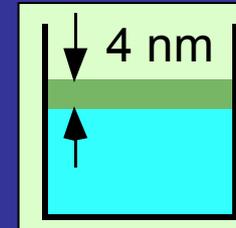
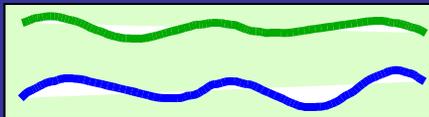


New Methods to Study Biomolecules at Liquid Surfaces

Mark Schlossman
University of Illinois at Chicago

Nanoscale aqueous films on aqueous subphases

*Coupled film fluctuations
probe interactions across film*

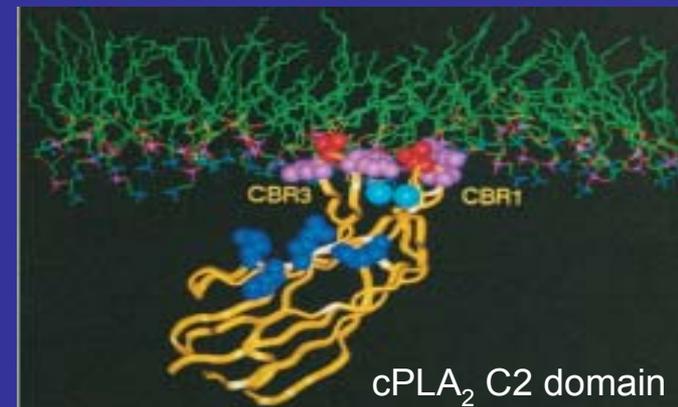


Protein assembly in films



Protein-lipid interactions

New analysis method for x-ray reflectivity



Collaborators and Funding Sources

Current members of Research Group working on these projects

Sai Venkatesh Pingali (graduate student)

Sarka Malkova (graduate student)

Former members of Research Group who worked on aqueous thin films

Dr. Ming Li (post-doctoral, currently Professor, Institute of Physics, Beijing)

At Argonne National Laboratory (aqueous thin films) Dr. David Chaiko

At UIC (lipid-protein interactions) Professor Wonhwa Cho, Fei Long, Robert Stahelin

Experiments were performed at:

X-ray beamline X19C (National Synchrotron Light Source, Brookhaven National Lab)

Dr. Aleksey Tikhonov

Gratefully acknowledged support:

NSF (Division of Materials Research and Division of Chemistry)

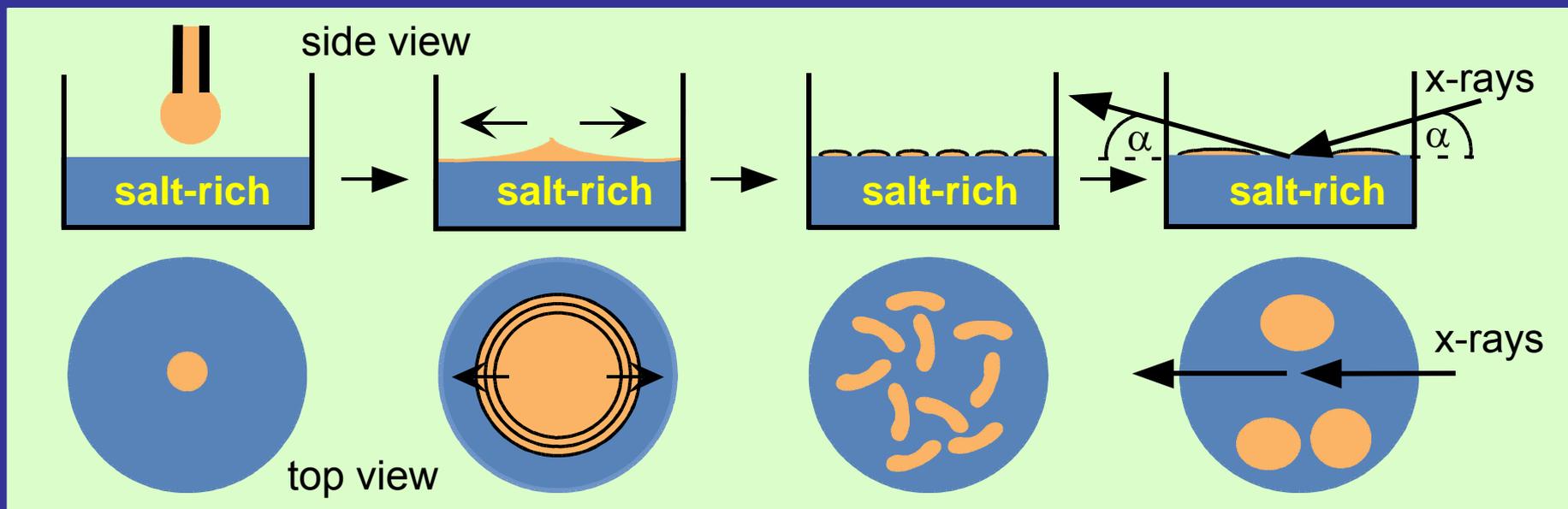
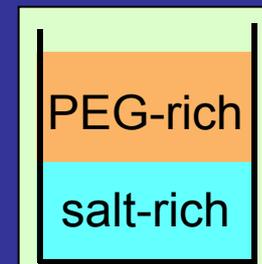
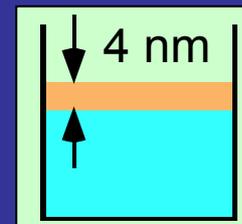
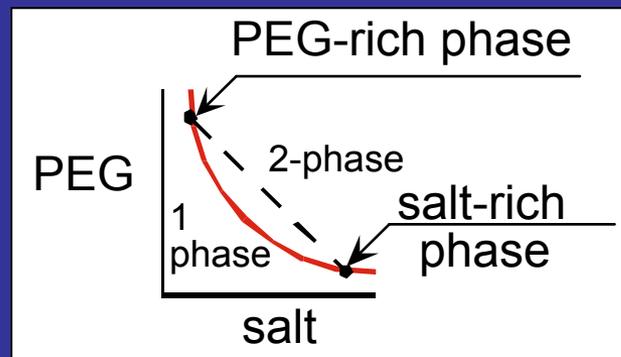
DOE (Chemical Technology Division, Argonne)

