Biomimetic Lipid Membranes for the Study of Membrane Protein Structure

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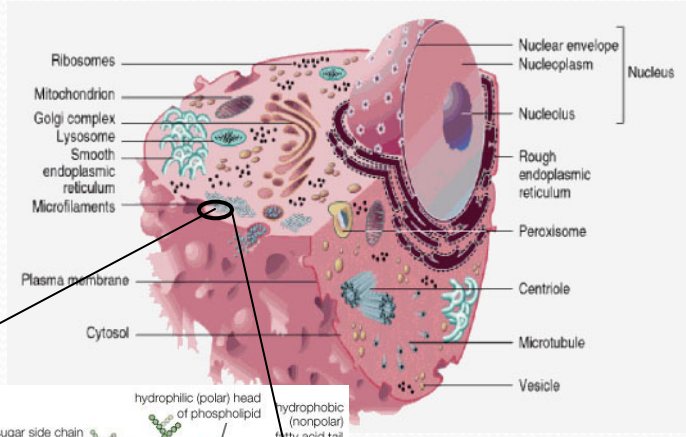
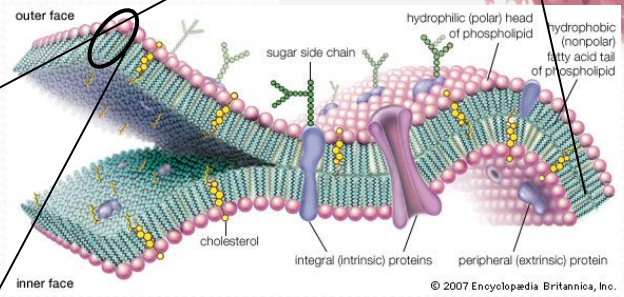
CHRNS NIST

- **Goal**: Prepare model oriented lipid bilayers with incorporated membrane proteins to be studied with x-ray and neutron diffraction.
  - **Part I**: Study lipid membranes and the effect of charge and pH on sample alignment and phase behavior.
  - **Part II**: Study how the antimicrobial peptide, Gramicidin A, resides in the lipid membranes.

