Sphingolipids/Cholesterol Membranes

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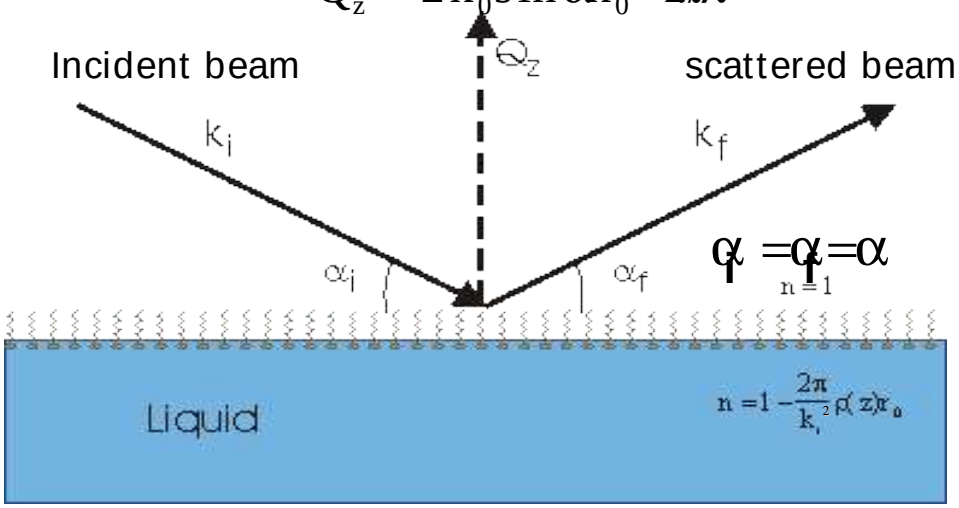
Outline

- **Sphingosine, sphingomyelin and ceramide monolayers**
- **Cholesterol and cholesterol/lipid mixtures in oriented bilayer systems**

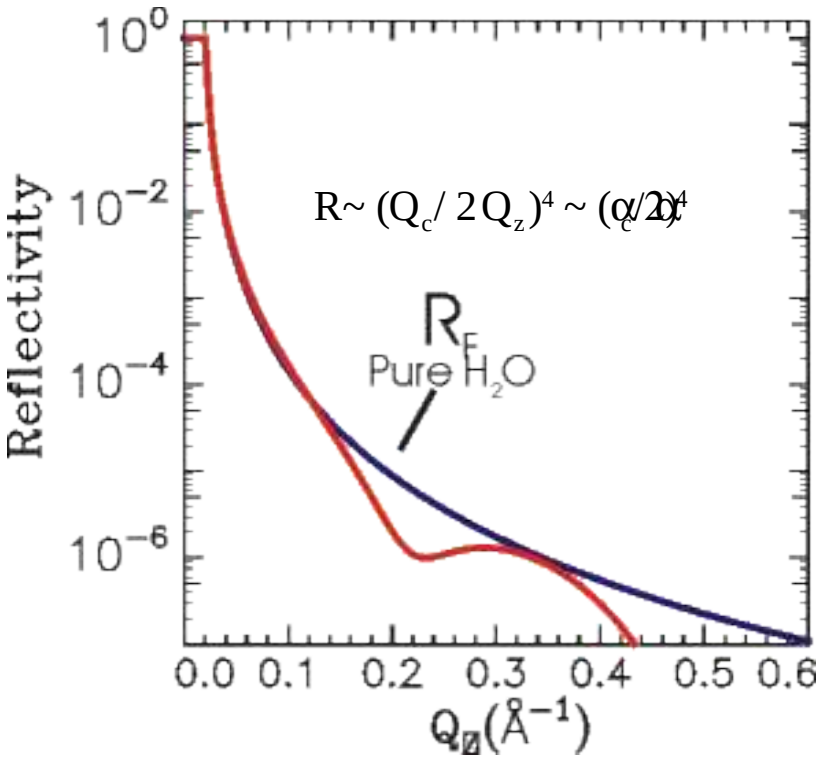
Reflectivity

$$Q_z = 2k_0 \sin\alpha \quad k_0 = 2\pi/\lambda$$



What is measured?

Reflected beam versus momentum transfer



First X-ray reflectivity from water (or any liquid) was measured by Pershan's group at Harvard Phys Rev. Lett. 54, 114 (**1985**).

Master Formula

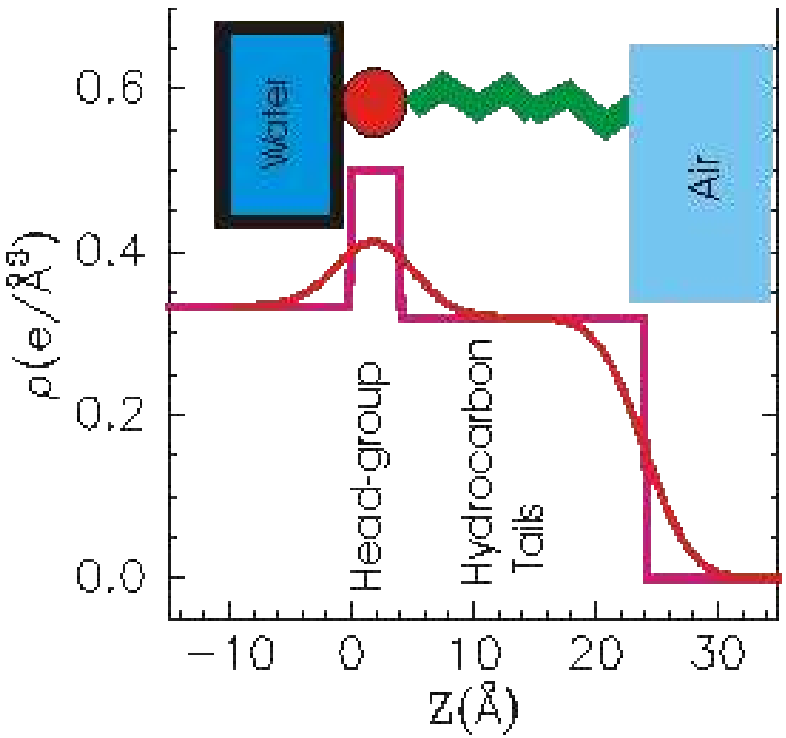
- **Exact solution – matrix method**
- **Born- Approximation**

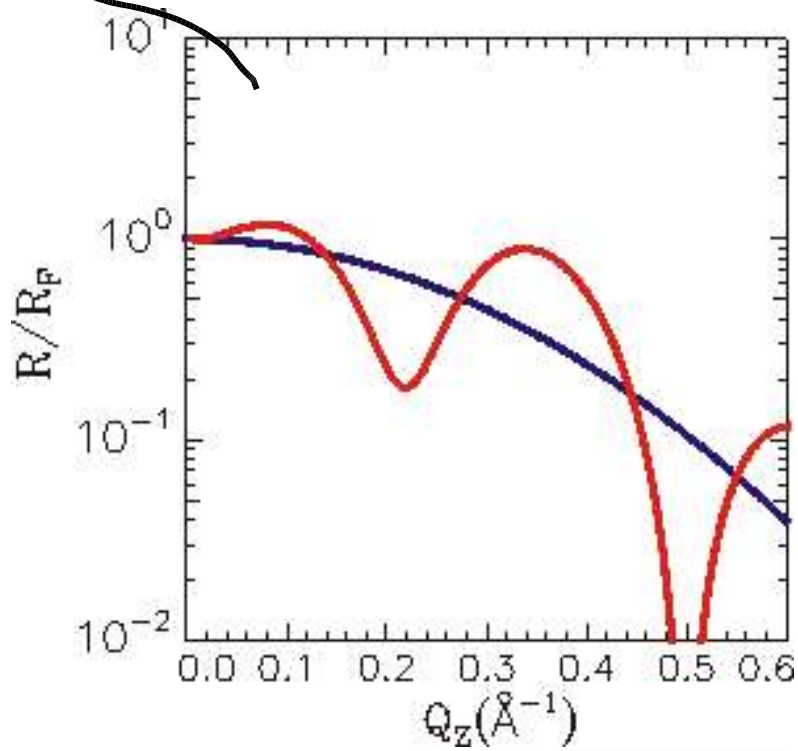
$$R(Q_z) = R_F(Q_z)\left|\frac{1}{\rho_s}\int \frac{d\rho}{dz} e^{iQ_z z} dz\right|^2 = R_F(Q_z)\left|F(Q_z)\right|^2$$

Electron Density Across the Interface

From the reflectivity, the scattering length density (Neutron) or electron density (X-rays) can be determined and correlated with the structure at the interface.

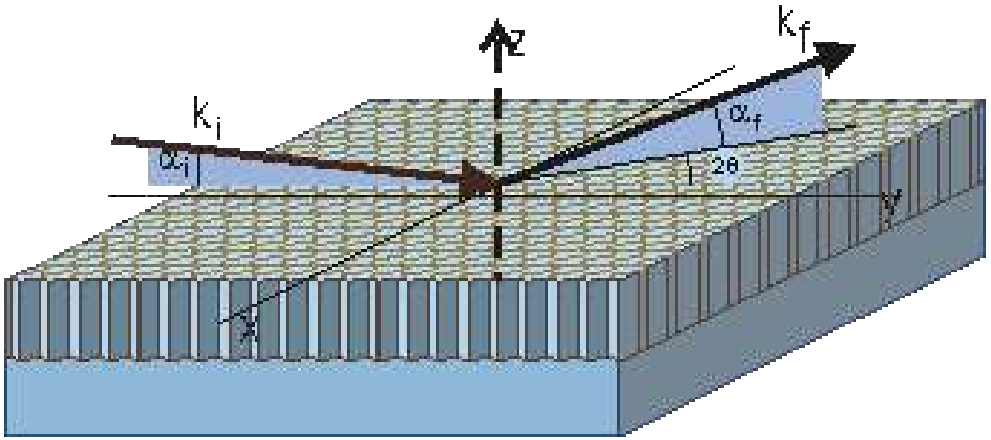
The reflectivity results are best represented as the **normalized reflectivity**, that is, measured reflectivity, **R** divided by the calculated reflectivity from the ideally flat surface $\mathbf{R_F}$.



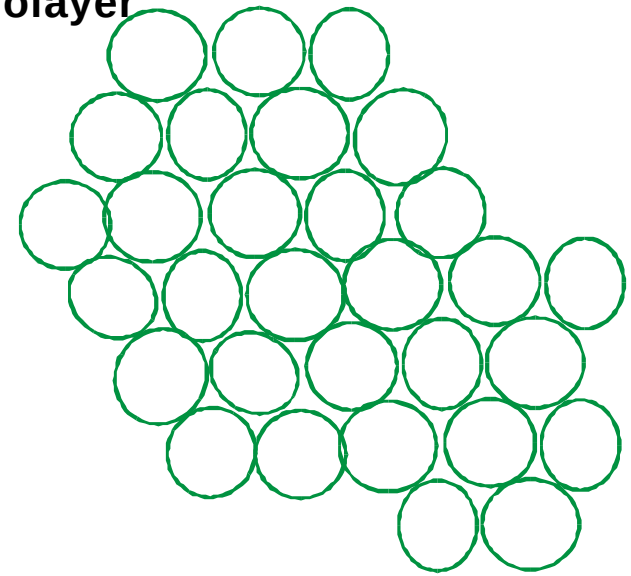


Inplane Structure – Crystalline or Disordered

- Grazing Incidence X-ray Diffraction (GIXD) - 2d crystallography
- Diffuse Scattering
- Liquid structure factor



Top view of an ordered monolayer



Incident beam, k_i is kept below the critical angle creating an evanescent wave with finite penetration depth into the bulk (approximately 100A) enhancing signals from the surface.

An ordered 2D system gives rise to rod like Bragg reflections that contain information on the electron density along the z-axis of the ordered objects. The total cross section for scattering from a 2D system is in general very small and X-ray flux from synchrotron sources is required.