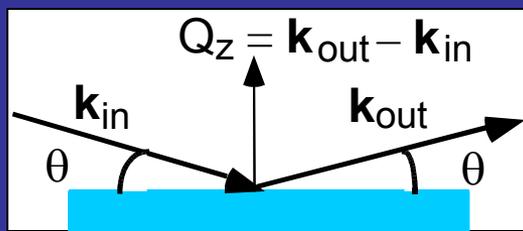
ACS-Petroleum Research Fund

Nanoscale Aqueous Films on Aqueous Subphases

Aqueous biphasic solutions

Polyethylene Glycol
(MW 3400 or 8000)
Potassium Phosphates
 K_2HPO_4 or
 $K_2HPO_4:KH_2PO_4$ (3:2)
Water

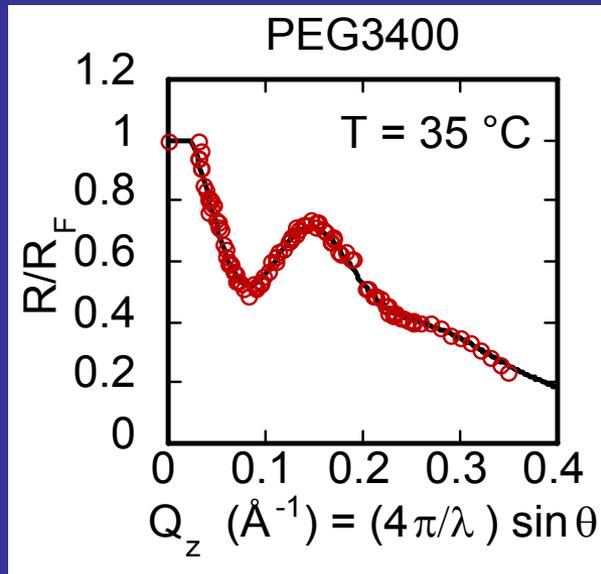
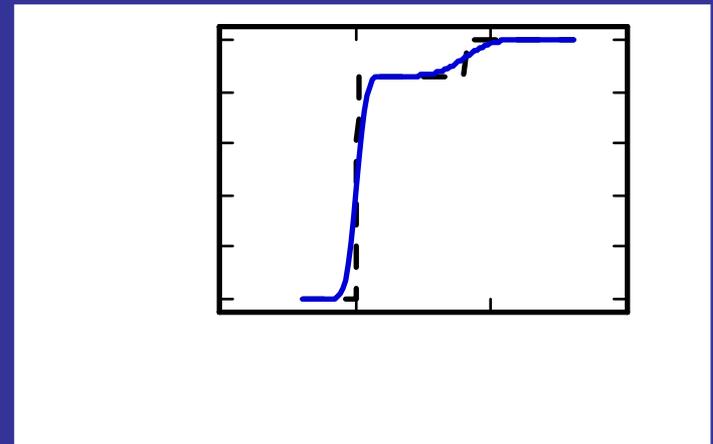




X-ray Reflectivity

Probe of Film Thickness and Interfacial Width

$$\frac{R(\theta)}{R_F(\theta)} = \left| \frac{1}{\rho_\infty} \int dz \frac{\partial \langle \rho(z) \rangle}{\partial z} e^{iQ_z z} \right|^2$$



Film thickness $d = 42 \text{ \AA}$

Interfacial widths:
 $\sigma_{\text{PEG-rich/vapor}} = 2.9 \text{ \AA}$
 $\sigma_{\text{salt-rich/PEG-rich}} = 7.9 \text{ \AA}$

