c$ represents the rescaled critical pore radius. We note that even for $\tilde{r} < \tilde{r_c}$, given sufficient time, there is a finite probability of the membrane crossing the energy barrier and bulging out due to thermal fluctuations. However, for a realistic bending modulus $\kappa = 20k_BT$ at room temperature, the barrier becomes large ($\sim 100k_BT$ for $\tilde{r} = 1.7$), making such a process highly unlikely within a cell's lifetime.Thus $\tilde{r}_c$ provides a reasonable estimate of the minimum pore size for spontaneous membrane bulging. Using an estimated turgor pressure $P = 1$ atm, we find $l_p = (\kappa/P)^{1/3} \approx 9$ nm, and a critical pore radius of $r_c = \tilde{r}_c \times l_p \approx 18$ nm. This size is larger than the typical pore size of a highly crosslinked cell wall [19], and corresponds to a distance of ~ 10 glycan strands measured along the long axis of the cell.

To better understand the results in Fig. 2, we note that for small θ, $E_{\rm bend} \sim \theta^2$ while $E_{\rm press} \sim -\theta$. For $\tilde{r} < \tilde{r_c}$, the local minimum at small θ is given by the balance between these two contributions. More precisely, expanding to order θ^3 for small θ, $\tilde{E} \approx 2\theta^2 - \tilde{r}^3/4\left(\theta+\theta^3/6\right)$. For small $\tilde{r}$, one can ignore the θ^3 term to determine the minimum in $\tilde{E}$ at $\theta \approx \tilde{r}^3/16$. Since the position of this minimum grows as $\tilde{r}^3$, as $\tilde{r} \to \tilde{r}_c$ the θ^3 term becomes important and overcomes the positive curvature at the minimum. Also note that as $\theta \to \pi$, *i.e.* when the bulge approaches a complete sphere, the energy is negative and diverges as $-(4/3)[\tilde{r}/(\pi-\theta)]^3$.

To determine the value of $\tilde{r}_c$ analytically, we note that for $\tilde{r} > \tilde{r_c}$ the slope of the curve for E versus θ is negative for all $0 < \theta < \pi$. Thus, when $\tilde{r}$ equals the critical value $\tilde{r}_c$, the maximum value of the slope $\partial\tilde{E}/\partial\theta$ equals zero.

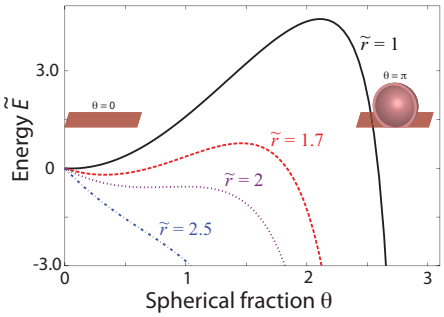

FIG. 2. (Color online) Energy (in units of $\pi\kappa$) as a function of the bulge angle θ for several values of the dimensionless pore radius $\tilde{r} = r/l_p$. The minimum value of $\tilde{r}$ that allows bulges to form spontaneously is $\tilde{r} \approx 2$. Schematics indicate the membrane conformation at low and high values of θ.

The slope is given by

$$\frac{\partial \tilde{E}}{\partial \theta} = 4\sin\theta - \frac{\tilde{r}^3}{(1+\cos\theta)^2}, \tag{7}$$

and the condition $\partial\tilde{E}/\partial\theta \leq 0$ yields $\tilde{r}^3 \geq 4\sin\theta(1+\cos\theta)^2$. Therefore, at the critical pore radius, $\tilde{r}_c^3$ equals the maximum value of the right-hand side, whose extrema are given by the condition

$$(1+\cos\theta)^2(3\cos\theta - 2) = 0. \tag{8}$$

One trivial solution, $\theta=\pi$, is a saddle-point corresponding to a complete bulge. The other solution, $\cos\theta = 2/3$, is a maximum and determines the critical pore radius $\tilde{r}_c \approx 2.02$, which agrees well with our previous estimate.