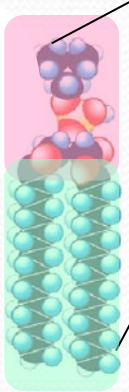
Cells



Membranes



Lipids

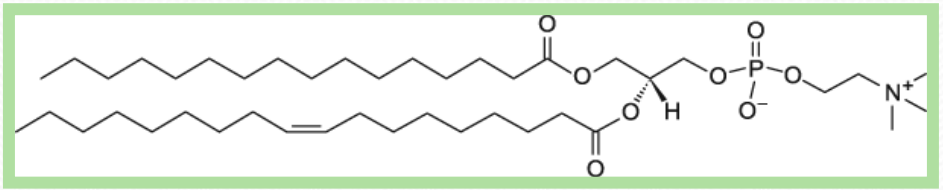


polar headgroup

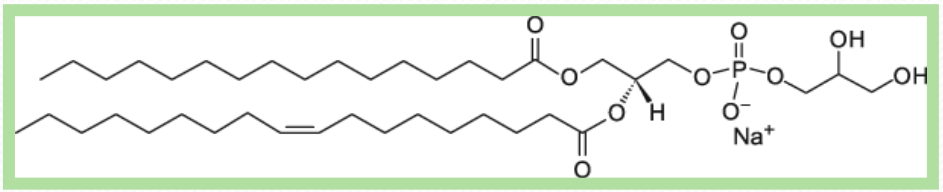
oily chains

Comparison of Lipid Structures

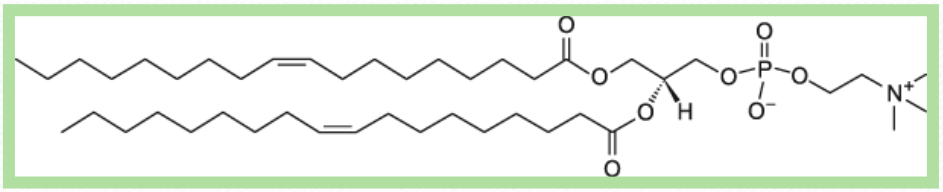
POPC



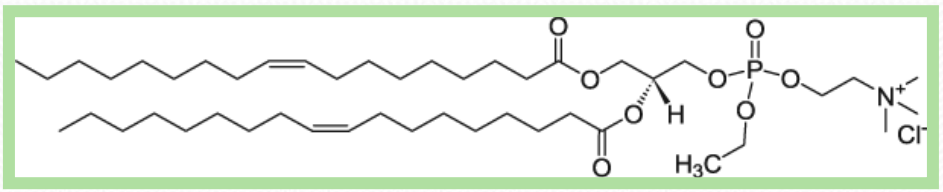
POPG



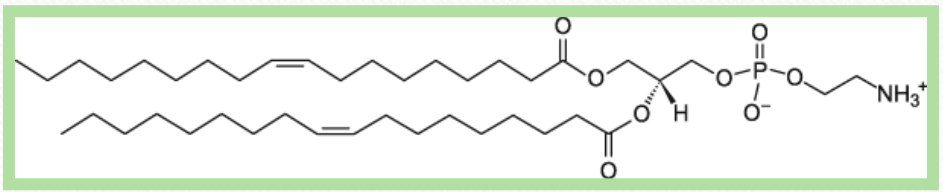
DOPC



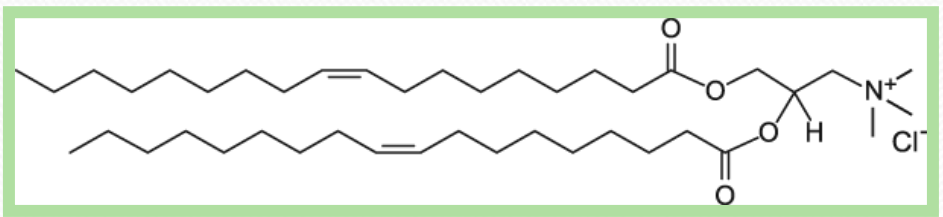
EDOPC



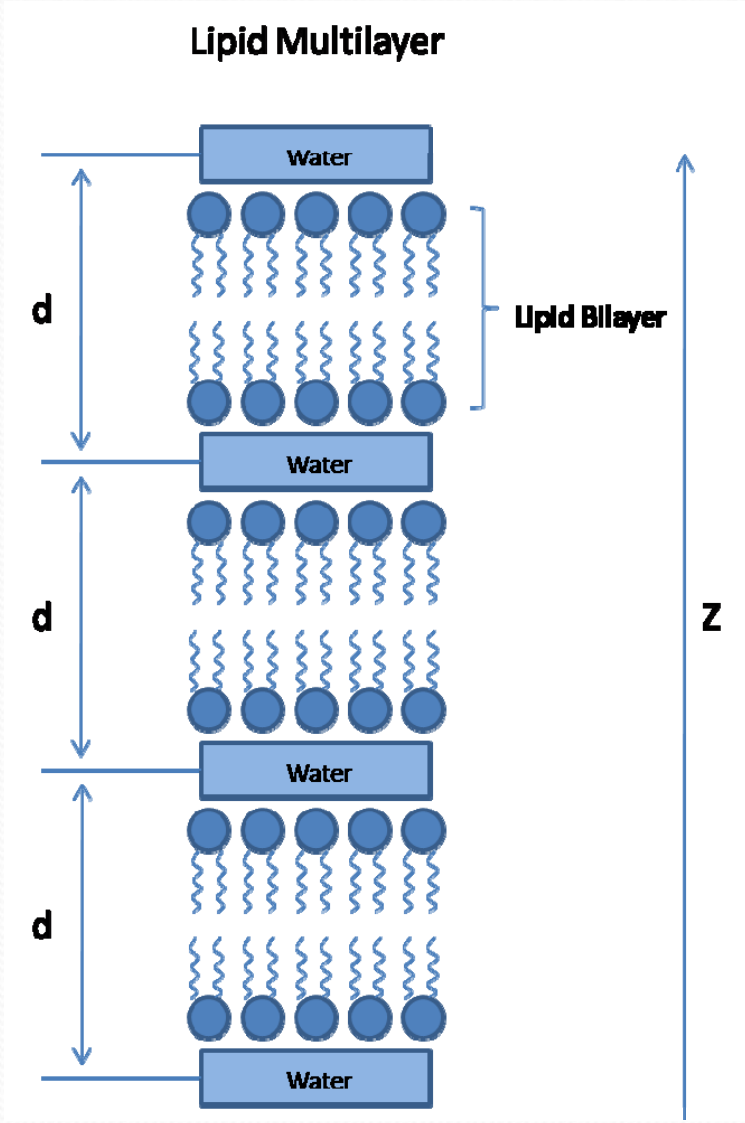
DOPE



DOTAP

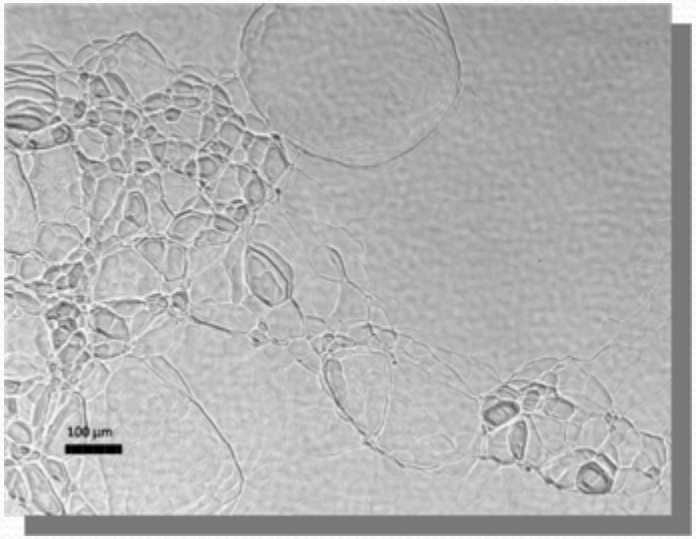


