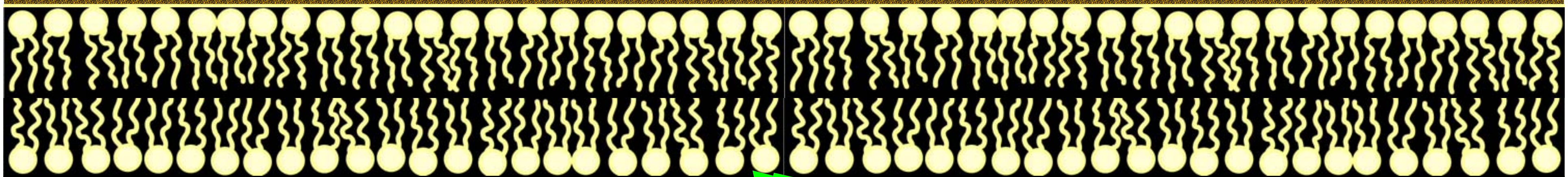
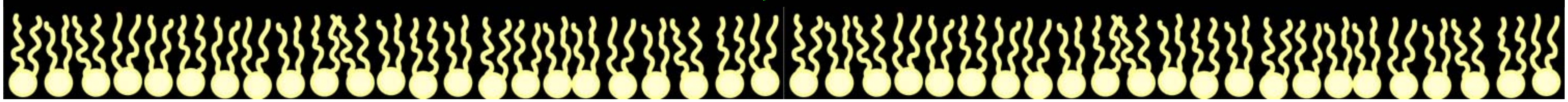


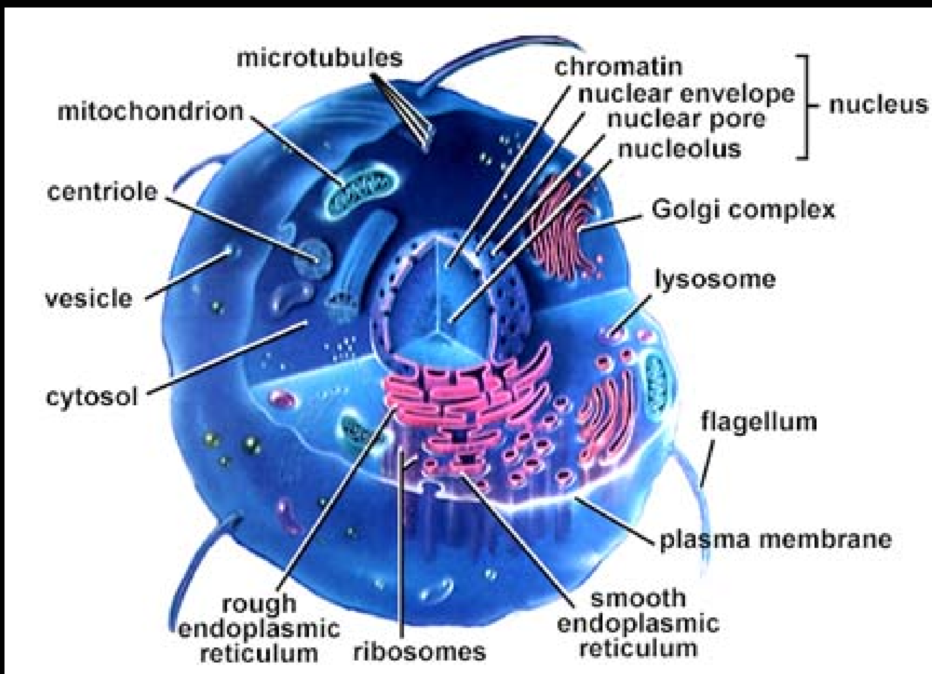
Structural Analysis of Phospholipid Membranes and Toxin Assault:

Neutron/X-ray Scattering Methods Open a Window
To Understanding Membrane Structure, Lipid Domains,
and Toxin Invasion

Tonya Kuhl
UC DAVIS

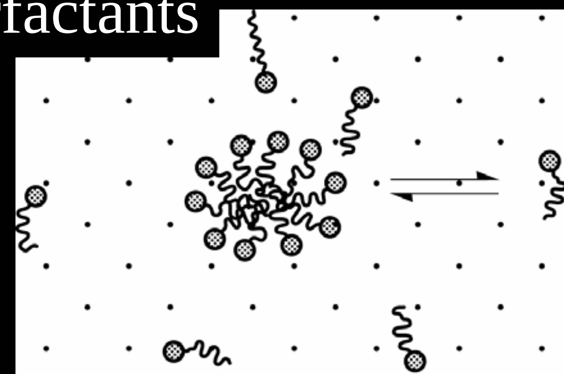


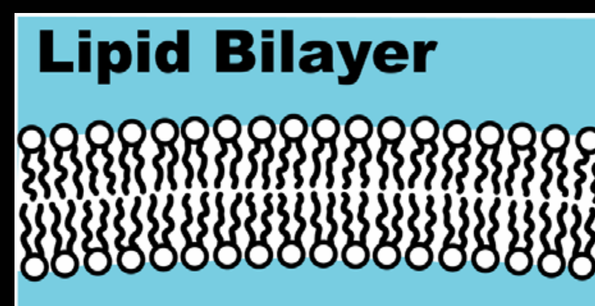
Biophysics of the Cell Membrane



Self-Assembly

surfactants

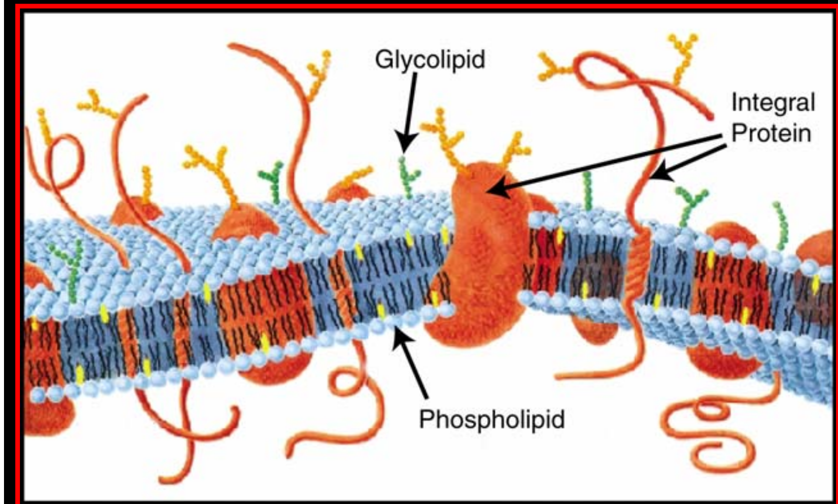


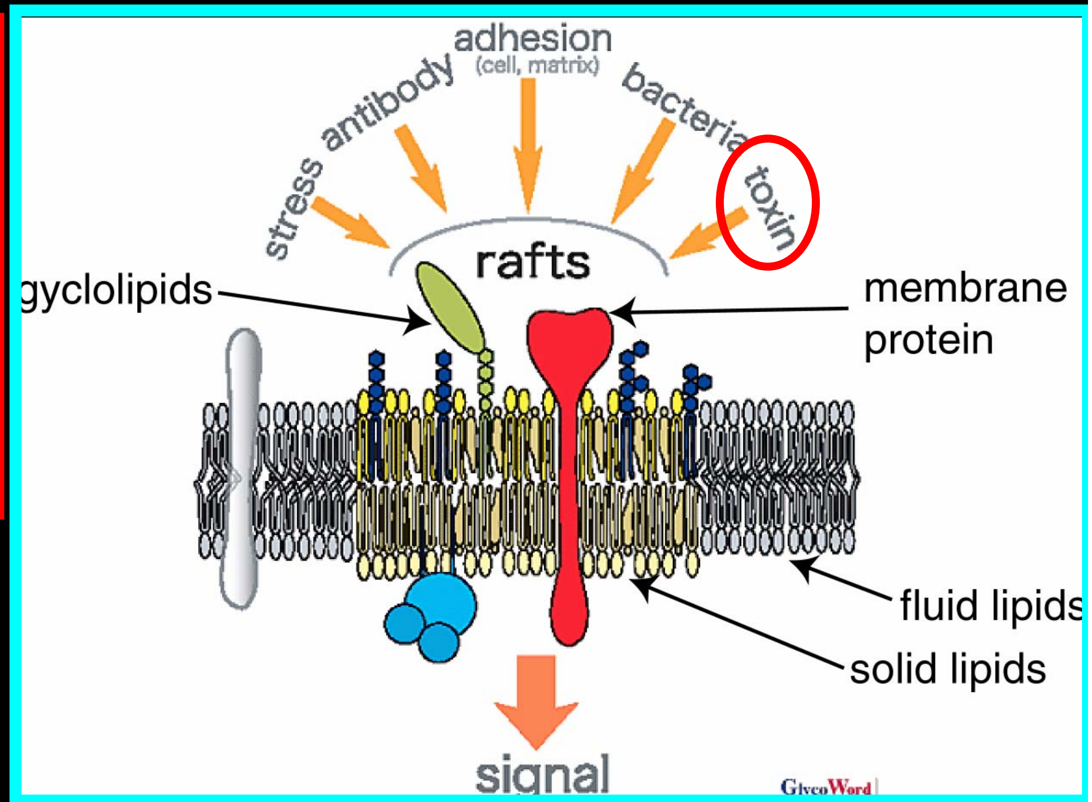


Membranes are where the ACTION takes place!

Homogeneous vs Heterogeneous

Fluid mosaic model vs. Lipid domains – "rafts" Since 2000, over 2500 articles



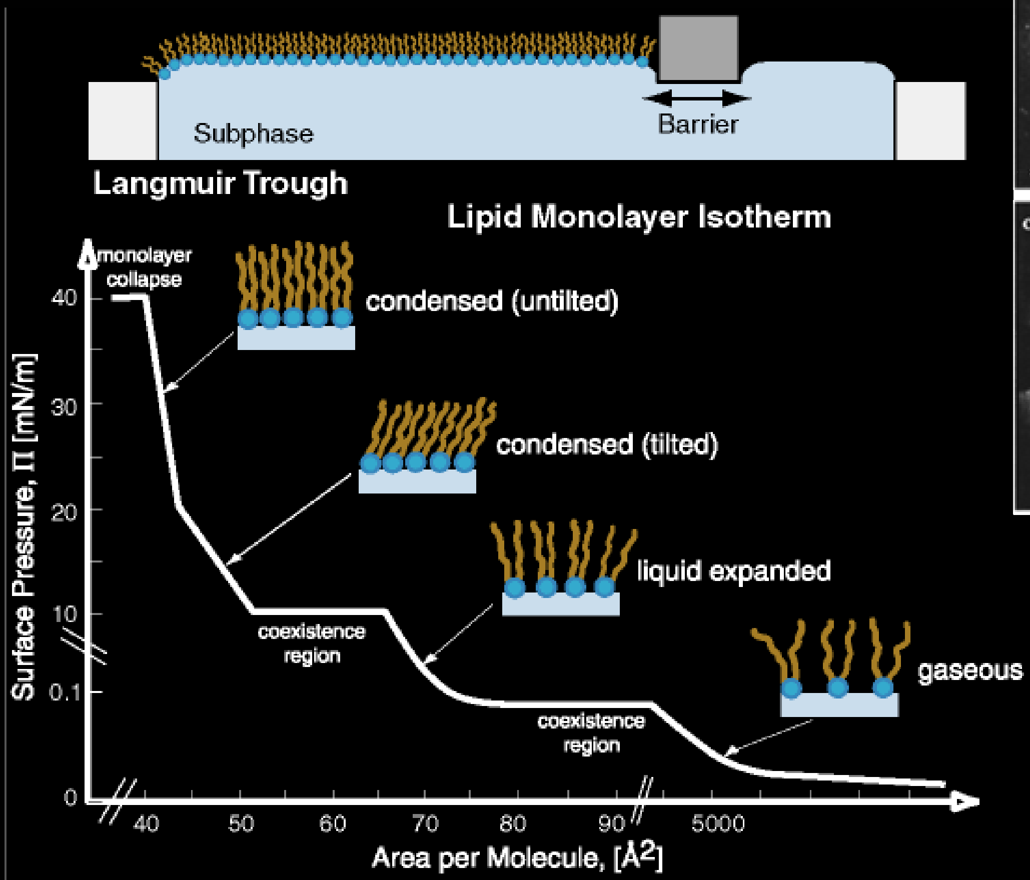


Cell membranes are no longer thought of as simply passive 2-D liquids.

