EUROPEAN SPALLATION SOURCE

Studying biological surfaces with neutrons

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Instrument Scientist – Neutron Reflectometry

www.europeanspallationsource.se

NBIA4, November 10, 2014

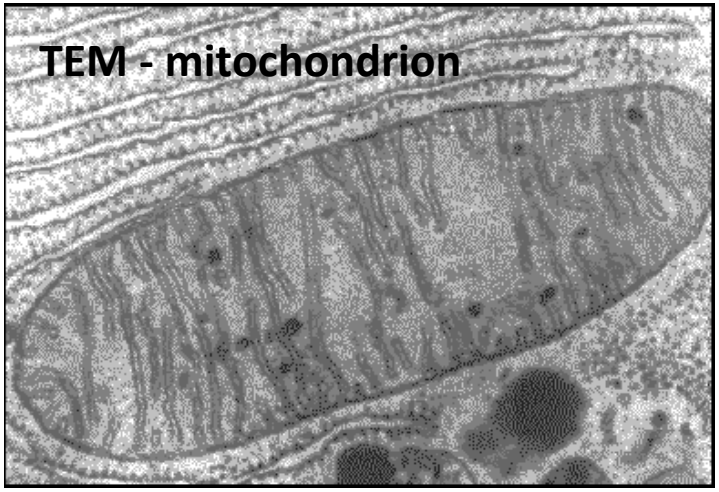
Cell membranes are the fabric of life

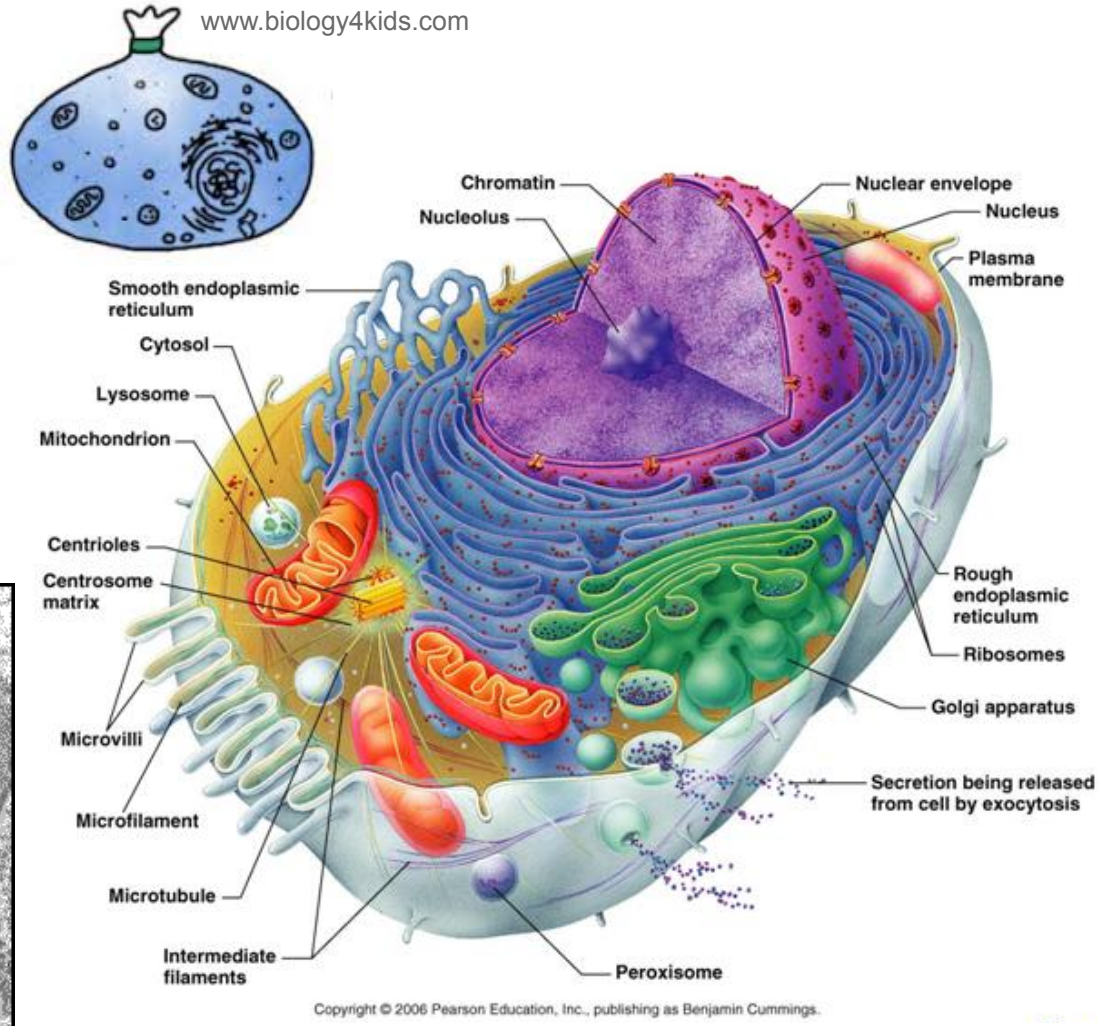
- The cell membrane controls traffic in and out of cell = metabolism, defence against intruders, response to drugs etc.
- Organelles within the cell are defined by membranes

⇒ COMPARTMENTALISATION

- allows functional specialisation
- maintains diverse protein biochemistry

Cells membranes are very small:





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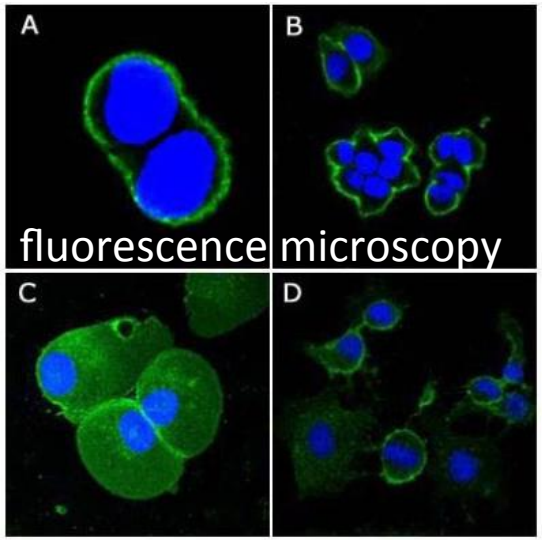

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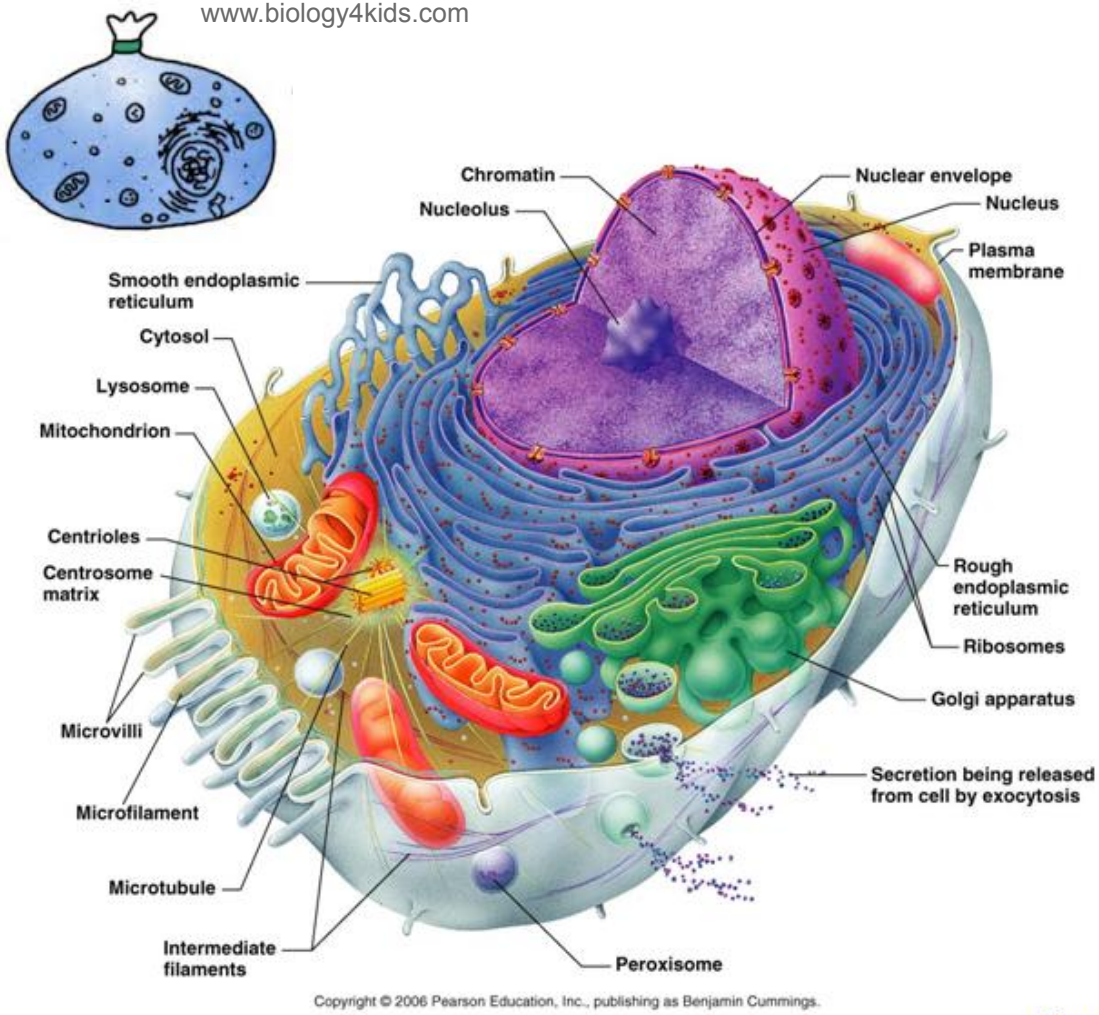

