Correlating Gramicidin Ion-Channel Formation to Artificial Membrane Dynamics

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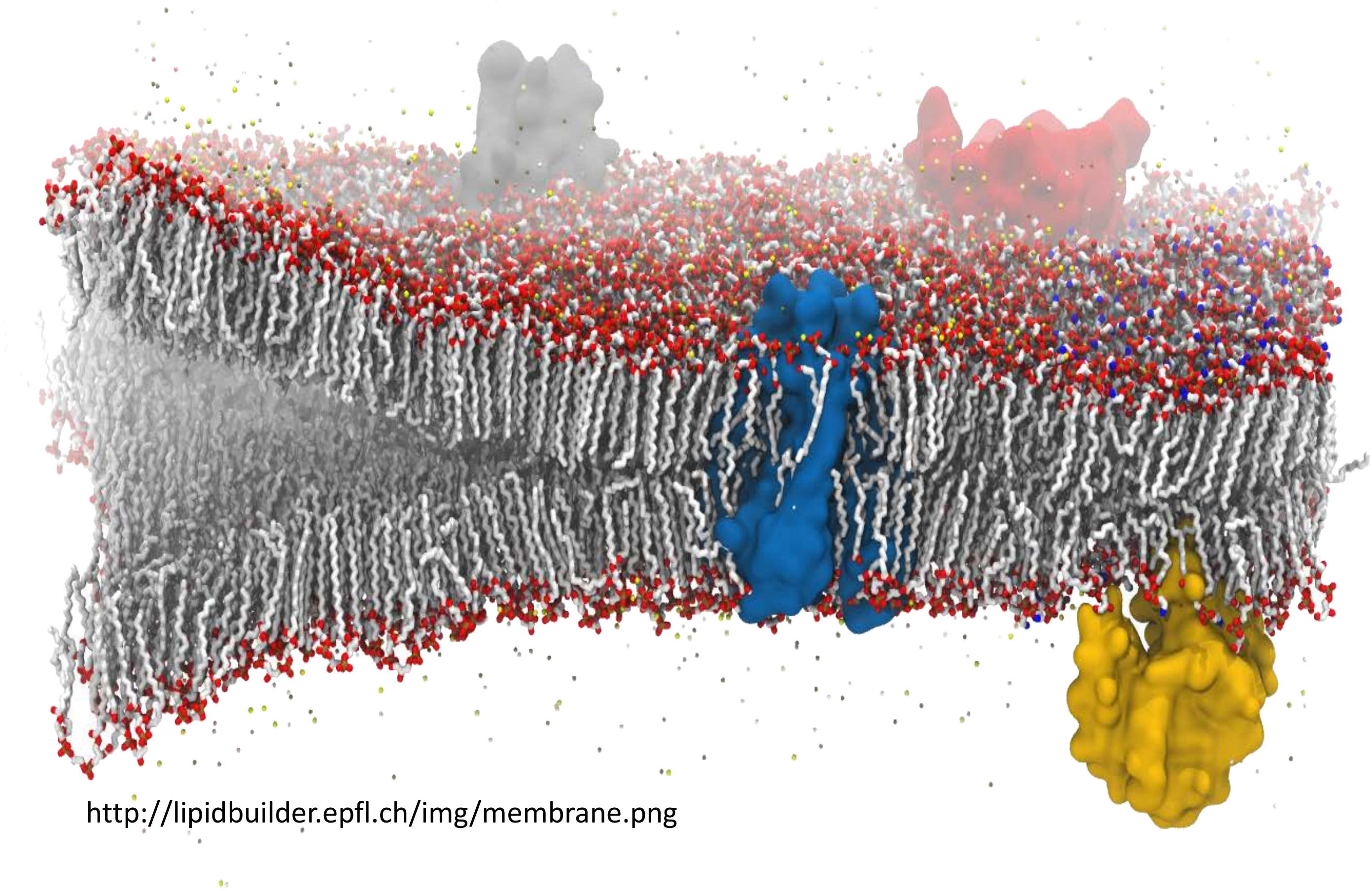
Lipid Membranes



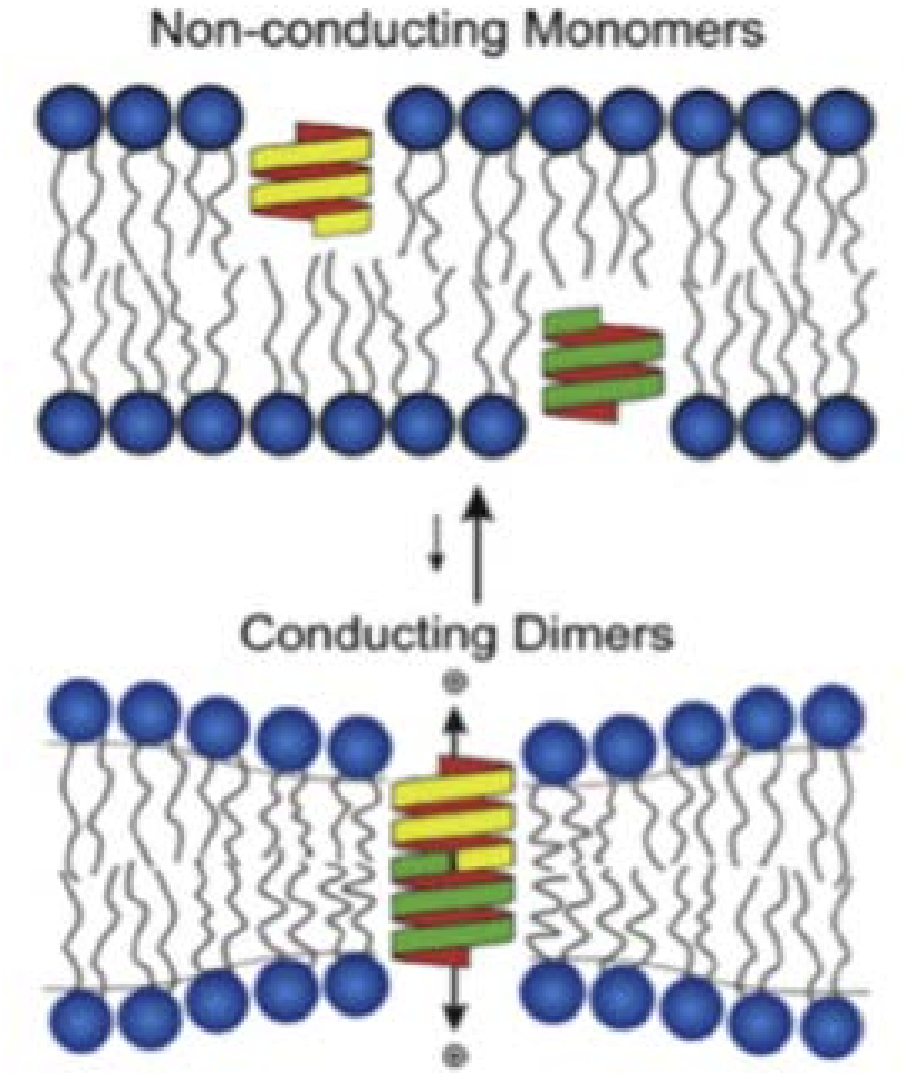
http://lipidbuilder.epfl.ch/img/membrane.png

Cell membranes contain an equal ratio of proteins to lipids. Lipid-lipid ratios are rigidly maintained.

Lipid and protein composition determines membrane structure and dynamics.

Cell function and disease have a direct link to nanoscale membrane dynamics and macroscopic structure

Gramicidin

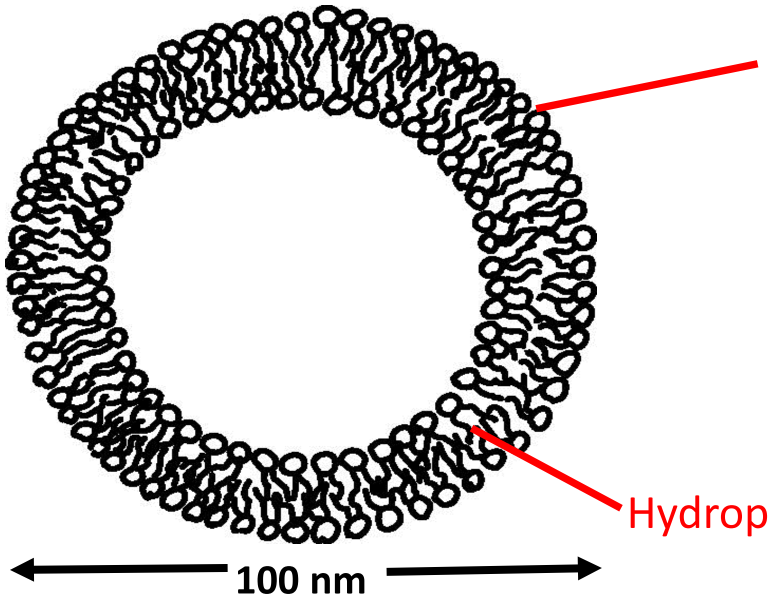


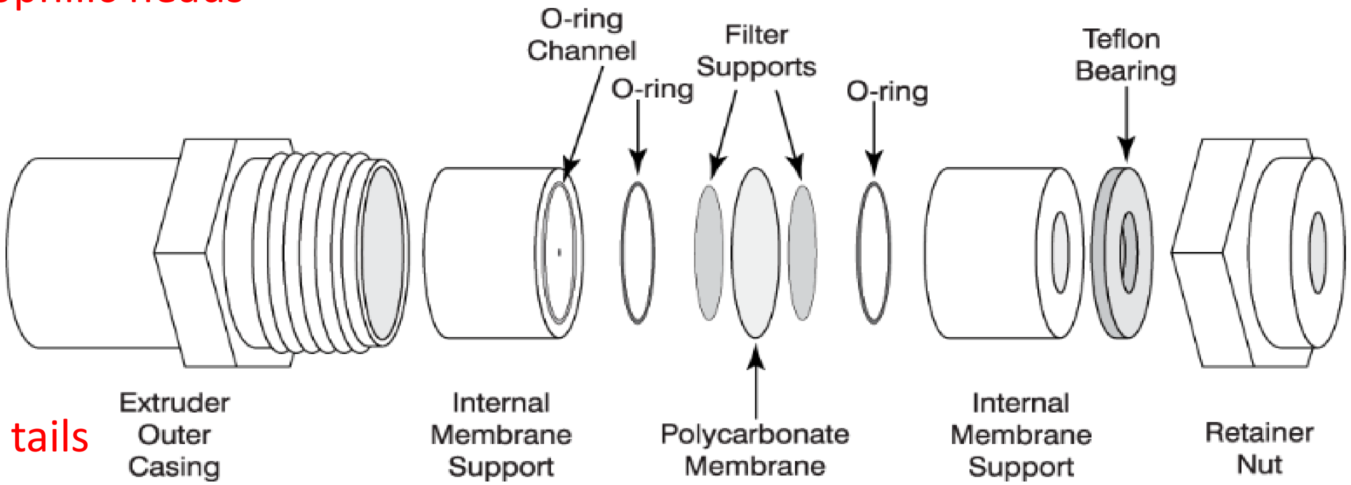
Beaven et al. Gramicidin A Channel Formation Induces Local Lipid Redistribution I: Experiment and Simulation

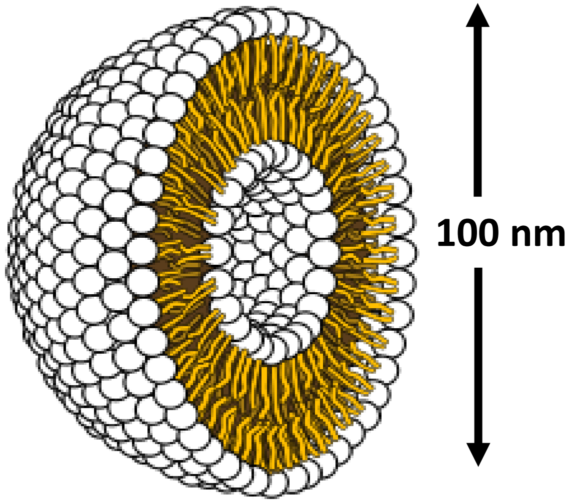
Gramicidin channels provide a unique combination of advantages that sets them apart from other channels.