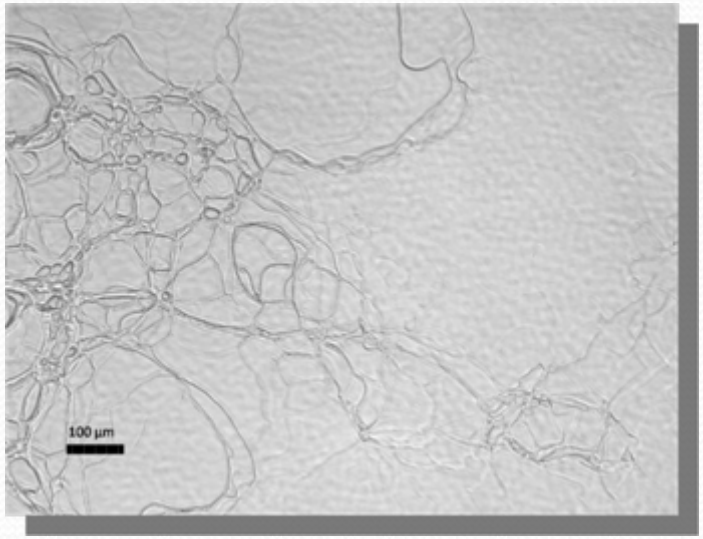
Introduction: Lipid Structure

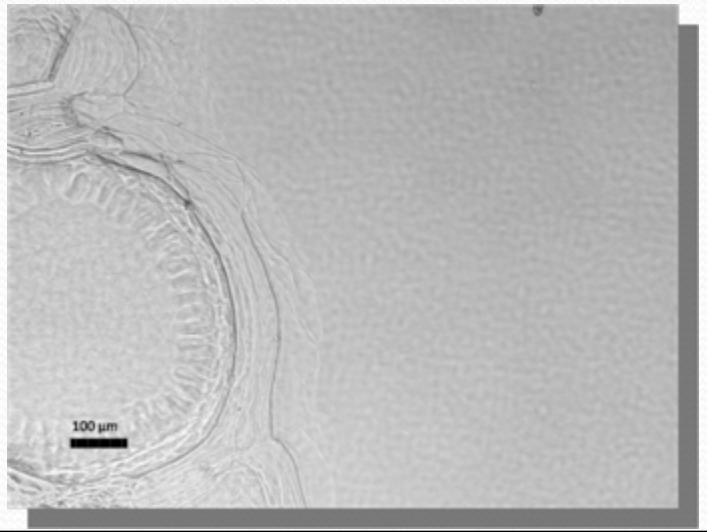


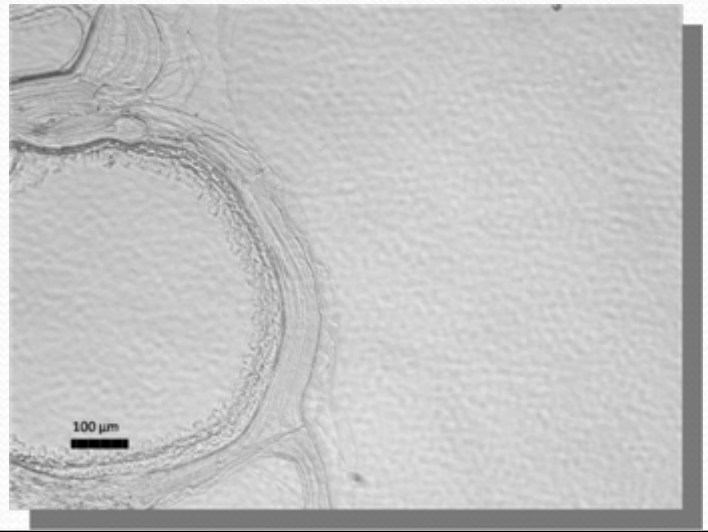
Microscope: PCPG (80:20)

Before Hydration **After Hydration**



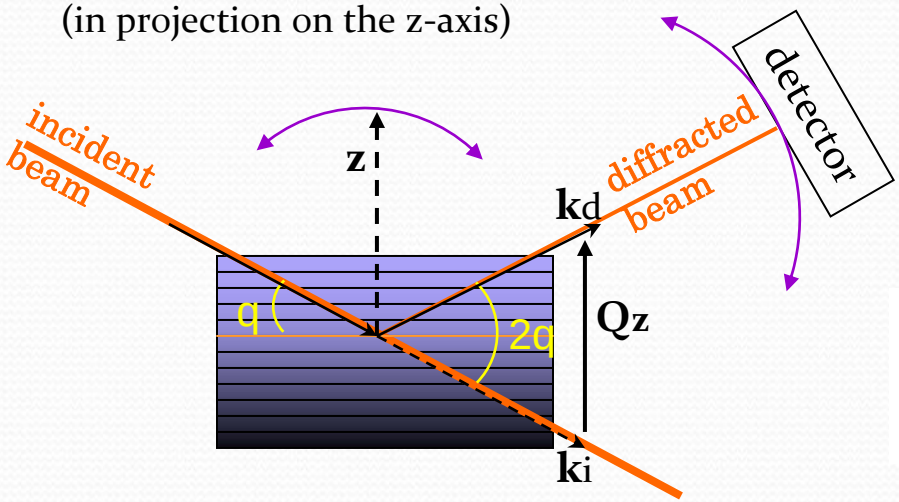




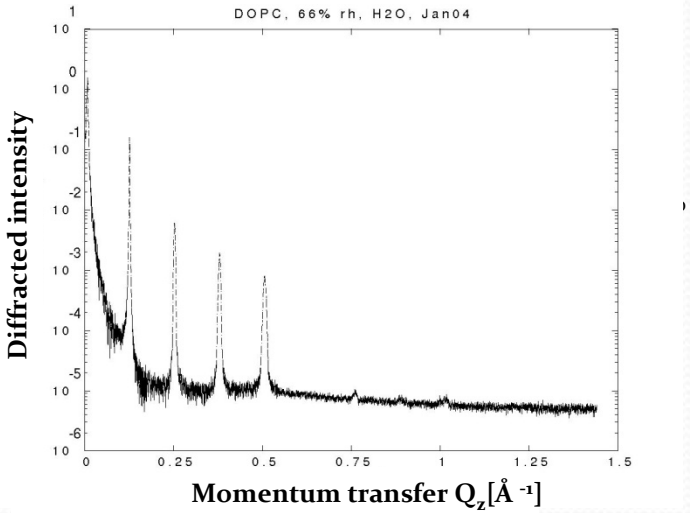


Measuring diffraction from oriented lipid multilayers

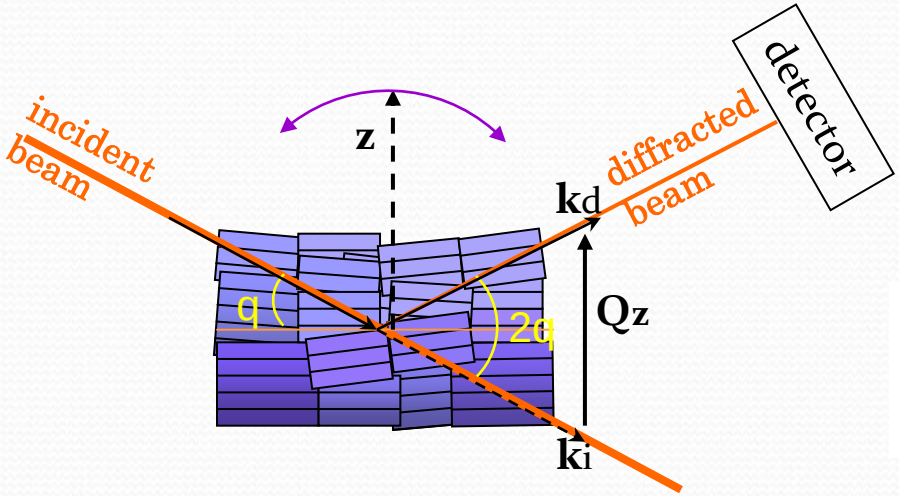
- θ– 2θ scans for determining lipid membrane structure (in projection on the z-axis)