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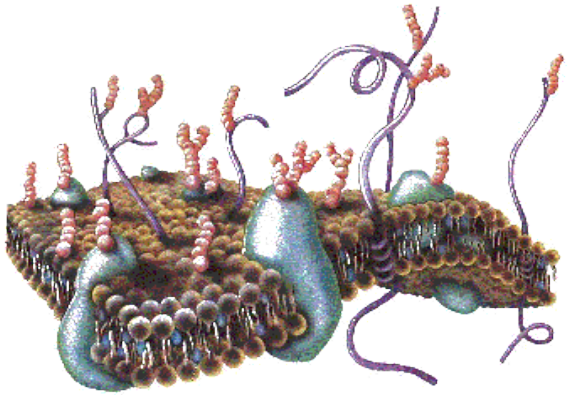
Cells membranes are also very complex assemblies of lipids and proteins:



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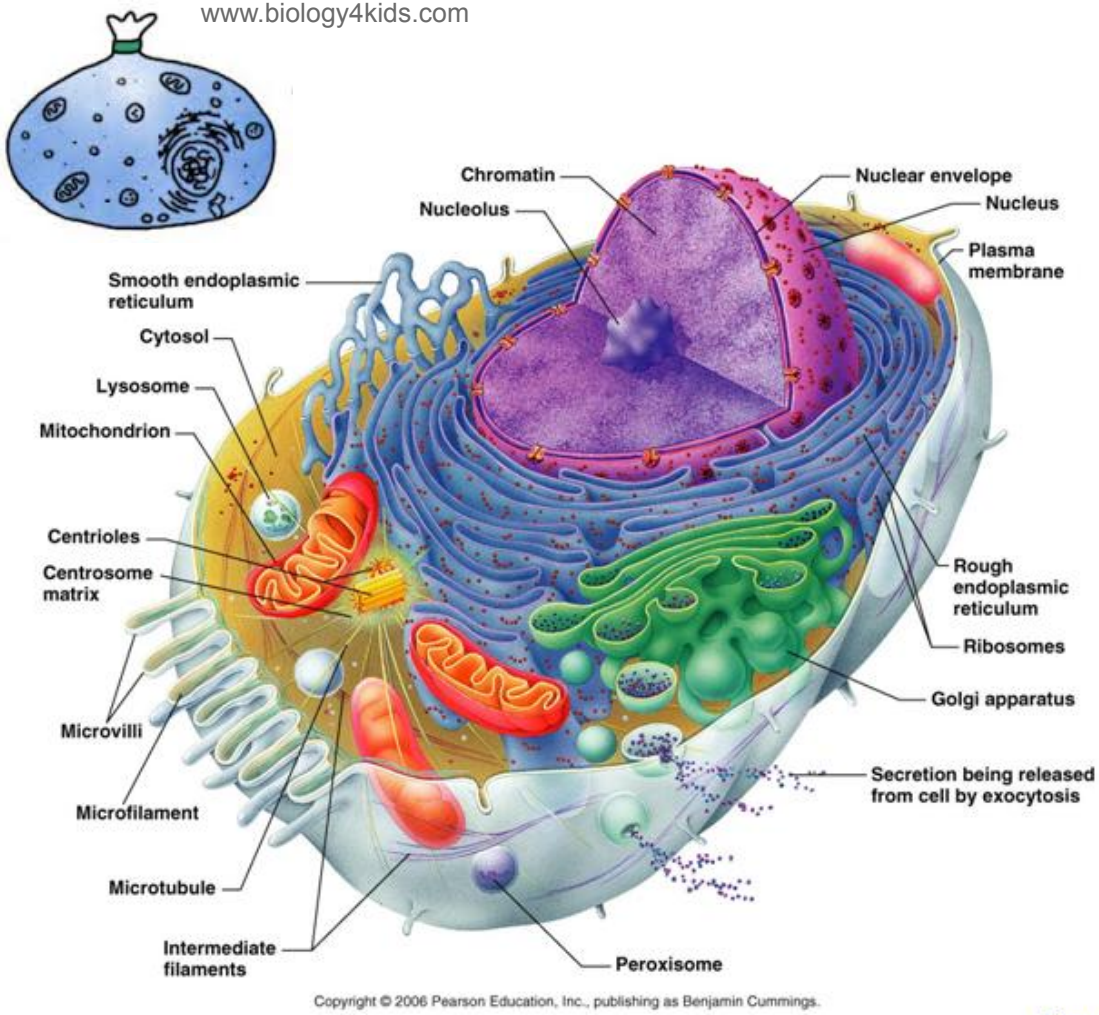
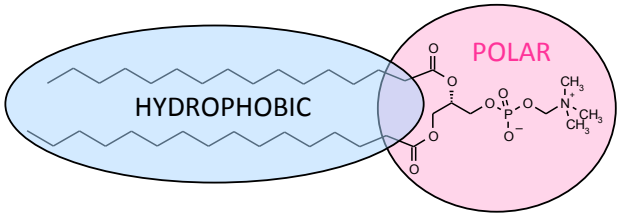
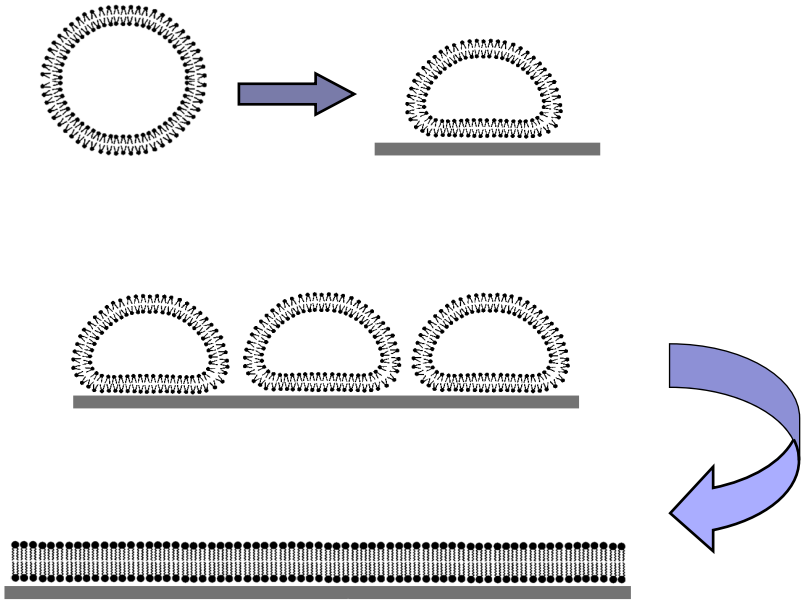


Modeling cell membranes

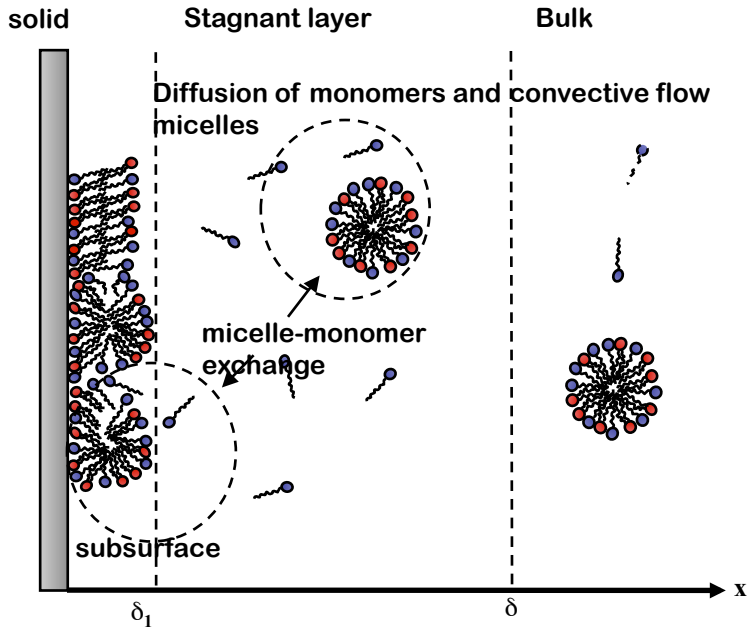
- Cell membranes very small, complex and require an aqueous environment
- Lipids are amphiphilic and self-aggregate
- Model membranes of can be built by lipid self-assembly
- Allow investigation of structure, role of components etc.