Rod- Scans at 2D Bragg Reflections

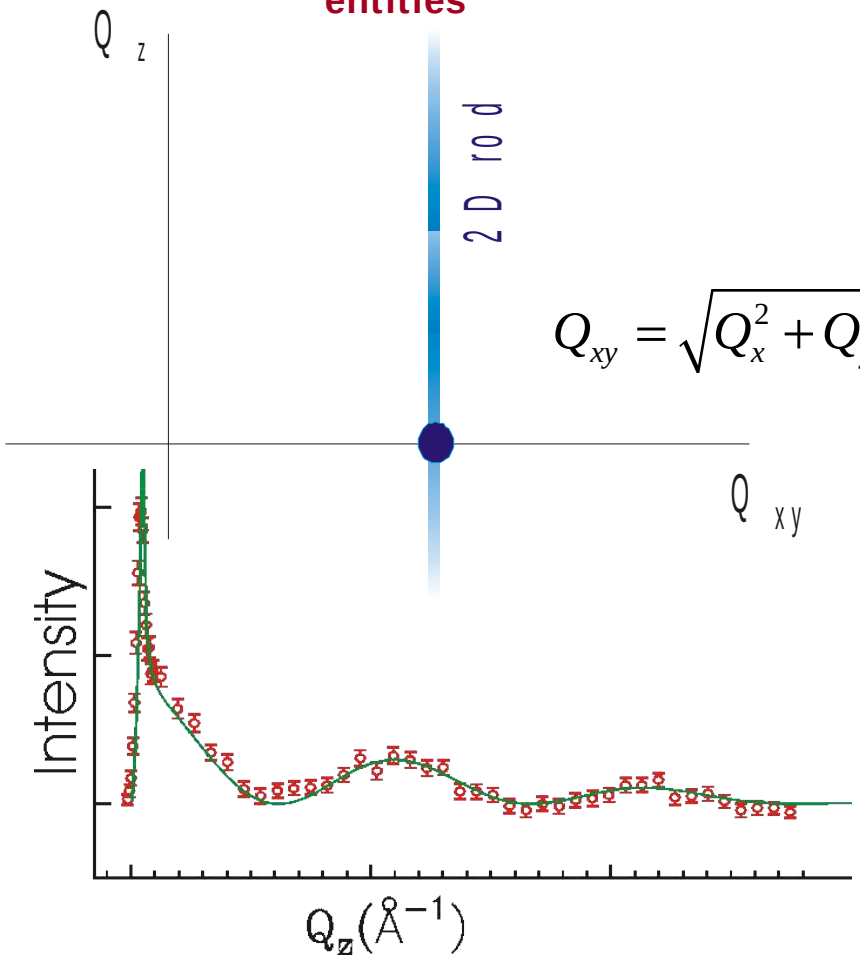
Multiple scattering DWBA

2D Structure Factor

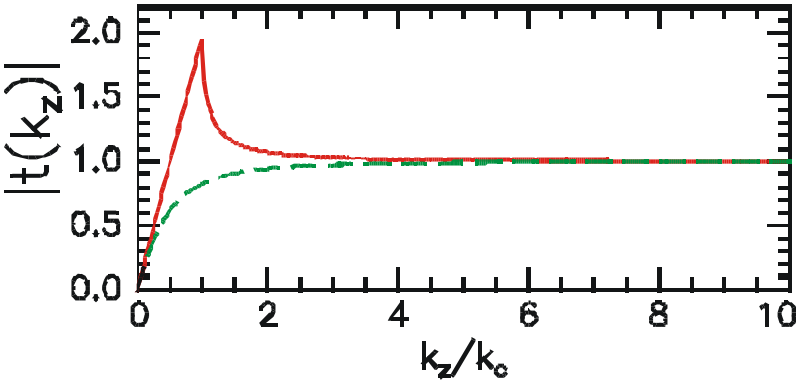
$$I_{2D}(Q_{xy},Q_z) \sim |F(Q_{xy})|^2 \, |f(Q_z)|^2 \, |t(Q_z)|^2$$

Molecular form Factor

Objective: Determine the shape of the diffracting entities

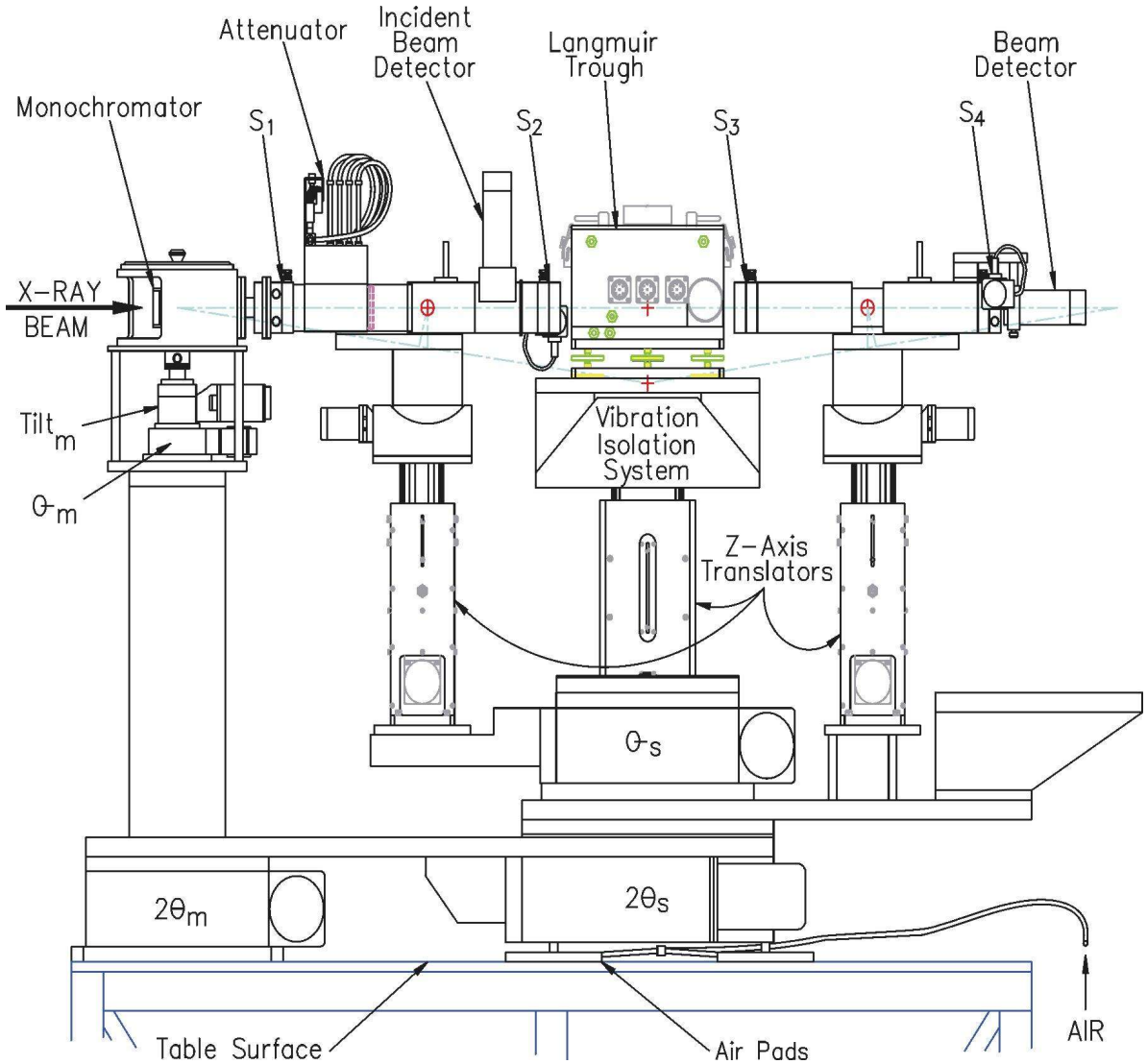


$$Q_{xy} = \sqrt{Q_x^2 + Q_y^2}$$



The peak due to the Fresnel transmission function indicates the top most layer is being probed.

Liquid Surfaces Diffractometer



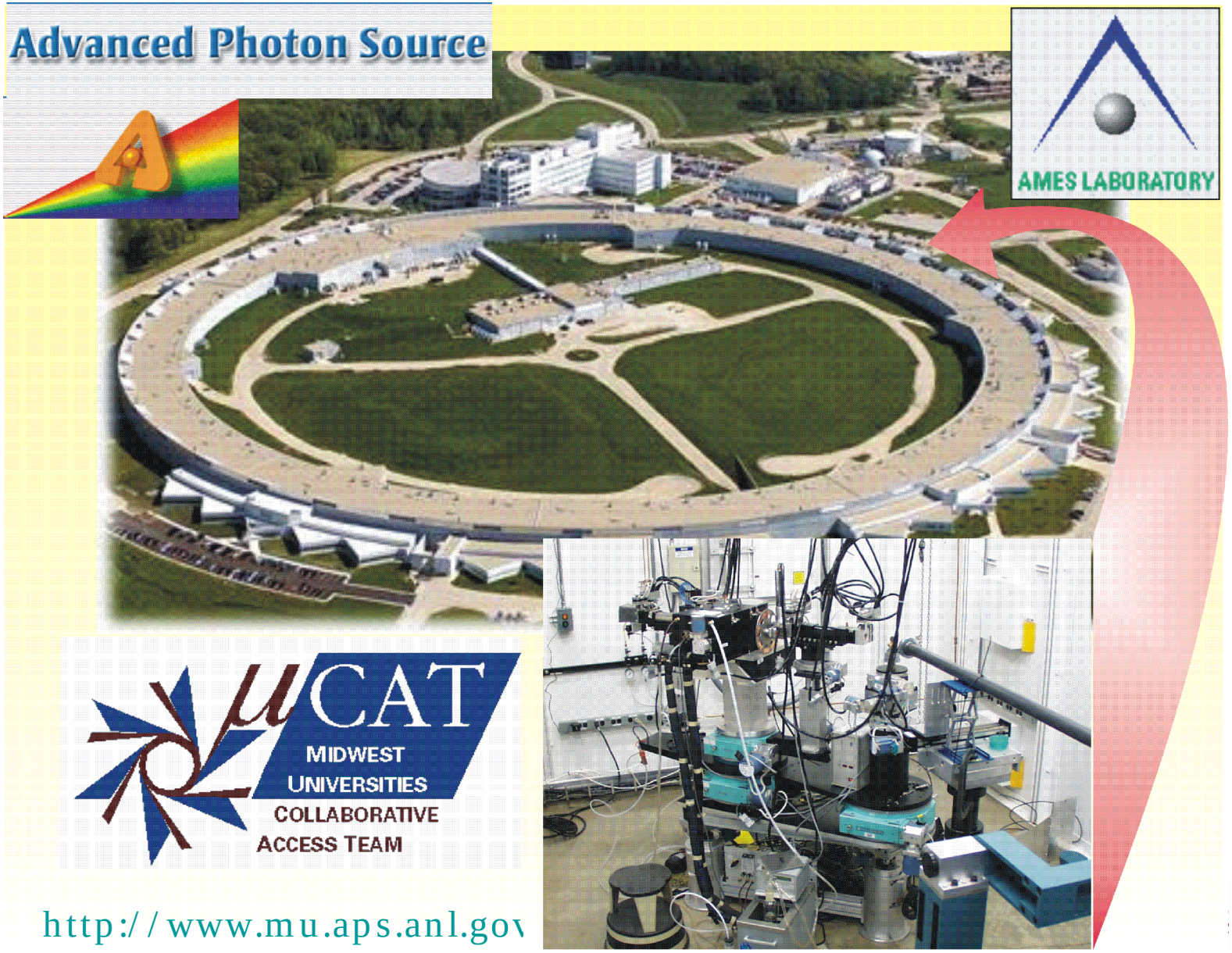
Built with the help of Mike Harper and Terry Herrman at ISU

IOWA STATE UNIVERSITY

AMES LABORATORY

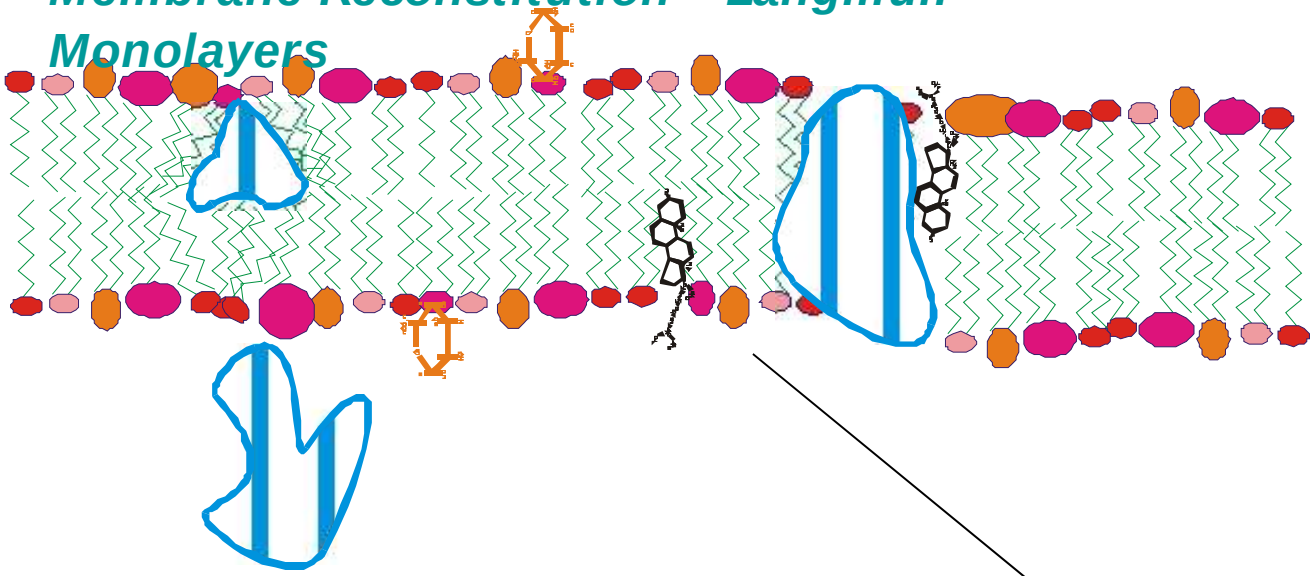
The Ames Lab – Liquid Surfaces Diffractometer (LSD)

www.aps.anl.gov

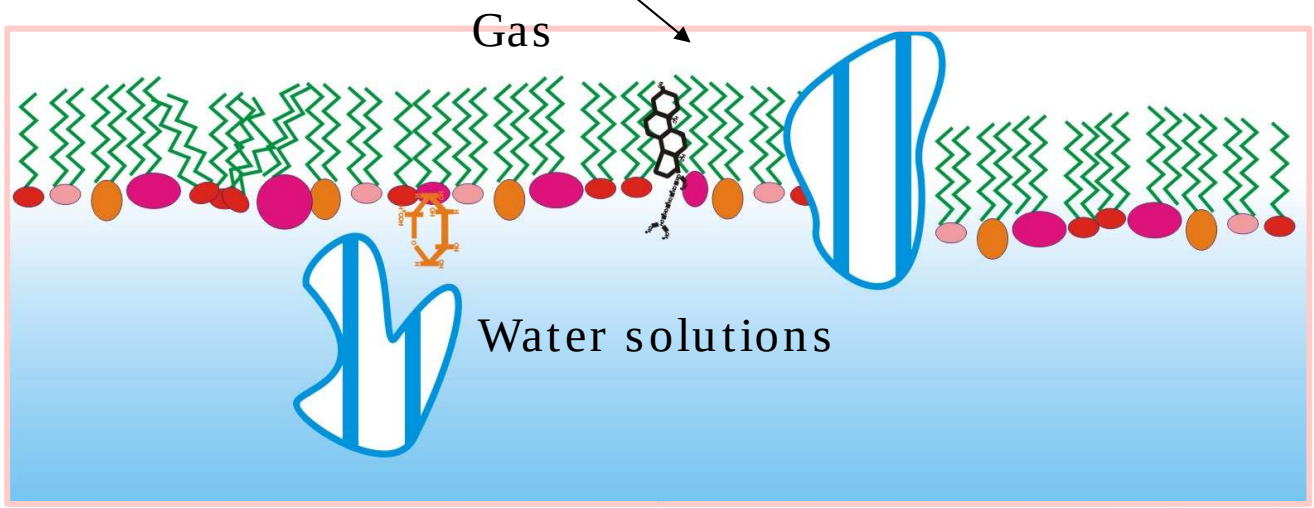


Motivation

Membrane Reconstitution – Langmuir Monolayers



Biomimetic - Membranes



Sphingolipids

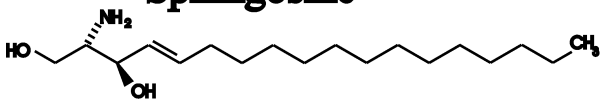
Function

Regulating Cell proliferation

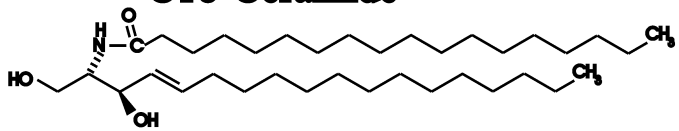
Cell programmed death, apoptosis, cell aging and differentiation