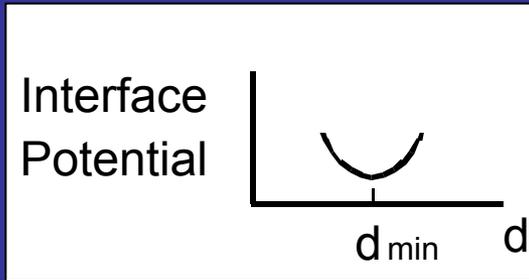
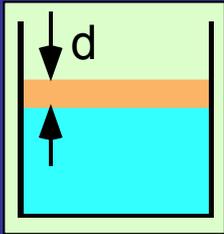
Capillary wave theory $\rightarrow \sigma^2 \propto \frac{k_B T}{\gamma}$

$$\rightarrow \frac{\sigma_{\text{salt/PEG}}^2}{\sigma_{\text{PEG/vapor}}^2} \approx \frac{\gamma_{\text{PEG/vapor}}}{\gamma_{\text{salt/PEG}}}$$

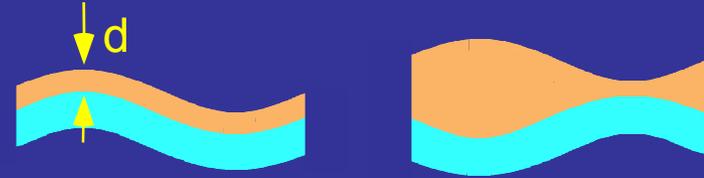
7.42 \approx 7.37 from measurements

Interfaces are nearly like bulk interfaces
 \rightarrow thin layer of bulk material

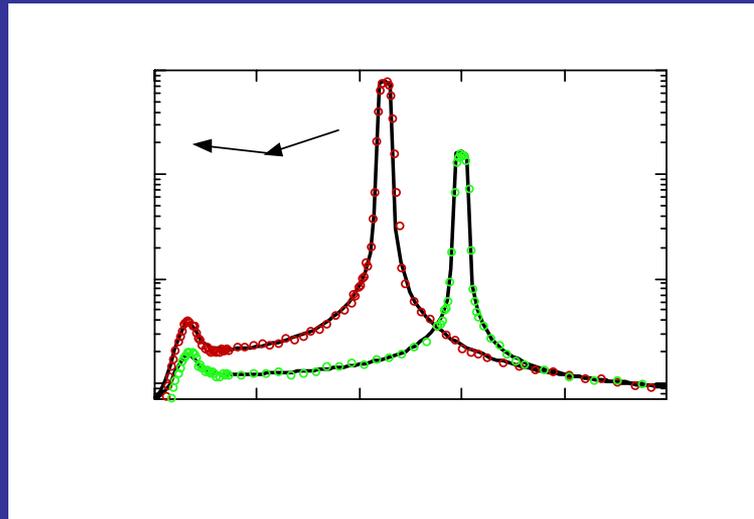
Film Fluctuations



Coupling of capillary waves on both interfaces



$$H = \int d^2s \left\{ \sum_{i=1,2} \left[\frac{1}{2} \gamma_i \left(|\nabla \zeta_i|^2 + \left(\frac{\zeta_i}{\xi_{l,cw}} \right)^2 \right) \right] + \frac{1}{2} B (\zeta_1 - \zeta_2)^2 \right\}$$

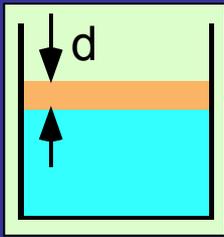


Coupling is intermediate between stacks of flexible lipid membranes and stacks of stiff lamellar liquid crystals

$$B = 1.4_{-0.8}^{+1.6} \times 10^{11} \text{ J / m}^4$$

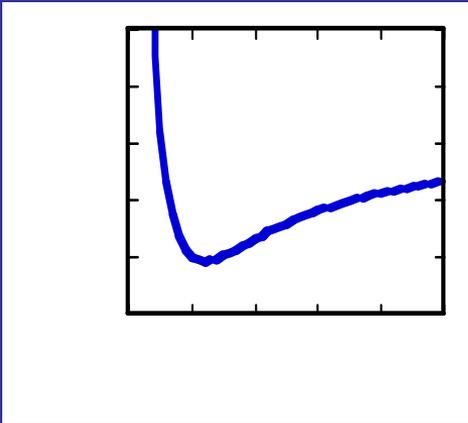
(PEG:K₂HPO₄&KH₂PO₄:H₂O -- 13.2:28.9:57.9 wt%)

Film Free Energy



$$F(d) = \gamma_{\text{salt-rich/PEG-rich}} + \gamma_{\text{PEG-rich/vapor}} + \Delta G(d)$$

Postulate $\Delta G(d) = S^p \exp[(d_o - d) / \Lambda] - A / 12\pi d^2$



Assume a value for d_o ($1\text{\AA} < d_o < 3\text{\AA}$),
 can determine other 3 unknowns
 by combining our tension and x-ray data -
 $S^p = 0.0187 \text{ J/m}^2$; $\Lambda = 2.9 \text{ \AA}$; $A = 8 \times 10^{-23} \text{ J}$

