
Composition space modeling for biological neutron reflectometry

Frank Heinrich

Carnegie Mellon University, Pittsburgh PA and NIST Center for Neutron Research, Gaithersburg MD

Introduction

20%



80%



Carnegie Mellon

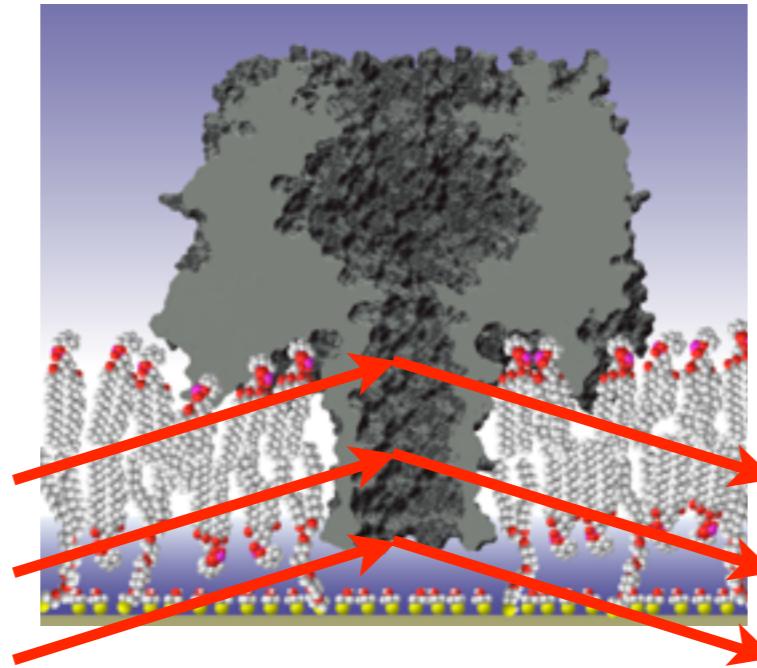
Physics Department,
Pittsburgh PA



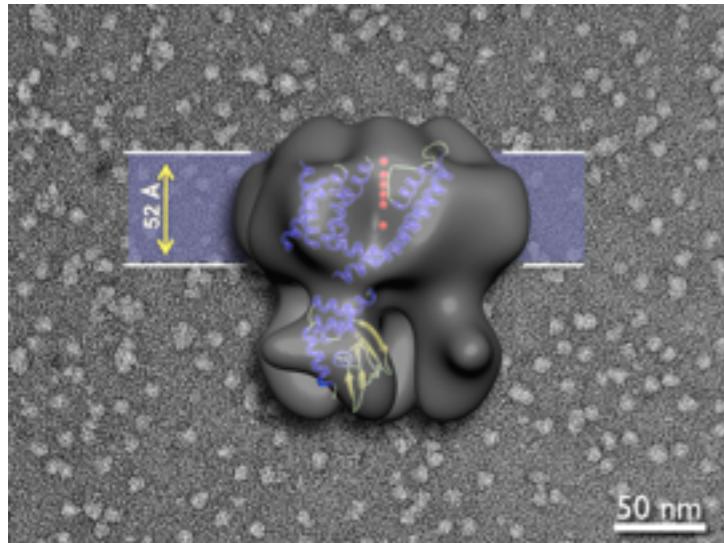
NIST Center for Neutron Research,
Gaithersburg, MD

Neutron Reflectometry for Protein Metrology

Neutron Reflectometry

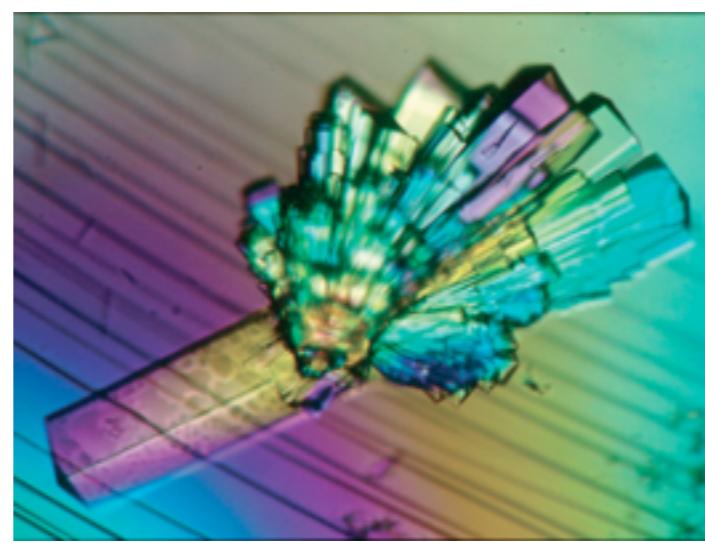


Cryo-Electron Microscopy



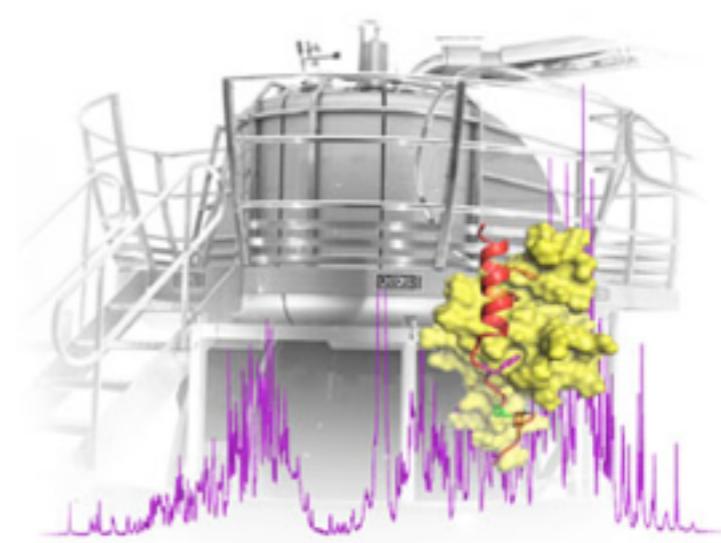
Chiu, P.-L. et al. *Structure/Folding and Design*, 15:1053
(2007)

Protein Crystallography



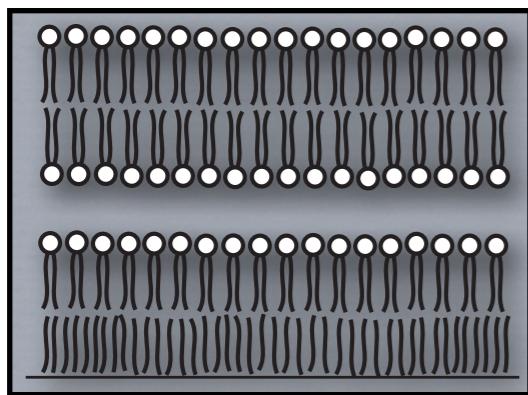
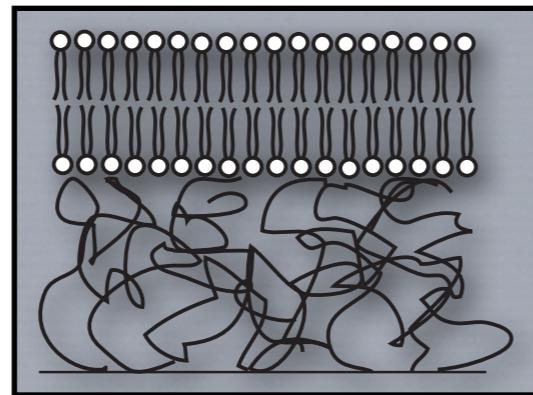
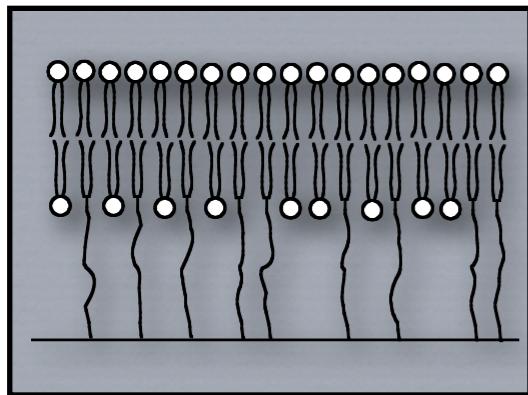
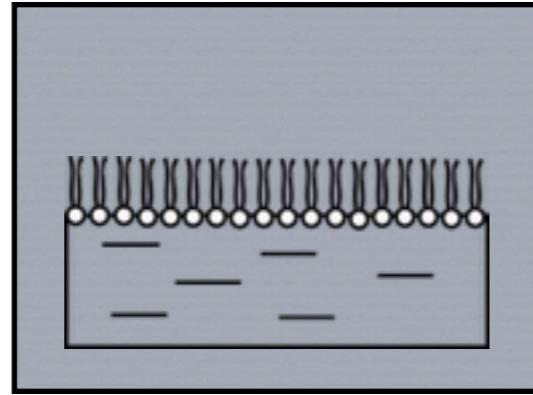
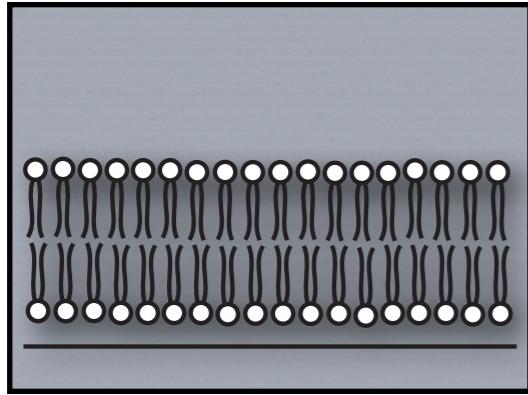
[http://hasylab.desy.de/user_info/available_instruments/
x_ray_protein_crystallography/index_eng.html](http://hasylab.desy.de/user_info/available_instruments/x_ray_protein_crystallography/index_eng.html)

Nuclear Magnetic Resonance



[http://www.bioch.ox.ac.uk/aspsite/printable.asp?
pageid=434](http://www.bioch.ox.ac.uk/aspsite/printable.asp?pageid=434)

Membrane Model Systems

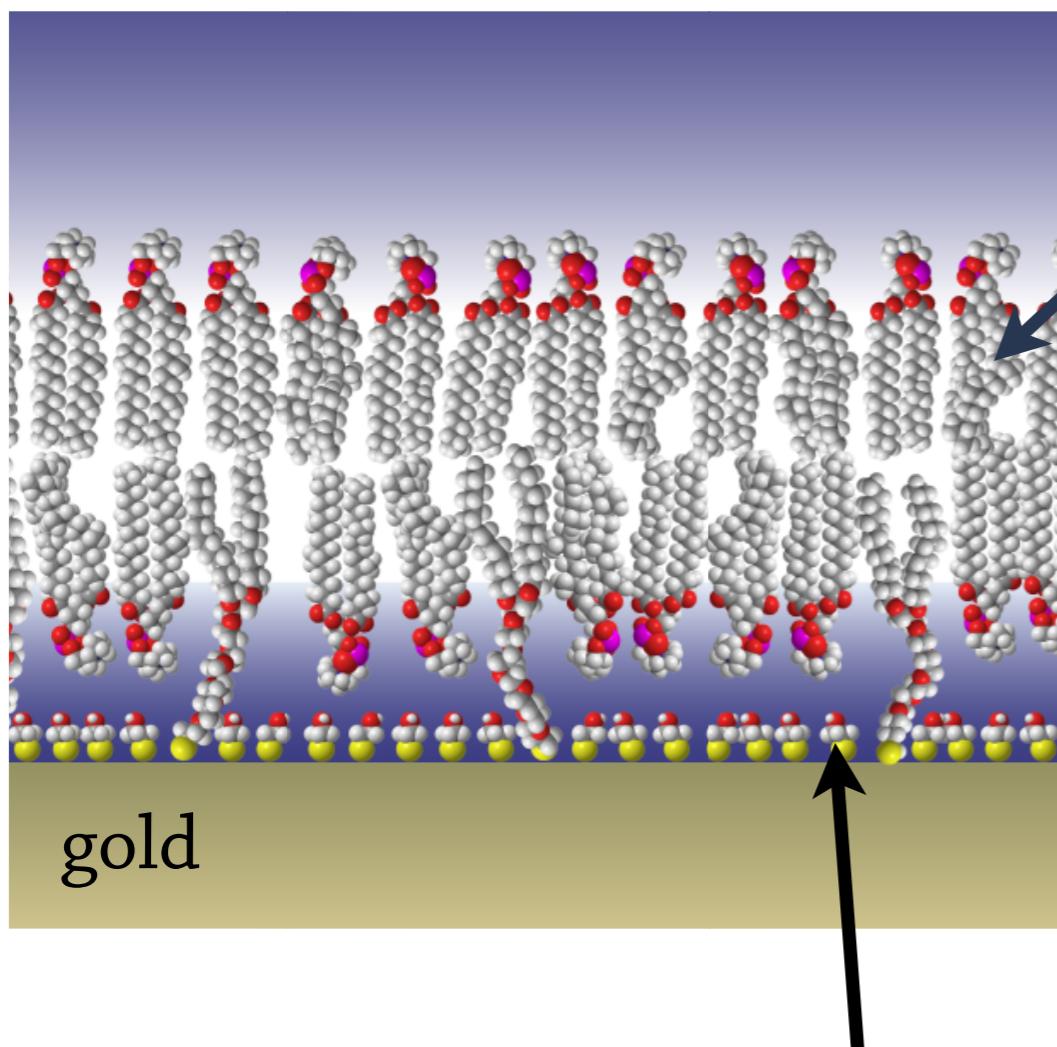


supported
membranes

new model systems:

Dave Hoogerheide
Dan Scott
Chuck Majkrzak

Sparsely tethered bilayer lipid membrane



Lipid bilayer (PA, PC, PG, PS, PI, PIP, chol.)