- Structure of the bilayer-spanning channel is known
- It’s ion permeability is well known and can be modified
- Lipid-protein interaction is universal in nature.

Lipid-Protein Vesicles Were Made via Extrusion







https://www.wur.nl/en/show/MSc-Cell-membranes-breaking-the-barrier-using-nanoparticles-1.htm
https://en.wikipedia.org/wiki/Lipid_bilayer#/media/File:Phospholipids_aqueous_solution_structures.svg

https://avantilipids.com/divisions/equipment-products

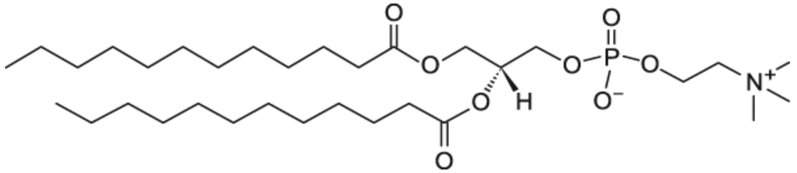
Density

Partial specific volume (v_s) was determined from measurements taken of the lipid solution density by the following equation:

$$v_s = \frac{1}{\rho_0}\left(1 - \frac{\rho_s - \rho_0}{c}\right)$$

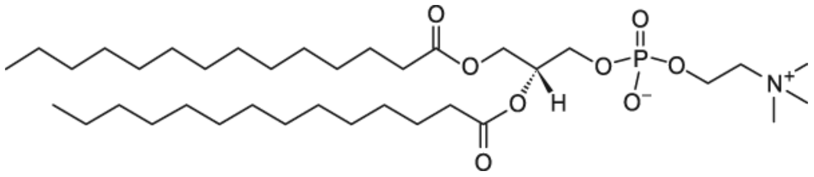
Volume per lipid molecule (V_L) was determined from measurements taken of the lipid solution density by the following equation:

$$V_L = \frac{v_s}{N_A}\sum x_i M_i$$



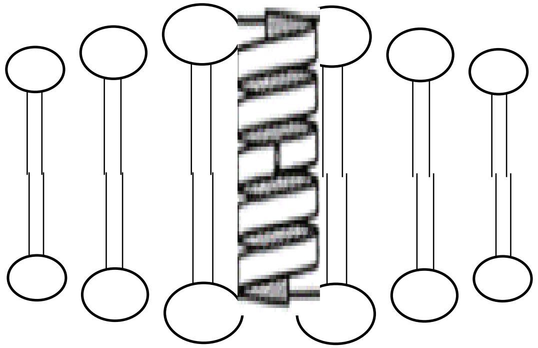
DLPC
A 12-C Lipid

1,2-dilauroyl-sn-glycero-3-phosphocholine

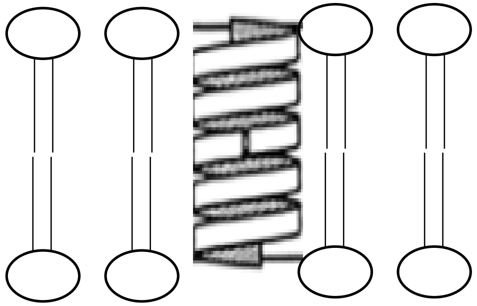


DMPC
A 14-C Lipid

1,2-dimyristoyl-sn-glycero-3-phosphocholine

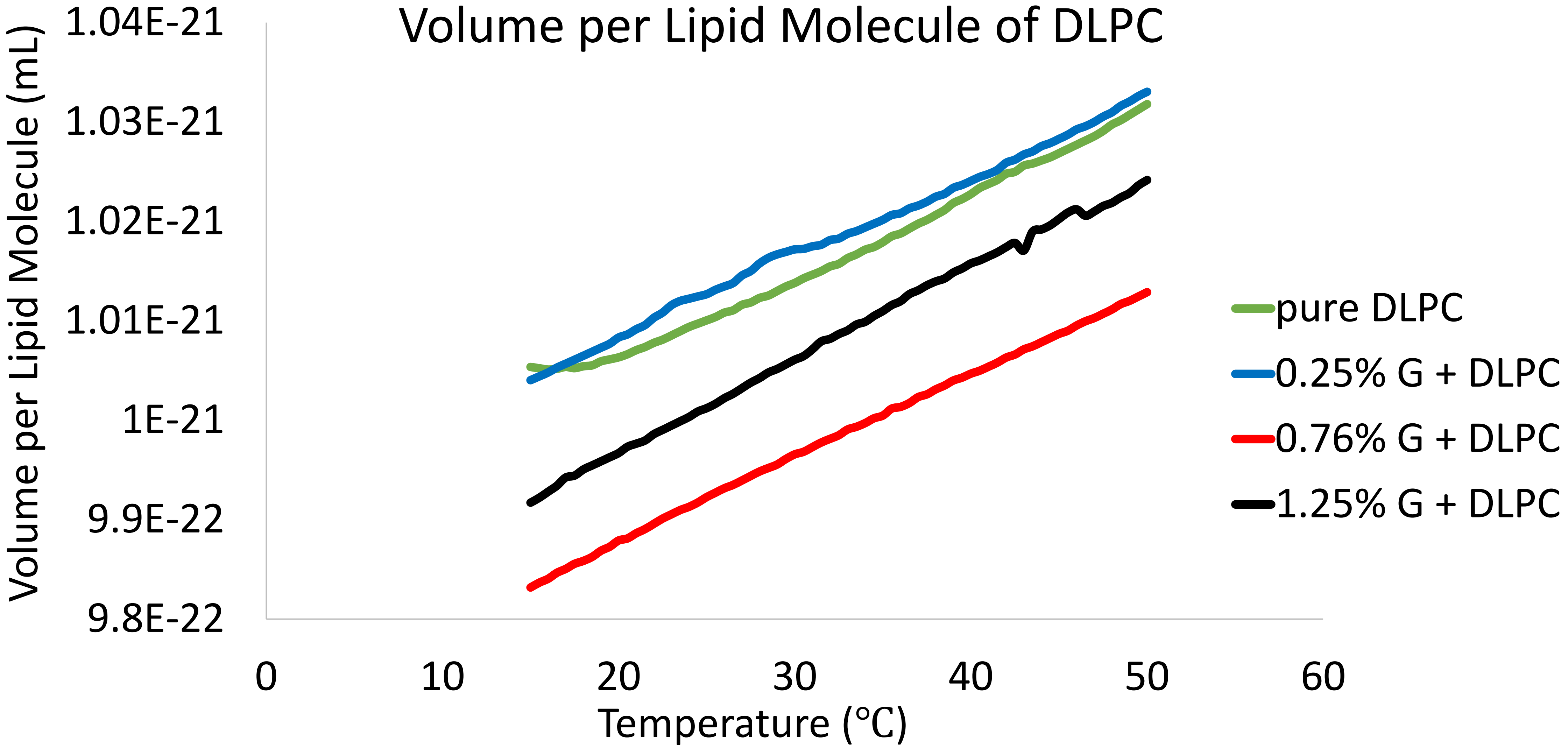


HYDROPHOBIC MISMATCH

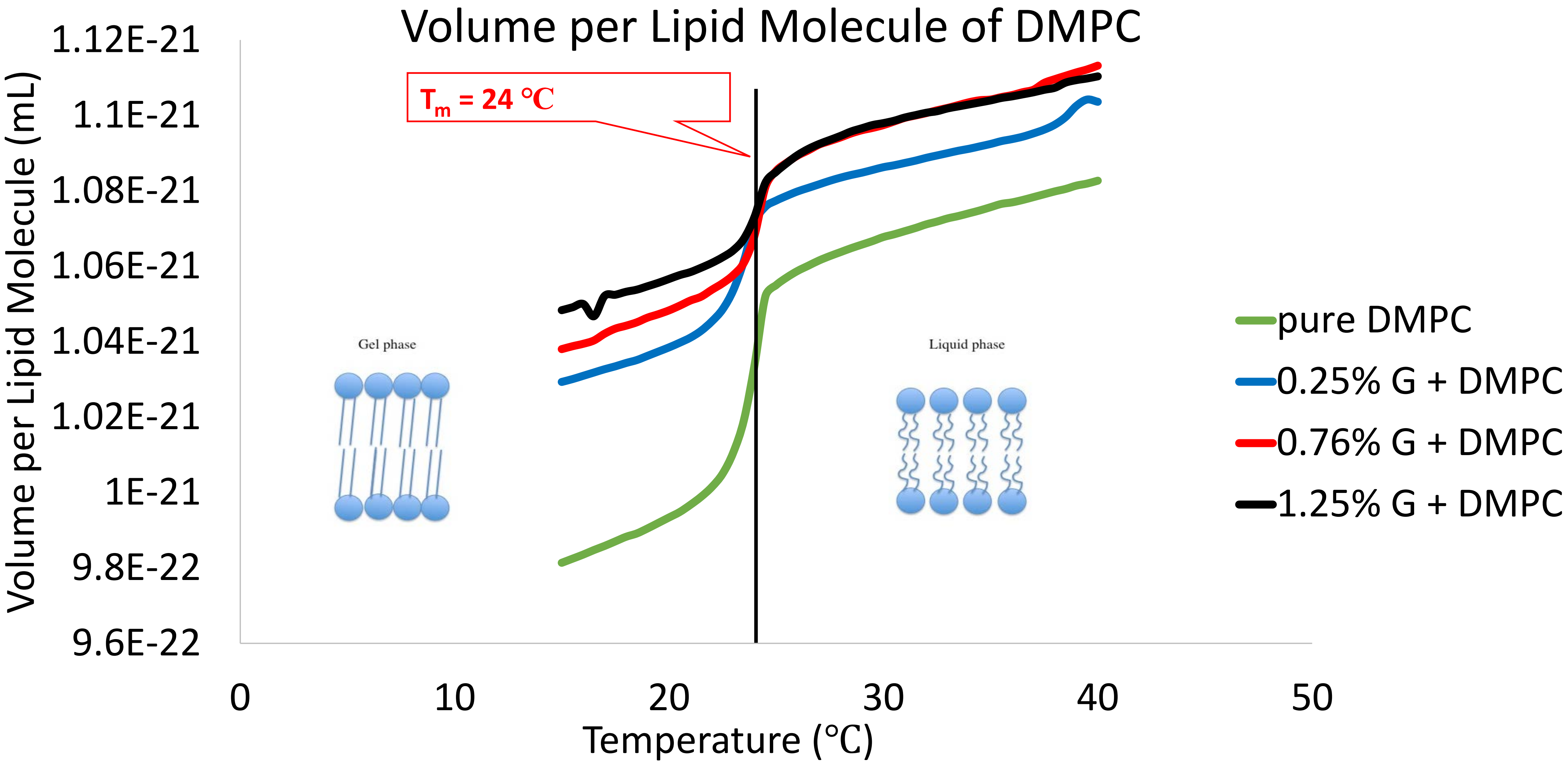


https://ac.els-cdn.com/S0005273607001848/1-s2.0-S0005273607001848-main.pdf?_tid=b0f57ec4-9014-4a1f-9c21-dbea993914cf&acdnat=1533184309_7906058a99389690d5d5c648b174e460

DLPC


Volume per Lipid Molecule of DLPC
Volume per Lipid Molecule (mL)
1.04E-21
1.03E-21
1.02E-21
1.01E-21
1E-21
9.9E-22
9.8E-22
0
10
20
30
40
50
60
Temperature (℃)
pure DLPC
0.25% G + DLPC
0.76% G + DLPC
1.25% G + DLPC

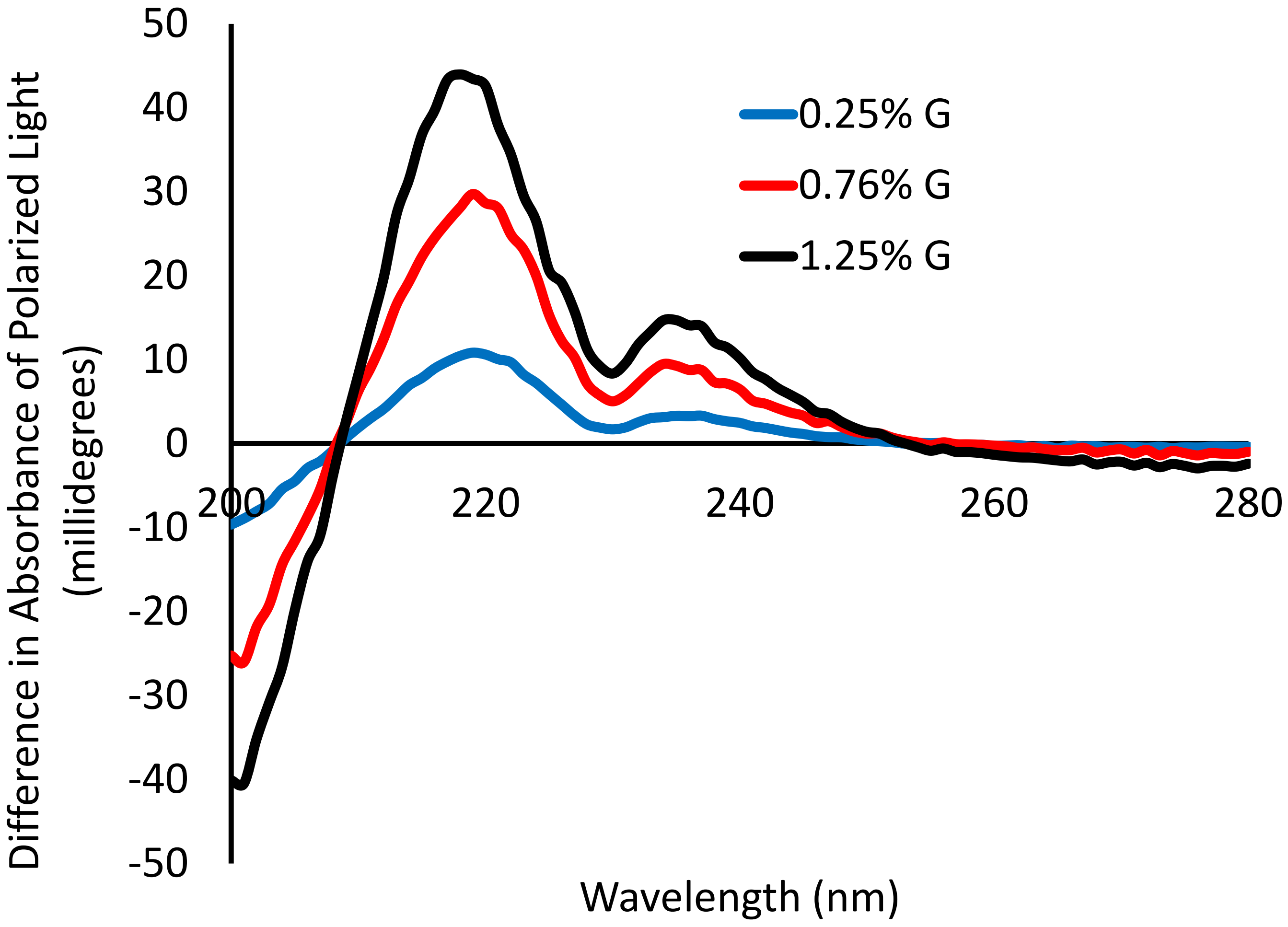
DMPC

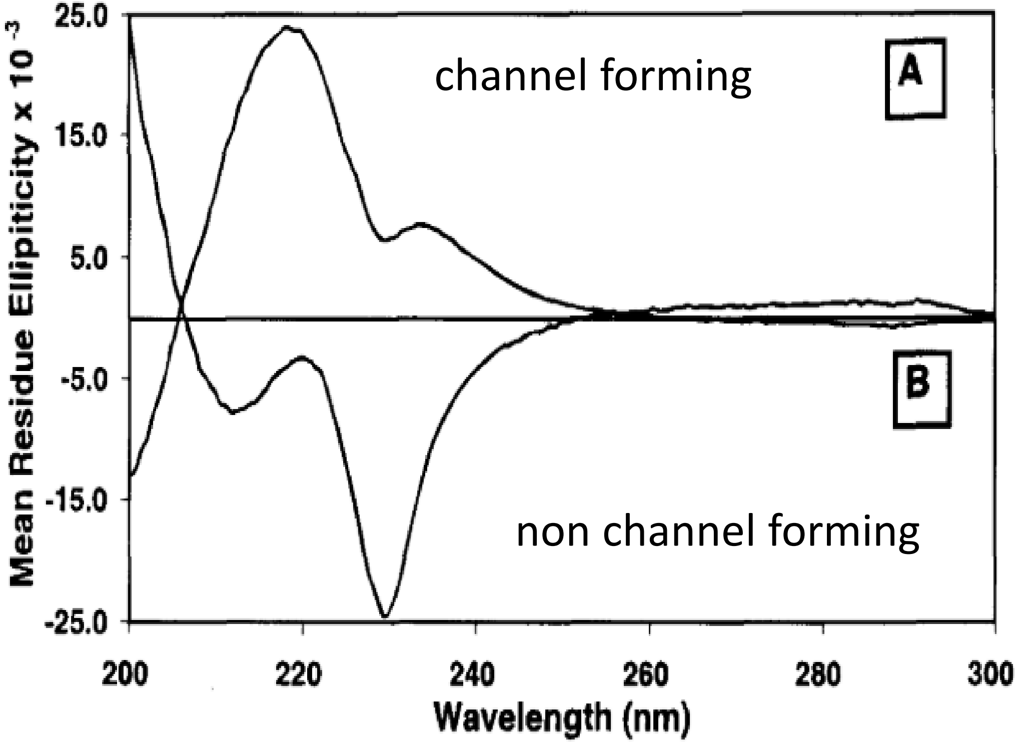


https://phys.libretexts.org/LibreTexts/University_of_California_Davis/UCD%3A_Biophysics_241_-_Membrane_Biology/Membrane_Phases/The_Gel_Phase

Gramicidin Conformation in 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC)

Circular Dichroism: Gramicidin β-helical Structure





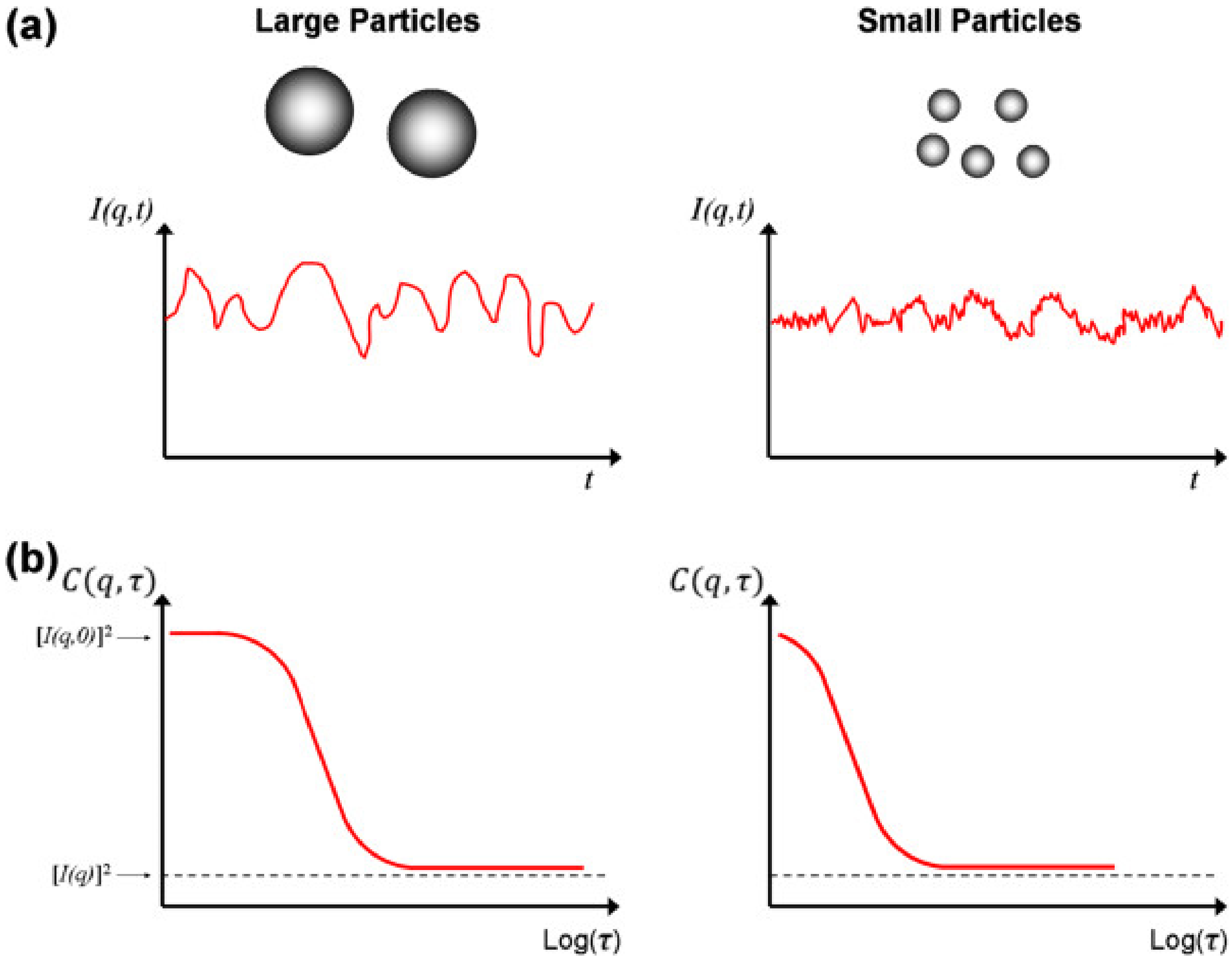
Koeppe and Andersen, Annu. Rev. Biophys. Biomol. Struct. 1996, 25: 231-258