S^p is essentially the spreading pressure of the film

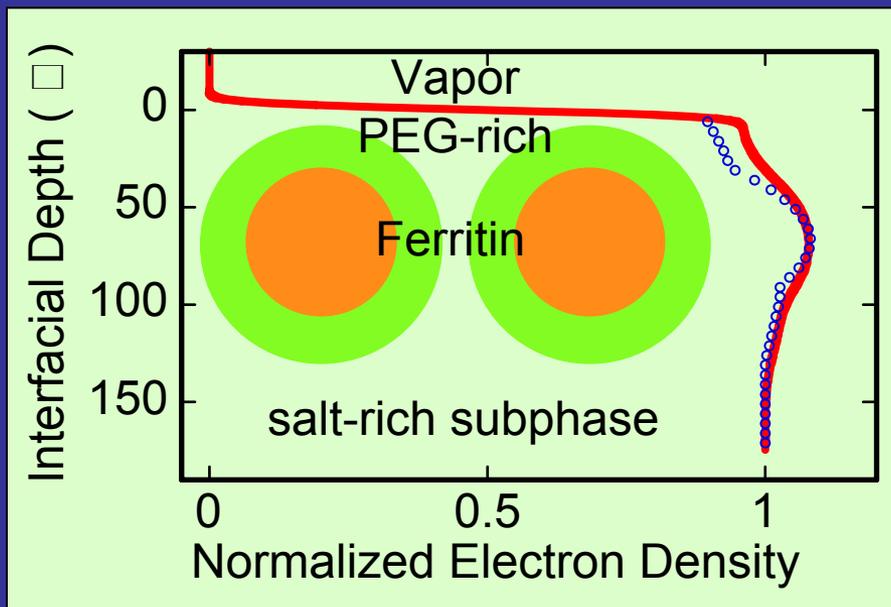
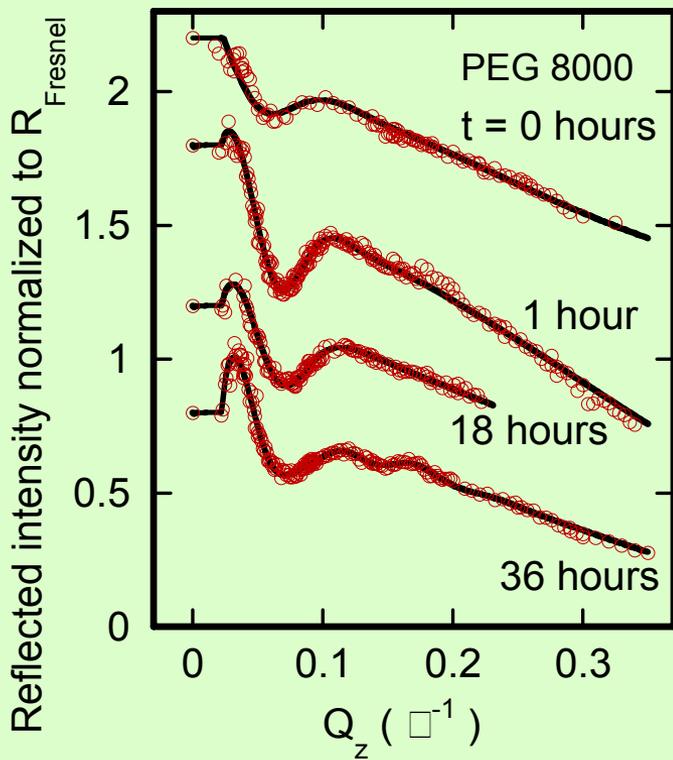
Decay length Λ - compare to calculation of entropic repulsion of two interacting membranes with tension (Diamant 2002)

$$\Lambda = \left[\frac{k_B T (\rho_{m,1} + \rho_{m,2})^2}{4\pi(\gamma_1 \rho_{m,2}^2 + \gamma_2 \rho_{m,1}^2)} \right]^{1/2} = 2.3 \text{ \AA}$$

Hamaker constant A is an effective constant, $A = A_{\text{PEG-rich/PEG-rich}} - A_{\text{PEG-rich/salt-rich}}$
 Attraction of upper phase for lower phase is only slightly different than attraction for itself \rightarrow thins film to coexist with macroscopic lenses.

Similar to predictions by Brochard-Wyart et al. Langmuir 7, 335 (1991)

Insertion of Ferritin Proteins into Thin Film



Measured electron density is similar to values calculated from known structure placed at the interface.

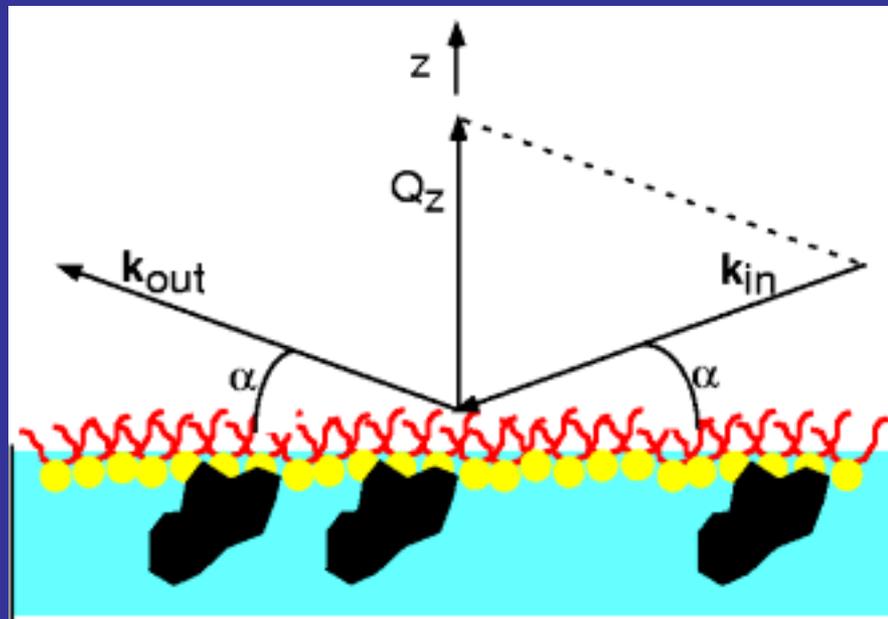
→ Ferritin does not denature at interface due to presence of thin film, contrary to its behavior and that of most proteins at the water surface.

