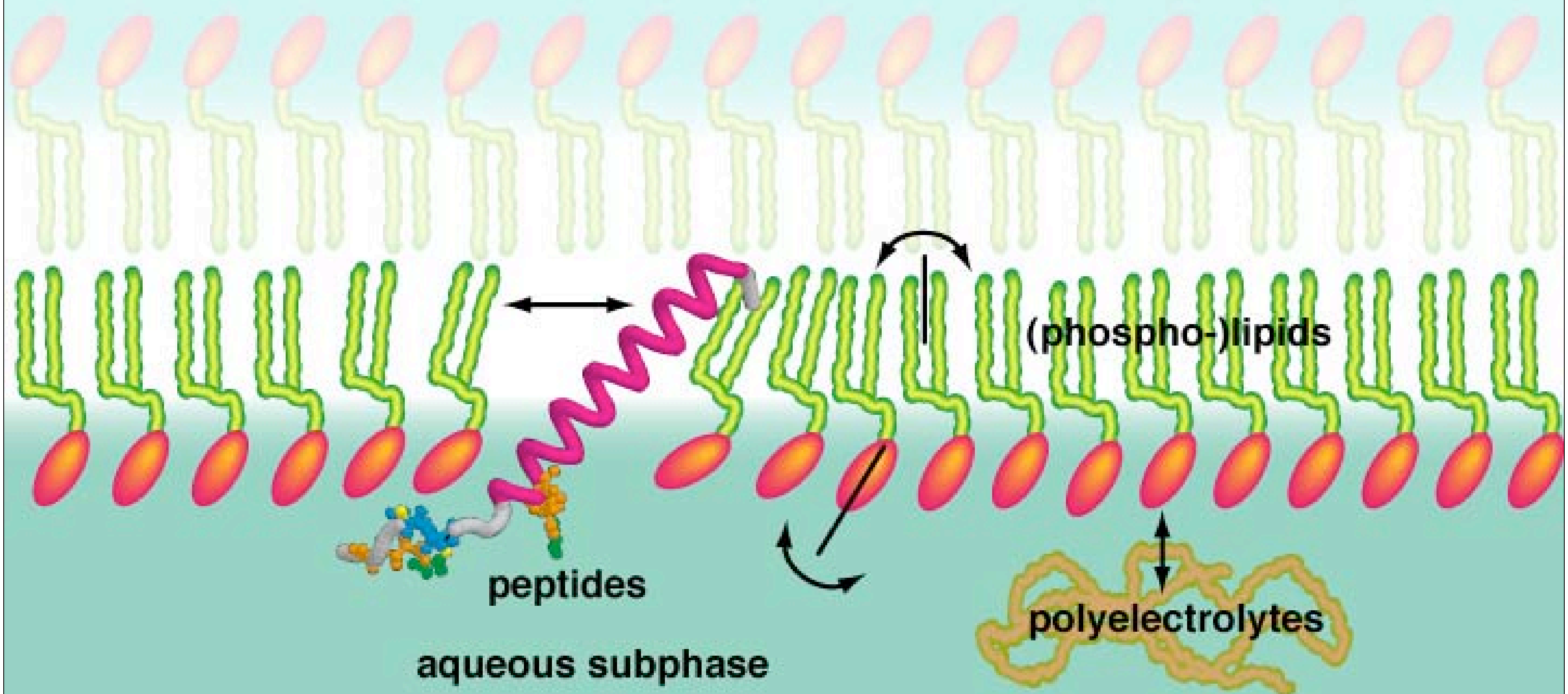
Surface-Sensitive Neutron Scattering as a Tool for the Molecular-Scale Characterization of Biomimetic Membranes



M. Lösche, *CNBT at the NIST Center for Neutron Research & Physics Dept., Carnegie Mellon University, Pittsburgh, PA*

CNBT Cold Neutrons for Biology and Technology

A Biotechnology Research Partnership
Funded by
National Center for Research Resources
RR14812

Joins high-performance computing with a unique neutron diffractometer/reflectometer…

Principal Biotechnology Research Partners

University of California at Irvine

National Institute of Standards and Technology

University of Pennsylvania

Johns Hopkins University

Collaborating Partners

Rice University

Duke University

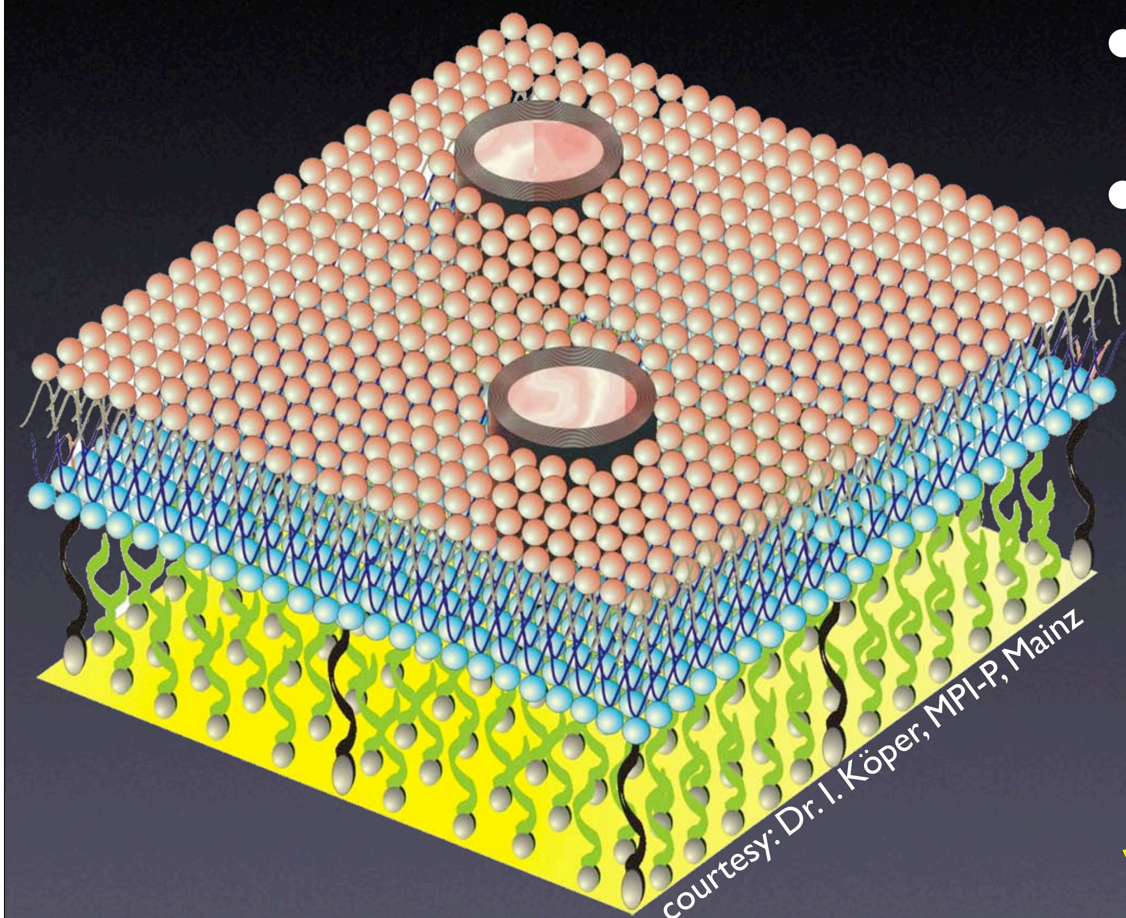
Carnegie Mellon University

Los Alamos National Laboratory

NIH (NIAAA)

… for structural studies of membrane-active proteins in their native environment, including experiment-validated studies of dynamics

Tethered Bilayer Lipid Membranes (tBLMs)