SAM of Thiol-(EO)_x-(C_{14+x})₂
tether with β-mercaptoproethanol

- Homogenous preparation over large areas possible
- Fluid bilayer is stable for more than a week
- No limitation in type of lipids used for bilayer
- Water-filled sub-membrane space decouples the bilayer from the substrate

**Carnegie Mellon University
Research Showcase @ CMU**

Dissertations

Theses and Dissertations

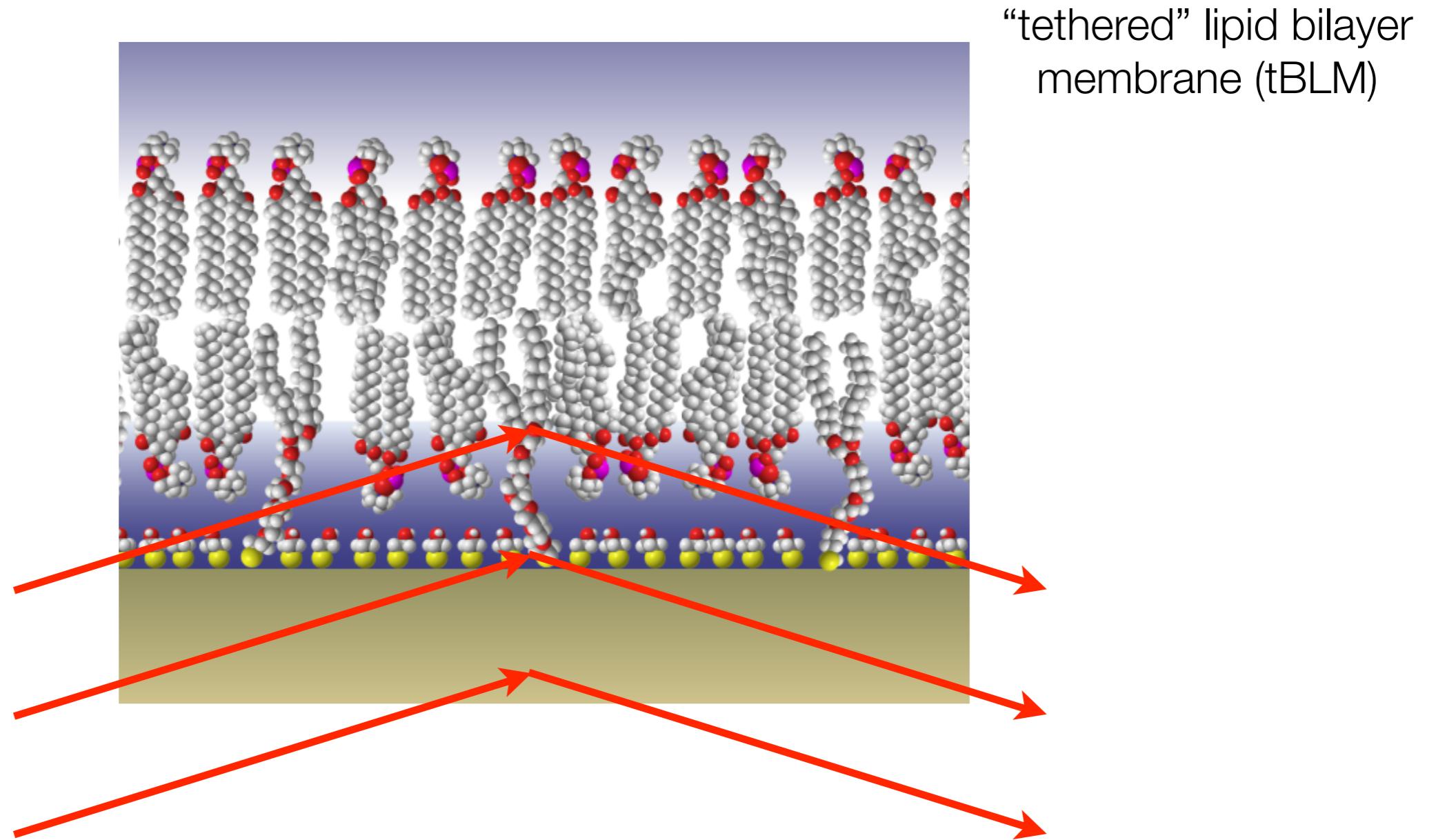
12-2013

Designing polymer-tethered membrane-nanoparticle composites

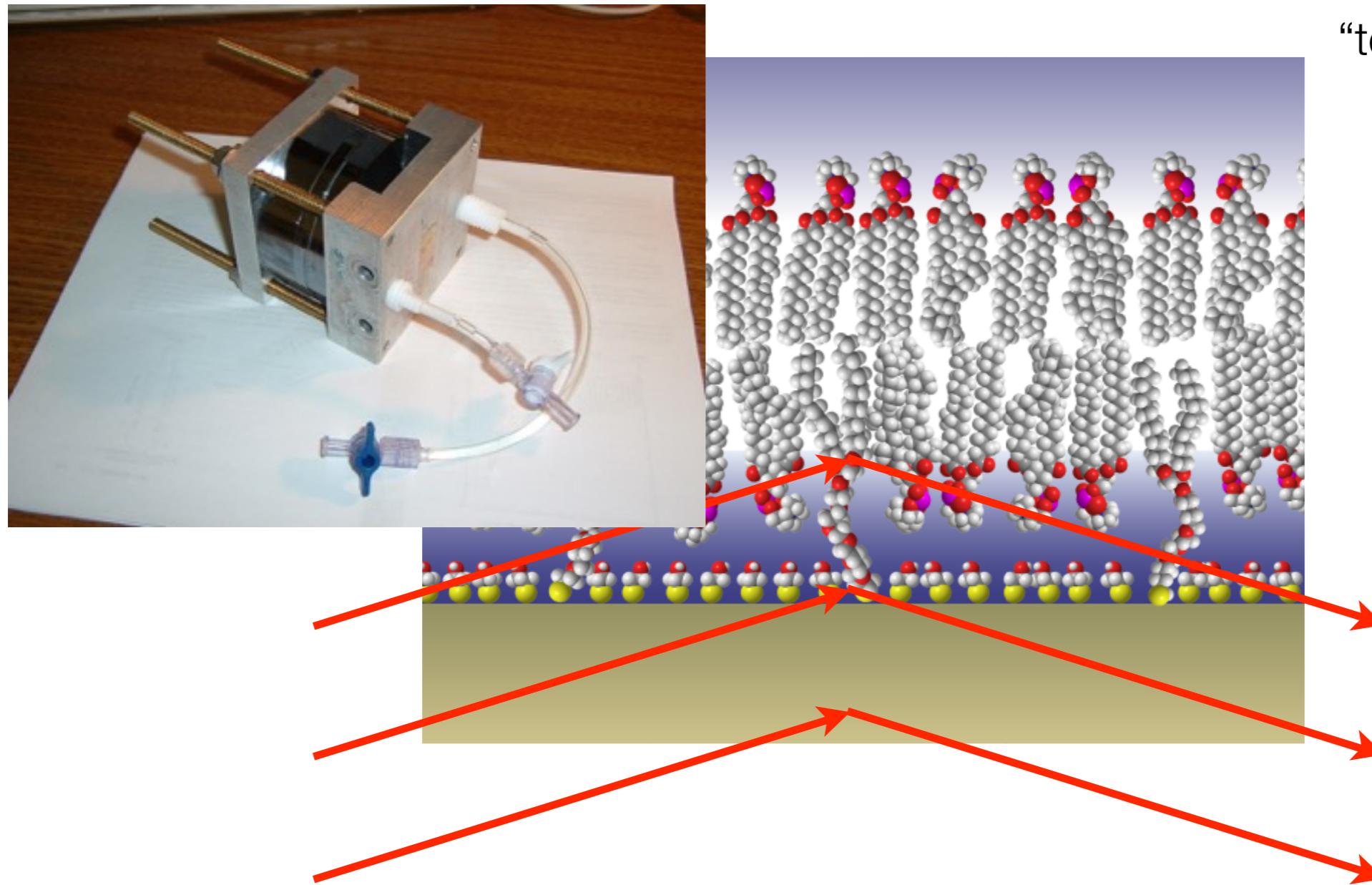
Mingyang Hu

Carnegie Mellon University, mingyang@cmu.edu

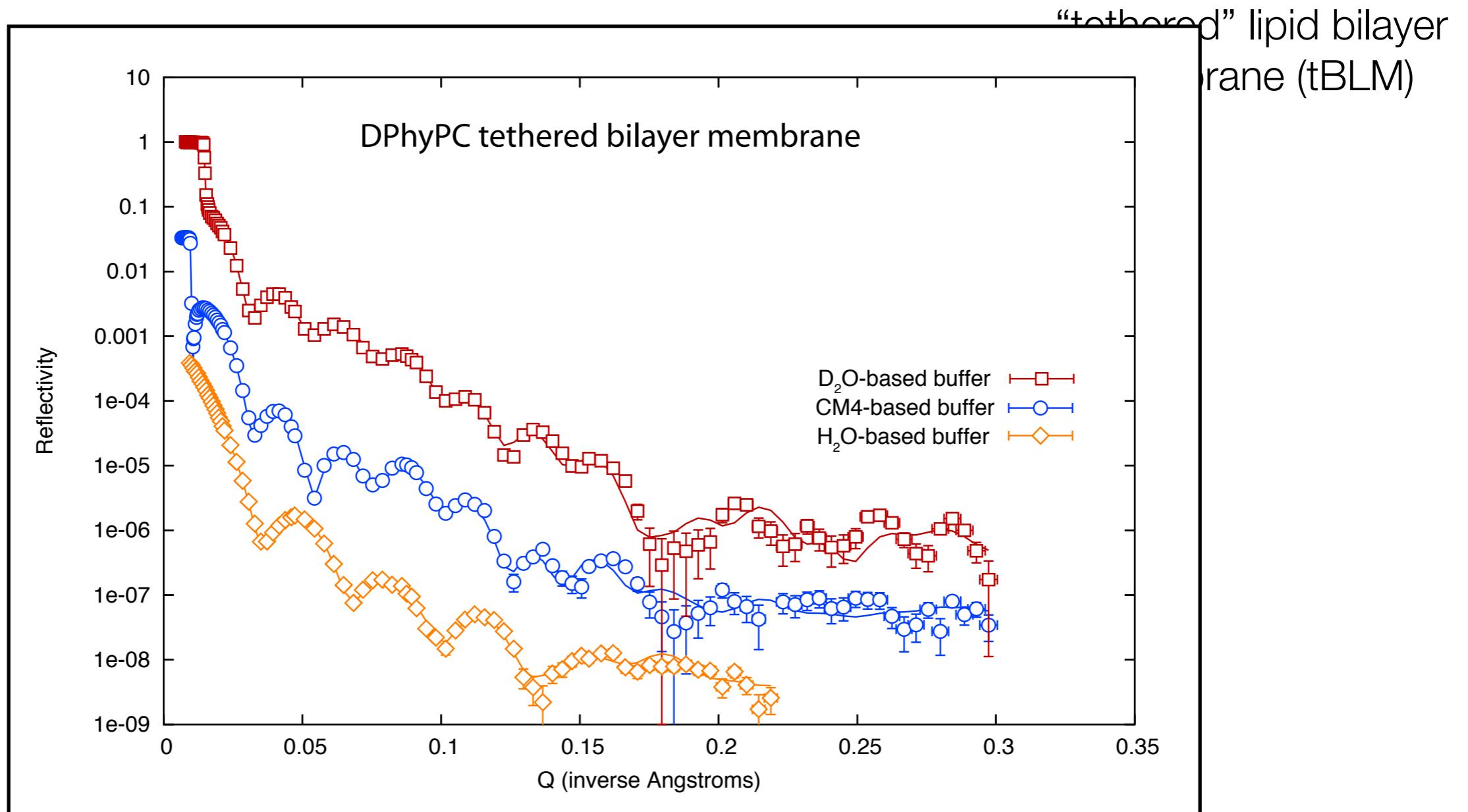
NR from surface-stabilized membranes (pre-2006)



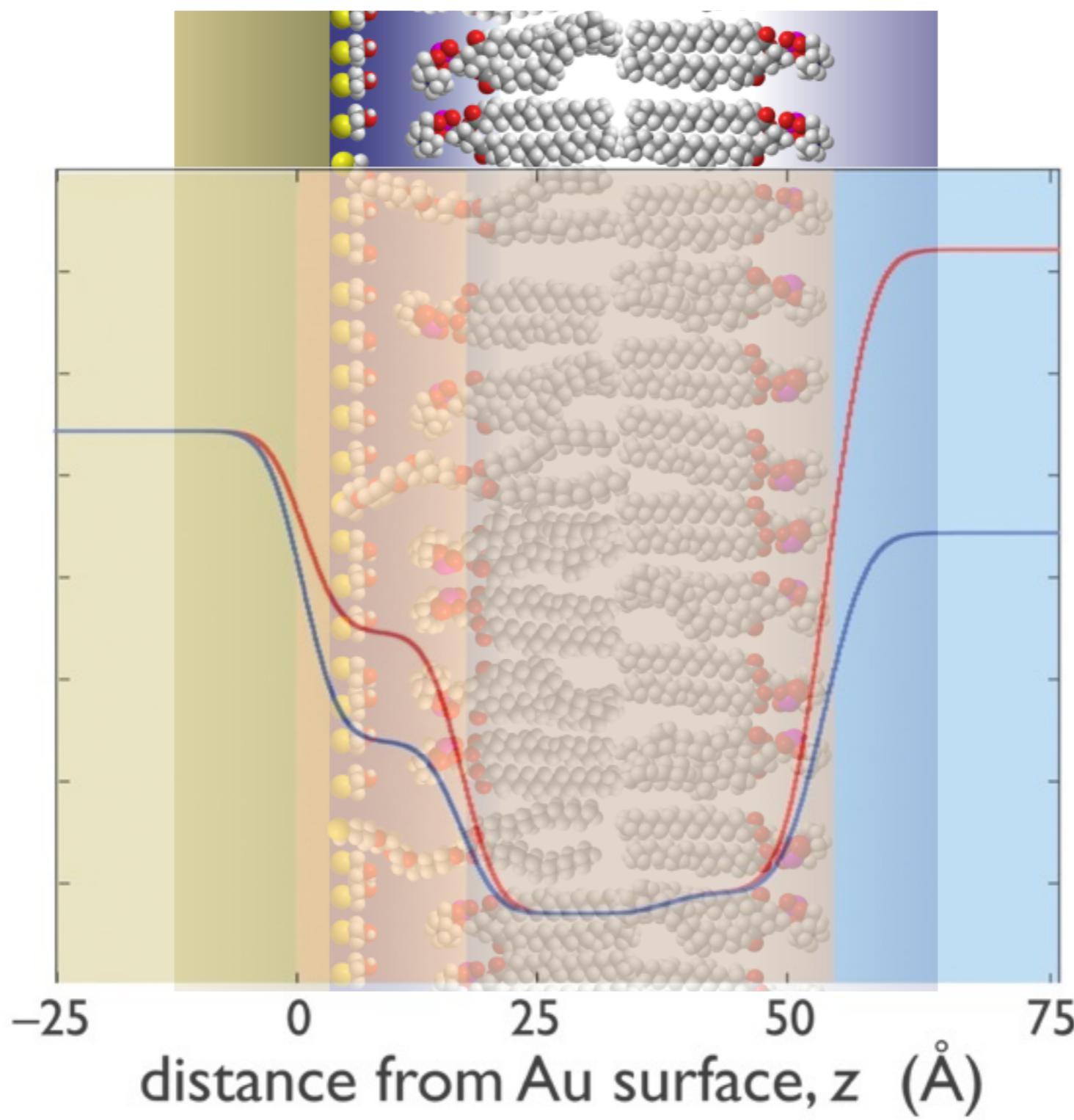
NR from surface-stabilized membranes (pre-2006)



NR from surface-stabilized membranes (pre-2006)

