Osmotic critical phenomena of semipermeable unilamellar vesicles

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Motivated by biological protocols that require transfer of large pieces of DNA into cells, we here review several aspects of osmotic swelling and lysis of semipermeable unilamellar vesicles. Swelling involves a continuous phase transition from a fluctuating flaccid state to a maximum volume turgid state, without a divergent correlation length and with exponents that violate the hyperscaling relation. At the point of rupture, fluctuation contributions to surface and line tensions can support or suppress thermal generation of stable pores. The transfer of water through the membrane in response to osmotic pressure changes can compress, swell, rupture, and fuse vesicles: semipermeable unilamellar vesicles exhibit diverse osmotic critical phenomena.

I. INTRODUCTION

Lipid membranes form functional barriers between cells and their environments. These soft structures facilitate energy gradients essential to metabolism, compartmentalize incompatible chemistries, and enable differential transport of molecules. Many biological protocols require import into cells of large macromolecules such as DNA, but DNA cannot pass through membranes. Therefore, diverse protocols transiently permeabilize membranes: these include electroporation, sonoporation, physical puncture of the membrane, and numerous methods of chemoporation.

Bacterial genome transplantation involves the uptake of entire *Mycoplasma mycoides* donor chromosomes by *Mycoplasma capricolum* recipient bacteria. [1] The protocol involves treatment of recipient cells with 0.1 M calcium chloride ($CaCl_2$) at 4 °C, then treatment of donor genomes and recipient cells with 5% polyethylene glycol (PEG) buffer at 37 °C. A small fraction of recipient cells uptake a chromosome and survive the ordeal.

The donor genomes (1.08 Mb, about 1 micron radius of gyration in solution) and recipient cells (about 1 micron in diameter, single lipid bilayer and no cell wall) are about the same size. How does the genome enter recipient cells? Fluorescence microscopy revealed calcium chloride condensed the donor DNA onto recipient cells, and the osmotic pressure of the PEG buffer compressed the genomes and cells (Figure 1). The yield of transplanted cells exhibits sensitive dependence on chemical concentrations and incubation durations and temperatures. Gradient centrifugation studies suggested the frequency of genome uptake depends on recipient cell size.

Under what conditions do external osmotic pressure changes open pores large enough to transmit whole chromosomes? How do fluctuations of the membrane influence the osmotic critical behavior?

We model the lipid bilayer as a semipermeable membrane. To pass through a lipid bilayer, water molecules must overcome a 20 k_BT energy barrier [2], and specialised membrane proteins can reduce the energy barrier to 8 k_BT [3]. Therefore, water can permeate the barrier over reasonable time scales, whereas larger or charged solutes cannot permeate the barrier over comparable time scales.

The Helfrich Hamiltonian

$$\mathcal{H} = \int dudv\sqrt{g}\left(\sigma + \kappa_H H^2 + \kappa_K K\right), \tag{1}$$

describes membrane curvature energy, where u and v parametrize the surface, g is the determinant of the metric tensor, H and K are the mean and Gaussian curvatures, invariants of the curvature tensor, and the κ variables are their moduli. [4] We added the term σ to include the surface tension.

We model the membrane as N particles of diameter a. We can model the fluctuations of the membrane in a basis of spherical harmonics [5, 6]: we constrain the membrane area and expand in modes around the sphere with maximum volume per surface area.

II. SWELLING

IIa. Analysis

We express the partition function of the vesicle in a Gibbs canonical ensemble as

$$\mathcal{Z}(T, p_0, Q, N) = \int dV Z_V(T, V, N) Z_S(T, V, Q) e^{-p_0 V/T},$$

where Z_V represents the canonical partition function of the N monomers that compose the vesicle, Z_S represents the canonical partition function of the Q cytoplasmic solute particles trapped within the vesicle, and p_0 represents the osmotic pressure outside the vesicle. [7] We need not specify Z_S: if $f(Q/V)$ denotes the free energy of the cytoplasmic particles as a function of their concentration, then $Z_S = e^{-\beta F} = e^{-\beta Q f(Q/V)}$. If, however, we model the cytoplasmic particles as an ideal gas, then $Z_S = \left(V/\lambda^3\right)^Q / Q!$, where $\lambda = h/\sqrt{2\pi m k_B T}$ is the thermal wavelength and m is the mass of a solute particle.