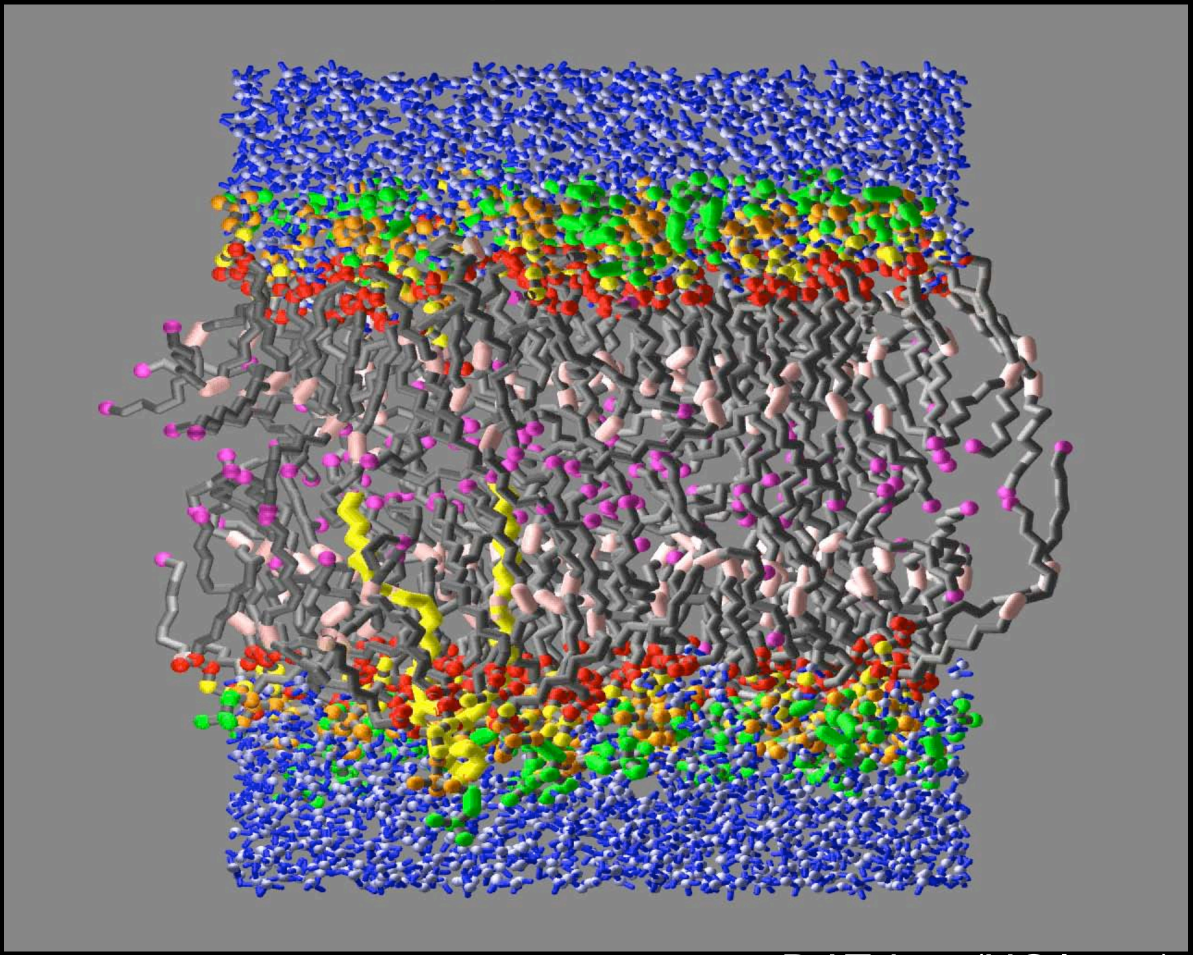


- comprehend biological processes
- utilize self-assembly for technical applications

→ are they indeed what we *think* they are?

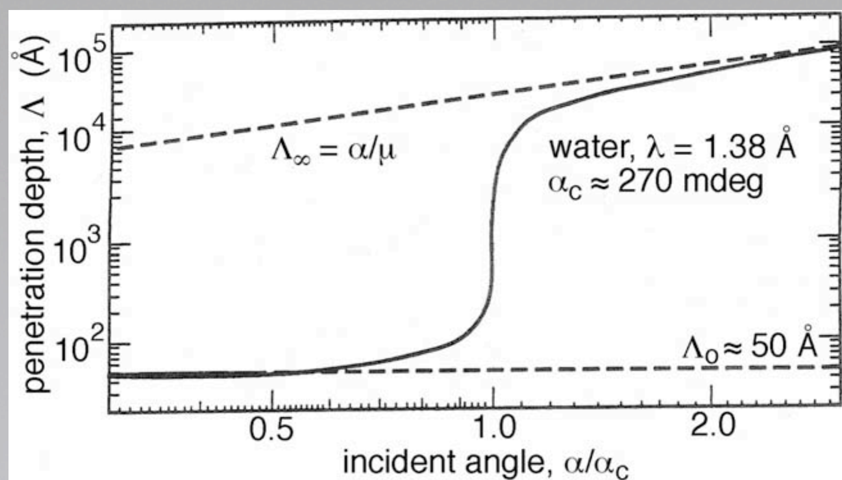
A Characteristic of Biomembrane Systems:
High Thermal Disorder!

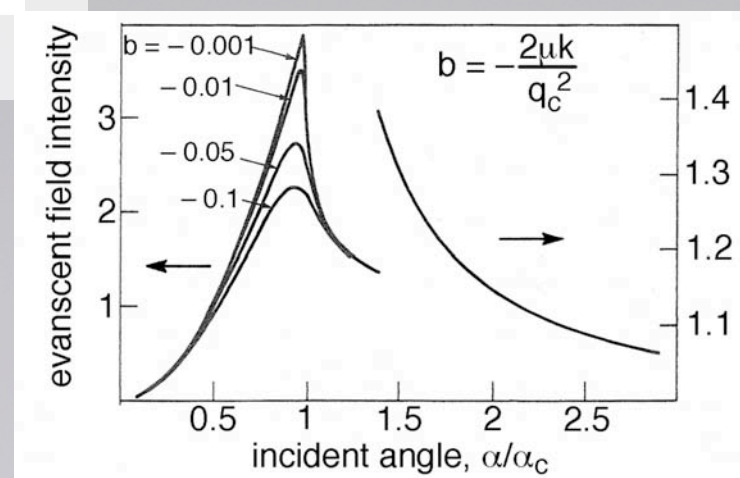
MD simulation: DOPC bilayer membrane



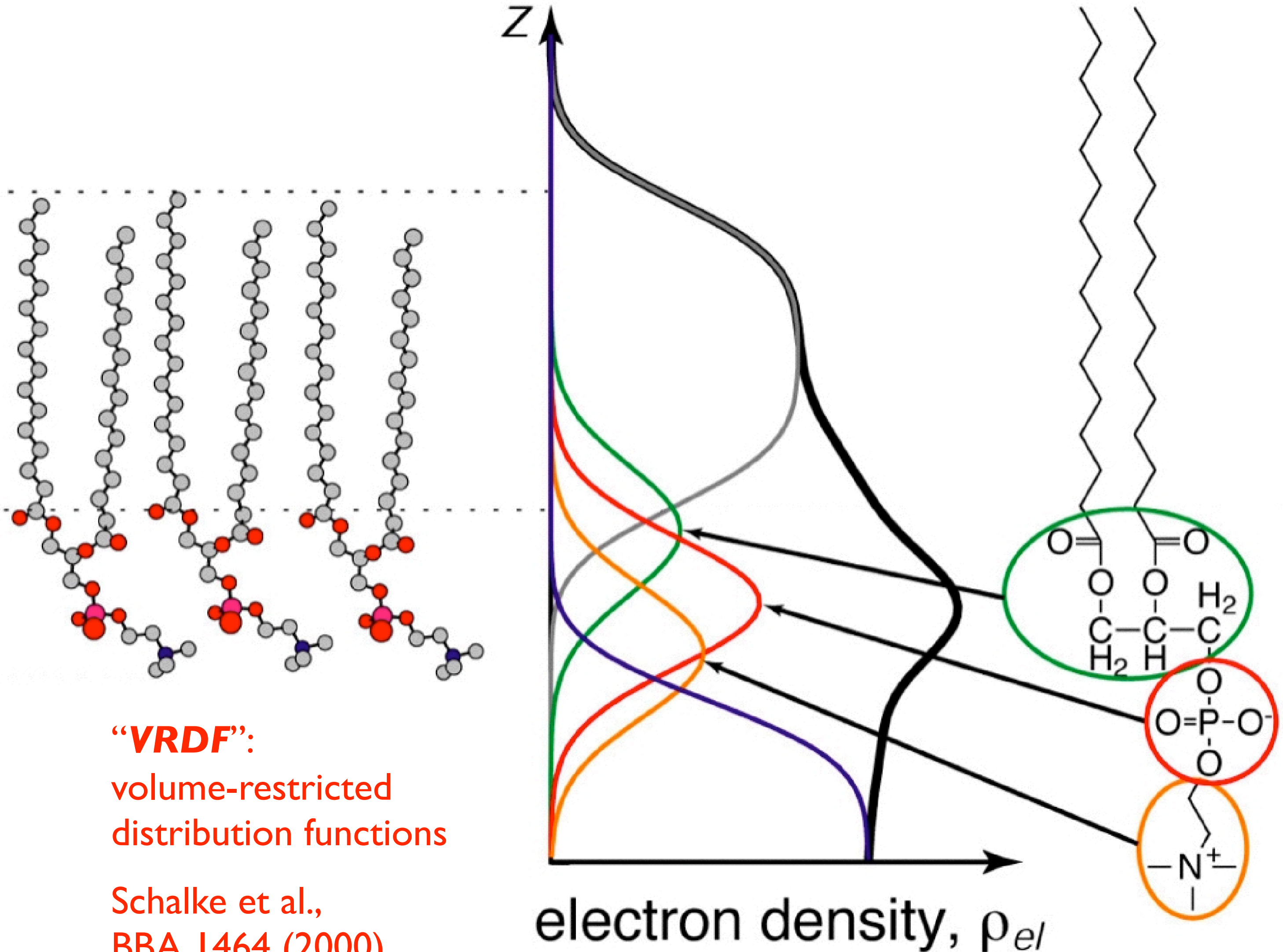
courtesy D J Tobias (UC Irvine)

