Investigation of the Dynamic and Diffusional Properties of Supported Membranes on Bioinspired Materials using HRMAS NMR

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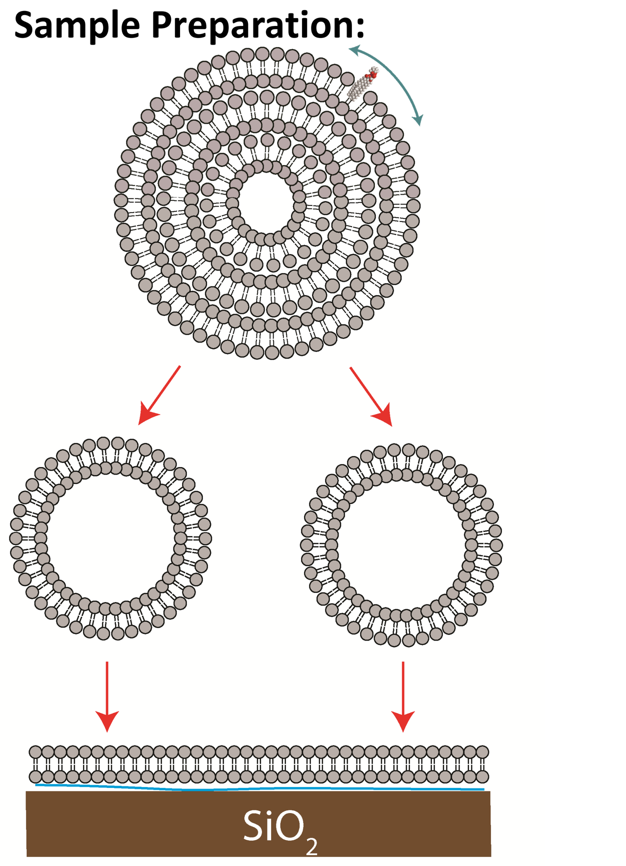
Photograph by Sarah K. McIntyre

Abstract/Introduction

This poster describes recent summer efforts to characterize lipid bilayers deposited on solid supported silica substrates. Supported lipids have received considerable attention due to their potential bio- and medical applications.[1-5] Lipid liposomes themselves are used as drug carriers in several commercial products, and through the rupture and fusion of liposomes formed from the sonication of lipid multilamellar vesicles (MLVs), they become attached to the silica surfaces to form a supported lipid bilayer (SLB). The SLBs are used as cell membrane mimics to investigate several cellular properties including membrane dynamics, protein-lipid interactions, surface-lipid interactions, and the participation of the lipid bilayer in intra-and extracellular processes.[6] Static ^{31}P nuclear magnetic resonance (NMR) was used to directly determine whether the liposomes attached to the silica substrate by investigating curvature disruptions in the bilayer structure. MLVs and SLBs of DOPC and DMPC were compared using ^{1}H, ^{31}P, ^{13}C, and 2D Nuclear Overhauser Effect Spectroscopy (NOESY) magic-angle spinning (MAS) NMR. Lateral translational diffusion of lipids in the membrane and the diffusion of water through the membrane was investigated using pulse field gradient (PFG) high-resolution magic-angle spinning (HRMAS) NMR diffusion experiments. MAS and HRMAS NMR improve spectral resolution by removing dipolar, chemical shift anisotropy (CSA), and magnetic susceptibility interactions described by the inhomogeneity dipolar Hamiltonians. By spinning the rotor at an angle the magic angle 54.7°, the Hamiltonians become zero, and the interactions are removed. Diffusion constants for the water and lipid environments in MLVs and SLBs were compared to determine how the spatial motion of the species was affected when the lipids attached to a solid support. These results provide insight into the fluidity and motion of the lipid bilayer as well as a new method to study the diffusion of the membrane using PFG HRMAS NMR.