Metabolism – sphingomyelin cycle

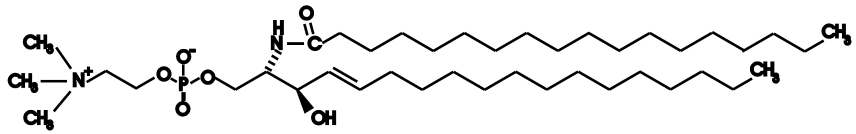
Sphingosine



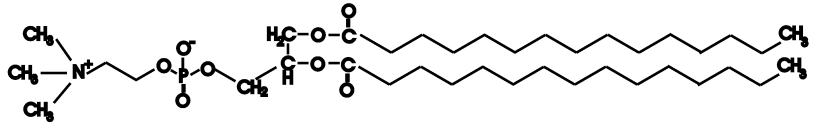
C18-Ceramide



C18-Sphingomyelin



DiPalmitoyl-phospatidyl-choline (DPPC)



(in animals)

Skin, hair , etc.

Increased ceramide levels are associated with cell programmed death

Myelin sheath – nerves, brain

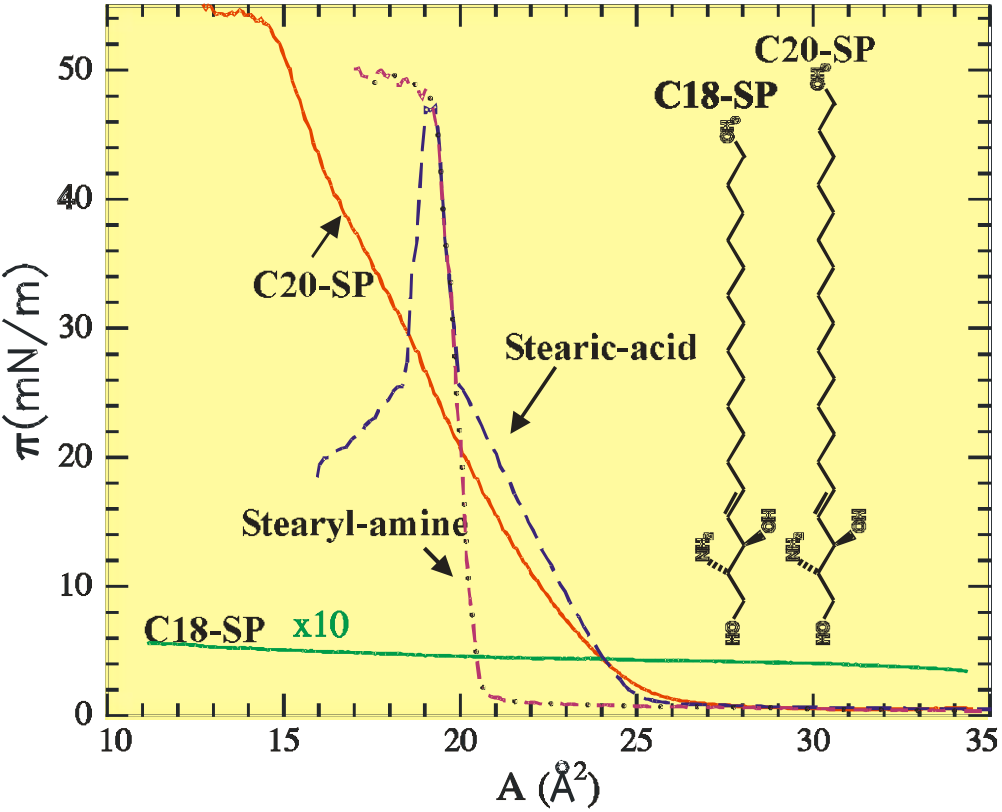
Protein rafts

Hannun, Y.A. *Science* **274,** 1855 (1996).

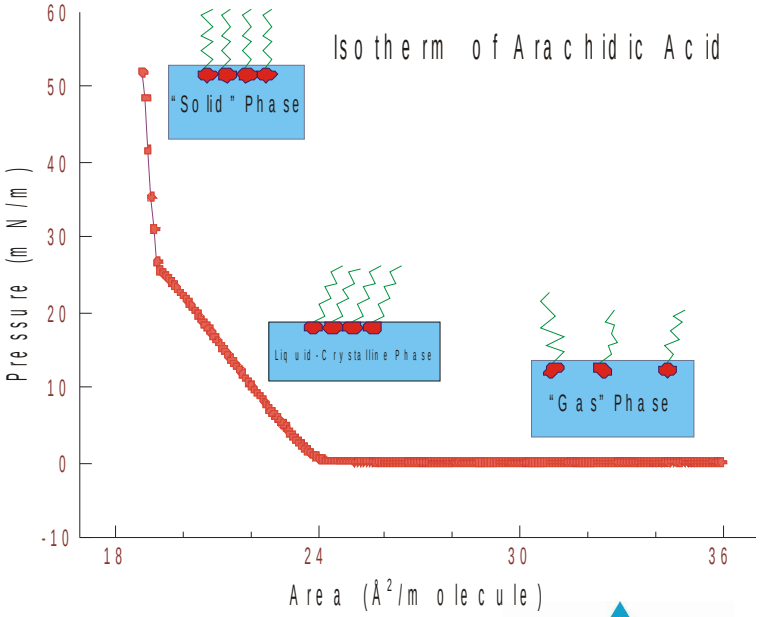
Simon & Ikonen, *Nature* **387**, 569 (1997)

Spiegel, and A. H. Merrill, *FASEB J* **10,** 1388 (1996).

π *A* isotherm



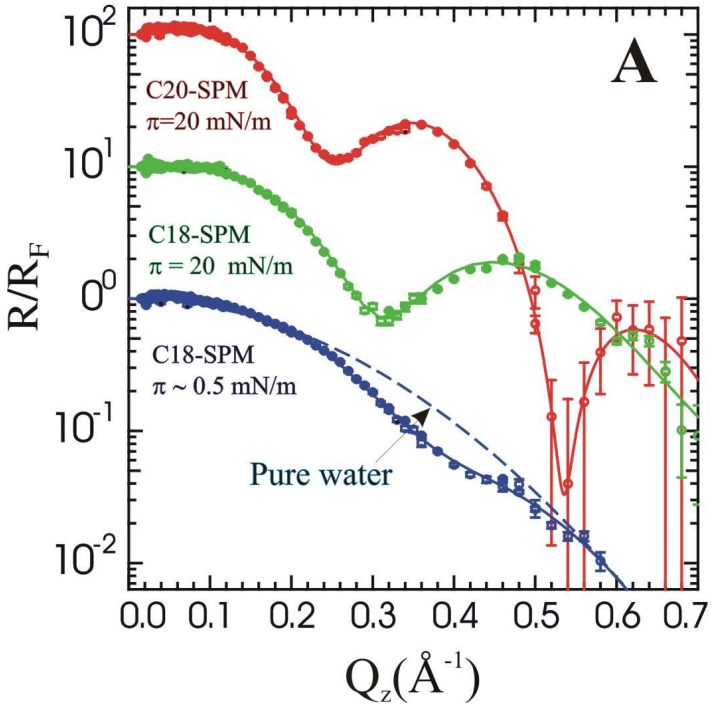
C18 sphingosine does not form a *regular* monolayer –Highly soluble in water - messenger activity?

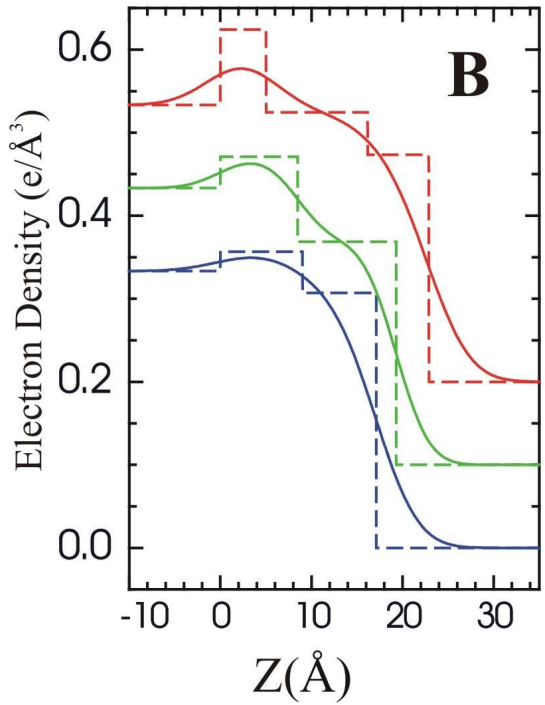


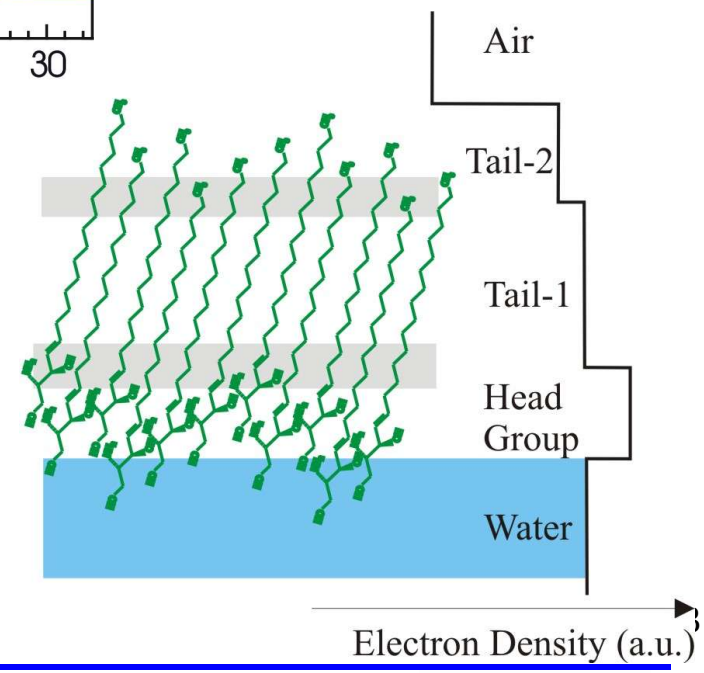
C18 sphingosine is natural - does not form a regular monolayer

C20 sphingosine is synthetic - forms a monolayer.

C18- SP Residues Show in Reflectivity







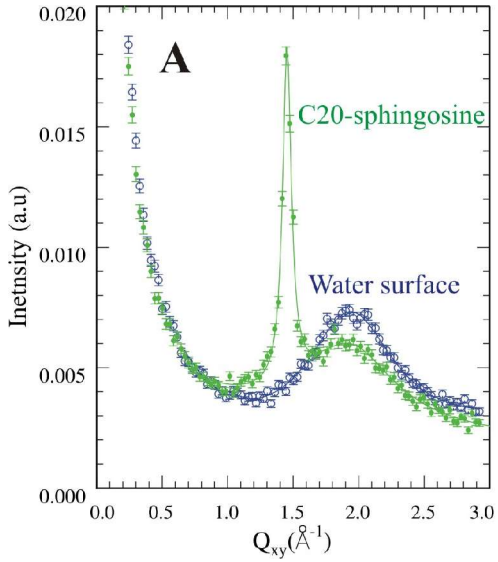
Vaknin, *J. Am. Chem. Soc.* (2002).

