Neutron Reflectometry for Studying Membrane Proteins

NCNR Summer School 2008

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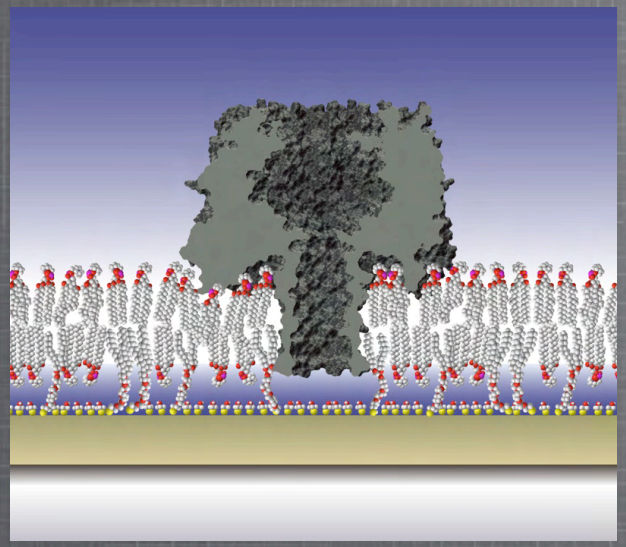
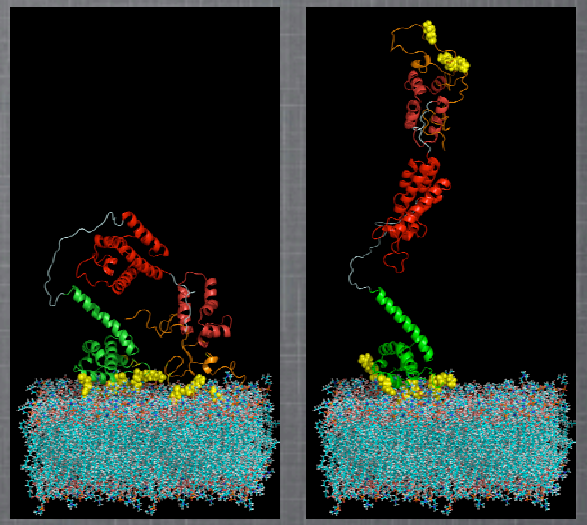
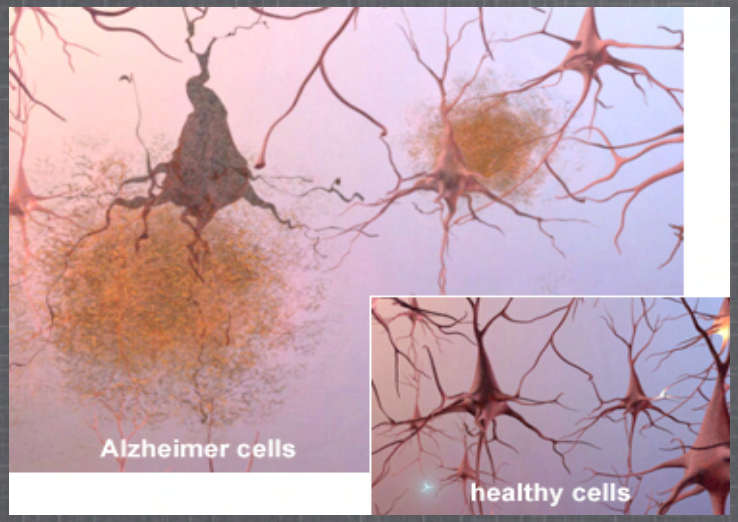
Motivation



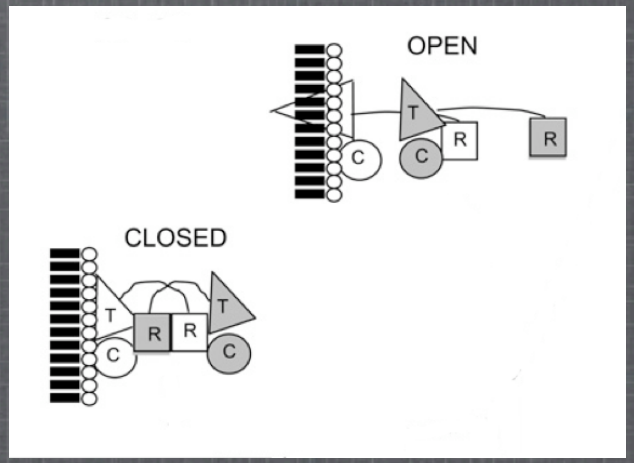
Structure



Conformation and Conformational Changes



Impact on Membranes



from :Kent et al. *Biophys J* (2008) vol. 94 (6) pp. 2115-27

Part 1. Membrane Model Systems for Neutron Reflectometry.

Part 2. Protein interactions. Examples from NCNR research.

Lipid model systems

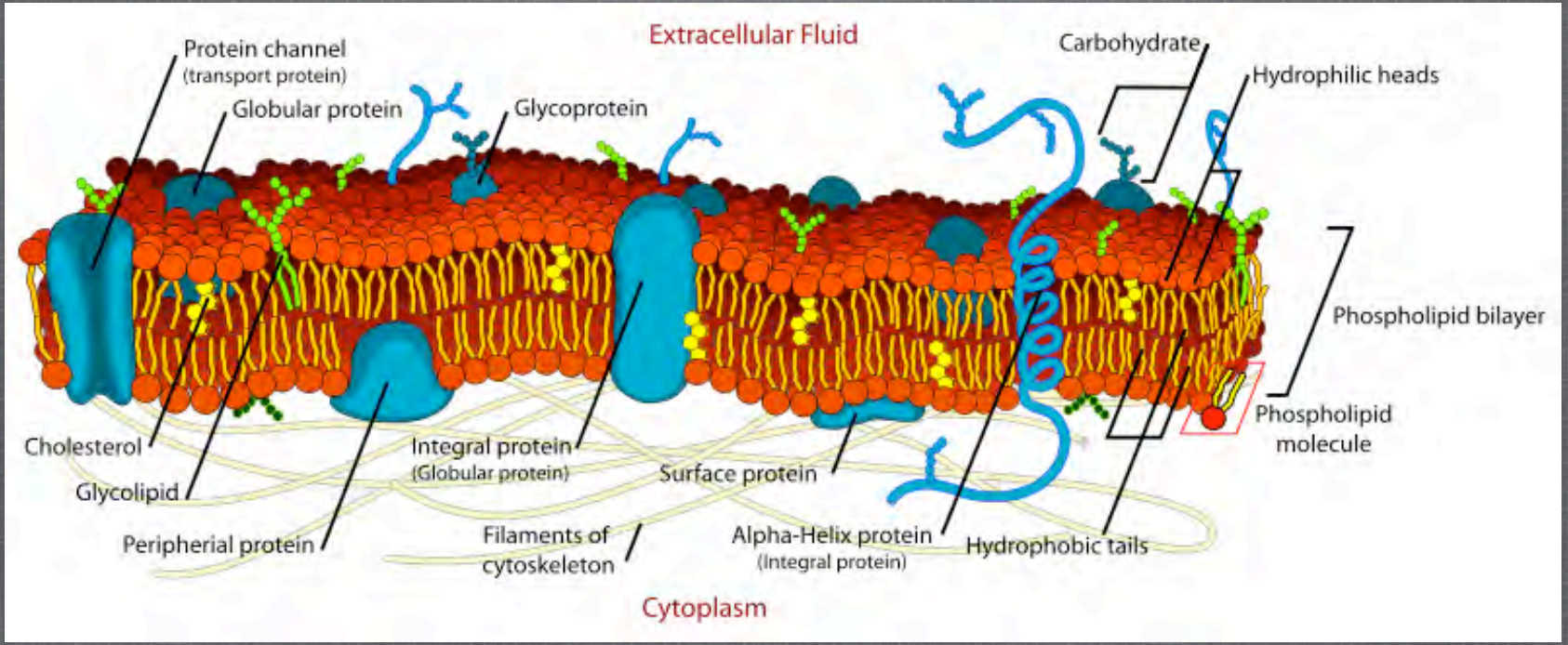
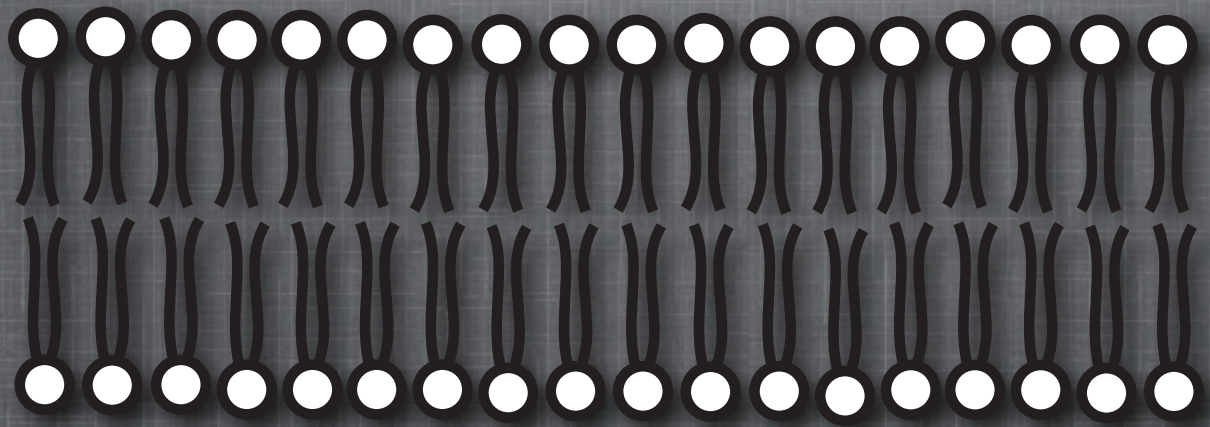
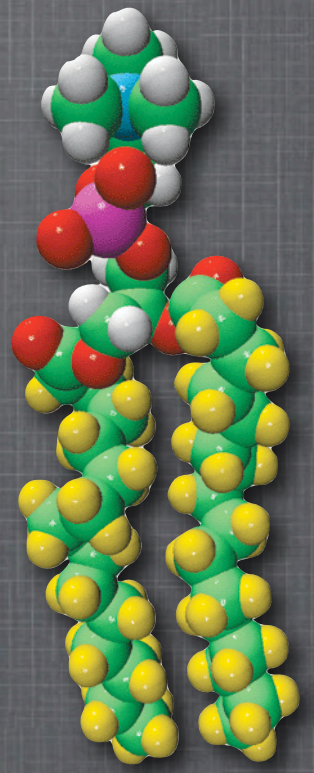


Illustration of a biological cell membrane. From www.wikipedia.org.

Lipid model systems

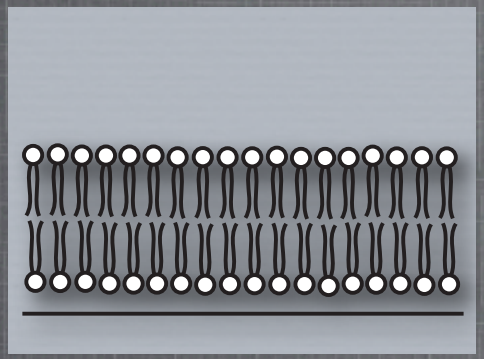




d62-DPPC

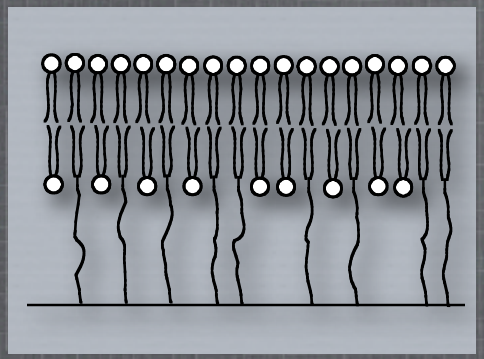
www.avanti-lipids.com.

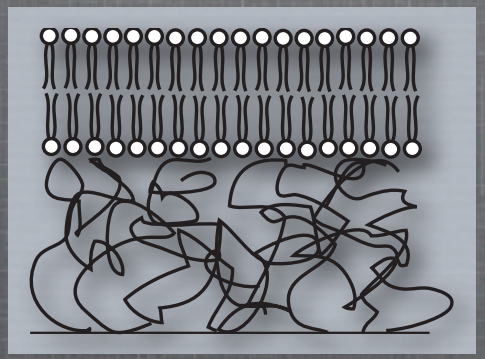
Solid supported membrane model systems



Bare solid support

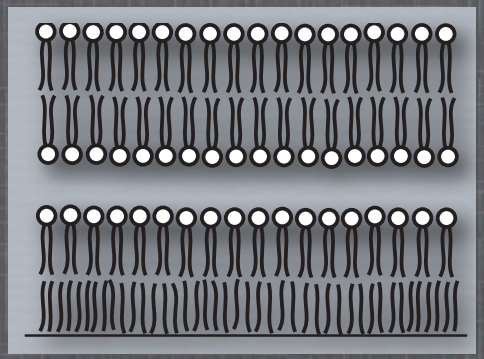
Hanna Wacklin, Robert Thomas,
Langmuir 23:7664 (2007)
ISIS/ Ansto





Tethered / cushioned

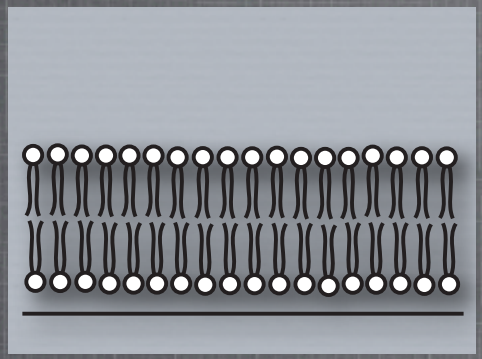
D. McGillivray et al., Biointerphases 2:21 (2007)
NIST
J. Y. Wong, Biophysical Journal 77:1445 (1999)
Los Alamos

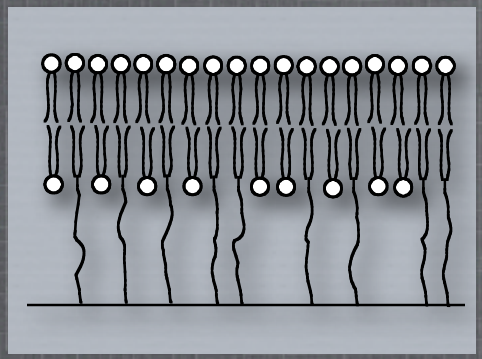


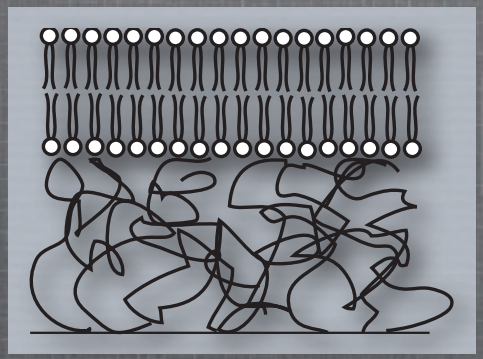
Floating

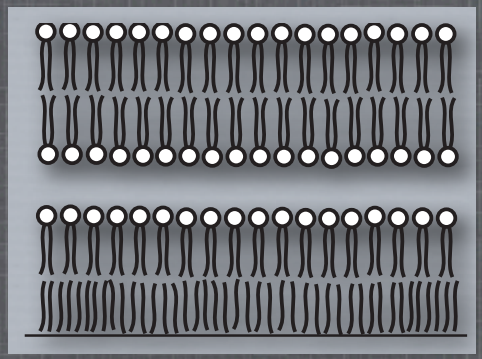
G. Fragneto et al., Europhys Lett. 53:100 (2001)
ILL