Experimental

Sample 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

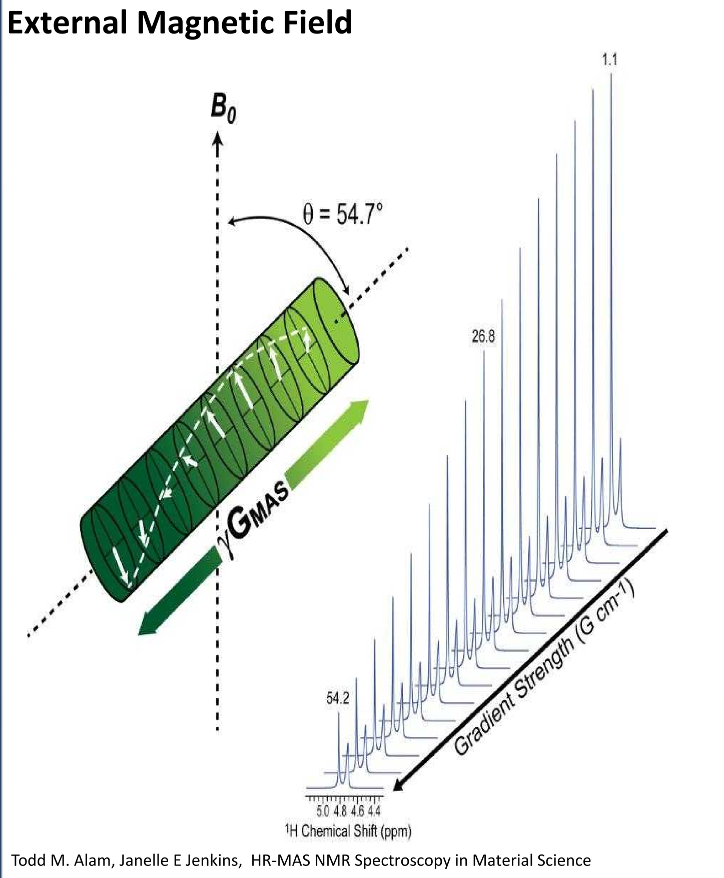
Supported Lipid Bilayer (SLB):

- 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

HRMAS NMR Spectroscopy:

^{1}H and ^{31}P experiments were performed on a Bruker Avance-I 400 Spectrometer using a 4mm MAS probe at 400.162 and 161.989 MHz, respectively. Diffusion and transverse spin-spin relaxation time (T_2) experiments were performed on a Bruker Avance-III Spectrometer at 600.135 MHz using a 4mm HRMAS probe at 298 K. A stimulated bipolar sequence with a gradient ramp containing 64 values and 32 scans was used to obtain diffusion coefficients. The self-diffusion coefficients (D) were determined from the signal decay using the signal intensities after gradient pulses where I and I(0) are the integrated signal intensities, D is the translational self-diffusion coefficient, γ is the gyromagnetic ratio, g is the gradient strength, δ is the duration of the gradient pulse, and Δ is the diffusion time between pulses. Relaxation times were obtained using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence.

External Magnetic Field



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

Supported Bilayer Equation[7]

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

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

Inhomogeneity Dipolar Equations

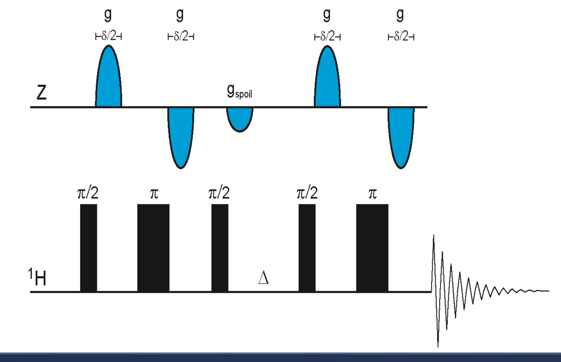
$$B(r_i, \theta_i, \phi_i, t) \sim \sum \frac{M_j}{r_{ij}^3}\left[\frac{1}{4}(3cos^2\beta - 1)(3cos^2\beta'_{ij} - 1)\right]$$