We next investigate the effect of membrane surface tension, represented by the energy contribution

$$E_{\rm surf} = \sigma A(\theta) = 2\pi\sigma r^2(1-\cos\theta)/\sin^2\theta.$$

Note that at small θ, this term is proportional to $\sigma\theta^2$, aside from a constant energy. Thus for positive σ (corresponding to an energy cost for drawing out membrane), the scaling of $\tilde{E} \propto \theta^2$ remains the same for small θ. The basic form for the E versus θ curves is similar, though the additional energy cost due to $E_{\rm surf}$ increases the critical pore radius. By defining the dimensionless surface tension $\tilde{\sigma} = \sigma/(\kappa P^2)^{1/3}$, the rescaled bulge energy can be written as

$$\tilde{E} = 4(1-\cos\theta) - \frac{1}{3}\left(\frac{\tilde{r}}{\sin\theta}\right)^3(2-3\cos\theta+\cos^3\theta) + \frac{\tilde{\sigma}\tilde{r}^2}{\cos^2(\theta/2)}. \tag{9}$$

For $\tilde{r} \geq \tilde{r}_c$, the condition $\partial\tilde{E}/\partial\theta \leq 0$ can expressed in the form $0 \leq \tilde{r}^3 + a\tilde{r}^2 + b\tilde{r} + c$ with $a = -2\tilde{\sigma}\sin\theta$, $b = 0$, and $c = -4\sin\theta(1+\cos\theta)^2$. Treating $\tilde{r}^3 + a\tilde{r}^2 + b\tilde{r} + c = 0$ as a cubic equation in $\tilde{r}$, its discriminant, $\Delta = 18abc - 4a^3c + a^2b^2 - 4b^3 - 27c^2$, can be shown to be negative definite in the range $0 < \theta < \pi$ for $\sigma \geq 0$. Negative Δ implies one real and two complex conjugate roots, hence the inequality can be written as $0 \leq (\tilde{r} - \tilde{r}_1)\left[\tilde{r}^2 + \alpha\tilde{r} + \beta\right]$, where $\tilde{r}_1$ corresponds to the real root, and $\tilde{r}_1$, α, and β are functions of θ. Since the other two roots are complex, $f(\tilde{r}) = \tilde{r}^2 + \alpha\tilde{r} + \beta$ cannot cross the x-axis (corresponding to $f = 0$), and thus we find $f > 0$ for all real $\tilde{r}$ and for $0 < \theta < \pi$. The sign of $-\partial\tilde{E}/\partial\theta$ is thus the same as the sign of $\tilde{r} - \tilde{r}_1$; for $\tilde{r} < \tilde{r}_c$, $\tilde{r} - \tilde{r}_1$ changes signs as a function of θ while for $\tilde{r} > \tilde{r}_c$, $\tilde{r} - \tilde{r}_1$ is greater than zero for all θ $(0 < \theta < \pi)$ (see Fig. 2). At $\tilde{r} = \tilde{r}_c$, the minimum value of $(\tilde{r}_c - \tilde{r}_1)$ is zero, implying that the critical radius can be obtained by maximizing $\tilde{r}_1$ as a function of θ. In Fig. 3, we plot $\tilde{r}_c$ as a function of $\tilde{\sigma}$. Notice that the dependence of $\tilde{r}_c$ on $\tilde{\sigma}$ is weak at small values of $\tilde{\sigma}$. For a typical lipid bilayer membrane with a rupture tension of 10^{-2} N/m [18] and $\kappa = 20k_BT$ and a turgor pressure of $P = 1$ atm, $\tilde{\sigma} \approx 10$ at rupture. Therefore, given that we previous an unbulged bacterial membrane to be at low surface tension relative to rupture, inclusion of surface tension does not dramatically alter our previous estimate of the critical pore radius.

If the spontaneous curvature of the membrane is nonzero, the bending energy of the bulge becomes [20],

$$E_{\rm bend}(\theta) = 4\pi\kappa(1 - RC_0)(1-\cos\theta). \tag{10}$$

Rewriting R as $r/\sin\theta$ and introducing a dimensionless spontaneous curvature $\tilde{C}_0 = C_0 l_p$, we obtain the rescaled energy $\tilde{E}$. By calculating the slope $\partial\tilde{E}/\partial\theta$, and proceeding as above, we obtain $\tilde{r}_c$ as function of $\tilde{C}_0$, as shown in the inset of Fig. 3. The reduction of $\tilde{r}_c$ at nonzero $\tilde{C}_0$ becomes significant only when $\tilde{C}_0 > 1$, *i.e.*, when the spontaneous radius of curvature $1/C_0$ is smaller than $l_p \approx 9$ nm. Although this is smaller than the radius of curvature of typical bacterial phospholipids, the plasma membrane is a multicomponent membrane, such that aggregation of components with high intrinsic curvature in the pore region could drive membrane bulging by reducing the critical pore radius.