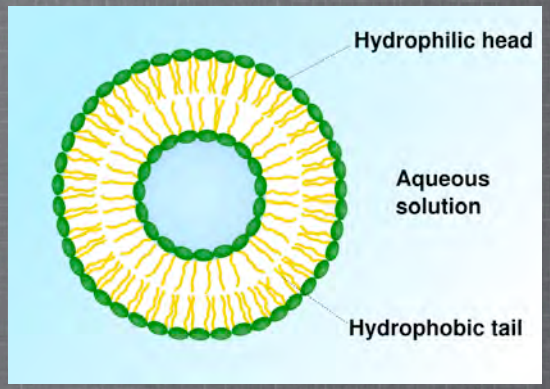
Solid supported membrane model systems

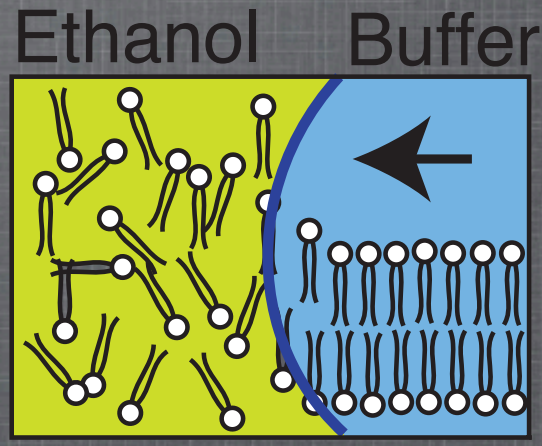


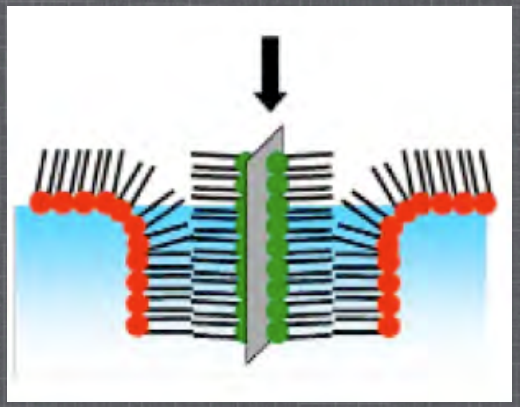




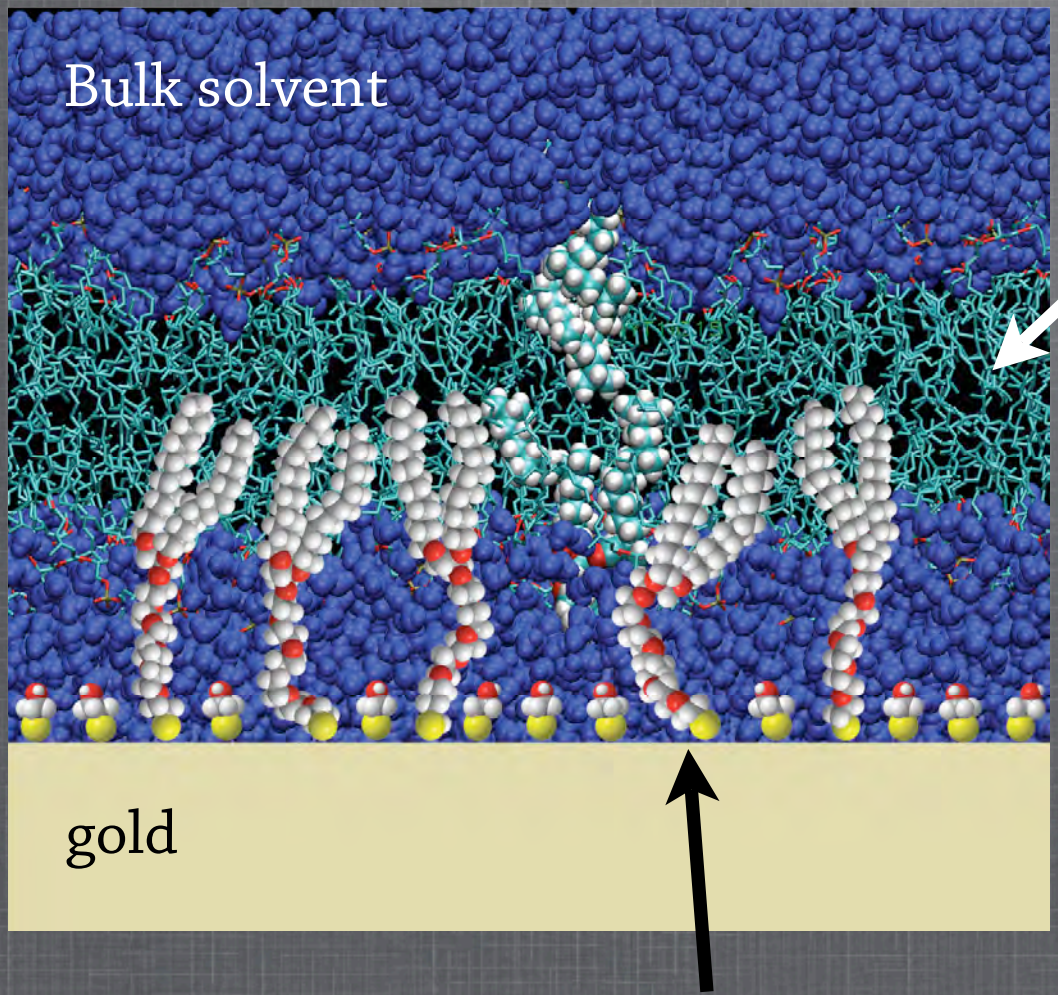








The sparsely-tethered bilayer lipid membrane



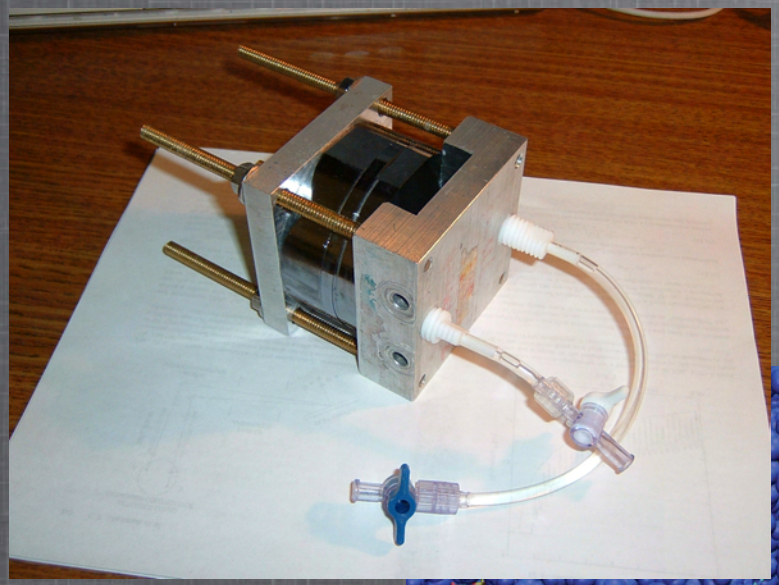
Lipid bilayer (PC, PG, PS, chol.)

Thiol-$(EO)_6$-$(C14)_2$ tether with β – mercapto ethanol

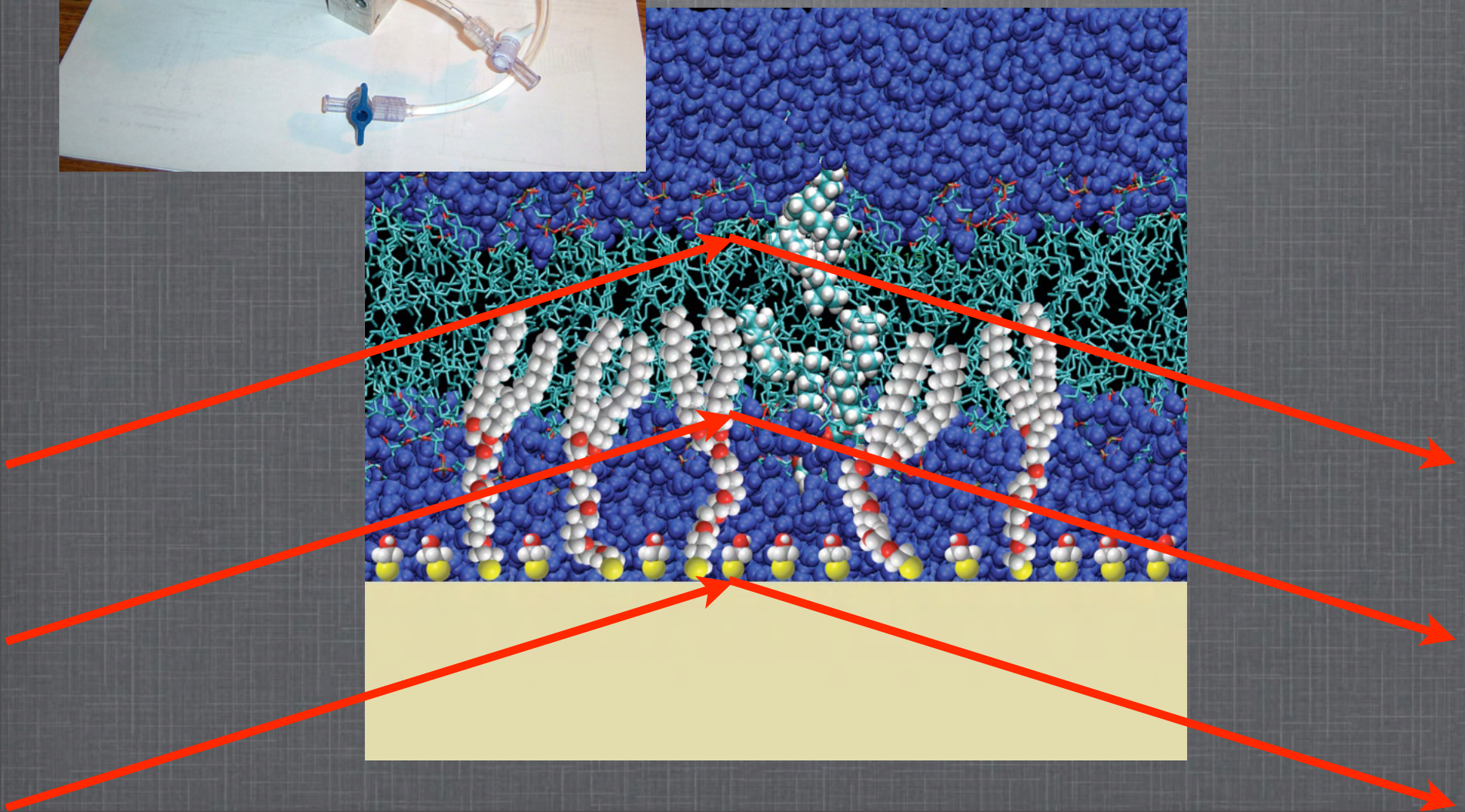
- Homogenous preparation over large areas possible
- Fluid bilayer is stable for more than a week
- No limitation in type of lipids used for bilayer
- Water-filled sub-membrane space decouples the bilayer from the substrate

McGillivray et al., *Biointerphases* 2:21 (2007)

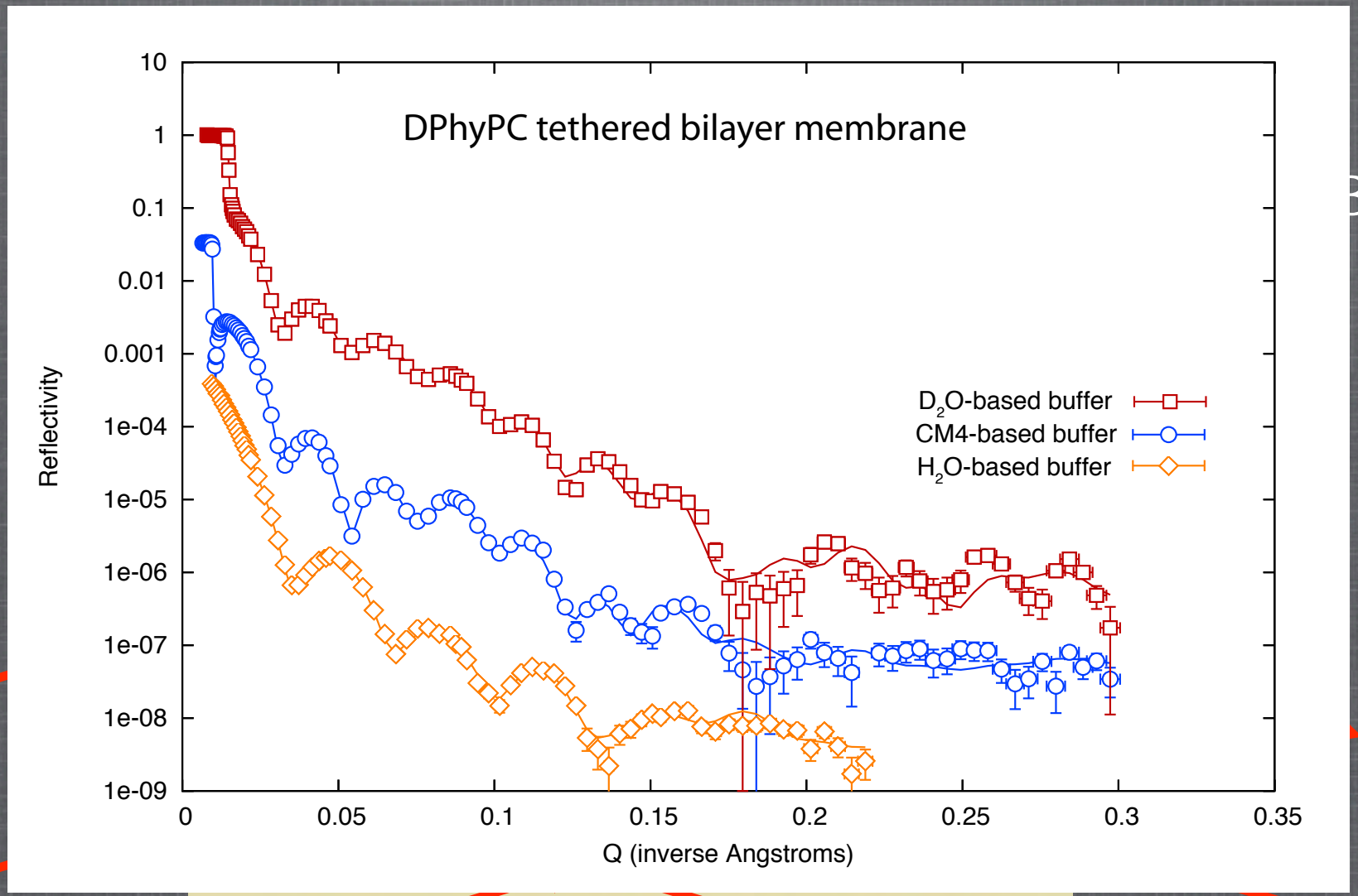
NR from surface-stabilized membranes



"tethered" lipid bilayer membrane (tBLM)