0

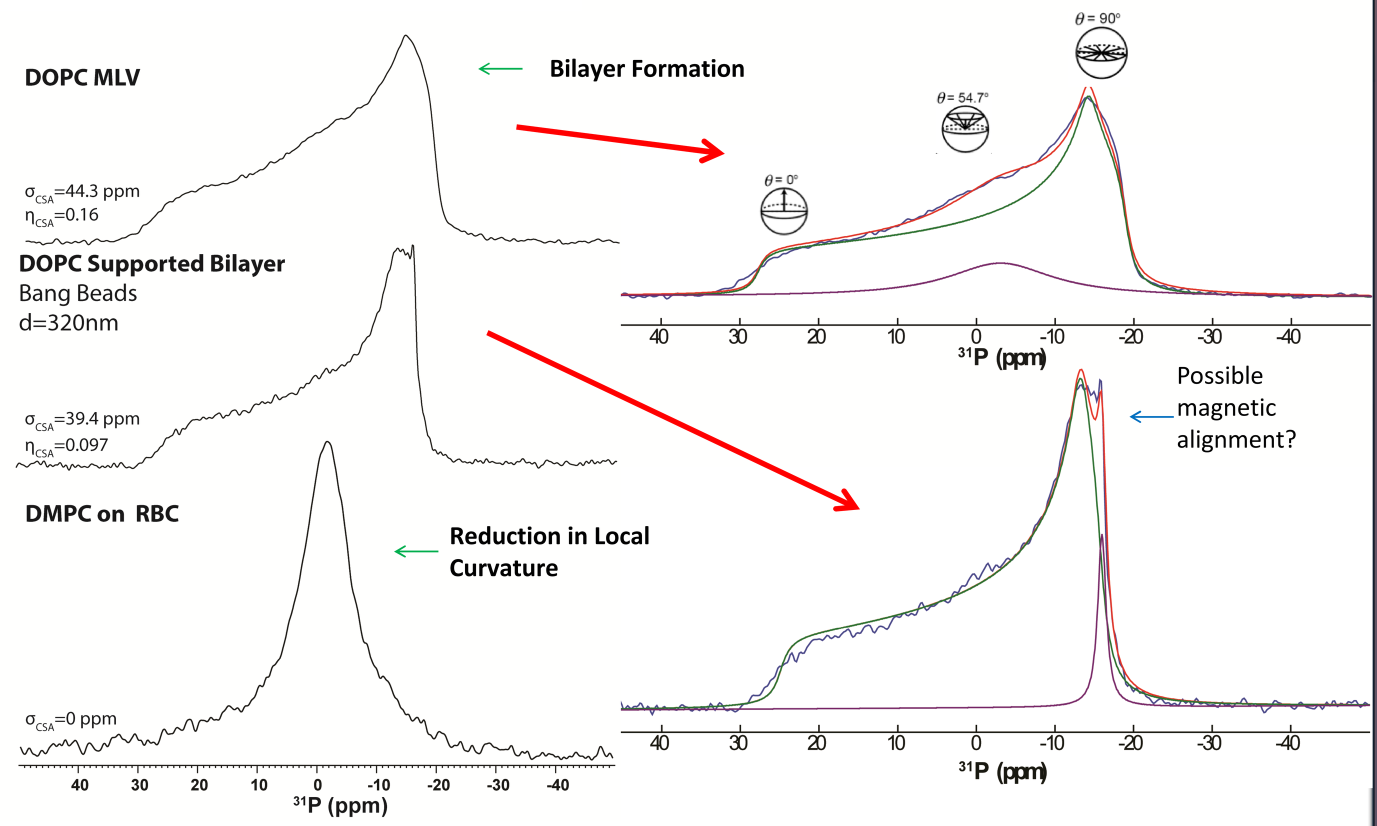
$(3cos^2\beta - 1) = 0$ when $\beta = 54.7°$