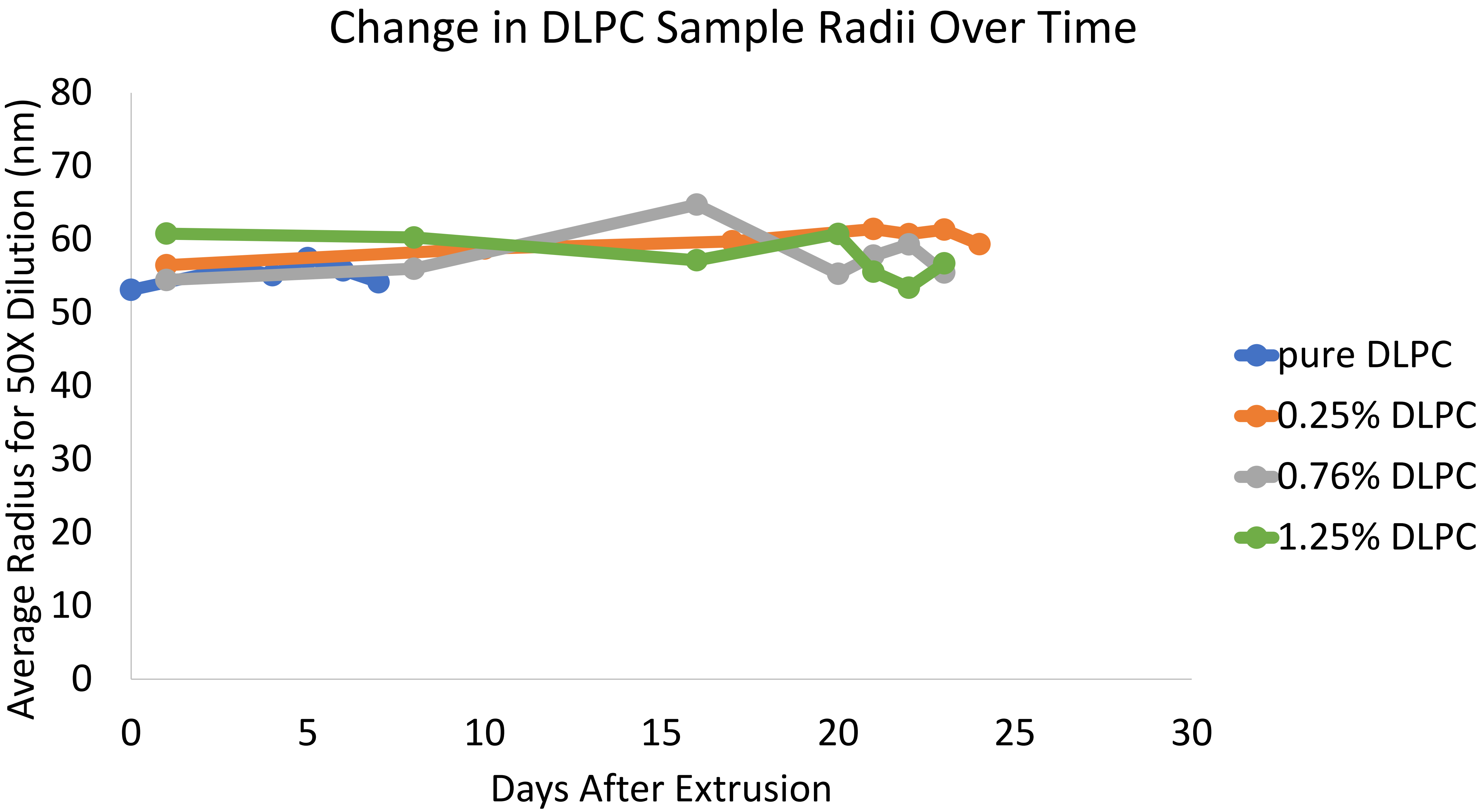
Dynamic Light Scattering (DLS)

A technique used to measure the hydrodynamic radius of nanoparticles suspended in solution.

Particle size can be determined by measuring the random changes in the intensity of light scattered from a suspension or solution.

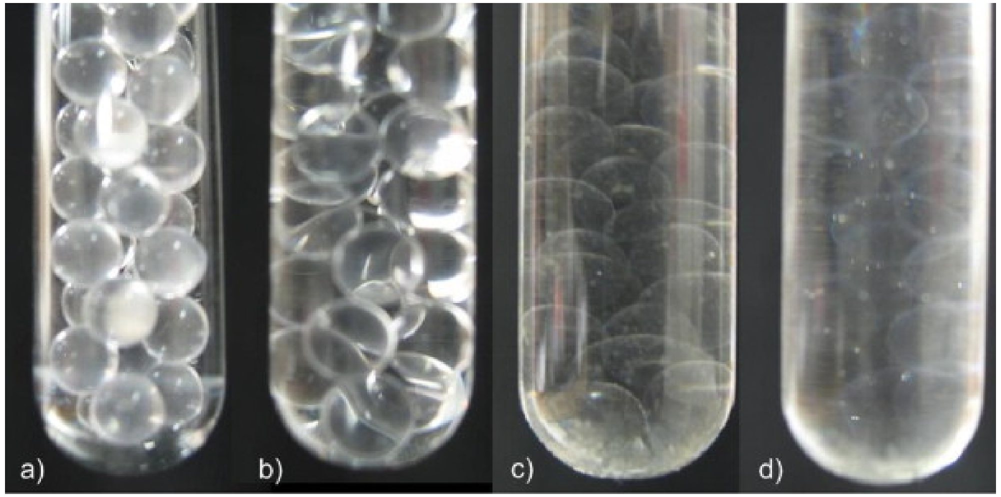
Used to determine the size of our artificial membranes



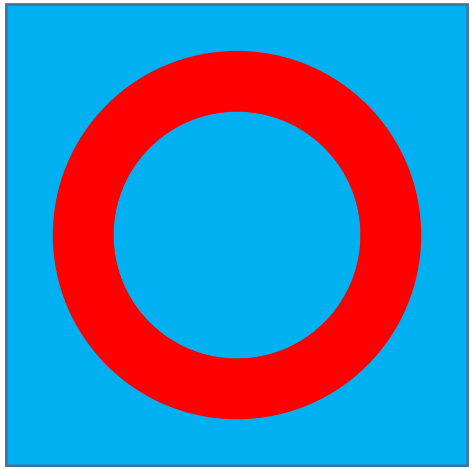

Change in DLPC Sample Radii Over Time
Average Radius for 50X Dilution (nm)
0
10
20
30
40
50
60
70
80
Days After Extrusion
0
5
10
15
20
25
30
pure DLPC
0.25% DLPC
0.76% DLPC
1.25% DLPC

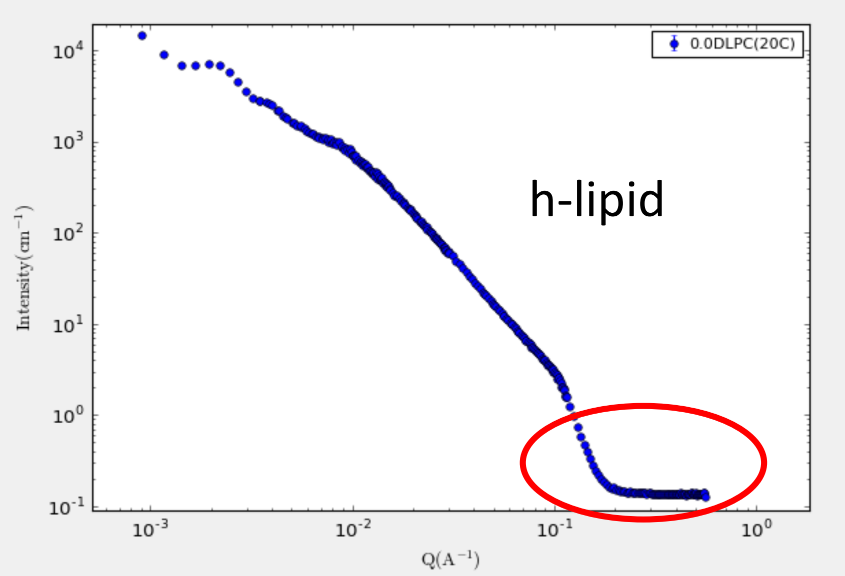
Small Angle Neutron Scattering (SANS)

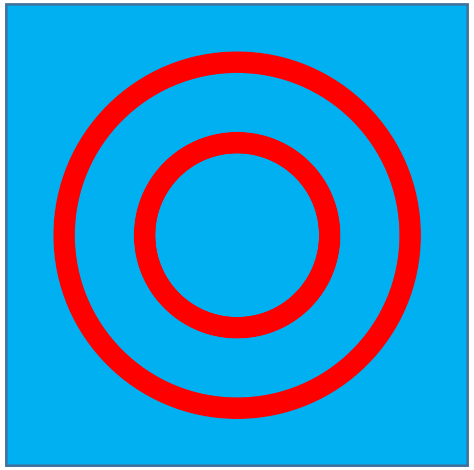
Information about membrane structure is gleaned from contrast between deuterated lipid tails and solvent compared to head groups.