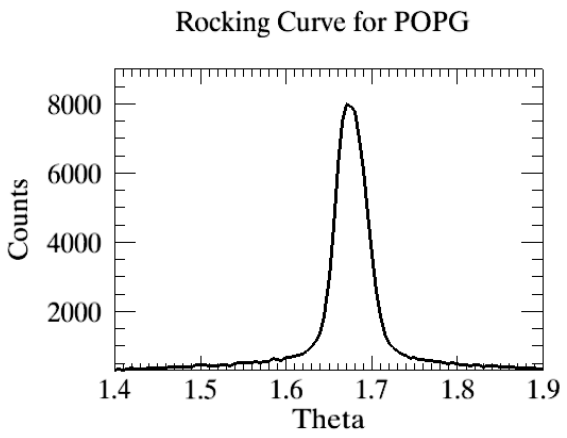


$$Q_z = \frac{4\pi}{\lambda}\sin(\theta) = n\frac{2\pi}{d}$$

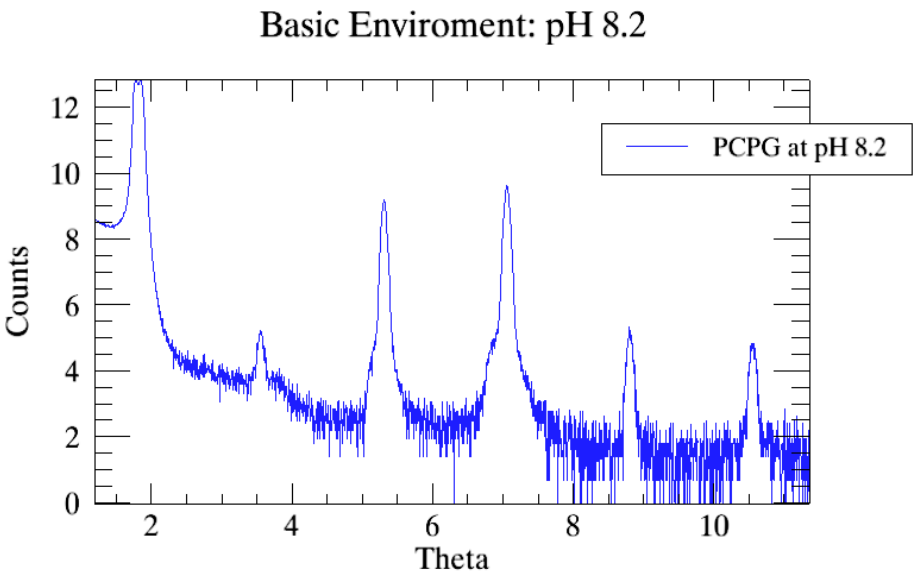


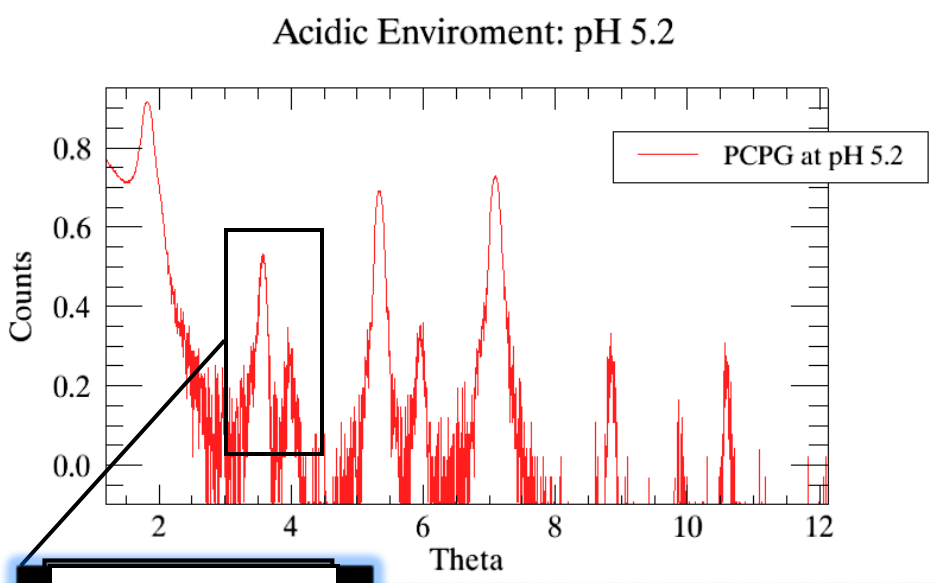
- θ scans for determining the degree of orientation