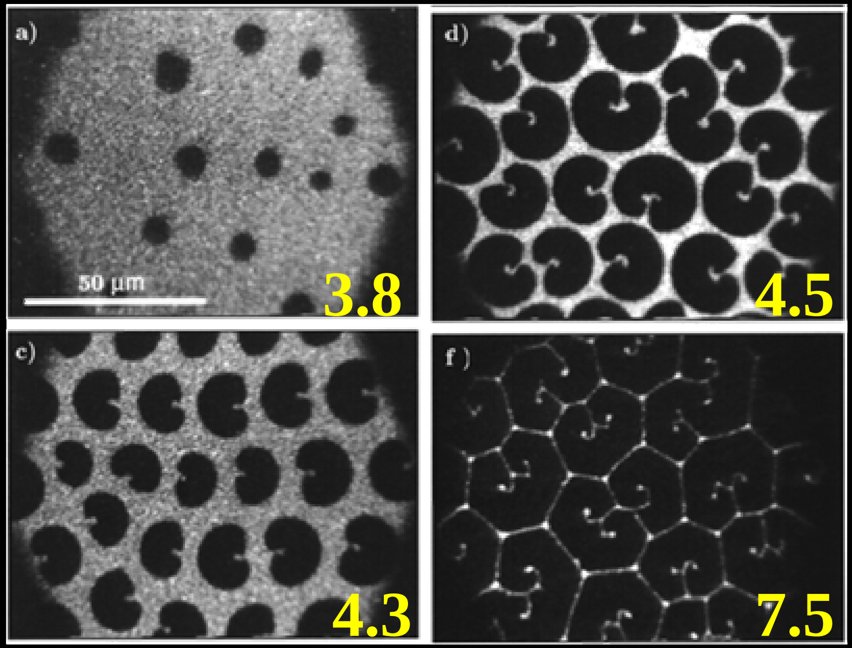
cell polarity, protein trafficking, **signal transduction**

Lipid Monolayers

-Single Molecular Layer
-Control Membrane components



Fluorescent Microscopy

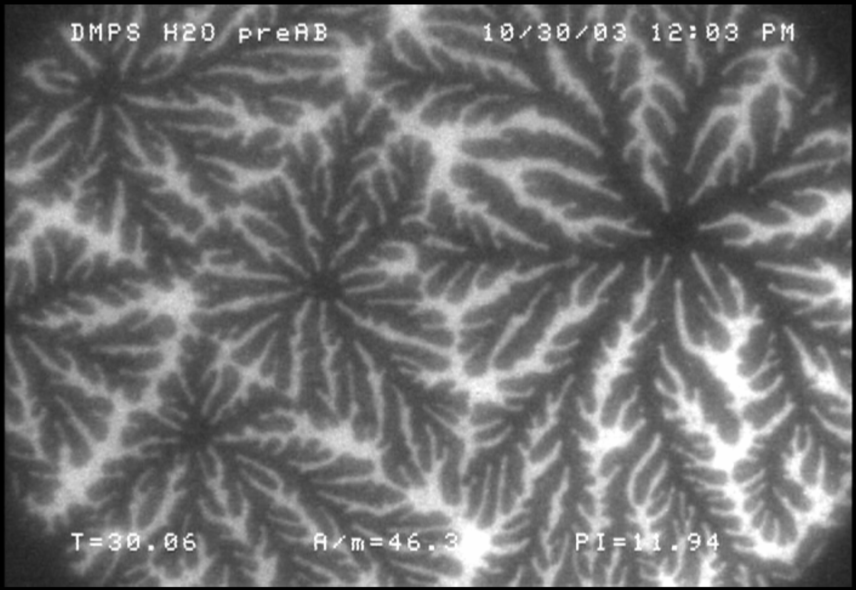


Di-16-PC = DPPC

Fluorescence vs Brewster Angle Microscopy

DMPS lipids

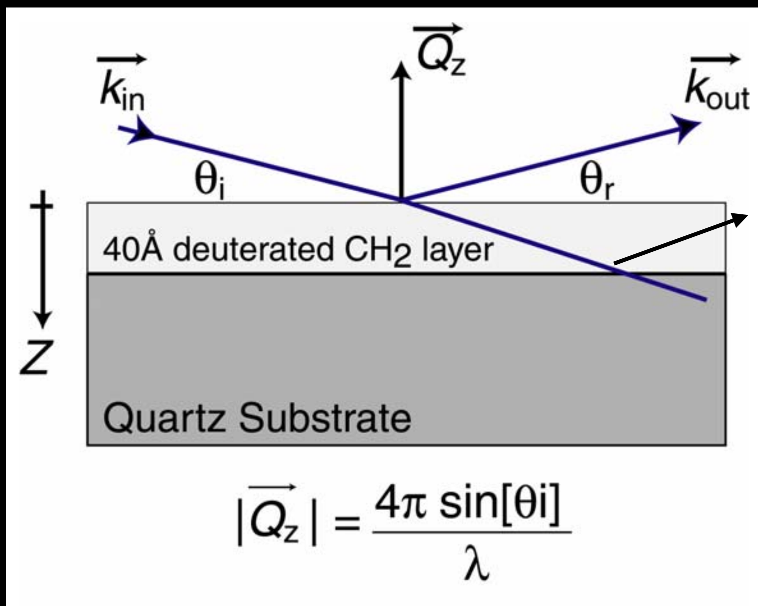
Fluorescence



Brewster Angle

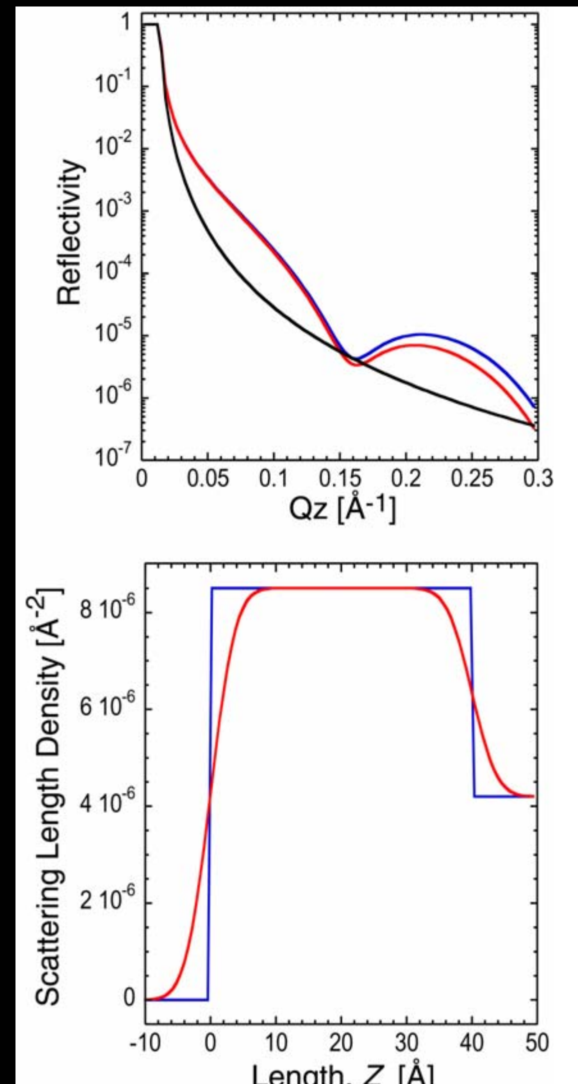
Maximum resolution ~1 μm

Reflectivity [Neutrons and X-rays]



$\theta_i = \theta_r$

Elastic



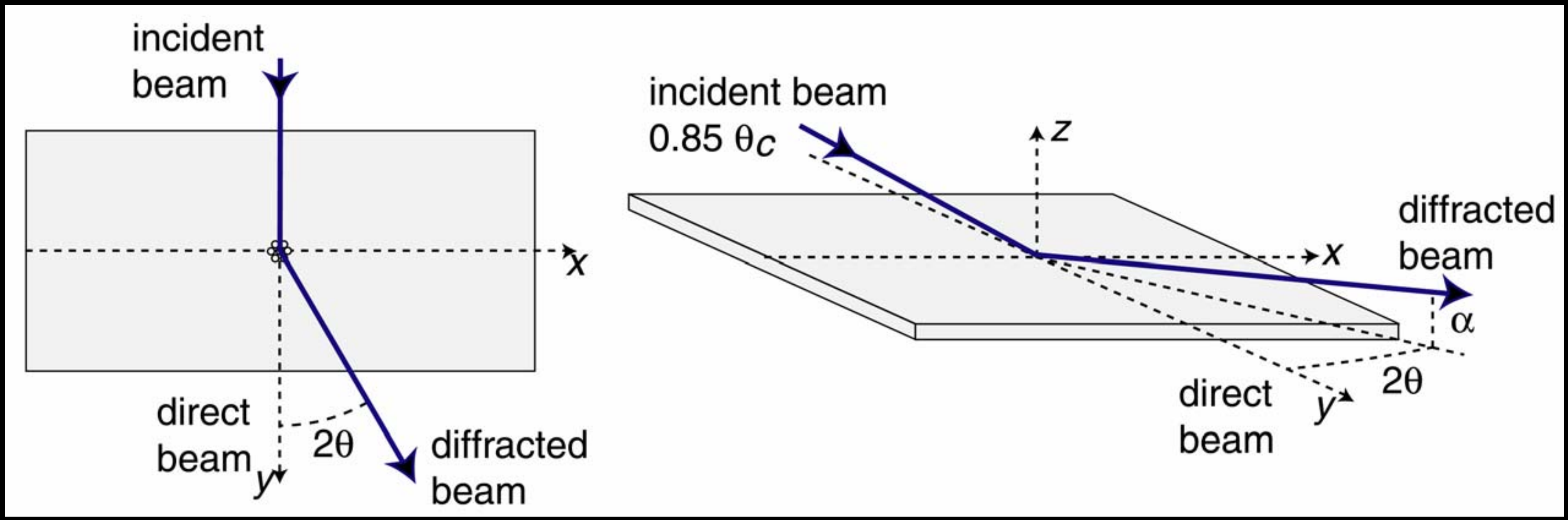
Measures:

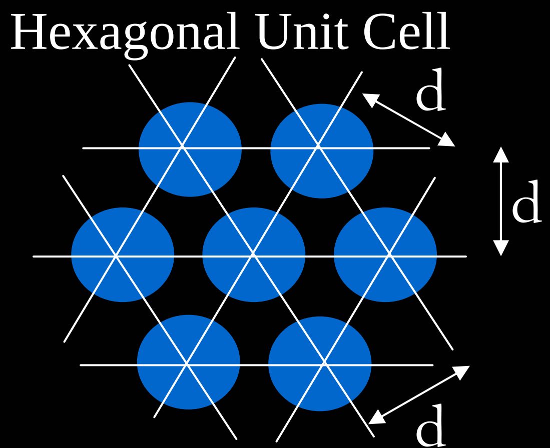
average density structure **normal** to the interface.