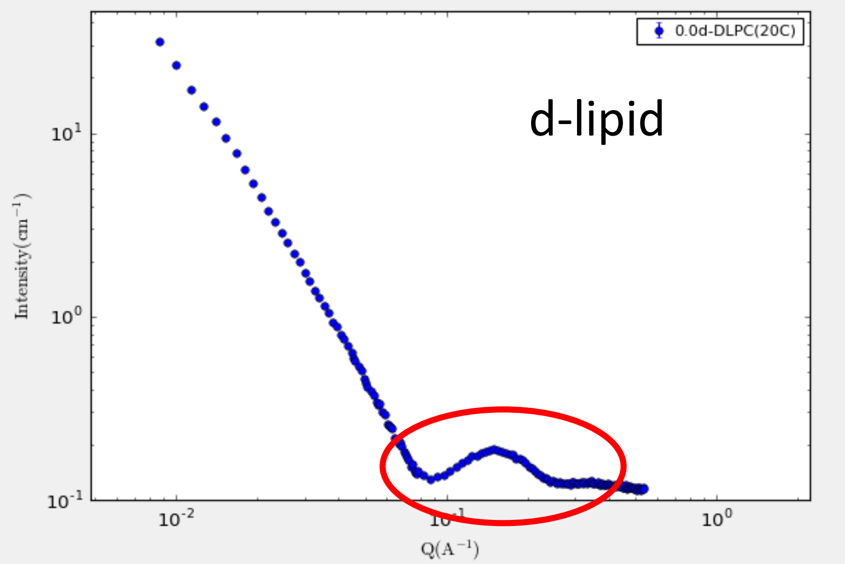


Y.A. Hassan, E.E. Dominguez-Ontiveros / Nuclear Engineering and Design 238 (2008) 3080–3085

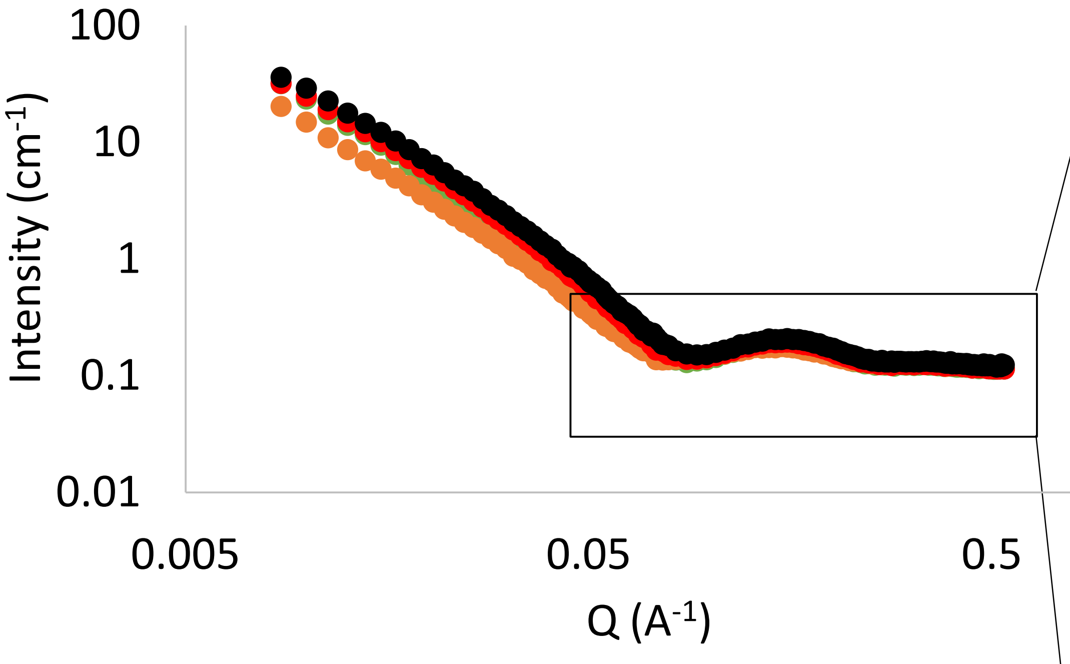


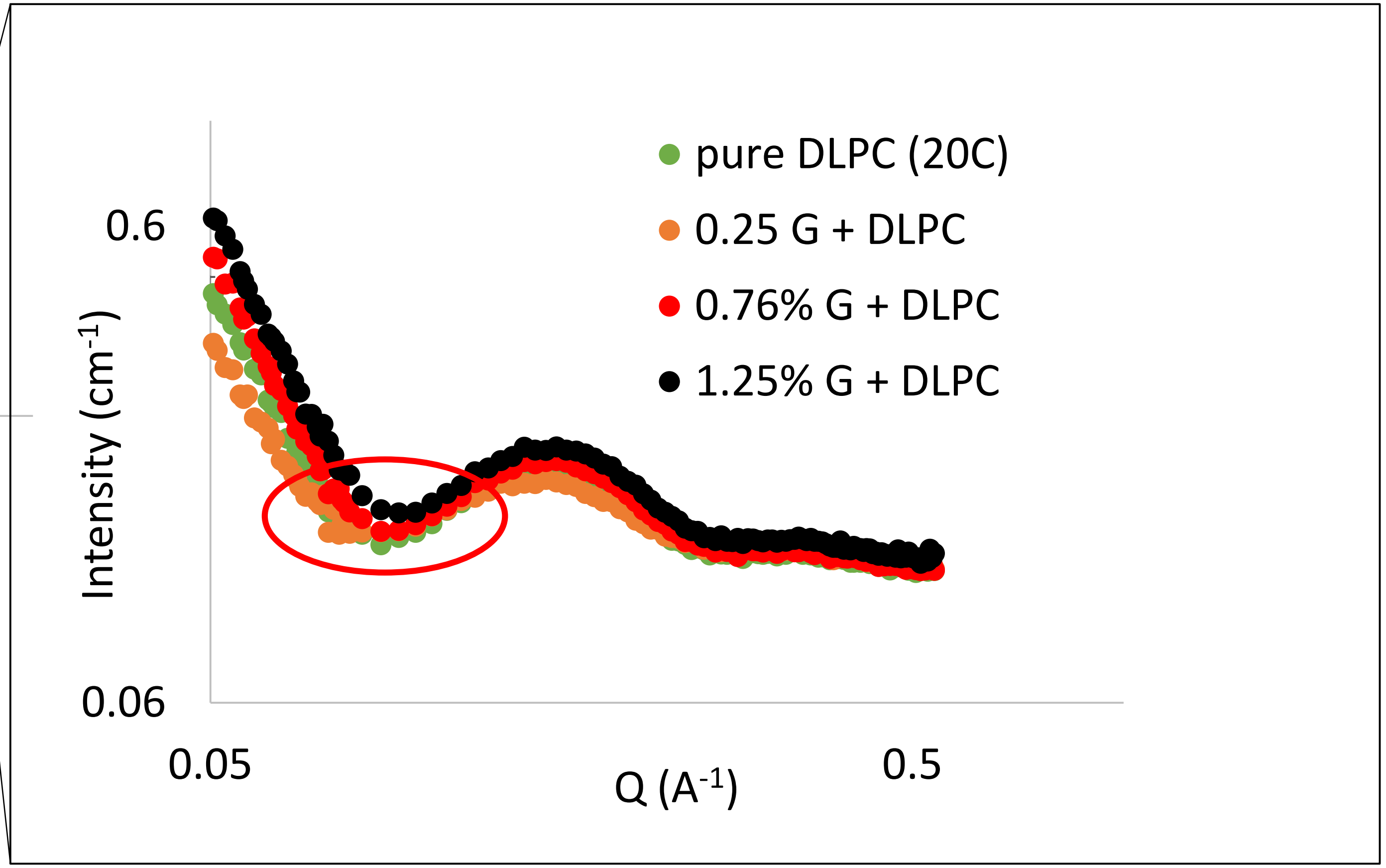


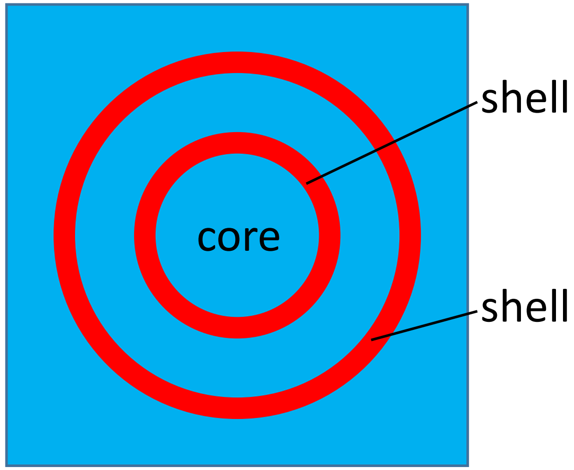




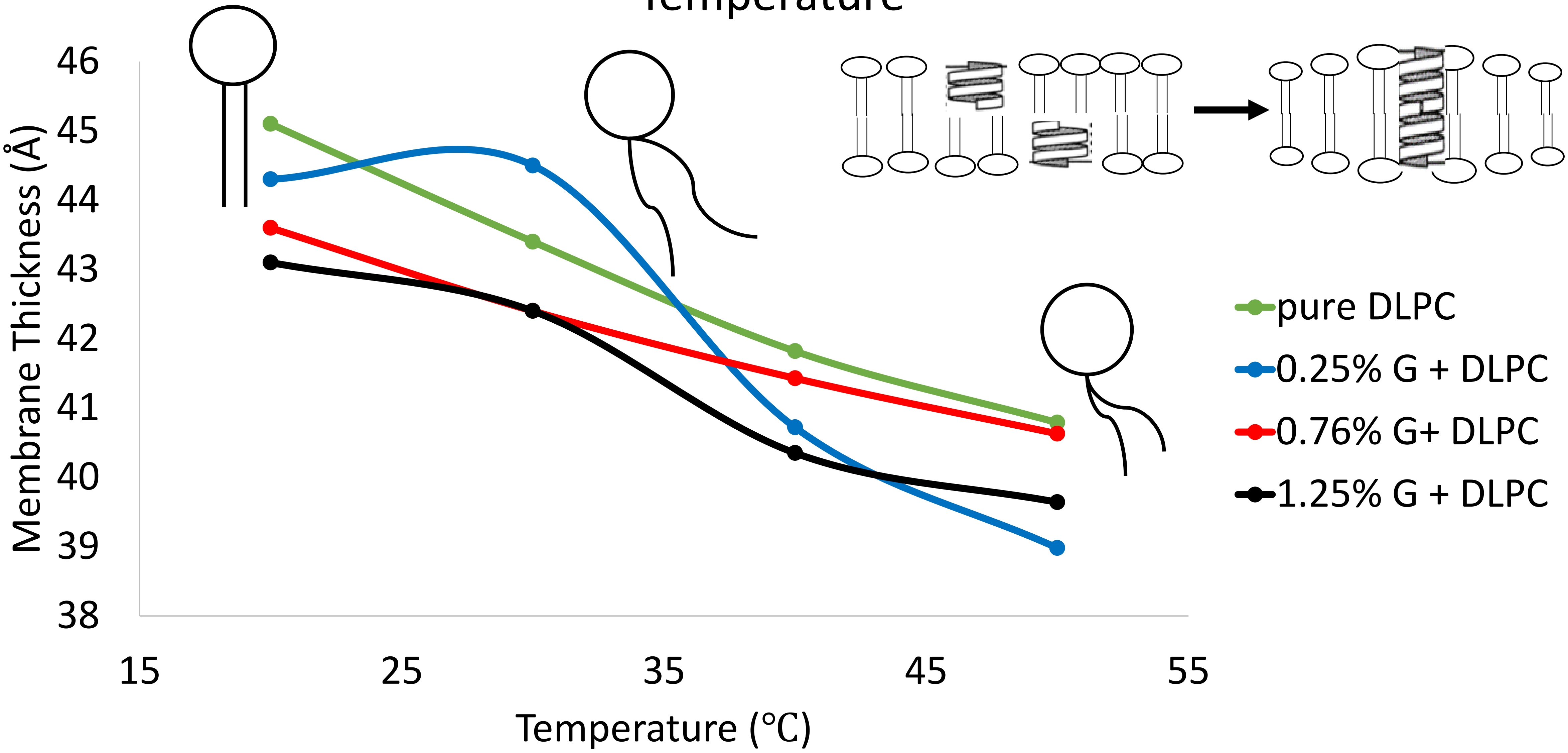
Raw SANS Data for Deuterated DLPC Samples (20°C)