Protein Adsorption to Lipid Monolayers on Aqueous Surfaces

Peripheral membrane proteins important for cell signaling, biocatalysis. Interactions with the membrane are poorly understood.

Studies of protein adsorption to Langmuir monolayers of saturated lipids using a variety of techniques, including x-ray reflectivity, are common.

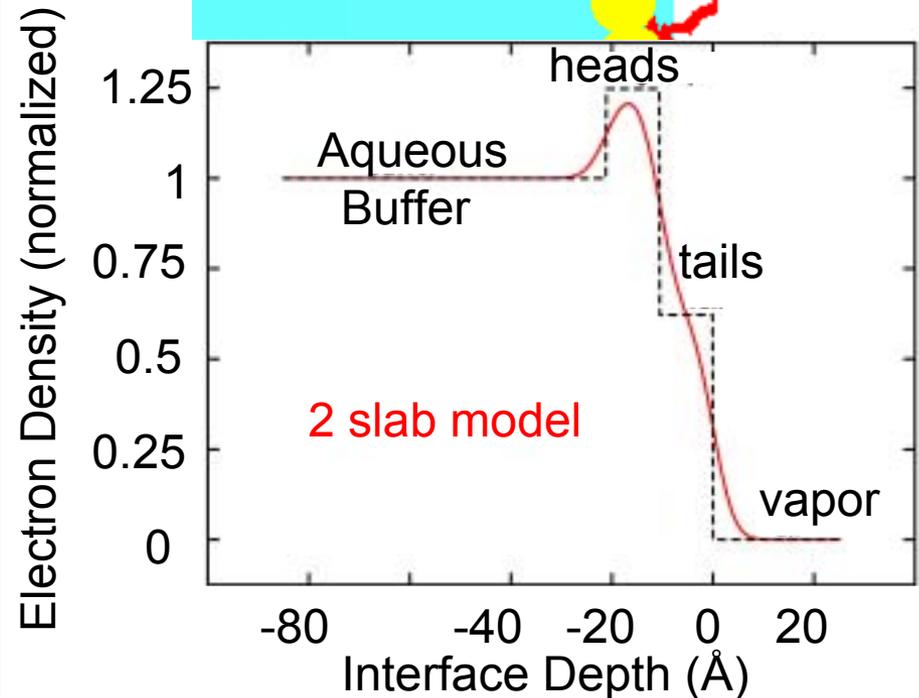
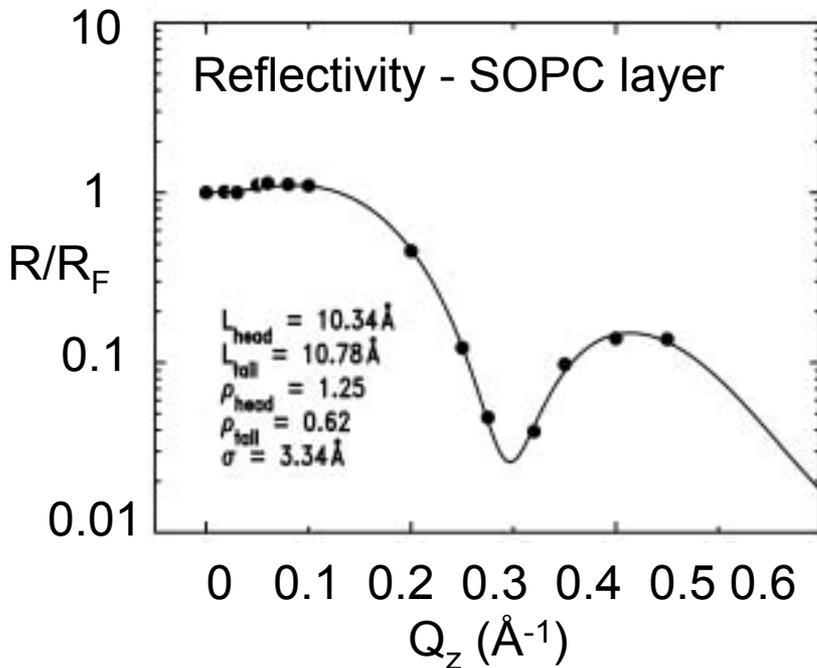
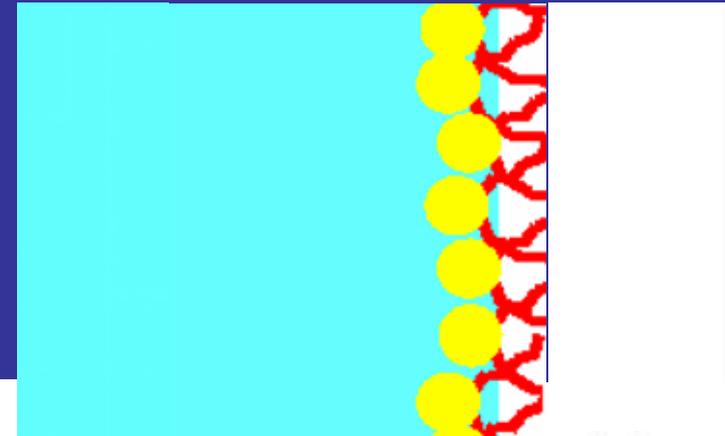
We present a study using unsaturated lipids and a new analysis technique that, under appropriate conditions, provides more information than the standard slab model.