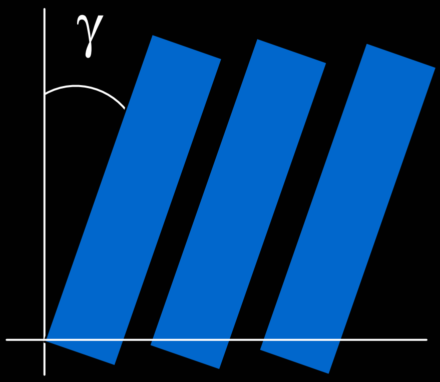
(layer thickness, density and roughness)

X-ray Grazing Incidence Diffraction

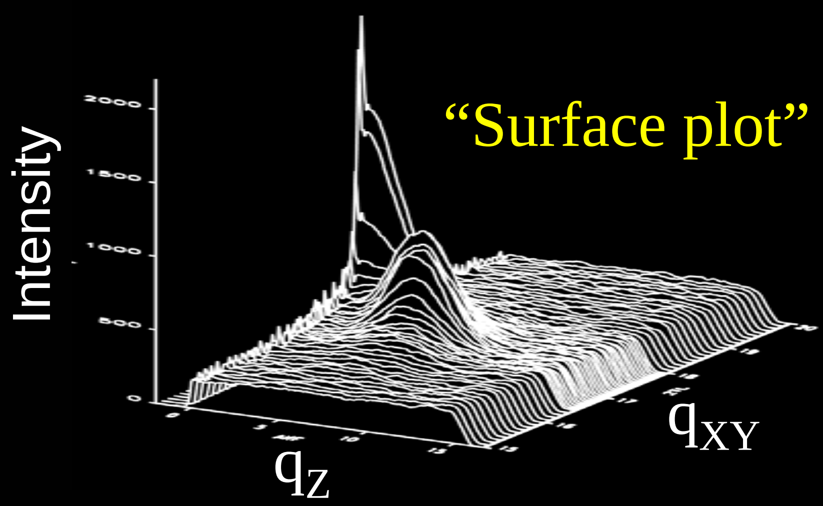
G I D

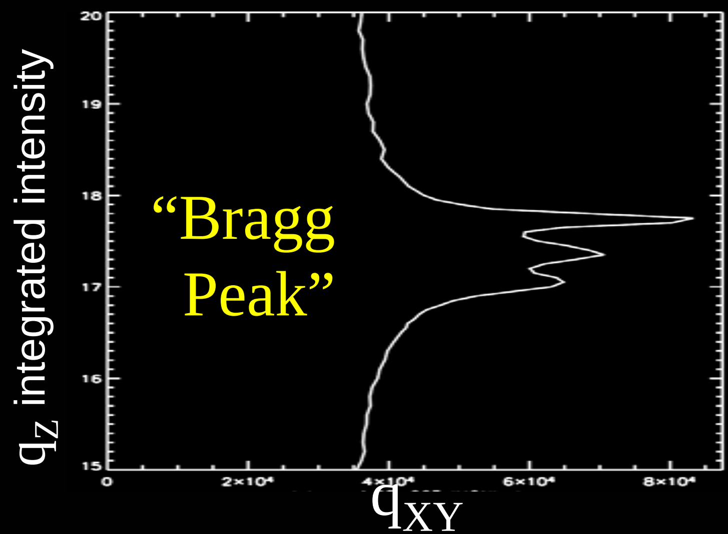


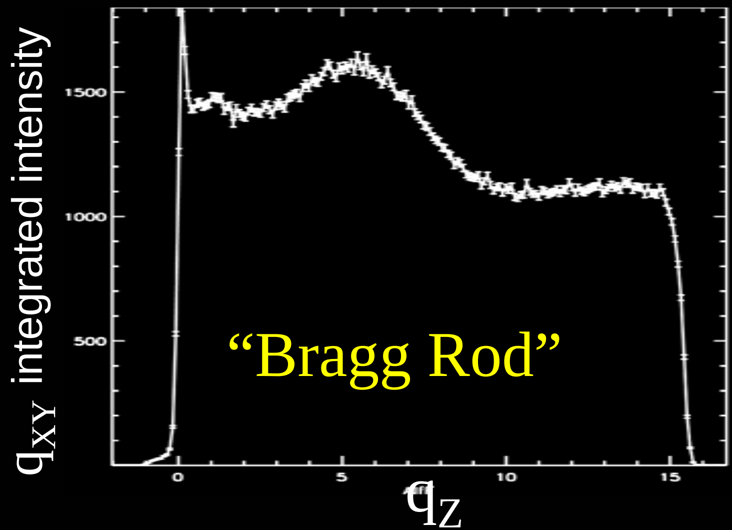




Typical GID Data







Measures:

Molecular arrangement for ordered parts of molecules

Obtain correlation lengths

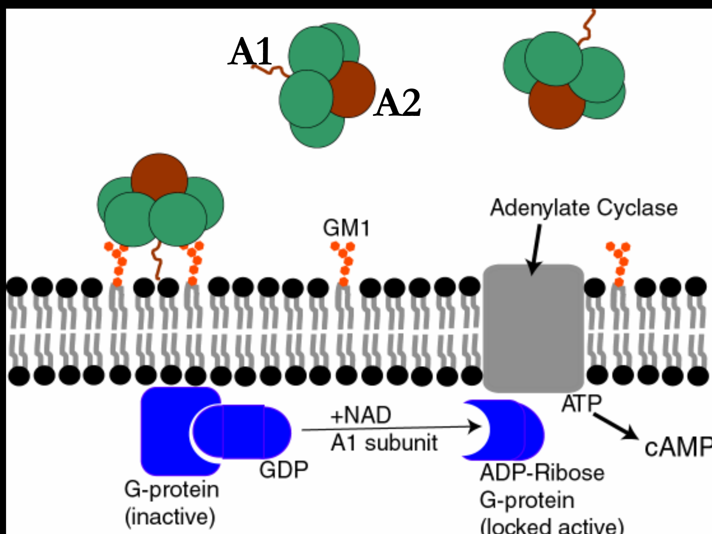
Examples

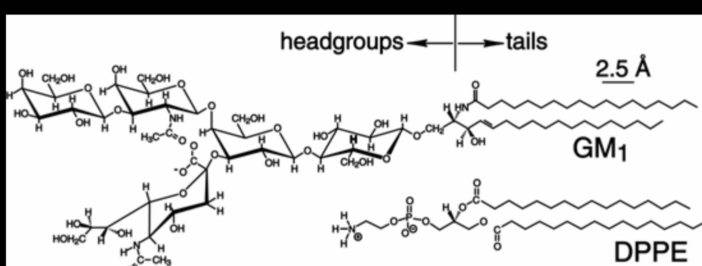
1. Monolayer Studies - Cholera Toxin
   1. Neutron scattering
   2. X-ray scattering
2. Bilayer Studies

Cholera Toxin Assault

Cholera is caused by a comma-shaped bacterium, *vibrio cholerae*

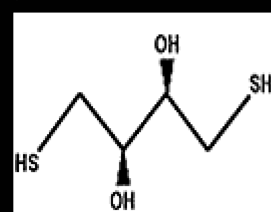
** Over 1 million deaths annually in third world countries.



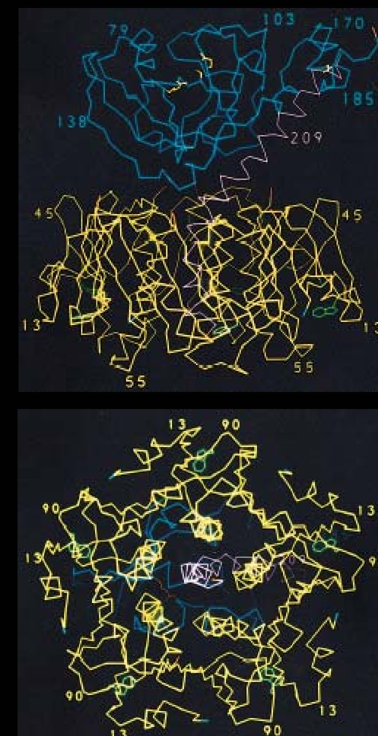


-Binding

-Proteolytic cleavage (192:194)
-Disulfide reduction
(cys187=cys199)
(Dithiothrietol (**DT**) addition)



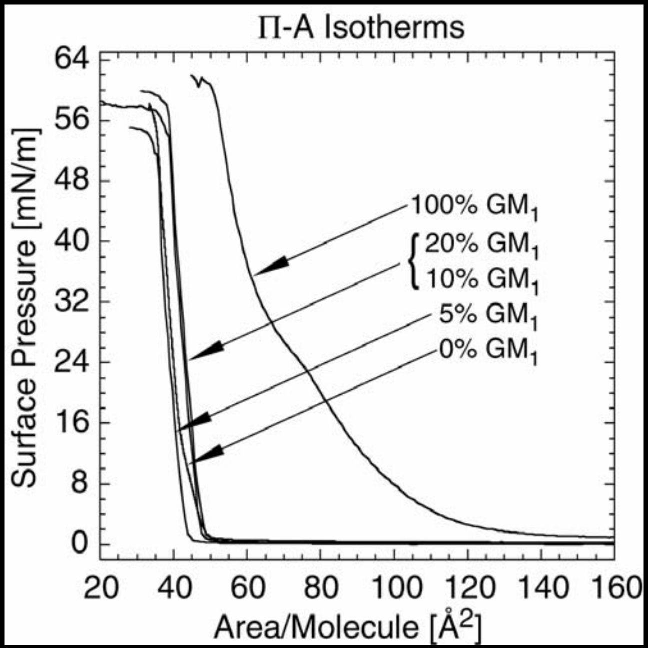
-A1 peptide penetration
-**Mechanism of membrane penetration remains unknown**