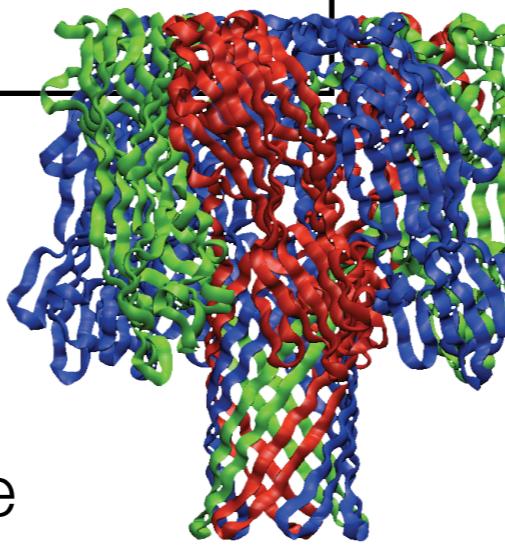
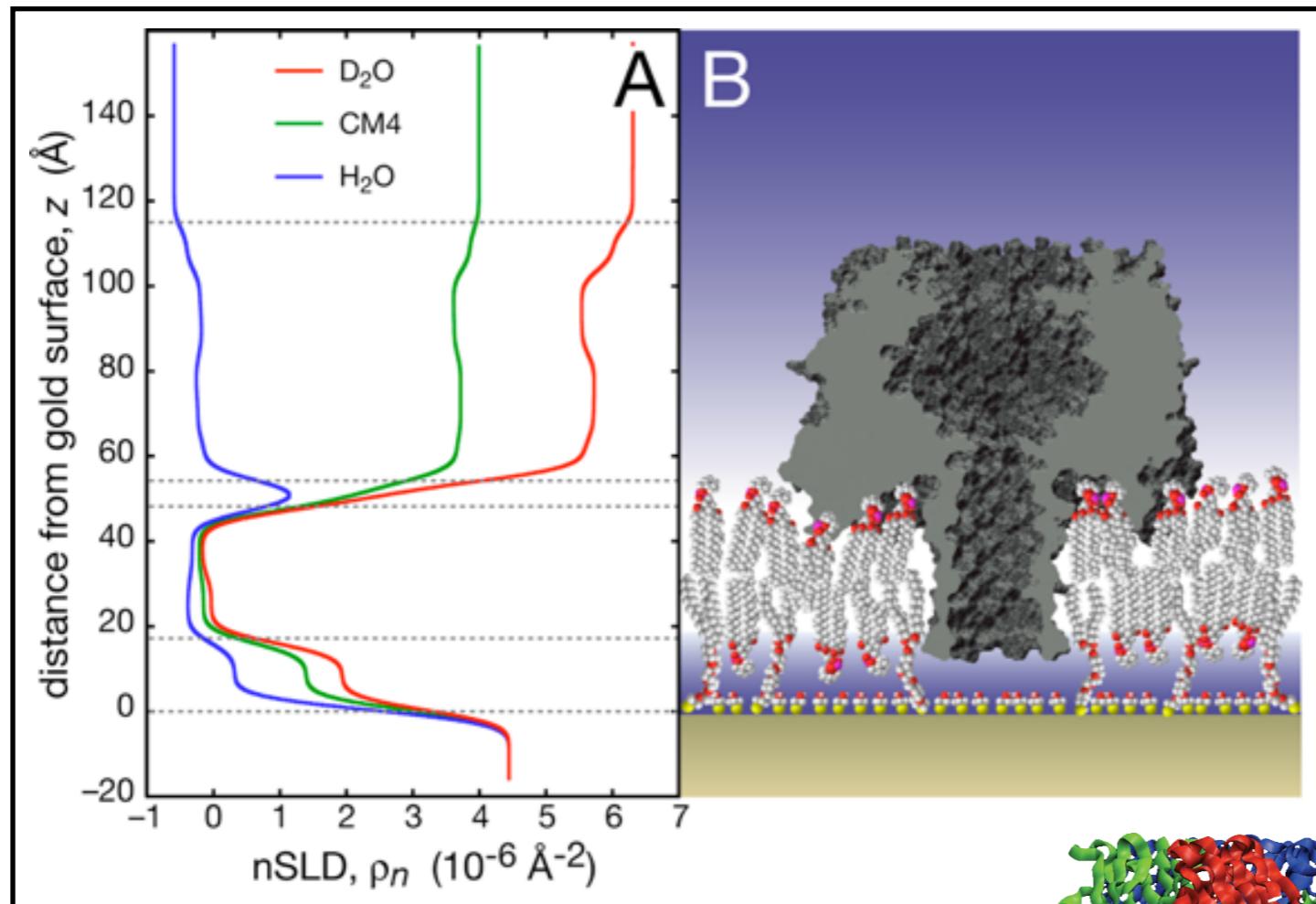


NR from surface-stabilized membranes (pre-2006)

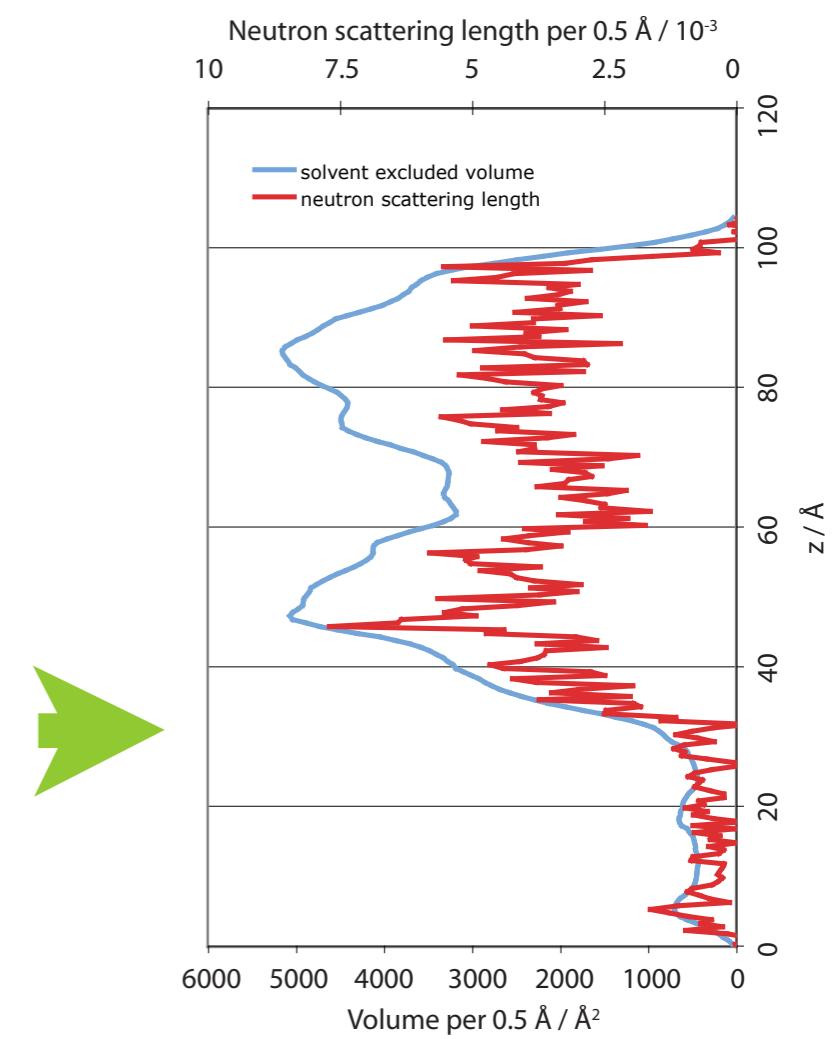


interpret nSLD
distribution *in terms of*
molecular structure

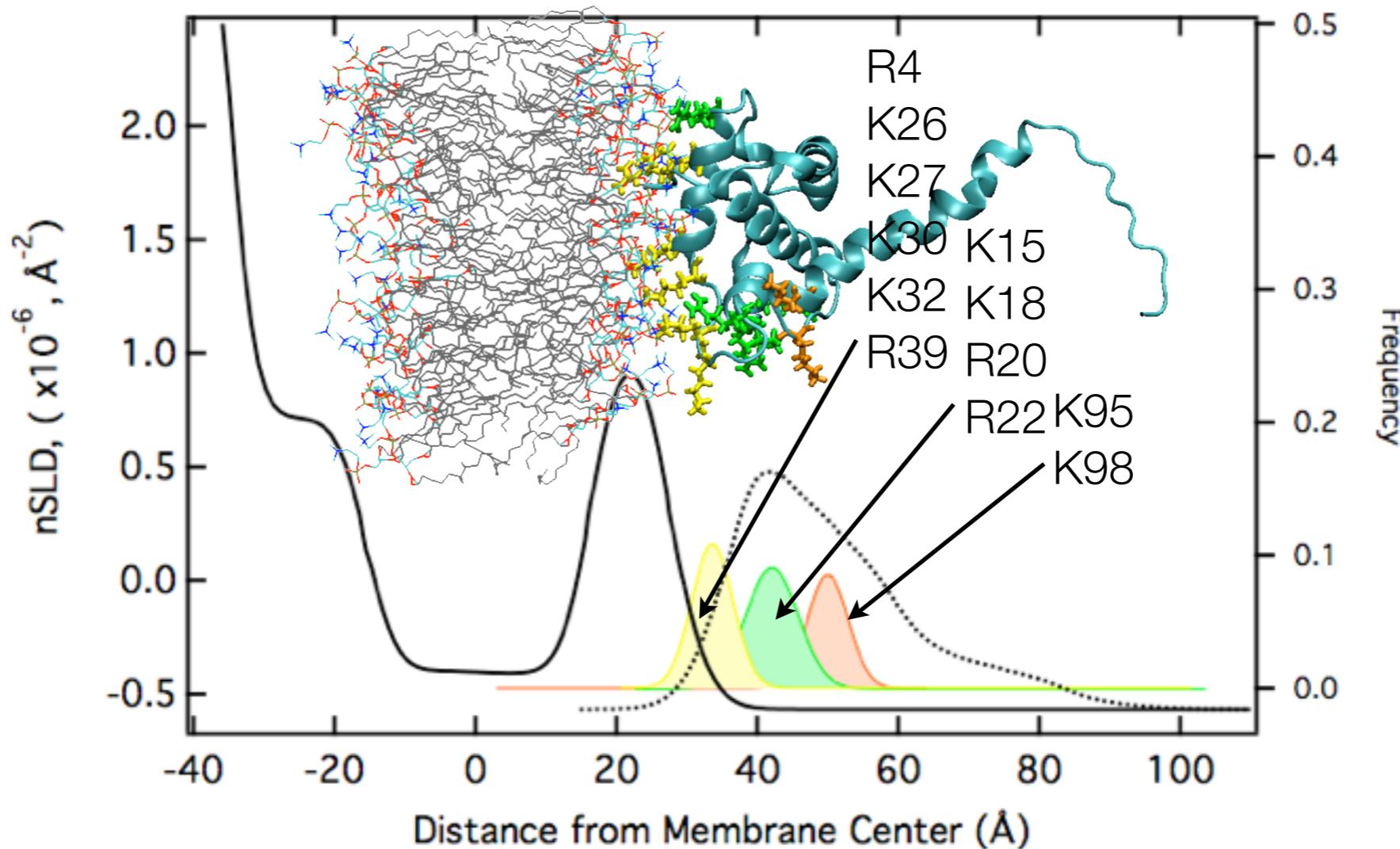
Overcoming the slab model, using high-resolution structures (2006)



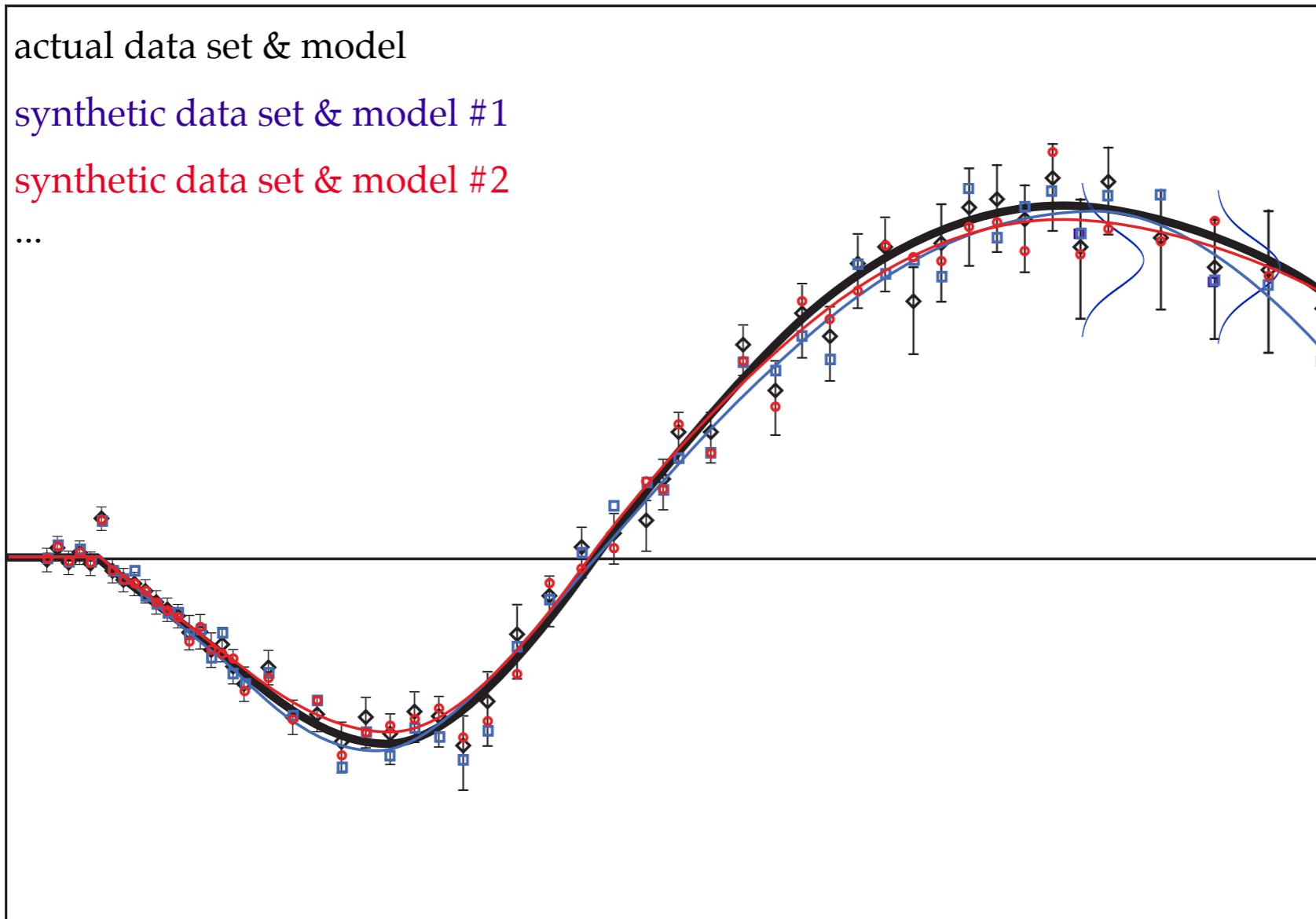
Requires microslicing of nSLD profile



Emerging Sub-resolution information



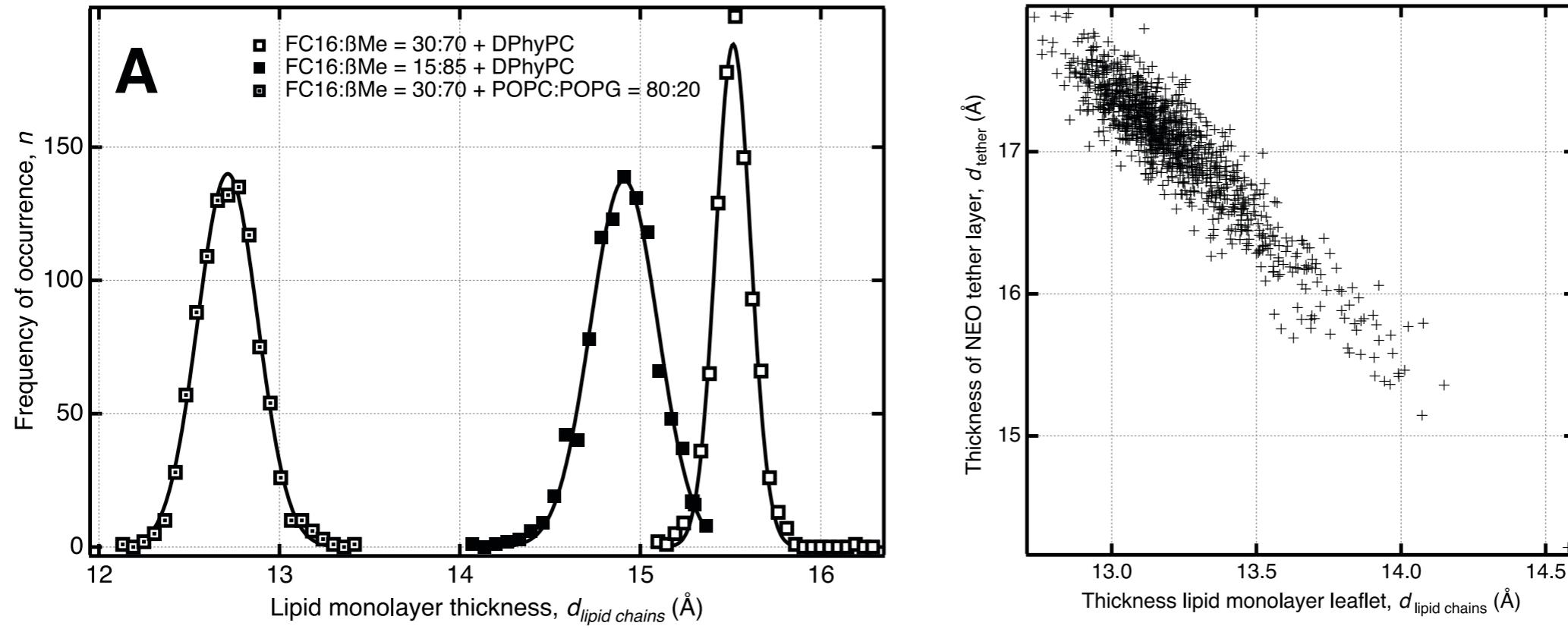
Reliable confidence intervals (2008)



Simulation and fit
of n=1000
reflectivity curves
as random normal
deviates from the
original data.