Reflectivity from an Unsaturated Lipid Monolayer -

SOPC on aqueous buffer (20 mM HEPES, pH 7, 0.1 mM KCL, 0.1 mM CaCl₂)

Successive addition to 25 mN/m
Stable for 8 hours
for maximum x-ray exposure
of 0.4×10^8 photons/s mm²



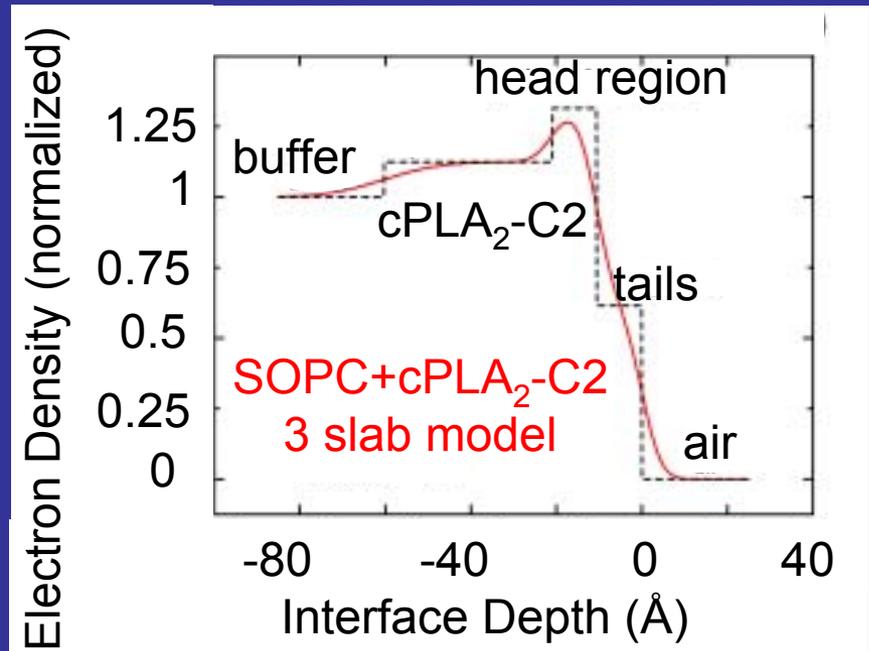
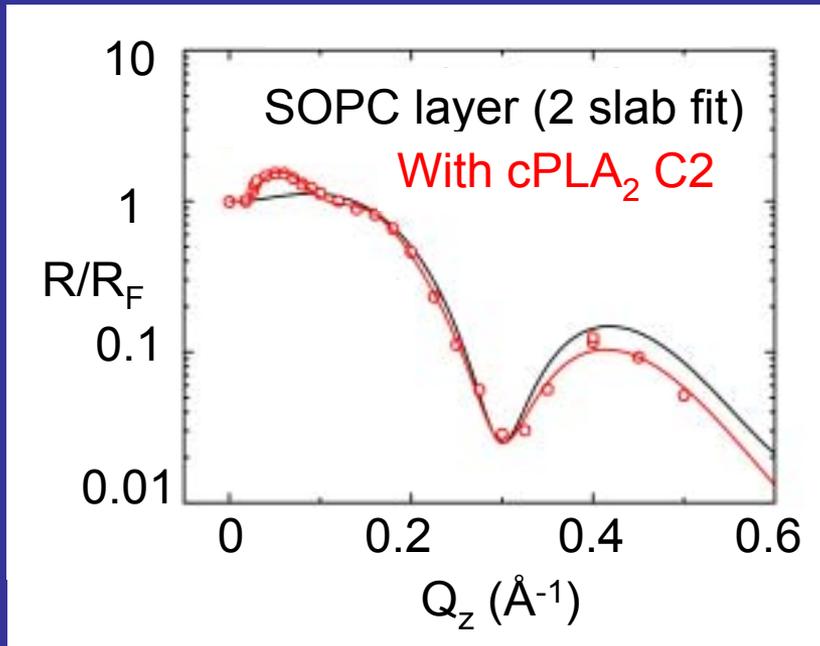
cPLA₂-C2 Domains Adsorbed onto SOPC Monolayer

Cytosolic phospholipase A₂ (cPLA₂) is an enzyme that catalyzes the production of arachidonic acid from membrane phospholipids.

cPLA₂ consists of 2 domains,

the C2 domain is responsible for membrane binding.