As the concentration of solute particles increases, or as the external osmotic pressure decreases, the vesicle swells and approaches its maximum volume $V_{\max} \sim a^3 N^{3/2}$. The partition function Z_V of the membrane particles is

$$Z_V = \int \mathcal{D}\mathcal{R}\, e^{-\beta\mathcal{H}[\mathcal{R}]}\delta\left(V - V\left[\mathcal{R}\right]\right),$$

where $\mathcal{R}$ represents the configuration of the membrane, $V[\mathcal{R}]$ represents the volume of the configuration, and the Hamiltonian $\mathcal{H}$ represents the energy of the configuration and includes bending and surface contributions. We expand $V[\mathcal{R}]$ in a basis of spherical harmonics [5, 6] and represent the delta function in terms of an integral as

$$\delta\left(V - V_{\max}\right) = \frac{1}{2\pi}\int dp\, e^{ip\left(V - V_{\max} + \sum_n c_n |u_n|^2\right)}.$$

We approximate $e^{-\beta\mathcal{H}[\mathcal{R}]} \approx e^{-\beta\mathcal{H}[V_{\max}]}$ and integrate over the configurations as $\int \mathcal{D}\mathcal{R} \to \int \prod du_n$. Each Gaussian integral contributes $p^{-1/2}$. If the number of modes equals the number of particles, integration by parts gives $Z_V \sim \left(V_{\max} - V\right)^{\alpha N}$ with $\alpha = 1/2$. Haleva and Diamant [7] attribute the form of Z_V to (i) extensivity of the free energy for $V < V_{\max}$ and (ii) vanishing probability to find vesicles larger than the maximum size.

Substituting Z_V and Z_S into the full Gibbs partition function we obtain $\mathcal{Z} \sim \int dV e^{-\beta F}$, with $F = -\alpha N \ln\left(V_{\max} - V\right) + Qf(Q/V) + p_0 V$. [7] To solve for the vesicle volume, we minimize the free energy with respect to V and approximate V by its mean value $\langle V\rangle$

$$\frac{Q^2 f'\left(Q/\langle V\rangle\right)}{\langle V\rangle} - p_0\langle V\rangle = \frac{\alpha N\langle V\rangle}{V_{\max} - \langle V\rangle}. \tag{2}$$

We define an order parameter $M = 1 - \langle V\rangle / V_{\max}$ to describe how the vesicle approaches its maximum volume as the number of solutes Q increases or as the external osmotic pressure p_0 decreases. We follow Haleva and Diamant [7] and expand in terms of the small quantity $V_{\max} - \langle V\rangle$ to obtain

$$M = \frac{\sqrt{\left[s - t(q)\right]^2 + 4sg(q)} - s - t(q)}{2g(q)},$$

with $q = Q/V_{\max}$ the solute concentration at the maximum vesicle volume, $t(q) = q^2 f'(q)/p_0 - 1$ related to the ratio of the internal and external osmotic pressures, $g(q) = q^2\left[2f'(q) + qf''(q)\right]/p_0 > 0$ related to the derivative of the chemical potential with respect to Q (positive, provided the solutes remain below the concentration at which they precipitate/condense), and $s = \alpha N/(p_0 V_{\max}) \sim N^{-1/2}$ related to the ratio of the number of membrane monomers N to the critical number of solute monomers $Q_c = p_0 V_{\max}$. In the thermodynamic limit, $s \to 0$ and M approaches

$$\lim_{N\to\infty} M = \frac{|t| - t}{2g} = \begin{cases} -t/g, t < 0 \\ 0, t > 0 \end{cases}.$$

At the critical point $q_c = p_0$, $\langle V\rangle$ approaches a discontinuous first derivative. Haleva and Diamant [7] comment inclusion of fluctuations beyond the mean field approximation yields negligible corrections to M. M follows a scaling law in the neighborhood of the critical point, as

$$M/\Delta = g^{-1}\tilde{M}\left(t/\Delta\right), \text{ with } \tilde{M}(x) = \frac{\sqrt{x^2+1} - x}{2}.$$