Requires fit automation

Reliable confidence intervals (2008)



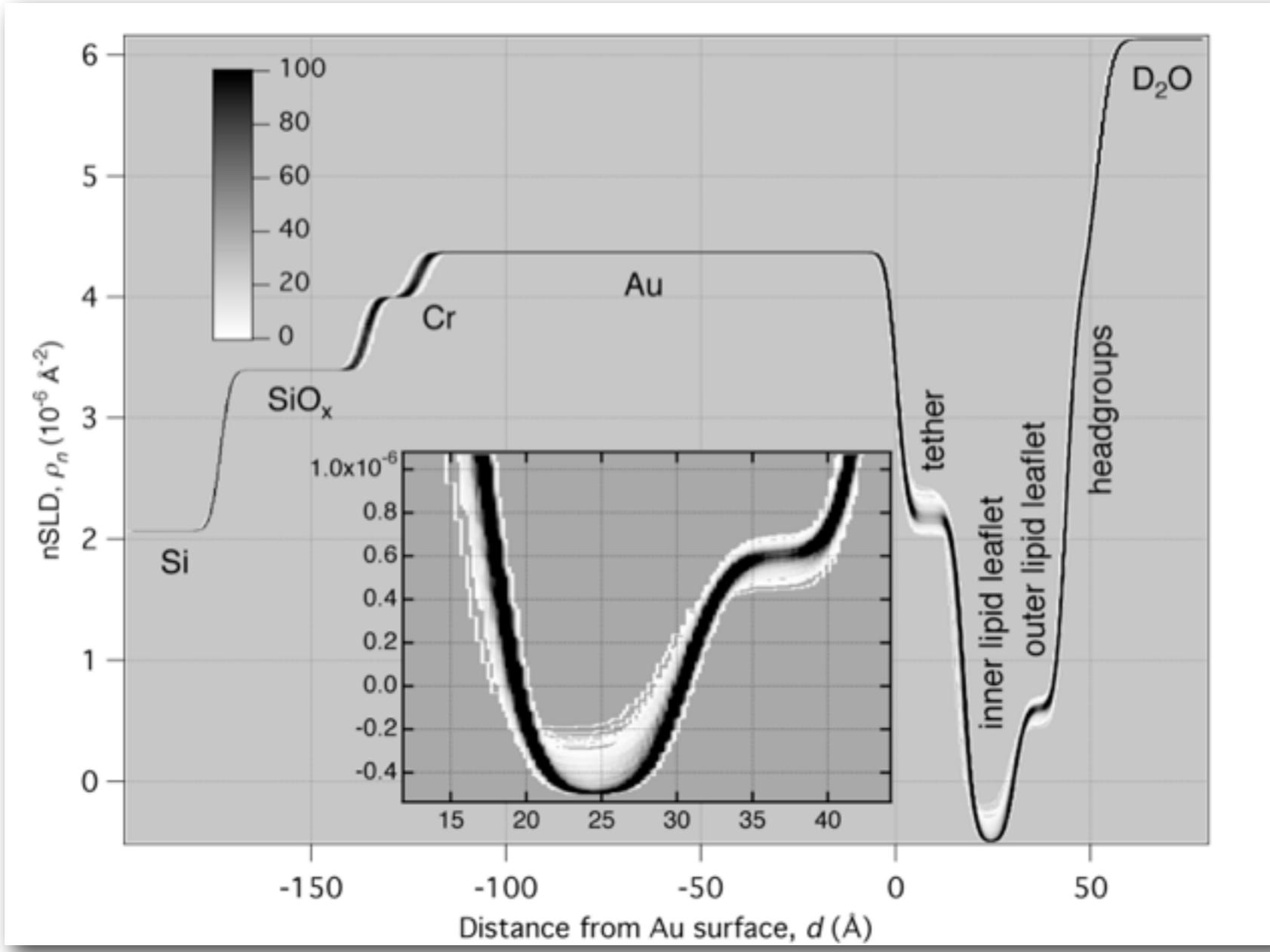
Monte Carlo Techniques

- Confidence intervals from parameter distributions
- Parameter correlations
- Model optimizations
- Paul Kienzle's MCMC

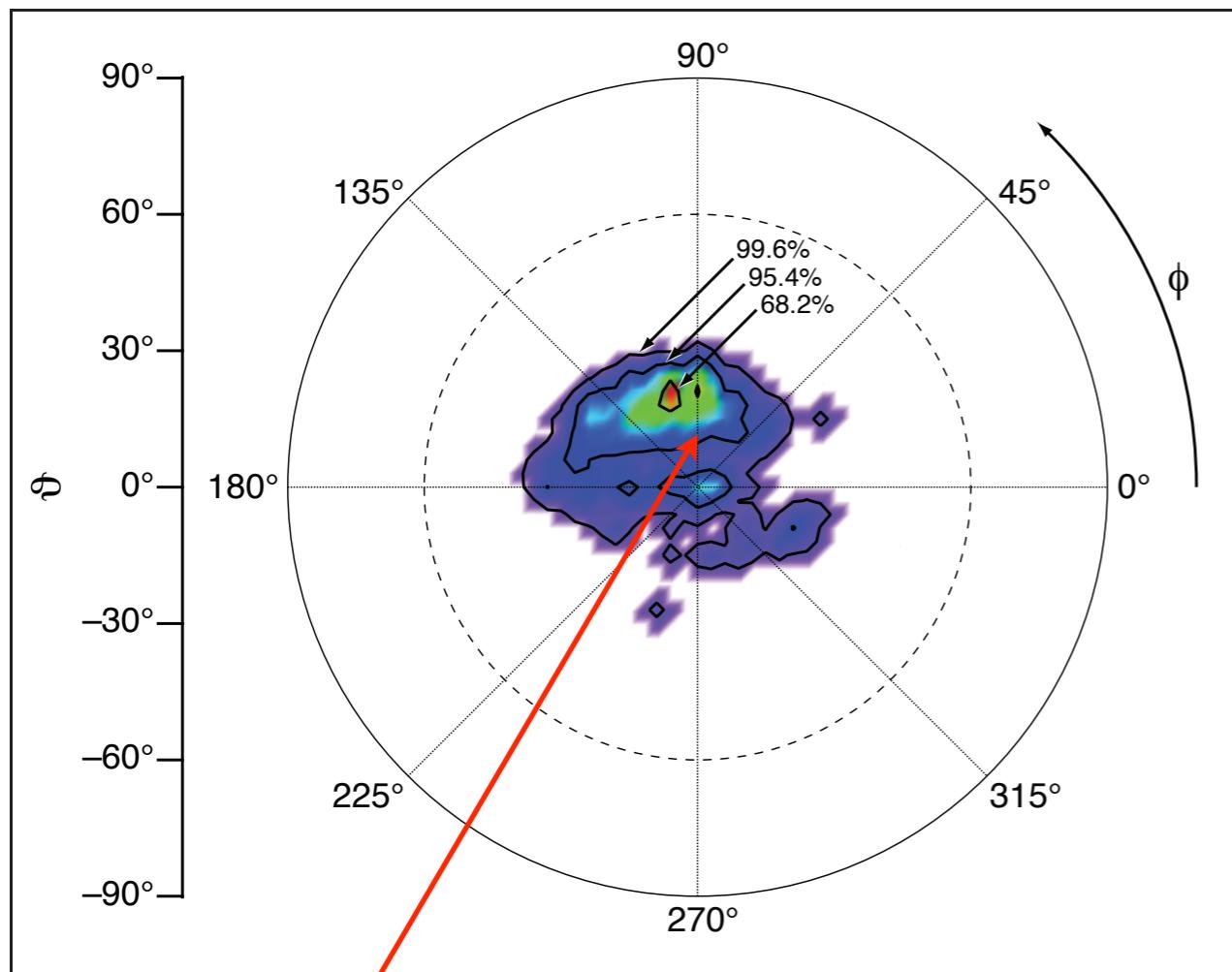
Heinrich, F. et al. Langmuir 25, 4219–4229 (2009).

Kirby, B. J. et al. Current Opinion in Colloid & Interface Science 17, 44–53 (2012).

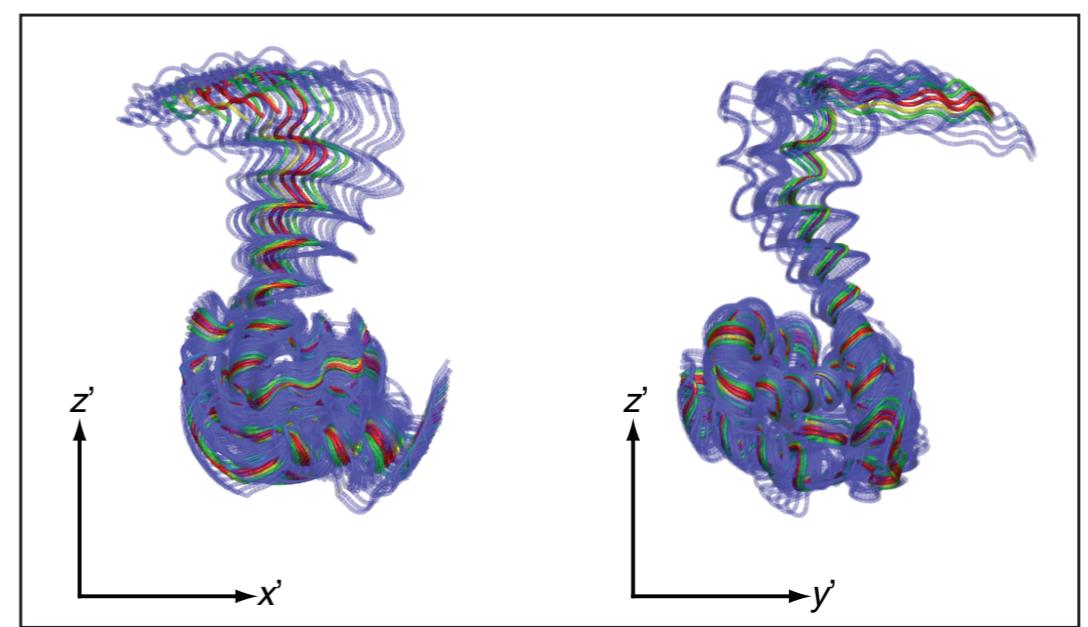
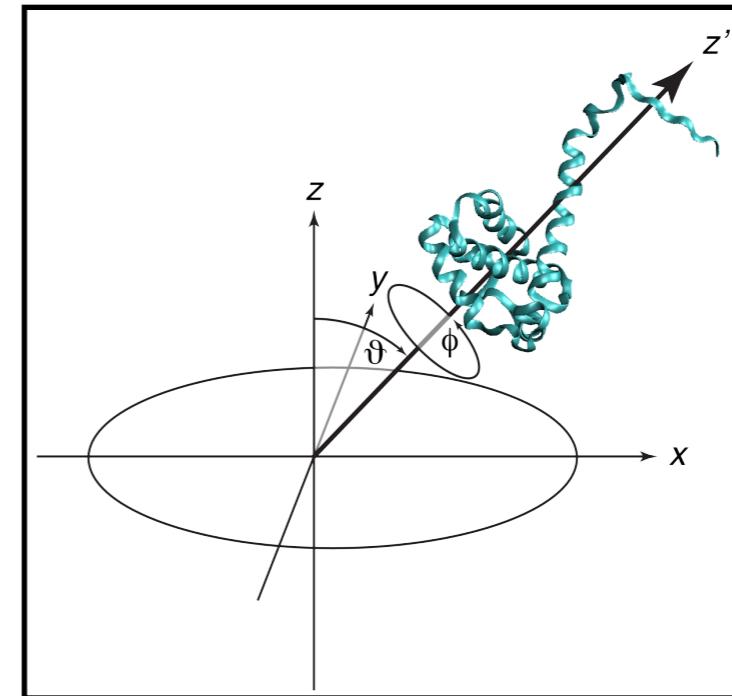
Uncertainties on nSLD profiles



Matrix membrane binding: Protein orientation on bilayer



orientation suggested
from crystal structure

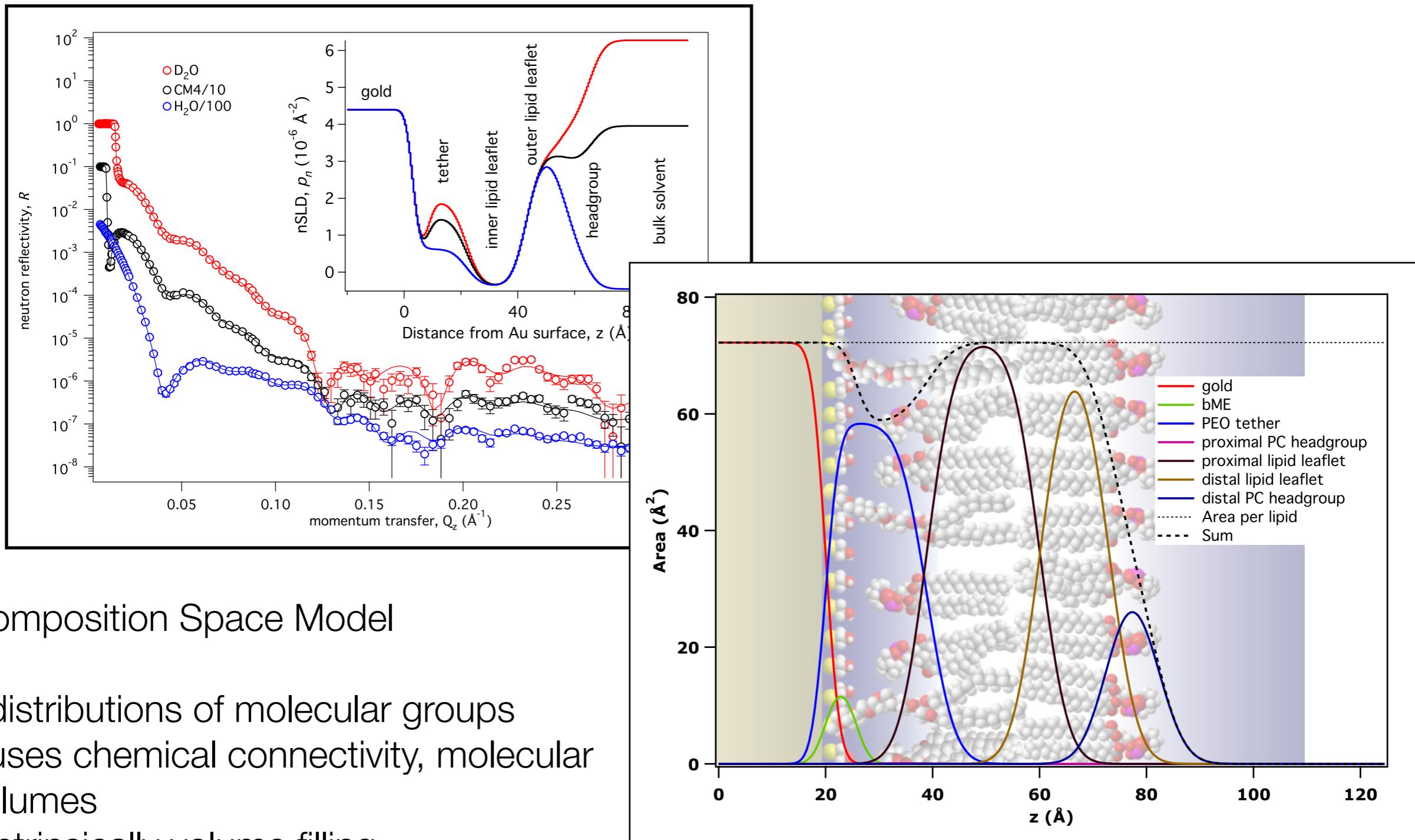


Impact

Reviewers are 99% happy with our data analysis.