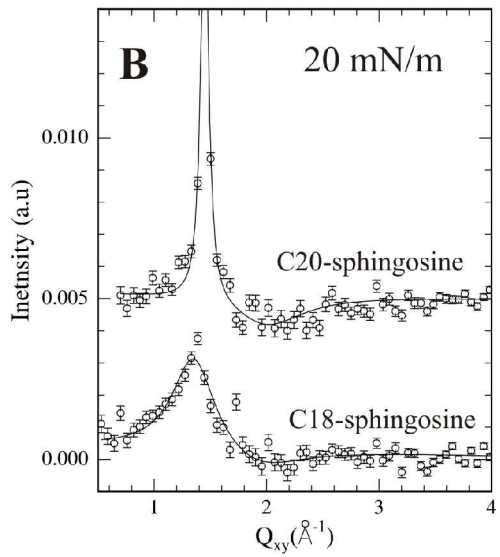
Short chain alcohols spread in excess – producing a meta- stable monolayer

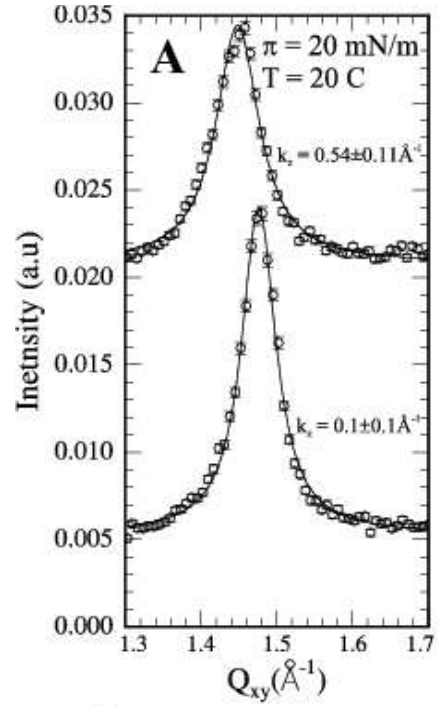
Berge et al, *Phys. Rev. Lett.* **73**, 1652 (1994).

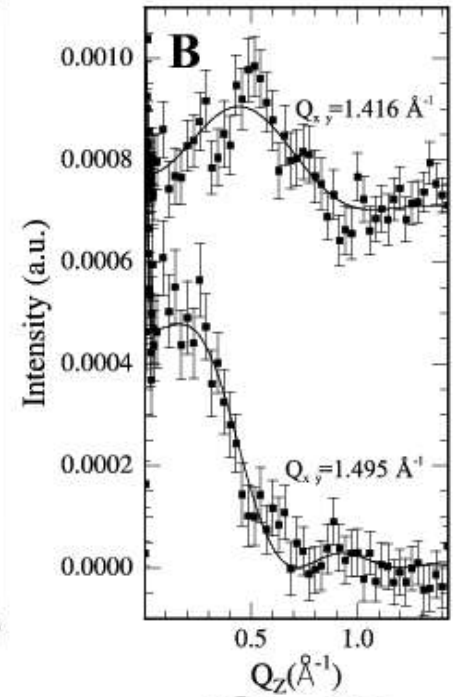
Short chain alcohols

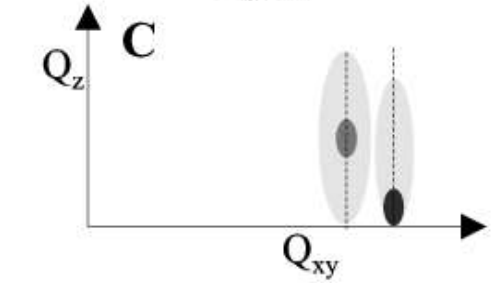
2D Diffraction (GIXD) - C18- SP and C20- SP Monolayers

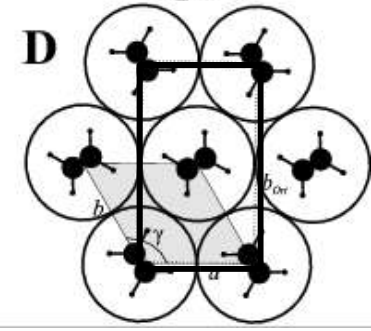












- C18- SP – forms a 2D liquid- like structure at the interface
- C20- SP forms a 2D rectangular lattice with tilted molecules. The rod scans indicate that only a portion of the tails is ordered – consistent with the corrugation (digitation) of chains (seen in reflectivity).

Relation to Function

Experimental Findings

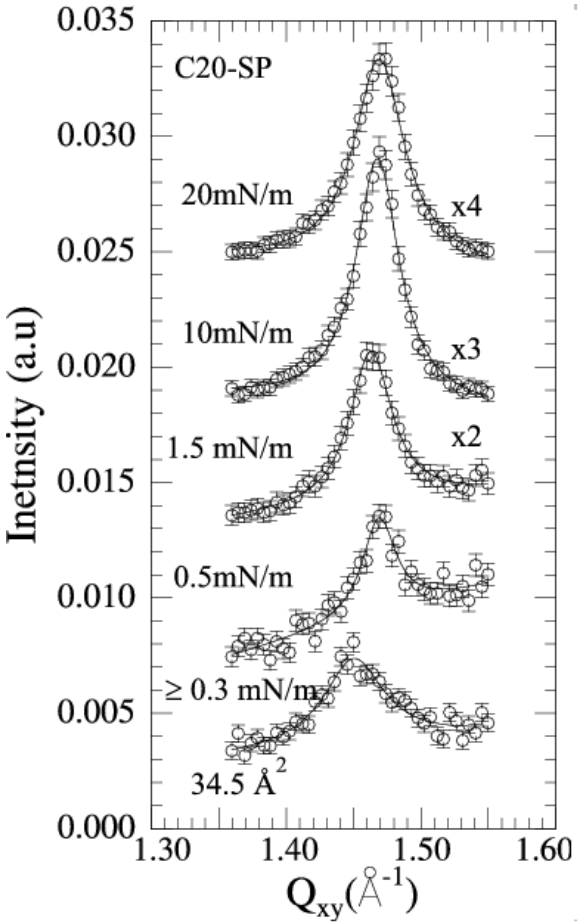
- C18-SP solubility
- Clustering
- Corrugated (digitated) surfaces

  - strong hydrogen bonding in the head-group region
  - strong affinity to water

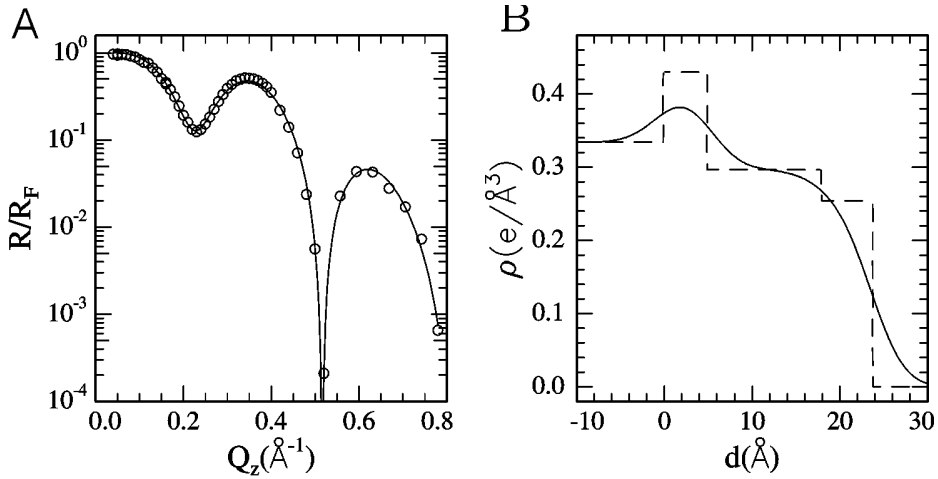
Implications

- **C18-SP is a secondary messenger – marginal marginal solubility –**
- **Protein rafts - Clustering due to strong binding at the head group. Corrugation at the tails - stronger binding with hydrophobic parts of the proteins.**

Sphingosine clusters at low densities – strong hydrogen bonding at the head group



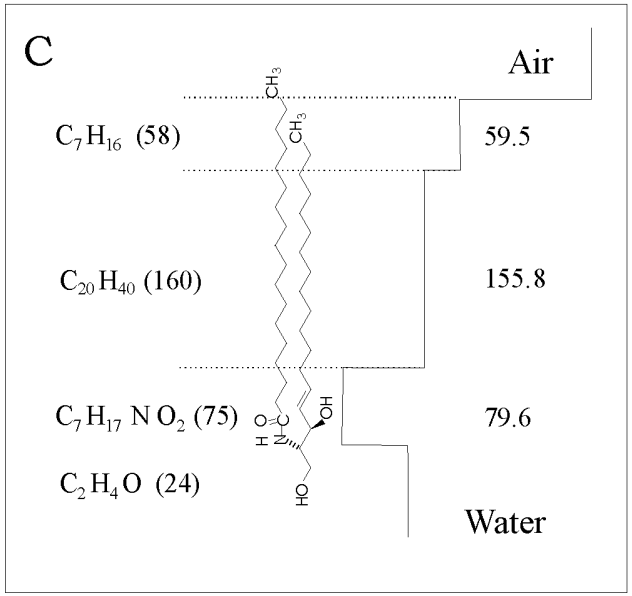
C18- Ceramide



Just like for sphingosine - 3 slabs are necessary to reconstruct the electron density. In general our studies as well as those of others on numerous kinds of lipids show that 2 slabs are sufficient.