Magic angle at which magnetic susceptibility goes to zero

Pulse Gradient Stimulated Echo with Biopolar Gradients

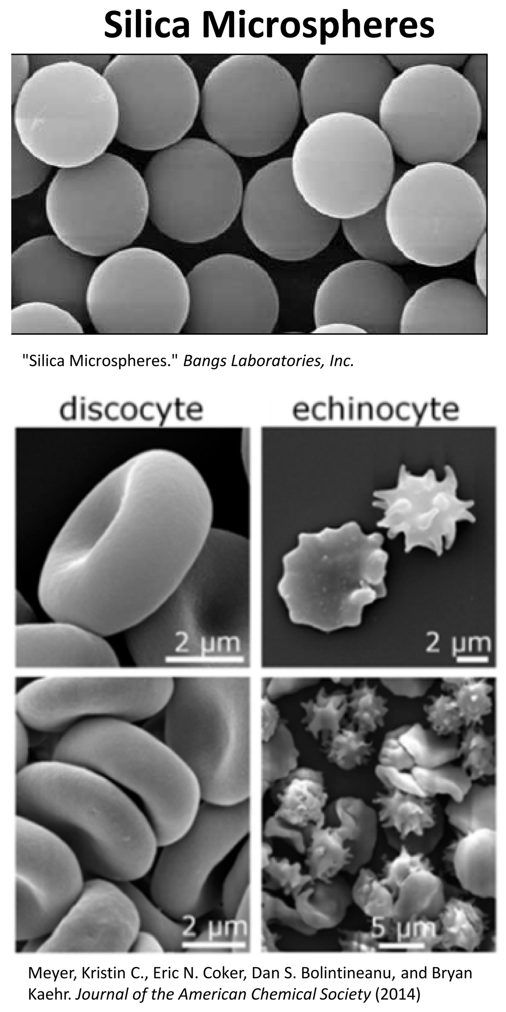


Bilayer Formation Confirmed using Static ^{31}P NMR



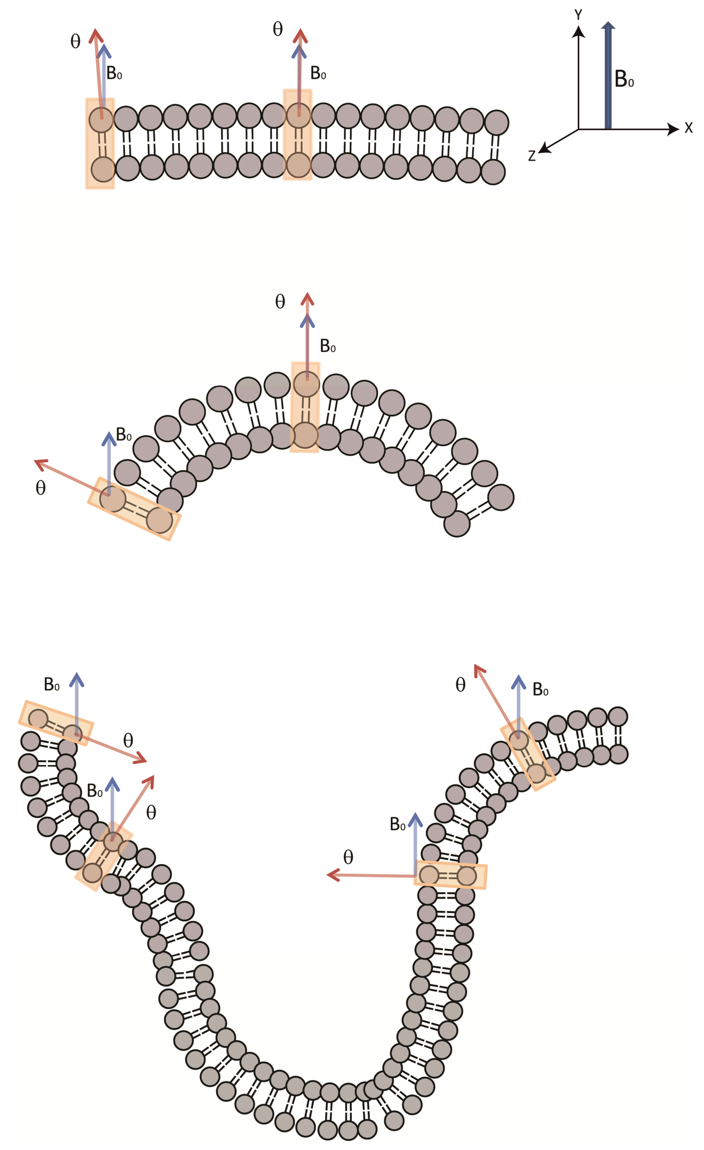
- ^{31}P chemical shift anisotropy (CSA) powder pattern analysis used to confirm bilayer structure

