RELAXATION OF A SIMULATED LIPID BILAYER VESICLE COMPRESSED BY AN AFM

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SUMMARY

The relaxation time of bilayer vesicles, uniaxially compressed by an Atomic Force Microscope (AFM) cantilever, exhibits a strong force dependence.

In water, phospholipids self-assemble to form closed membranes called *lipid bilayer vesicles* (Fig. 1). The lipids do this because they are amphiphiles: their tails are hydrophobic, but their heads are hydrophilic. The closed (vesicle) configuration, shown to the right in Fig. 1, is stable because it shields the (hydrophobic) tails from the surrounding water. At its foundation, the cell membrane is a lipid bilayer. In the context of the origin of life, that such closed membranes assemble spontaneously in nature is a very interesting fact.

Cells are very complicated mechanical objects—eukaryotic cells especially so—and vesicles have been attractive model systems for theoretical work, simulations and experiments.

SIMULATION SETUP

To investigate the viscoelastic properties of vesicles, we ran computer simulations wherein a vesicle is squeezed between two plates (Fig. 2).

This procedure is relevant to experiments [3,6] which use an Atomic Force Microscope (AFM) to poke and squeeze and stretch living cells and vesicles. Our focus is on the *dynamics* of stress relaxation in the membrane, rather than static properties.

We use coarse-grained molecular dynamics [1] in order to reproduce the basic characteristics common to all real bilayer membranes: thermal undulations, in-plane fluidity, intermonolayer friction, area compressibility and bending rigidity. The model (Fig. 2) consists of approximately 140,000 particles. Our vesicle is the same as was used in [2], its membrane composed of coarse-grained lipids having one hydrophilic 'head' particle and two hydrophobic 'tail' particles (see inset in Fig. 2). At 3000 lipids, the membrane area is large enough to achieve the macroscopic properties described by continuum models. Fig. 2 omits the outer fluid particles that surround our small unilamellar vesicle. On the scale of our simulations, we treat a rounded AFM tip as approximately flat. For giant vesicles, this corresponds to a tipless AFM cantilever. The vesicle is compressed via a step force applied uniformly to each bead in the upper crystal (AFM), whose motion is constrained to the direction normal to the substrate.

DYNAMICS

Time evolution of the (triangulated) area strain α after we activate the squeezing force is described as an exponential saturation

$$\alpha(t) = \alpha_{\infty}\left(1 - e^{-t/\tau}\right) \qquad (1)$$

(see Fig. 3). This viscoelastic creep response corresponds to the 'Kelvin-Voigt' model, or the more general

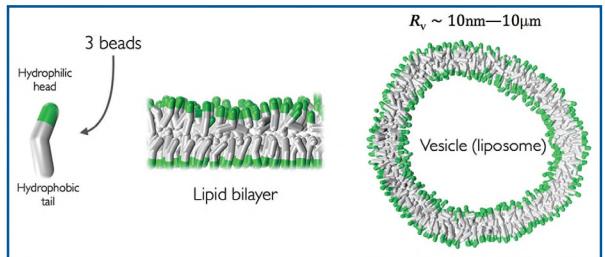

Fig. 1 Self-assembly of amphiphiles into closed quasi-spherical vesicles. In nature the closed configuration is entropically favourable because it minimizes the hydrophobic tails' exposure to the surrounding fluid. In our simulations the hydrophobic interaction is modelled by making exposure of the tails energetically costly. Thus in contrast to real vesicles, the simulated vesicle is stable because it is energetically favourable. A major benefit of the molecular dynamics approach to simulating bilayers (shown here) is that this method reproduces key properties of real bilayers —e.g. thermal undulations, in-plane fluidity, area compressibility, bending rigidity, ability to open and close pores, etc.

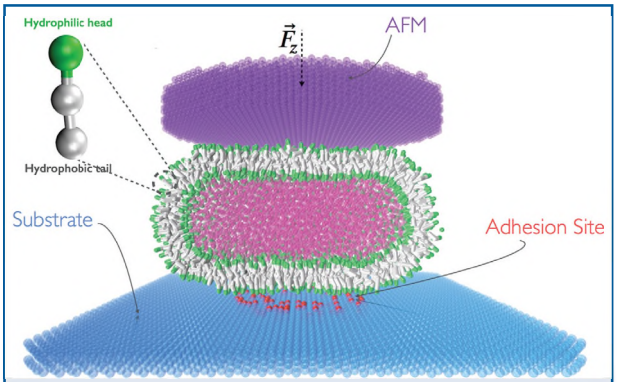

Fig. 2 Simulated vesicle undergoing parallel plate compression. In addition to the ordinary substrate particles, a bullseye of randomly distributed 'sticky' particles was placed at the centre of the substrate to ensure adhesion. Without this *adhesion site*, the vesicle would slip out from underneath the AFM. Coarse-grained lipid shown at upper left. (An analogous experimental setup was used by Schäfer et al.[6] to investigate *static* properties of giant liposomes.)

'Standard Linear Solid' model. In this model the relaxation time is $\tau \sim \eta/K$, where η is a viscosity and K is an elastic modulus.

RELAXATION TIME VERSUS TENSION

As it turns out, the relaxation time τ depends on the magnitude of the applied stress. In Fig. 4, we plot $\tau(\gamma)$—relaxation time versus surface tension. (The tension, estimated via the α-contribution to the work done compressing the vesicle, scales nearly linearly with the squeezing force.) The sharp rise in the vesicle's relaxation time at low force arises from the effect of entropic undulations on the area expansion.