Haleva and Diamant note we can express this scaling relation in the form $M \sim R^{-\beta/\nu}\tilde{M}\left(R^{1/\nu}t\right)$, and they computed similar exponents for the compressibility $\chi \sim R^{\gamma/\nu}\tilde{\chi}\left(R^{1/\nu}t\right)$, where $\chi = \partial M/\partial p_0$. Comparison to the previous scaling results gives $\beta = 1$, $\gamma = 0$, and $\nu = 2$. The hyperscaling relation $2\beta + \gamma = d\nu$ [8] is not satisfied for $d = 3$, the lack of equality related to a divergent length scale $\xi \sim a\left|t\right|^{-\nu}$, with the system near the phase transition if $R < \xi$. Haleva and Diamant explain the divergent ξ reflects a competition between different degrees of freedom, not correlations. They note the phase transition depends on the fixed solute number, which leads to a pressure difference and surface tension nonanalytic in the solute number Q. In contrast, the grand canonical ensemble with fixed chemical potential rather than particle number does not exhibit criticality.

In anticipation of section III, the saddle point result in equation 2 reflects a balance between the pressure difference across the membrane (left side of the equation) and a surface energy term (right side of the equation). [7] Thus, in terms of the order parameter M and the vesicle radius $R \sim aN^{1/2}$, we solve for the pressure difference Δp and surface tension σ via the Young-Laplace equation as

$$\begin{aligned} \Delta p &= \frac{\alpha N}{V_{\max} - \langle V\rangle} = \frac{\alpha N}{V_{\max} M} \sim a^{-3}N^{-1/2}M^{-1} \\ \sigma &= \frac{R_1 R_2}{R_1 + R_2}\Delta p \sim a^{-2}M^{-1}. \end{aligned}$$

IIb. Simulations

Lin et al. [9] investigated the membrane properties of swollen vesicles via dissipative particle dynamics (DPD) simulations. To increase simulation durations to hydrodynamic time scales in the microsecond range, DPD particles interact via soft potentials with predefined collision rules. A modified velocity Verlet algorithm advances the positions and velocities. The particles interact within cutoff radii through three sets of pairwise forces - repulsive, dissipative, and random - with interaction parameters a_{ij} related to the Flory-Huggins χ parameters, and force amplitudes tuned to satisfy the fluctuation-dissipation theorem. [10]

Lin et al. [9] equilibrated coarse-grained vesicles, then they injected water molecules to swell the vesicles. They

observed a decrease in bilayer thickness and in area densities of the lipid head groups. Haleva and Diamant [7] assumed the entropy approached a nonextensive value near the maximum vesicle volume. In contrast, Lin et al. found the configurational entropy of the lipid molecules increased as the vesicle approached its maximum volume. [9] In the flaccid state the lipid tails stacked and formed two ordered leaflets, whereas in the turgid state the tails interdigitated within a single leaflet, causing a loss in the orientational order. In future, it would be interesting to repeat the analysis of Haleva and Diamant with an expression for Z_V that does not approach zero as V approaches $V_{\max}$.

III. RUPTURE

As the internal osmotic pressure continues to grow relative to the external pressure, the probability of pore formation increases, driven by a competition between line and surface tensions. The energy of a pore is

$$E = \Gamma 2\pi r_0 - \sigma\pi r_0^2,$$

where Γ represents the line tension (due to exposure of hydrophobic tails or curvature of hydrophilic heads at the interface) and σ represents the surface tension. With Γ and σ both positive, a pore with radius larger than $r_c = \Gamma/\sigma$ will grow until the membrane ruptures. To achieve a pore with radius r_0, the system must overcome a nucleation energy barrier $\delta E = \pi\Gamma^2/\sigma$. Farago and Santangelo [11] commented the above model cannot sustain stable pores, though stable pores have been observed in experiments and in computer simulations. In support of stable pores, they noted pore opening relaxes the surface tension via increased area density of monomers and allows internal cytoplasmic solutes to escape [12]. Farago and Santangelo investigated the roles of membrane fluctuations and pore shape entropy. They showed that large enough pores have the same energy form as above, with renormalized Γ_{eff} and σ_{eff}. In particular, they expanded in a basis of modified Bessel functions and integrated over fluctuations. They made a step function approximation: the pore did not perturb modes with characteristic lengths much larger than the pore radius, and the pore reduced the effective size of the membrane for modes with characteristic lengths much smaller than the pore radius. They derived a thermal correction to the surface tension