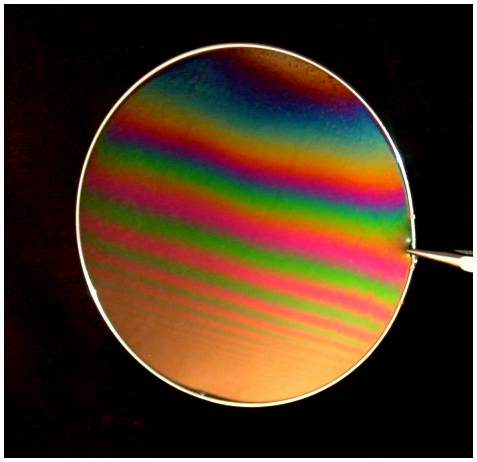
Vesicle adsorption



Surfactant reconstitution

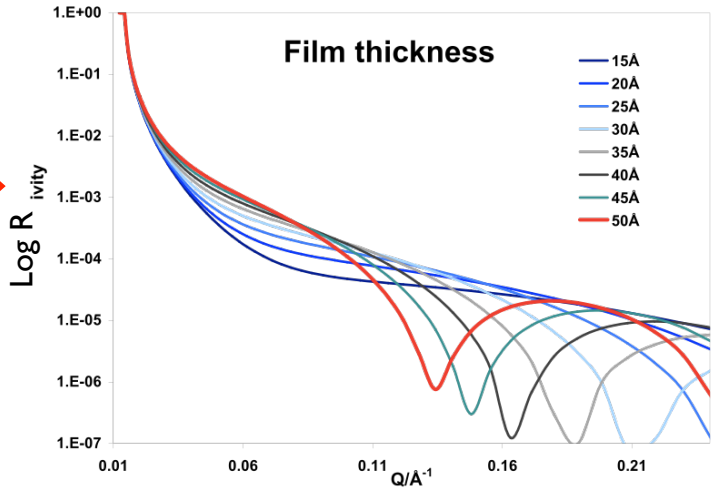


Reflecting neutrons off membranes



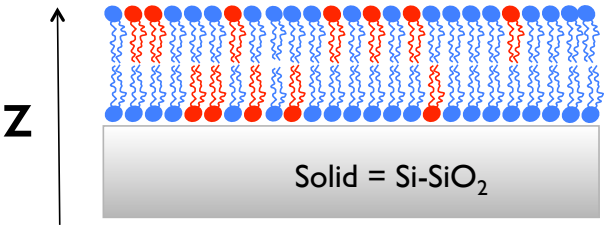
Neutron wavelength << light wavelength

⇒*nano*thin films e.g. lipid membranes (~ 5nm)



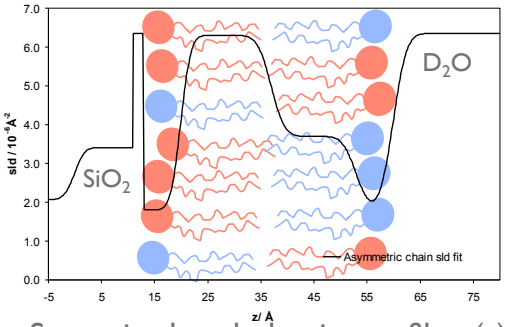
$Q = 4\pi\sin\theta/\lambda$

liquid = D_2O



Model structure of lipid membrane
= layered model of structure & composition

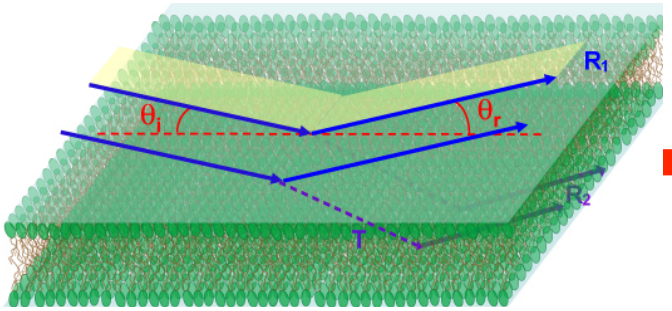
Optical Matrix modeling

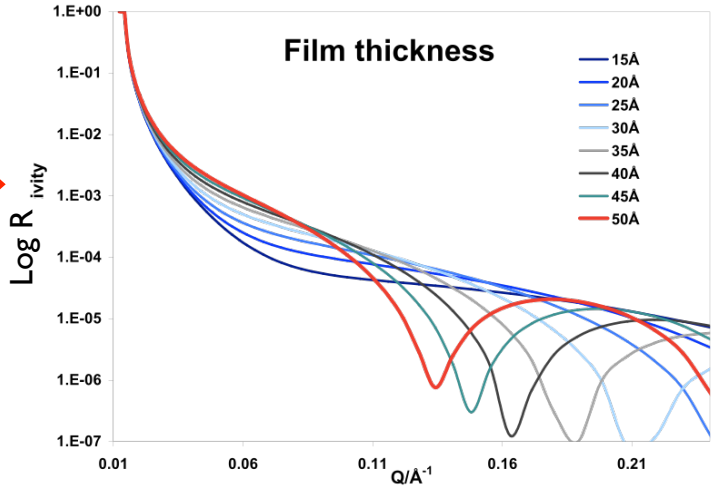


Scattering length density profile ρ(z)
= neutron refractive index profile

Reflecting neutrons off membranes

Thickness sensitivity ~2Å





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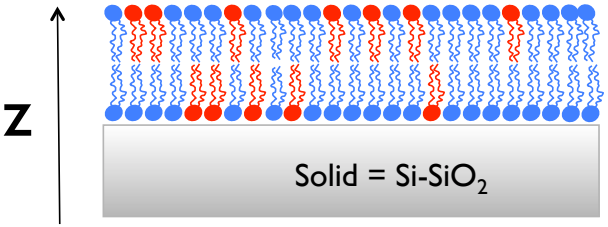

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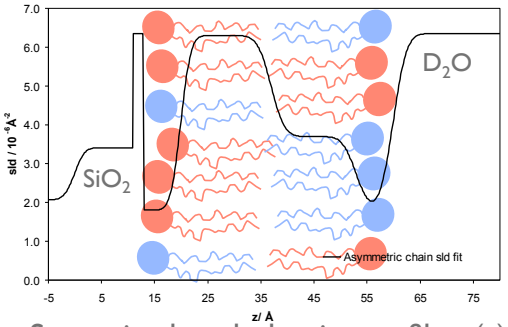

Scattering length density profile ρ(z)
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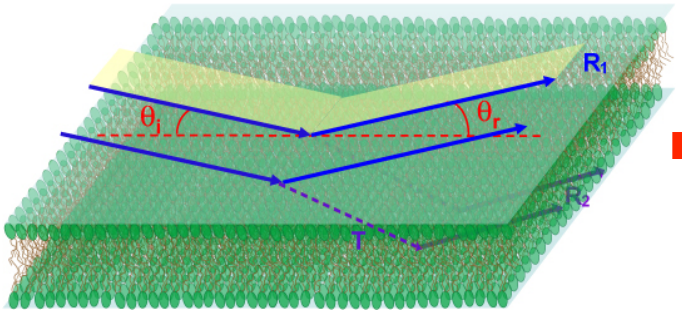
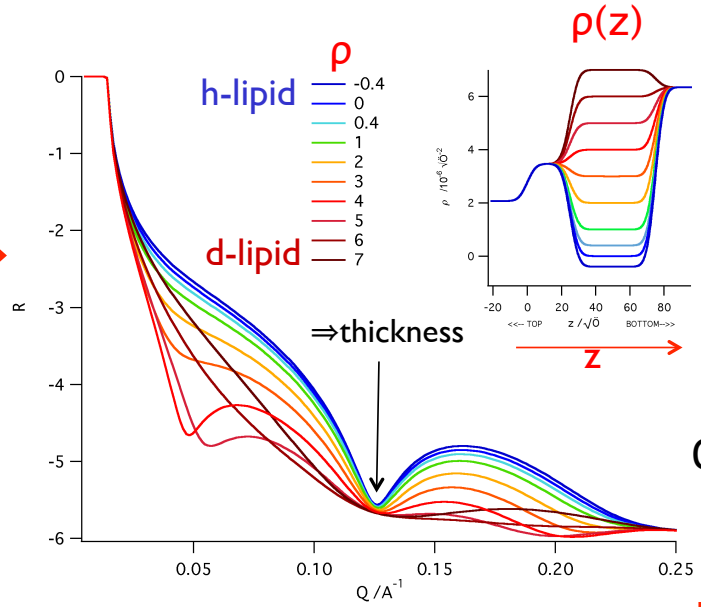
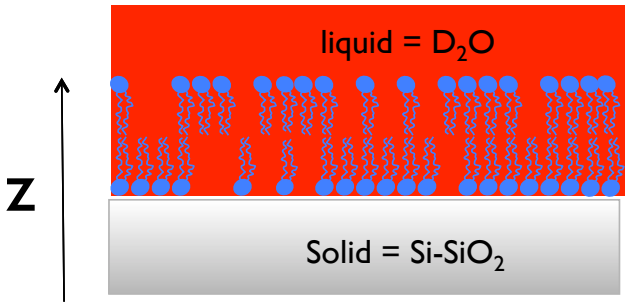
Reflecting neutrons off membranes

Composition sensitivity ~ 3-5 Vol%





Q = 4πsinθ/λ



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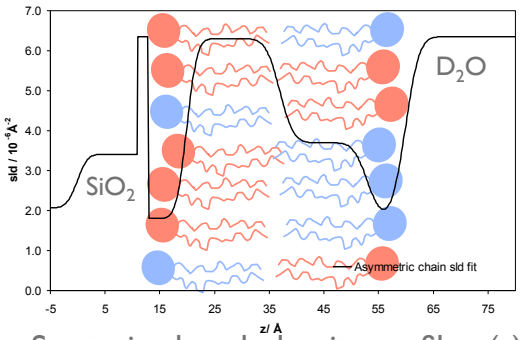

Scattering length density profile ρ(z)
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Reflecting neutrons off membranes

