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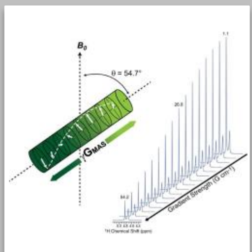

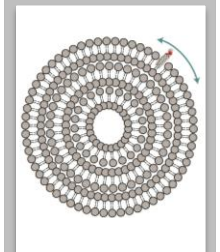

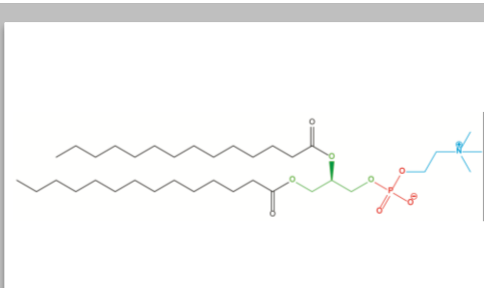

Lipid Bilayers on Silica Beads

A Study of the Fluidity and Diffusion of Lipids on Silica-Aluminum Beads using MAS and HRMAS NMR

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7/31/14

** Bachelor of Science in Chemical Engineering, UNM, May 2015*

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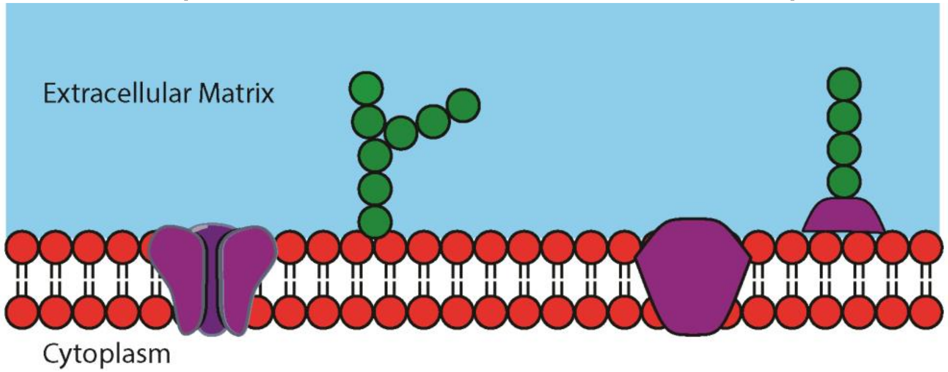
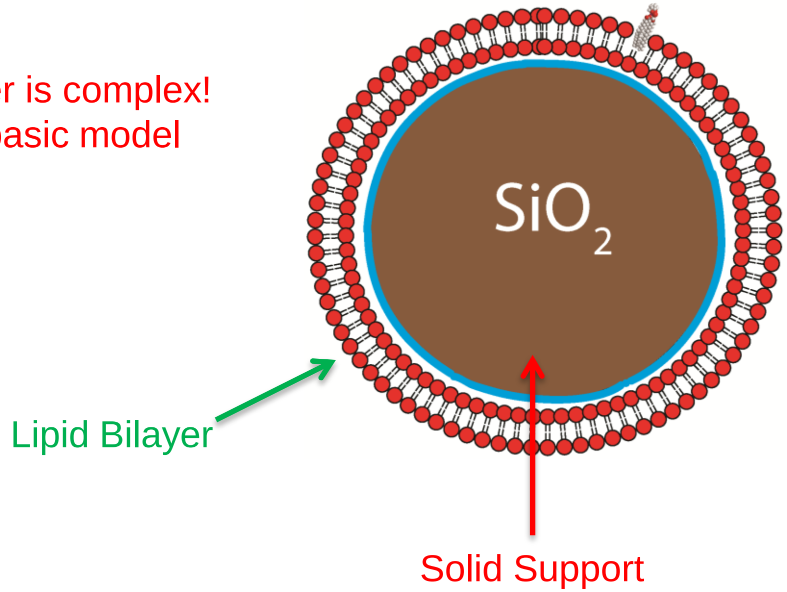
Importance of Lipid Bilayers

Basic unit of the cell membrane

- Raft formation
- Ion and molecule transportation
- Incorporation of membrane proteins
- Participation in intra- and extracellular processes



Lipid bilayer is complex!
Start with basic model



Biotechnological Applications[1]:

- The design of phantom cells
  - Study the role of cell adhesion in immunological recognition processes
  - Study the interplay of specific key-lock forces between receptors and universal forces (dynamic repulsion forces)
- Design of smart biosensors
  - Bilayer used as a thin electrical insulator
  - Bilayer used as a matrix for the incorporation of receptors (i.e. lipid-coupled antigens or antibodies)
  - Electrical monitoring of ligand binding
    - Gold-covered or SnO4/InO4- covered supports or metal-oxide-semiconductor field-effect transistors (MOSFETs)

[1] Sackmann, E. Science 271.5245 (1996)

Supported Bilayers on Solid Substrates

Solid substrates allow lipid bilayer investigation that is difficult in bulk solution