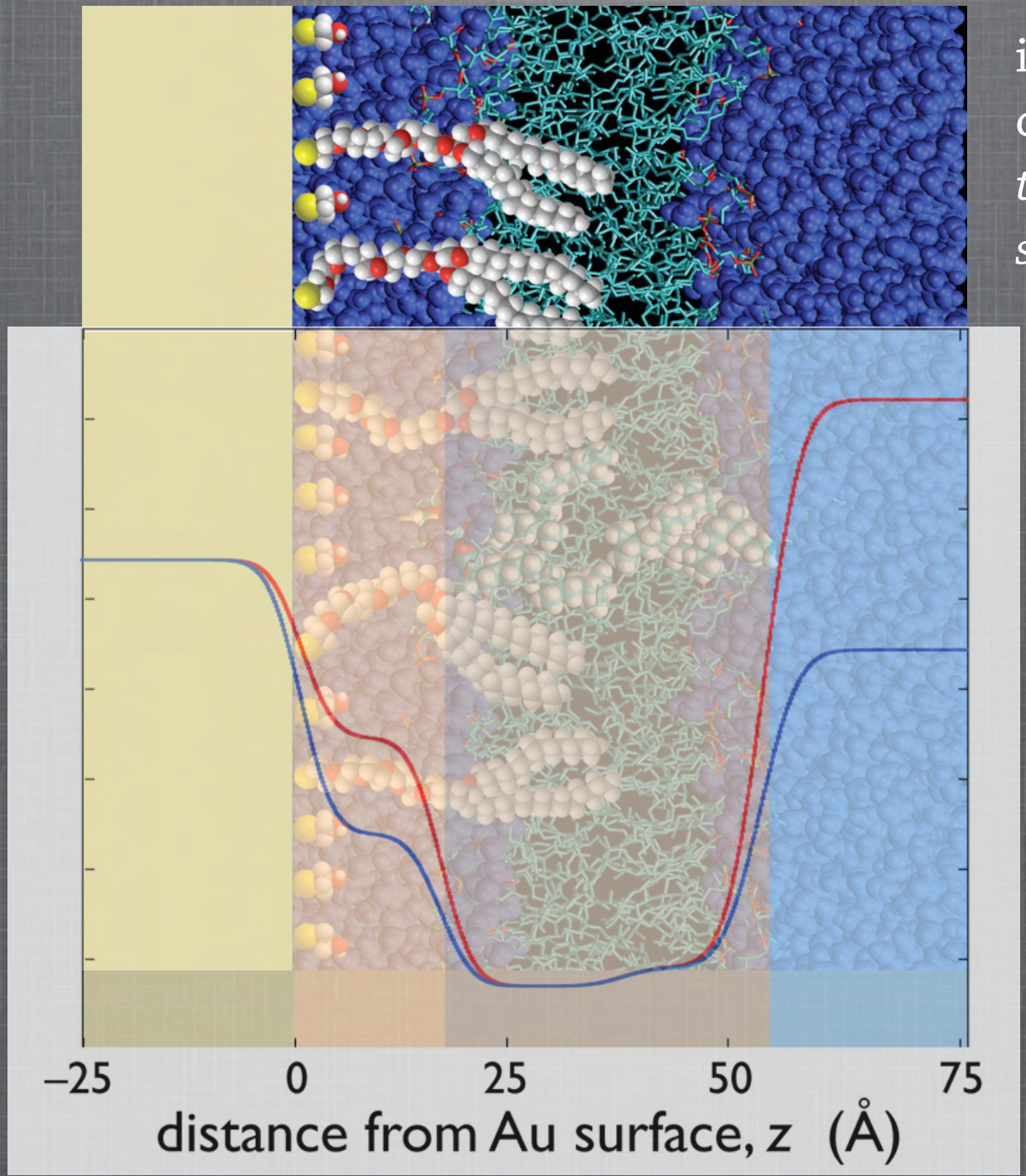


NR from surface-stabilized membranes



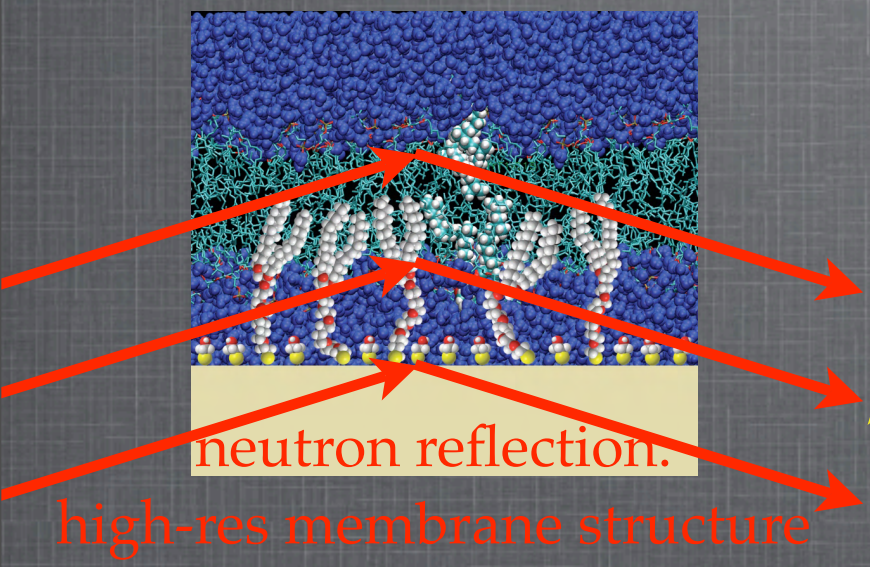
bilayer
BLM)

NR from surface-stabilized membranes

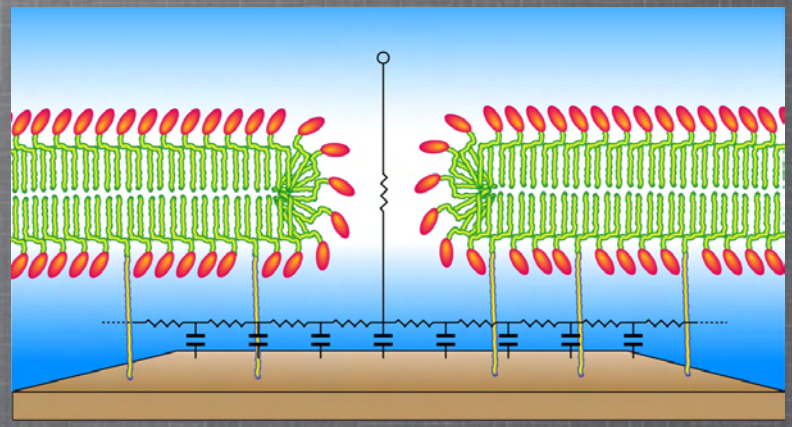


interprete nSLD distribution *in terms of molecular structure*

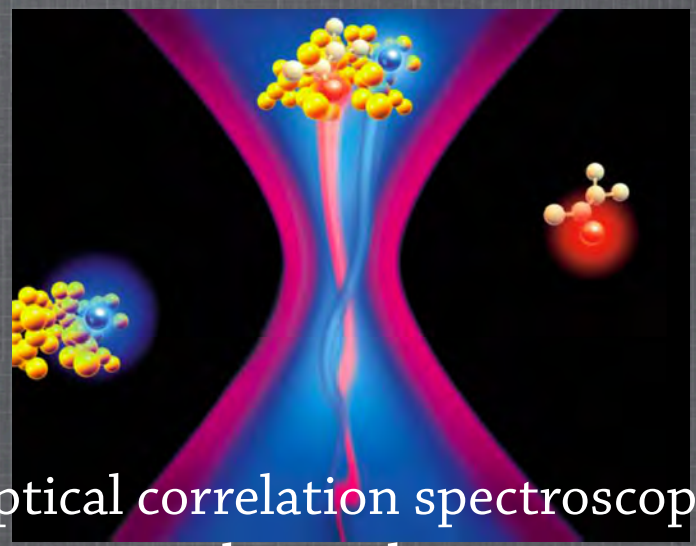
Complementary techniques



neutron reflection:
high-res membrane structure



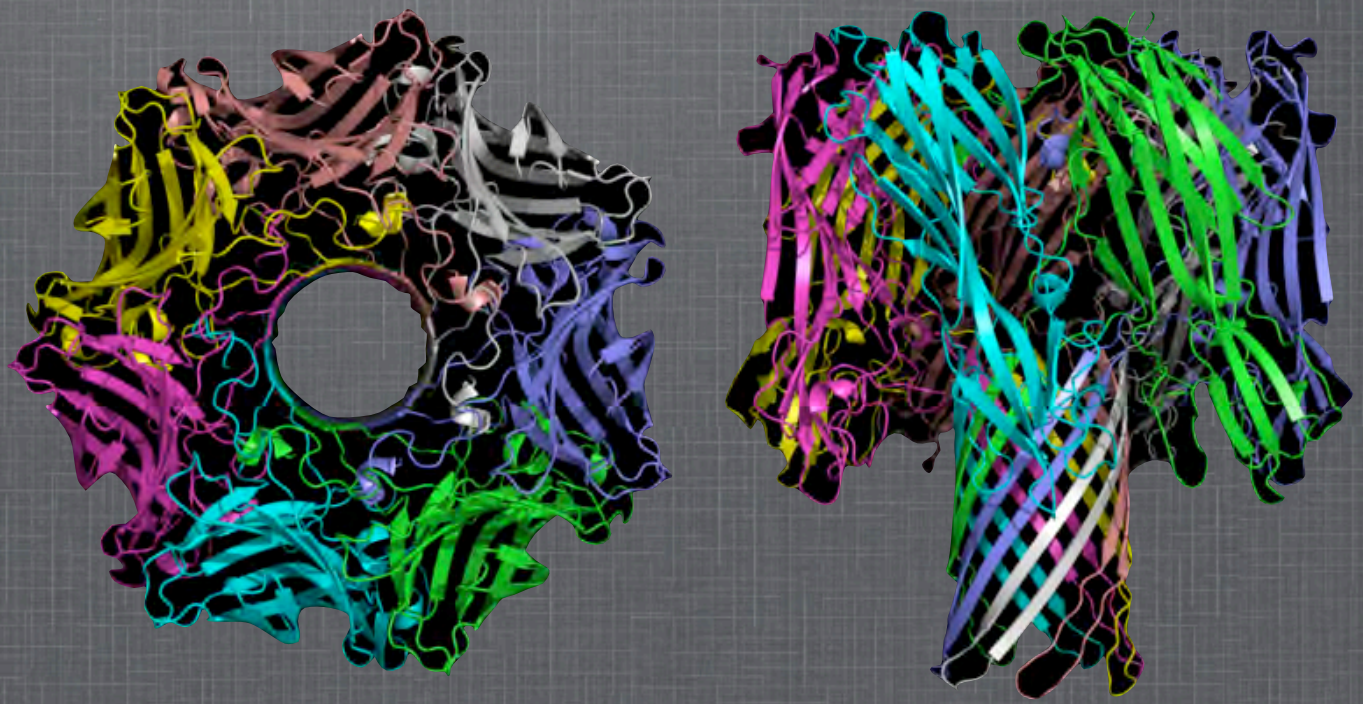
electrochemical spectroscopy:
ion barrier properties, function



optical correlation spectroscopy:
membrane dynamics

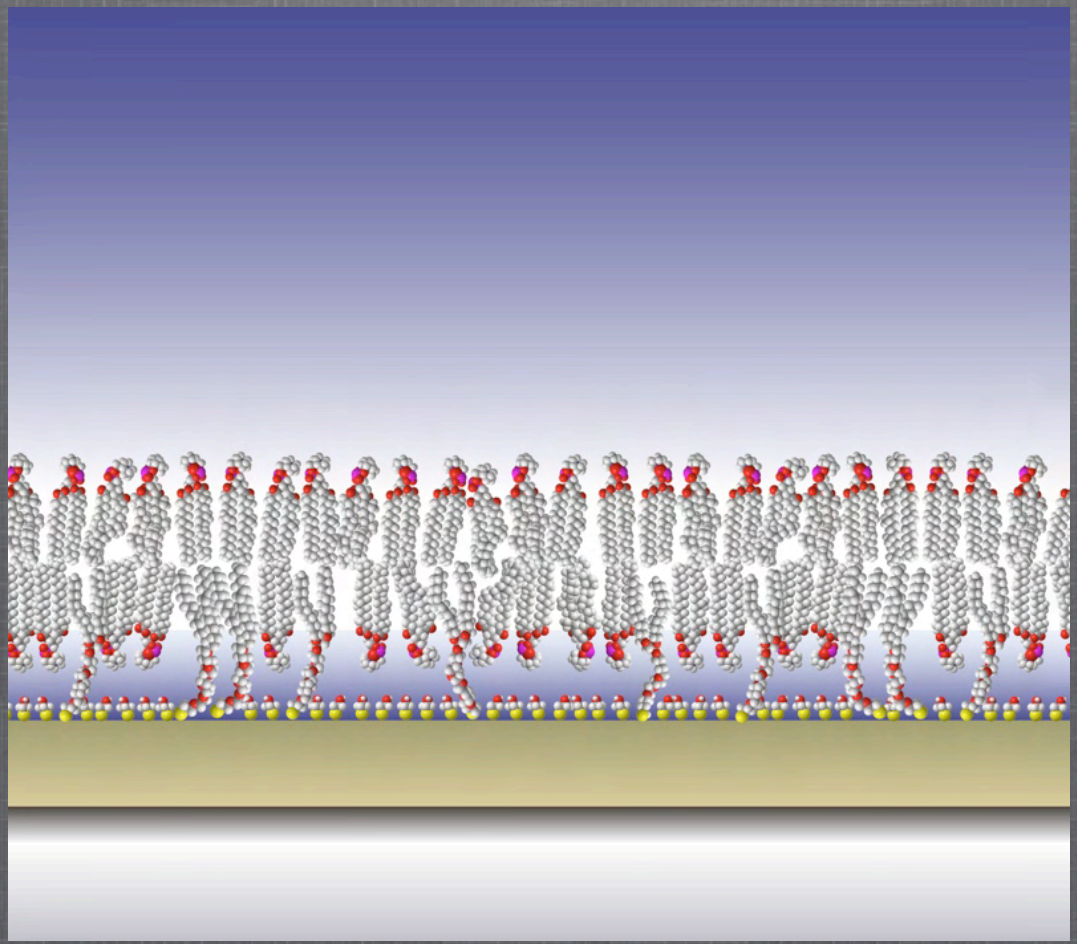
Controlled perforation of membranes: The toxin pore, α-hemolysin (αHL)

with:
Joey Robertson, John Kasianowicz, NIST;
Ilja Ignatiev, Gintaras Valincius, Institute of Biochemistry, Vilnius;
Duncan McGillivray, The University of Auckland, Auckland