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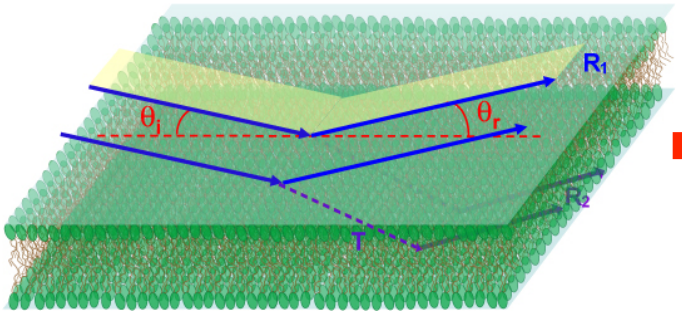
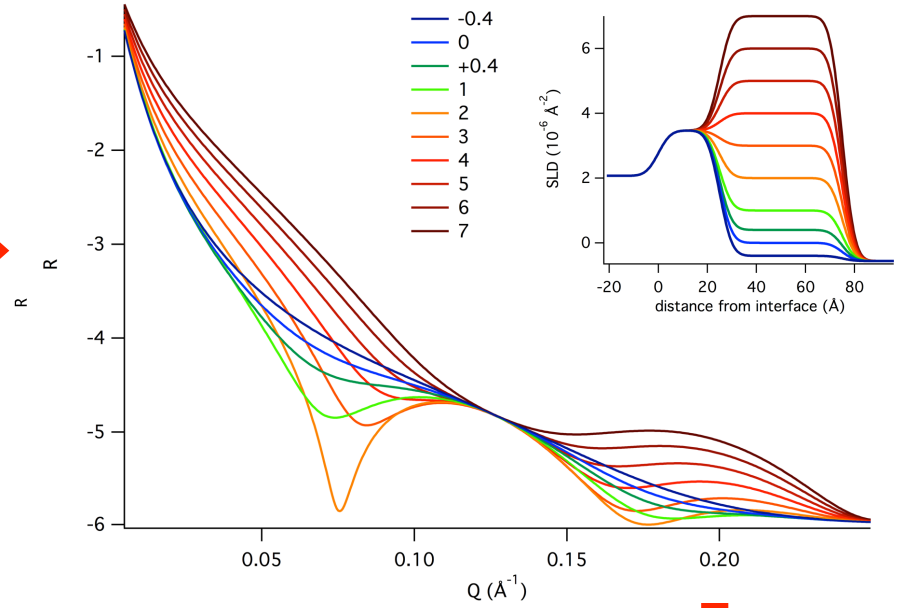
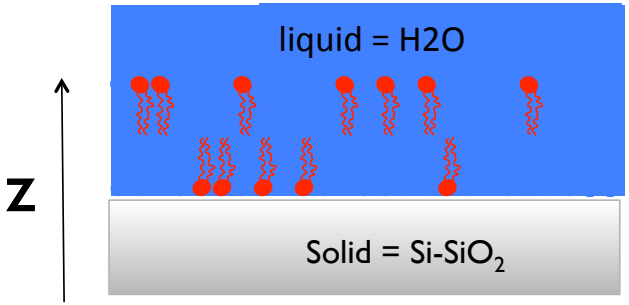
Contrast determines sensitivity







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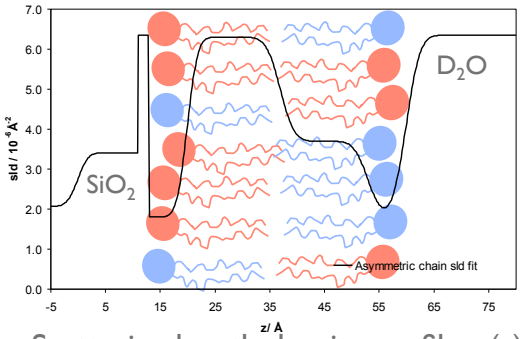

Scattering length density profile ρ(z)
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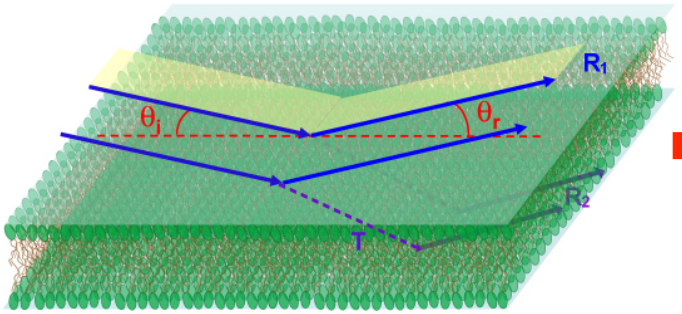
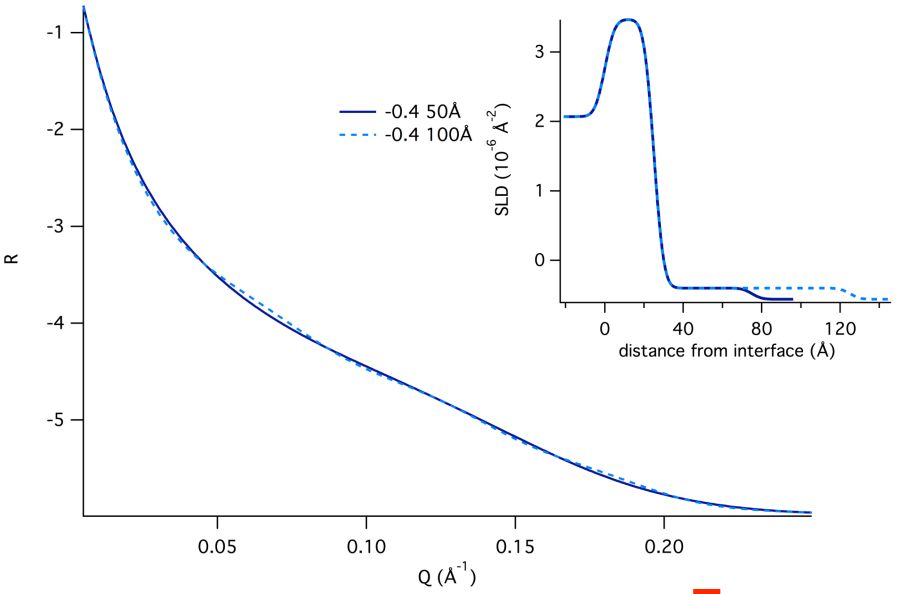
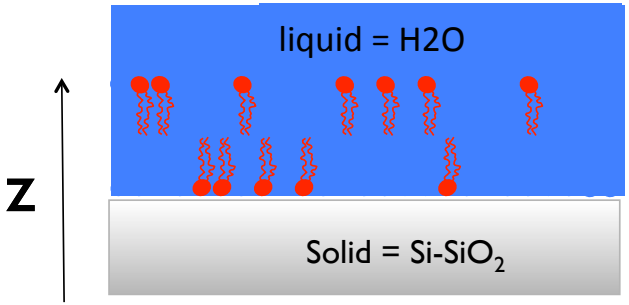
Reflecting neutrons off membranes

A poor contrast with H2O:







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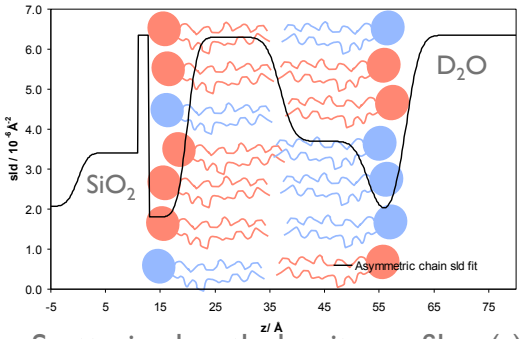

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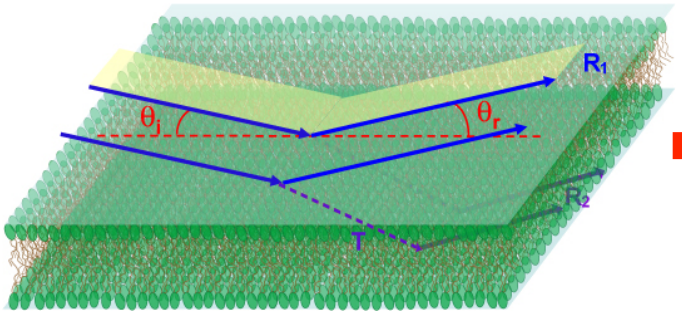
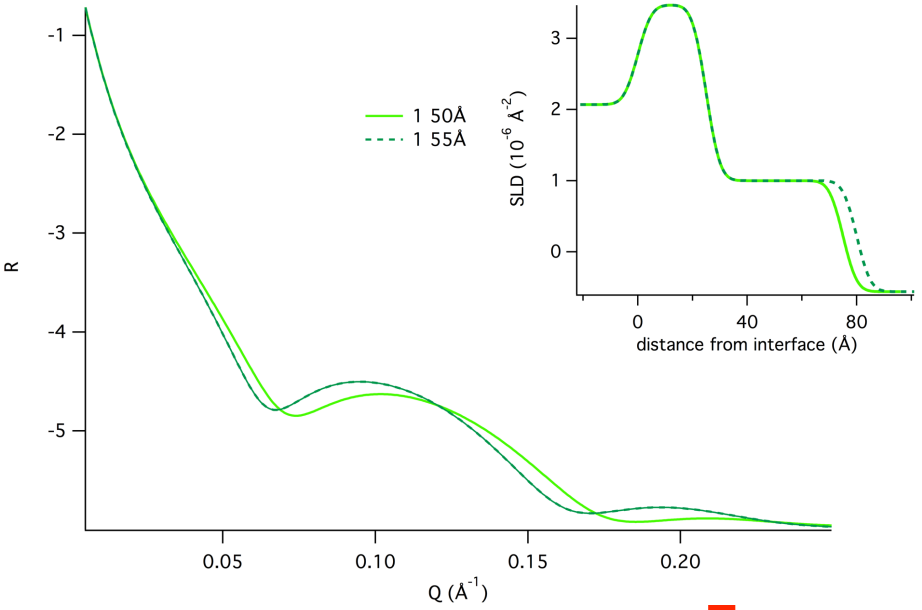
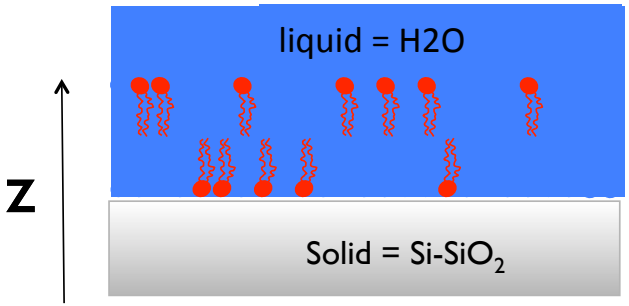
Reflecting neutrons off membranes