Surface-Sensitive Neutron and X-ray Scattering





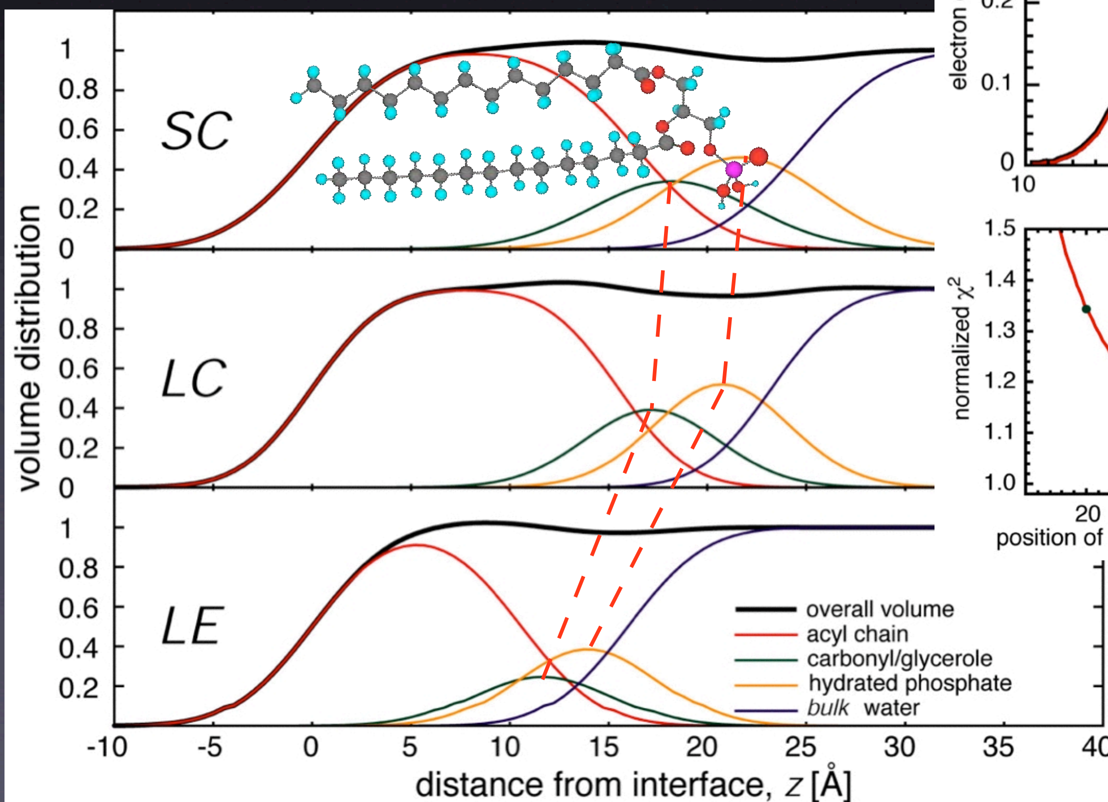
Composition-Space Refinement Modelling

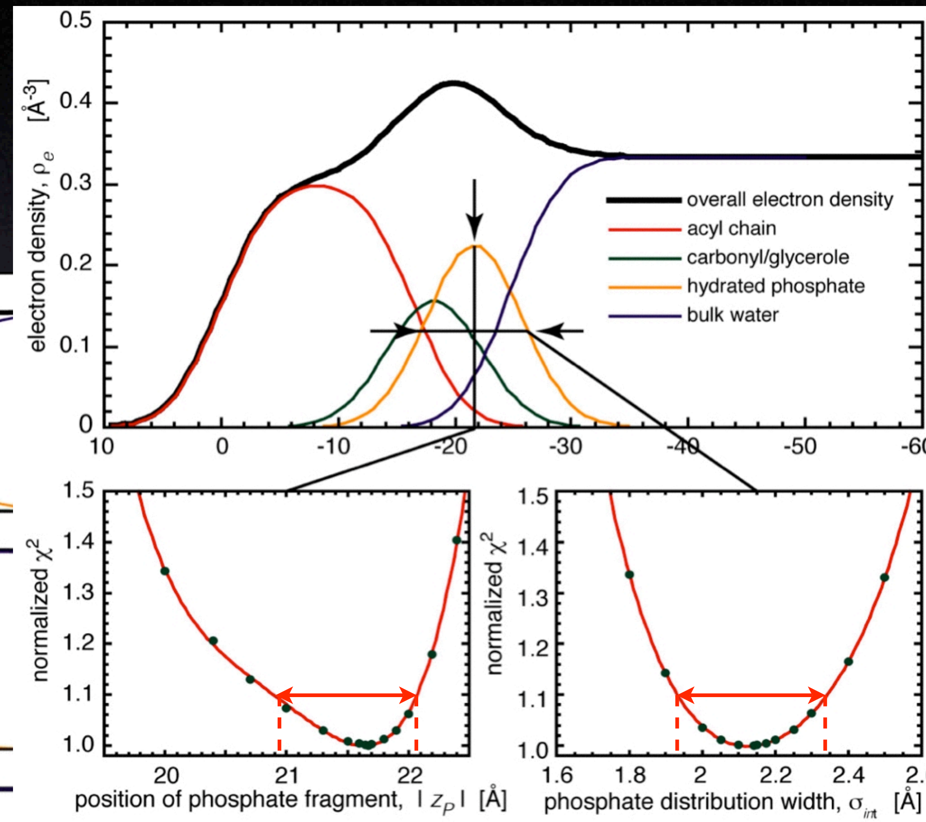


"***VRDF***":
volume-restricted
distribution functions

Schalke et al.,
BBA 1464 (2000)

Model Performance and Credibility: DMPA

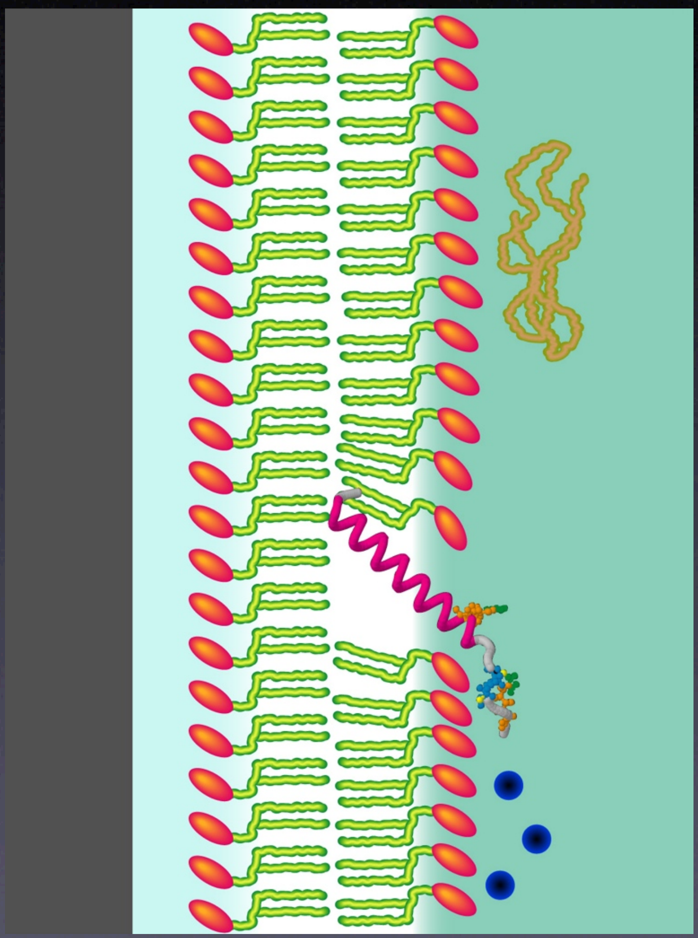




(x-ray characterization of Langmuir monolayers)

Solid-Supported Bilayer Membranes:

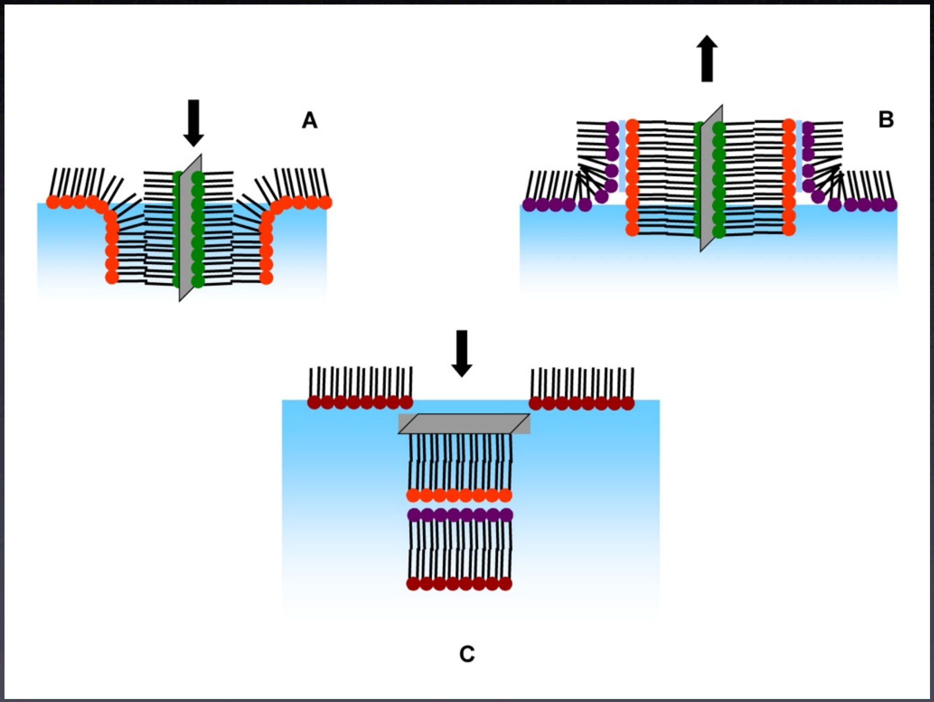
Variations on a common theme



- *Adsorbed bilayers (Tamm & McConnell – and many others): vesicle fusion, etc.*
- *Floating bilayers (Roser; Fragneto & coworkers): LB transfer*
- *Tethered bilayers (Knoll, Ringsdorf, Cornell – and many others): thiol (gold), silane (quartz) chemistries; PEG spacers, etc.*