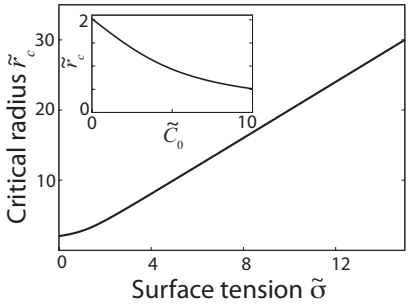

FIG. 3. Rescaled critical radius $\tilde{r}_c$ as a function of the rescaled surface tension $\tilde{\sigma}$. Inset: $\tilde{r}_c$ as a function of the rescaled spontaneous curvature $\tilde{C}_0$ at $\tilde{\sigma} = 0$.

III. CONCLUSIONS AND DISCUSSION

To conclude, we estimate that for an osmotic pressure differential of one atmosphere, there is a critical peptidoglycan pore radius of ~ 20 nm above which the membrane will spontaneously bulge through the pore. The critical pore radius scales as $P^{-1/3}$ so that at higher pressure the critical pore radius decreases. Since the critical radius is large compared to the distance between neighboring peptide and glycan strands in the peptidoglycan network (typically 2-4 nm), a critical pore in the cell wall would correspond to several adjacent broken or missing glycan chains. At the same time, the critical pore radius is small compared to the cell's radius of $\sim$0.5 microns.

In most animal cells, the cortex – a network of actin, myosin, and associated proteins – lies under the plasma membrane and determines the shape of the cell. The cortex enables the cell to resist externally applied stresses and to perform mechanical work. In many physiological conditions, transient, localized detachment of the cortex from the plasma membrane causes the formation of a bleb (a bulge in the plasma membrane), driven by local contractions of the actin cortex that push the cytosol outwards and generate a pressure difference across the membrane [7, 8]. A bleb will typically grow to a size of ~ 2 μm over a time scale of 30 seconds, and then retract over the subsequent two minutes. Our analysis can be directly carried over to model bleb generation. In the absence of surface tension, a typical pressure difference of around 100 Pa [7] would correspond to a critical radius of 200 nm, which is close to the mesh size of the actin cortex [7]. This indicates that surface tension of the plasma membrane in animal cells may play an important role in preventing bleb formation, by increasing the critical pore radius as shown in Fig. 3.

The related phenomenon of budding and vesicle formation provides an important means for protein sorting and trafficking. In intracellular trafficking, protein components of secretory vesicles, lysosomes, and the plasma membrane are sorted and directed to specific destinations via vesiculation of the Golgi complex [10]. In red blood cells, the plasma membrane is anchored at discrete locations to an underlying two-dimensional spectrin-actin network known as the membrane skeleton.Vesiculation has also been observed in red blood cells when cells are treated with amphipathic agents, under change of pH [11], during blood storage [13], or in diseased cells with defects in the membrane skeleton. During vesicle formation, the network does not fragment but instead retracts into the body of the cell [13]. Vesiculation can also be induced in healthy red blood cells during externally induced shape changes. While a red blood cell has the equilibrium shape of a biconcave disc, a variety of chemical agents can cause the cell to deform in a systematic manner to form invaginated shapes known as "stomatocytes" and spiculated shapes known as "echinocytes" [11, 12]. Increased concentration of echinocytic agents results in vesiculation and shedding of plasma membrane. In this case, budding and vesiculation are driven by spontaneous curvature rather than by pressure difference, but our general model of the energetics of a constrained membrane remains applicable.

In this Letter, we have demonstrated that a simple model for membrane energetics predicts a critical pore radius beyond which spontaneous bulging will occur, which is in reasonable agreement with the distribution of pores in a peptidoglycan network. Our model predicts that the critical pore radius will increase with increasing surface tension and decrease with increasing spontaneous curvature or turgor pressure, suggesting that bulging may depend on the lipid composition of the membrane and the metabolic state of the cell. The biological systems we have studied also mimic experimental devices to measure the mechanical properties of thin films by pressurizing the film over a pore in a etched silicon substrate [21]. Our results elucidate the minimum length scales at which bulging or budding can occur for the broad spectrum of biomembranes that are typically found coupled to an elastic matrix such as the cytoskeleton or cell wall, and provide a mechanistic explanation for the trajectory of a bacterial cell treated with cell-wall-acting antibiotics.

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