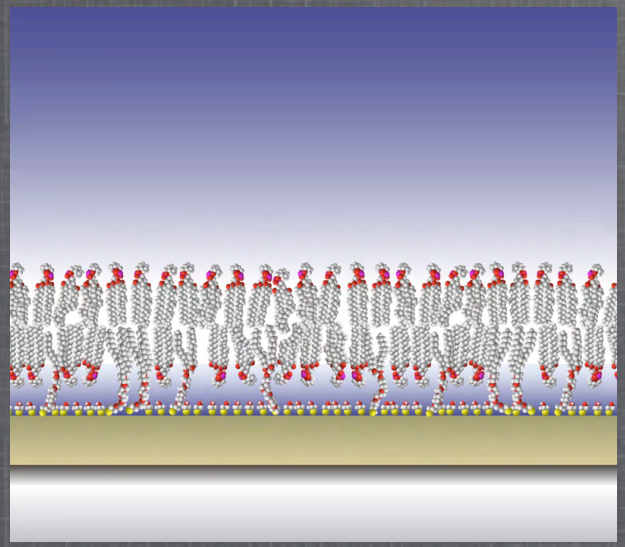


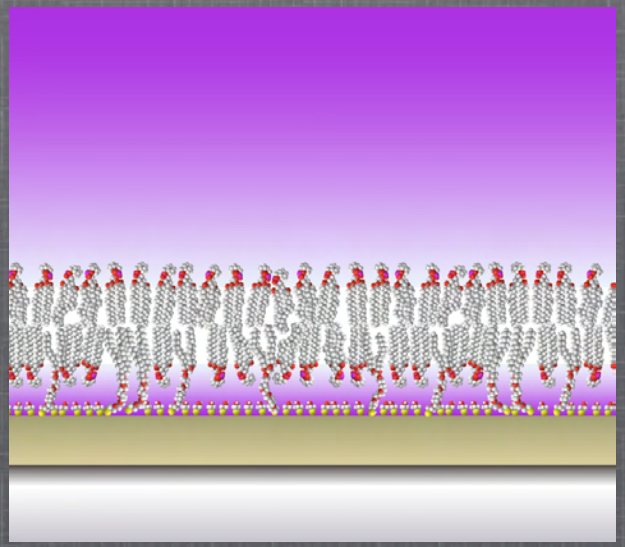
crystal structure: Song et al., Science 274 (1996); PDB: 7AHL