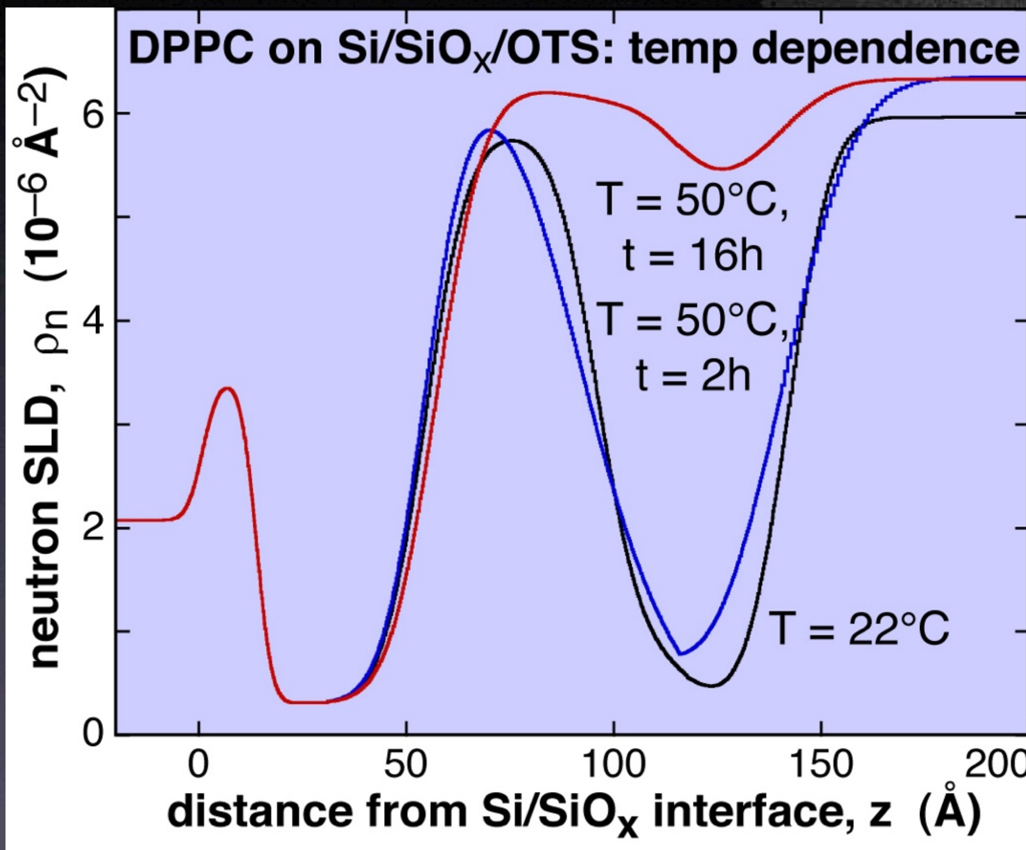
Neutron scattering:
– deeply penetrating
– non-destructive
– molecular sensitivity & resolution

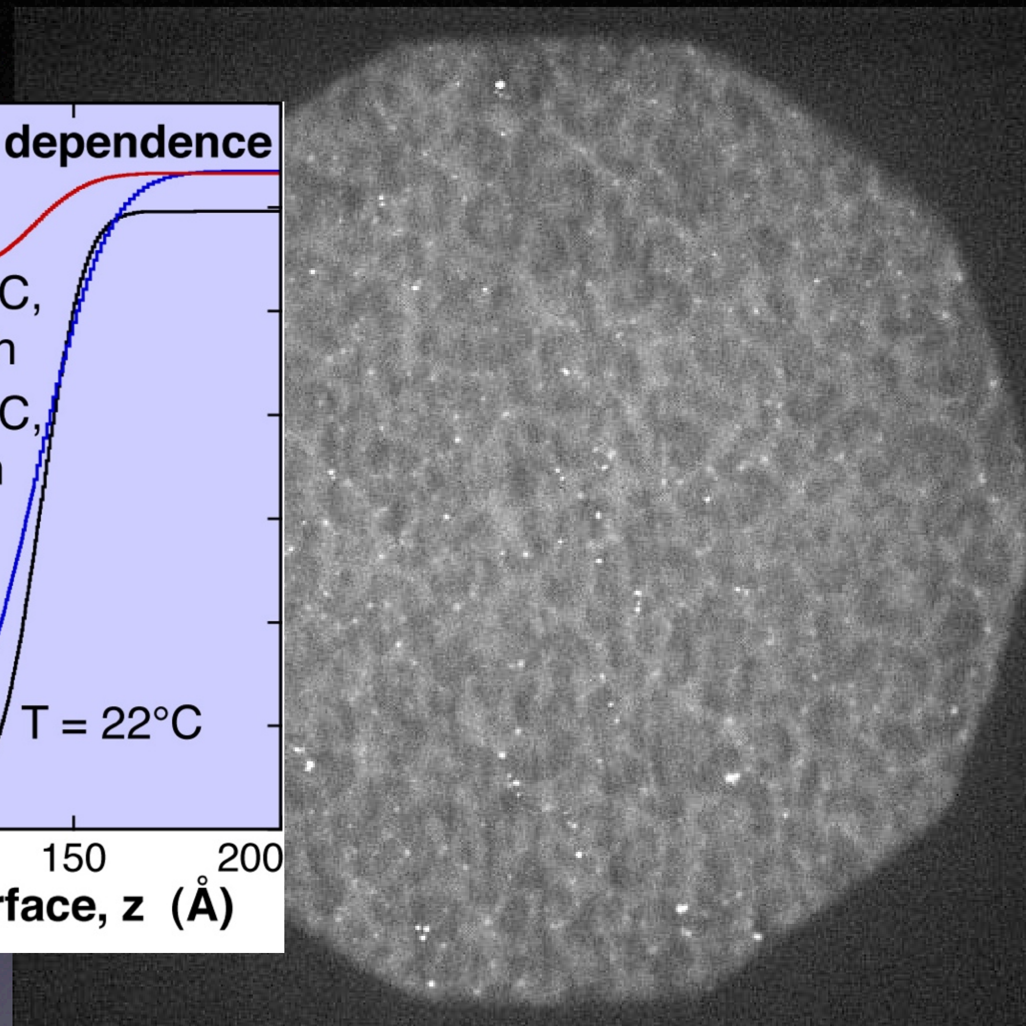
“Floating” Bilayers as Fluid Membrane Models



- *Step-by-step preparation (Langmuir trough)*
- *Retains undulation dynamics*
- *Transfer often incomplete*

“Floating” Bilayers as Fluid Membrane Models

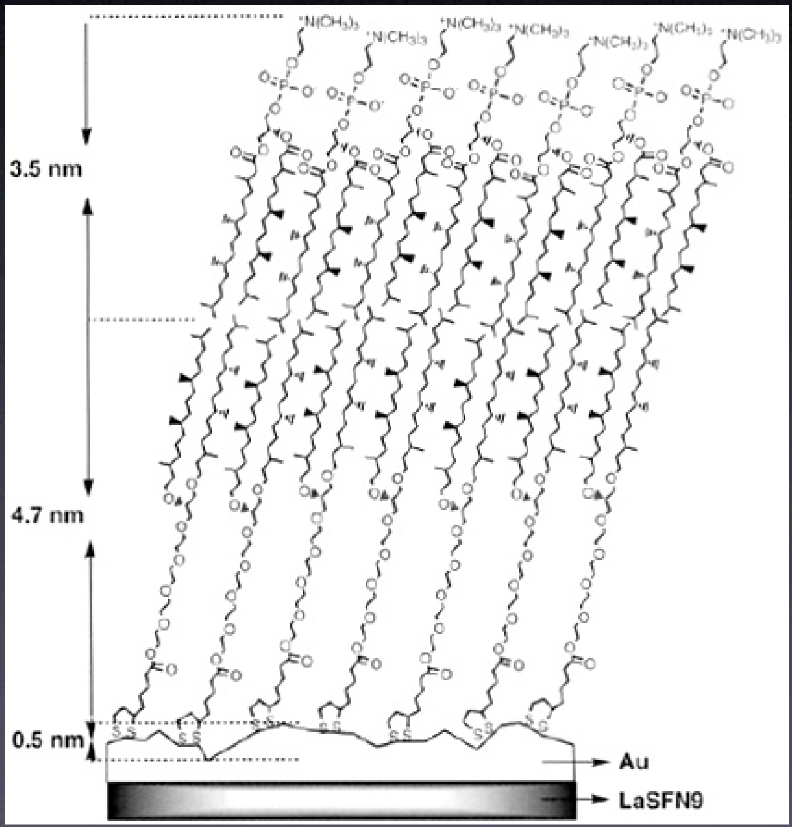




A. Kerth/D.J. McGillivray, unpublished

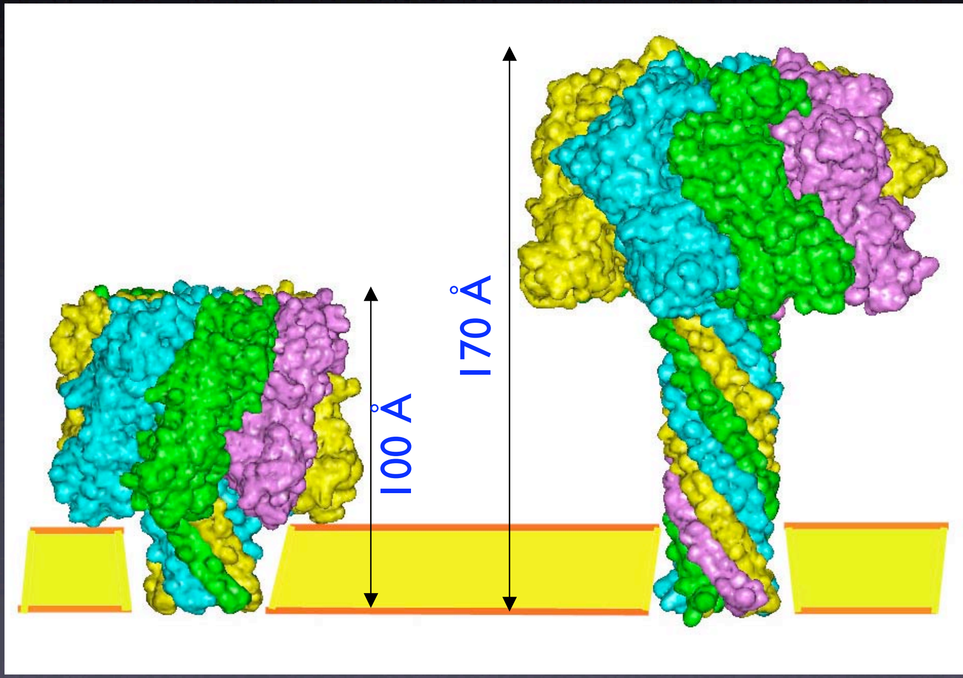
tBLMs and their Functionalization

Schiller et al., Angew. Chemie 42 (2003)



