

Cell Membrane Studies: Vesicle Deformation and Bilayer Defects

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Surface Morphology Effects on Vesicles

A primary membrane function is the active regulation of solute gradients across the lipid barrier. As a consequence, the extensive pore network and flippases may function to release inner leaflet compression and to regulate the interior volume to best accommodate the cellular profile with minimal bilayer defects. In our vesicle deformation study, we find that sucrose crossed the lipid bilayer via a transient-defect pore model with pore lifetimes of 10 μ s to 1 ms. Recent investigations have implicated static deformation to pressure changes within our system. The physical response of the bilayer to pressure has enormous implications for the cell membrane, membrane receptor complexes, and therefore, on cell and tissue health. Future membrane work will focus on perturbations to invoke deformation.

Lipid behavior...not simple

Gel Phase: tightly packed, low lateral mobility
 L_{β}

Liquid Ordered Phase: cholesterol, high lateral mobility, ordered
 L_{α}

Fluid Phase: disordered, high lateral mobility
 L_{α}

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Vesicle Deformation on Surface Topography

Vesicles cooled to 37 °C, placed on surface at 37 °C, Imaged at 20 °C

Pillar edge dip between pillars

XZ Plane
YZ Plane

(a) (b)

Analysis of Deformation

Quantification of Temperature Deformation Dependence

Temperature and Deformation

Vesicles full cooled placed on surface at 20 °C Imaged at 20 °C

Equatorial plane

Vesicles placed on surface at 55 °C Imaged at 20 °C

Contact with slide. Vesicle shows no deformation at surface

Contact area on surface

Vesicle on Complex Topography

Vesicle supported in Puramatrix, a three dimensional flexible peptide matrix, similar to a collagen extracellular matrix. (a) and (b) the vesicle image, in confocal 3D, (a), and the cut-aways, (b), reveals a highly deformed vesicle.

Vesicles cooled to 37 °C, placed on surface at 37 °C Imaged at 20 °C

Equatorial plane XZ
YZ

Contact area on surface

A_{lipid} → calculated vesicle surface area
 $A_{lipid} = 4\pi R^2$
 $V_c = \frac{4}{3}\pi R^3$
 V_f → calculated volume
 $\frac{V_c}{V_f} = V_{ratio}$

Temperature	V_{ratio} Range	V_{ratio} Mean
20 °C	1.0-1.078	1.01±0.030
37 °C	1.06-1.21	1.12±0.054
55 °C	1.13-1.26	1.18±0.066

Berndl, Käs, Lipowsky, Sackmann, and Seifert, *Europhys. Lett.* 1990, 13, 659-664

Analysis: Curvature Induced Defects



Raphael, Waugh, Svetina, and Zekš, *Physical Review E* 2001, 64, 051913.

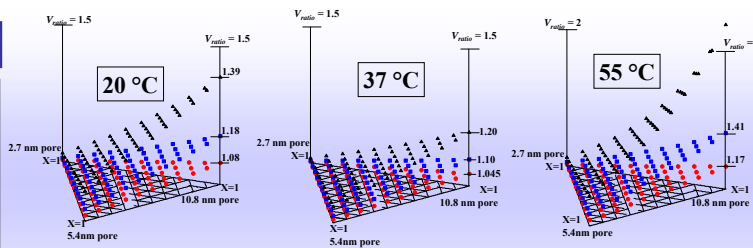
Net Diffusion Distance
 $\langle x \rangle = \sqrt{2Dt}$
 D = Diffusion Coefficient
 t = time
Net Diffusion Volume
 $V_{net} = \langle x \rangle^3 A_{pore}$

Modeling Parameters:

Vesicle $r = 8.9 \mu\text{m}$
Surface area = $995 \mu\text{m}^2$
Volume = $2950 \mu\text{m}^3$
Pore defect size:
2.7 nm
5.4 nm
10.8 nm

Lifetimes:
1 ms
100 μs
10 μs

Temperatures:
20 °C
37 °C
55 °C

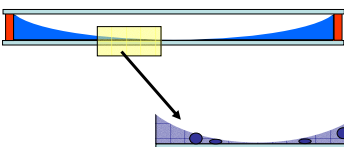


Vesicle deformation can only occur if the sucrose solute exits the vesicle. The bilayer is virtually impermeable to sucrose. However, transient, nanoscale pores would allow sucrose to cross the bilayer and the vesicle to deflate. We base our calculations on transient pores that are theorized to form due to mechanical stress. We assume that sucrose exits the vesicle at a rate similar its diffusion in free volume. From these assumptions, we model the deformation as a function of pore diameter and lifetime.

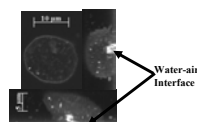
Lifetimes:
1 ms
100 μs
10 μs

Tailoring Vesicle Deformation

Capillary Forces squeeze vesicles at room temperature



Between two glass slides, a small amount of vesicle suspension is deposited. The capillary forces wick the liquid to the outer rim of the sample, which imparts pressure on vesicles caught between the air-water interface and the surface.



A vesicle is sandwiched between the two interfaces and over time changes its shape dramatically. Originally, it is an ellipsoid with a height of $\sim 8 \mu\text{m}$. After ~ 30 minutes, its height decreases to $\sim 2.5 \mu\text{m}$.

Original vesicle shape, $t = 0$

Vesicle shape, $t = 13 \text{ min}$

Vesicle shape, $t = 19.5 \text{ min}$

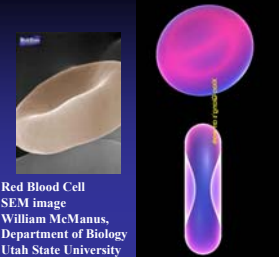
Vesicle shape, $t = 23 \text{ min}$

Vesicle shape, $t = 27 \text{ min}$

Glass-water Interface

Water-air Interface

Vesicle dimple formation as height decreases. It first appears at the water-air interface. Finally, it appear also at the glass-water interface.



Red Blood Cell SEM image
William McManus,
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Calculated membrane Deformation:
Q. Du, C. Liu and X. Wang, *J. Computational Physics*, 198, pp450-468, 2004.

The vesicle dimpling is similar to the shape of the red-blood cell.