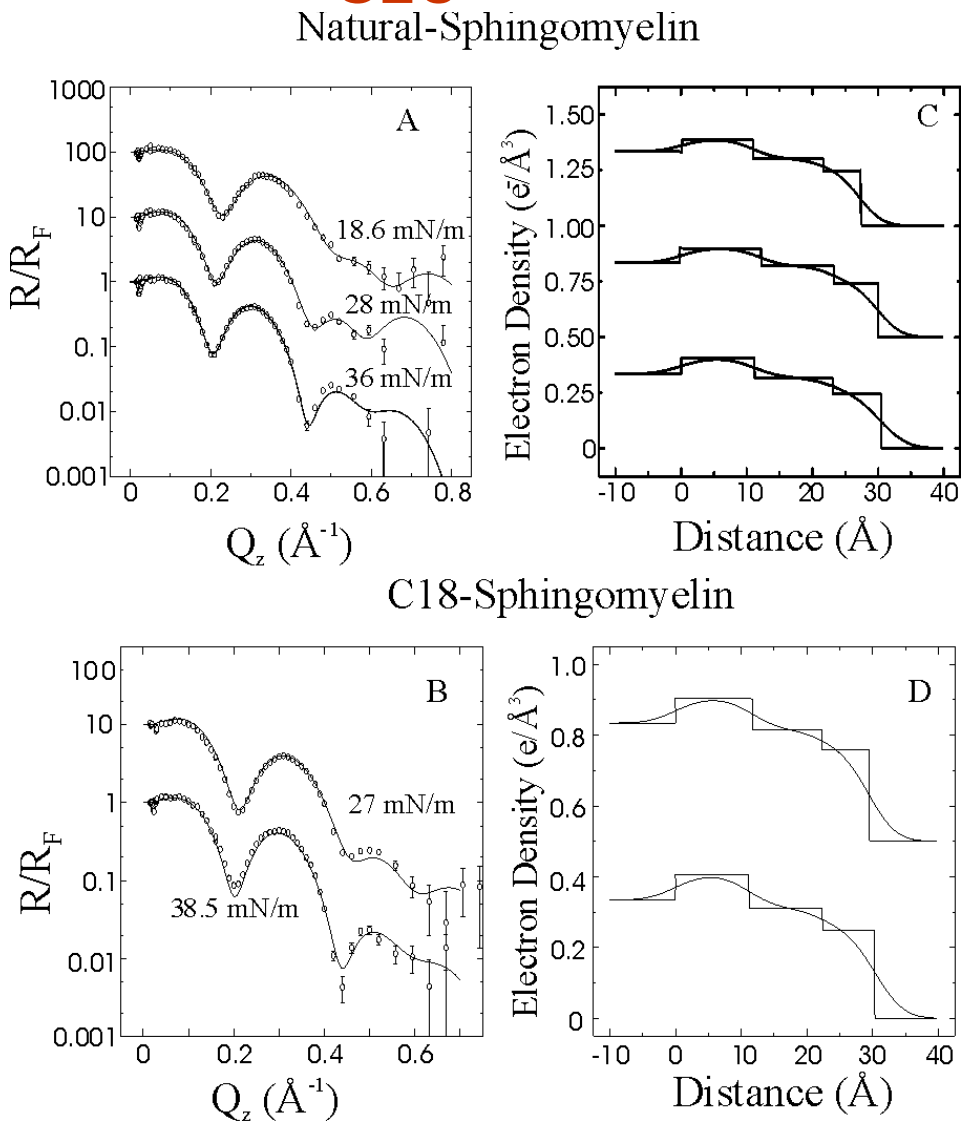
π=m N/ m

T = 18 C

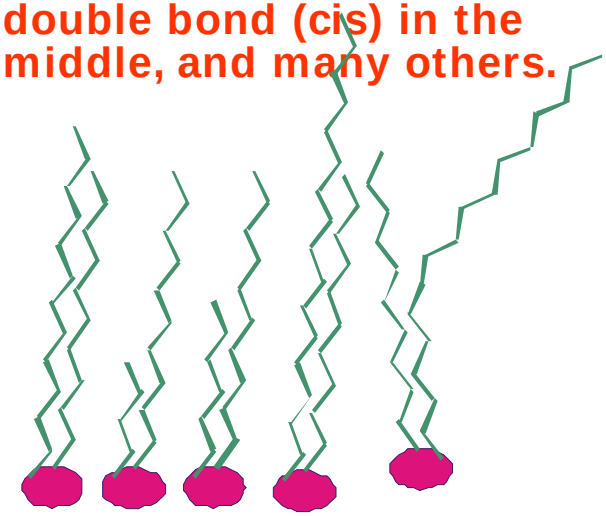


D. Vaknin, and M. Kelley, *Biophys. J* **79,** 2616 (2000).

Sphingomyelin Natural and C18



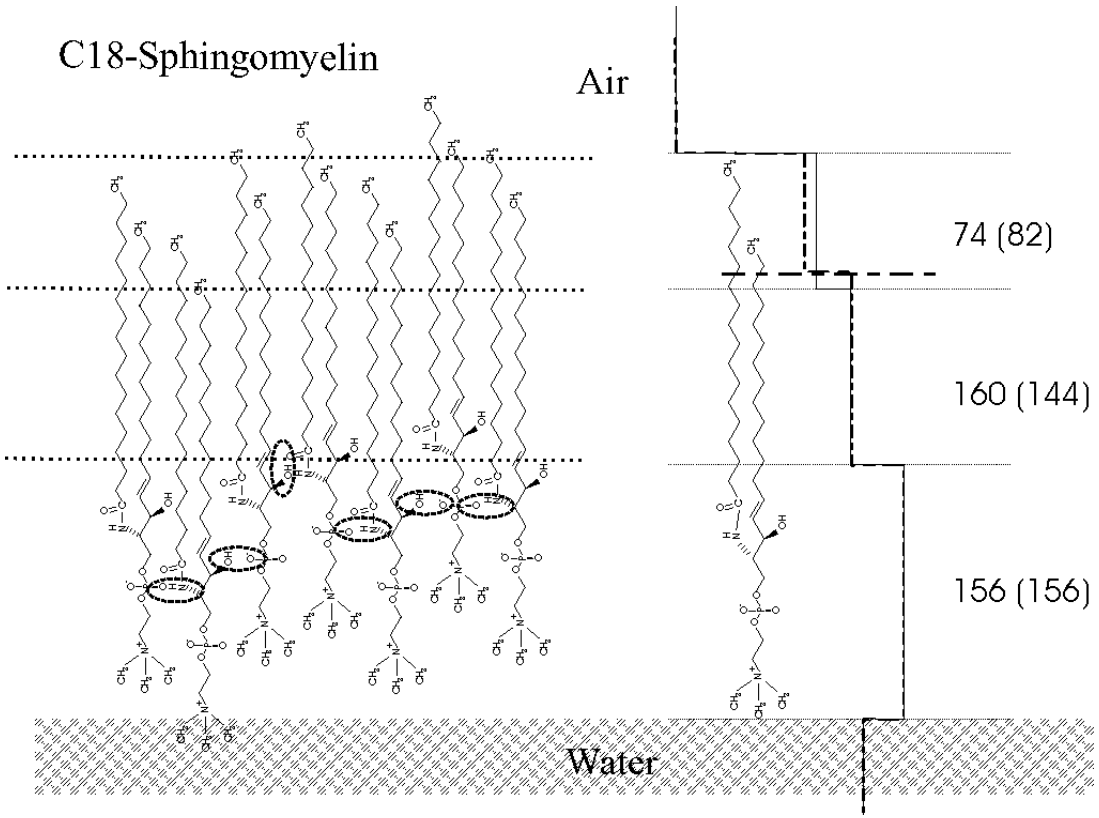
Natural Sphingomyelin (extracted from bovine brain) consists of a distribution of chain lengths (45% C18, 31% C24 with a double bond (cis) in the middle, and many others.



The two slabs at the air interface are associated with the chains $(CH_2)_n$

D. Vaknin, and M. Kelley, and B. M. Ocko, *J. Chem. Phys.* **115,** 7697 (2001).

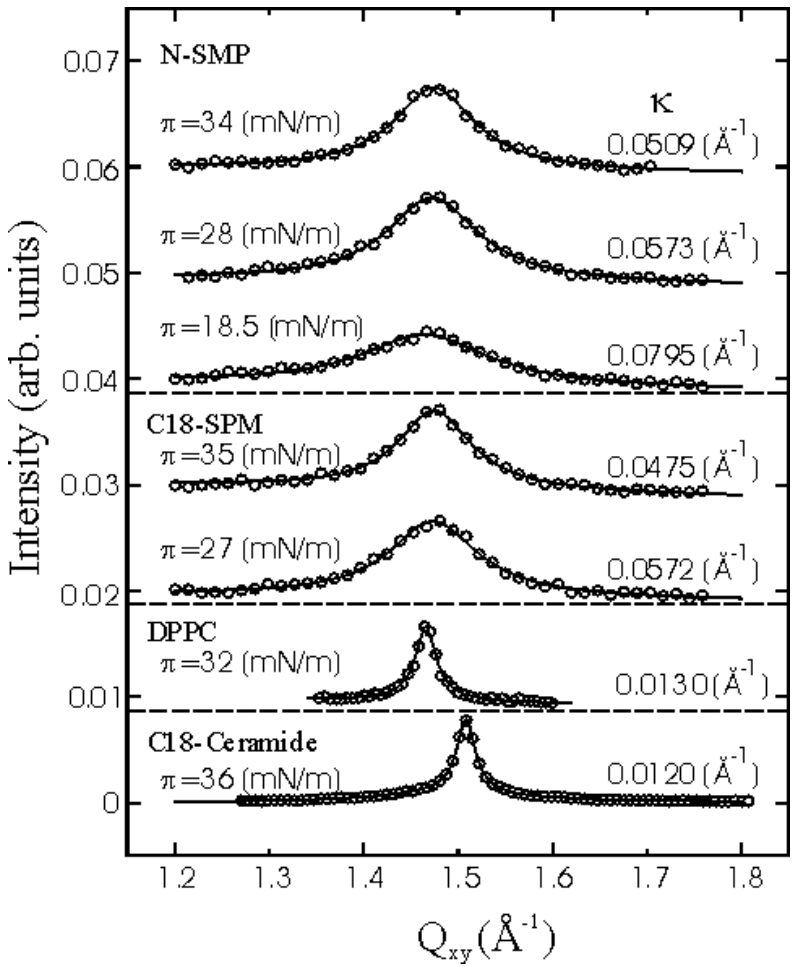
Corrugated Monolayers



<u>Hypothesis:</u> The digitation in the chains is simply a conformation to the staggering at the head group region, which is driven by the multiple sites that form hydrogen bonding.

<u>Relation to Function:</u> Ceramides are abundant in skin (dealing with harsh environmental conditions), myelin is an insulator in nerve cells (80%lipid in membrane). As a bilayer (a real membrane) one expects interdigitation of chains (strength, impermeability). Velcro effect of the bilayer.

Sphingomyelin – Short- range- inplane- order

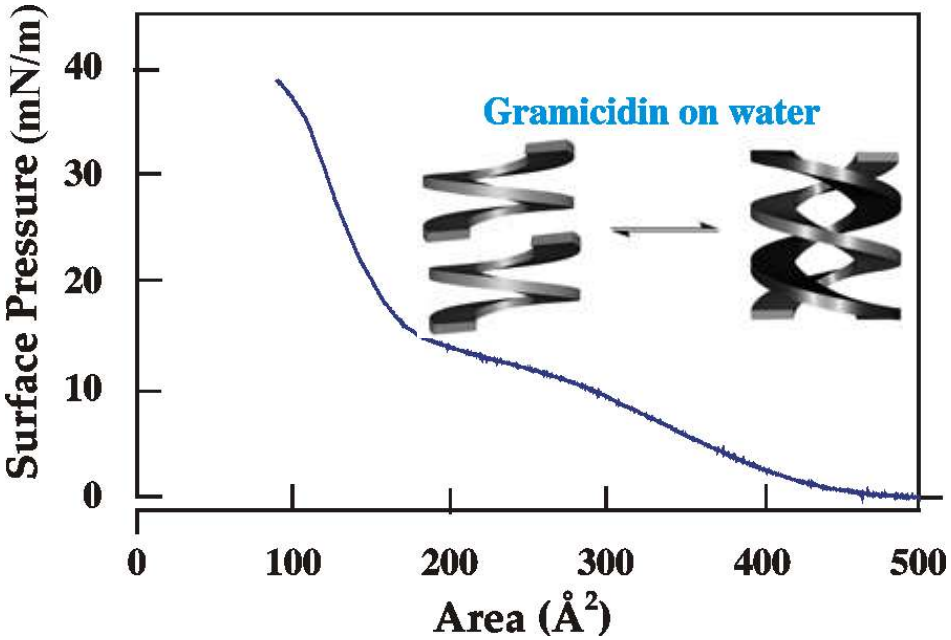


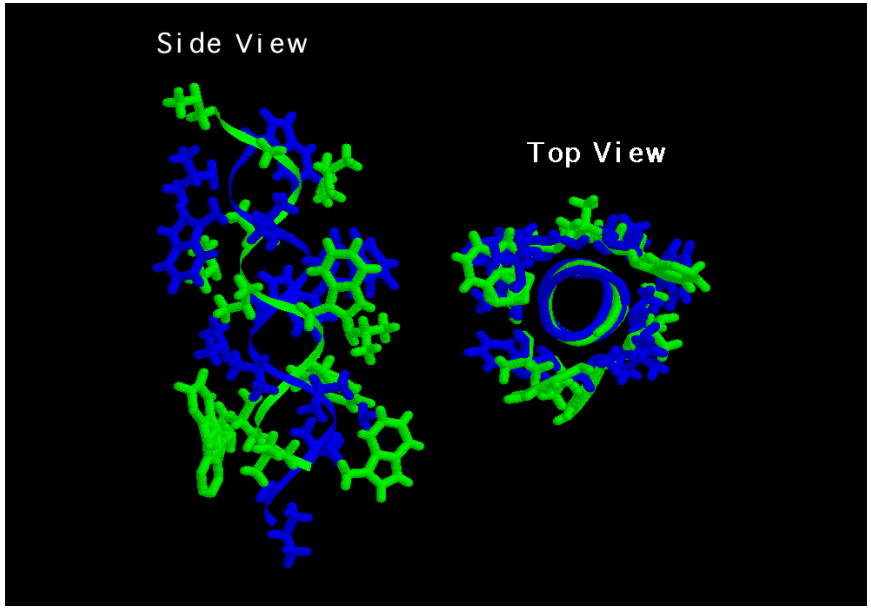
- **Chain length distribution does not play an important role in the organization of the film, the interaction at the head group region are more important.**
- **Molecules with double- bond chains (kink) phase separate from saturated chains.**

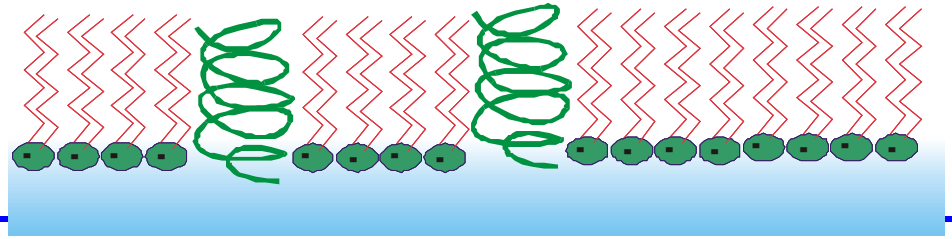
D. Vaknin, and M. Kelley, and B. M. Ocko, *J. Chem. Phys.* **115**, 7697(200

Simple membrane protein

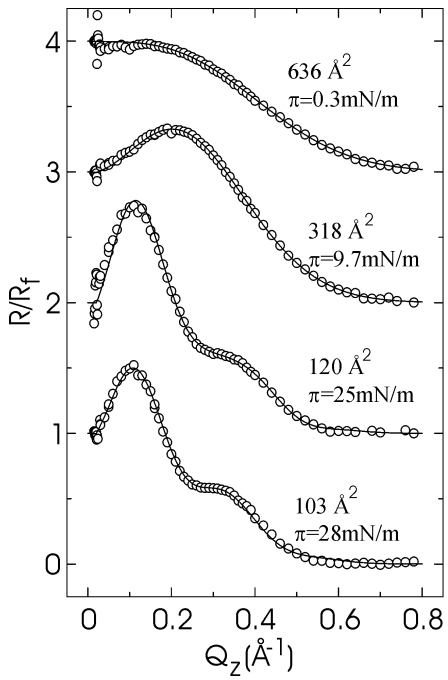
Linear gramicidin is a hydrophobic antibiotic polypeptide composed of 15 hydrophobic amino acids in D- and L- alternate conformers with the following sequence: formyl- L- X- gly- L- ala- D- leu- L- ala- D- val- L- val- D- val- L- trp- D- leu- L- trp- D- leu- L- trp- D- leu- L- trp- ethanolamine where X represents either valine or isoleucine