(collaboration with W. Knoll, J. Kasianowicz)

e.g., bacterial toxins:

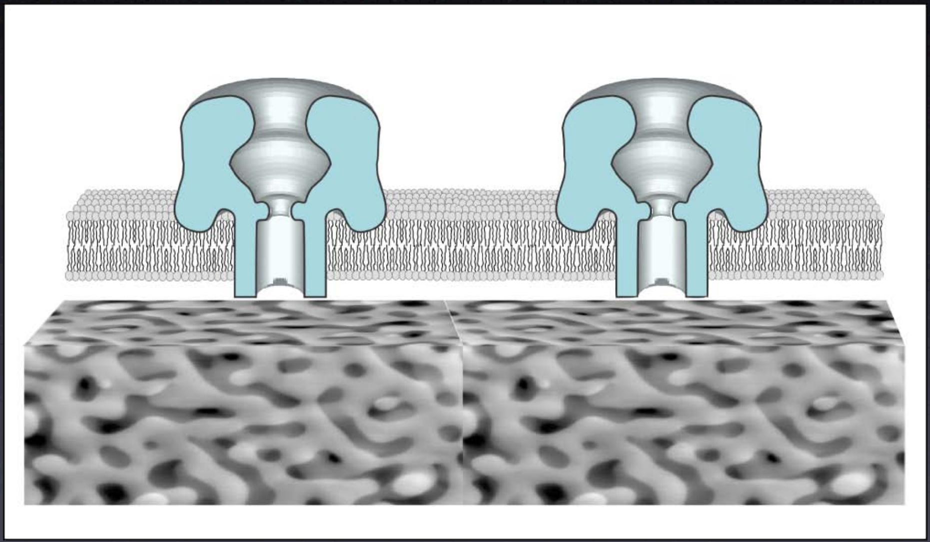


hemolysin
(Song et al.,
Science 1996)

B. anthracis PA63: putative membrane association
(Nguyen, J Biomol Struct Dyn 2004)

Bilaterally Accessible, Scaffolded *Fluid* Membranes

→ studies of signal transduction & amplification; biosensoric devices



active NSF-NIRT, together with

Mike Paulaitis (OSU ChemEng),
Jonah Erlebacher (JHU Mats Sci & Eng), and
John Kasianowicz (NIST Electron Elt Eng Lab)

scaffold structures:
porous gold, S-layer sheet crystals

goal:

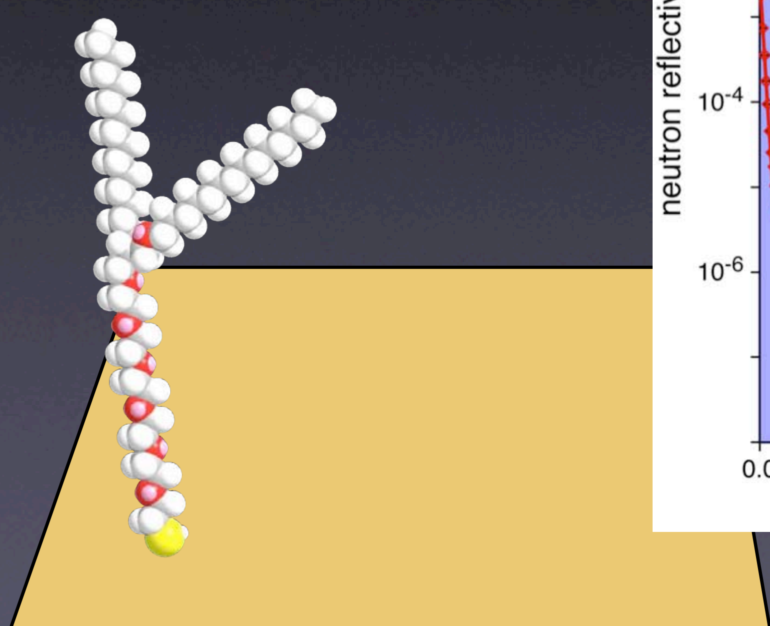
incorporation into stabilized membrane systems as a basis for

- molecular sensor systems
- pharmaceutical screening

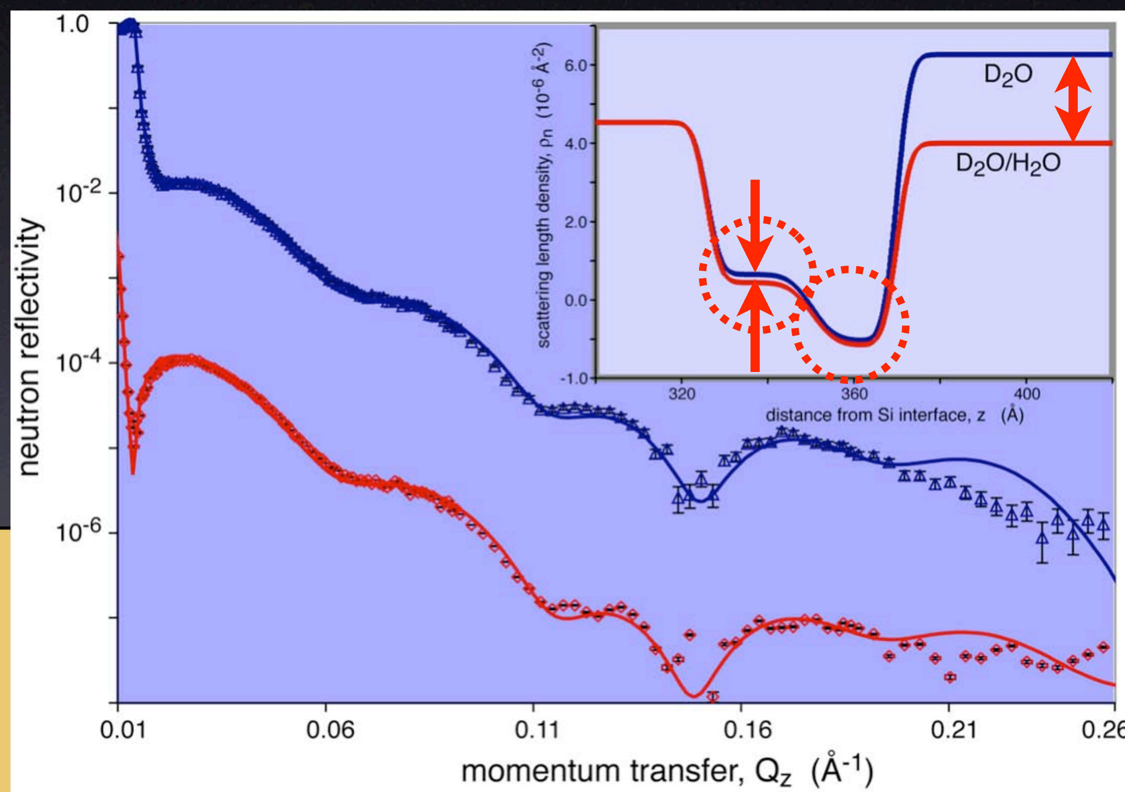
Tethered Bilayer Membranes
(on continuous gold only, so far)

WC14:

A thiolated oligo-EG dialkane for grafting on Au surfaces (synthesis: Dave Vanderah *et al.*)