Silica Microspheres

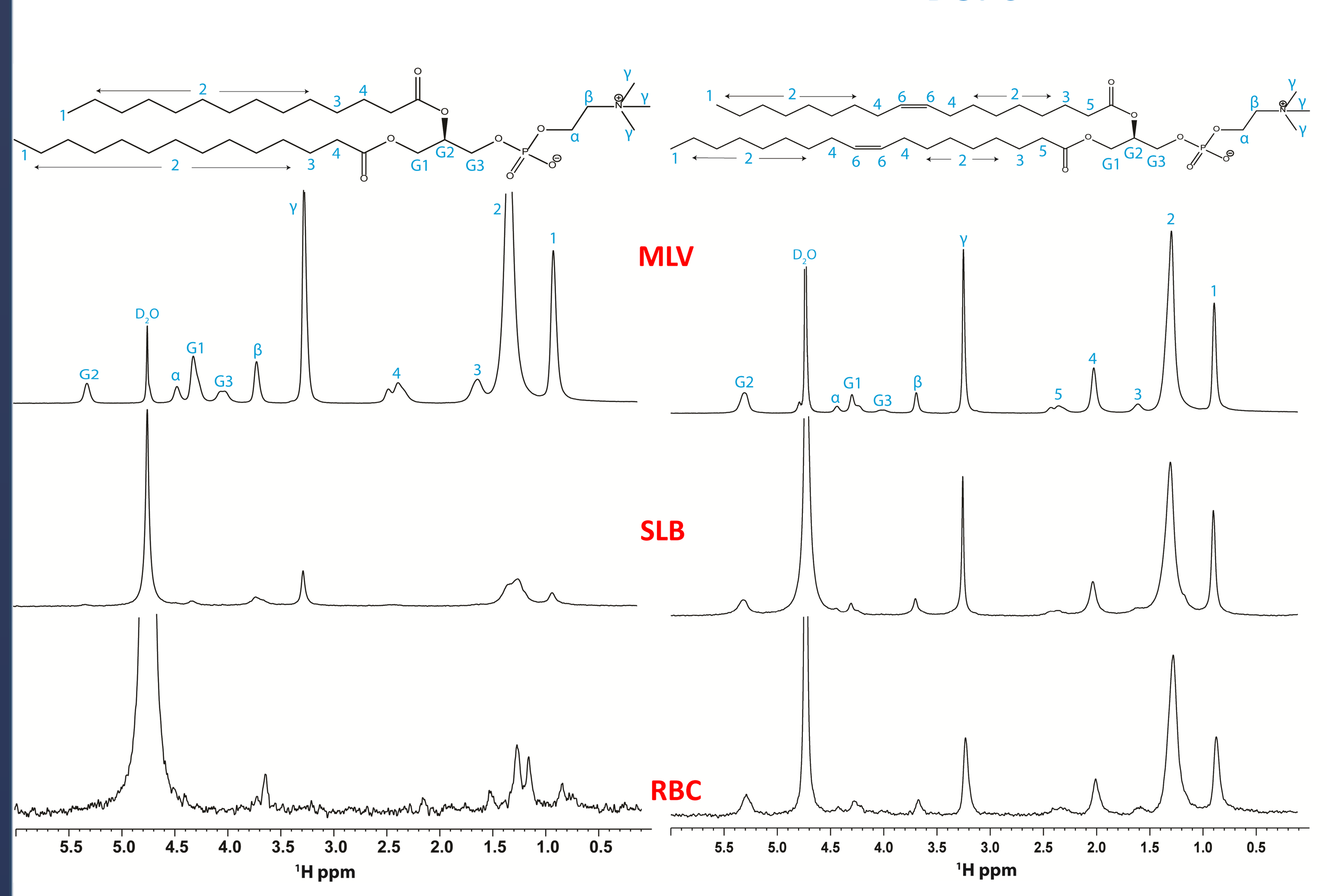


"Silica Microspheres." *Bangs Laboratories, Inc.*

Meyer, Kristin C., Eric N. Coker, Dan S. Bolintineanu, and Bryan Kaehr. *Journal of the American Chemical Society* (2014)