pH Effect on PG

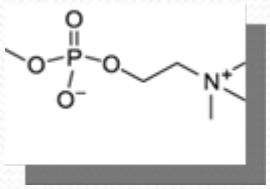




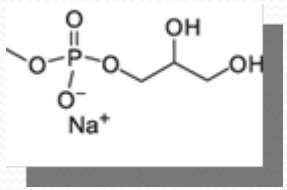
Additional Diffraction Peaks

$$Q_z = \frac{4\pi}{\lambda}\sin(\theta) = n\frac{2\pi}{d}$$

POPC



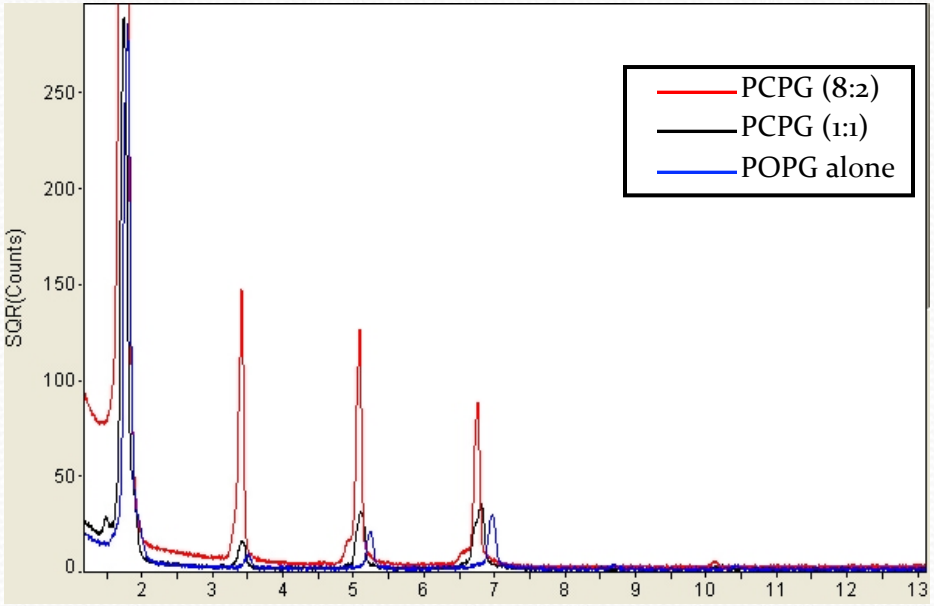
POPG



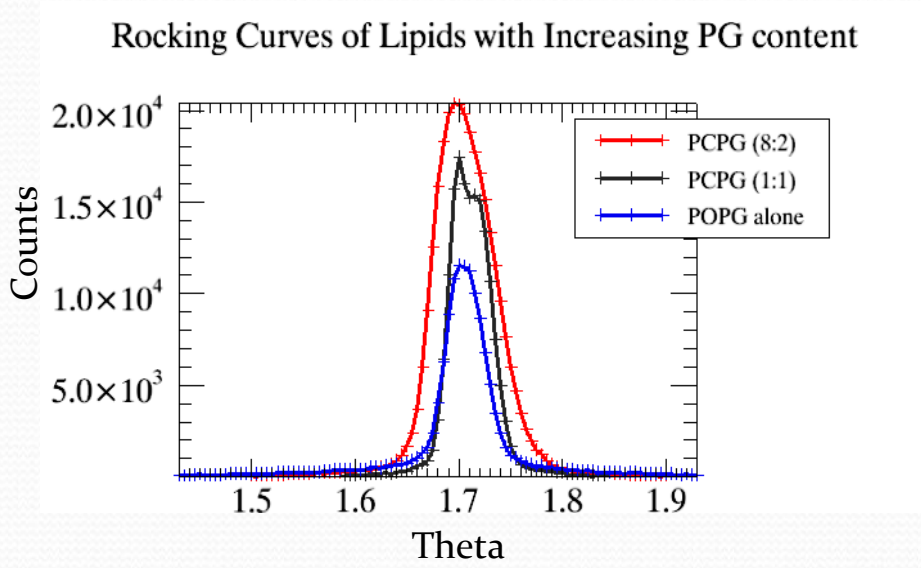
Phase separation of POPG from POPC in a acidic environment.

X-Ray Diffraction Results

PCPG(1:1) vs. PCPG(8:2) vs. POPG alone



Rocking Curve



Repeat Distances ➔

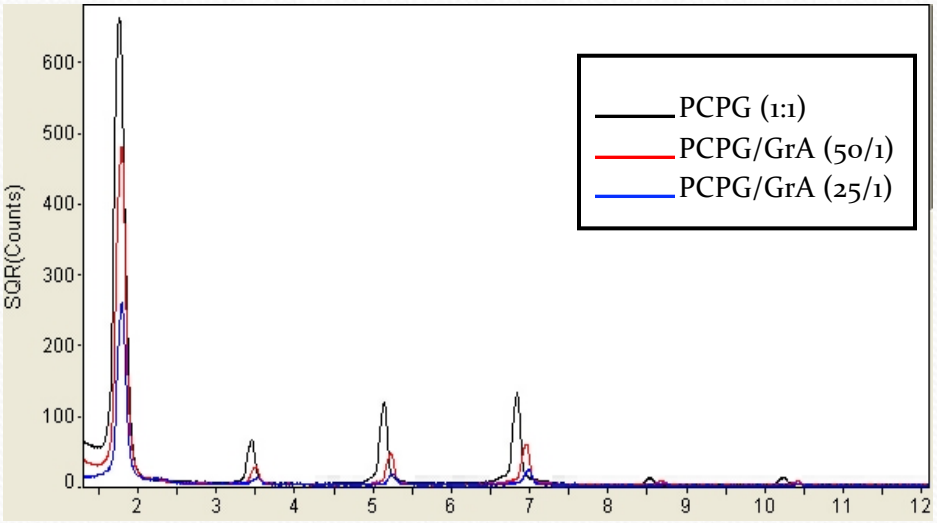
PCPG (8:2):	52.6 +/- 0.07 Å
PCPG (1:1):	52.1 +/- 0.05 Å
POPG alone:	50.7 +/- 0.05 Å

FWHM➔

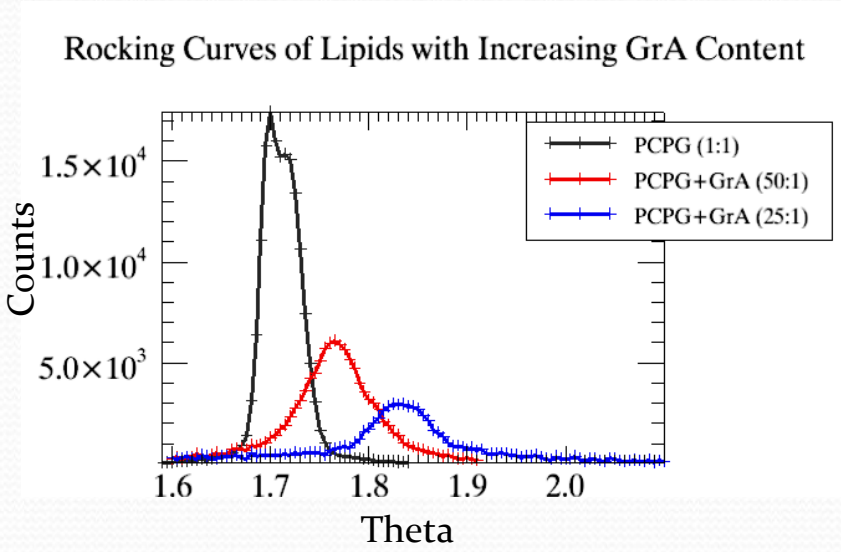
PCPG (8:2):	0.066 +/-0.003
PCPG (1:1):	0.044 +/-0.004
POPG:	0.041 +/-0.001