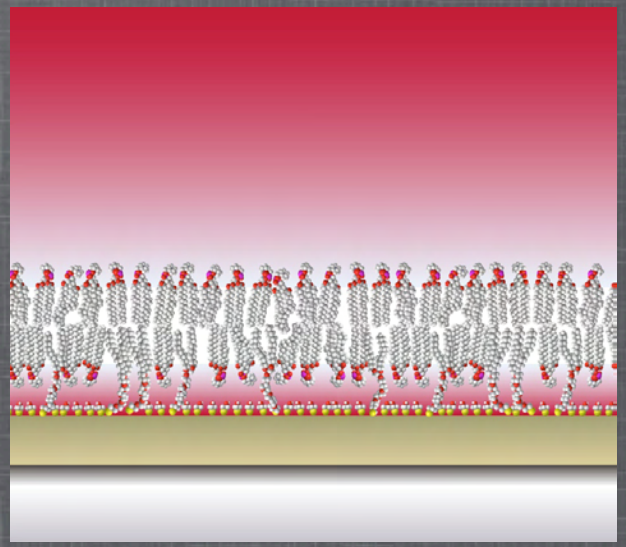
αHL NR measurements



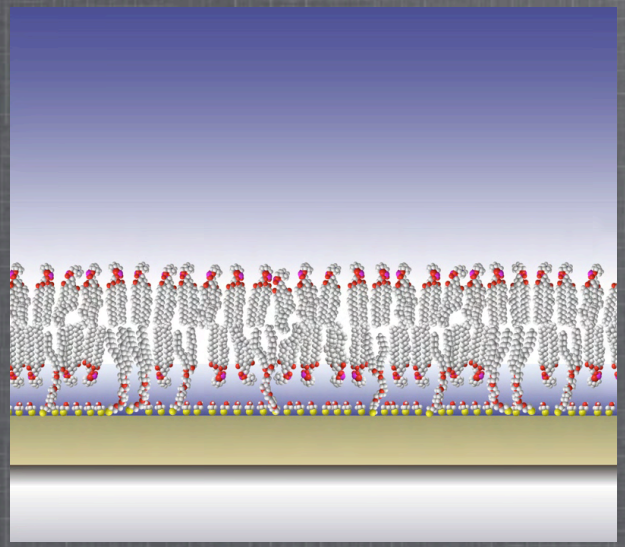
αHL NR measurements

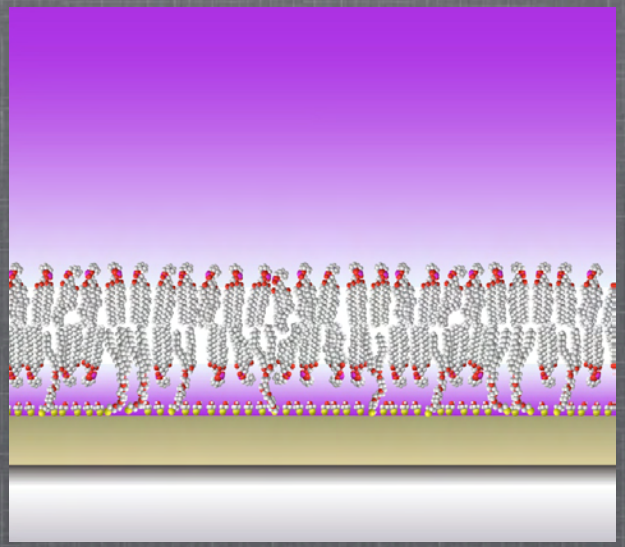


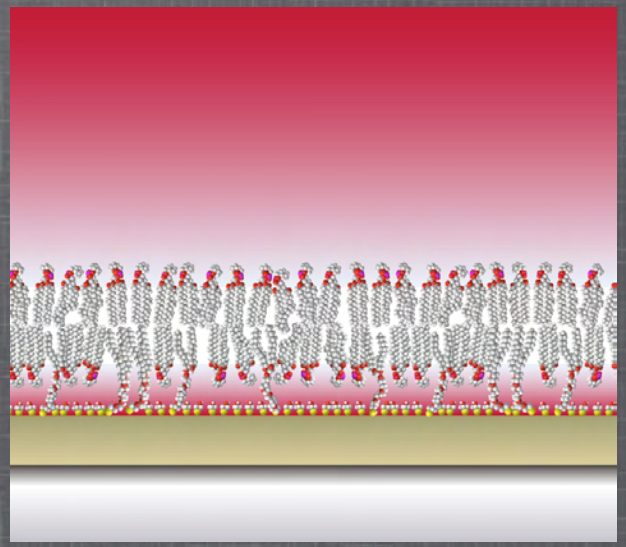




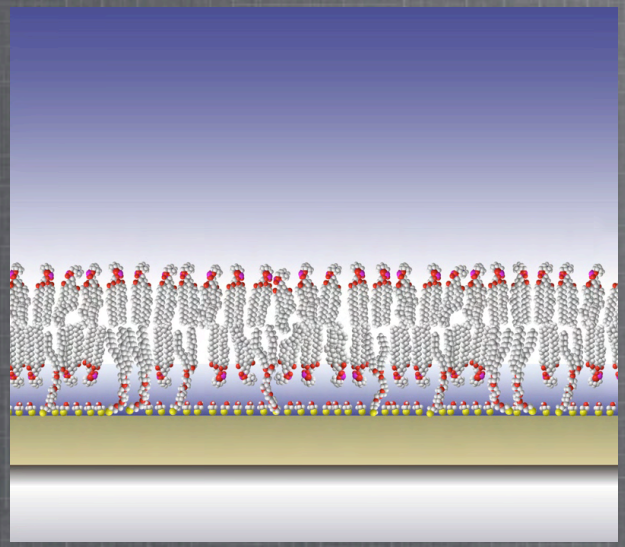
αHL NR measurements

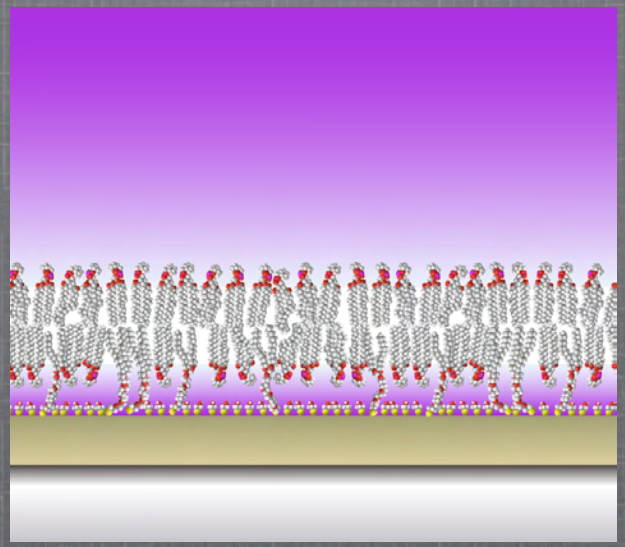


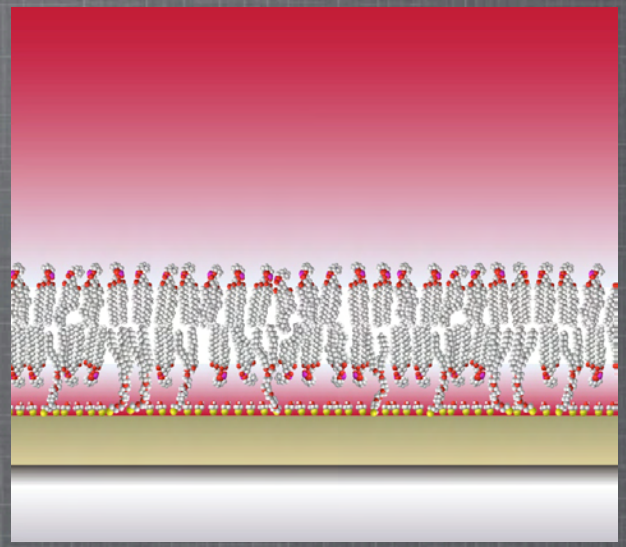


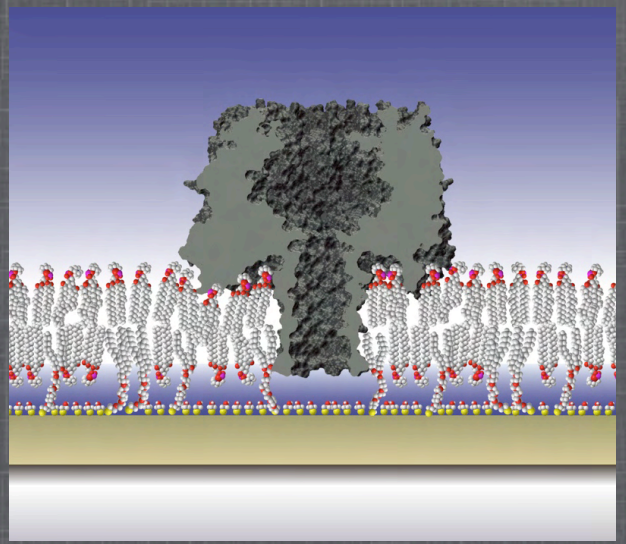


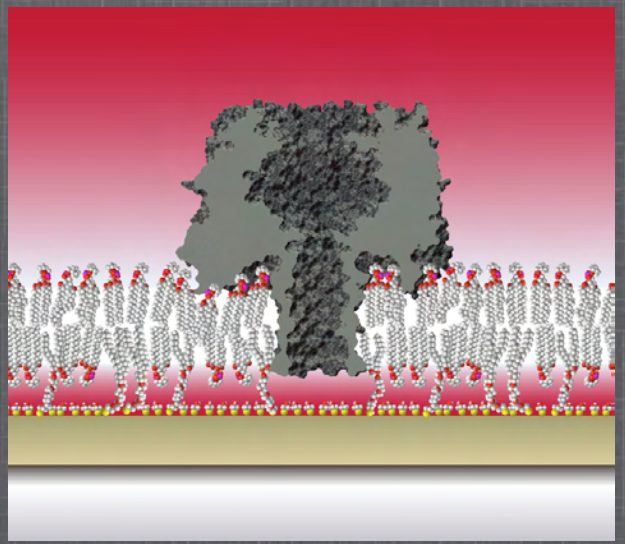
αHL NR measurements



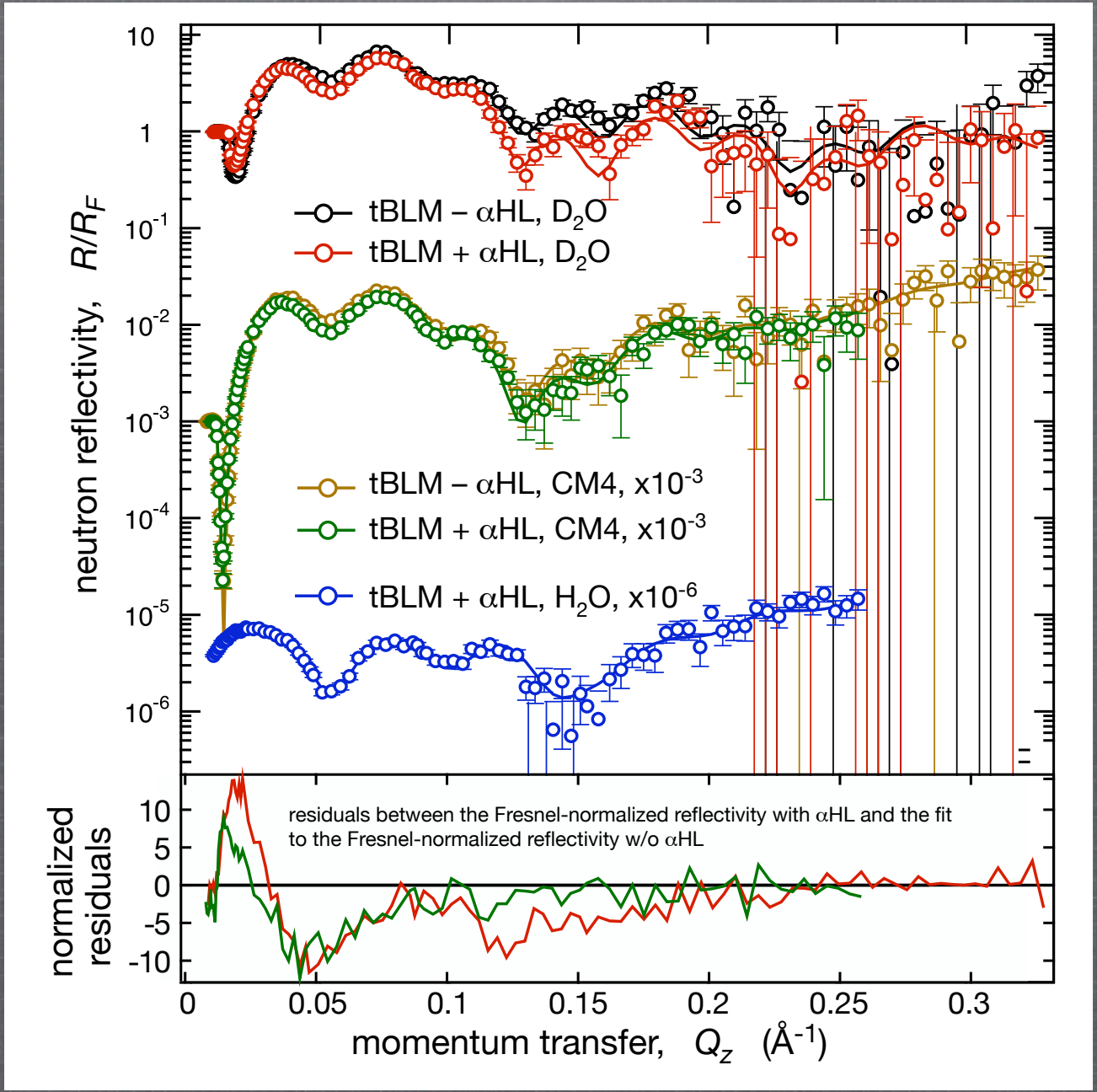




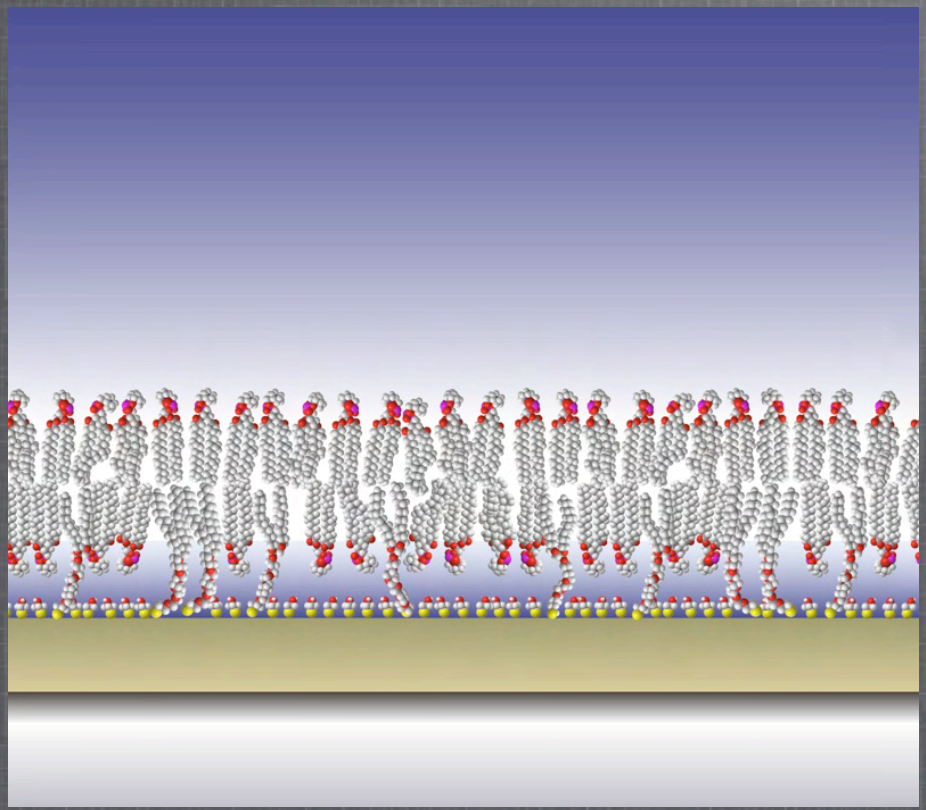


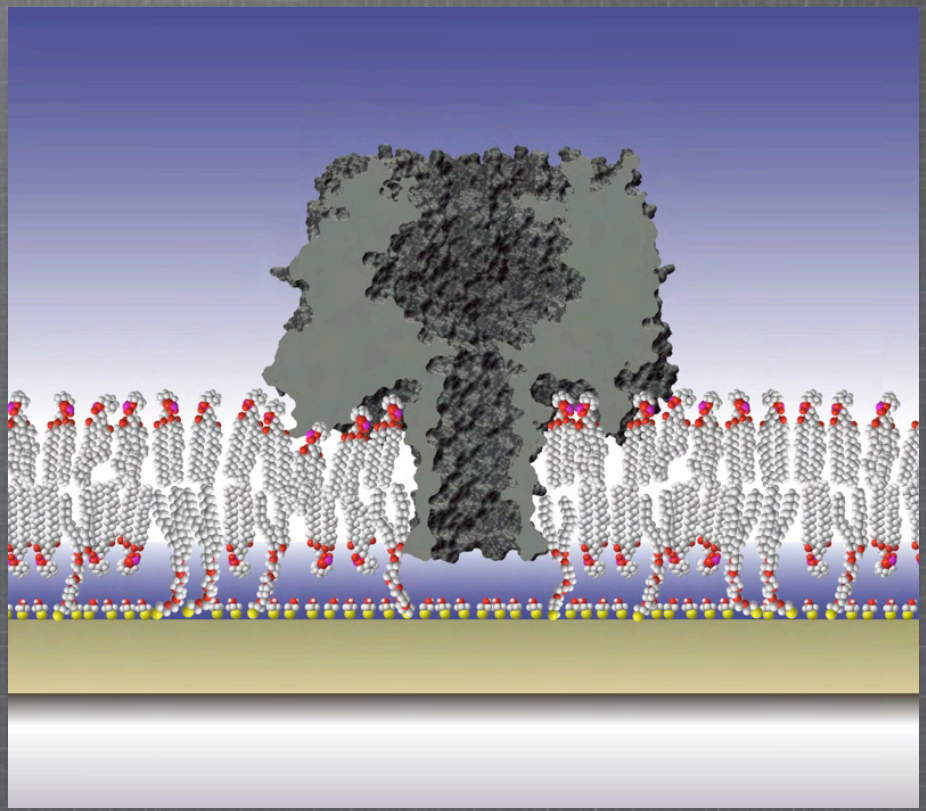


αHL reflectivity curves