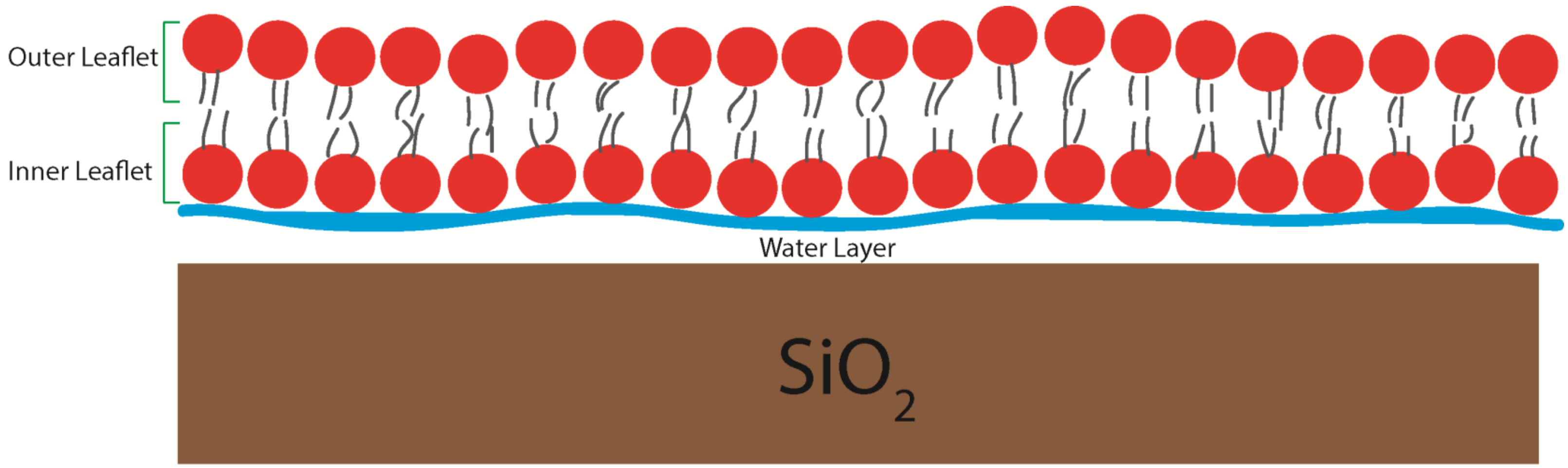
- Biological functionality must be preserved (i.e. lateral fluidity)
- Silica (SiO_2) provides the necessary balance of adhesion, repulsion, and hydration forces for fluidity

Lipid Investigation:

- Membrane fluidity
- Membrane structure disorder
- Diffusion constants of lipid bilayer
- Diffusion constant of water layer
- Physical properties of bilayer and solid substrate

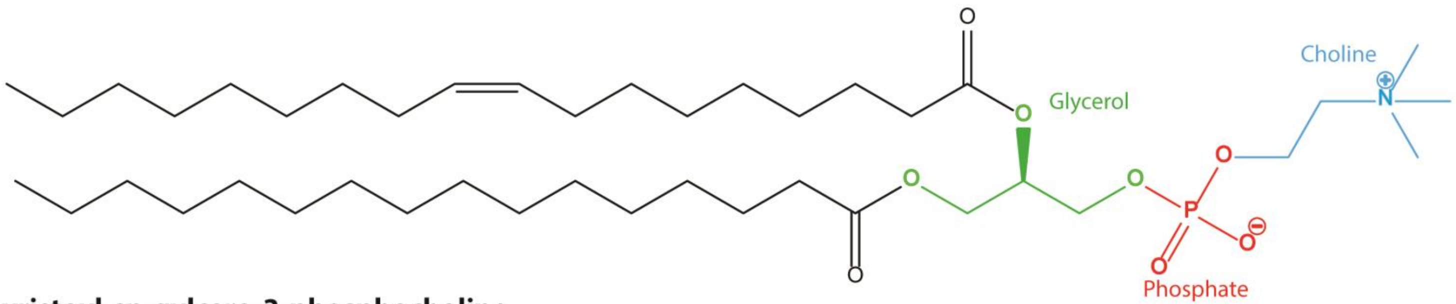
Solid Supports:

- SiO_2 with varying aluminum concentrations
- Porous vs. nonporous
- Spherical vs. non-spherical