- Monte Carlo Simulation Implementation in Motofit
- Implementation of MCMC in Refl1D

Molecular modeling (2009)



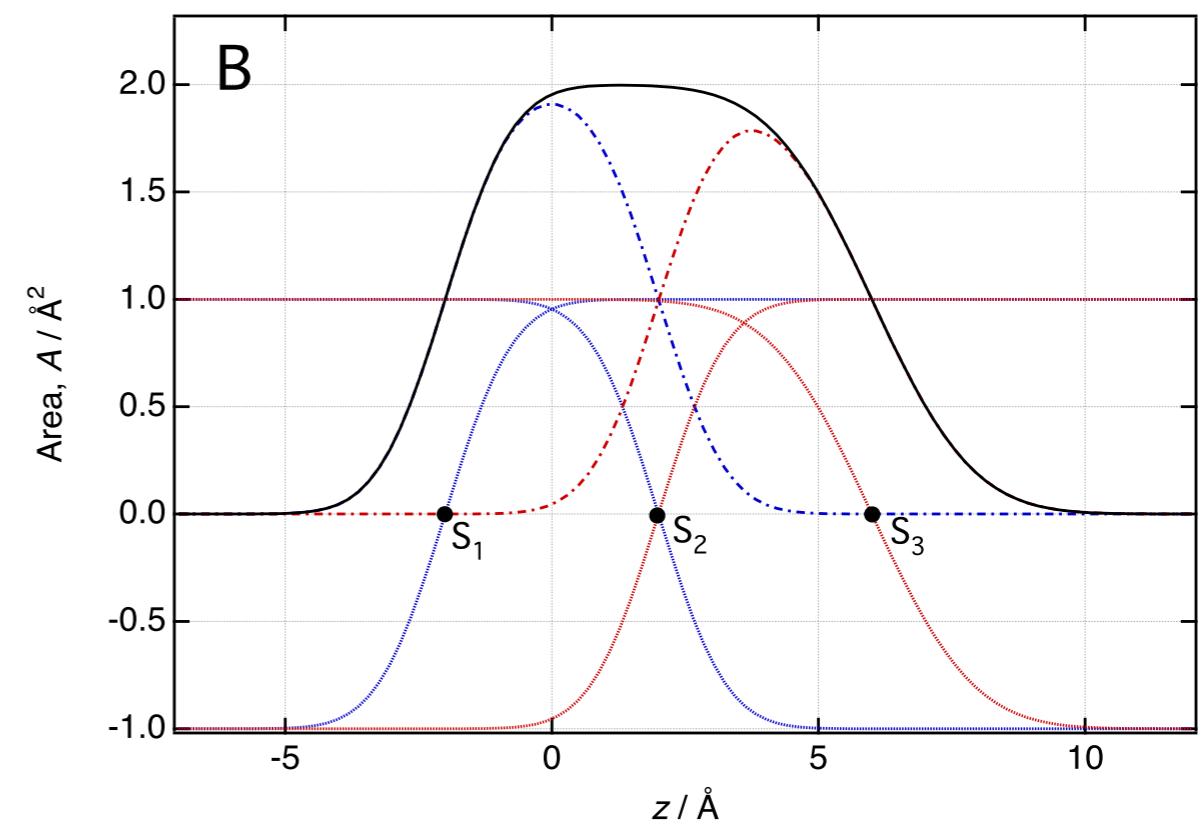
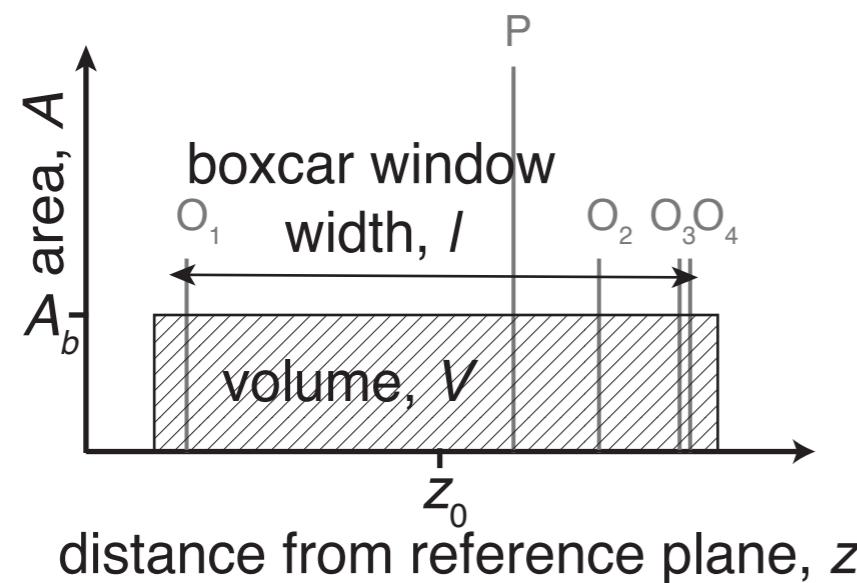
Composition Space Model

- distributions of molecular groups
- uses chemical connectivity, molecular volumes
- intrinsically volume filling

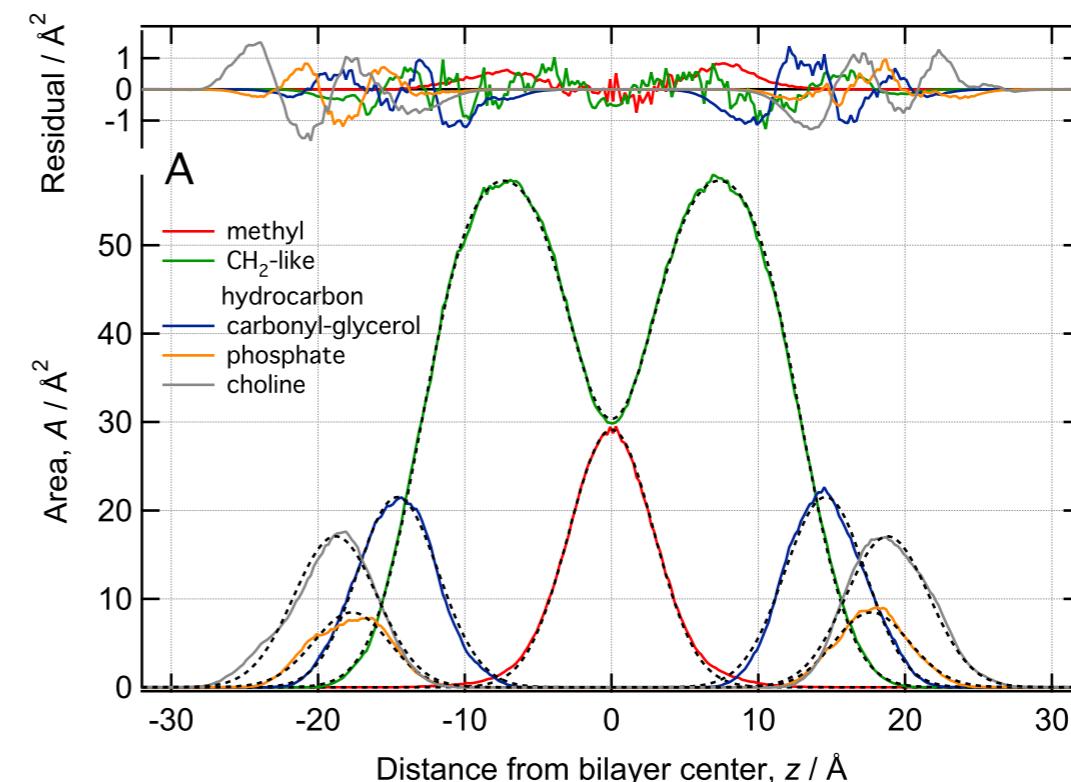
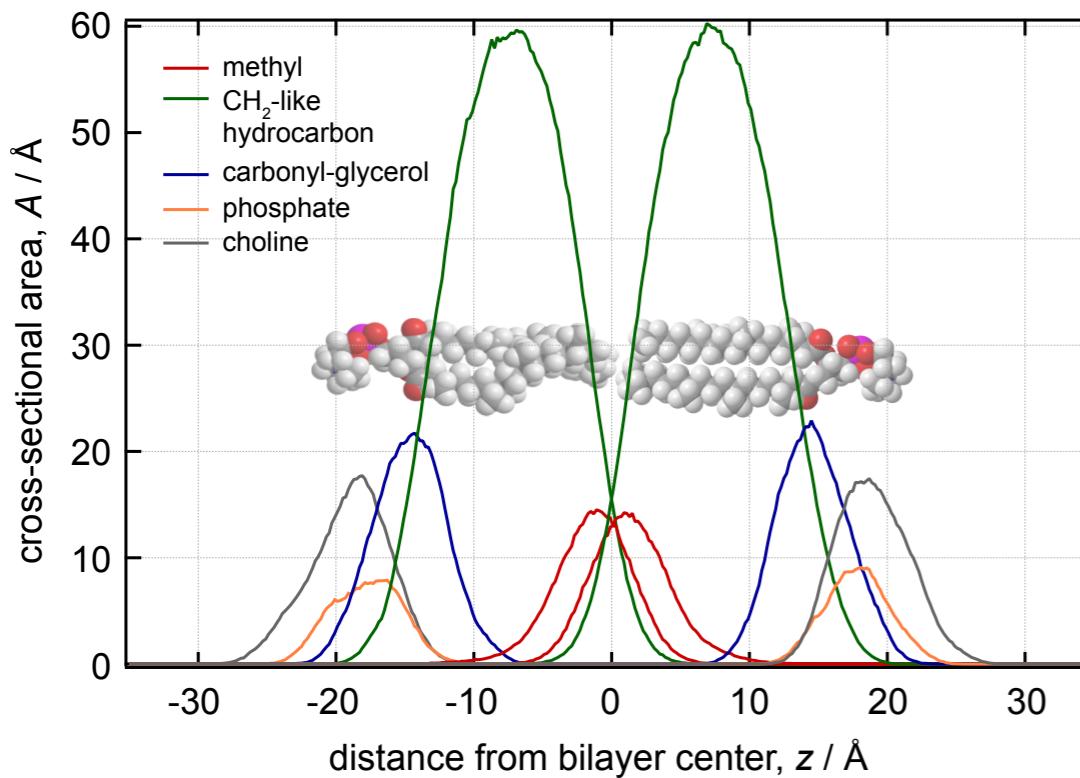
Overcoming Gaussian functions

We conclude from the above discussion that properly formed multilamellar fluid bilayers consist of a highly ordered lattice of thermally disordered unit cells, that the resolution is limited only by this unit-cell disorder, and that an appropriate representation of any multi-atom submolecular group is as a **Gaussian function**. The

Wiener, M. C., & White, S. H. (1991). Fluid bilayer structure determination by the combined use of x-ray and neutron diffraction. I. Fluid bilayer models and the limits of resolution. *Biophysical Journal*, 59(1), 162–173. doi:10.1016/S0006-3495(91)82208-1