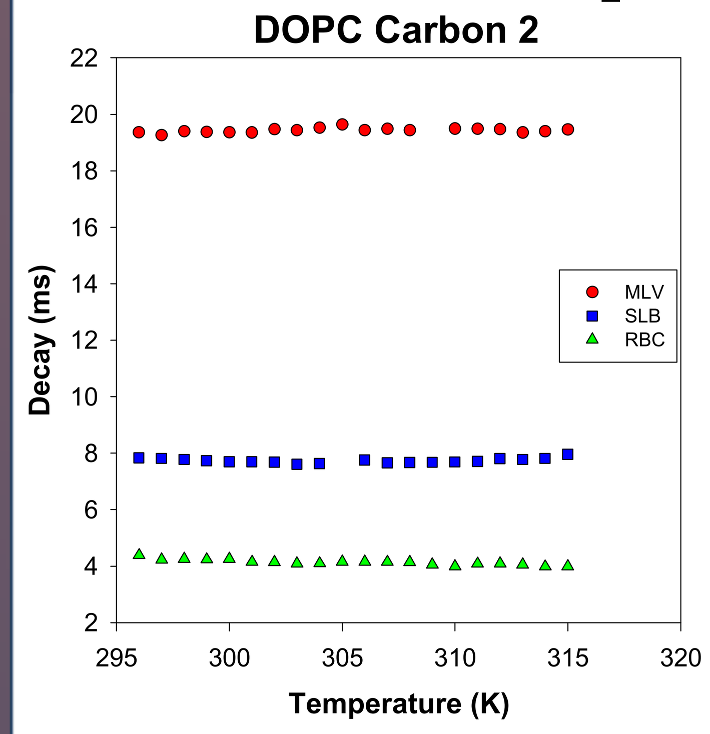


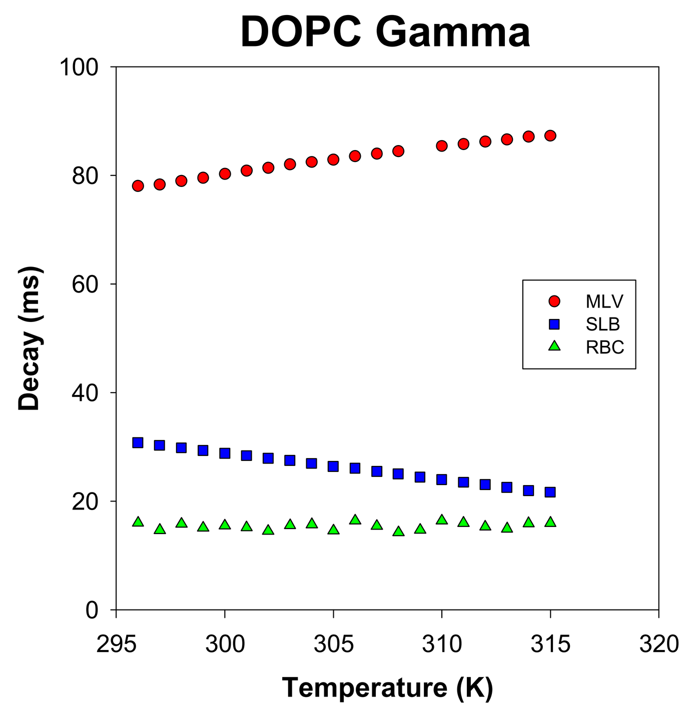
Investigation of MLV, SLB, and RBC using ^{1}H NMR



- Decreased fluidity, chain dynamics revealed by increased line widths due to ^{1}H-^{1}H dipolar coupling