X-Ray Diffraction Results

PCPG/GrA(50/1) vs. PCPG/GrA(25/1) vs. PCPG(1:1)



Rocking Curve



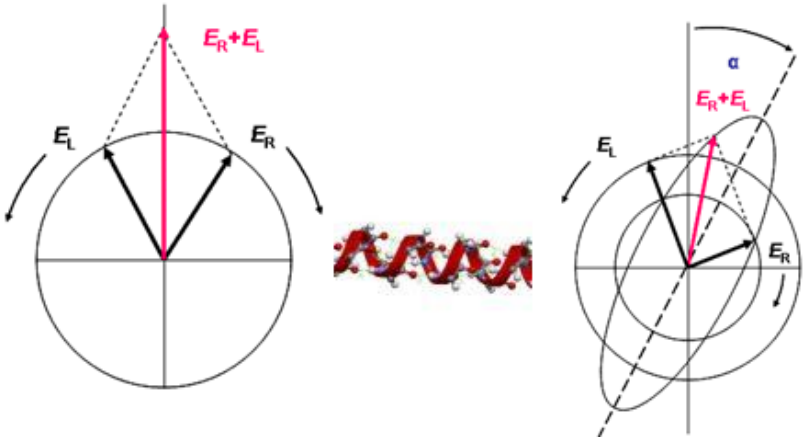
Repeat Distances ➔

PCPG (1:1):	52.1 +/- 0.05 Å
PCPG+GrA (50:1) :	49.2+/- 0.05 Å
PCPG+GrA (25:1) :	49.0 +/-0.05

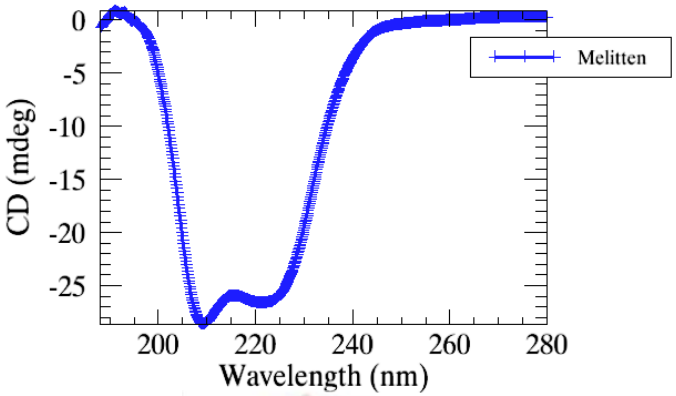
FWHM➔

PCPG :	0.044 +/-0.004
PCPG+GrA (50:1) :	0.072 +/-0.003
PCPG+GrA (25:1) :	0.076 +/-0.003

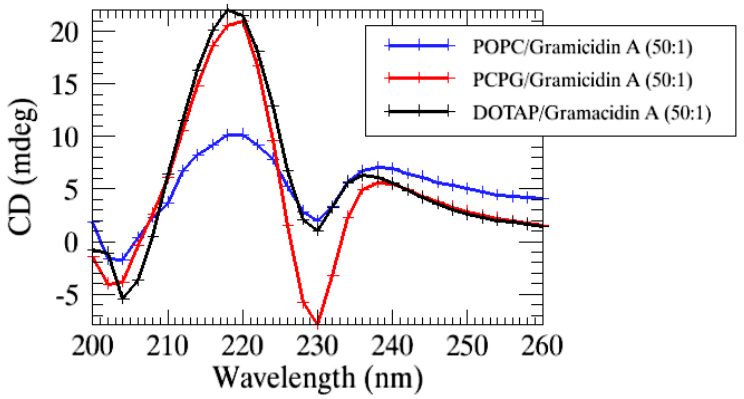
Circular Dichroism

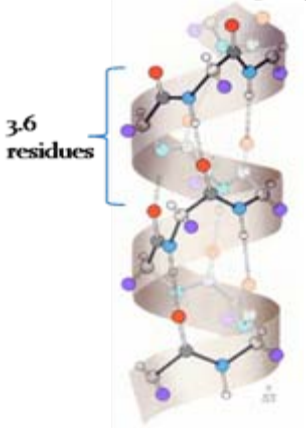


CD Signal for an Alpha Helix Configuration



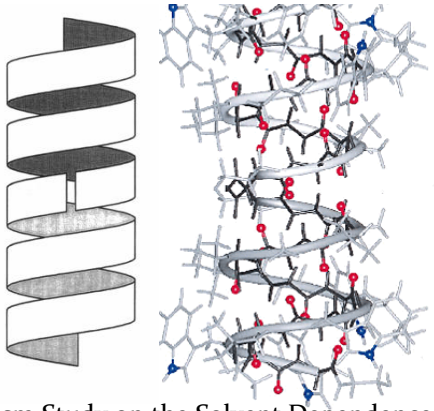
Gramicidin A in POPC, PCPG (1:1), and DOTAP