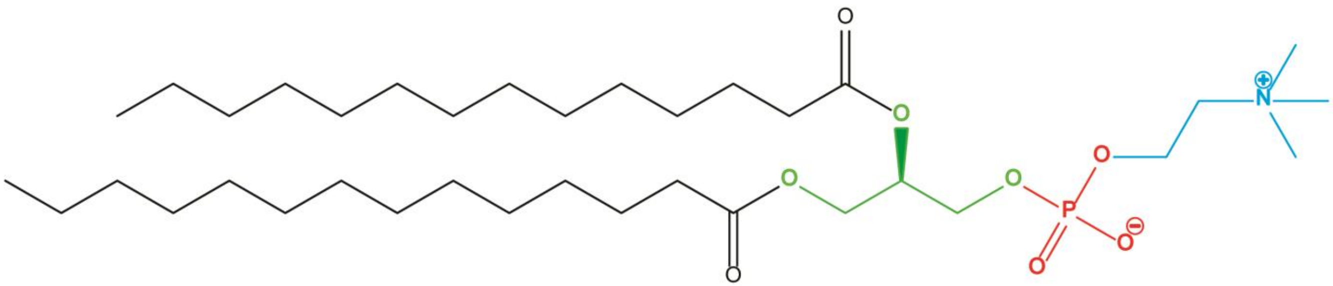


Investigated Lipids

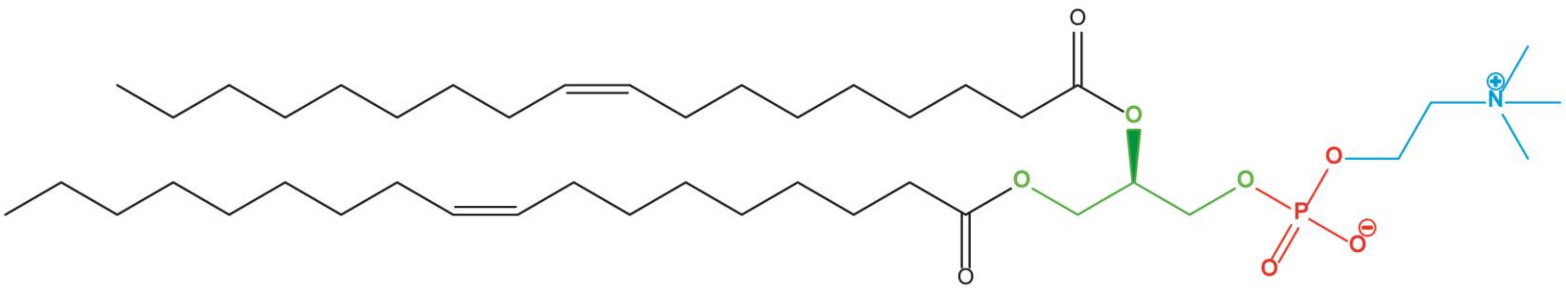
POPC = 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine



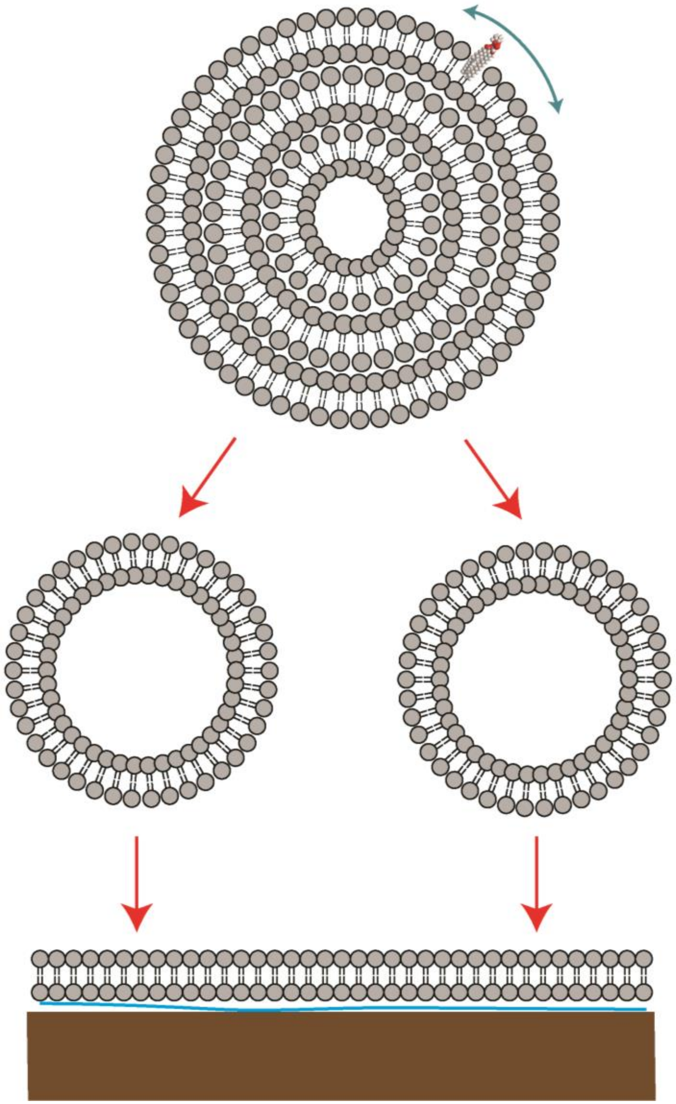
DMPC = 1,2-dimyristoyl-sn-gylcero-3-phosphocholine



DOPC = 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine



MLV and SLB Preparation



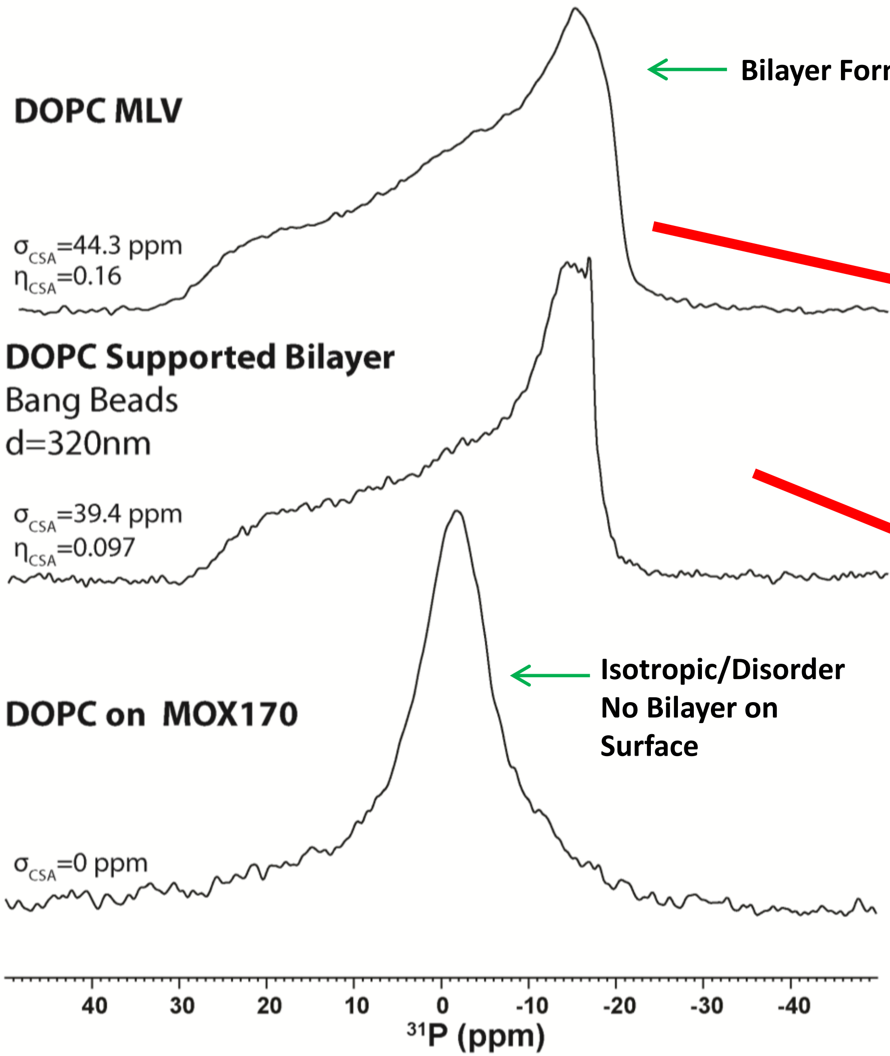
Multilamellar Vesicle (MLV):