$$\Delta\sigma = \frac{k_B T}{\pi\alpha l_0^2}\left[\alpha - 2 + \left(\frac{l_0}{\pi\xi}\right)^2 \ln\left[(\pi\xi/l_0)^2 + 1\right]\right],$$

where α represents a dimensionless constant of order unity, determined by numerical calculation of ΔF_s, $l_0 = L_p/\sqrt{N_0}$ is a minimum cutoff comparable to the bilayer thickness, L_p represents the radius of the system, $2N_0$ represents the number of modes, proportional to the number of particles, and $\xi = \sqrt{\kappa/\sigma_0}$ is a length scale inversely related to the surface tension. They conclude $\Delta\sigma < 0$, and for large ξ (small surface tension), the effective tension may be negative - in other words, the surface tension could prevent rather than encourage pore formation!

Farago and Santangelo next integrated over the pore shape and size to estimate line tension contributions. They evaluated the integral in a Fourier basis and assumed the surface tension was much less than the line tension per unit circumference to derive

$$\Delta\Gamma = \frac{bk_B T}{\pi l_0}\left[\ln\left(\frac{b\lambda^2\Gamma}{k_B T l_0}\right) - 2\right],$$

where b is a dimensionless constant of order unity and λ is the thermal wavelength. For typical bilayers at room temperature, $\Delta\Gamma < 0$. The decreased line tension decreases the pore nucleation energy barrier, whereas the decreased surface tension decreases the pore formation impetus.

IV. CONCLUSION

Semipermeable vesicles exhibit diverse osmotic critical phenomena such as swelling and rupture transitions, when the internal osmotic pressure exceeds the external pressure. We did not discuss the opposite region of the phase diagram, when high external pressures cause buckling or branching transitions [13], nor did we discuss how osmotic swelling may encourage vesicle fusion, observed by Lin et al. in DPD simulations [9]. Many experimental questions remain. For example, do the chromosomes get pushed into cells when the external pressure increases, or pulled into cells when the external pressure decreases? The rupture theory may help explain why small temperature or chemical changes influence the yield of transplanted cells, with line and surface tensions in a subtle competition, modulated by physicochemical parameters.

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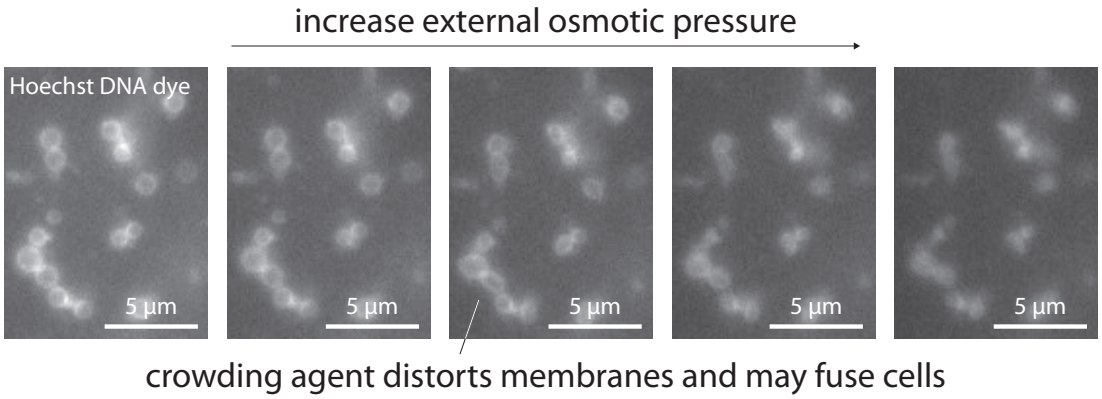

FIG. 1: Fluorescent *Mycoplasma mycoides* genomes condensed on *Mycoplasma capricolum* cells in microfluidic chambers. 5% polyethylene glycol MW 8000 solution enters the chambers and increases the external osmotic pressure. The cells and genomes are compressed, as the ring morphologies deform and the cell volumes decrease.