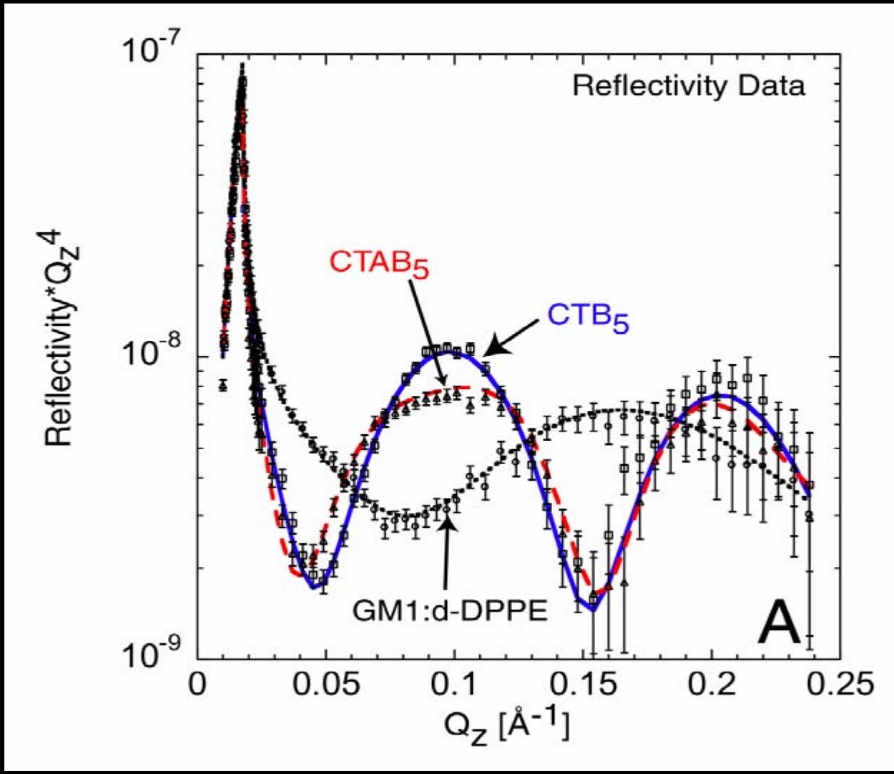


Zhang RG, et al, JMB (1995) **251**, 563–573

Neutron Reflectivity

CE Miller, et al, Biophys J. (2004) 86: 3700-3708





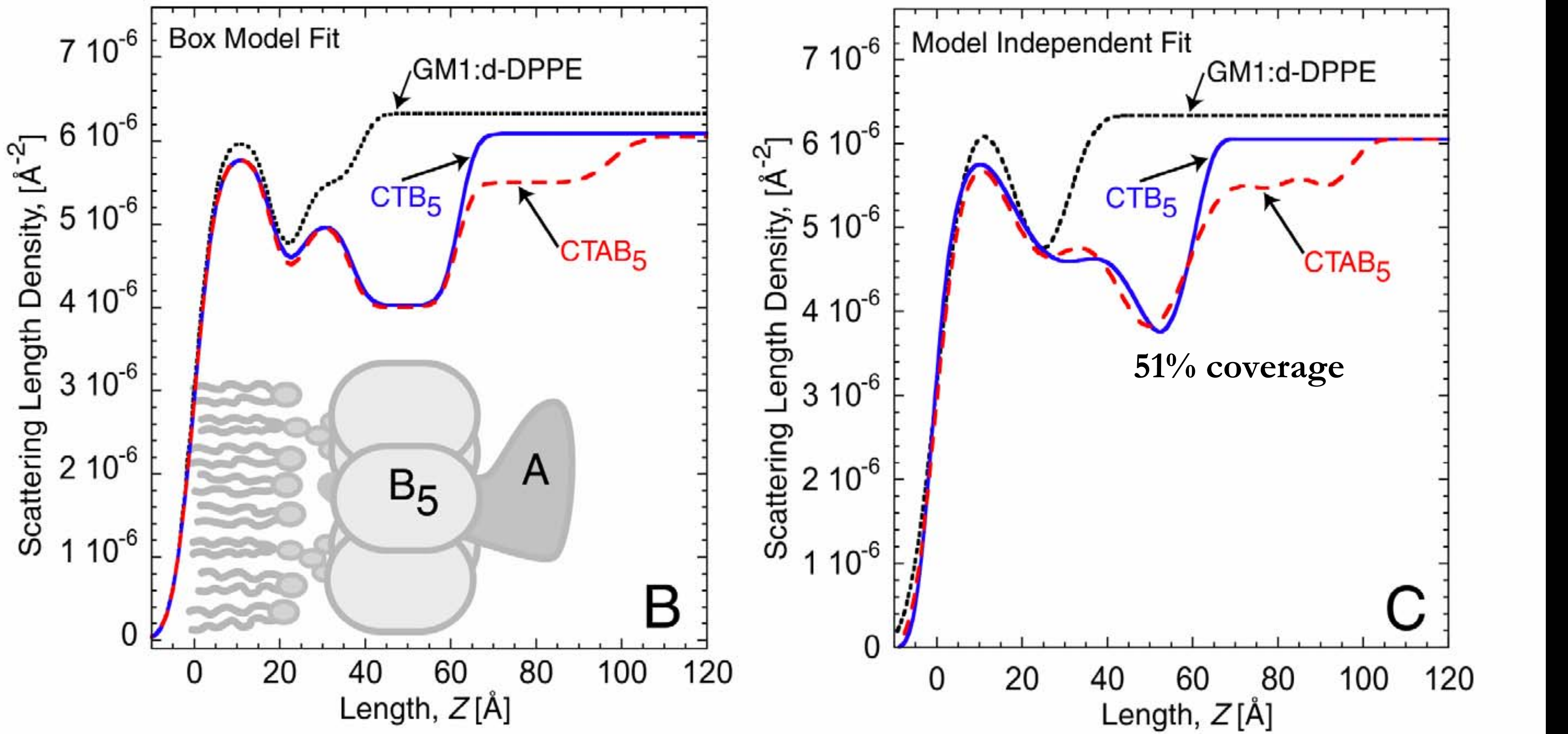
Data and fitted reflectivity curves

<u>Lipid Monolayer Composition:</u>

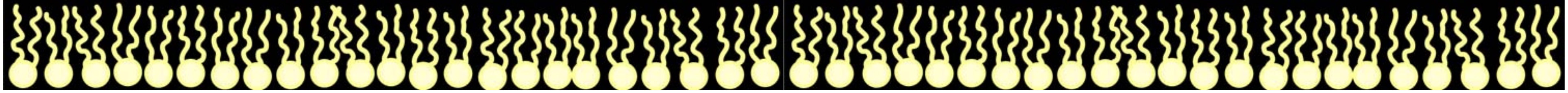
- 20% GM1 80% DPPE
- @ 20 mN/m (constant pressure)
- T=23°C

Neutron Reflectivity

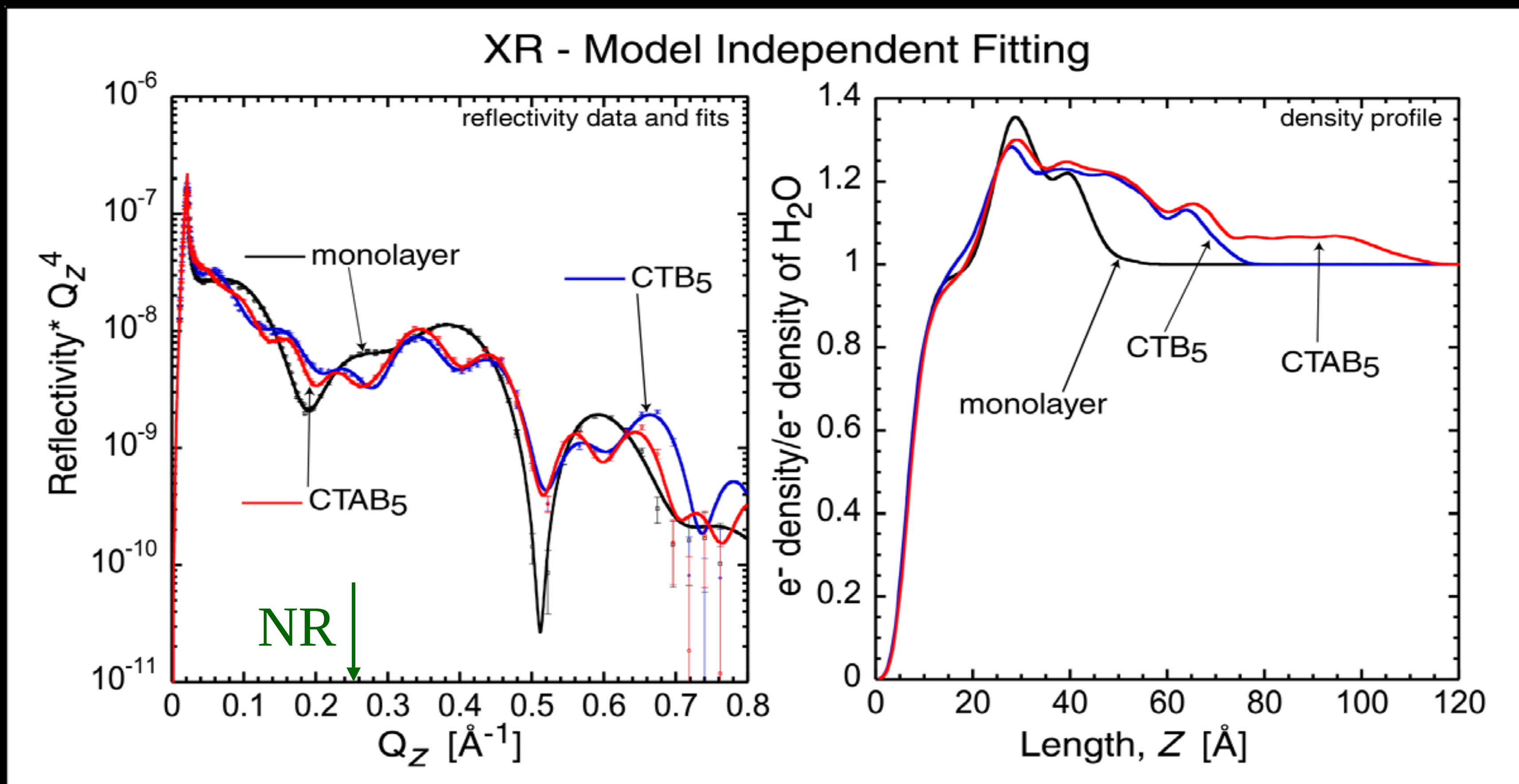
CE Miller, et al, Biophys J. (2004) 86: 3700-3708