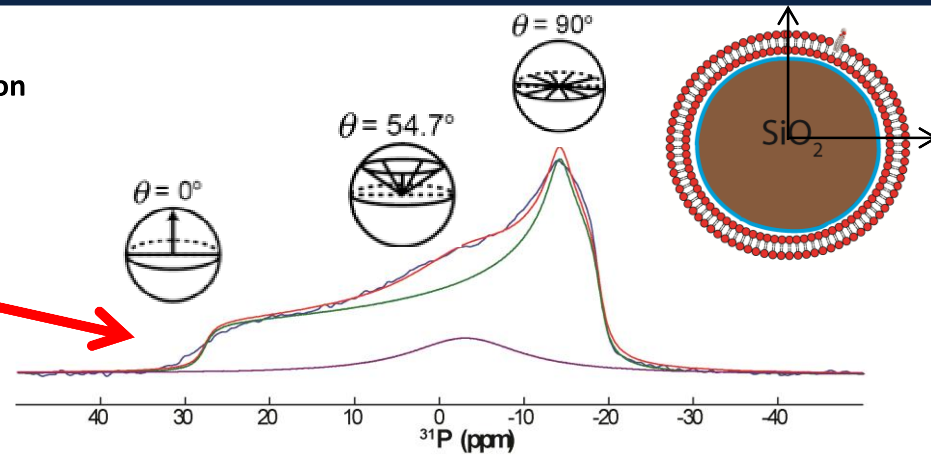
- Lipid film formed from rotary evaporation with chloroform
- Dispersed in Nanopure DI water
- Vortexed, freeze-thaw cycles to form MLV dispersion

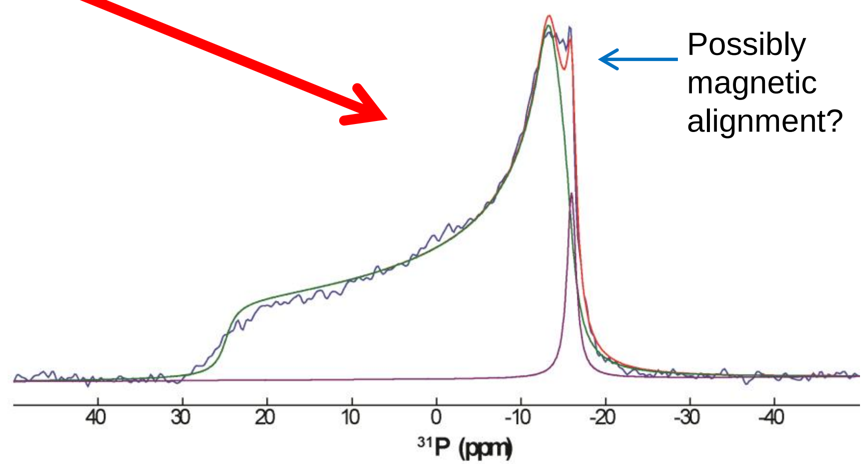
Supported Lipid Bilayer (SLB):

- Rod sonicated MLVs
- Mixed with lyophilized Bang beads (d=320nm, Bangs Laboratories, Inc.)
- Sample placed in water bath overnight and vortexed every hour
- Multiple washes and centrifugations to isolate SLBs