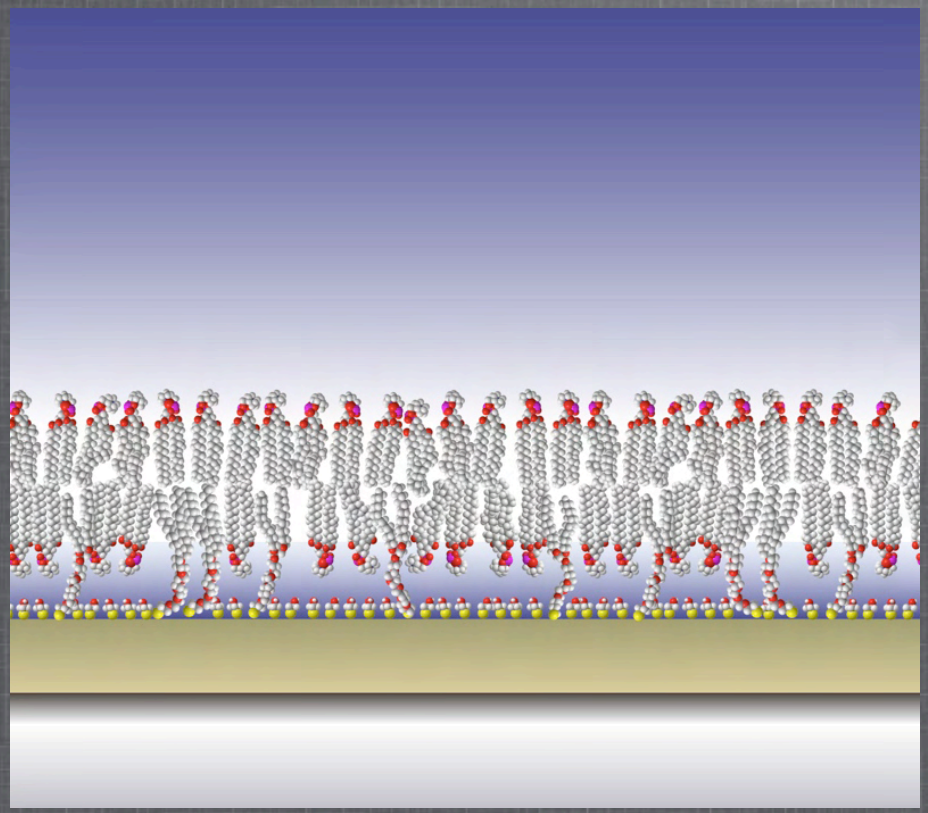


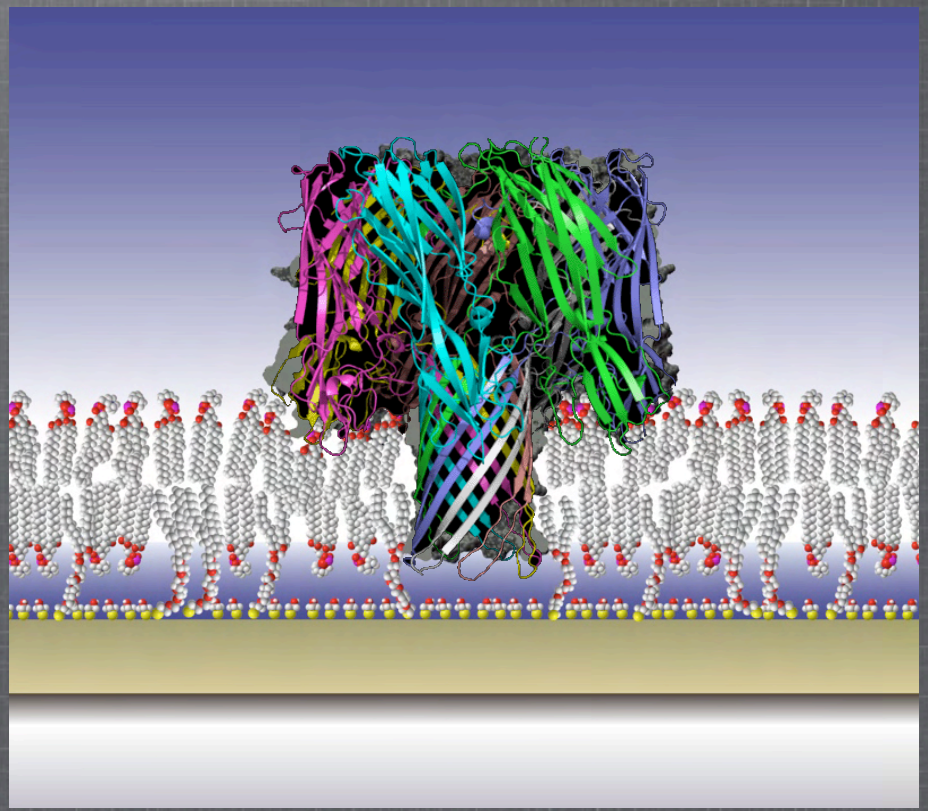
Proteins & composition space refinement





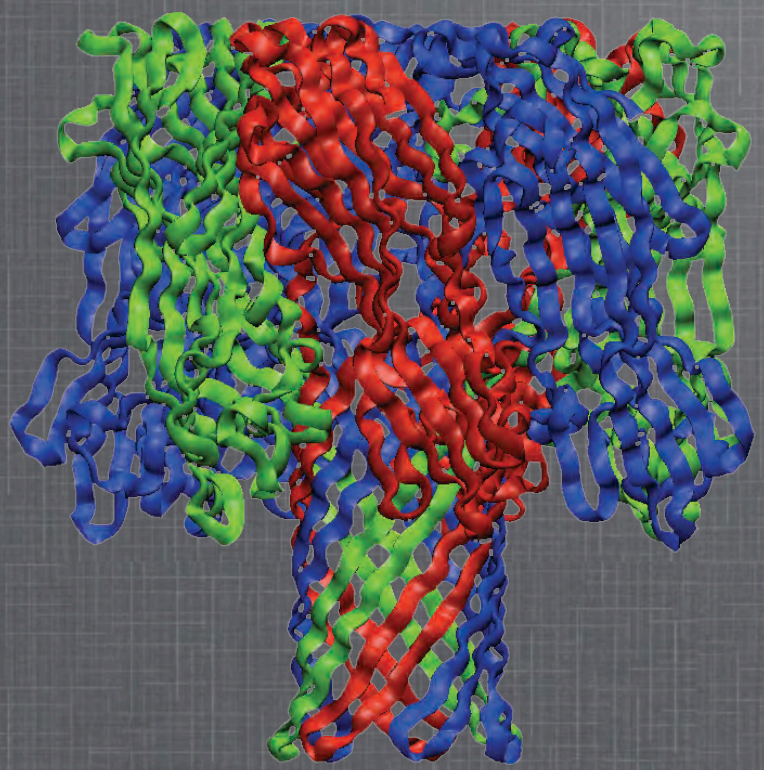
Proteins & composition space refinement

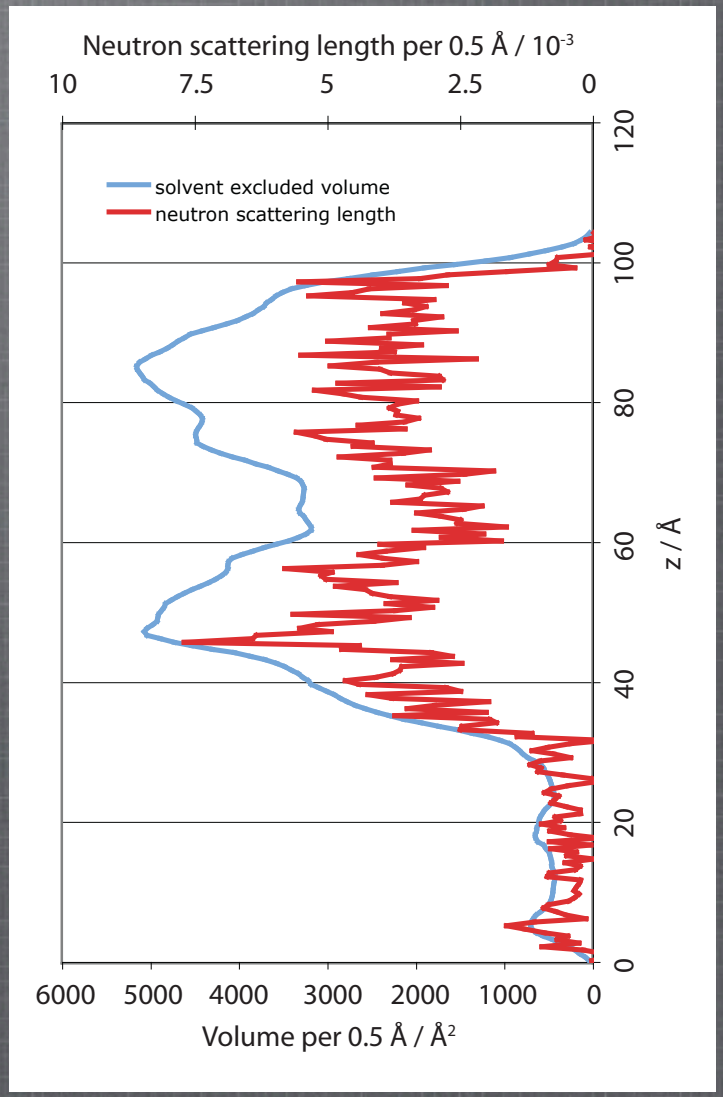




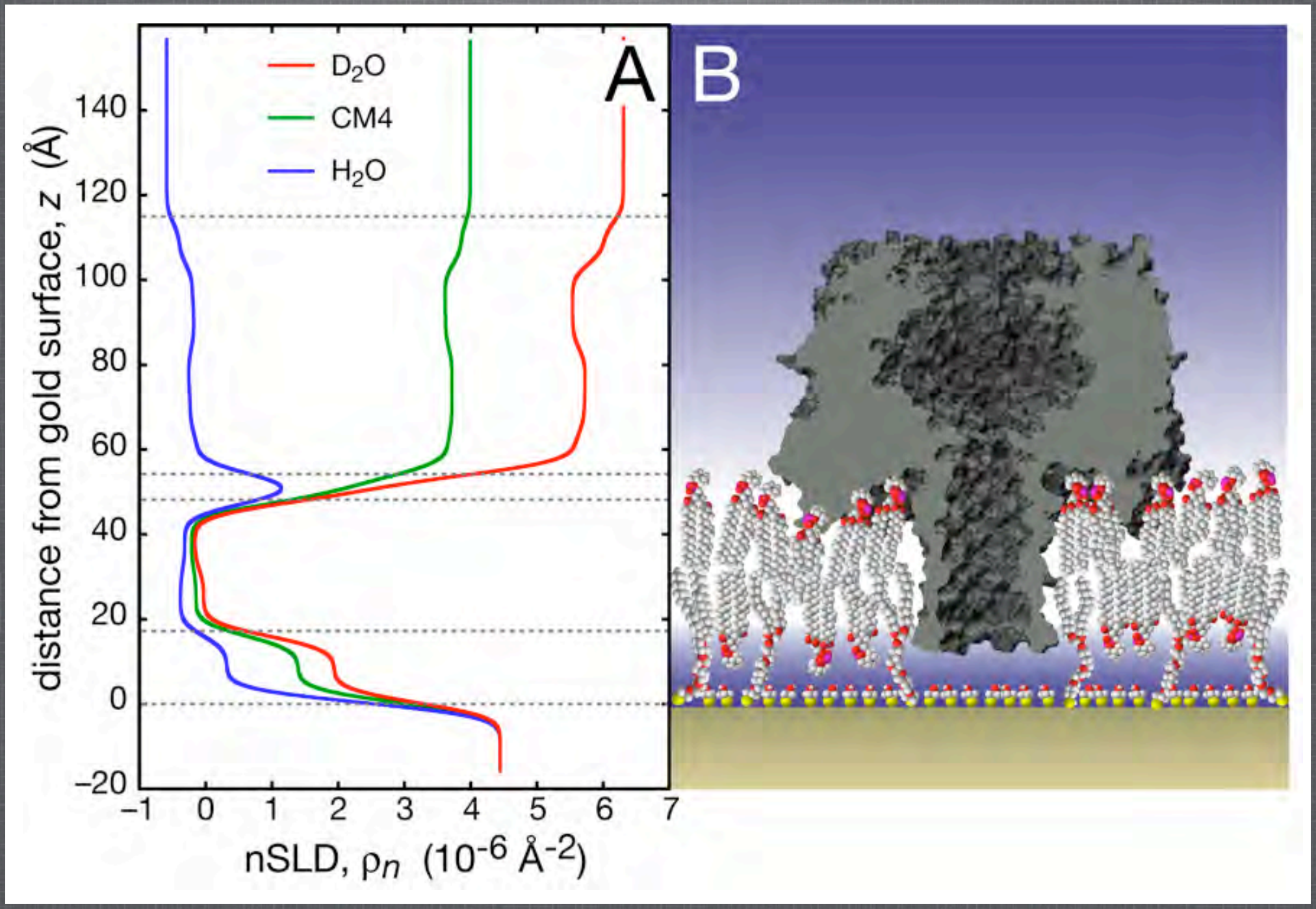
compare → determine changes in lipid conformation / hydration

αHL slicing

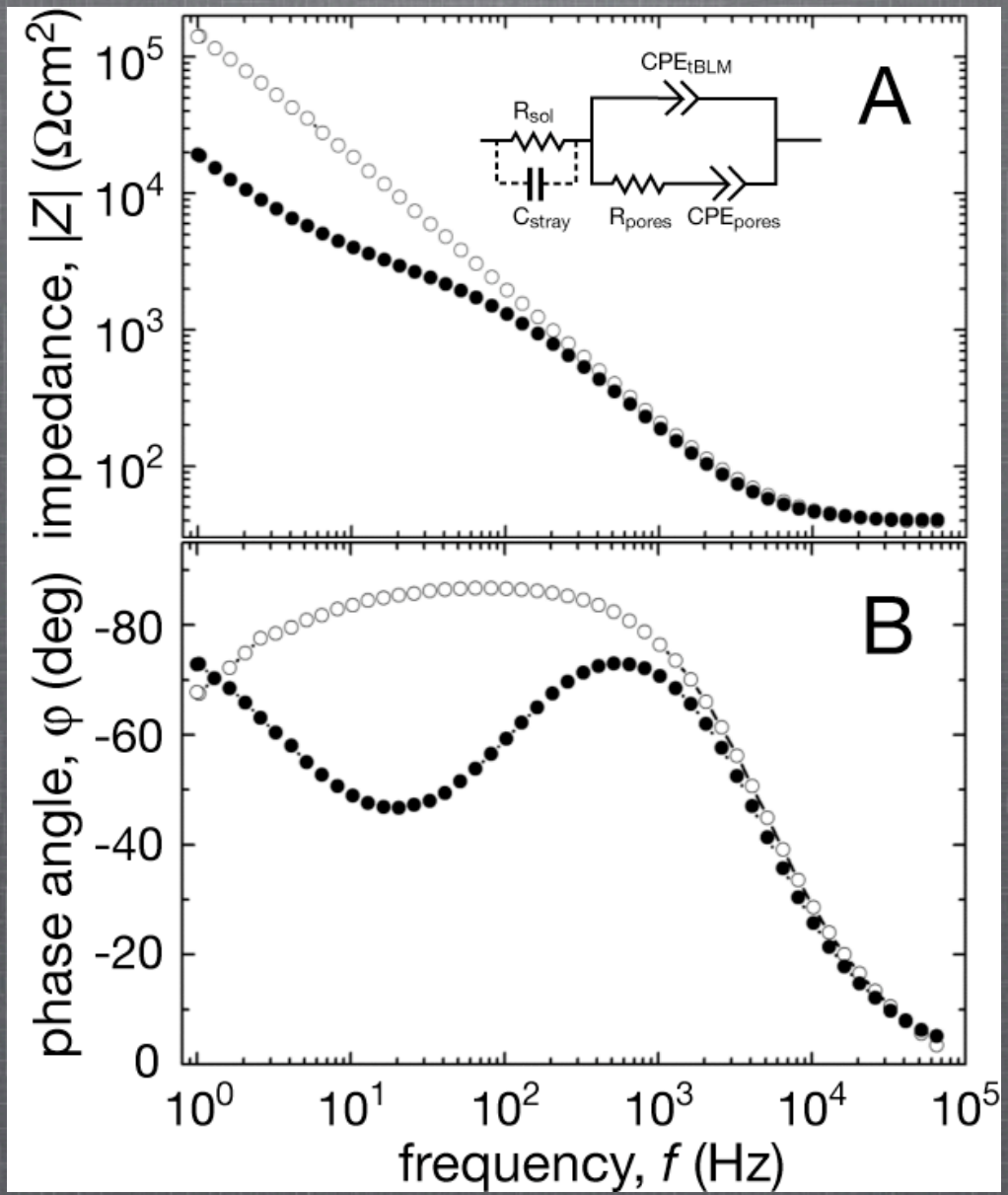



Neutron scattering length per 0.5 Å / 10-3
10
7.5
5
2.5
0
solvent excluded volume
neutron scattering length
120
100
80
60
40
20
0
z / Å
6000
5000
4000
3000
2000
1000
0
Volume per 0.5 Å / Å2