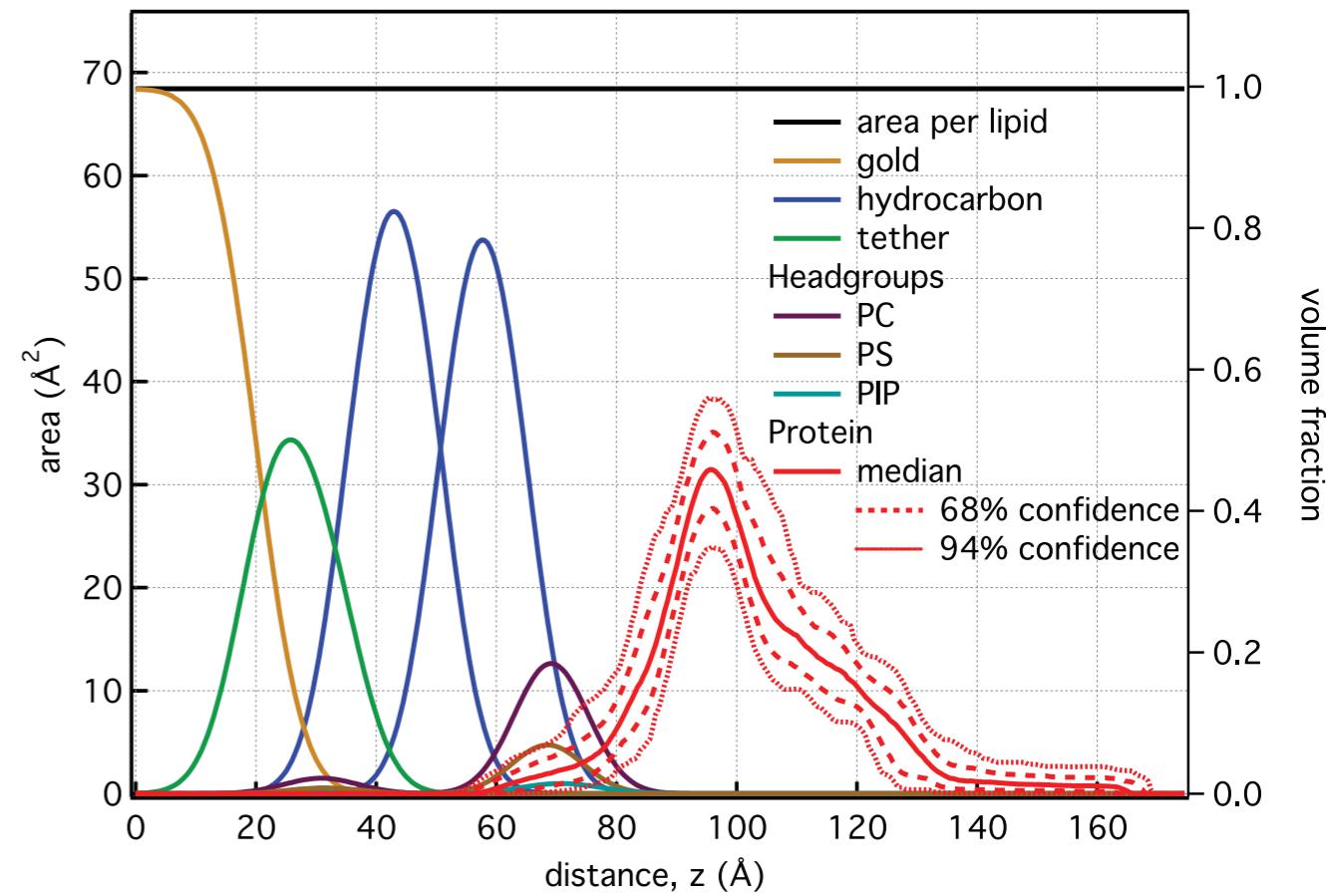
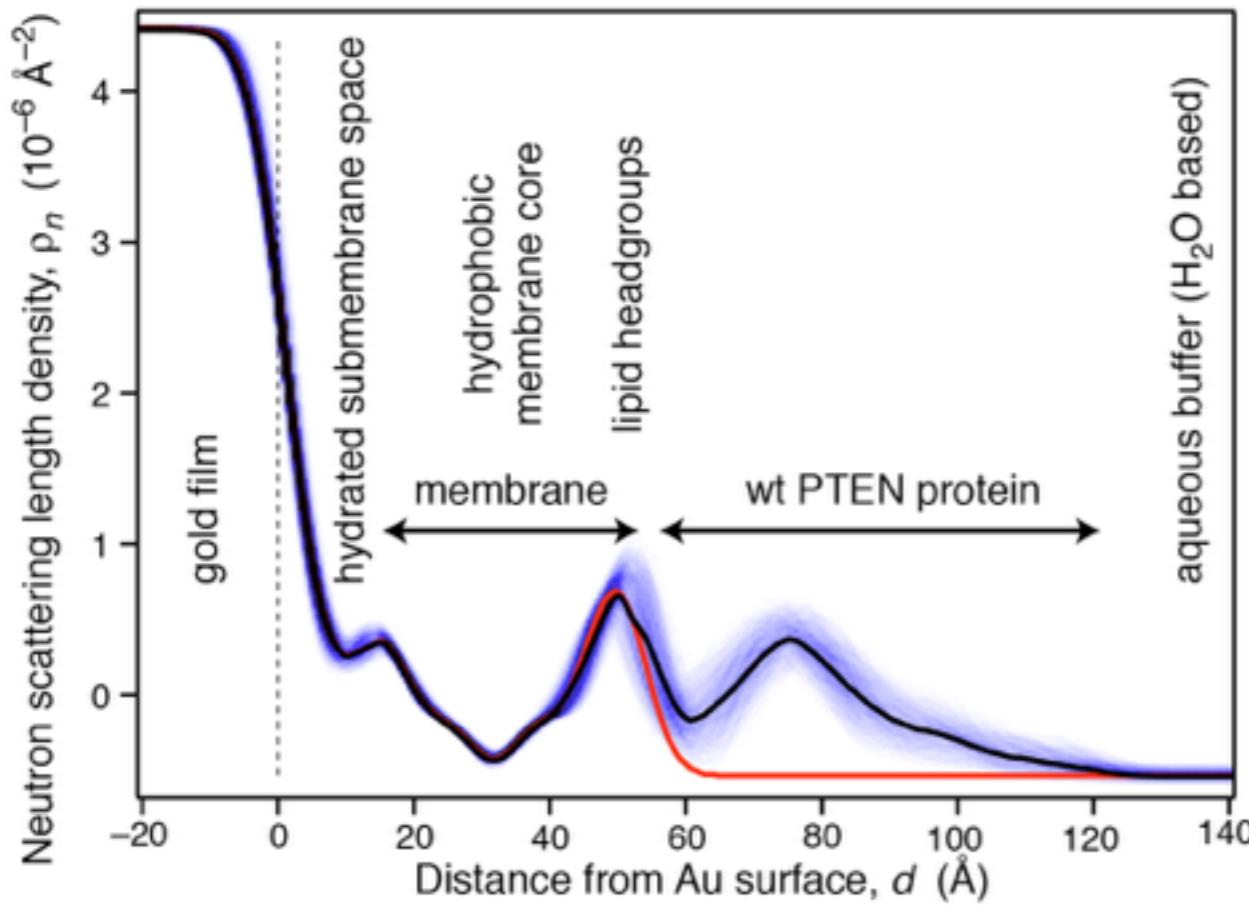
Continuous space model vs. MD simulation



Composition Space Model

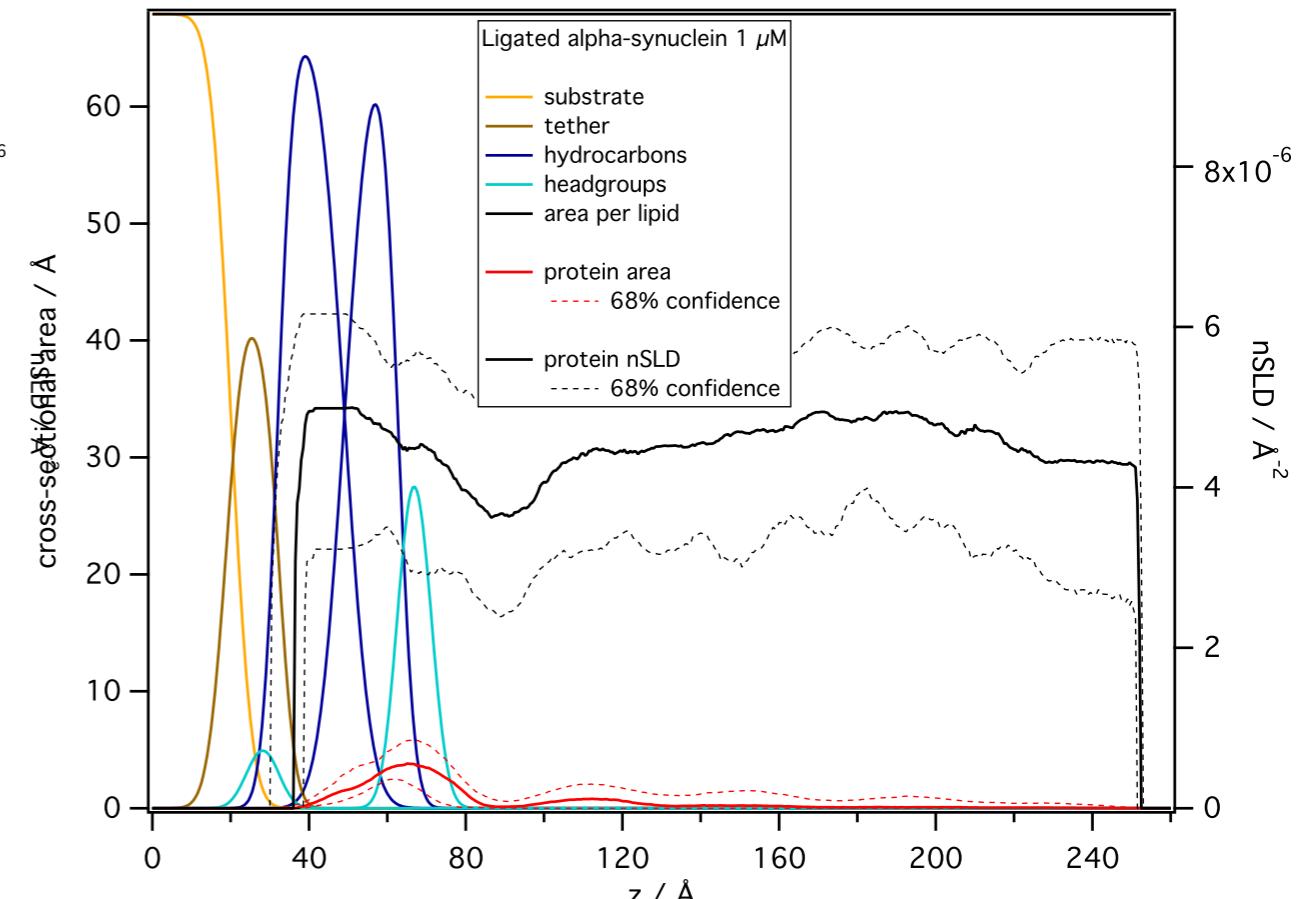
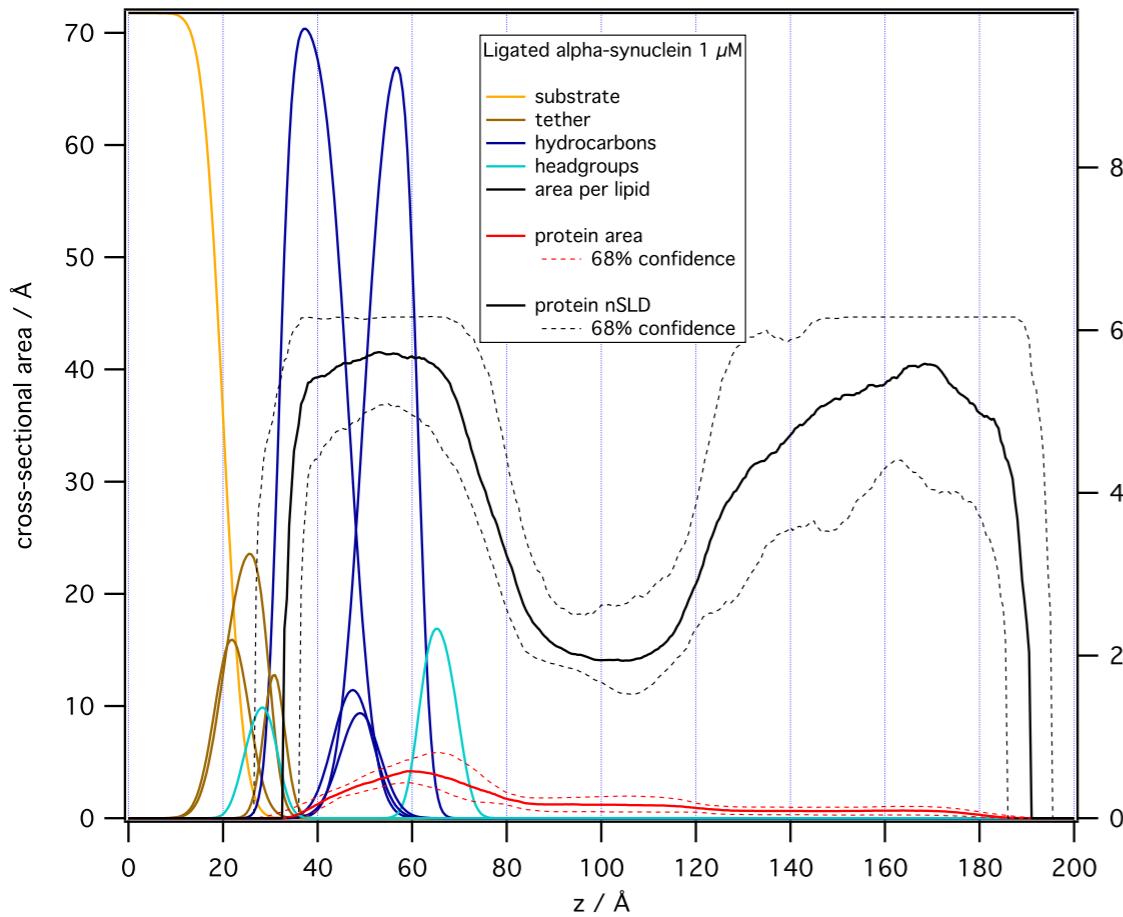
- exemplary tested against realistic sub-molecular distributions obtained from MD simulations

Molecular modeling of protein envelopes (2011)

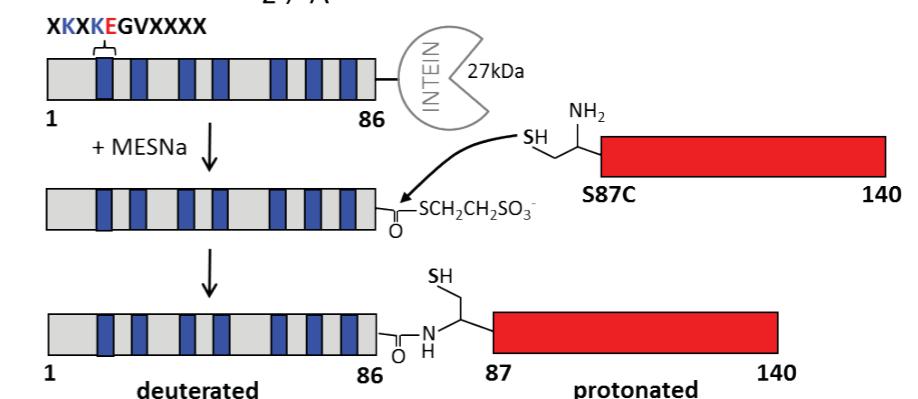


- Hermite Spline free-form model
- Monte Carlo uncertainty analysis
- proton exchange in headgroups and protein
- hydrocarbon replacement by protein affects headgroups

Modeling of Molecular Complexes (2013)