Only small contrast required for thickness sensitivity:







Model structure of lipid membrane
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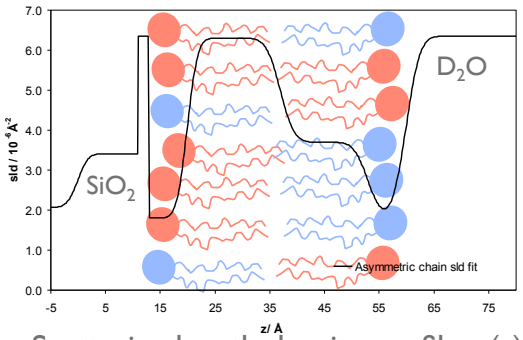

Scattering length density profile ρ(z)
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Summary:

- Reflectivity can distinguish thickness changes of 2-3Å
- Reflectivity can distinguish composition differences of 3-5 mol%
- Sensitivity = Contrast, achieved by deuterium labeling
- Each contrast is uniquely sensitive to components/ features that have good contrast
- Thickness sensitivity depends on contrast

Examples

- Lipid membrane structure
- Insertion mechanism of peptides in membranes
- Protein conformation at membrane surfaces
- Enzyme catalysis in a membrane

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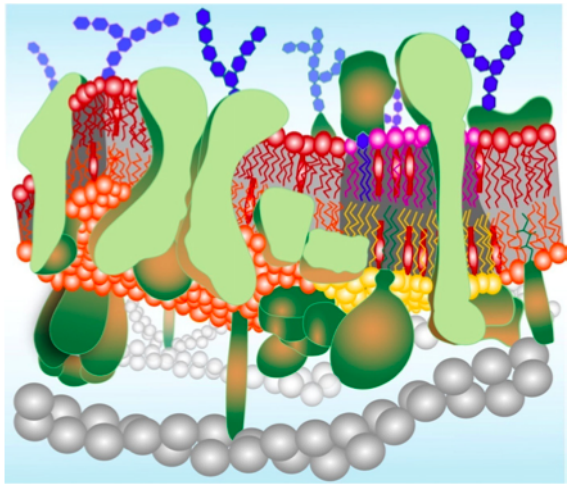
Membrane Asymmetry



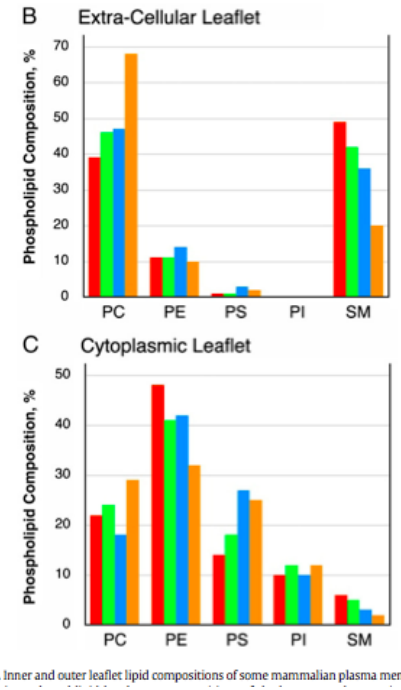
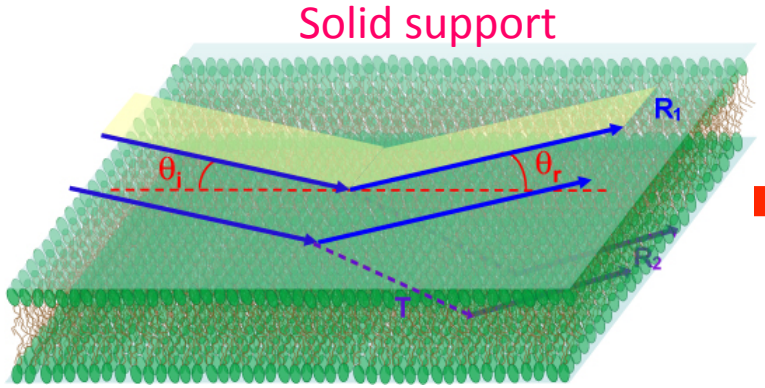


Fig. 2. Inner and outer leaflet lipid compositions of some mammalian plasma membranes.

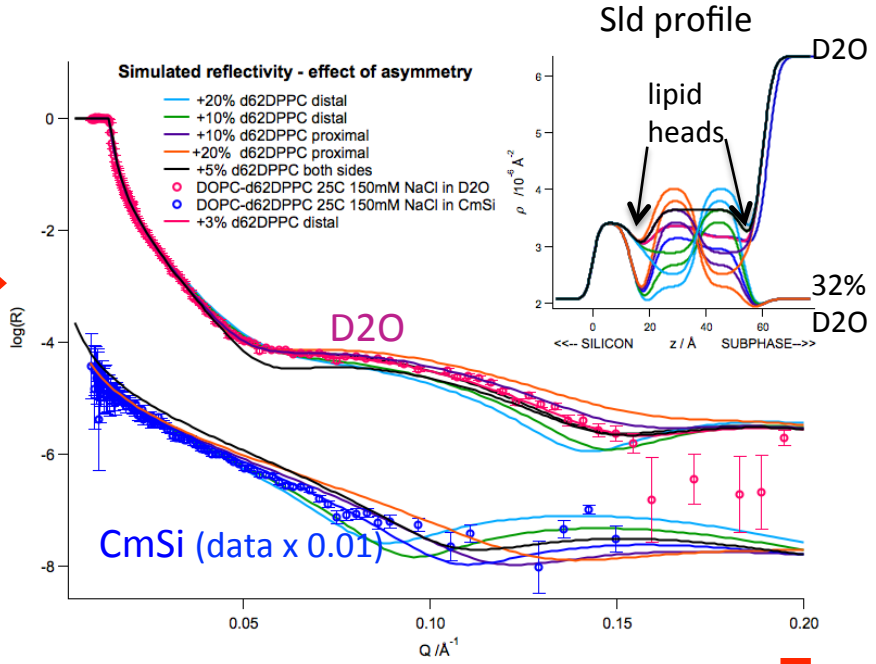
- Cell membranes are asymmetric in composition
- Natural asymmetry is maintained by lipid transporting proteins
- Role in cell biochemistry and signalling – e.g. loss of negatively charged PS asymmetry in cell death
- Lipid asymmetry may be induced by proteins or membrane curvature
- Can asymmetry be established by lipid self-assembly?

V.Kiessling, C.Wan, L.K.Tamm, BBA 1788 (2009), 64-71

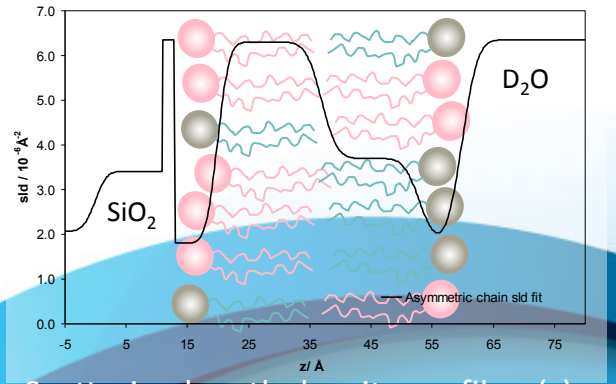
1. Membrane Structure



Buffer solution



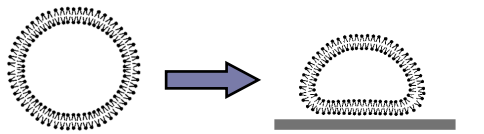
Monolayer structure can be resolved if the difference in layer composition is ~10% (with support structure as reference, using contrast variation)

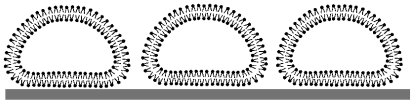


Scattering length density profile ρ(z)
= neutron refractive index profile

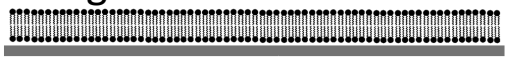
Lipid arrangement in model membranes

Vesicle adsorption



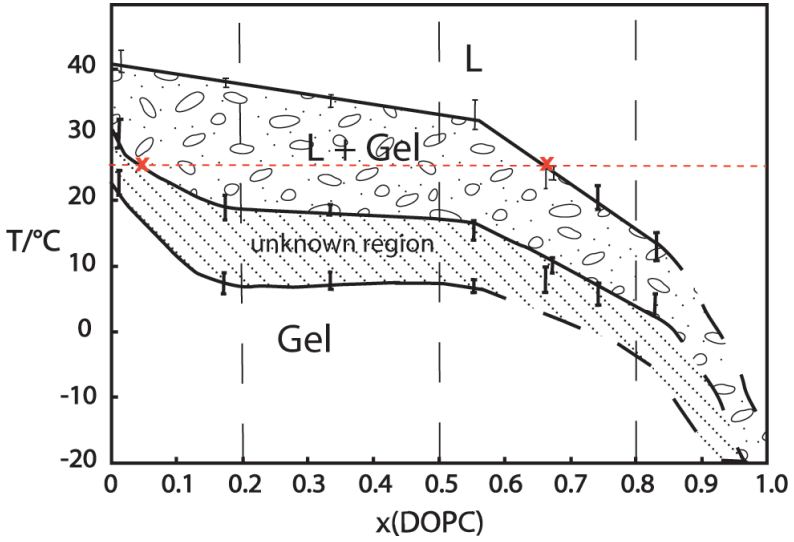


what happens to molecular arrangement?