αHL structure in stBLMs

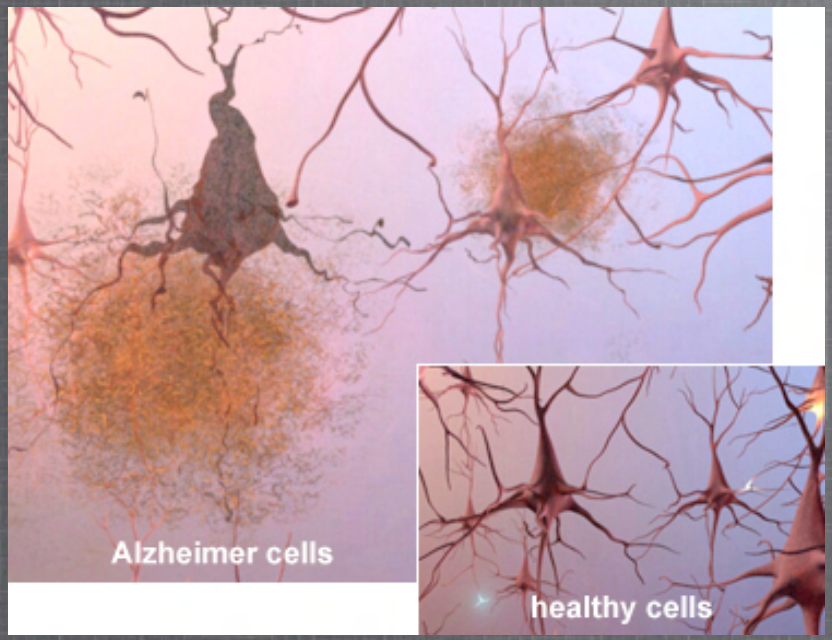


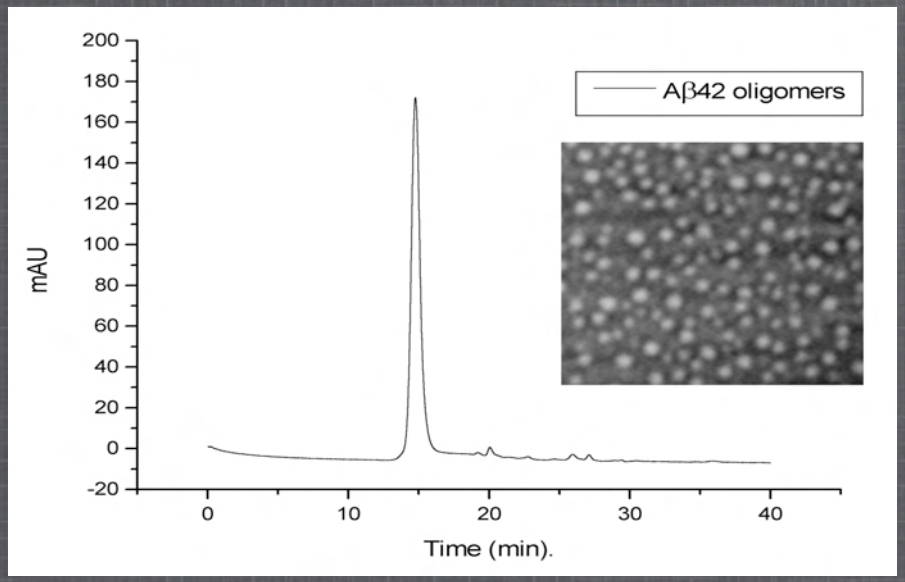
Functional incorporation shown with EIS

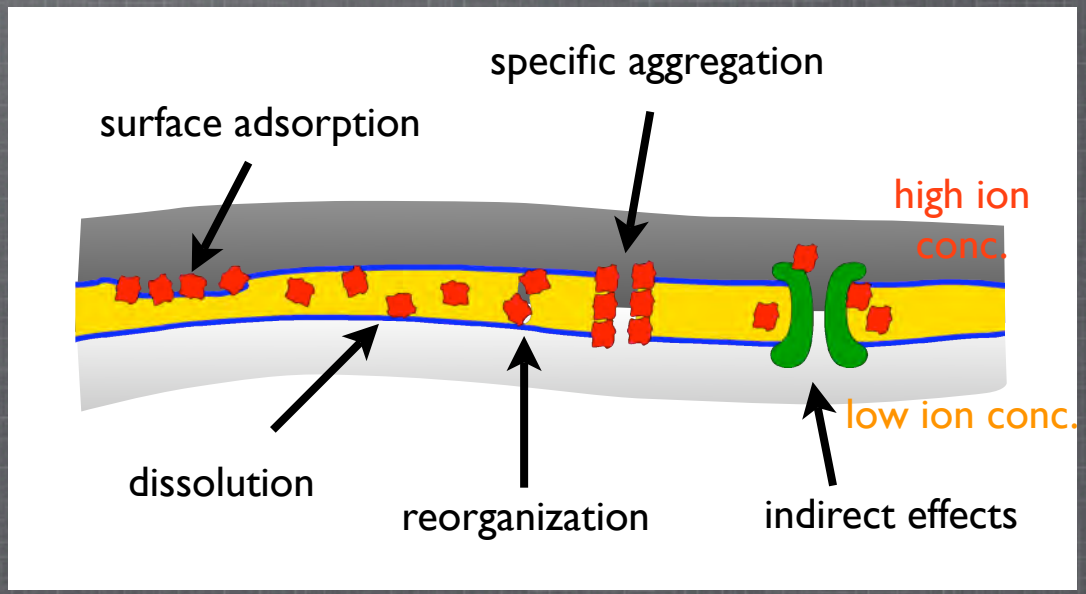


Effects on Bilayer Structure - Alzheimer's Disease

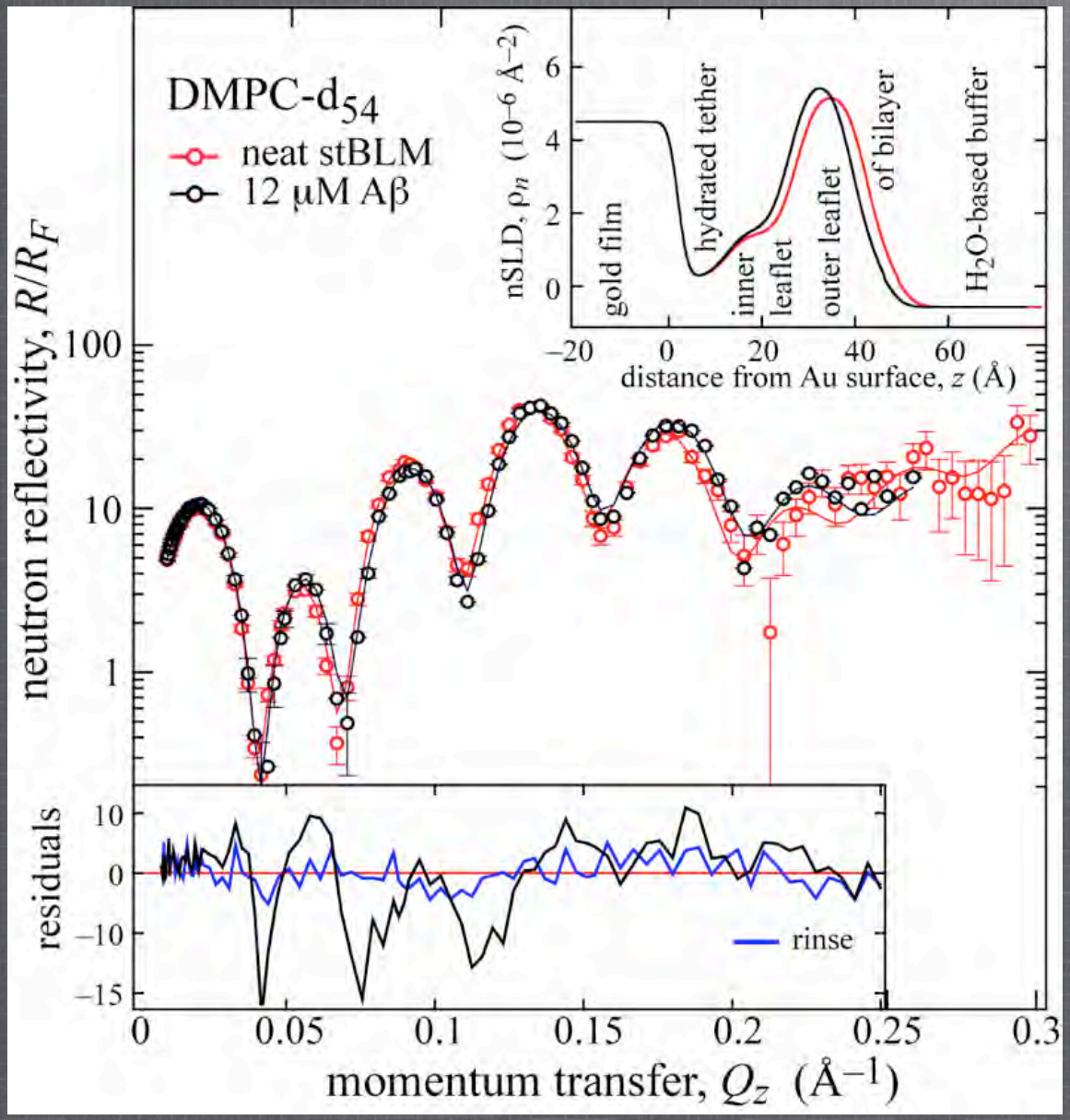
with:
Yuri Sokolov, Jim Hall, Charlie Glabe, UC Irvine;
Rima Budvytyte, Gintaras Valincius, Institute of Biochemistry, Vilnius; Duncan McGillivray, The University of Auckland, Auckland