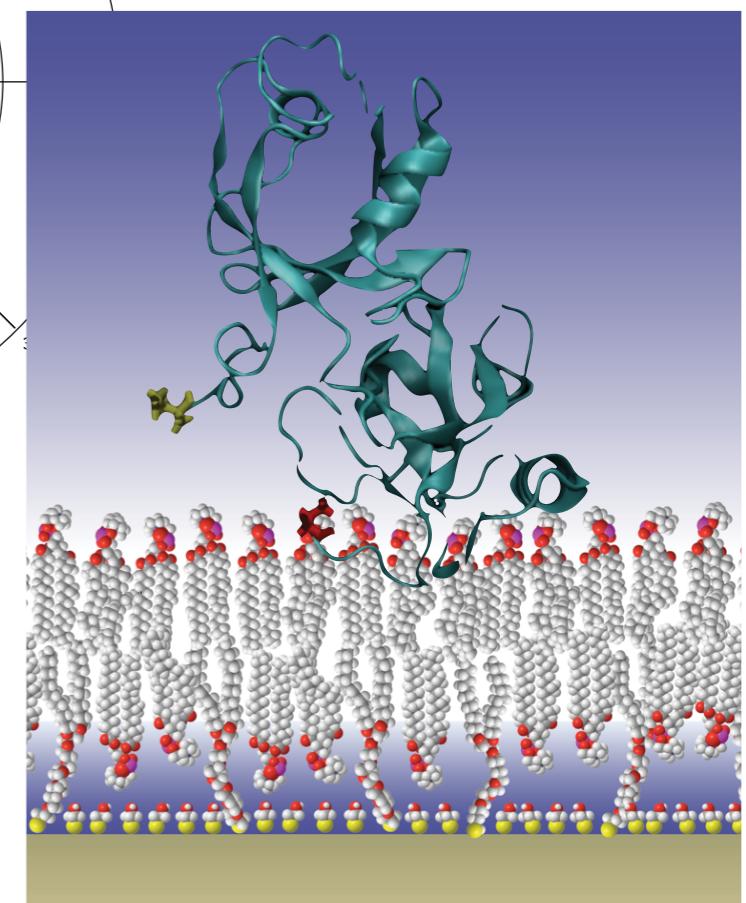
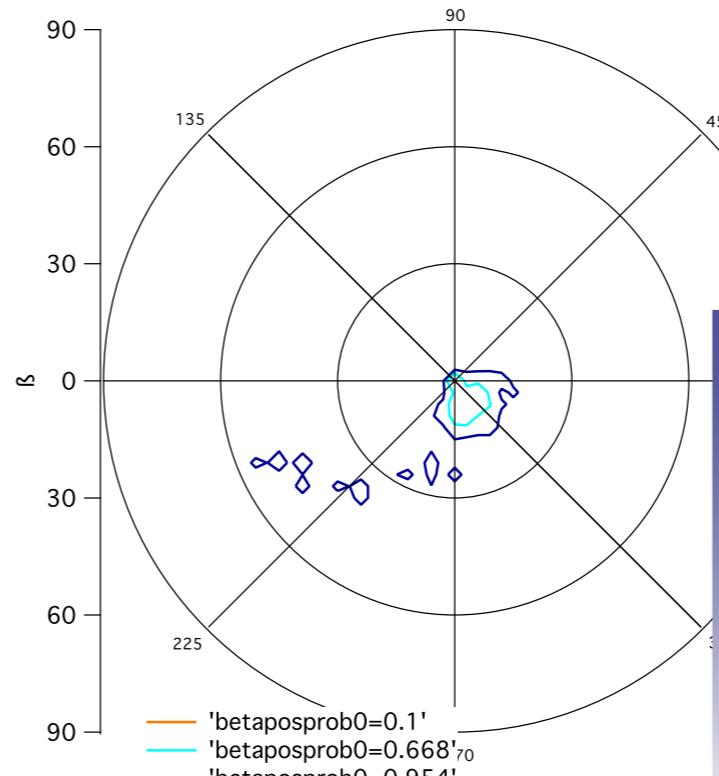
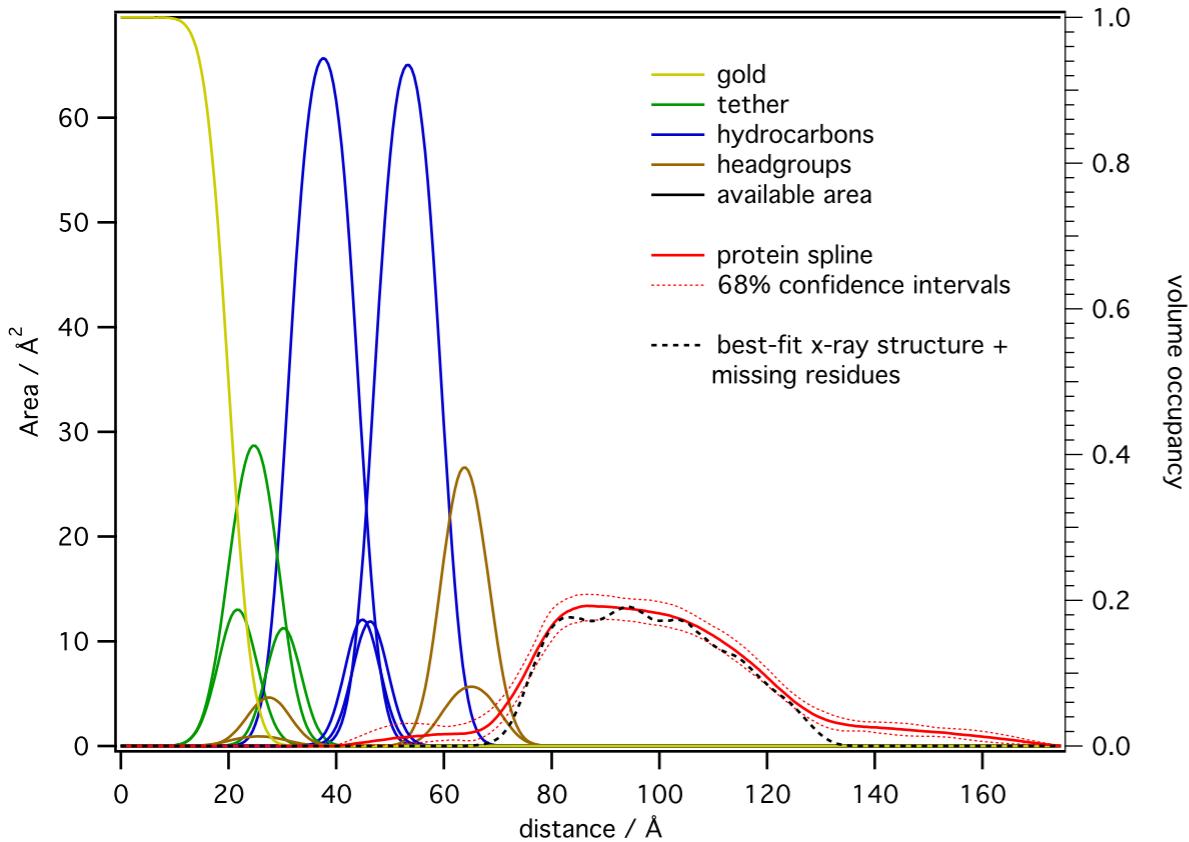


- Hermite Spline for volume occupancy and nSLD
- Monte Carlo uncertainty analysis
- proton exchange, hydrocarbon replacement



☞ allows for free-form modeling of entire interfaces (Dave H.)

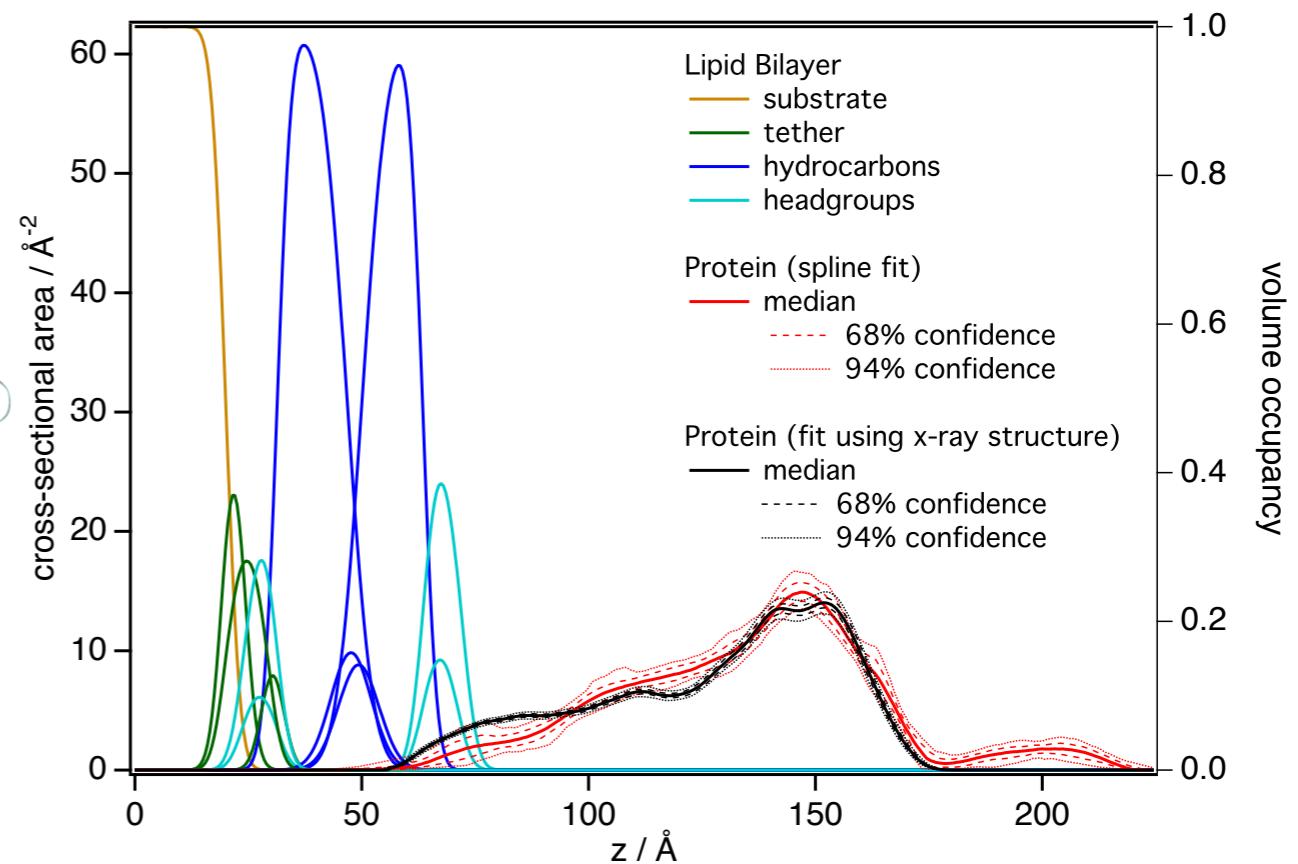
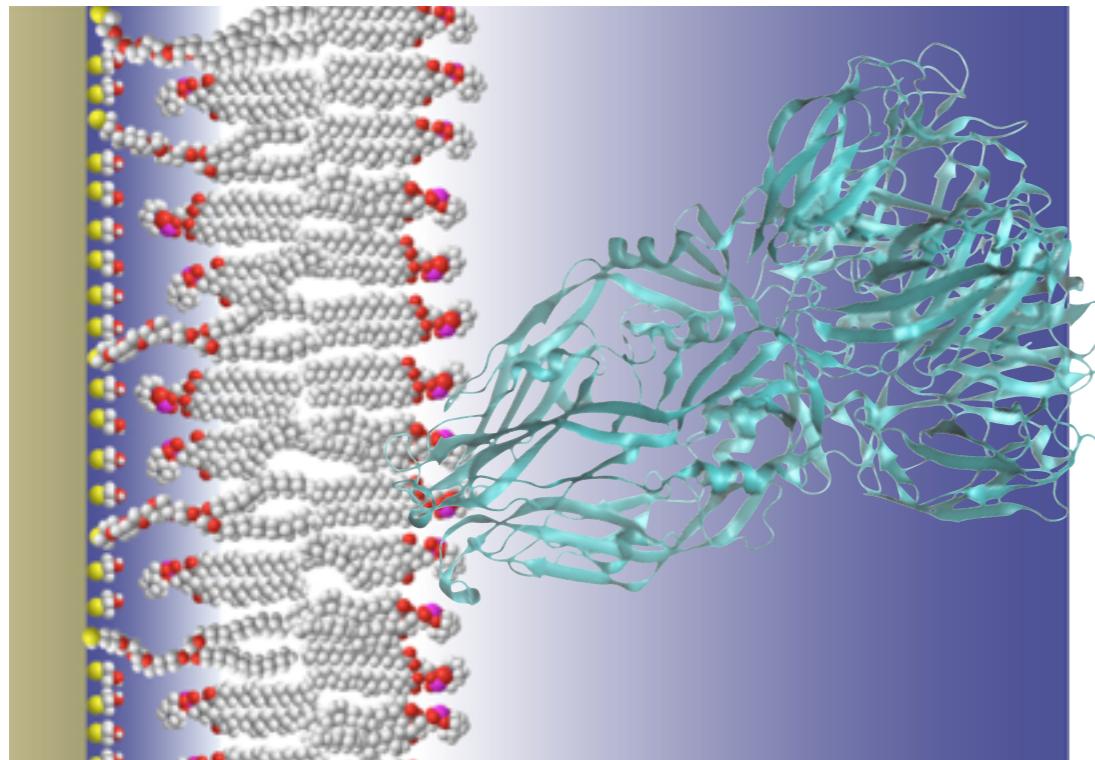
Example: GRASP 55



- in collaboration with Adam Linstedt, Hirsh Nanda, and Mathias Lösche, CMU
- main objective: differentiating between different structural models for GRASP55 self-dimerization to achieve membrane tethering in Golgi membrane stacks

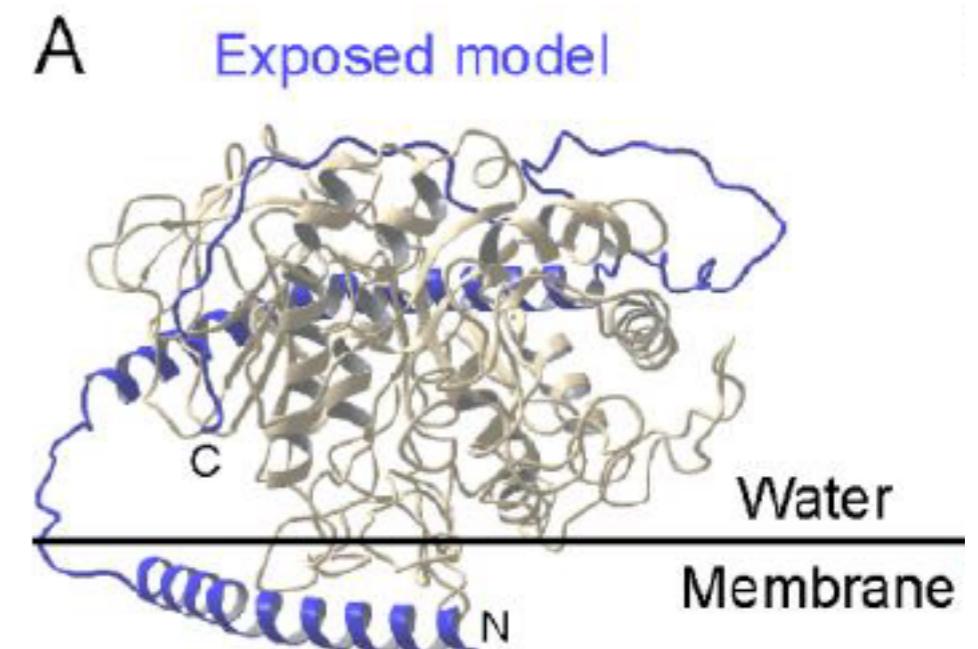
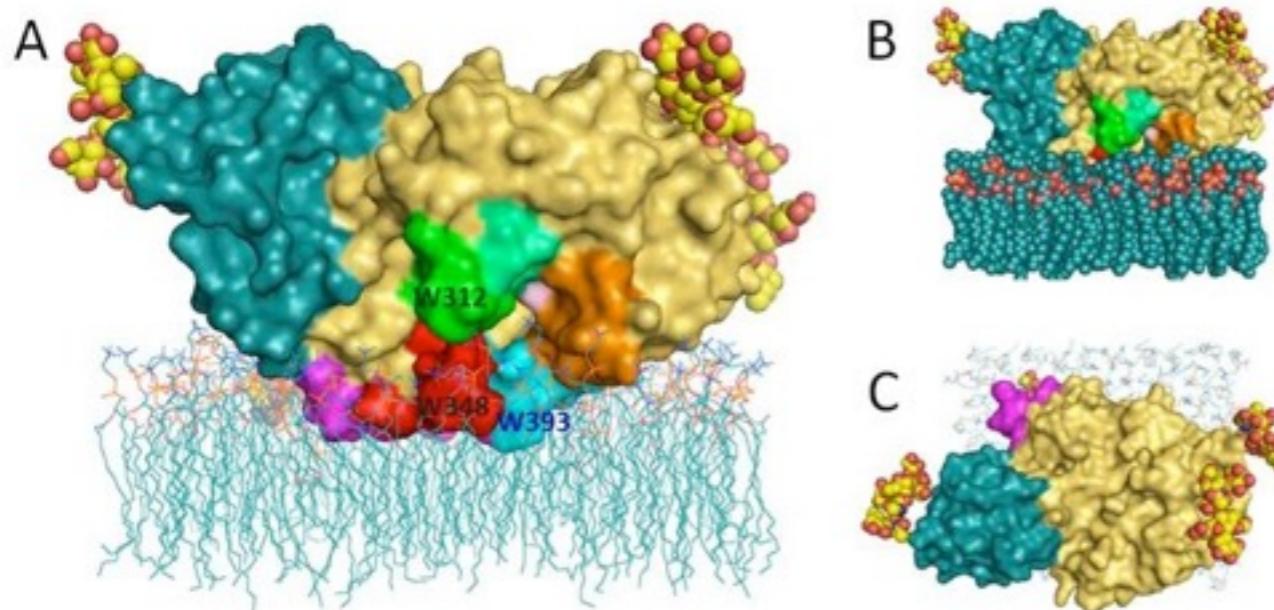
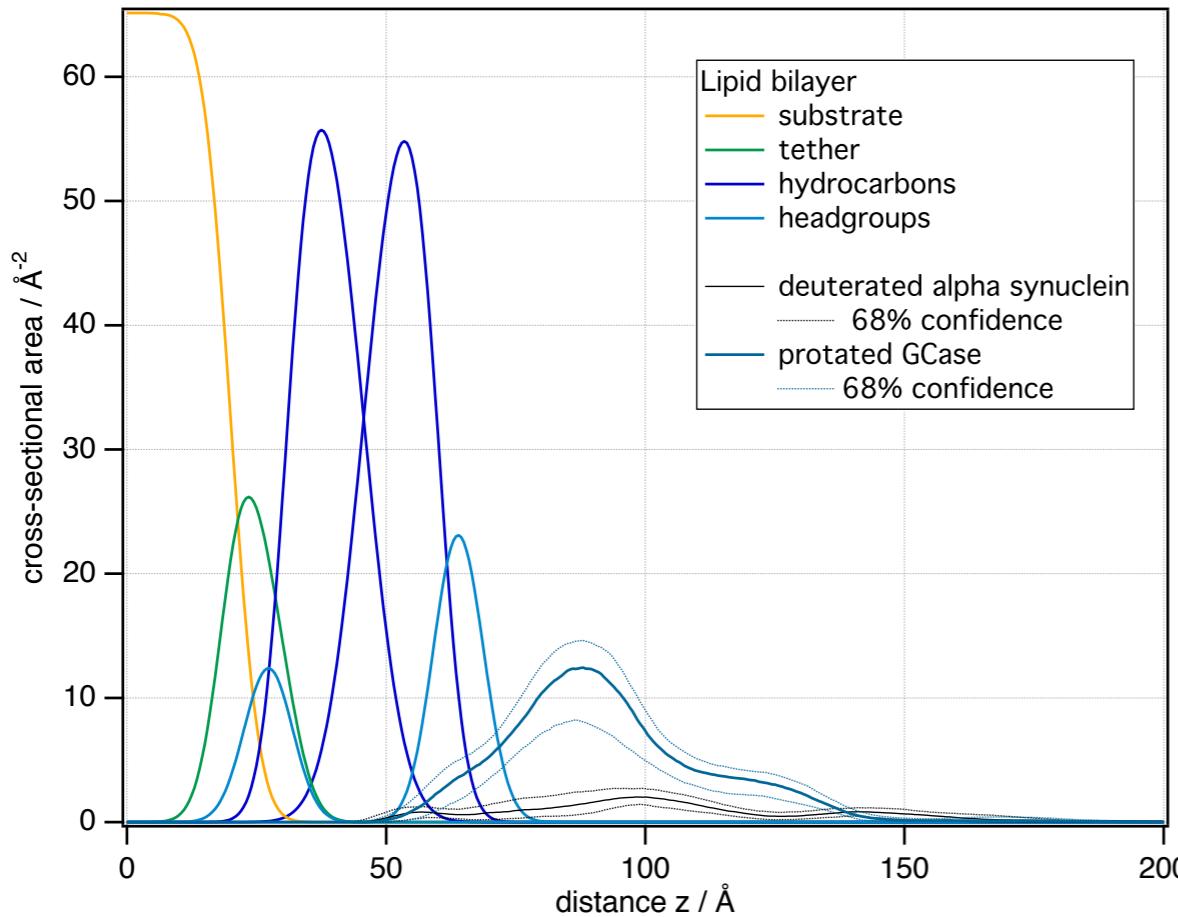
Frank Heinrich, Hirsh Nanda, Haw-Zan Goh, Collin Bachert, Mathias Lösche, and Adam D. Linstedt, Myristoylation Restricts Orientation of the GRASP domain on Membranes and is Critical to Promote Membrane Tethering. *Journal of Biological Chemistry* 289:9683 (2014), DOI:10.1074/jbc.M113.543561