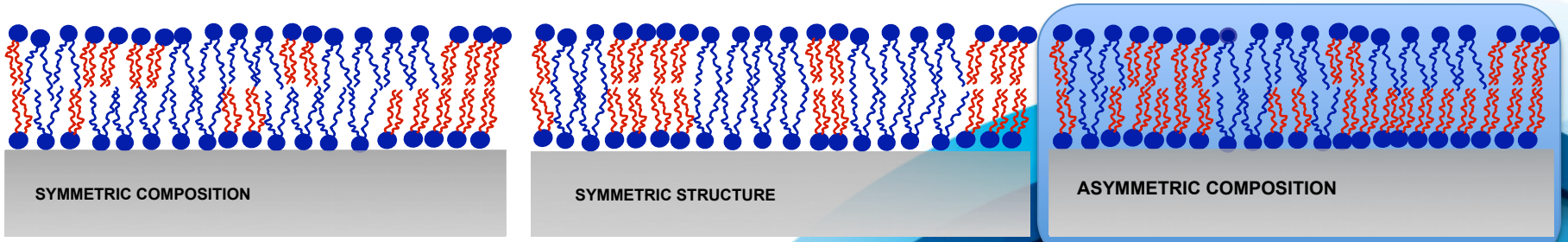


How is the lipid arrangement in vesicles transferred to surfaces?

Many lipid mixtures exhibit partial immiscibility: e.g. DOPC-DPPC:

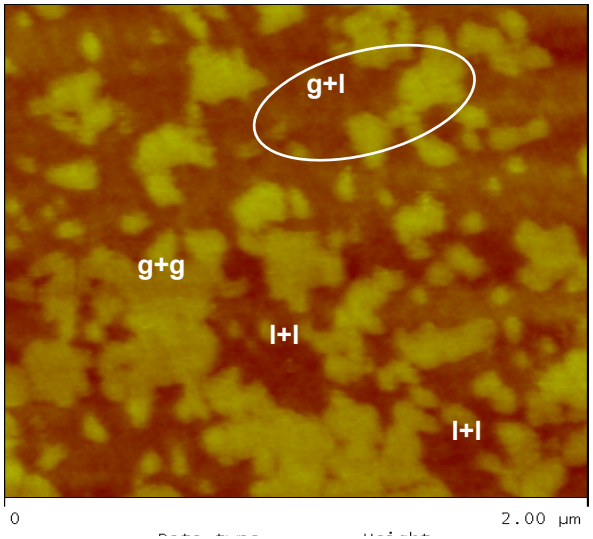


Three possibilities:



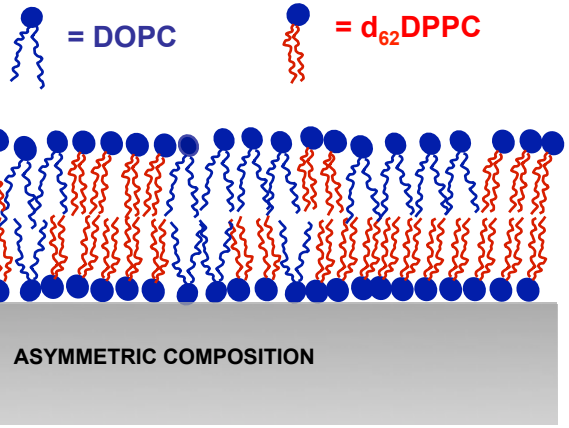
Lipid spreading by vesicle fusion:

Domain topography (AFM): 3 heights

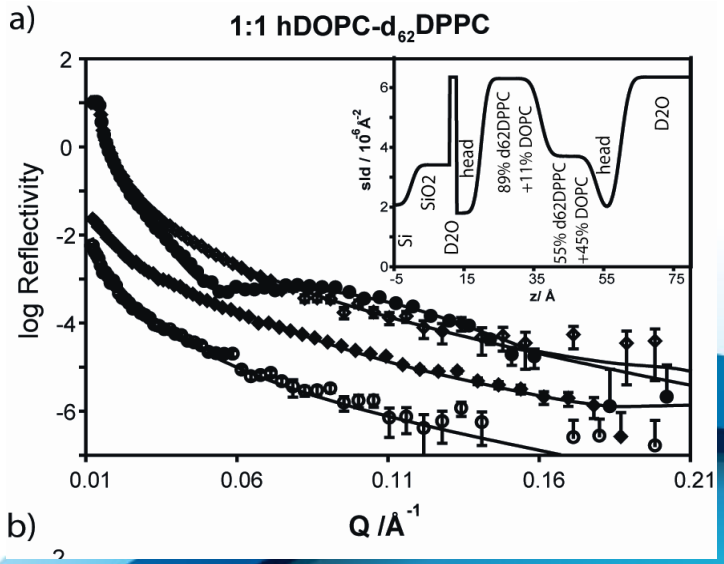


composition in water

x_{DOPC} vesicles	x_{DOPC}in	x_{DOPC}out	x_{DOPC}tot
0.2	0	0.08	0.04
0.5	0.11	0.45	0.28
0.8	0.2	0.7	0.45



1:1 hDOPC-d_{62}DPPC (50°C)



Wacklin & Thomas (2007), Langmuir 23, 7644-7651

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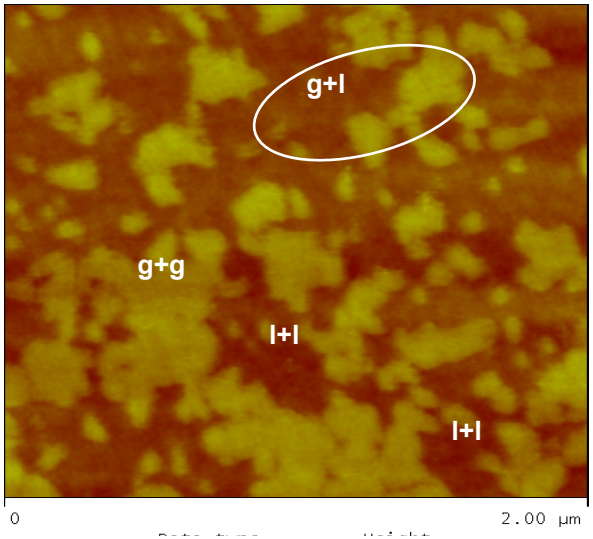

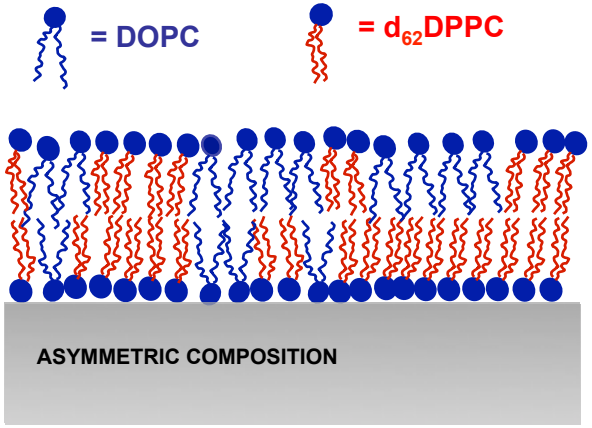

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0.5	0.11	0.45	0.28
0.8	0.2	0.7	0.45

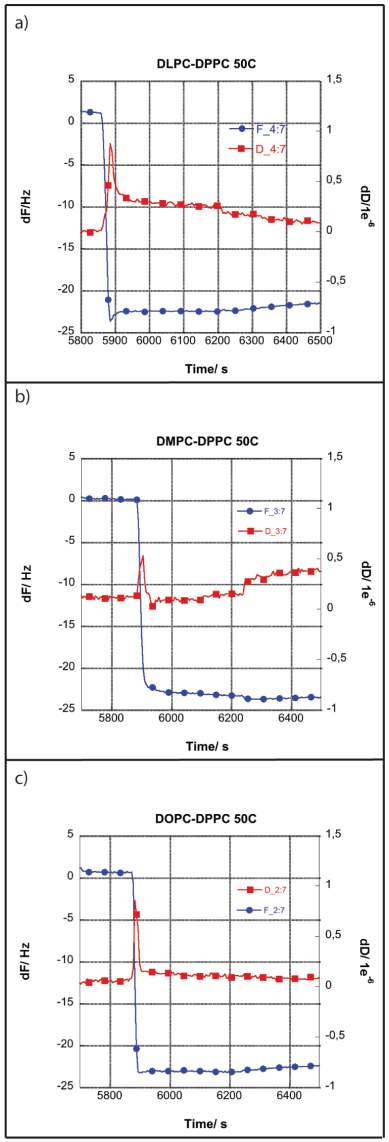
in 100mM NaCl solution

x_{DOPC} vesicles	x_{DOPC}in	x_{DOPC}out	x_{DOPC}tot
0.2	0.19	0.23	0.21
0.5	0.46	0.49	0.475
0.8	0.62	0.72	0.67