Similar C2 domains are present in many signaling proteins.



Can model with 3 slabs, but this model does not determine the penetration of the protein into the lipid layer or the angular orientation.

New Reflectivity Model for adsorbed cPLA₂-C2 domains

cPLA₂-C2 is not expected to change conformation upon binding

stable β sheet structure

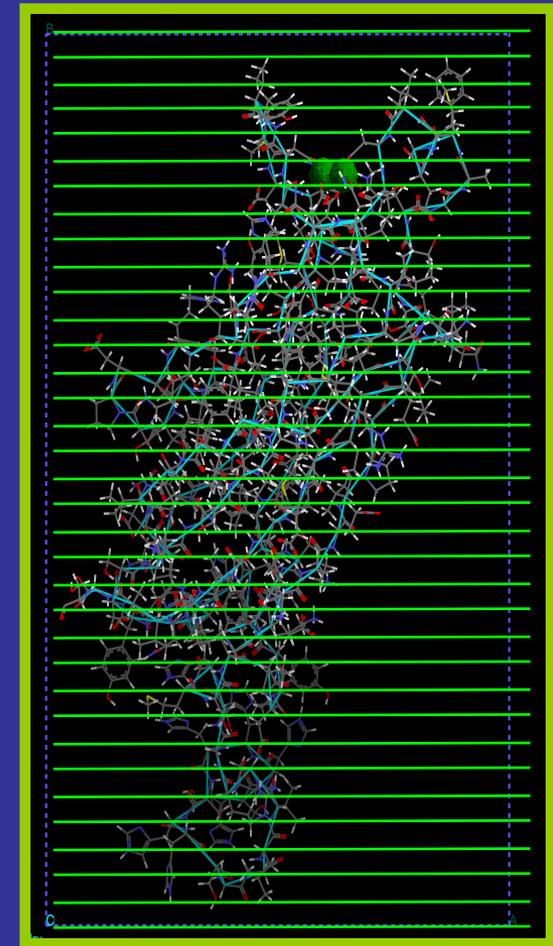
x-ray crystallography & NMR solution structures similar

EPR measurements demonstrate few changes upon binding

Malmberg et al. Biochemistry 42, 13227 (2003)

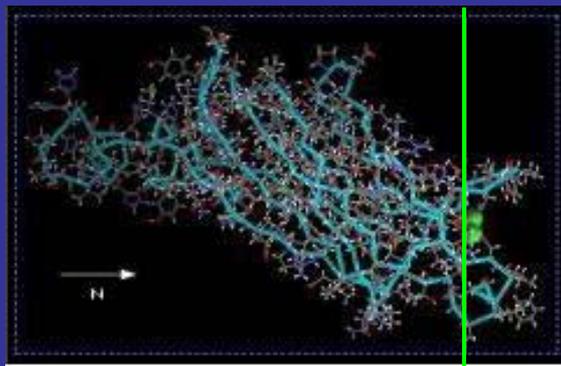
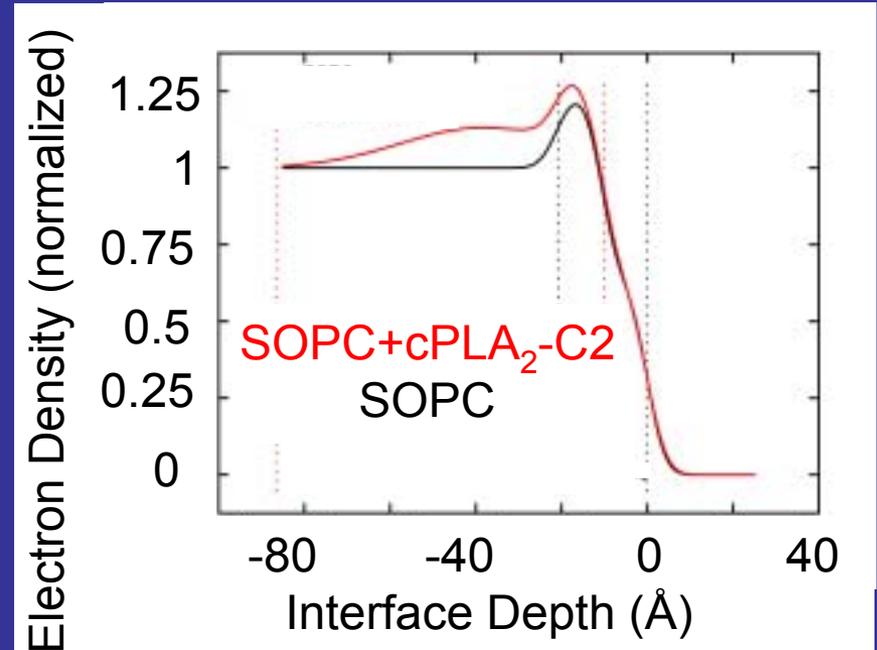
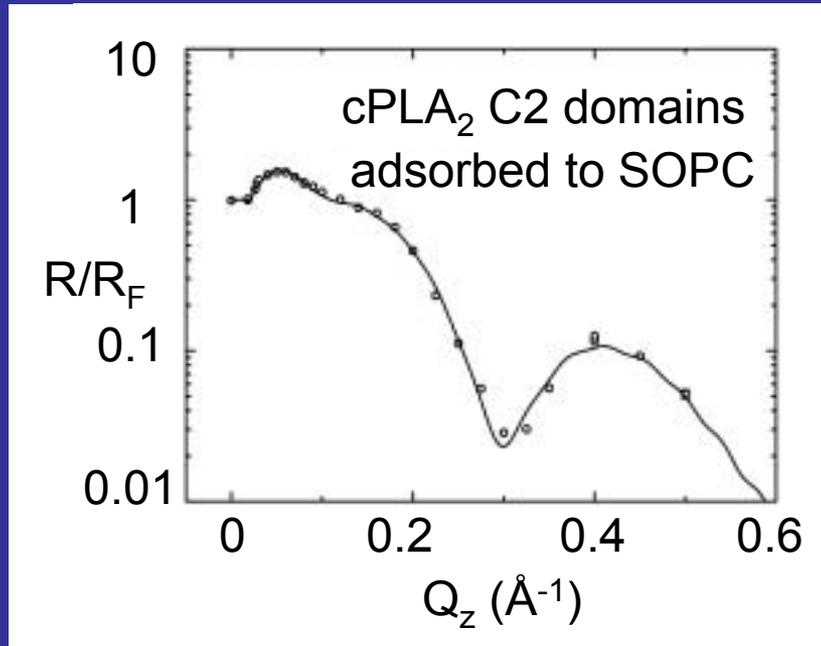
Model cPLA₂-C2 electron density
with x-ray crystallography structure
and use to fit reflectivity