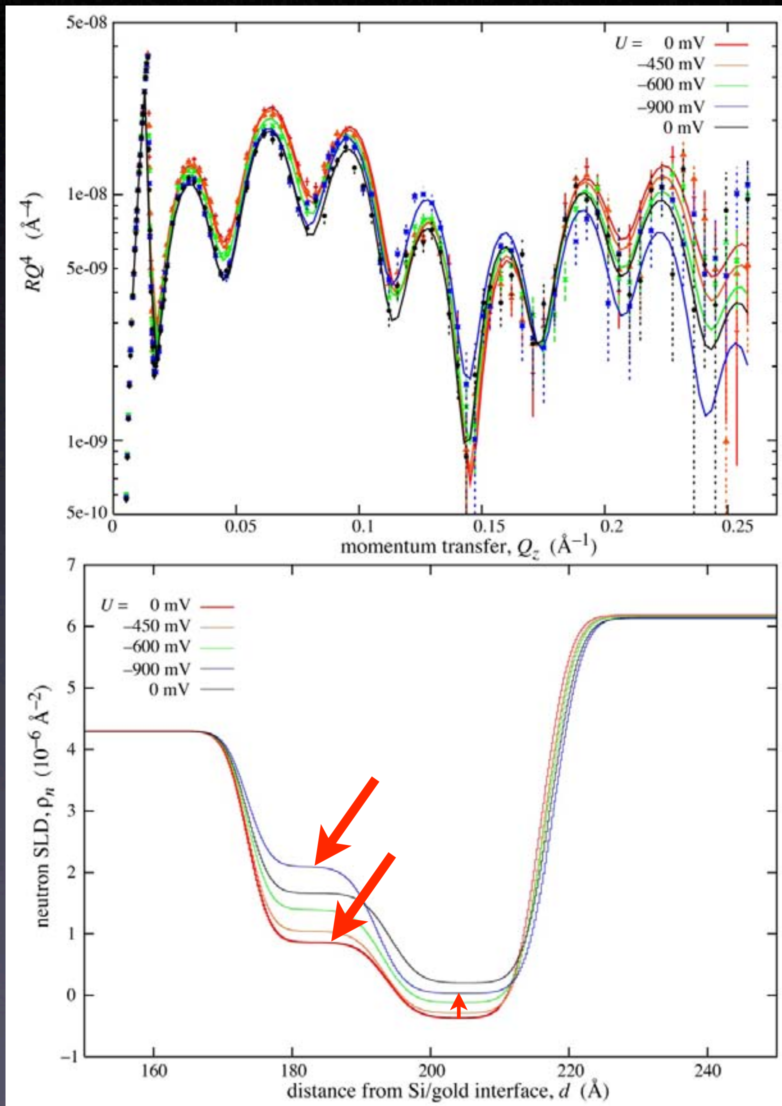
WC14 monolayer on Au (Si) under water



- extremely dense alkane packing
- << 10 vol% solvent in proximal space

Control of Hydration by Electric Field:

Structural assessment as a function of electrostatic potential

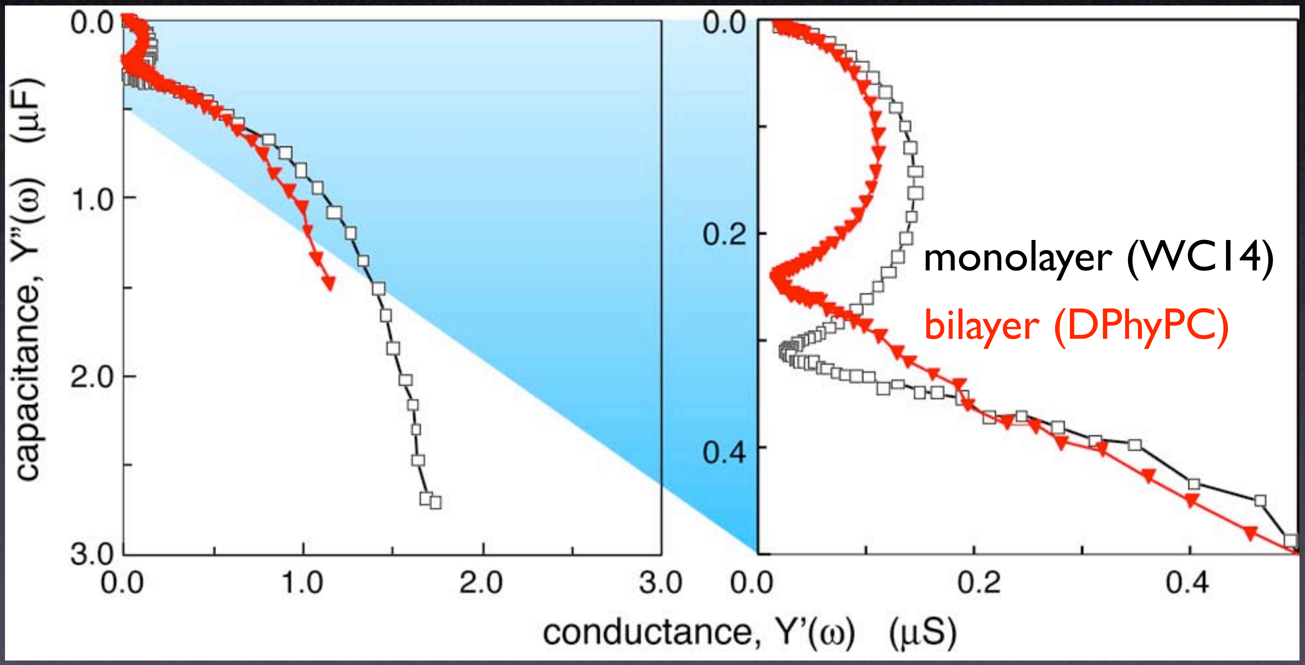


- DPTL monolayer under water
- "cleaning" of surface w/ positive pulse
- long-term stability at Φ ~ 4x10^8 V/cm
- submembrane space significantly more hydrated upon return to U = 0V
- (but alkyl region more disordered: hydration through creation of defects!)

Köper & McGillivray, unpublished data

Bilayer Completion: "Rapid Solvent Exchange"

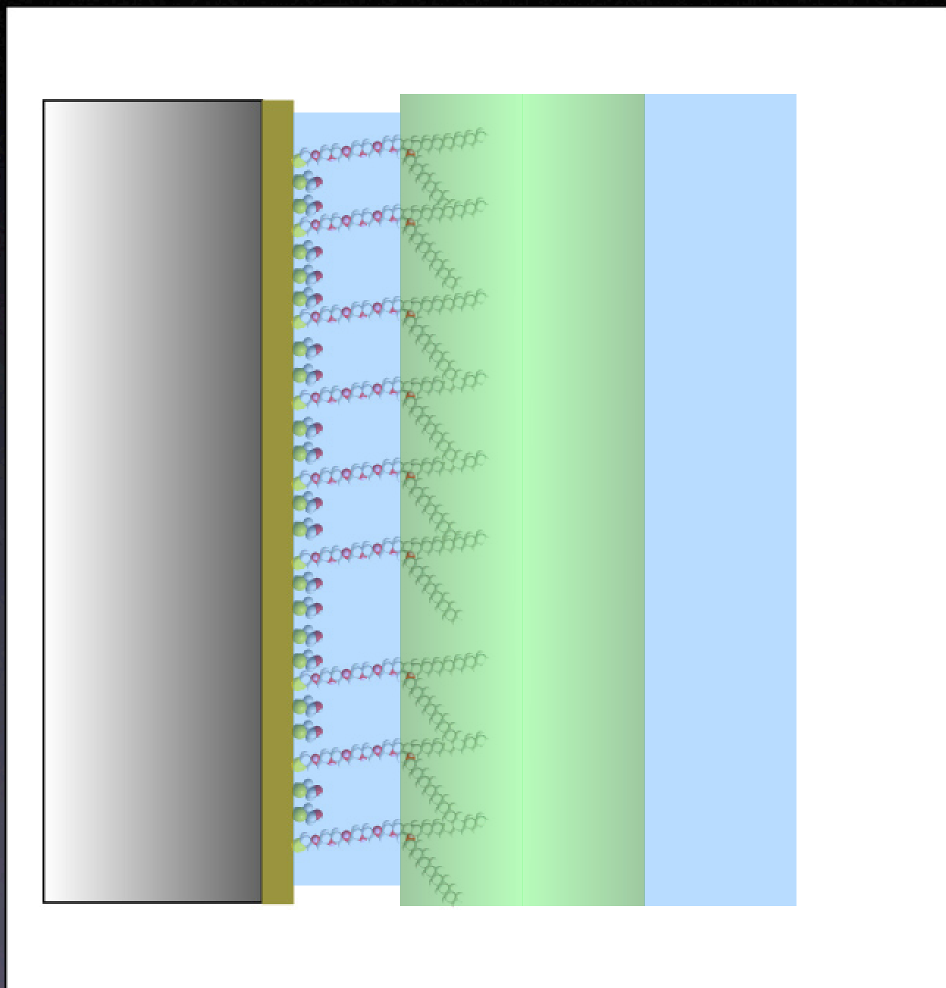
Nyquist diagrams of monolayer and bilayer



- capacitance (0.75 μF/cm^2) and conductance indicate bilayer formation
- EIS and NR: bilayer > 90% complete