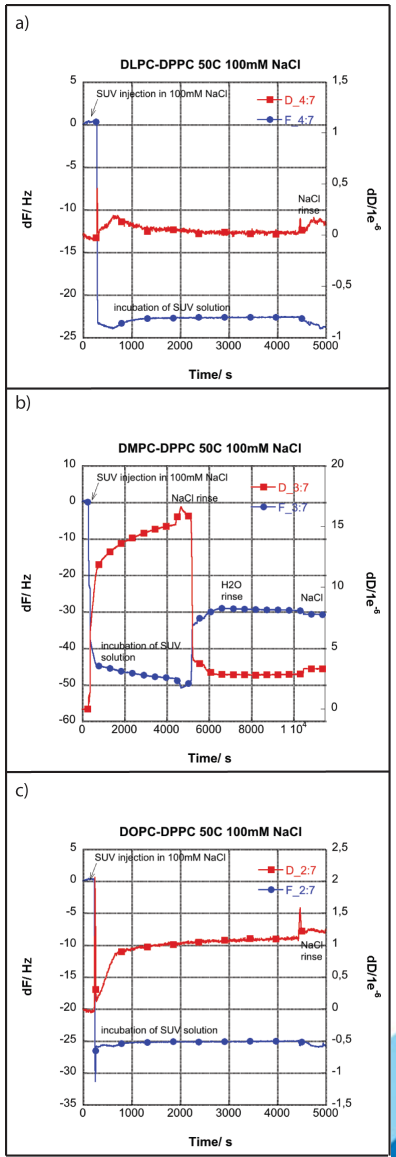
Wacklin & Thomas (2007), Langmuir 23, 7644-7651

Membrane formation – QCM-D

no salt



100mM NaCl



Membrane formation pathway depends on salt screening:

- in 100mM NaCl a large number of vesicles are in contact with the bilayer

⇒ Lipid exchange

Asymmetry result of competing effects:

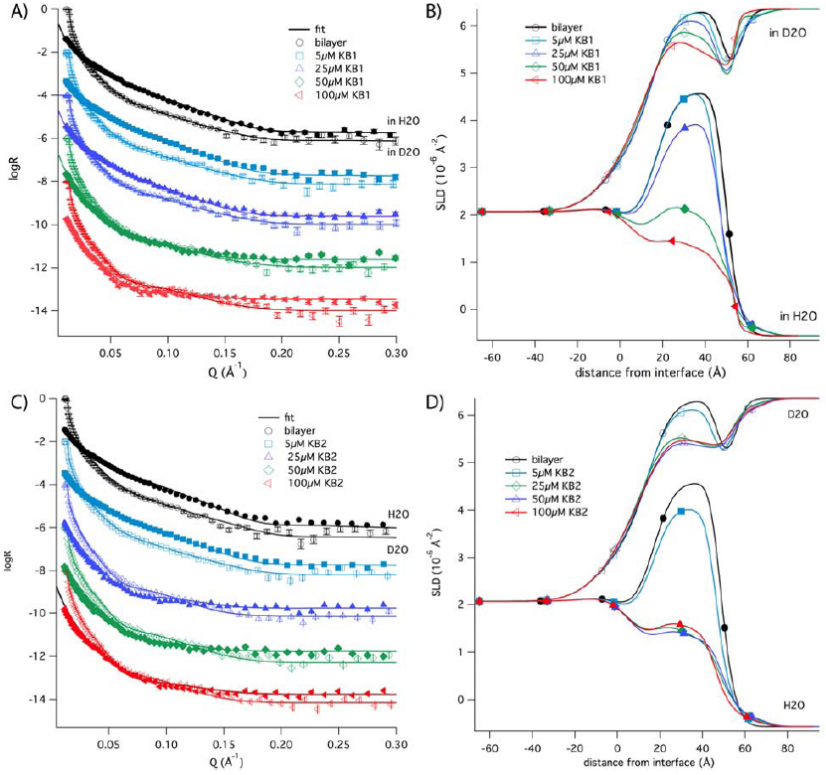
- Lipid flip-flop fast in incomplete bilayer
- Rate of vesicle fusion/ spreading increases with T

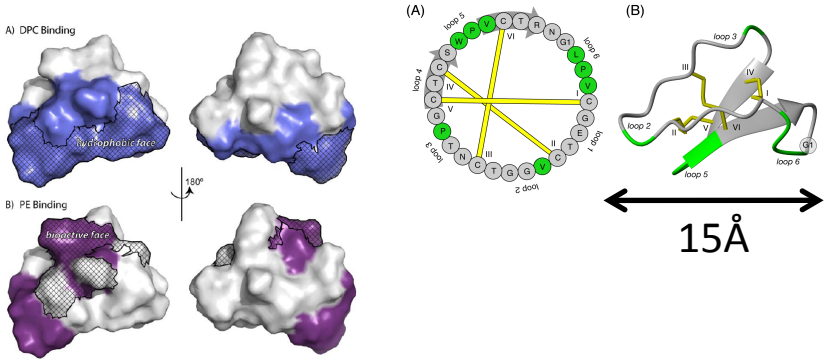
H.Wacklin, Langmuir, **2011**, *27* (12), pp 7698–7707

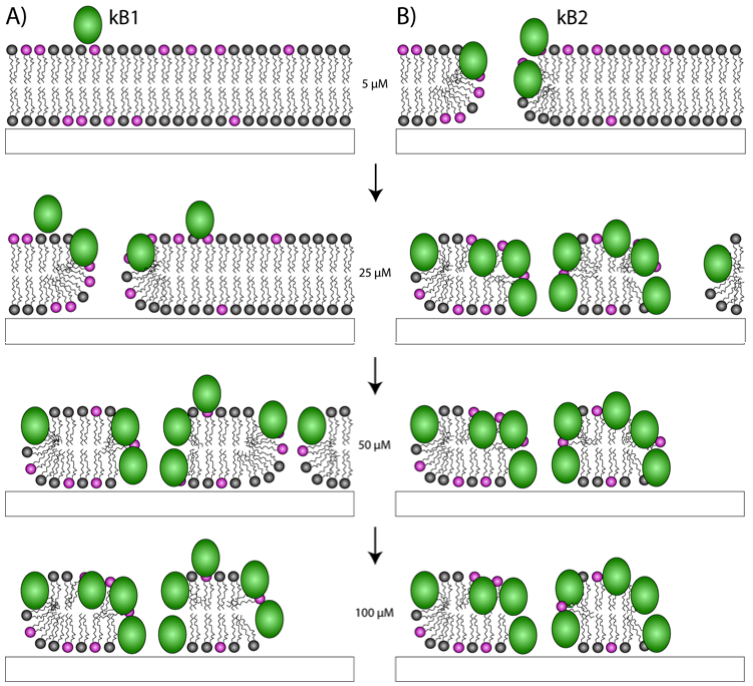
Kalata B1/B2 – plant cyclotides

Conan Wang/David Craik
Institute for Molecular Bioscience
UQLD Brisbane

- Circular peptides are found in many plants – kill insect larvae
- Compact structures with hydrophobic patch - oligomers in solution