- Select angular orientation
- Slice into 2Å layers
- Count # of electrons and
determine protein volume in layer
→ electron density in each layer
- Use this electron density profile to fit
x-ray reflectivity.
- Repeat for different orientations

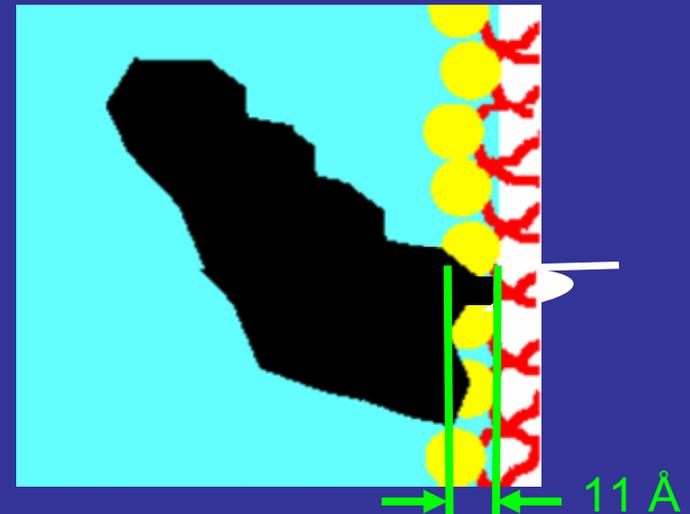


Fitting with New Model

Two fitting parameters per orientation: protein penetration & coverage



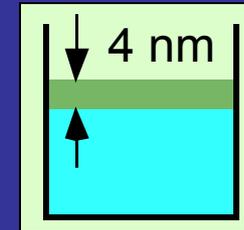
Subphase + cPLA₂-C2 | Lipid



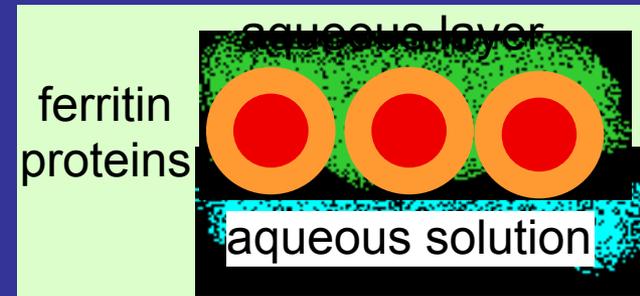
Best fit: penetration of 11 \AA and orientation shown

Summary

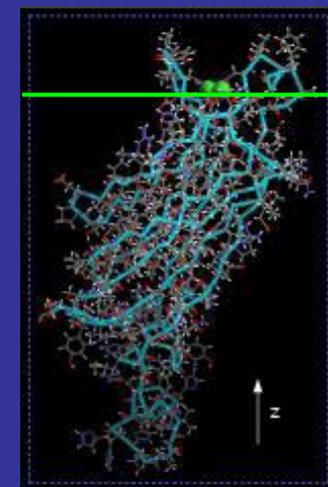
Creation of nanoscale aqueous films on top of aqueous subphases.
Can use x-ray scattering to study structure and interactions across these films.



Assembly of proteins in these films.



New method of analyzing x-ray reflectivity data for proteins adsorbed to lipid monolayers yields angular orientation of proteins and penetration distance into lipid layer.



Lipid

Subphase
&
cPLA₂-C2