We have fit the $\tau(\gamma)$ data using Equation 6, which we will now derive.

Thermal agitation excites undulations in the vesicle membrane. Due to these undulations, the surface area of a vesicle as measured in the lab will be less than the true surface area of its membrane. Hence a distinction is made between 'apparent' or 'projected' versus true surface area of the membrane. In 1984, Helfrich and Servuss (HS)[4] derived an expression connecting the relative change in a membrane's apparent area ΔA to its surface tension γ:

$$\alpha(\gamma) \equiv \left(\frac{\Delta A}{A}\right)_{\gamma>0} = \underbrace{\frac{k_B T}{8\pi\kappa} \ln\left(\frac{\frac{\zeta_\kappa}{A}+\gamma}{\frac{\zeta_\kappa}{a}+\gamma}\right)}_{\text{entropic}} + \underbrace{\frac{\gamma}{K_A}}_{\text{direct}}, \qquad (2)$$

where K_A, κ, A and a are the membrane's area compressibility modulus, bending rigidity, unstressed area and area per lipid, respectively. k_B is Boltzmann's constant, T is the temperature,

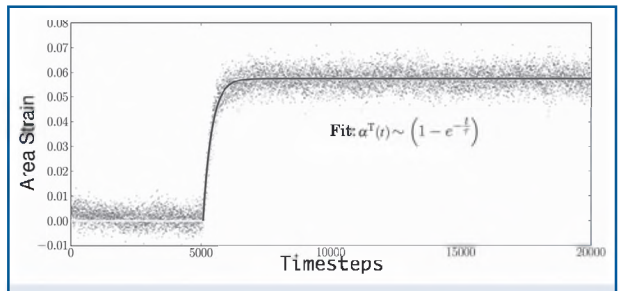

Fig. 3 Ensemble fit to creep response of the bilayer's triangulated area. For each value of the applied force, data from multiple simulations are fit as one time-series. This helps to reduce the uncertainty on the relaxation time, by reducing the influence of noise from any particular simulation on the fit.

and ζ depends on membrane shape. (E.g. $\zeta = \pi^2$ for a planar membrane, and for a sphere $\zeta = 24\pi$.) For small $\Delta(\cdot)$ we know that

$$\Delta(strain) = \frac{1}{K}\Delta(stress) \approx \left(\frac{\partial(strain)}{\partial(stress)}\right)\Delta(stress). \qquad (3)$$

For a stretching membrane $strain = \alpha$ and $stress = \gamma$, so that

$$\frac{1}{K} \equiv \frac{\partial(strain)}{\partial(stress)} = \frac{\partial\alpha}{\partial\gamma} \qquad (4)$$

defines the stiffness of the *apparent* surface.

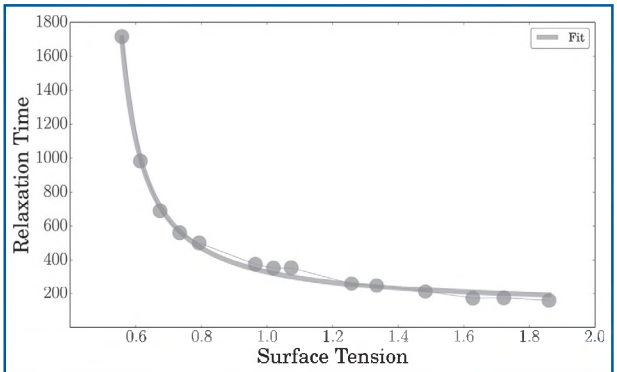

Fig. 4 Relaxation time versus surface tension, fit to Equation 6. Derived out of the HS model, $\tau(\gamma)$ (Equation 5) leads to a correct description of the force dependence of the relaxation time. (The tension scales nearly linearly with the squeezing force. Figure axes are in dimensionless units.)

From the HS model and linear viscoelasticity theory, we derive the relaxation time

$$\tau(\gamma) \sim \eta\left(\frac{1}{K}\right) \approx \eta\left(\frac{\partial\alpha}{\partial\gamma}\right) \approx \eta\left(\frac{1}{K_A} + \frac{\frac{k_B T}{8\pi\kappa}}{\frac{\zeta_K}{A} + \gamma}\right). \quad (5)$$

A phenomenological form consistent with both the low and high tension limits of 5 is

$$\tau \approx C_1 + \frac{C_2}{C_3 + \gamma}, \quad (6)$$

where C_1 is the high-tension asymptotic limit and $C_1 + \frac{C_2}{C_3}$ is the finite limit as $\gamma \to 0$. Going a step further, in Fig. 4 we have fit the entire $\tau(\gamma)$ curve with this function, which succeeds as a phenomenological model. Though the fit extends beyond small $\Delta\gamma$, it yields a reasonable estimate of η/K_A.

CONCLUSION

The relaxation time depends on the magnitude of the applied stress, increasing sharply in the limit of low stress. This is caused by entropic undulations in the bilayer. Equation 5 predicts a *finite maximum relaxation time, proportional to the membrane's surface area*, so the effect should be *stronger* in cell-sized systems. Moreover, since undulations have been observed in real vesicles and cells, the force-dependence should be present in them as well. The connection between our vesicle's relaxation time, entropic undulations and the applied stress may help to explain the wide variability of relaxation (and recovery) times reported for cells [3,5].

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