Same decrease in density of tails

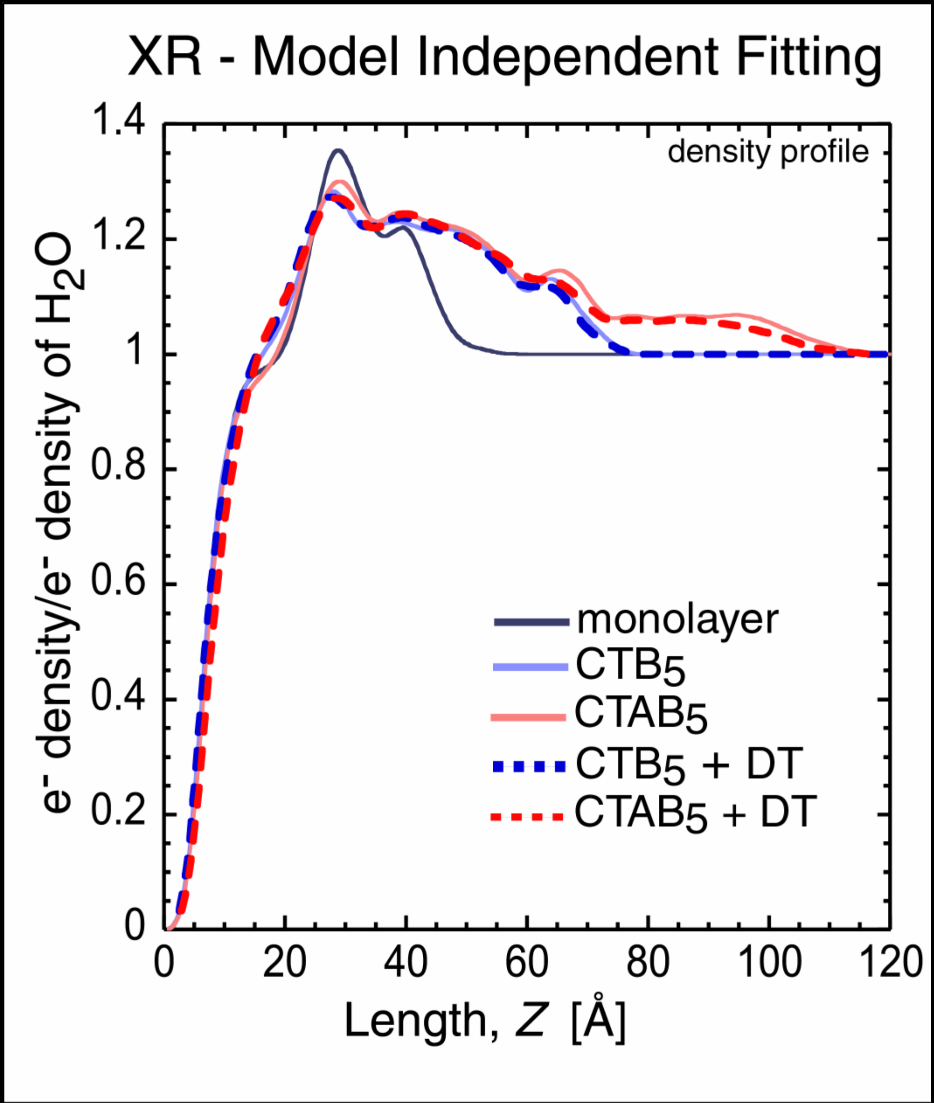


X-ray Reflectivity



CE Miller, et al, Colloids and Interfaces B (2005)

Toxin Activation



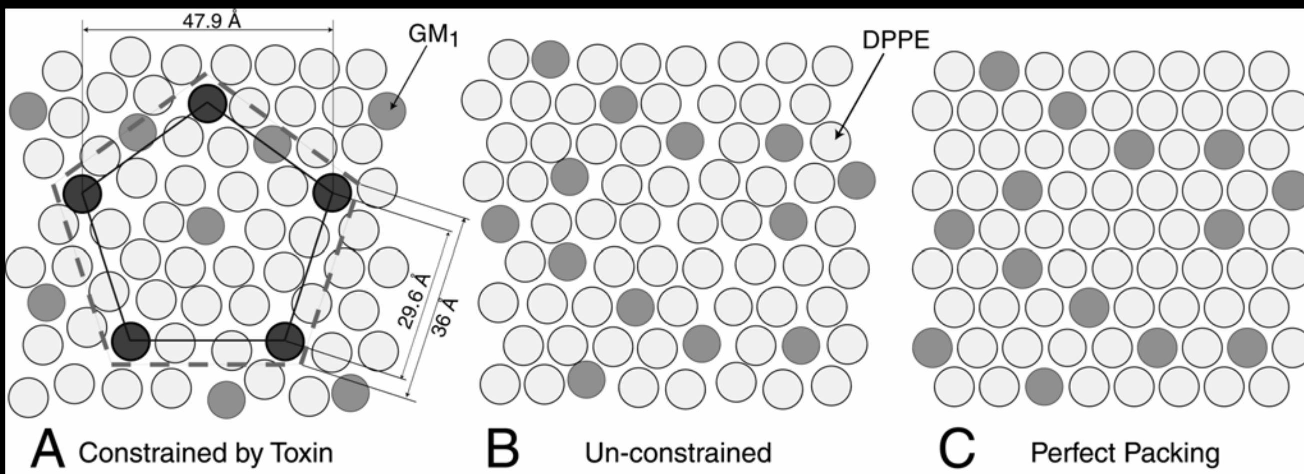
Enzymatic Cleavage:

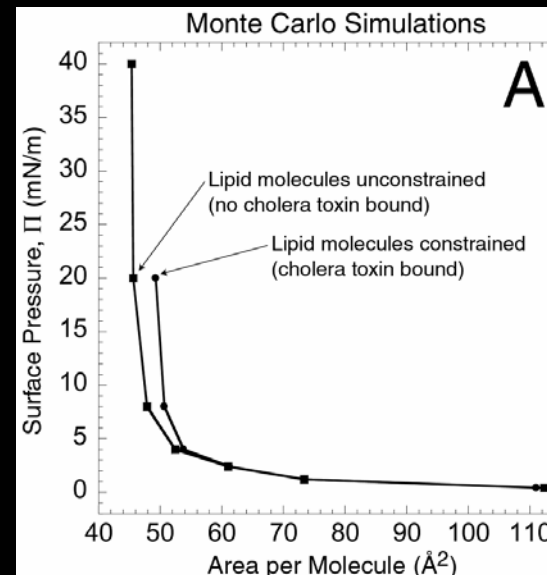
- tail density increases
- implies active role of CTB_5 *not* just binding to the cellular receptor GM_1
- @ 20 mN/m (constant pressure)

Monte Carlo Simulation [Roland Faller]

Modeling the binding of cholera toxin to a lipid membrane by a non-additive two-dimensional hard-disk model

Faller R., **Kuhl** TL. *Soft Materials. 1(3):343-352, 2003*





Simulation:

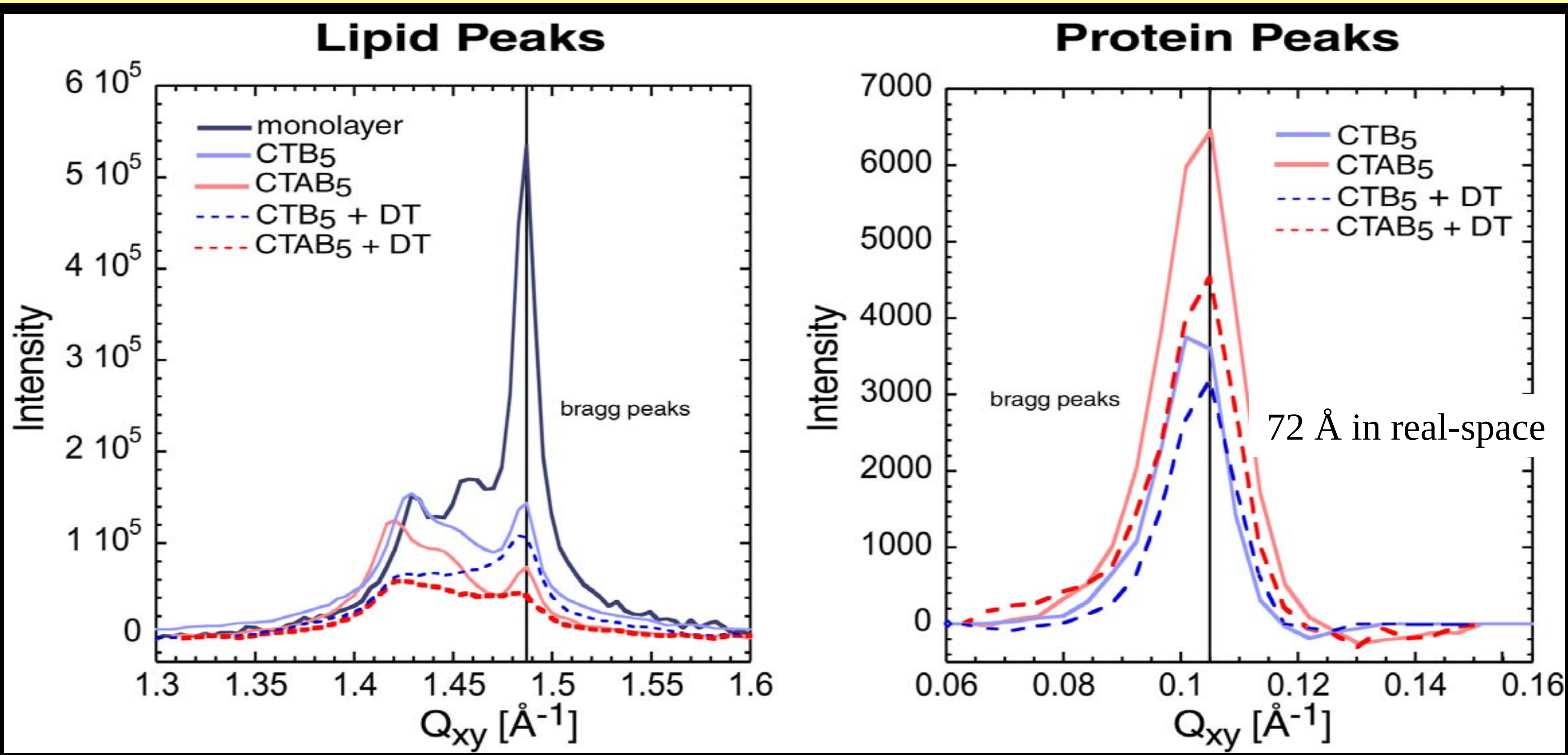
7 % increase due only to geometrical constraints imposed by toxin binding

Measurement:

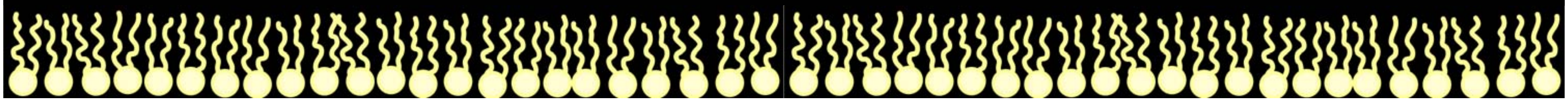
8±5 % increase for both $CTAB_5$ and CTB_5 binding for constant pressure (20mN/m)

geometrical constraints imposed by toxin binding lead to a decrease in lipid packing density.