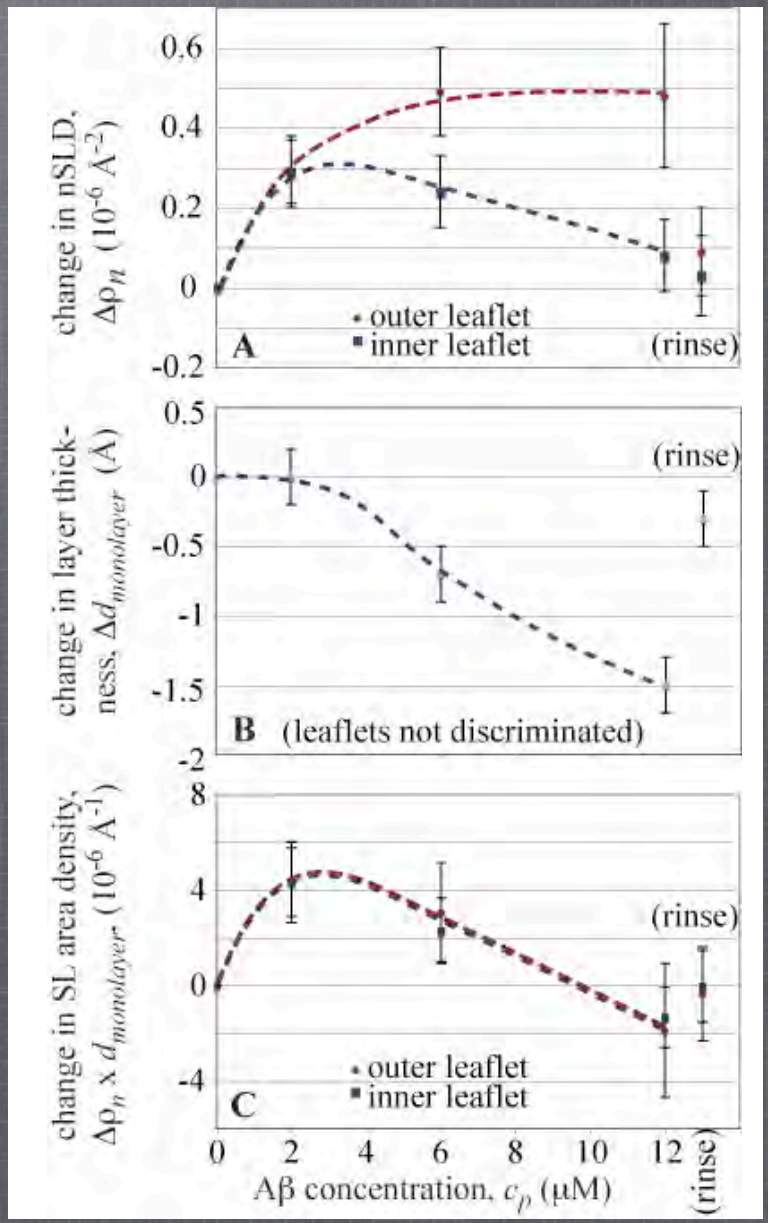






Aβ oligomer effects on stBLMs: NR

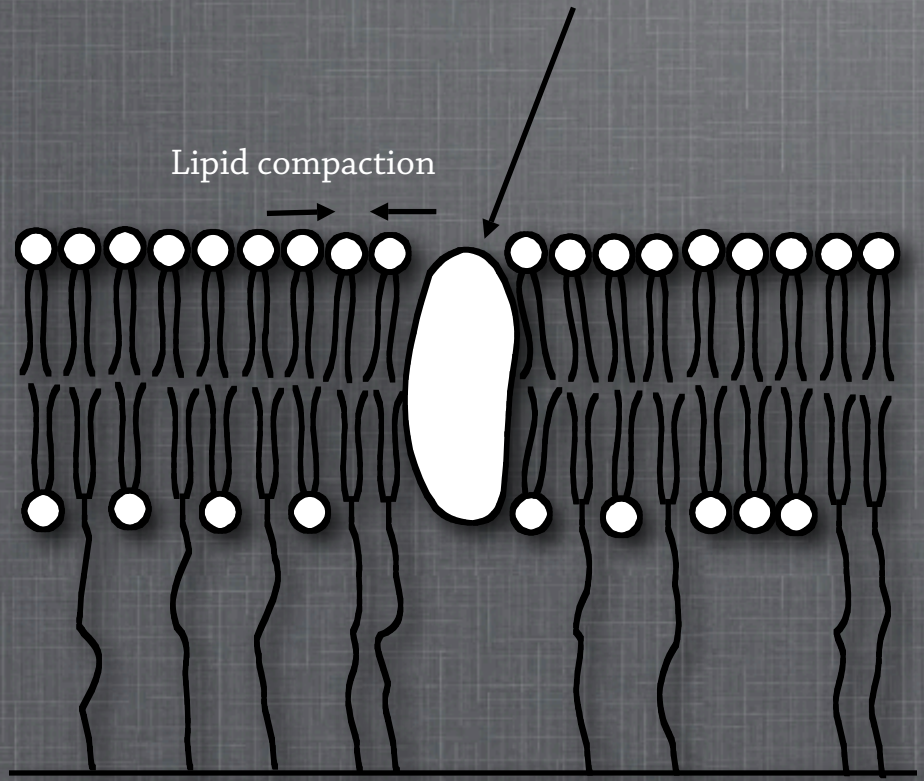


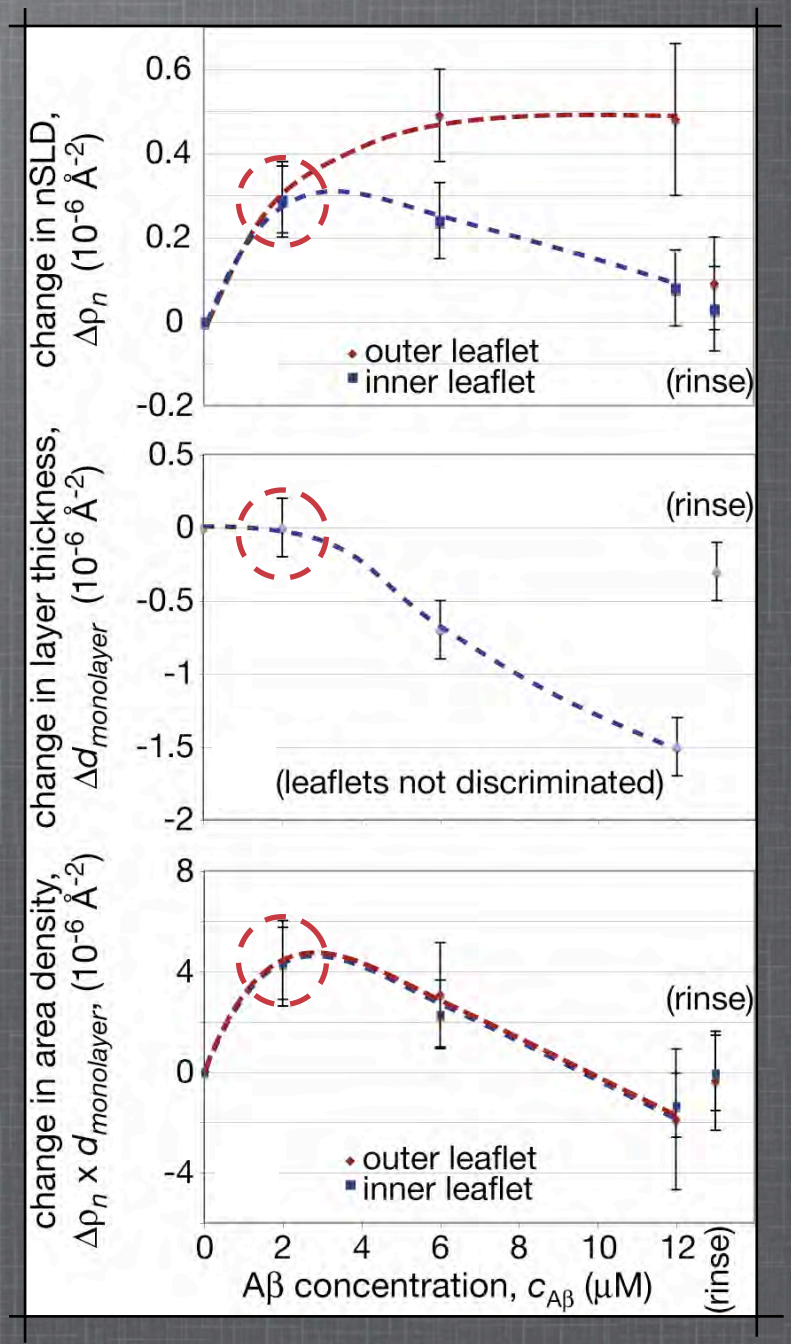


Valincius *et al.*, *Biophys. J., in press*

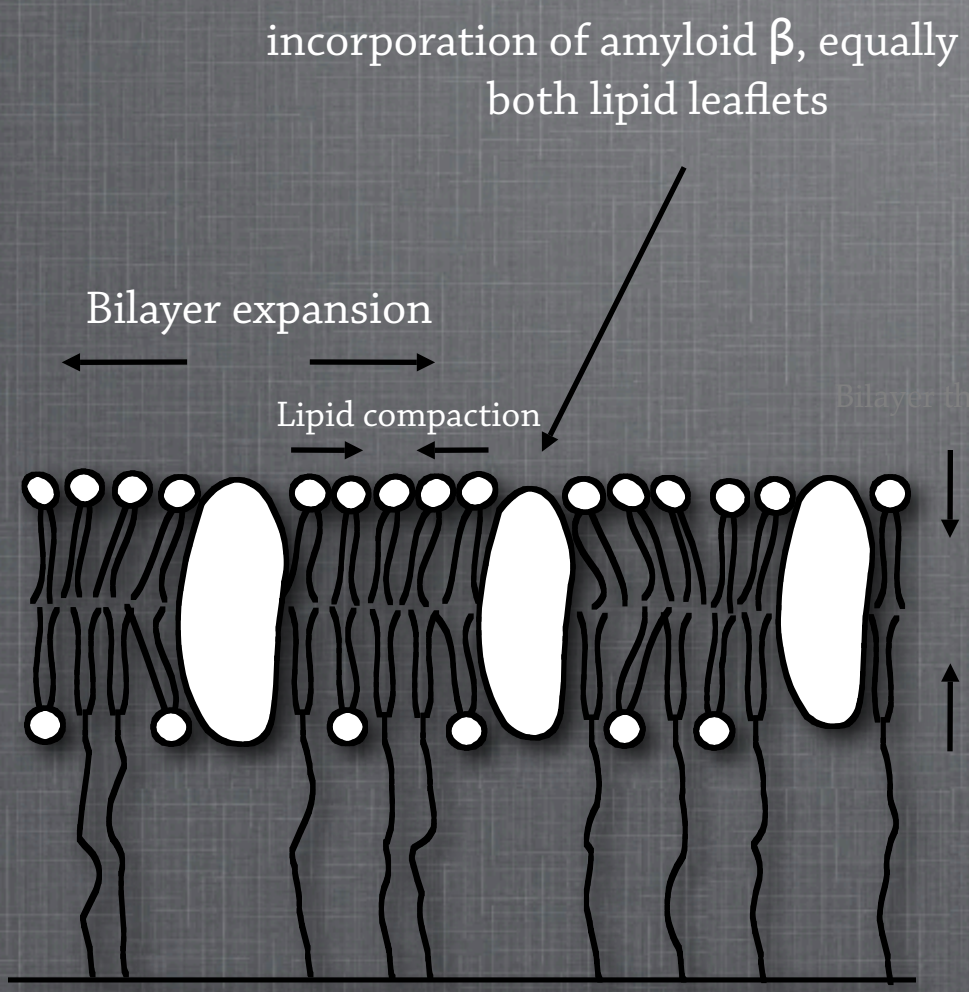
2 μM Aβ oligomers

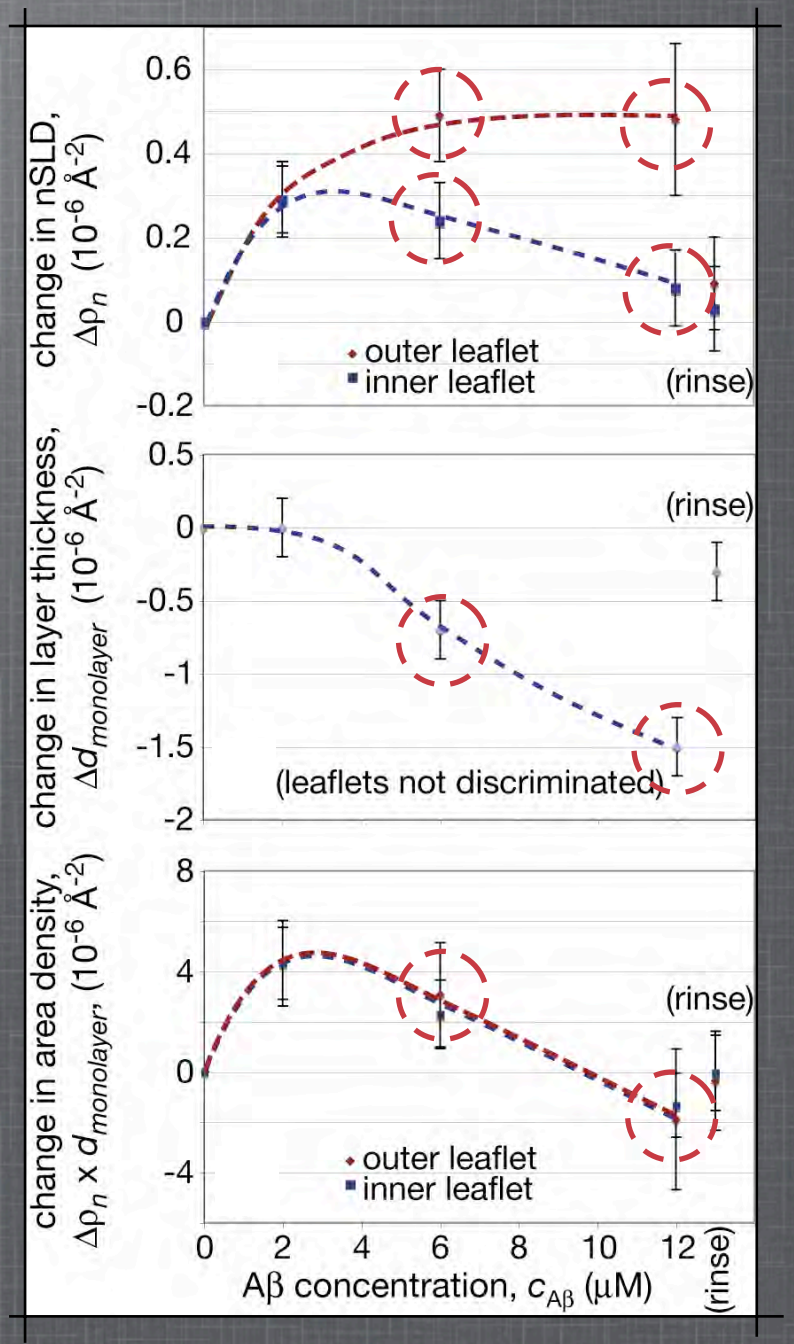
14%±5% incorporation of amyloid β,
equally into both lipid leaflets





6 μM and 12 μM Aβ oligomers



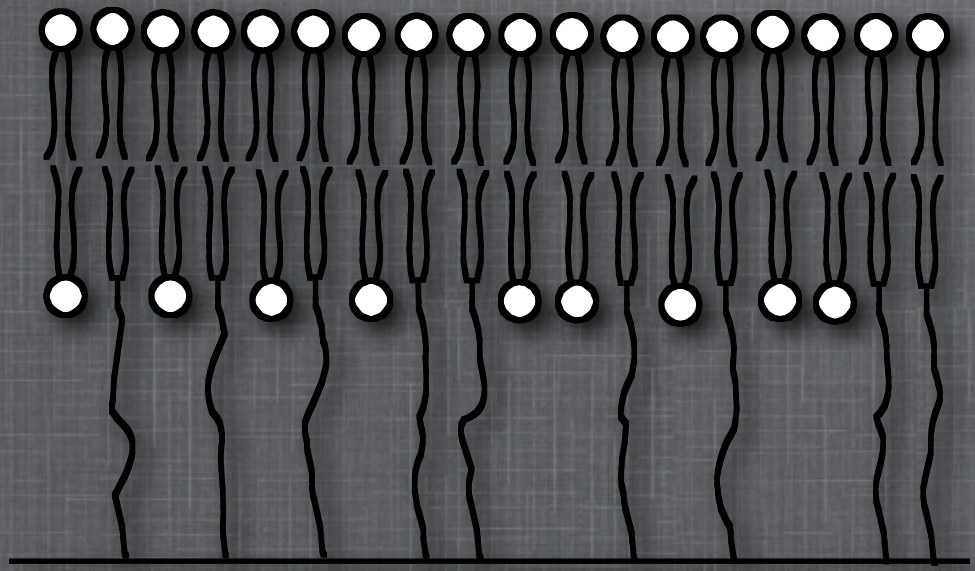


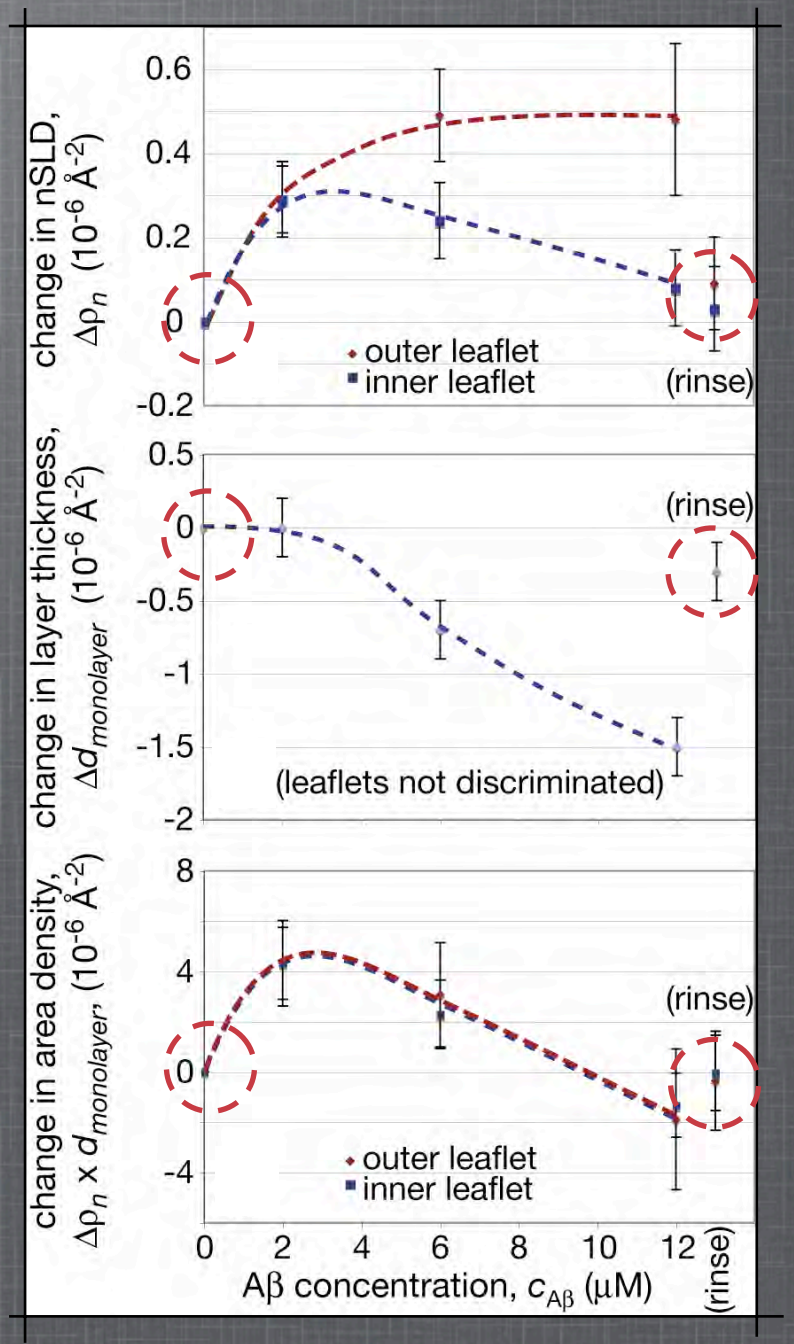
Buffer Rinse

Almost complete reversal of amyloid β impact within minutes.

↓

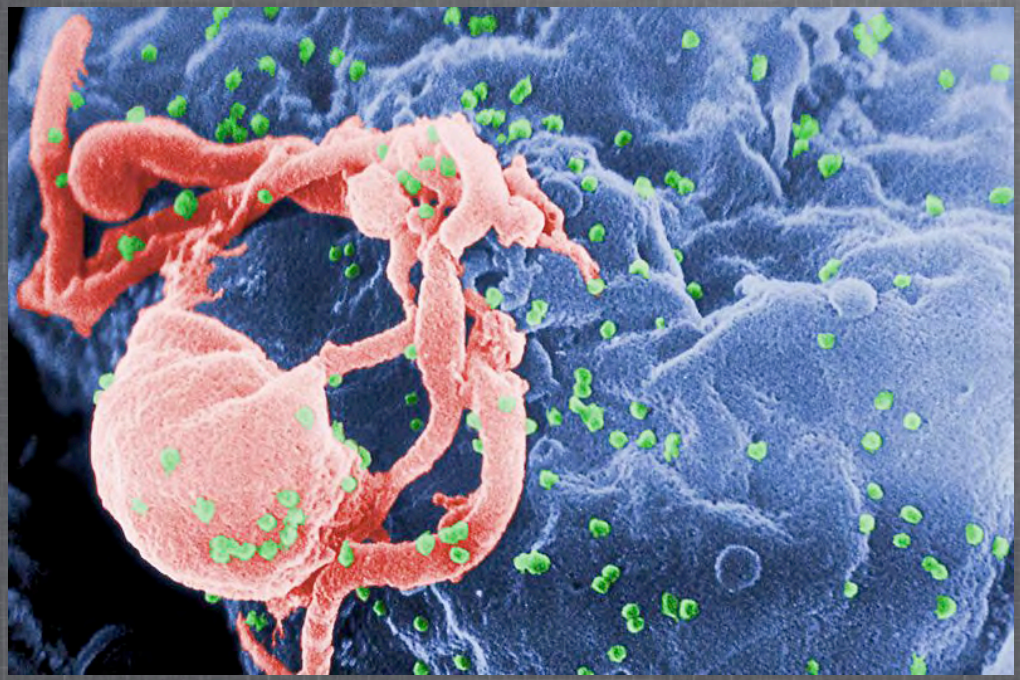
Fast equilibrium between bound and unbound state.



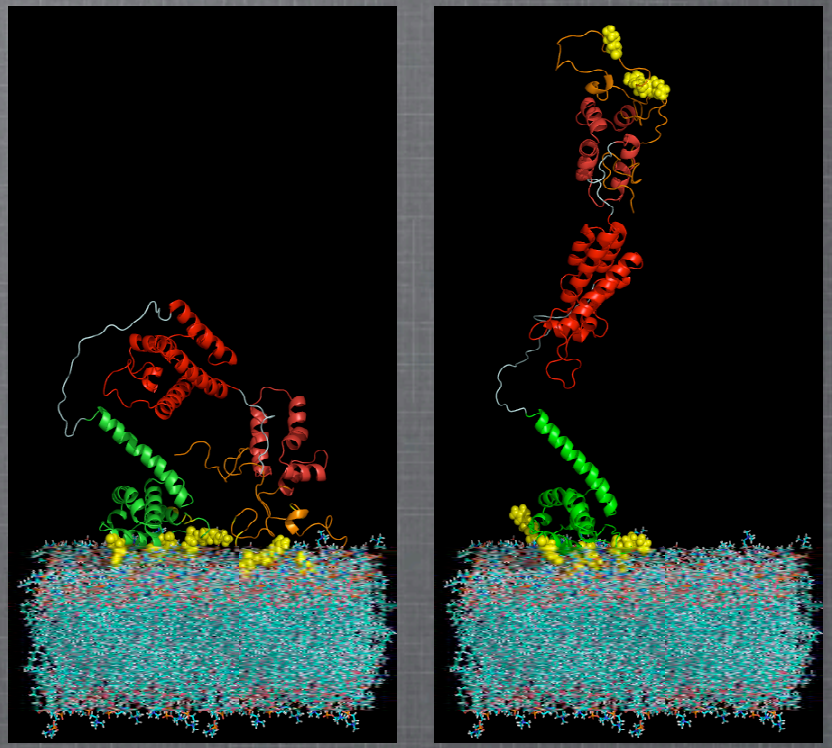


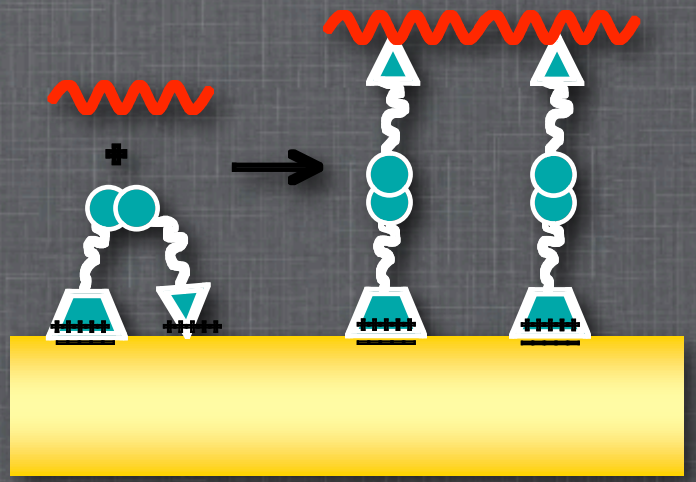
HIV-Gag interaction with tBLMs

with:
Sid Datta, Alan Rein,
National Cancer Institute, Frederick;
Susan Krueger, Joseph Curtis,
NIST, Gaithersburgh;



CDC/ C. Goldsmith, P. Feorino, E. L. Palmer, W. R. McManus





Acknowledgments

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Thank you very much
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Carnegie Mellon

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