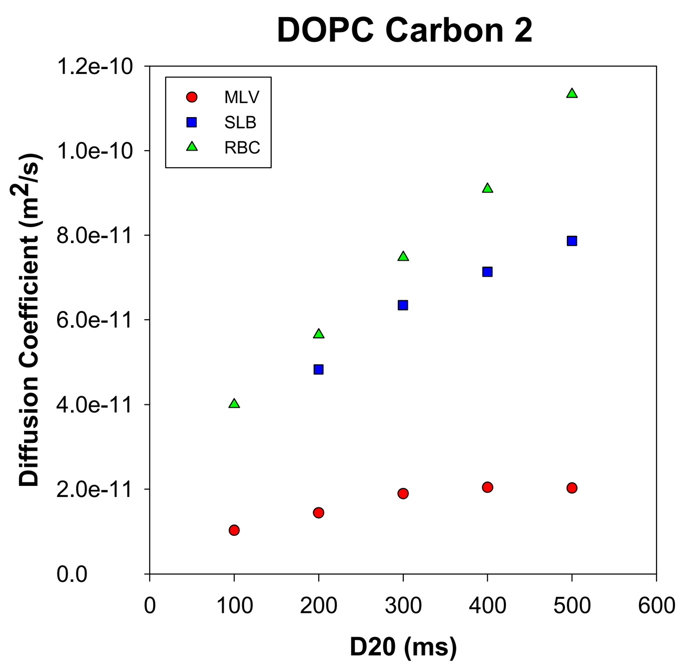
T_2 Relaxation Results

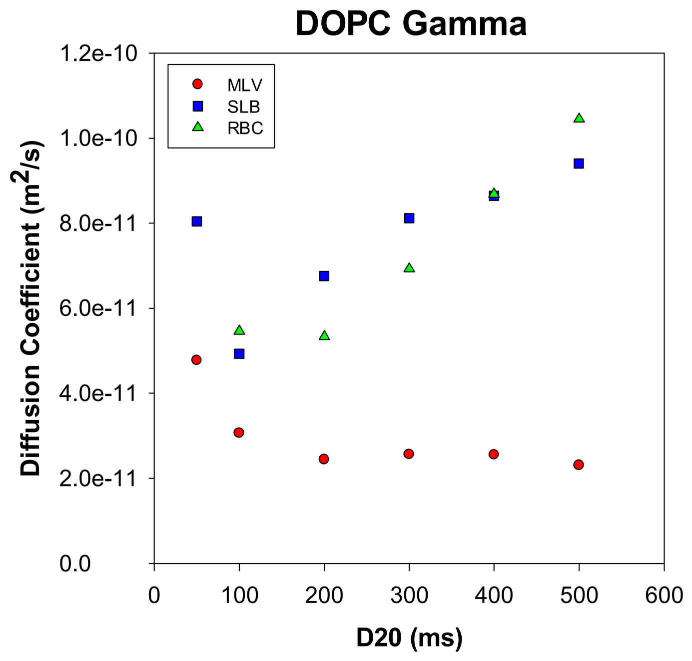


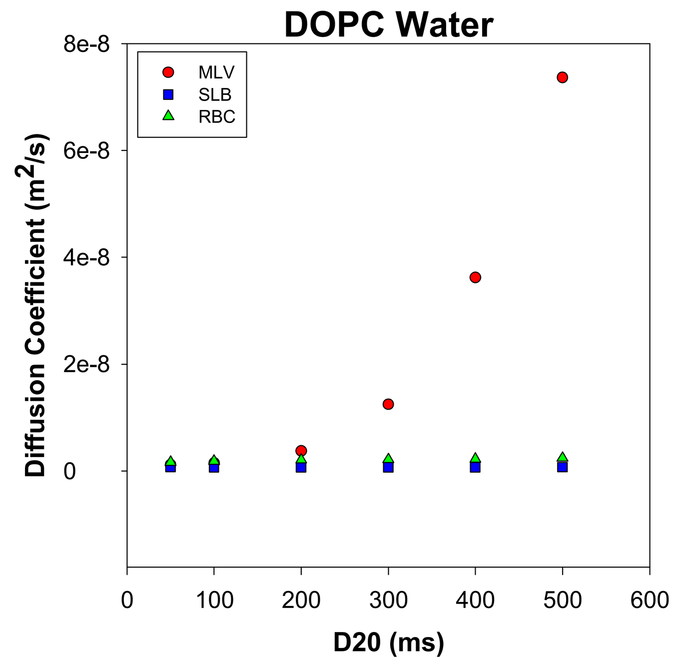


- Relaxation for MLV takes at least twice as long as a bilayer deposited on a substrate
- DMPC has trends similar to that of DOPC, although DMPC on SLB and RBC have closer relaxation times than DOPC

Diffusion Results







Conclusions

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