GID – Toxin Activation



	Monolayer	CTB	CTB+DT	CTAB	CTAB+DT
Coherence Length	660 Å	340 Å	237 Å	357 Å	193 Å
Tilt Angle	24º	46º	53º	42º	45º

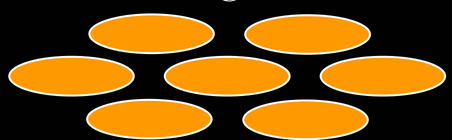


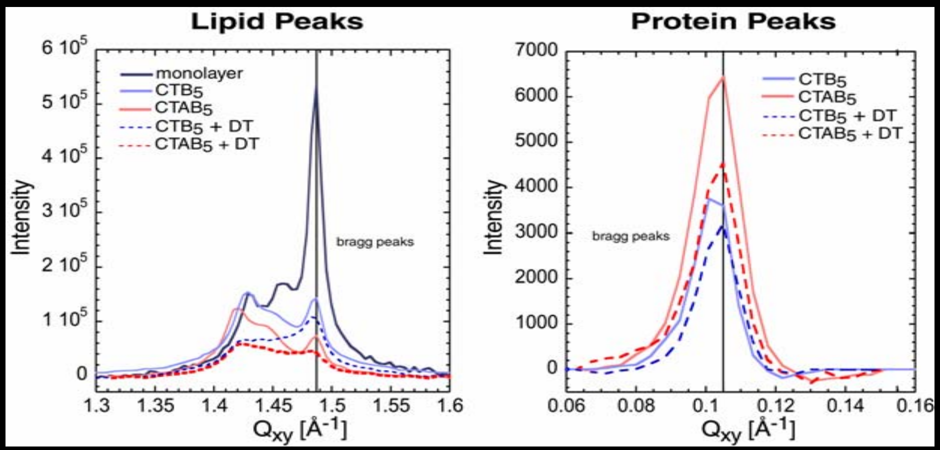
Summary of Findings

-Cholera Toxin disturbs lipid packing beneath it
-CTB behaves similar to activated CTAB
-X-rays Show that CTB penetrates into lipid layer before activation
-See scattering from lipid layer and protein layer simultaneously

-Studies inconsistent with Protein Crystallography
    -See changes in lipid structure when CT bound

Distorted Hexagonal Unit Cell

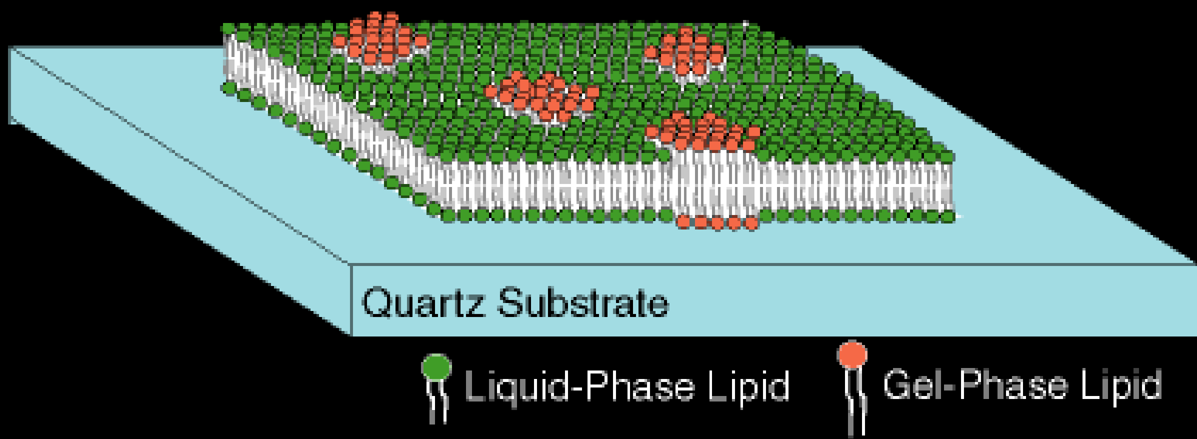




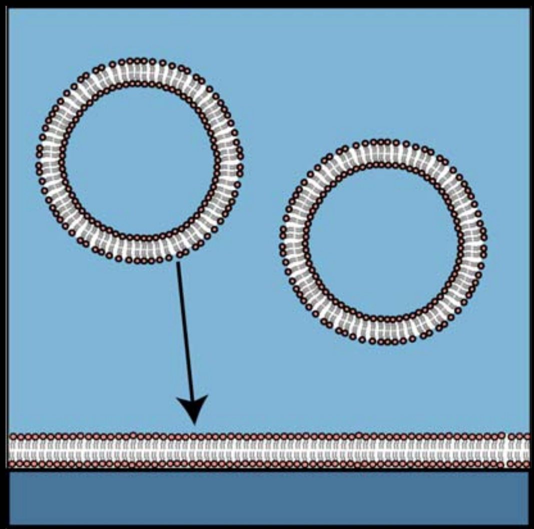
Examples

1. Monolayer Studies - Cholera Toxin
   1. Neutron scattering
   2. X-ray scattering
2. Bilayer Studies

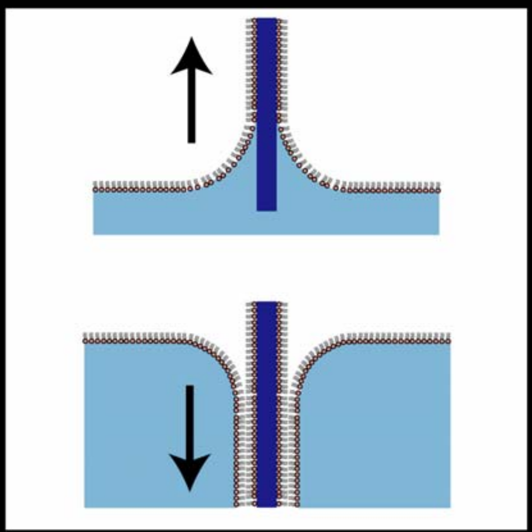
Lipid Bilayers



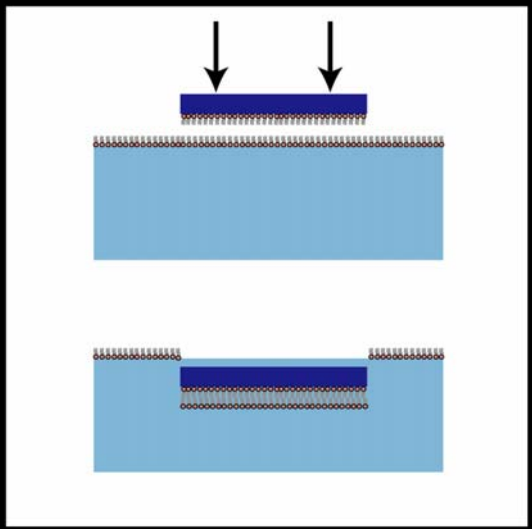
Vesicle Fusion



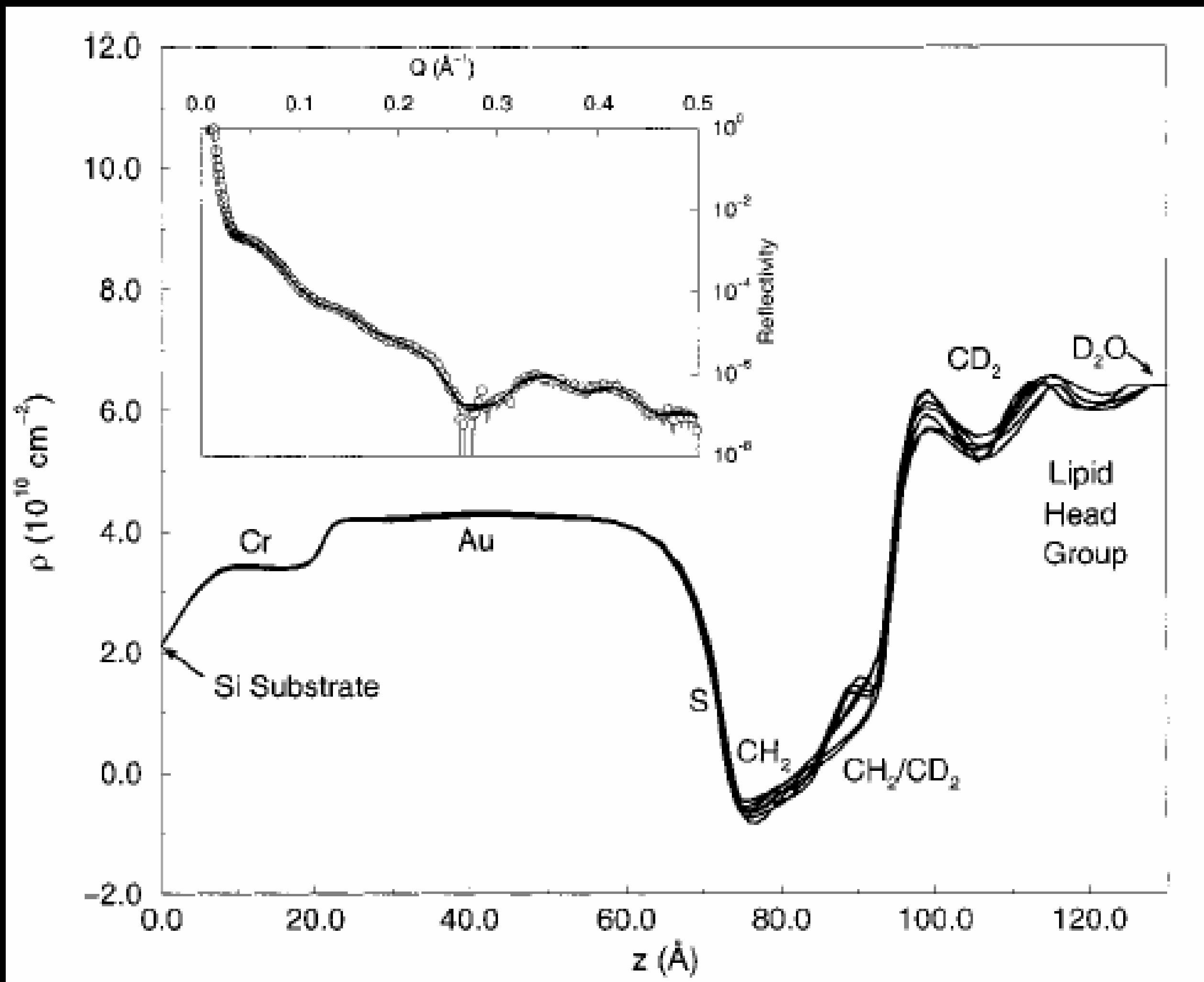
Langmuir-Blodgett (LB)



Langmuir-Schaffer (LS)