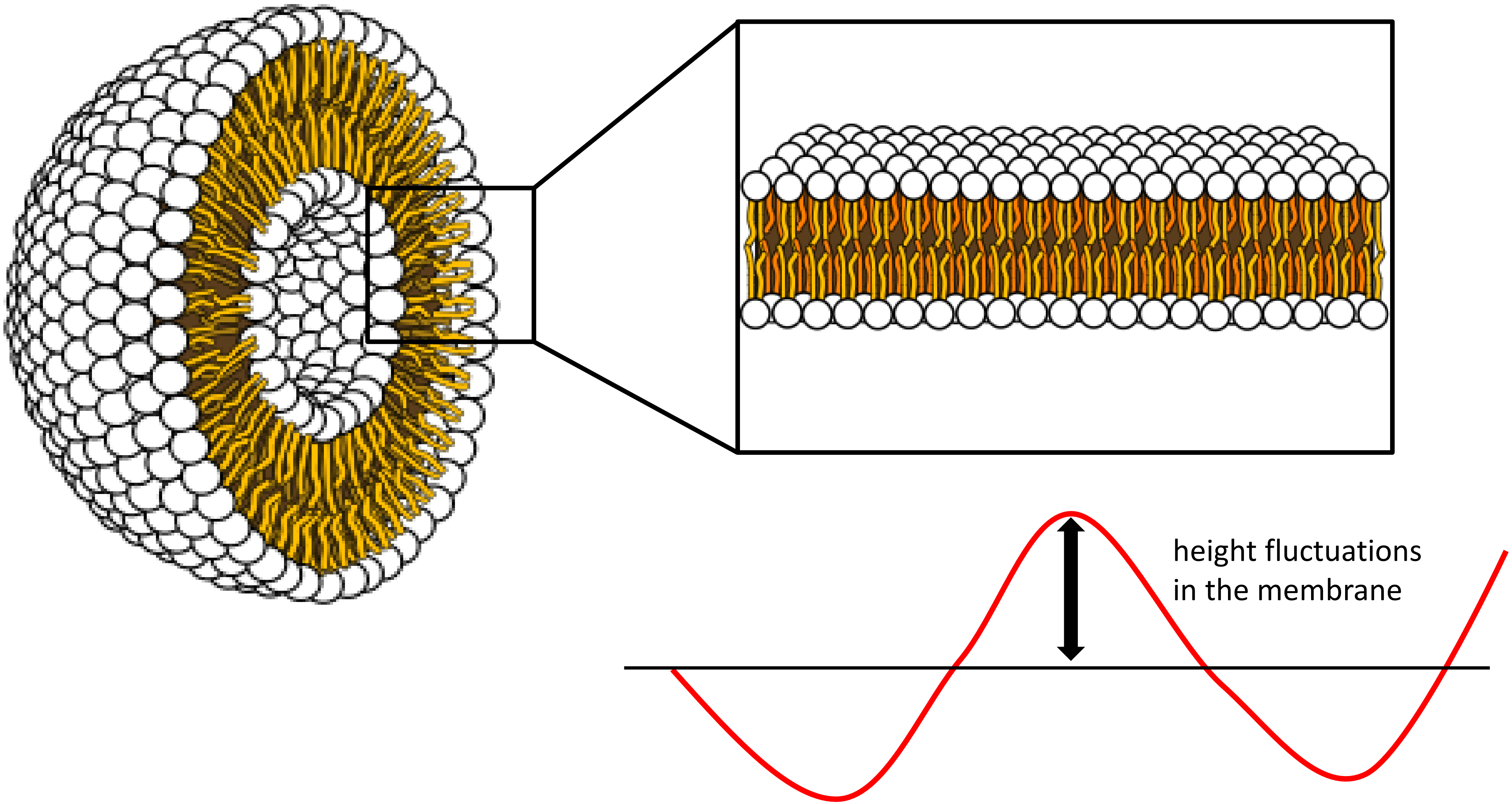




Membrane Thickness Decreases with Increasing Temperature



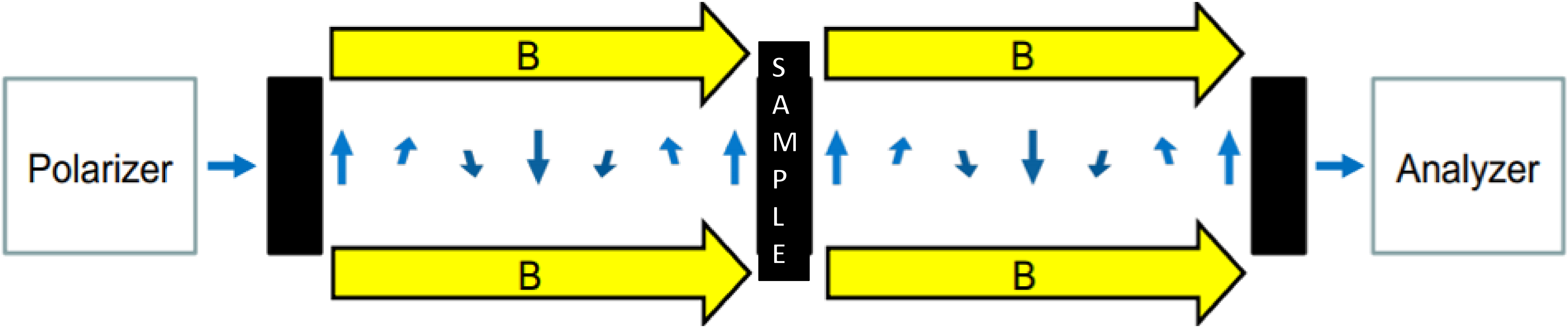
https://ac.els-cdn.com/S0005273607001848/1-s2.0-S0005273607001848-main.pdf?_tid=b0f57ec4-9014-4a1f-9c21-dbea993914cf&acdnat=1533184309_7906058a99389690d5d5c648b174e460



height fluctuations in the membrane

https://upload.wikimedia.org/wikipedia/commons/c/c6/Phospholipids_aqueous_solution_structures.svg

Neutron Spin Echo (NSE)



- Takes advantage of a neutrons Larmor Precession
- Differences in precession are analyzed
- Basic information about the structure and dynamics of the matter

https://neutrons.ornl.gov/sites/default/files/Zolnierczuk_Introduction_to_NSE2.pdf

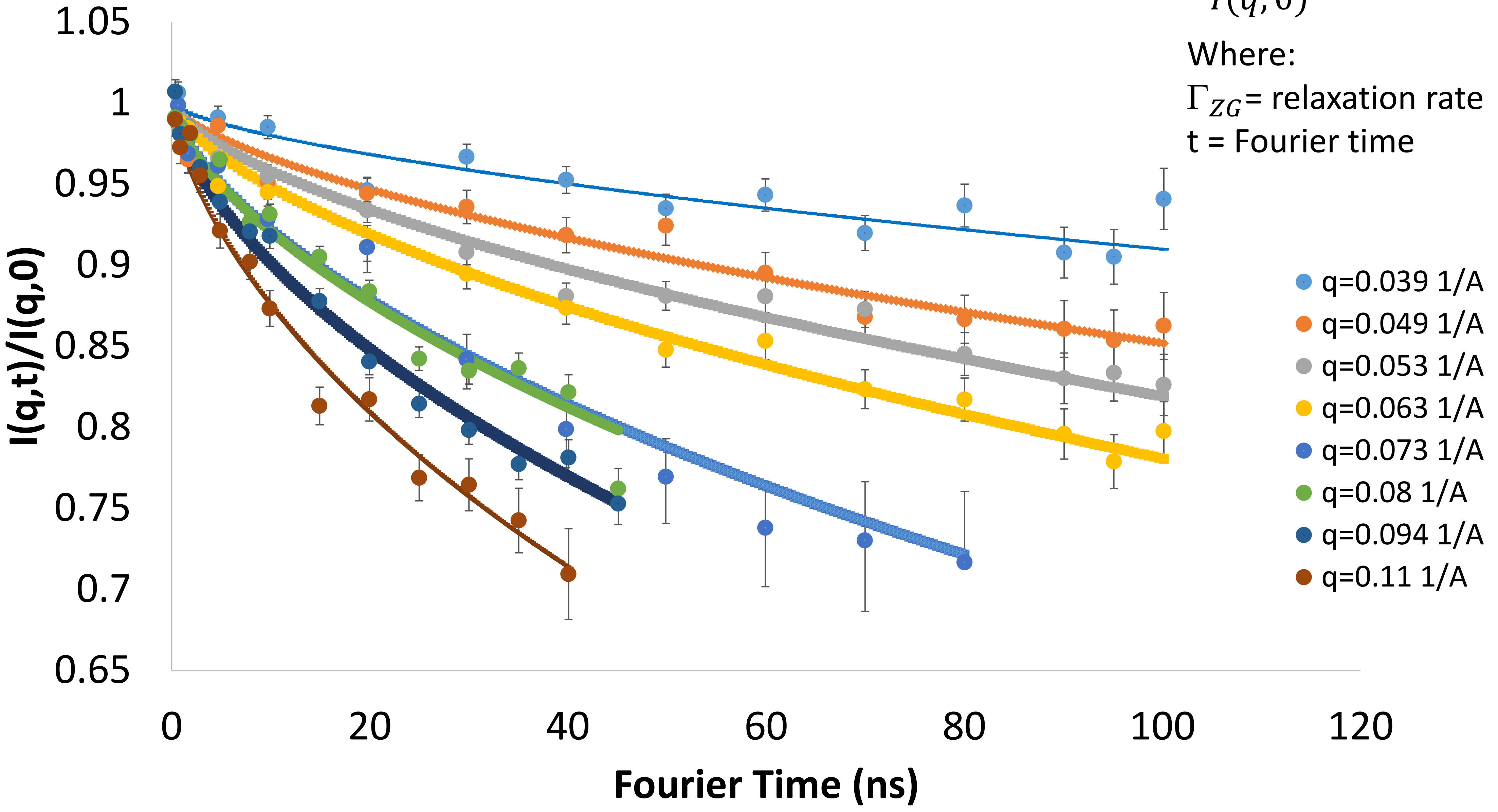
q-t correlation for pure DLPC

$$\frac{I(q,t)}{I(q,0)} = \exp[\,-(\Gamma_{ZG}t)^{\frac{2}{3}}]$$

Where:

Γ_{ZG}= relaxation rate

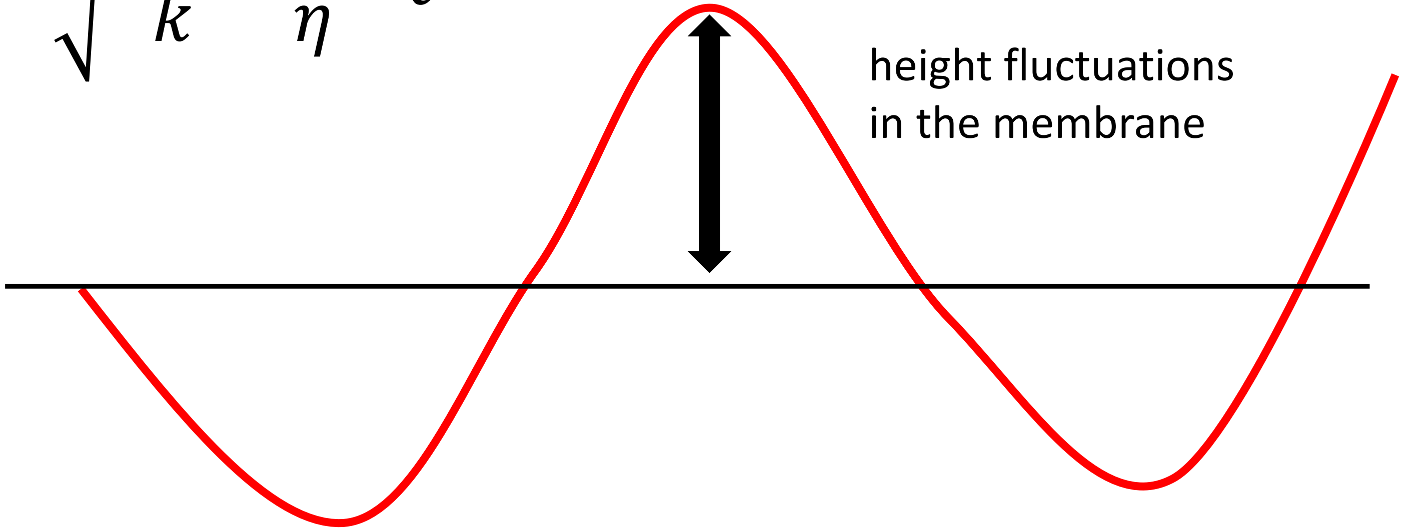
t = Fourier time



RELAXATION RATE

Collective height fluctuations can be used to quantify membrane elastic bending modulus from NSE experiments with the following equation:

$$\Gamma_{\mathrm{ZG}} = 0.0069 \sqrt{\frac{k_{\mathrm{B}}T}{k}} \frac{k_B T}{\eta} Q^3$$



Where:

Γ_{ZG} = relaxation rate

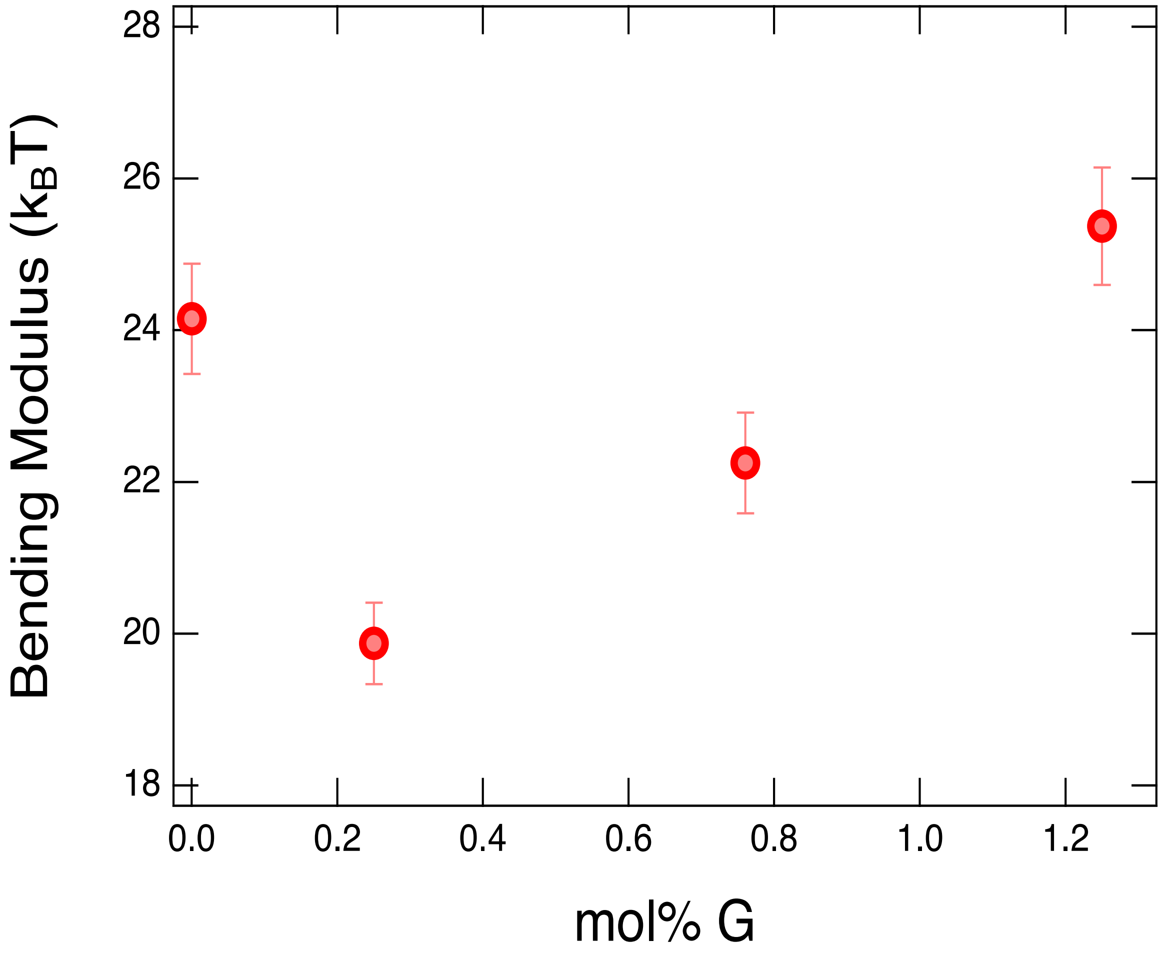
k_{B} = Boltzmann constant

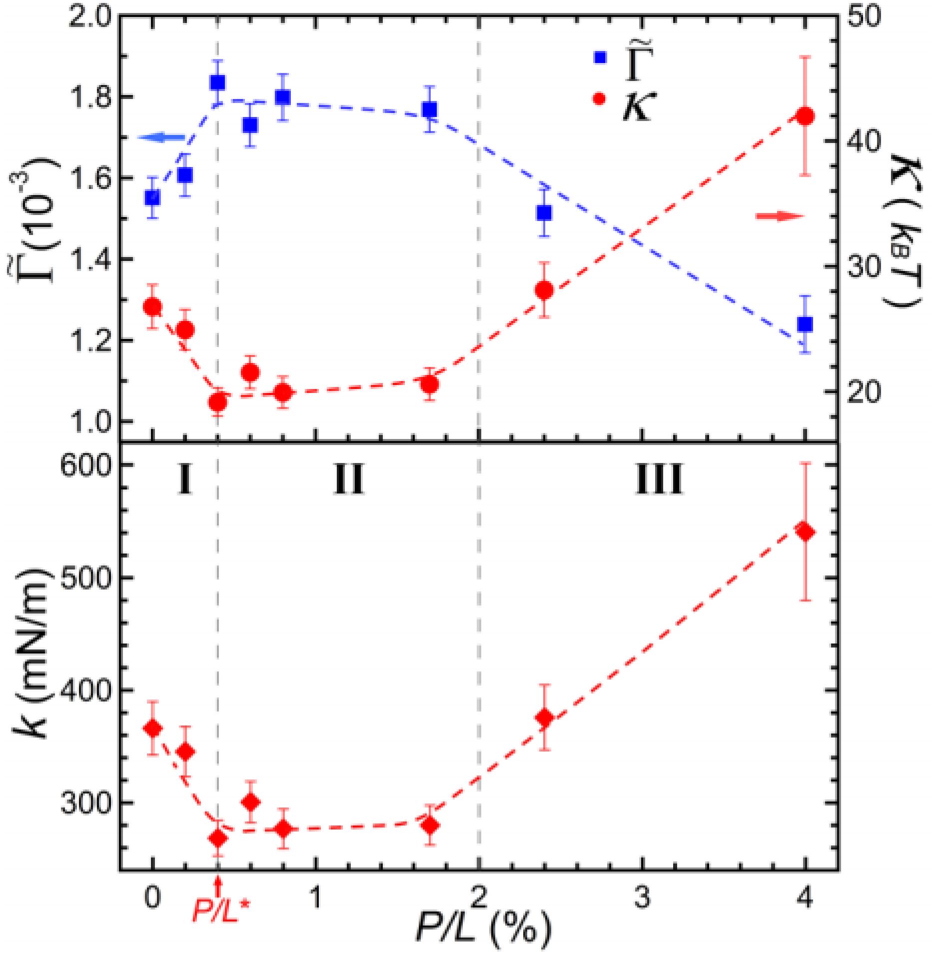
k= Bending modulus

η= solvent viscosity

NSE Results are Similar to Previously Conducted Experiments

Membrane Elasticity Increases

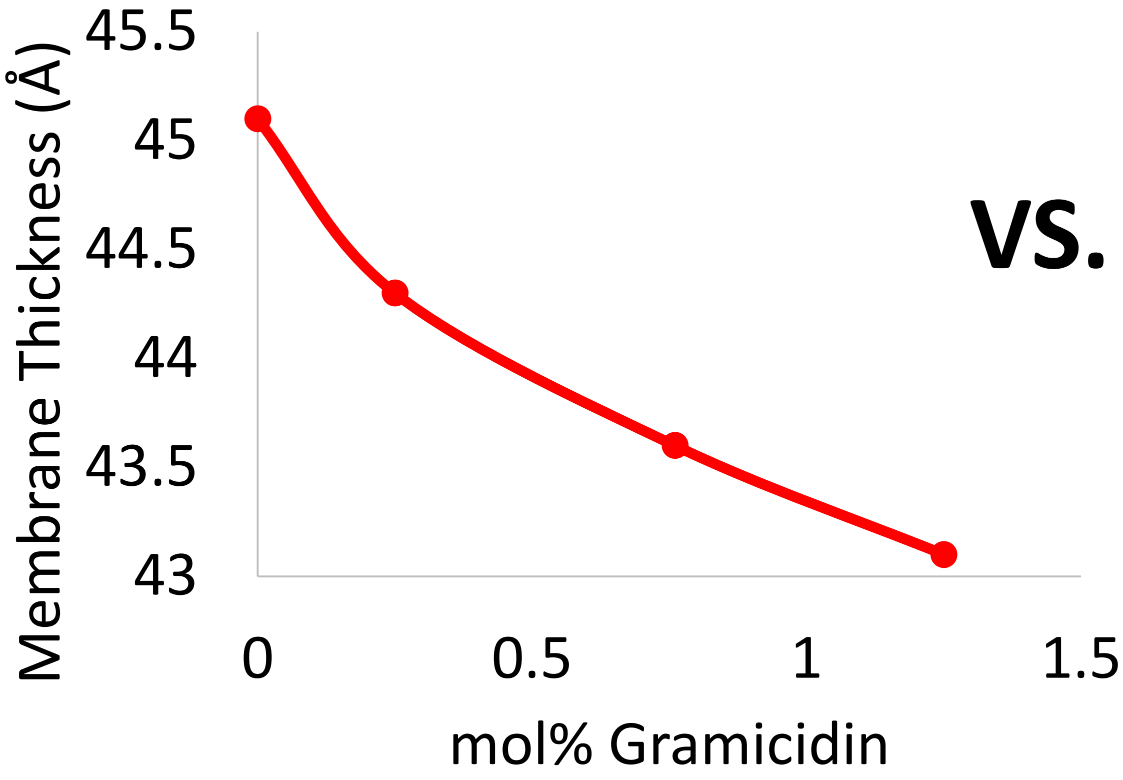




Lee et. al. Phys. Rev. Letter, 2010, 038101

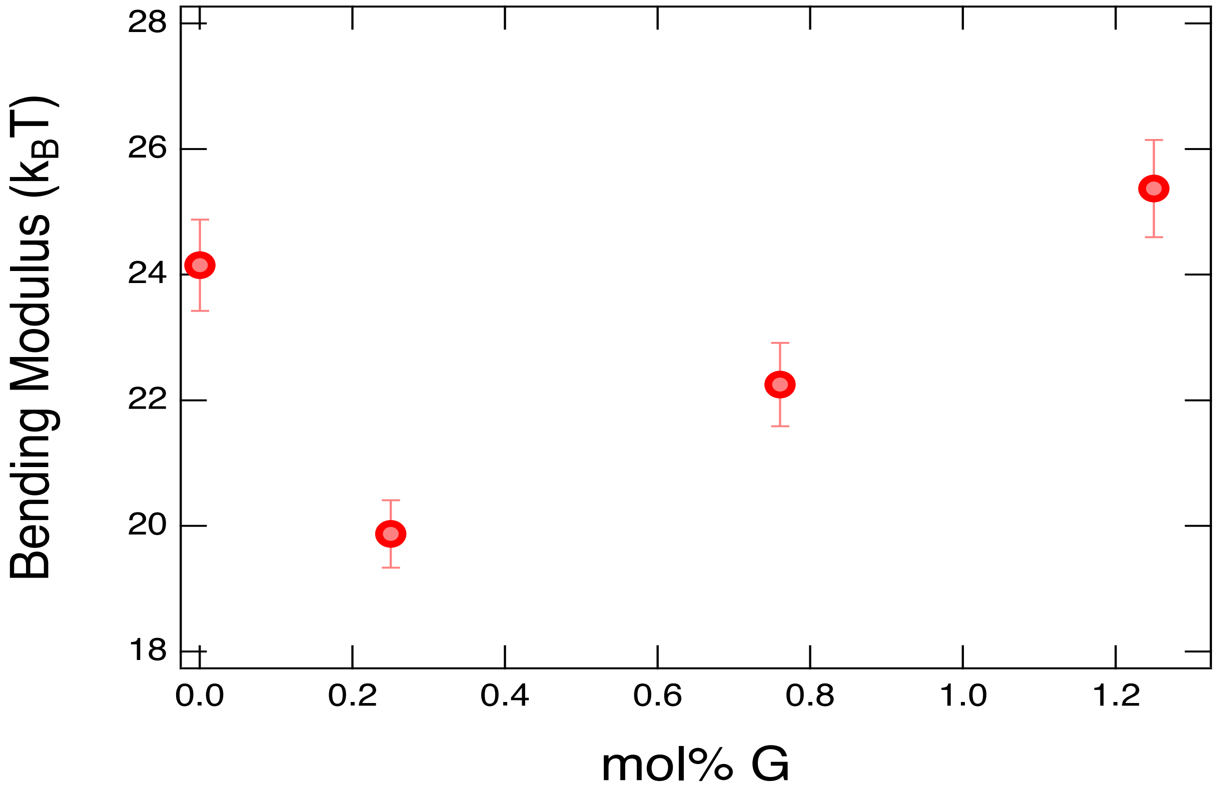
Contrasting Trends

Membrane Thickness Decreases with Increasing mol% Gramicidin



VS.

Membrane Elasticity Increases with Increasing mol% Gramicidin



FUTURE WORK

We can solve for the Area Compressibility Modulus ($\mathrm{K_A}$) with the Thin Sheet Theory:

$$\mathrm{K_A} = \frac{\beta K}{d_t^2}$$

Where:

$\mathbf{K_A} = \textit{area compressibility modulus}$

$\boldsymbol{\beta} = \textit{coupling constant between membrane leaflets}$

$\boldsymbol{K} = \textit{bending modulus}$

$\boldsymbol{d_t} = \textit{membrane thickness}$

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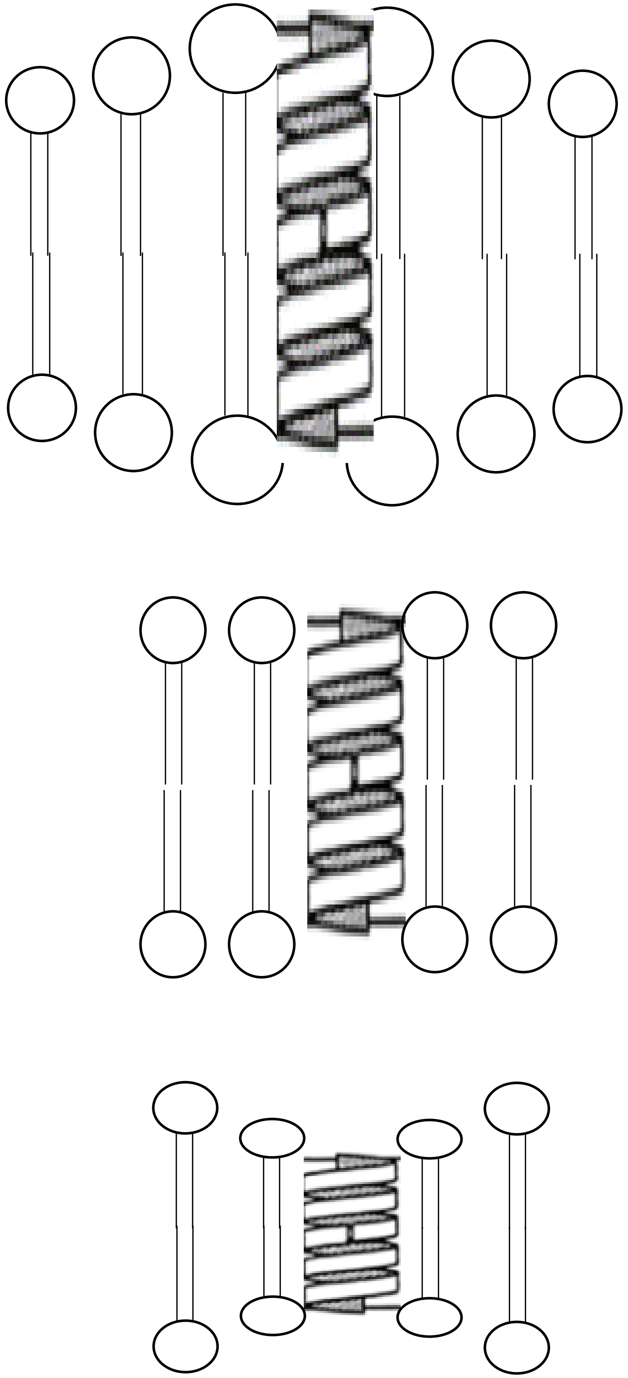
- Dr. Michael Zhang
- Dr. Joseph Dura and Dr. Julie Borchers
- NIST SURF director Dr. Brandi Toliver
- NCNR staff
- My fellow SURFers
- Center for High Resolution Neutron Scattering

NSF CHRNS CENTER FOR HIGH RESOLUTION NEUTRON SCATTERING NIST

This investigation was sponsored by NIH/NIGMS MARC U*STAR T34 HHS 00001 National Research Service Award to UMBC

FUTURE WORK



We can solve for the Area Compressibility Modulus ($\mathrm{K_A}$) in two ways:

Thin Sheet Theory

$$\mathrm{K_A} = \frac{\beta K}{d_t^2}$$

Statistical Mechanics

$$\mathrm{K_A} = \frac{k_B T}{\sigma_A^2 A_0}$$

Which can be combined as :

$$K = \frac{k_B T}{\beta \sigma_A^2} \frac{d_t^2}{A_0}$$

Where:

$\boldsymbol{d_t} = membrane\ thickness$

$\boldsymbol{A_0} = lipid\ head\ area$

$\boldsymbol{= \frac{V_L}{d_t}}$

$\boldsymbol{\beta} = coupling\ \ constant$

$between\ membrane\ leaflets$

$\boldsymbol{k_B} = Boltzmann\ Constant$

$\boldsymbol{\sigma_A} = \ fractional\ area\ change$

$\boldsymbol{K} = bending\ modulus$