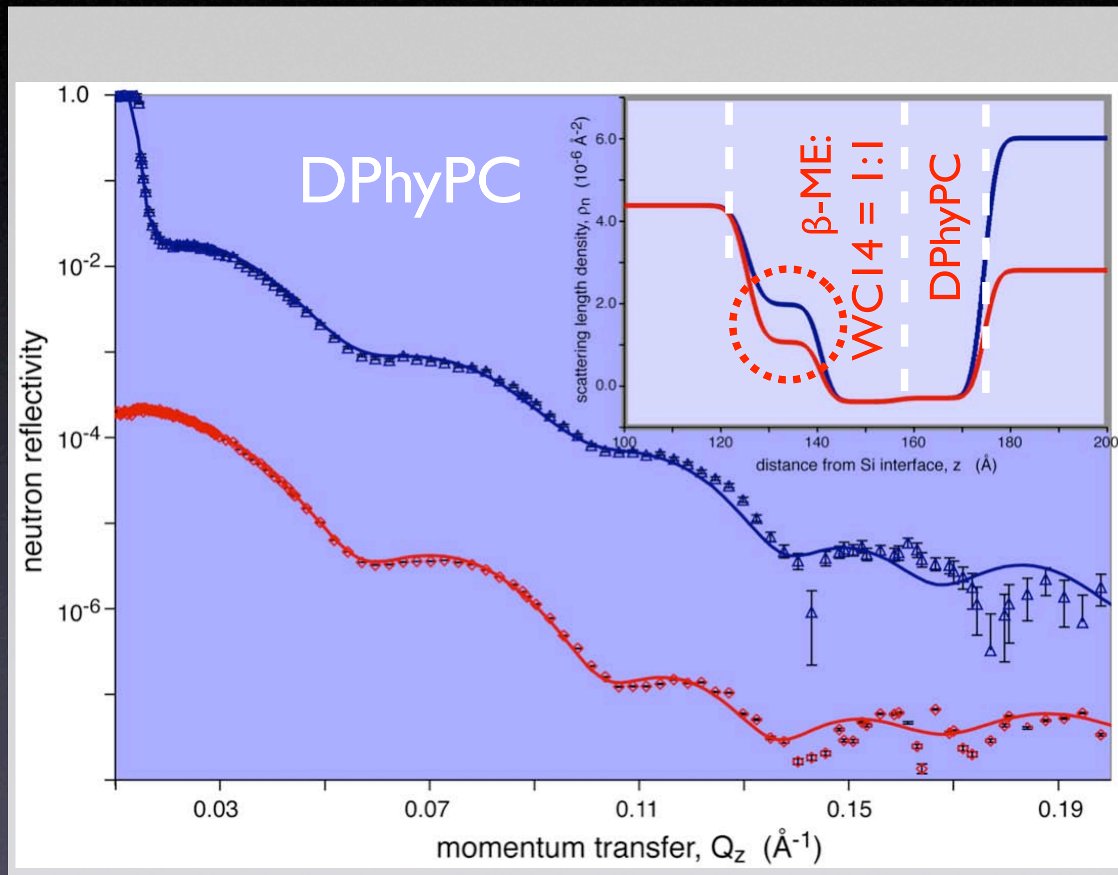
"*rapid solvent exchange*":
(Cornell *et al.*, *Nature* 387, 1997, 580)

Backfilling: β-Mercaptoethanol



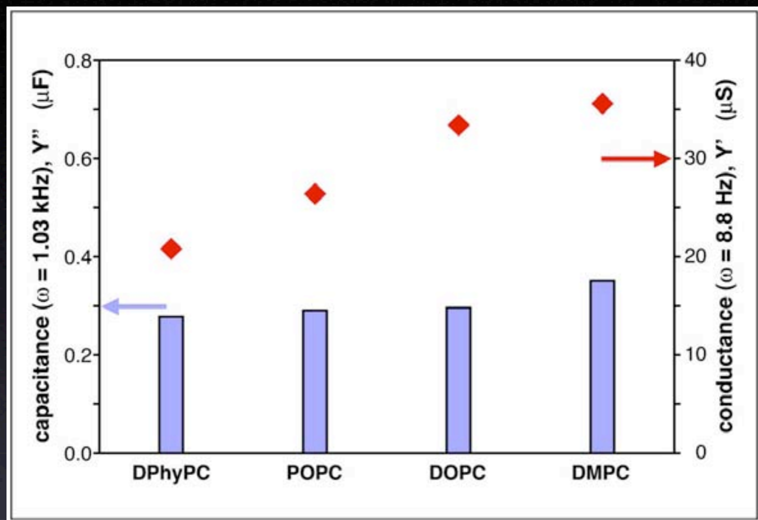
- does *not* work with vesicle fusion (low contact angle of the monolayer)
- works *well* with rapid solvent exchange

tBLM's Based on Mixed Monolayers of WC14 and β-ME

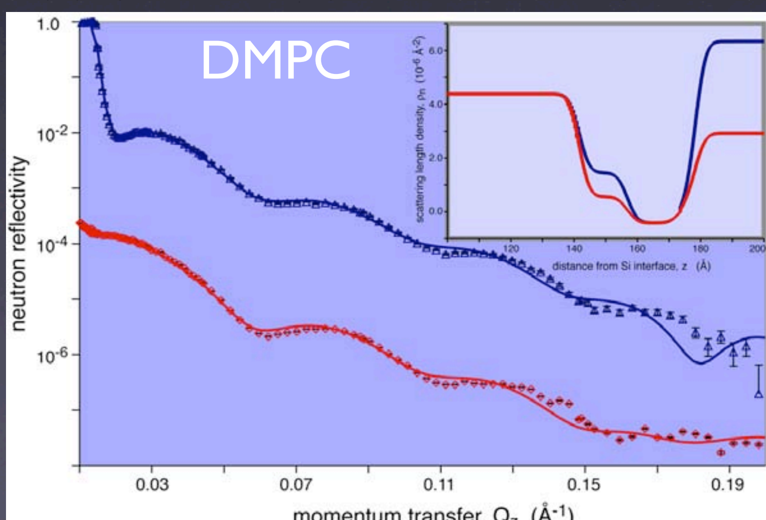


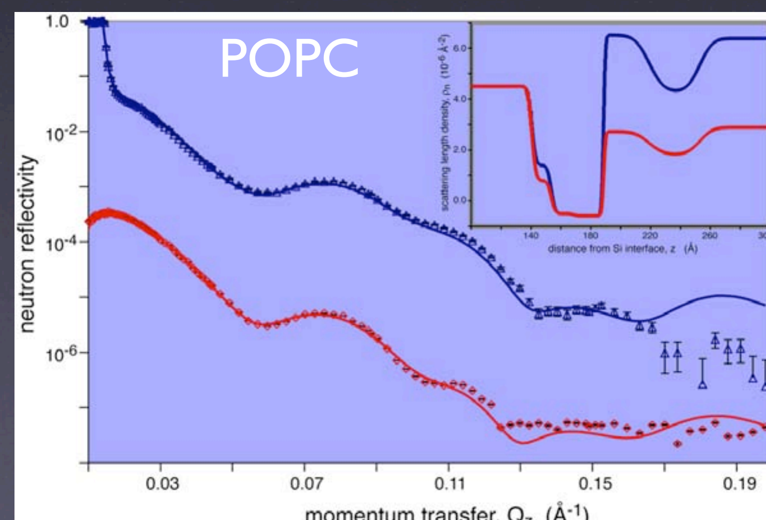
- Y',Y" of mixed monolayer >> pure WC14
- Y',Y" of bilayer (DPhyPC) based on mixed monolayer ~ as for pure WC14
- β-ME introduces solvent in proximal space (> 50% solvent by vol.)

Dependence on Cover Lipid



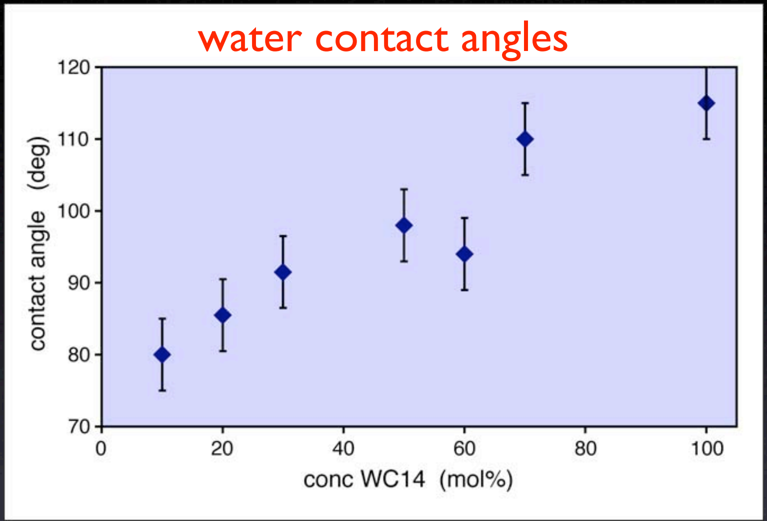
- DPhyPC, DMPC, POPC, DOPC all form complete bilayers on WC14/β-ME (1:1)
- Y'' almost invariant for all PC's
- Y' depends significantly on lipid
- POPC forms partial overlayer