High-Resolution NR

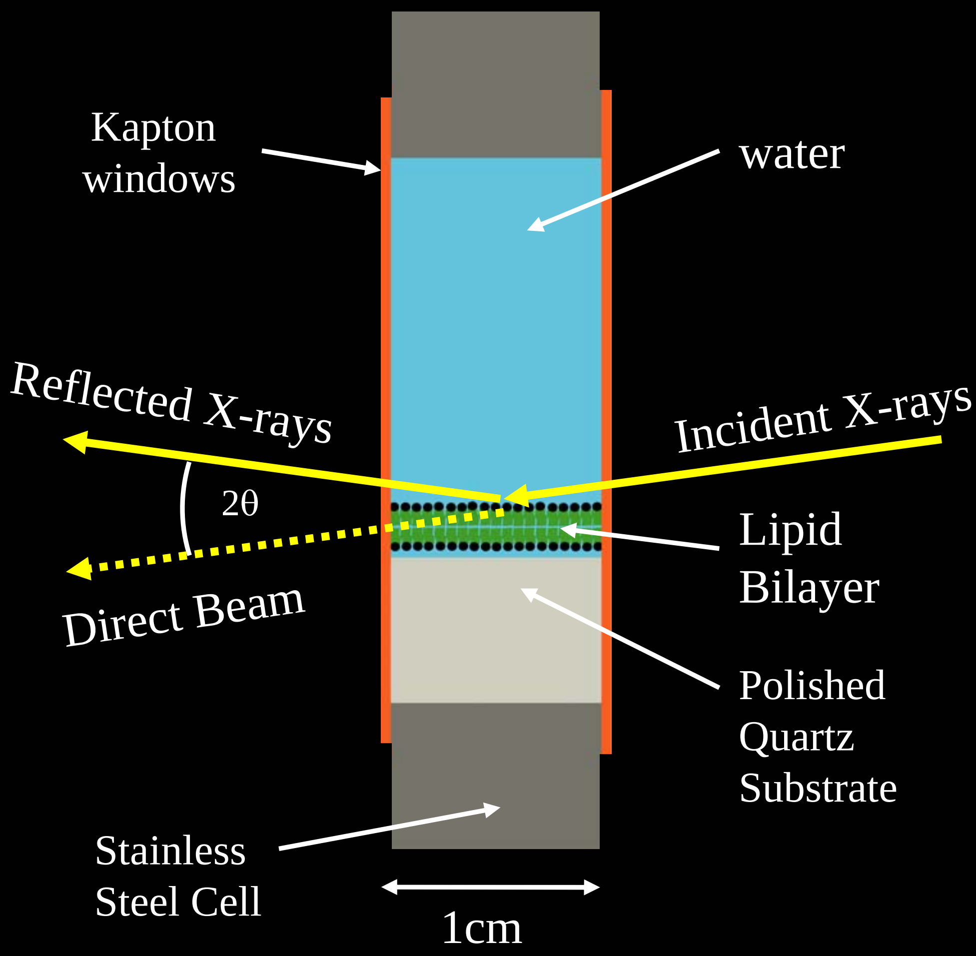


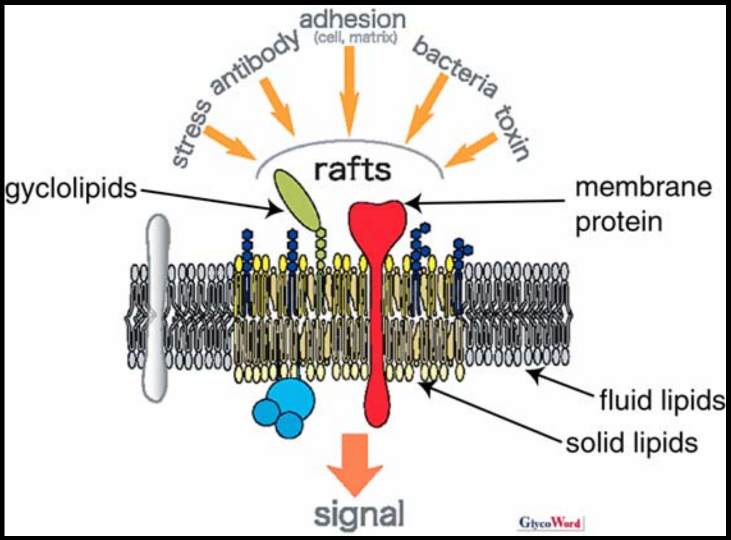
S. Krueger *et al*
Langmuir (2001)
17, p511-521

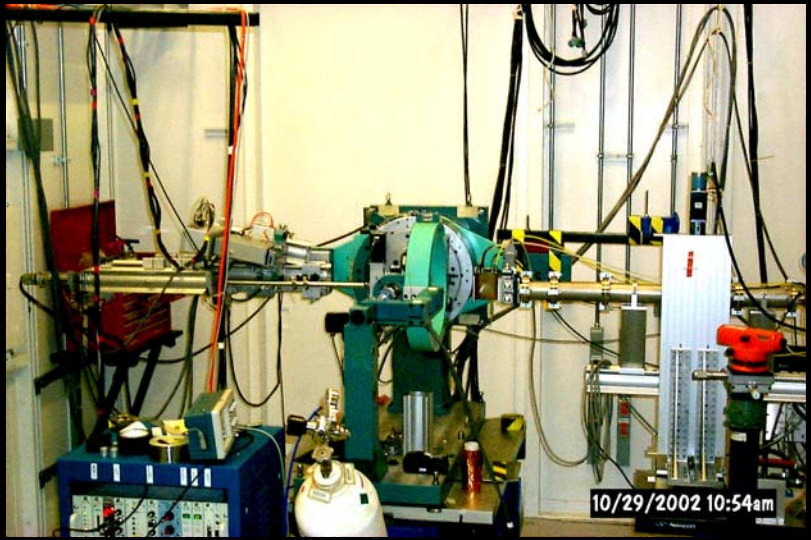
Qz ~ 0.5 Å^-1

requires
Au - Alkane Thiol

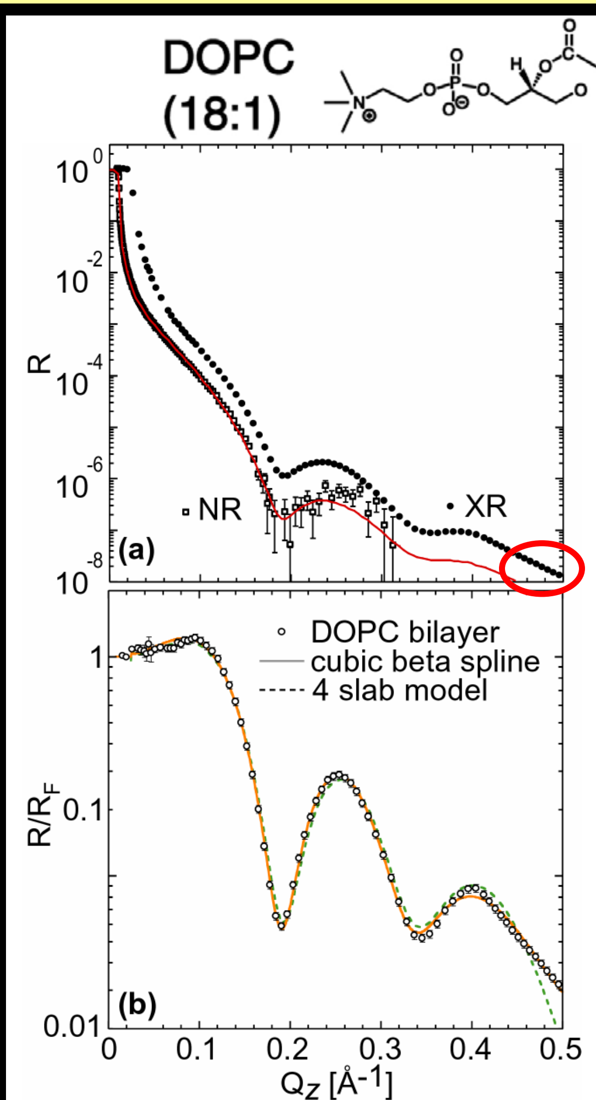
X-ray Scattering from the Solid-Liquid Interface

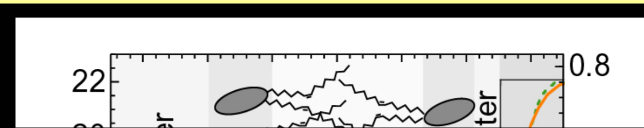




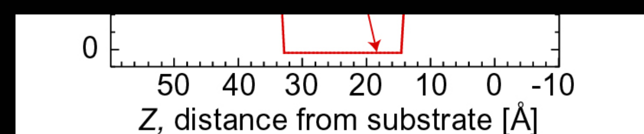


Comparison to Neutron Reflectivity





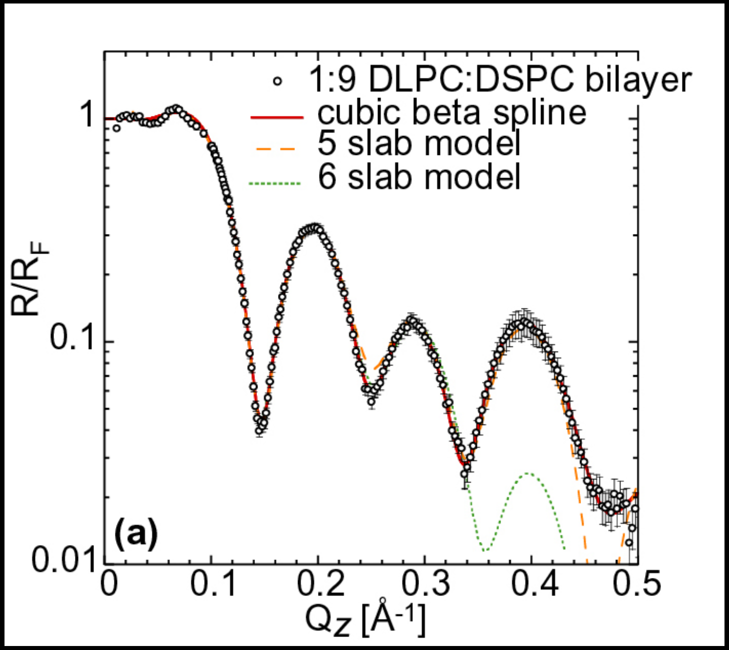
Neutrons	X-rays
High contrast	Low contrast
High penetration	Low penetration
No Beam Damage	Beam Damage
Less Abundant	More Abundant
Lower Resolution	Higher Resolution
~ 3 hour scan time	~10 min. scan time

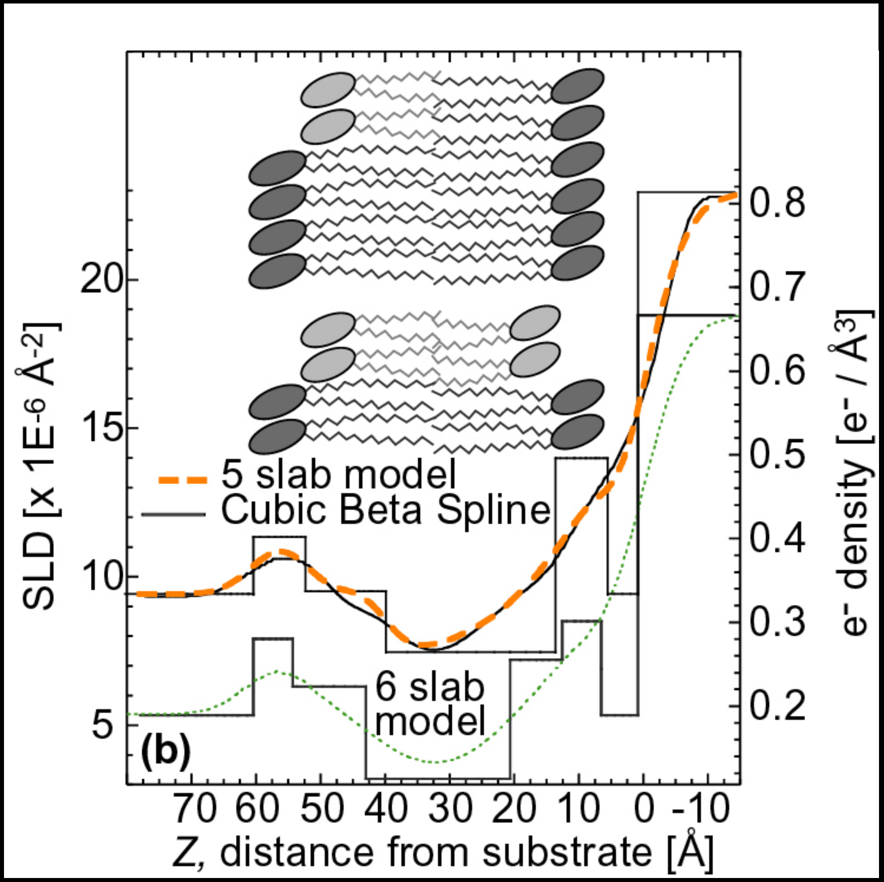


Leaflet Segregation

(1:9) DLPC [12:0]:DSPC [18:0] bilayer

Formed by vesicle fusion on quartz.