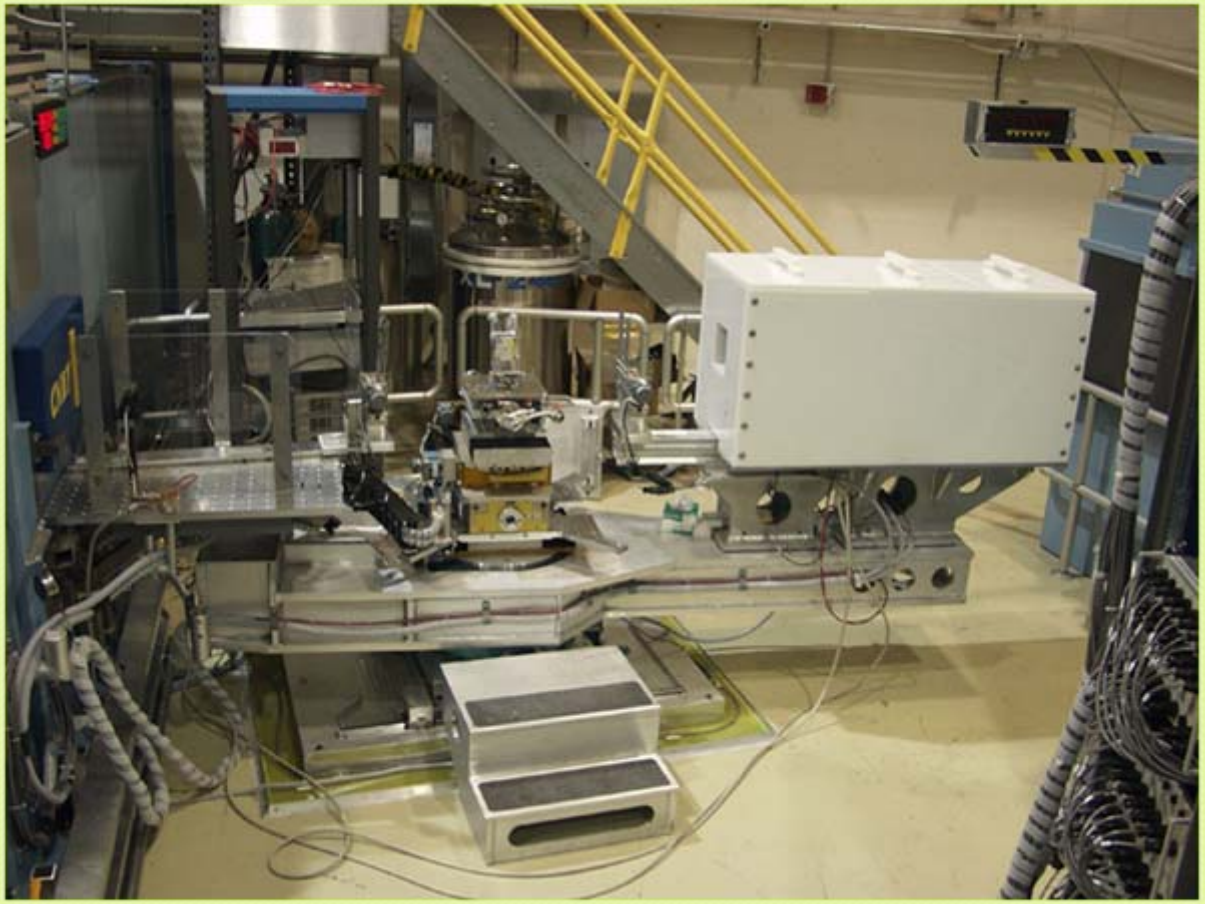
α-Helix

β6.3-Helix



Killian, J. A., et al. "The Membrane as an Environment of Minimal Interconversion. A Circular Dichroism Study on the Solvent Dependence of the Conformational Behavior of Gramicidin in Diacylphosphatidylcholine Model Membranes." Biochemistry 27.13 (1988): 4848-55.