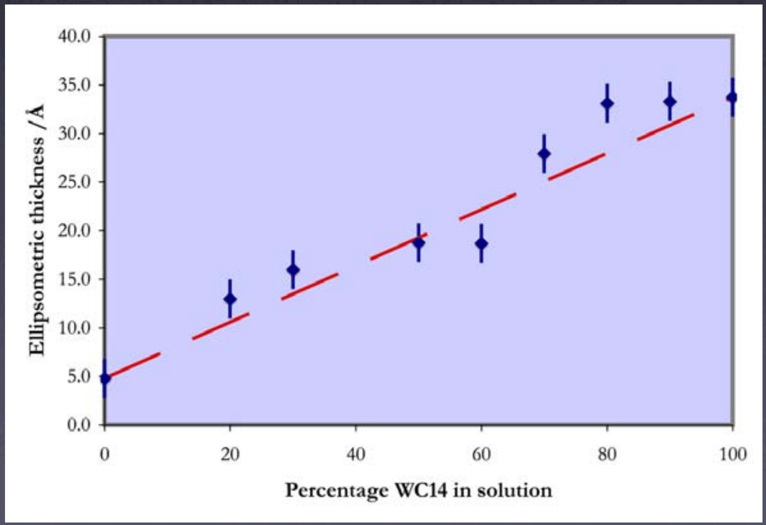


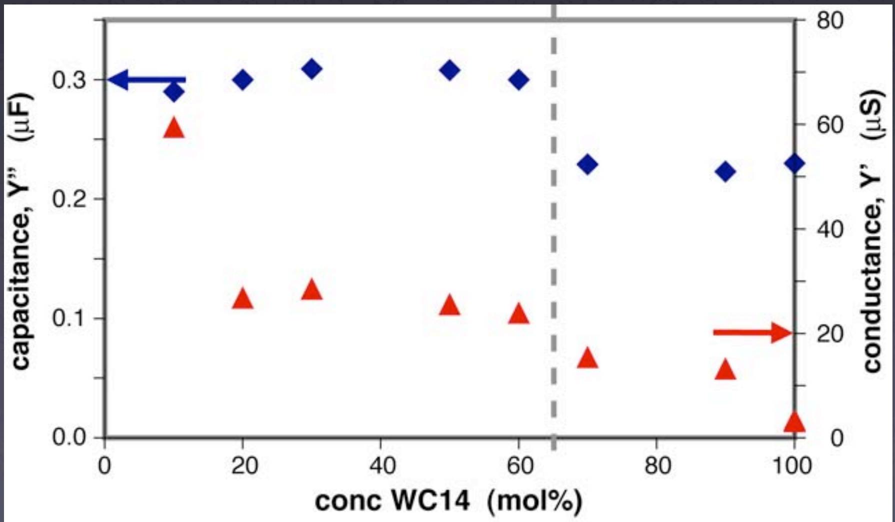


Influence of Backfiller Concentration

- *surface* concentration controlled by WC14:β-ME ratio in *deposition* solution
- significant amounts of WC14 on surface even at low solution conc
- partial fill-in of surface-tethered monolayer during solvent exchange
- structural/functional transition as a function of WC14 conc

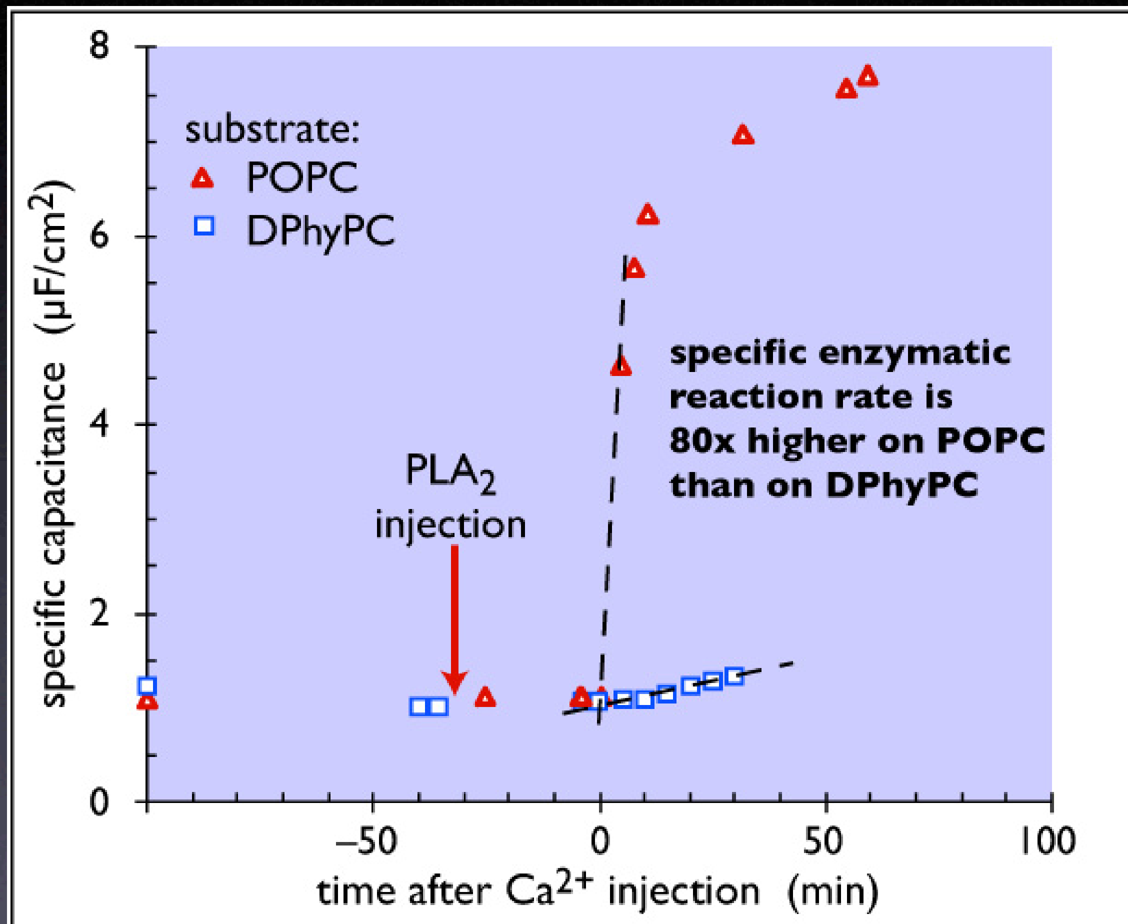






Challenging with Phospholipase PLA_2:

A Sensitive Amplifier of Local Defects



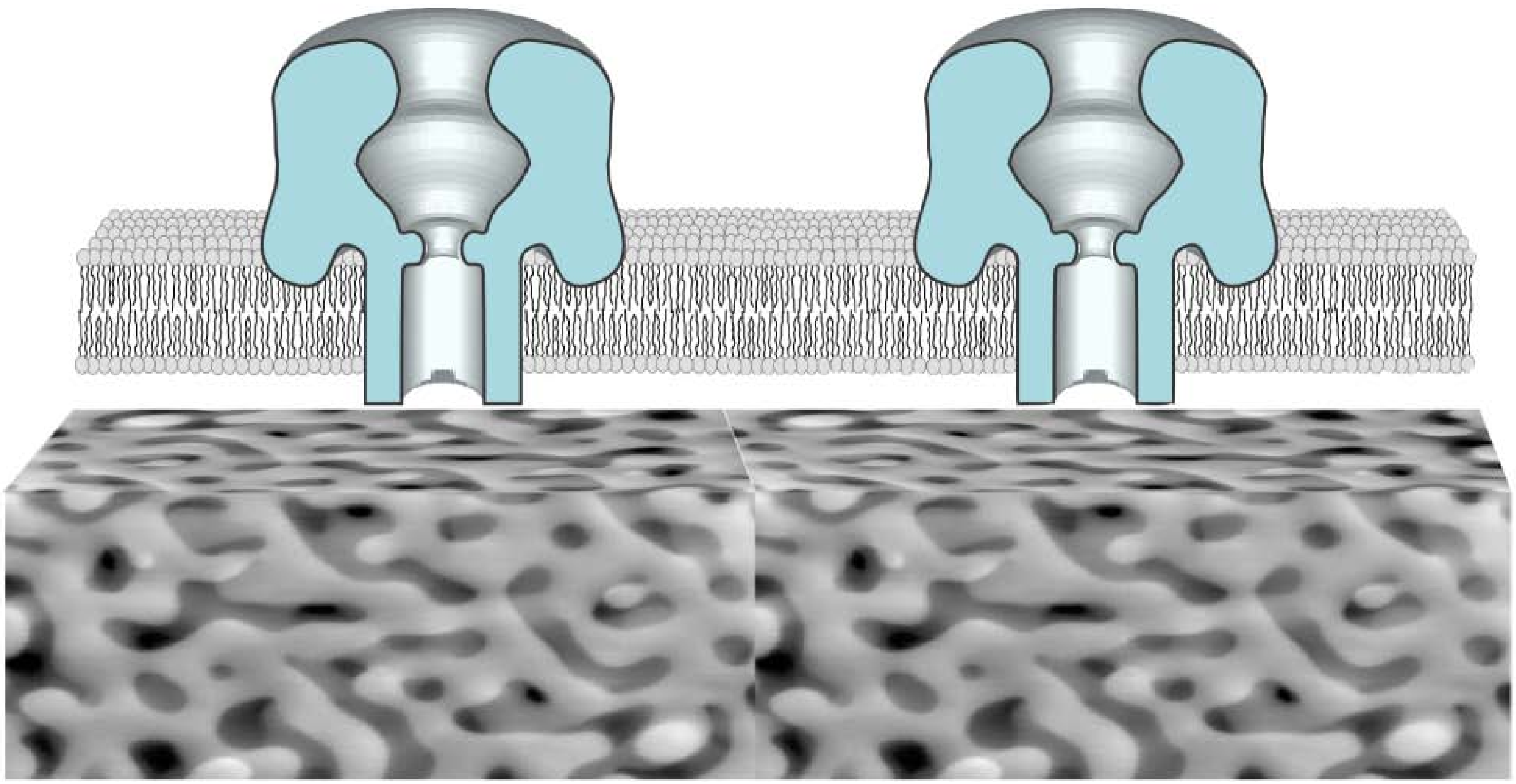
resistance against PLA_2 depends on cover lipid:

- well-ordered DPhyPC largely insensitive
- fluid POPC immediately degraded

Valincius *et al.*, submitted

Functionalization with PA63: DPhyPC

work in progress toward:



Conclusions

- Neutron scattering is adequate for non-destructive, molecular-scale characterization of molecular interface architectures
  - Optimization of tethered membrane structures using EIS and neutron scattering: Membrane properties controlled by backfilling and lipid chemistry
    - Densely grafted SAM insufficiently hydrated in submembrane space
    - Electric field induces water (by introducing membrane defects)
    - “Backfilling” more gentle method to introduce hydration
    - “Rapid solvent exchange” works well on backfilled SAM
    - Great flexibility in choice of lipid for bilayer completion
    - Magnetic contrast variation: Investigation of minute structural details
    - PLA_2: A sensitive amplifier of membrane surface defects

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