Bilayer Formation Confirmed using Static ^{31}P NMR

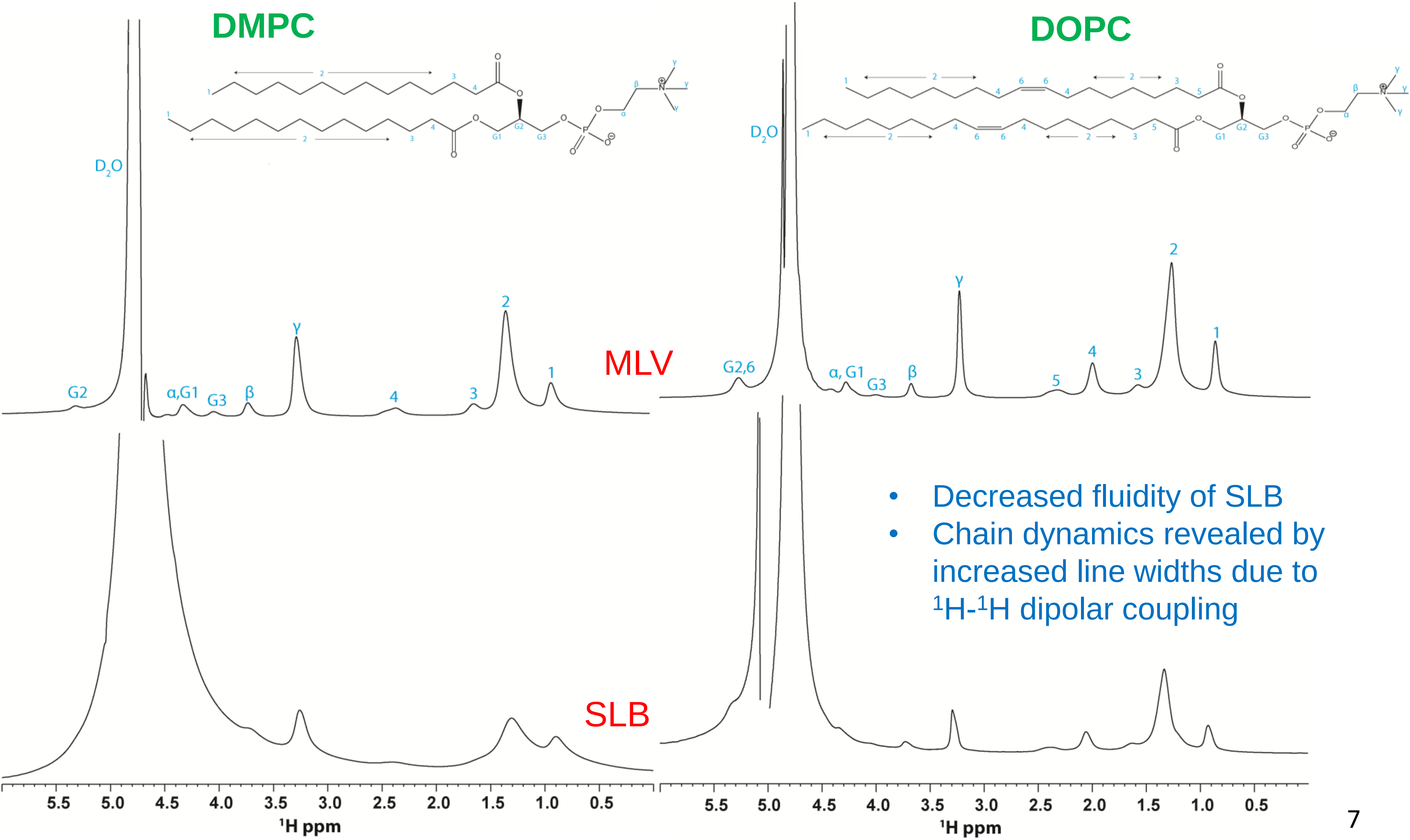






^{31}P CSA powder pattern used to confirm bilayer structure

Investigation of MLV and SLB using ^{1}H NMR

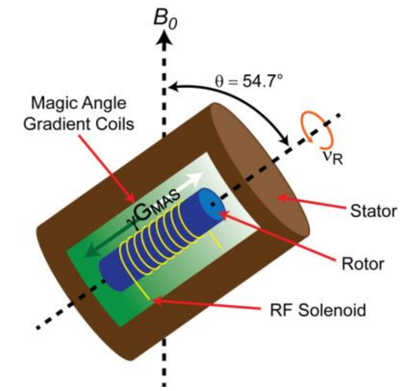


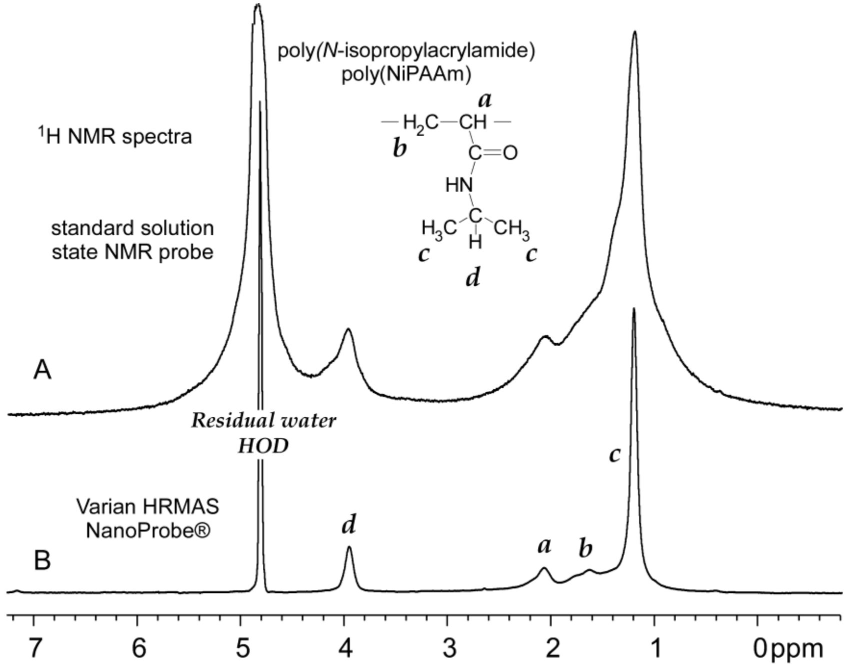
- Decreased fluidity of SLB
- Chain dynamics revealed by increased line widths due to ^{1}H-^{1}H dipolar coupling