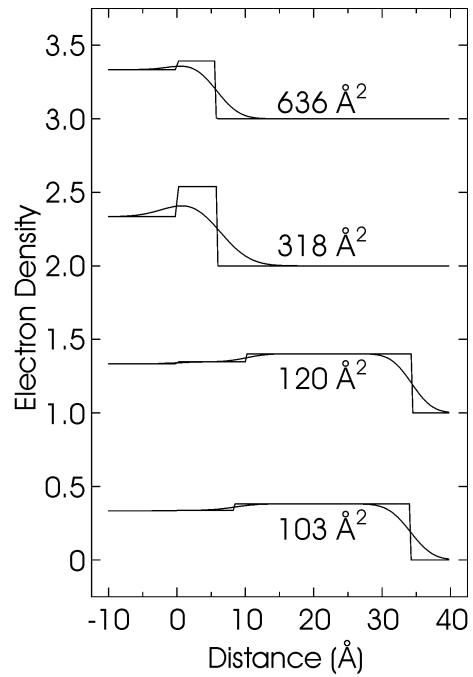


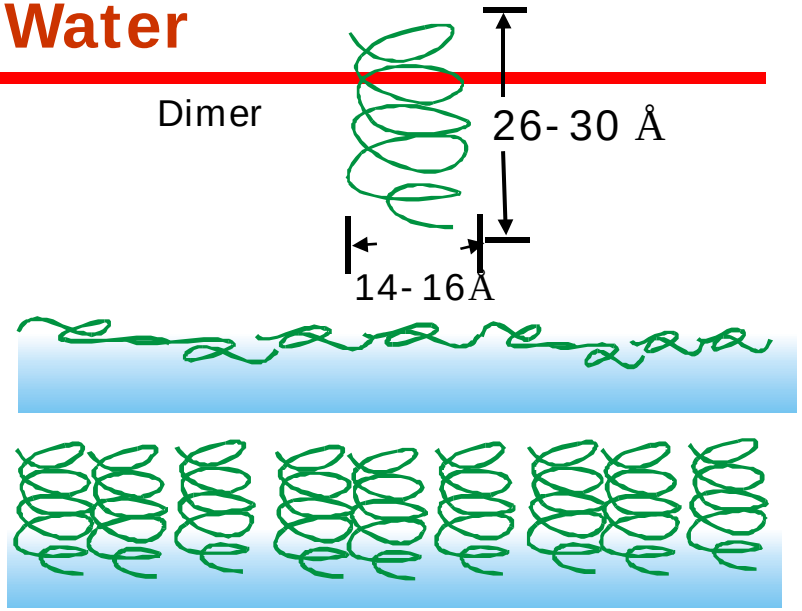




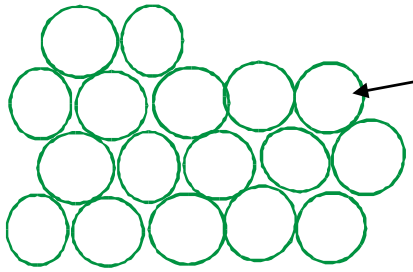
Gramicidin on Water

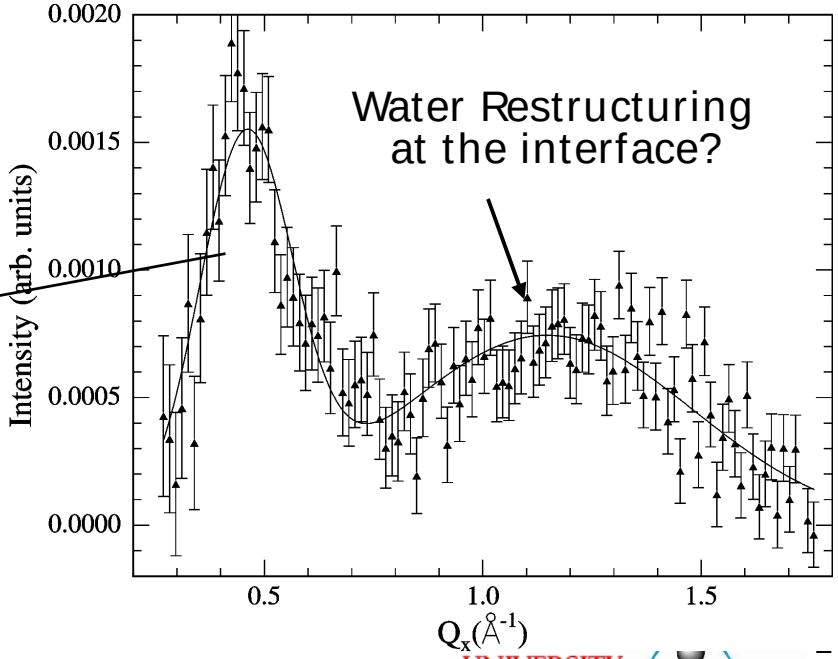




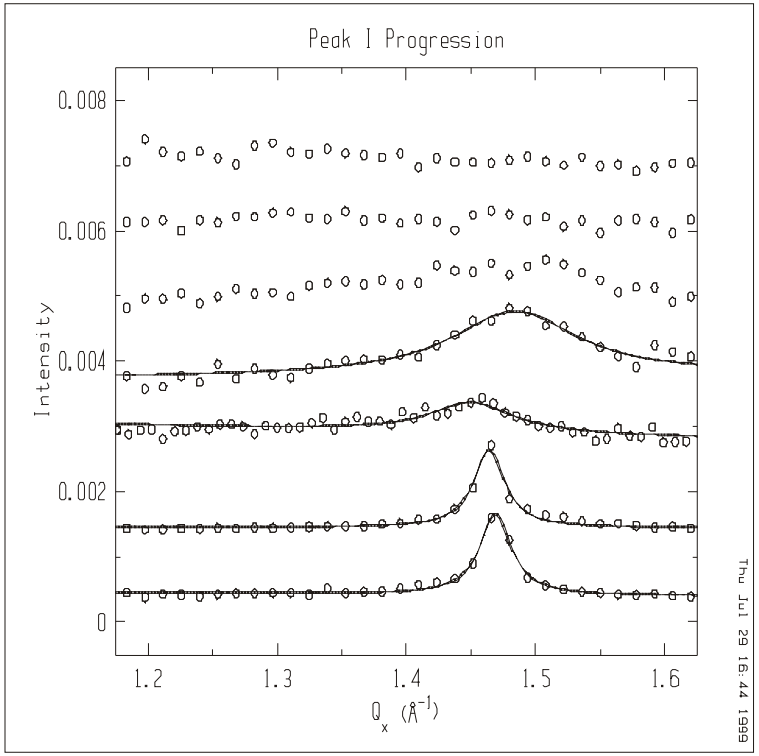


Short range in-plane order



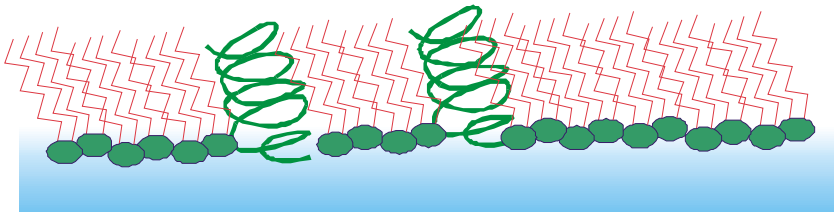


DPPC- Gramicidin on water 2D Diffraction from Chains

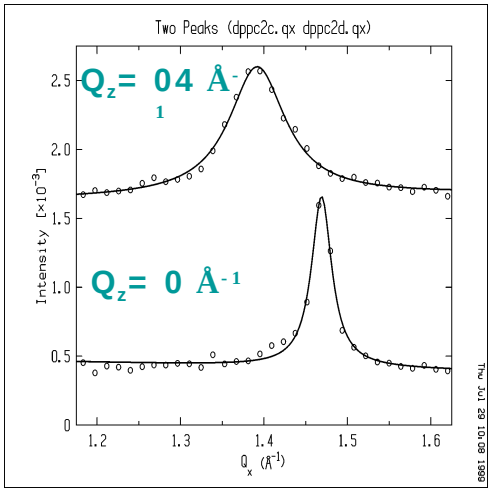


DPPC/ Gramicidin

0%
10%
25%
50%
75%
90%
100%

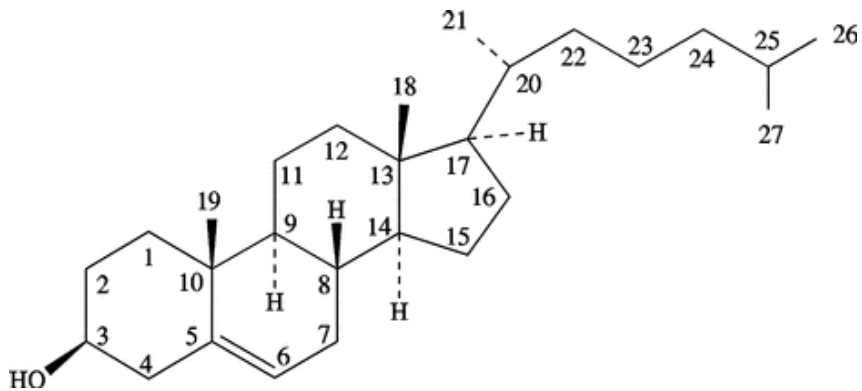


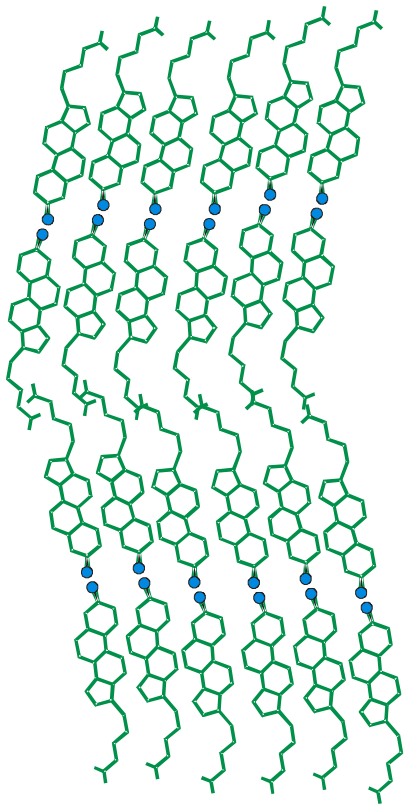
DPPC only



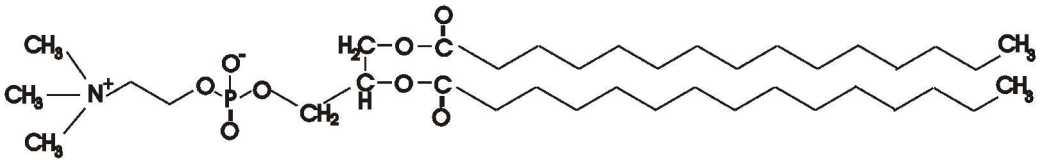
Broadening of peak and its disappearance with increase in G concentration indicate complete miscibility of DPPC/ Gramicidin; no phase separation

Oriented Bilayers – Cholesterol/ Lipid Mixtures

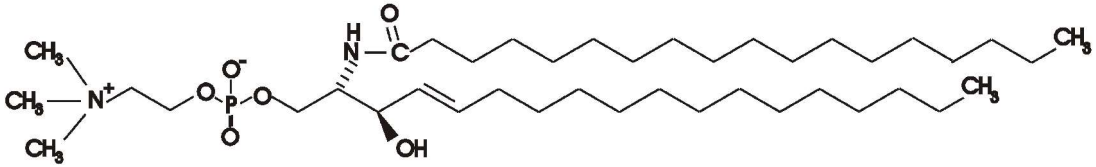




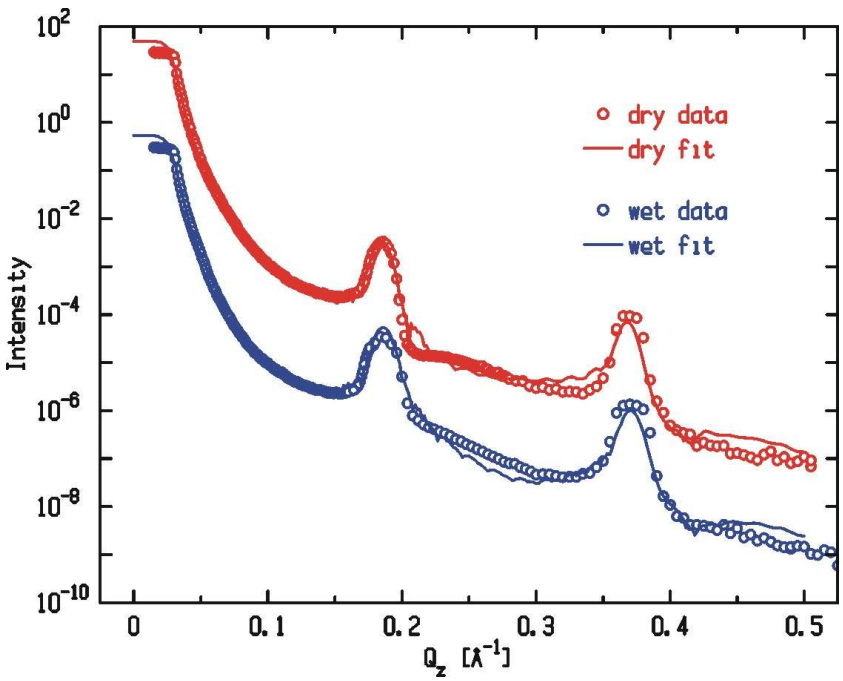
DiPalmitoyl-phospatidyl-choline (DPPC)