Dengue envelope protein



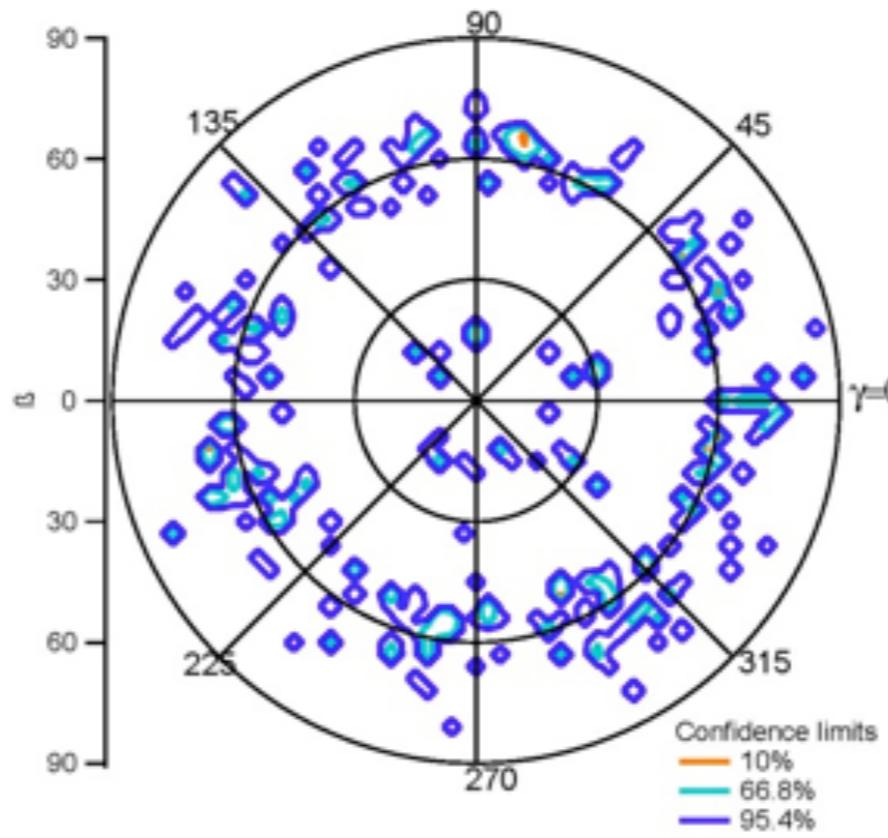
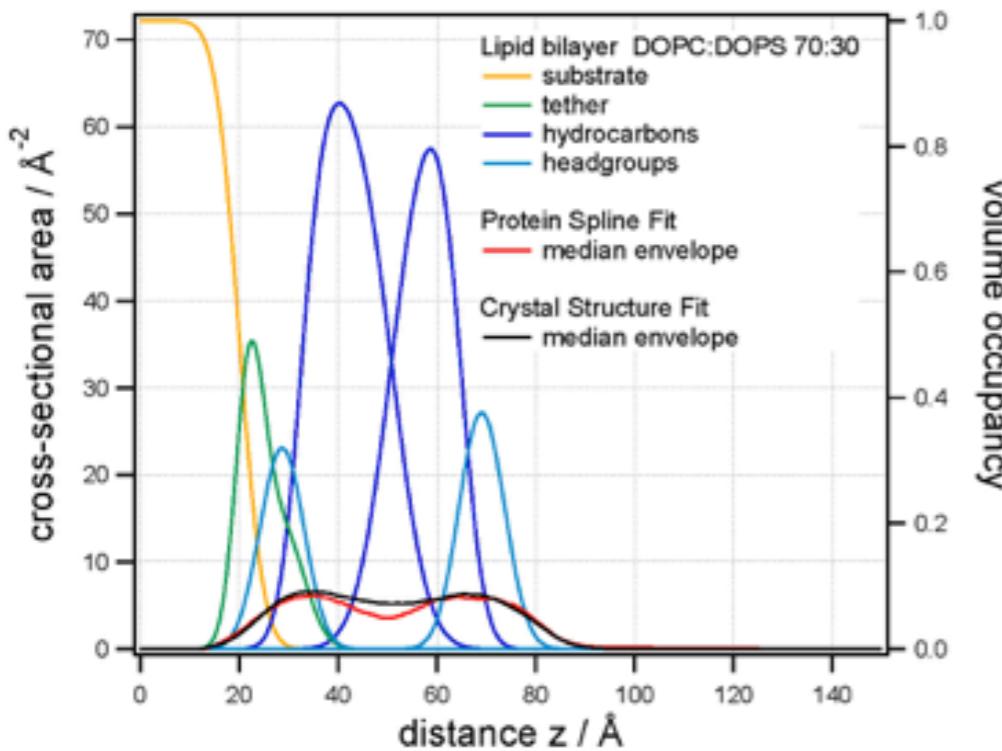
- in collaboration with Mike Kent,
Bulent Akgun, Sandia National
Laboratories
- main objective: insertion depth of the
protein into the bilayer

GCase / syn complex

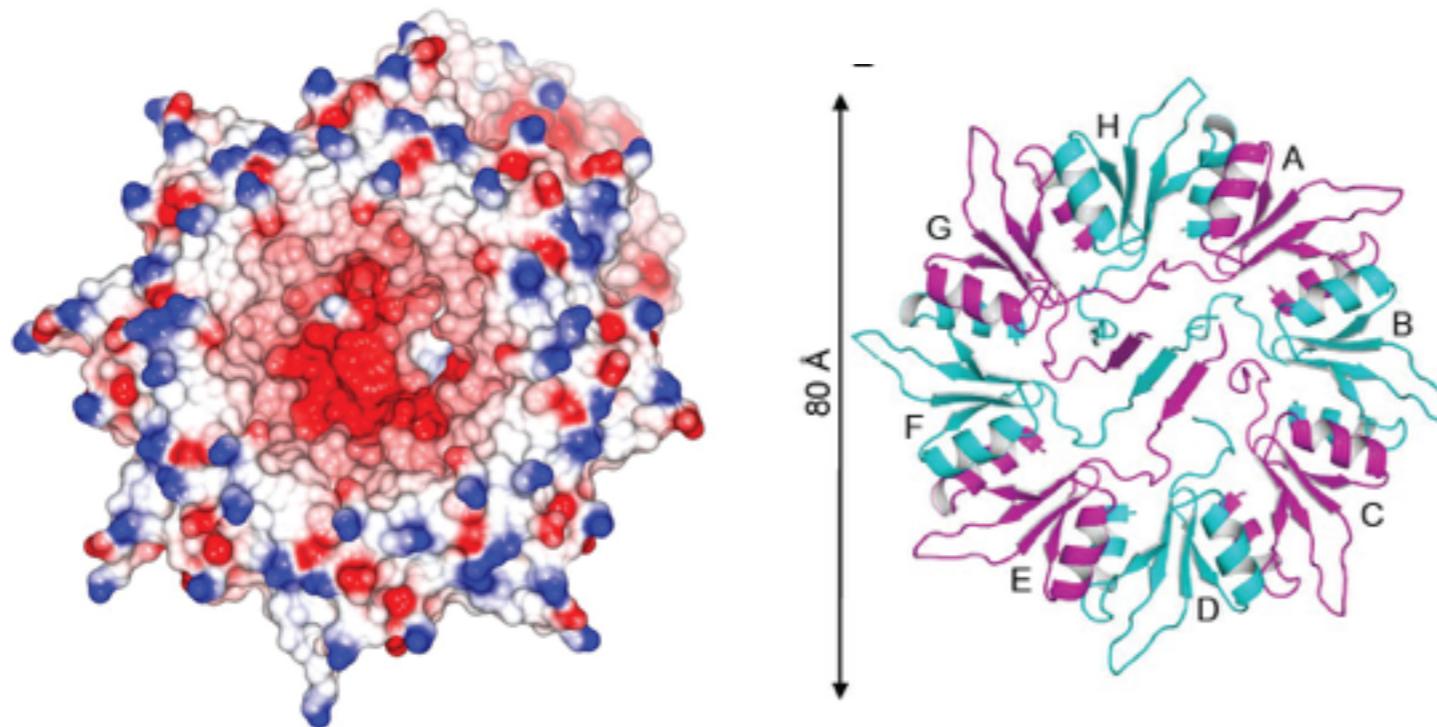


Thai Leong Yap, Zhiping Jiang, Frank Heinrich, James M. Gruschus, Candace M. Pfefferkorn, Marilia Barros, Joseph E. Curtis, Ellen Sidransky, and Jennifer C. Lee,
Structural Features of Membrane-bound
Glucocerebrosidase and α -Synuclein Probed by
Neutron Reflectometry and Fluorescence
Spectroscopy, submitted to JBC, (2014)

PlyCB



manuscript in preparation



- in collaboration with Daniel Nelson, IBBR / UMD
- medical relevance
- Grant target

Impact outside of NIST

almost none

What do we do about it?

Active applied projects (2014):

- Dengue
- Diphtheria toxin
- Gaucher's disease
- GRASP
- HIV Gag
- Ionic Liquids
- Macrocycles
- Neurotransmitter
- Parkinson's disease
- PKCa
- PlyC
- RSV & MLV
- Shiga toxin
- PTEN Tumor suppressor
- T-Cell receptor
- Vagolysin

What do we do about it?

Biochimica et Biophysica Acta 1838 (2014) 2341–2349



Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem



Review

Zooming in on disordered systems: Neutron reflection studies of proteins associated with fluid membranes[☆]



Frank Heinrich, Mathias Lösche *

Physics Department, Carnegie Mellon University, Pittsburgh, PA, U.S.A.
NIST Center for Neutron Research, Gaithersburg, MD, U.S.A.



Methods

Available online 27 October 2014

In Press, Uncorrected Proof — Note to users



Membrane association of the PTEN tumor suppressor: Neutron scattering and MD simulations reveal the structure of protein–membrane complexes

Hirsh Nanda^{a, c}, Frank Heinrich^{a, c}, Mathias Lösche^{a, b, c},

What do we do about it?

THE JOURNAL OF
PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Structure and Stability of Phospholipid Bilayers Hydrated by a Room-Temperature Ionic Liquid/Water Solution: A Neutron Reflectometry Study

Antonio Benedetto,^{*,†} Frank Heinrich,^{‡,§} Miguel A. Gonzalez,^{||} Giovanna Fragneto,^{||} Erik Watkins,^{||,⊥} and Pietro Ballone^{⊗,#}

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[‡]Department of Physics, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States

[§]Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States

^{||}Institute Laue-Langevin, Grenoble, France

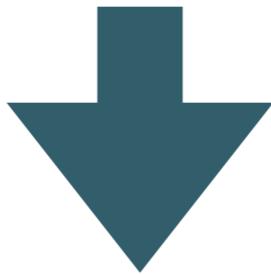
[⊥]Lujan Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, United States

[⊗]Center for Life Nano Science @Sapienza, Istituto Italiano di Tecnologia (IIT), 00161 Roma, Italy

[#]Department of Physics, Universita' di Roma "La Sapienza", 00185 Roma, Italy

Modeling Challenges and Future

Maximum model complexity for current data has been reached

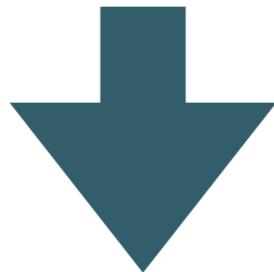


We need better data!

- Low-background sample cell development (project with Chuck, prototype 2014)
- CANDOR (2017)

Modeling Challenges and Future

Maximum model complexity for current data has been reached

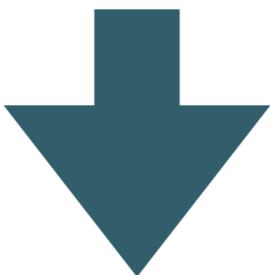


We need to supplement our data

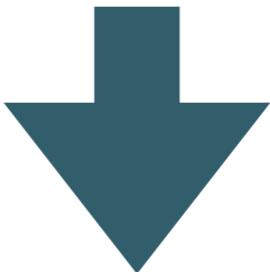
- Hybrid / integrative modeling techniques.
- MD simulations (new project Hirsh).

Modeling Challenges and Future

We are working above capacity. We have more projects than happy customers.



We think about automating model optimization (one of the biggest time sinks)



Need theoretical framework for what constitutes an optimal model. Started project in 2014 using information theory (with CMU and Paul Kienzle)

Acknowledgments

Paul Kienzle, NIST
Dave Vanderah, NIST

Thank you very
much
for your attention.



National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

Carnegie Mellon