HRMAS PFG NMR

High resolution magic angle spinning (HRMAS) pulsed field gradient (PFG) nuclear magnetic resonance (NMR)

- For liquid/solid samples (lipids) HRMAS produces improved resolution and sensitivity
- Resolution is improved because the Hamiltonians describing the dipolar, CSA and magnetic susceptibility interactions contain an orientational component $(3cos^2\theta - 1)$
    - θ is the angle between the rotor spinning axis and the magnetic field
    - At the "magic angle" these interactions vanish





Inhomogeneity Dipolar Equations

$$B(r_i, \theta_i, \phi_i) = \sum_i \frac{M_j}{r_{ij}^3} \frac{1}{2} (3cos^2\theta_{ij} - 1)$$

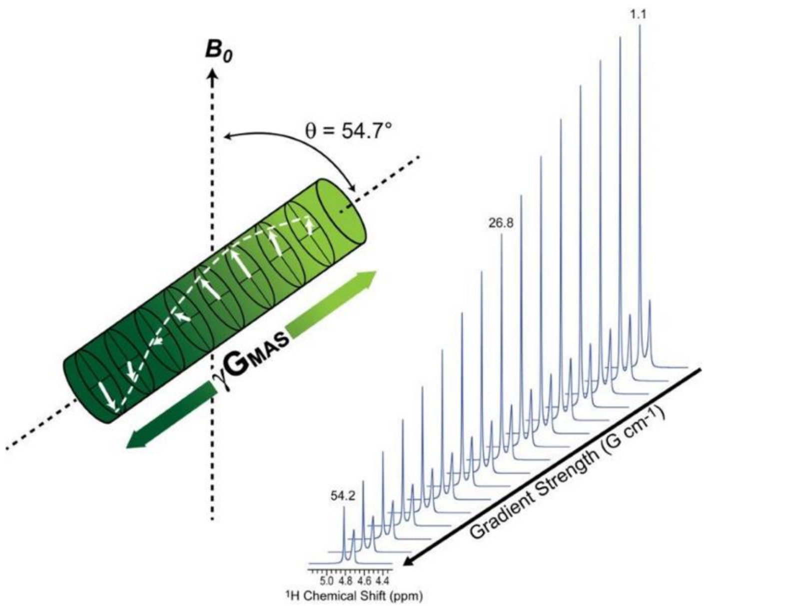
0

$$B(r_i, \theta_i, \phi_i, t) = \sum \frac{M_j}{r_{ij}^3} [\frac{1}{4}(3cos^2\beta - 1)(3cos^2\beta'_{ij} - 1) + \frac{3}{4} sin2\beta sin2\beta'_{ij} \cos(\omega_R t + \phi_{ij}) + \frac{3}{4} sin^2\beta sin^2\beta'_{ij} \cos(2\omega_R t + 2\phi_{ij})]$$

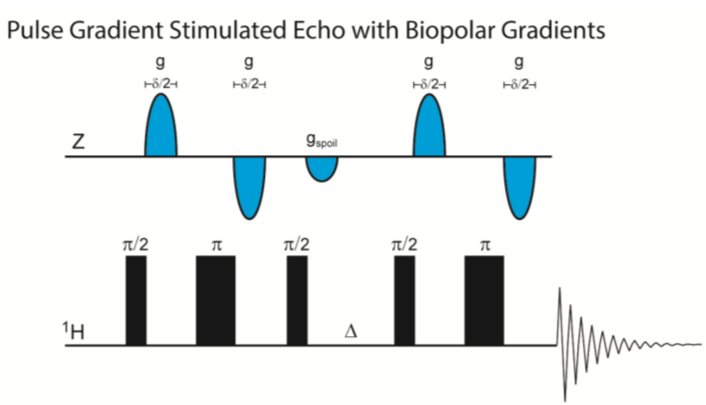
$(3cos^2\beta - 1) = 0$ when $\beta = 54.7°$

HRMAS PFG NMR

With the gradient coil along the magic angle, PFG experiments can be performed under MAS conditions



Todd M. Alam, Janelle E Jenkins, HR-MAS NMR Spectroscopy in Material Science



During PFG diffusion experiments, the application of a gradient "tags" a spin with a phase that is related to its spatial position