Water cushion 5 - 6 Å

AFM → 18 Å difference between lipid domains (quenched)

More Simple Model → Better fit → XR has adequate resolution to distinguish

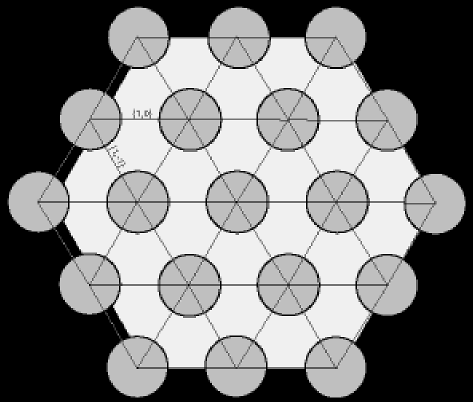
C.E. Miller *et al*, *Physical Review Letters*, 94, 238104 (2005)

GID?

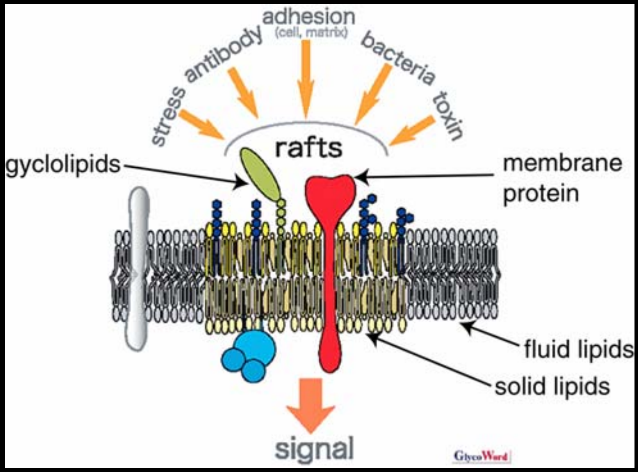
NO GID observed yet for single lipid membrane…………

Local in-plane structure of a bilayer has never been observed.
ONLY monolayers………what about membrane domains???

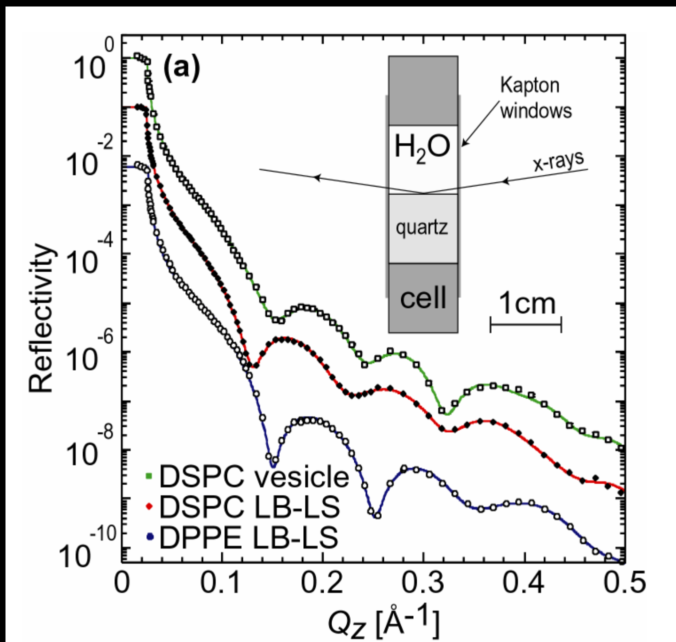
Useful Information:
Are domains between leaflets coupled?
Are cell membranes crystalline in regions?
What are the sizes of these scattering domains?

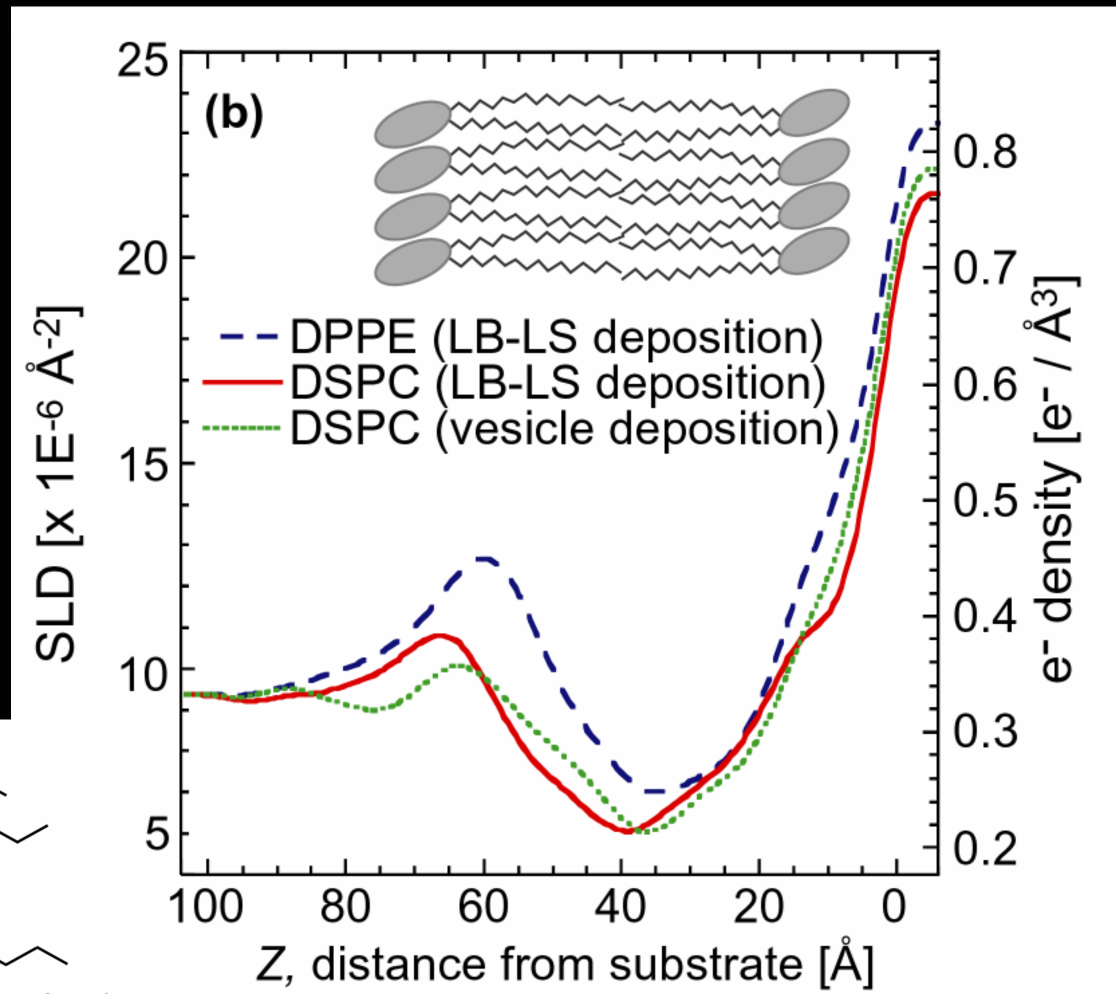


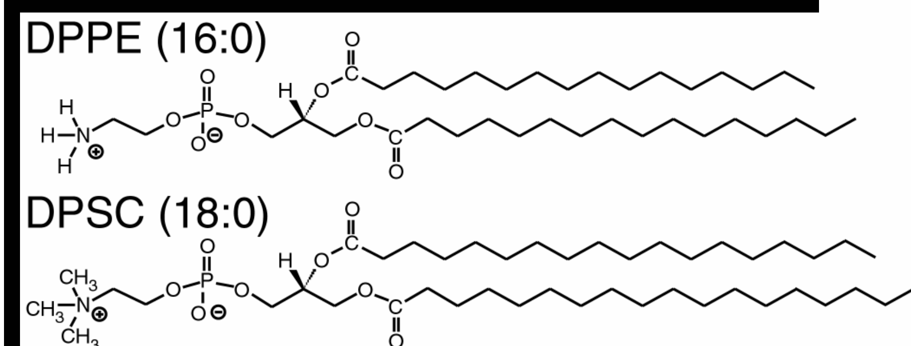
In-plane structure



Model Bilayers – Pure Solid Phase PC and PE







Beam damage lower than expected

GIXD from Solid-Liquid Interfaces

It is possible to observe GID at the solid-liquid interface

For the first time local in-plane structure of a bilayer has been observed.

Useful Information:
Are domains between leaflets coupled?
Are cell membranes crystalline in regions?
What are the sizes of these scattering domains?

Collaborations

Collaborators

- Chad Miller — Biophysics - UC Davis
- Jaroslaw Majewski — Los Alamos Neutron Science Center
- Kristian Kjær — Physics Department, Risø National Laboratory, Denmark
- Marcus Weygand — Physics Department, Risø National Laboratory, Demark
- Roland Faller — Chemical Eng Dept - UC Davis
- Sushil Satija — National Institute of Standards and Technology

Support

- SEARLE Scholars Foundation
- UC-CARE – Los Alamos National Laboratory
- Manual Lujan Jr. Scattering Center – Los Alamos National Laboratory
- National Institute of Standards and Technology

Summary

General:

- X-ray and neutron reflectometry provide complimentary pieces of structural puzzle
- X-ray GID gives in-plane and out-of-plane structure of crystallites

Specific:

- Higher resolution of X-ray reflectivity allows subtle structural features to be resolved - leaflet segregation, water, etc
- First GIXD measurements of single membranes

Future:

- Lipid Rafts?