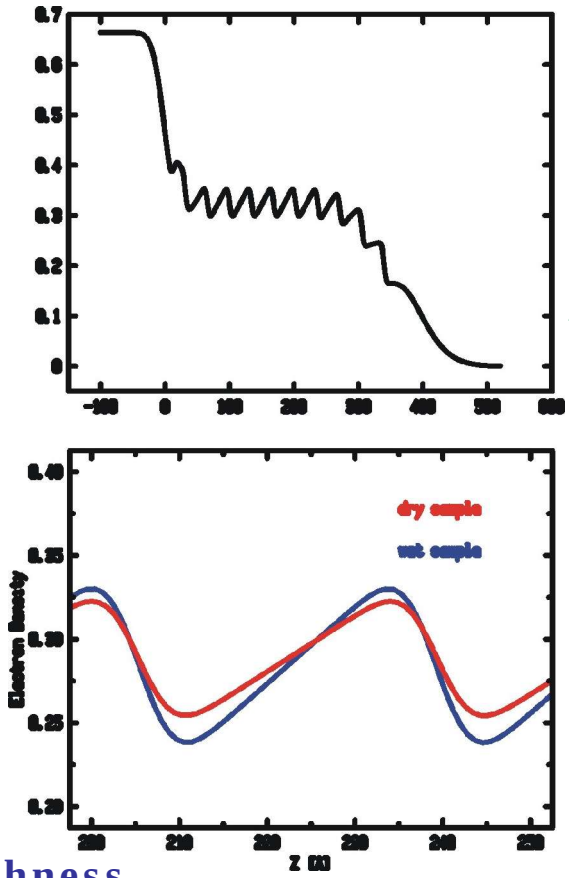


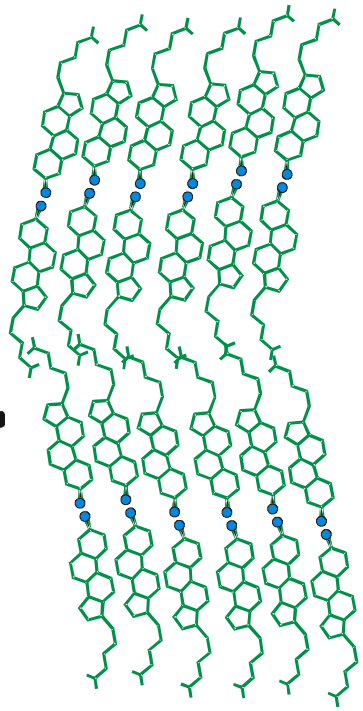
C18-Sphingomyelin



Oriented Bilayers of pure cholesterol



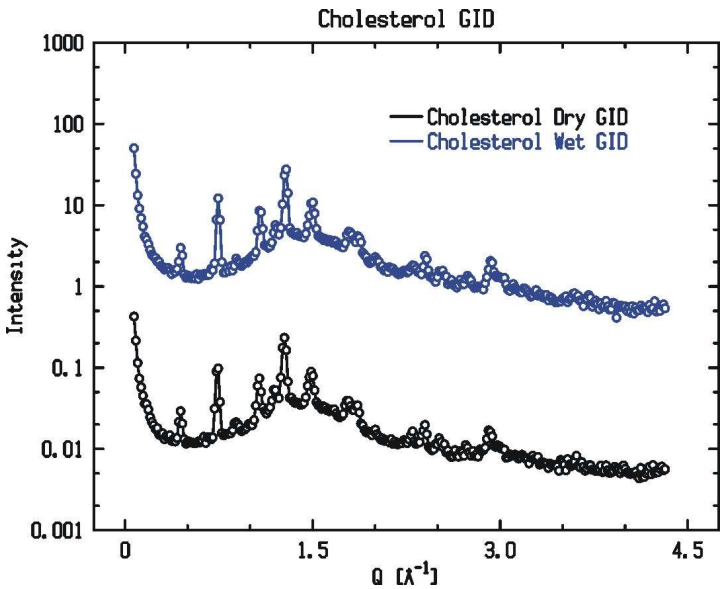


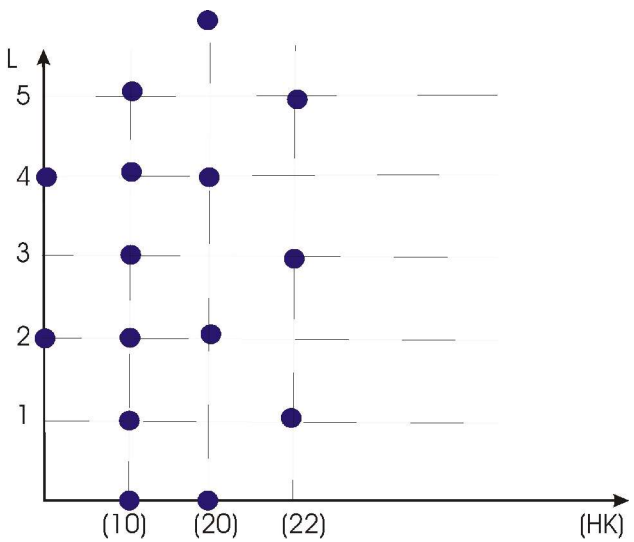


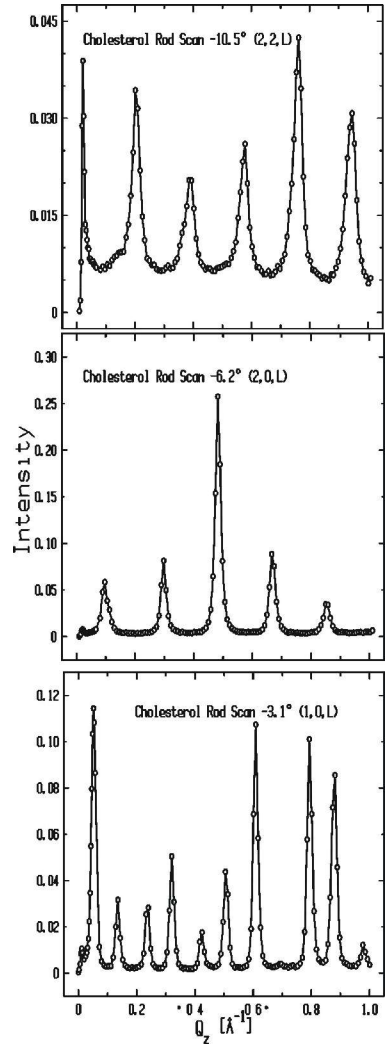
Spin Coated samples on glass flat (roughness ~ 1Å)

- **1000 rpm (10 to 11 layers).**
- **Chloroform solution 700 µL**
- **50/50 is molar ratio. No sonicate was used.**
- **Electrical shaker is used to dissolve the materials in chloroform.**

Crystalline Cholesterol







a = 14.1564⊕(14.172Å)

b = 10.521Å (10.481Å)

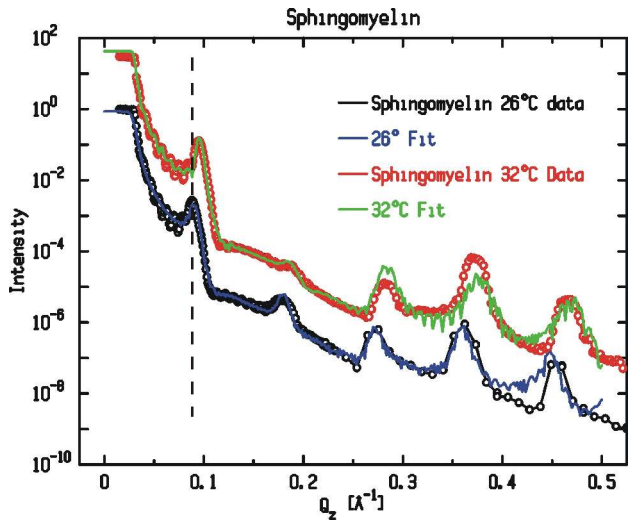
c = 68.4Å (34.209Å)

$\gamma \approx 90$deg.

Shieh, Hoard and Nordman "The structure of Chlesterol" Acta Cryst. B **37**, 1538 (1981).

- **Cholesterol Readily crystallizes**
- **New stacking found**
- **Very hard to hydrate (impossible)**

Oriented- Bilayers of Sphingomyelin

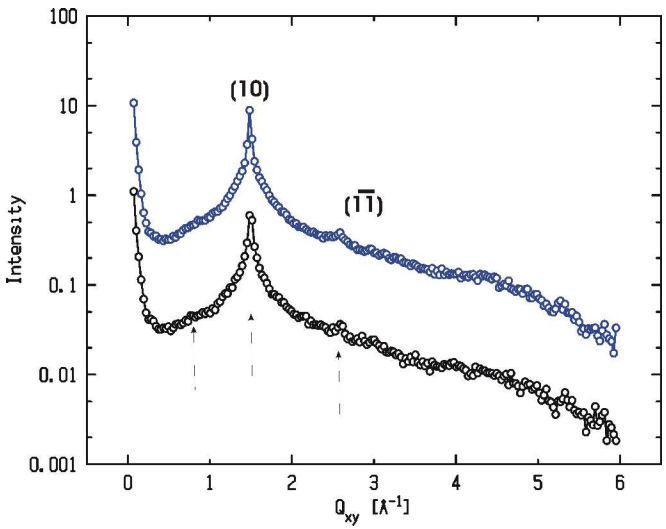


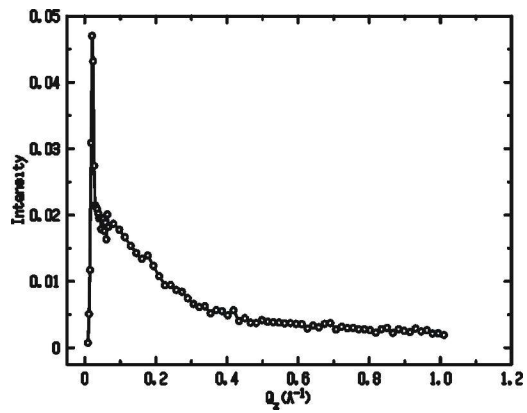
2D - Hexagonal

a = 4.837⊕

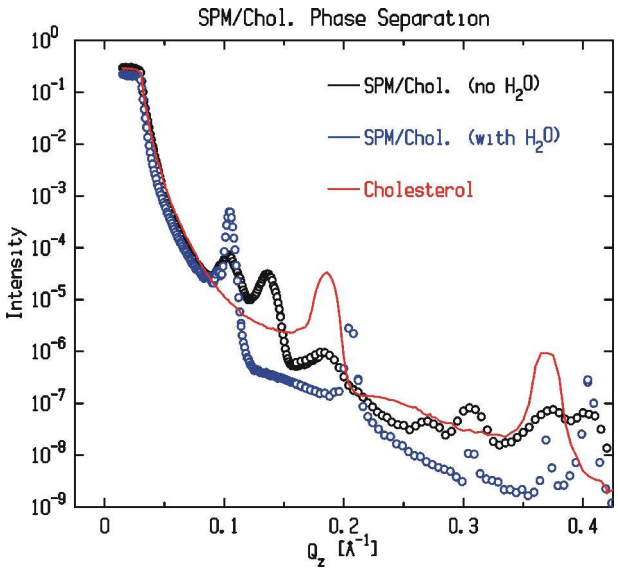
c = 62.8 (dry) - 69.8Å

- **No interlayer correlations**
- **Sensitive to humidity**



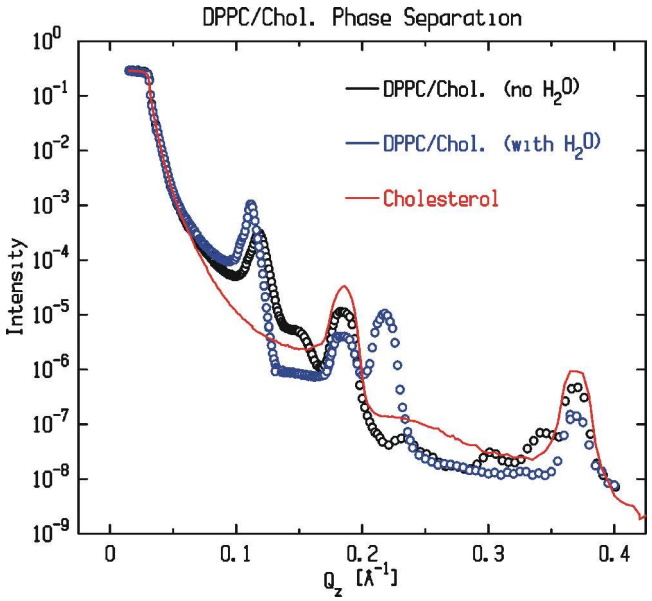


Oriented- Bilayers of SPM/ Chol and DPPC/ Chol



SPM/ Chol

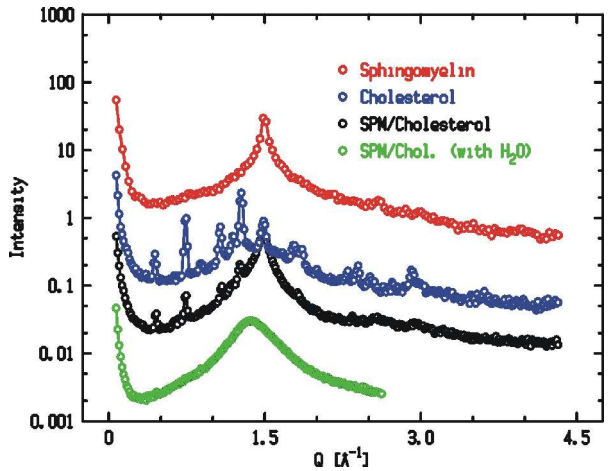
- **Dry conditions – phase separate**
- **Wet conditions - mixed**

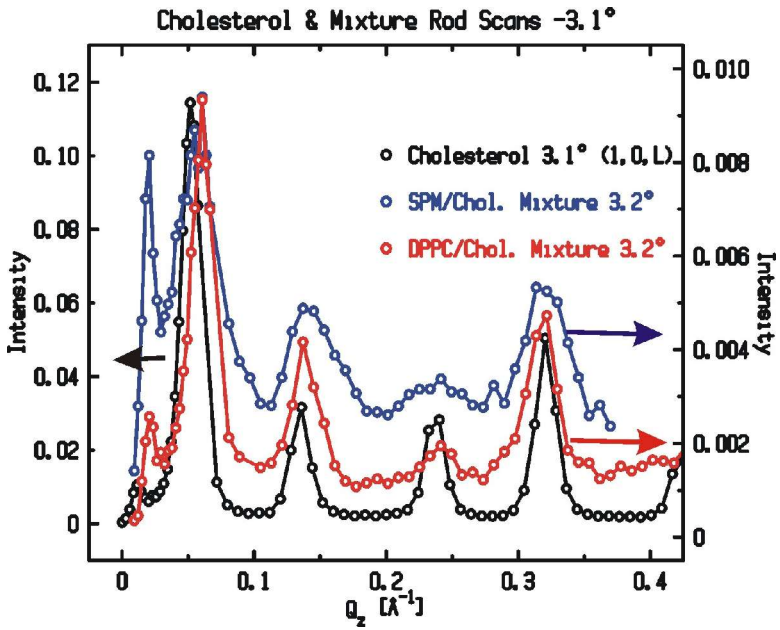


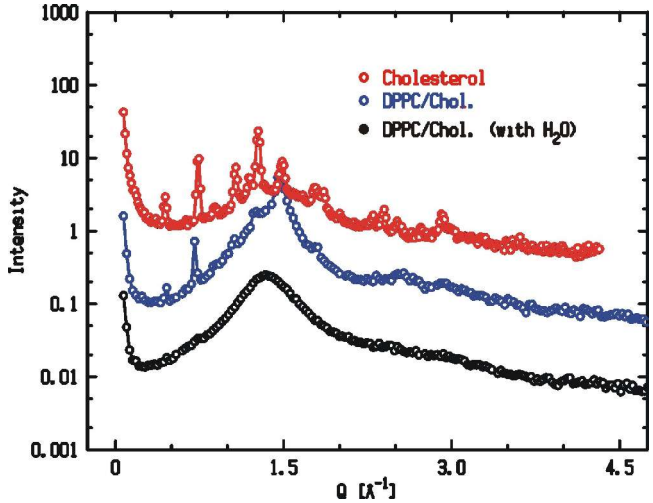
DPPC/ Chol

- **Dry conditions – phase separate**
- **Wet conditions – phase separate**

Oriented- Bilayers of SPM/ Chol and DPPC/ Chol







Conclusions and Future Directions

- Surface properties of membrane constituents can (might) shed light on their functions.
- Interfacial phenomena can be monitored and characterized on the molecular length scale - Mimic the immune system, biomineralization processes, ion- concentrations.
- Future work –
- Mixed monolayers – different lipids, cholesterol, and proteins – constructing a leaflet of a membrane.
- Transferring those half membranes to solid support to build membranes.
- Varying conditions of subphase – Temperature, pH, ionic concentrations etc.