If the spin diffuses (changes spatial position) during Δ

- The dephasing is not refocused
- Signal intensity decreases

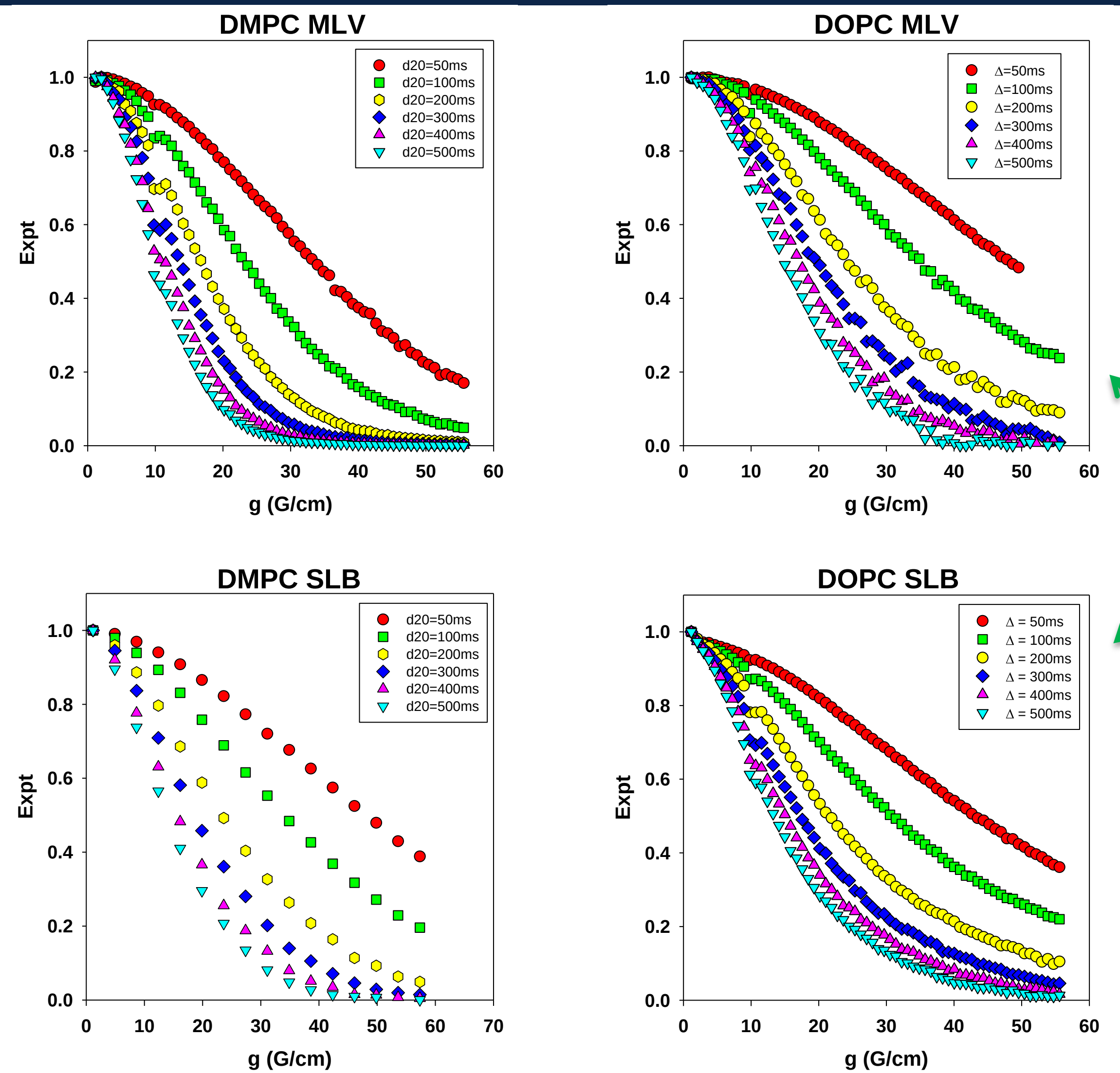
The loss in signal intensity with increasing gradient strength is related to the self-diffusion rate in the supported bilayer equation

$$ln\left(\frac{I}{I_0}\right) = -\frac{2}{3}kD + \frac{2}{45}(kD)^2$$

$$k = 4\gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)$$

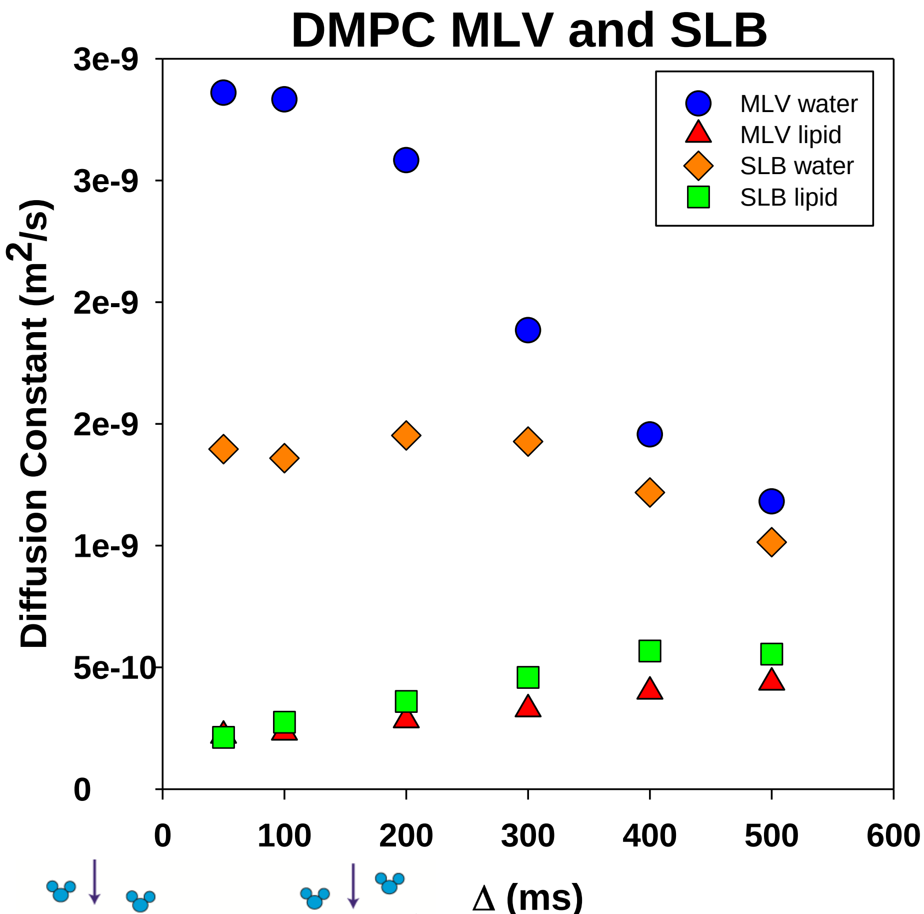
I=integrated signal intensities
γ=gyromagnetic ratio
g=gradient strength
δ=gradient pulse length
Δ=diffusion time between pulses
D=translational self-diffusion coefficient