Neutron Diffraction



Advanced Neutron Diffractometer/Reflectometer
NIST Center for Neutron Research

What we measure

Intensity(n) = $1/n^2 N^2 |F(n)|^2$

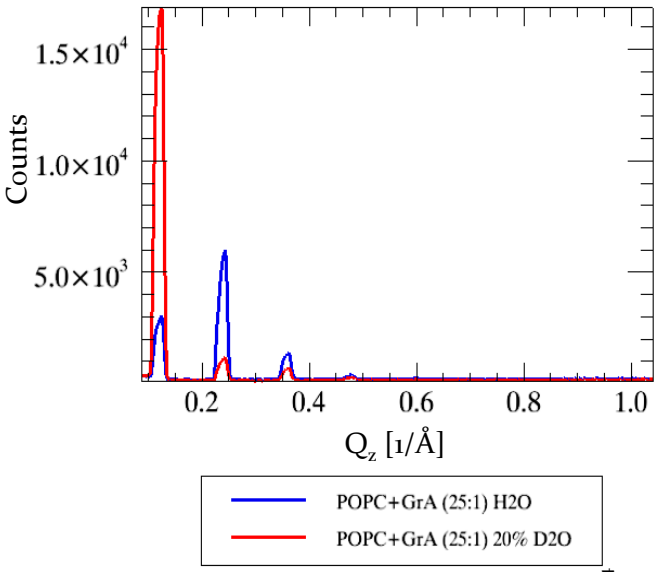
for large number of bilayers, N

Fourier Sum

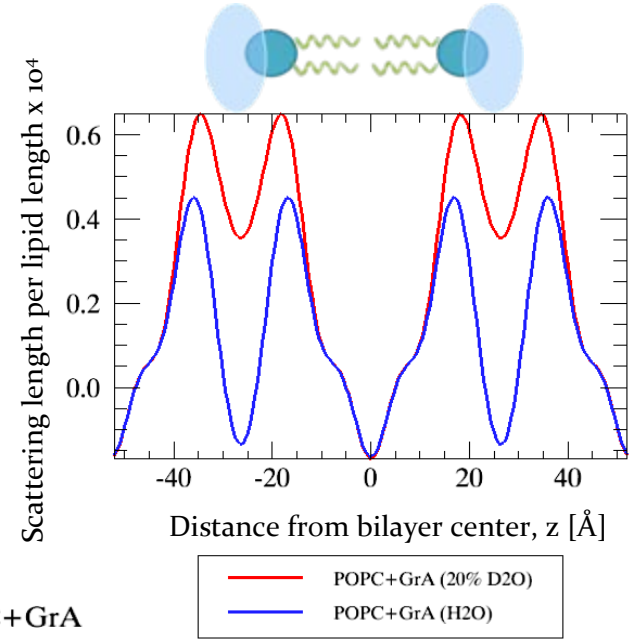
What we want to determine

$$\rho(z) = \frac{1}{d}\sum_n |F(n)| \cos(\frac{\quad}{d})$$

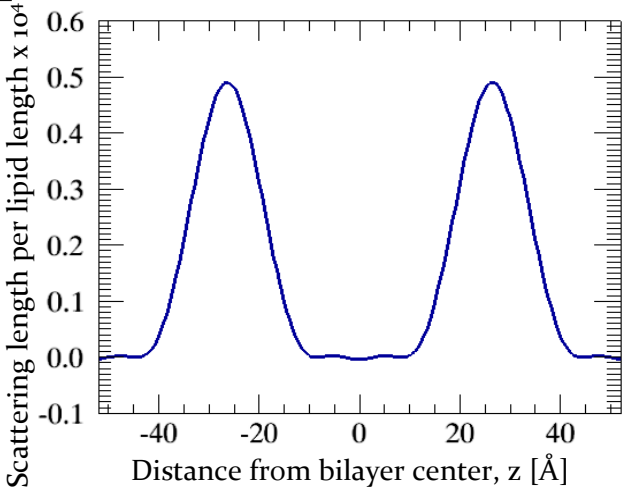
POPC+Gramicidin A (25:1) (H20 vs. 20% D2O)



SLD Profiles for POPC+GrA in 20% D2O and H2O



Water Distribution for POPC+GrA

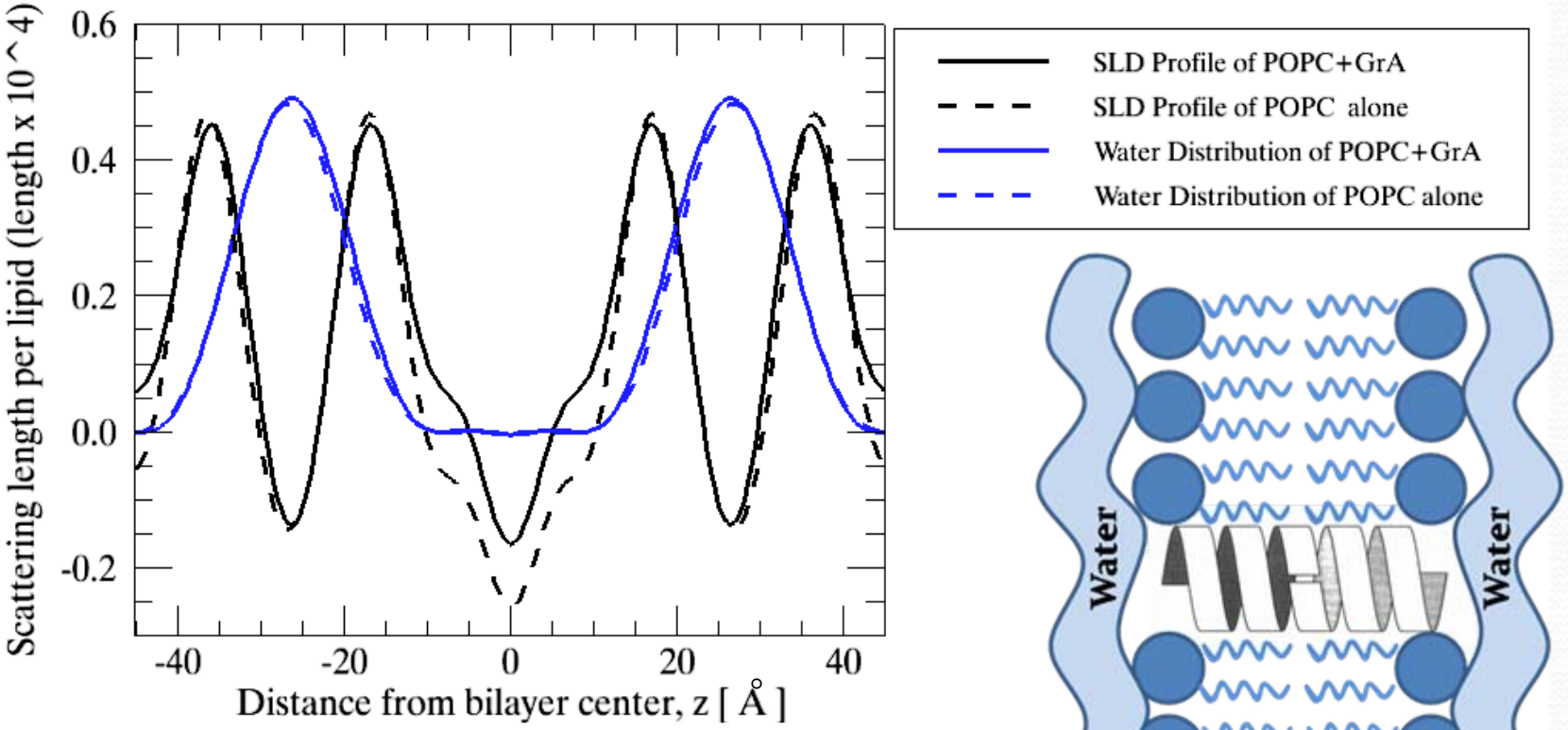


$\rho(z)_D - \rho(z)_H$

Neutron Diffraction Data

Neutron Diffraction Data

SLD Profiles and Water Distributions for POPC+GrA and POPC alone



d=53.5 +/- 0.08 Å(POPC alone)
d=52.8 +/- 0.05 Å (POPC+GrA (25:1))

Conclusions

- Improve on sample quality by hydration and the amount of charged lipid.
- Solvent pH plays an important role in the structure and properties of a lipid membrane.
- Neutron Diffraction and CD data indicate that Gramicidin A is incorporated into the membrane.
  - Suggests transmembrane, channel forming conformation.

Acknowledgements

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- NCNR
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- Dr. Julie Borchers
- University of Southern Mississippi

NCNR SURF

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