Acknowledgements

Sphingolipids and DHDP

Michael S. Kelley (ISU)

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Specific bindings/ Anomalous reflectivity

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Kristian Kjaer (Riso, Denmark)

Jen Als- Nielsen (Riso, Denmark)

Memebrane proteins

Christian Salesse (Quebec, Canada)

Judith Gallant (Quebec, Canada)

Hugo Lavoie (Quebec, Canada)

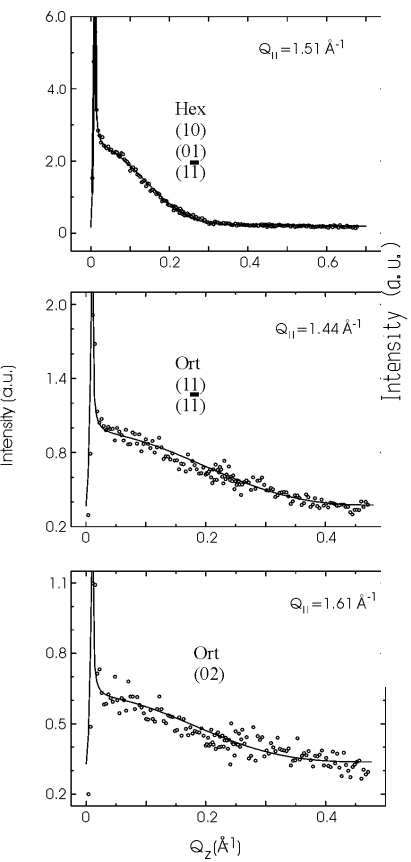
Engineers

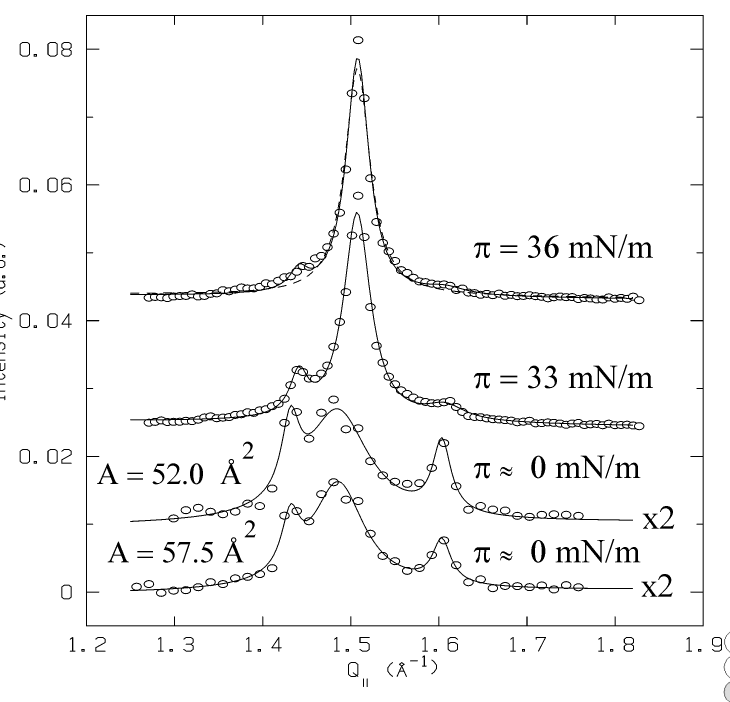
Michael Harper (ISU)

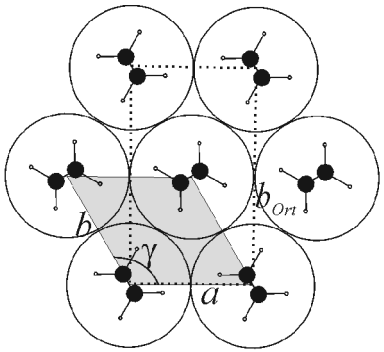
Terrance Herrman (ISU)

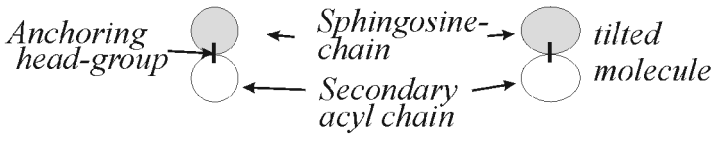
Ceramide forms spontaneously 2D crystals that nucleate at zero surface pressure, suggest strong binding energies between molecules. At low pressures the reflectivity can be fitted as an incoherent sum of water domains + monolayer domains

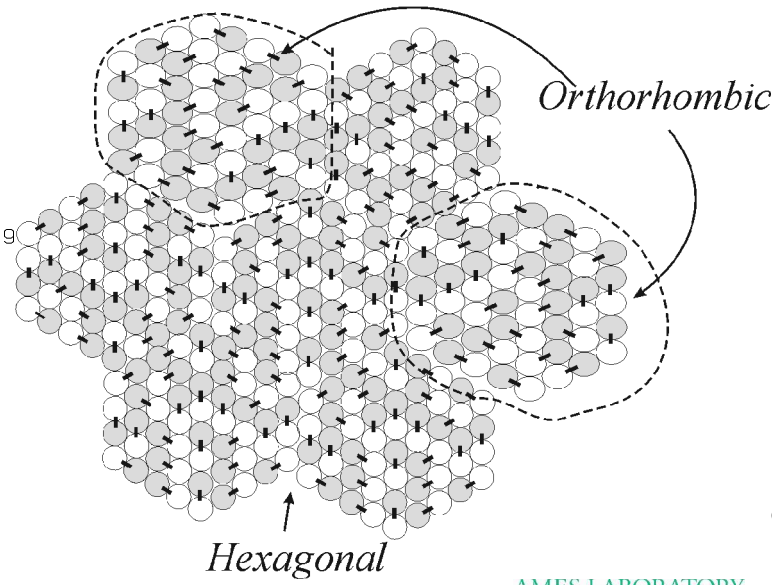
Bragg reflection is due to the ordering of single chains although pairs of chains are coupled to single head group











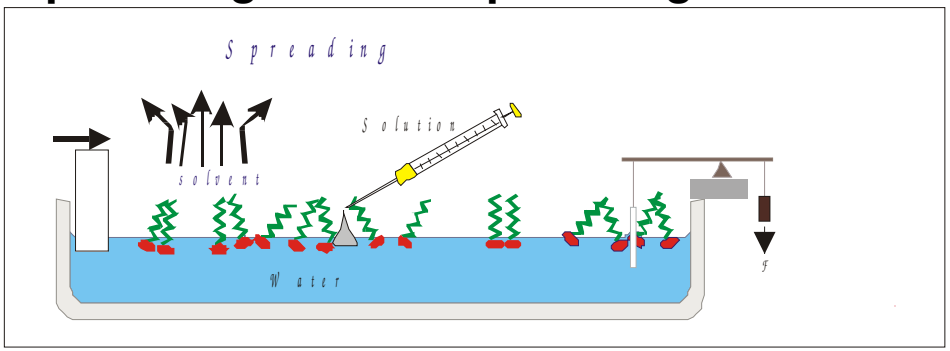
D. Vaknin, and M. Kelley, *Biophys. J* **79,** 2616 (2000).

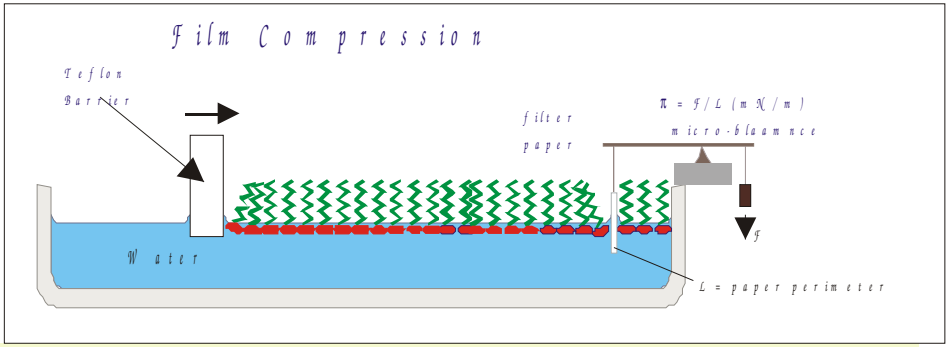
Langmuir Monolayer

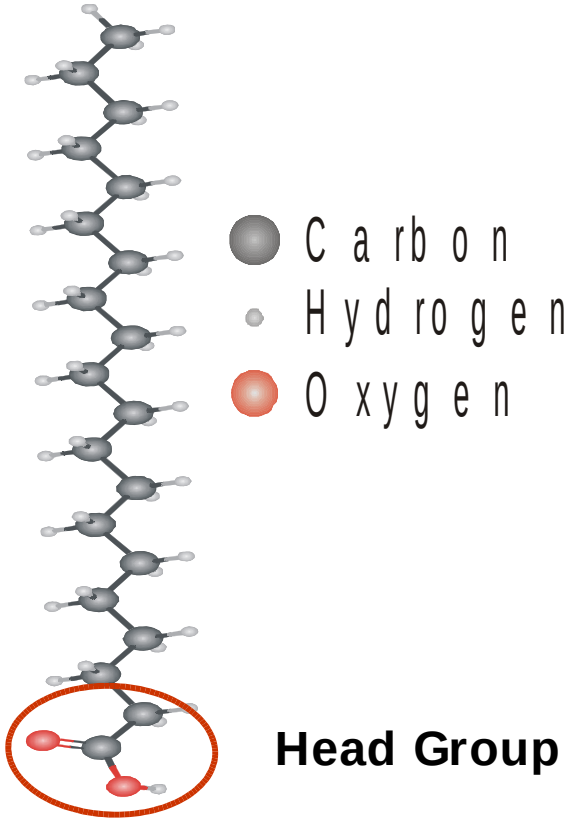
Langmuir monolayer - a monomolecular (amphiphilic) film at the air/water interface

Typical molecules - fatty-acids, -alcohols, lipids, and others

Spreading and Compressing







2D Physics

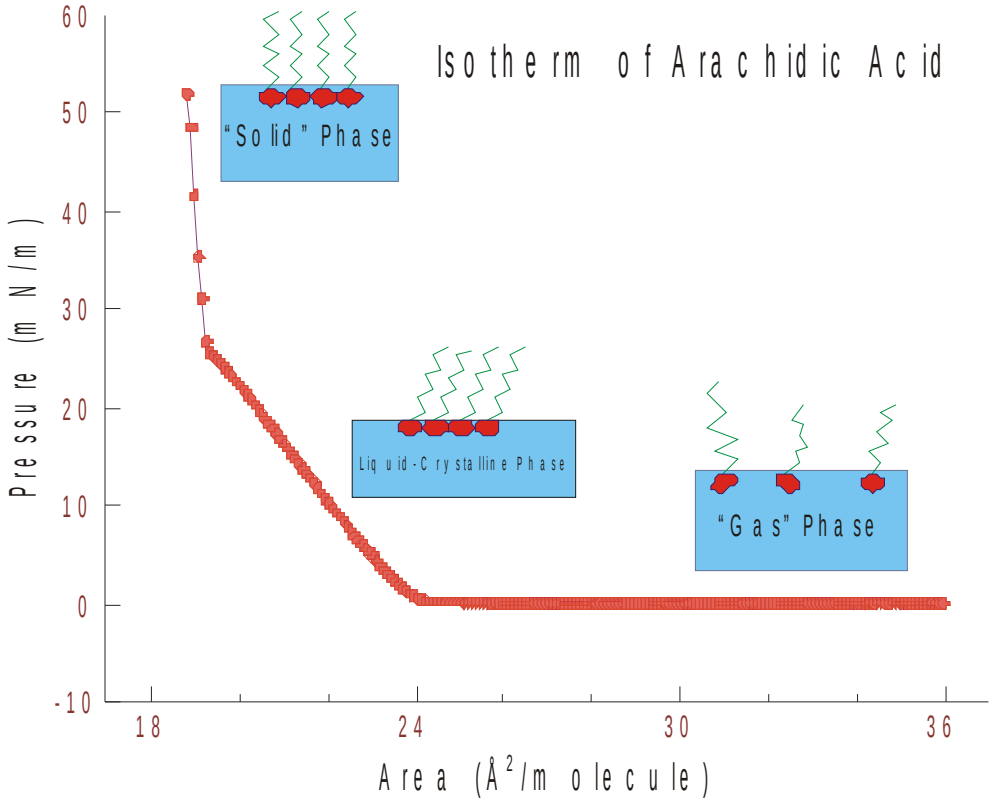
Structure and phase transitions in Langmuir monolayers

Kaganer et al., *Rev. Mod. Phys.* **71**, 779 (1999).

Structural Competing Techniques

Techniques:

- Ellipsometry
- Fluorescence microscopy
- Brewster Angle Microscope
- IR spectroscopy
- AFM
- others

Structure on molecular-length-scale

- X-ray scattering
- Neutron scattering