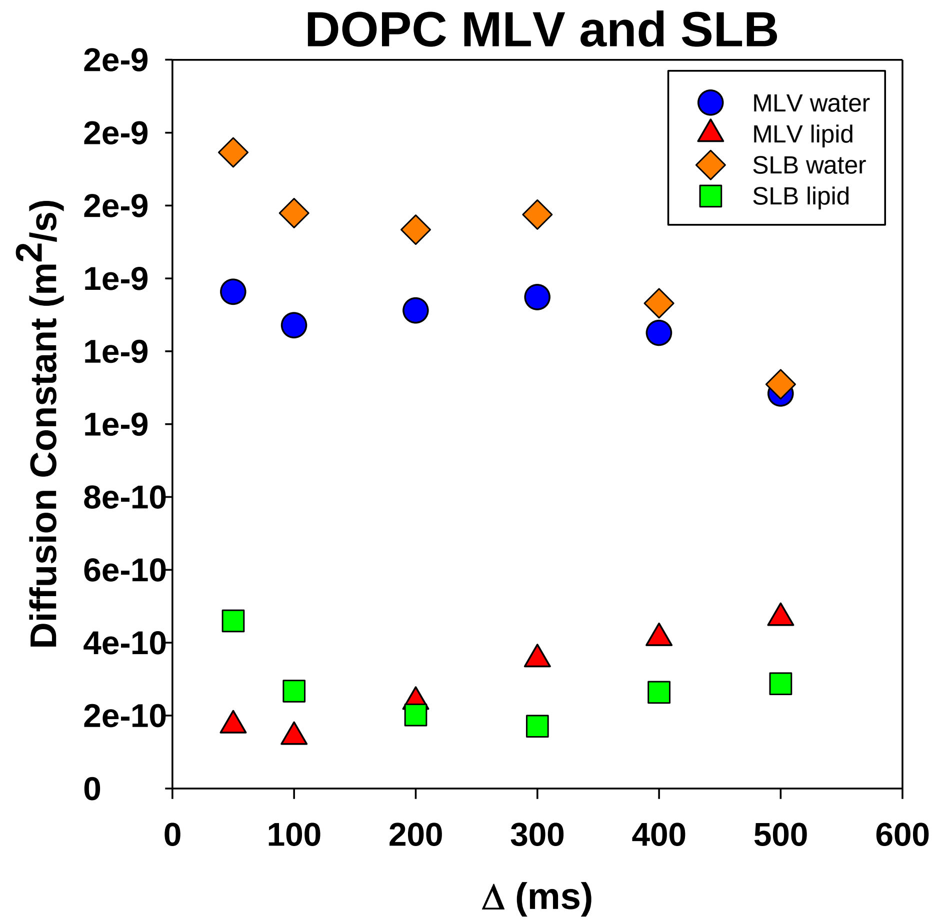
PFG NMR Diffusion

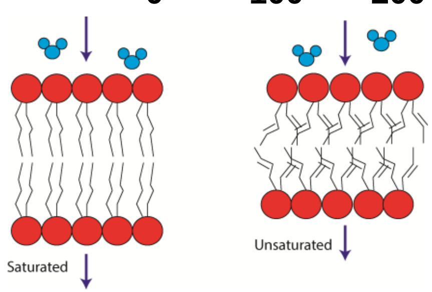


- Decay of signal intensity is a measure of diffusion rate
- Diffusion rates for MLVs are similar to SLBs
- DMPC diffuses faster than DOPC for MLV and SLB

MLV and SLB Lipid Diffusion







- Lipids diffuse an order of magnitude slower than water
- Lateral diffusion of lipids and diffusion of water are similar for MLVs and SLBs

Conclusions

- DMPC and DOPC were successfully deposited on silica beads to form SLBs
- PFG HRMAS NMR was used to determine the diffusion rates for MLVs and SLBs
- Lipids diffused an order of magnitude slower than water as predicted
- Diffusion constants for water and lipid didn’t change significantly when the lipids attached to the bead
- First NMR measurement of lipid lateral diffusion on SLBs!!

Future Directions and Acknowledgements

Future Directions

- Attach POPC to Bang beads
- Collect 1D and 2D, including diffusion data, for POPC
- Form SLBs with different types of beads (nonporous vs. porous, spherical vs. globular)
- Investigate diffusion of lipid MLVs and SLBs as a function of temperature
- Study T1 spin-lattice relaxation and T2 spin-spin relaxation for lipid MLVs and SLBs
- Study the properties of the water layer between the silica and the lipid bilayer

Acknowledgements

- Todd Alam
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- Elisa Friedmann
- James Thomas
- Vitaliy Dernov

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