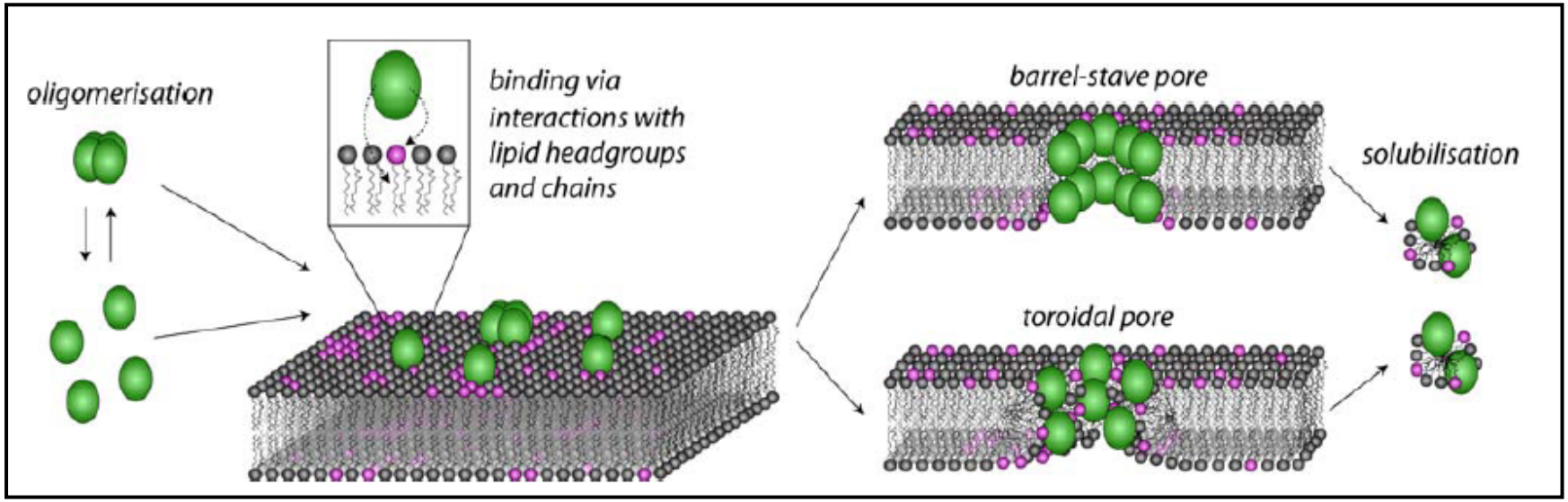






Kalata B1/B2 – plant cyclotides

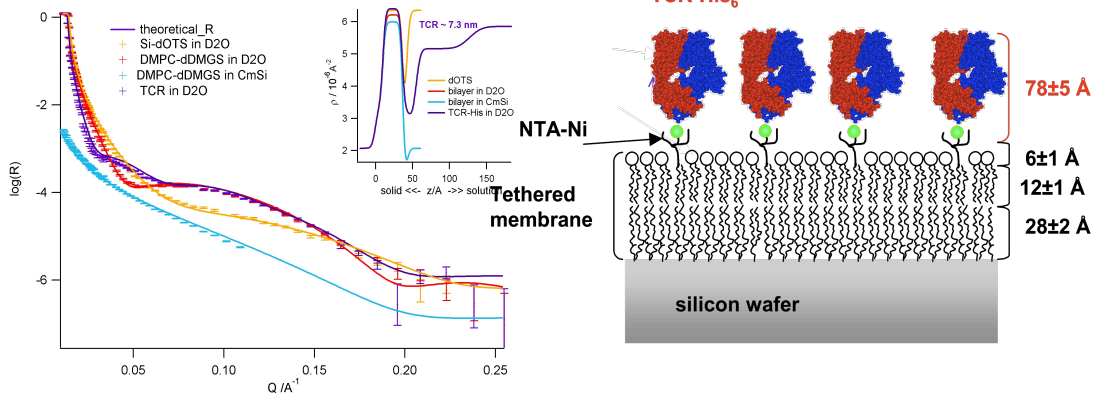
- *No correlation between the number of peptides* across the lipid bilayer during membrane insertion and pore formation – no organised peptide oligomers
- *A toroidal* pore structure involving both lipids and peptides

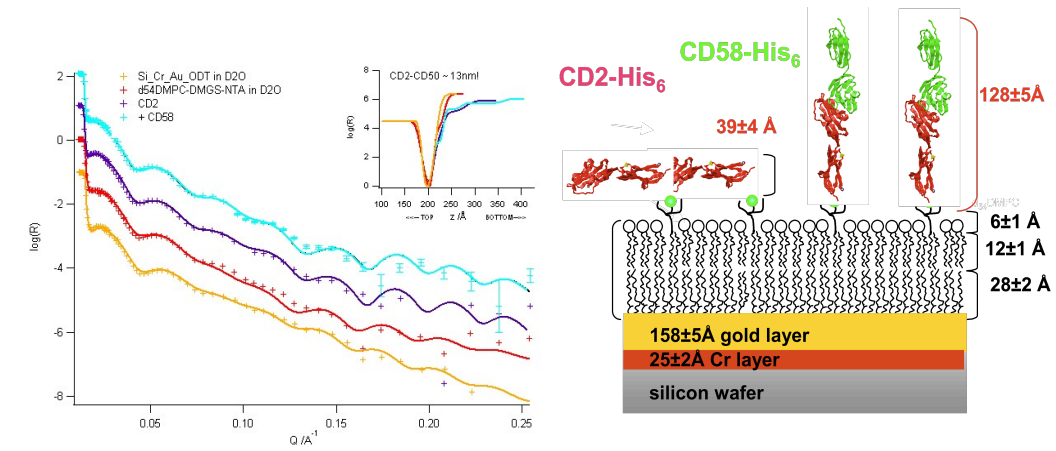


"Cyclotides Insert Into Lipid Bilayers to Form Membrane Pores and Destabilize the Membrane Through Hydrophobic and Phosphoethanolamine-specific Interactions" C.K.Wang, H.P.Wacklin, D.J.Craik (2012), JBC 287 (52) 43884-43898.

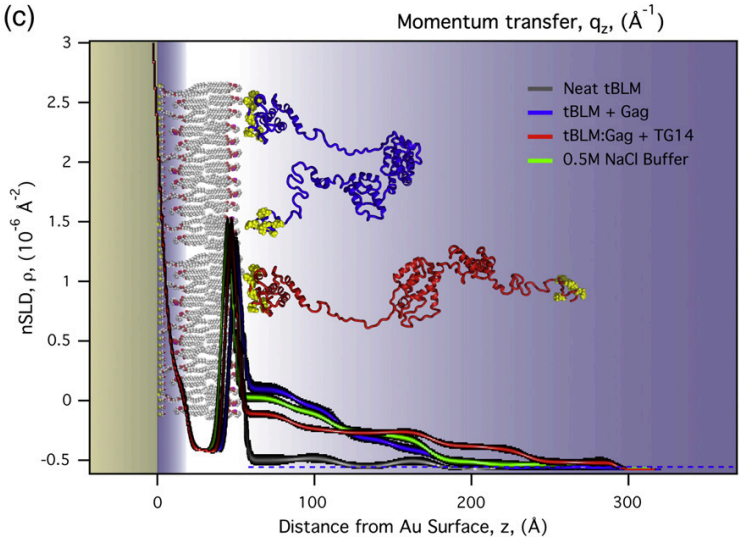
Protein conformation

Protein receptor extension from membrane





Conformational changes:

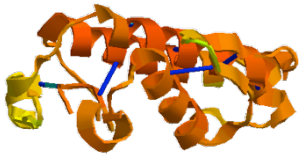


- HIV1-Gag fusion peptide conformation at membrane surface
- Conformational change upon binding DNA

Datta, S. A. K., Heinrich, F., et al. ,Journal of Molecular Recognition (2011) Volume 24, Issue 4, pages 724-732

A. van der Merwe, University of Oxford

PHOSPHOLIPASE A_2

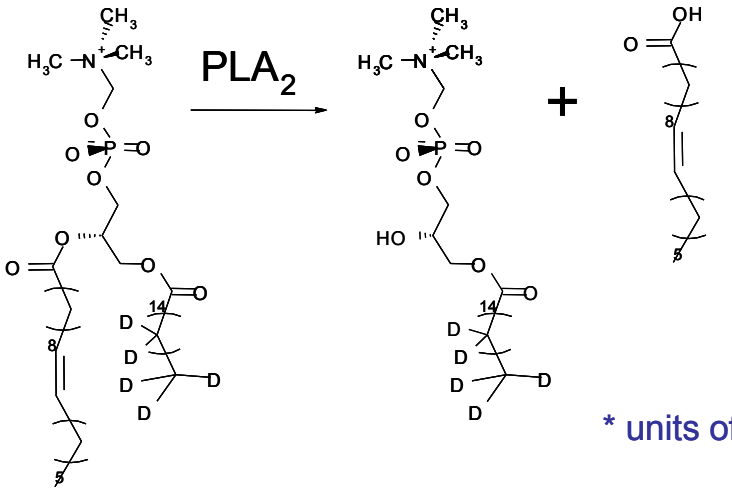


Naja mossambica mossambica

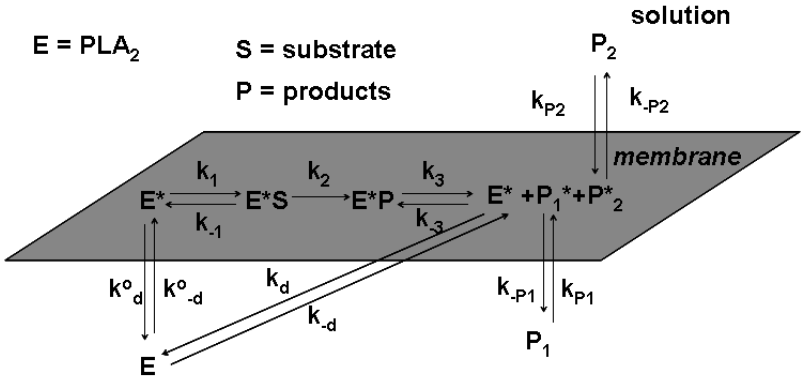
Breakdown of phospholipids:

d_{31}-POPC ⇒ d_{31}-lyso-PC + oleic acid

ρ = 3.17 ρ = 6.44 ρ = - 0.2*



* units of 10^{-6} Å^{-2}



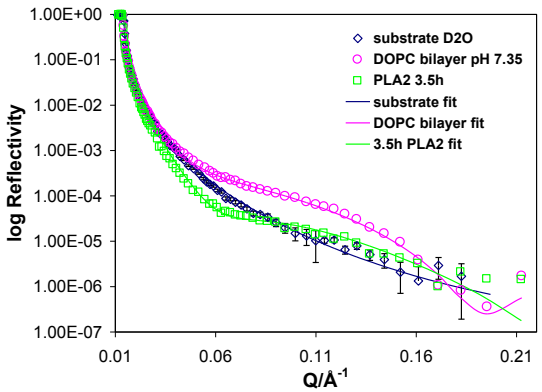
Interfacial enzyme catalysis: *a collective interaction* with membrane

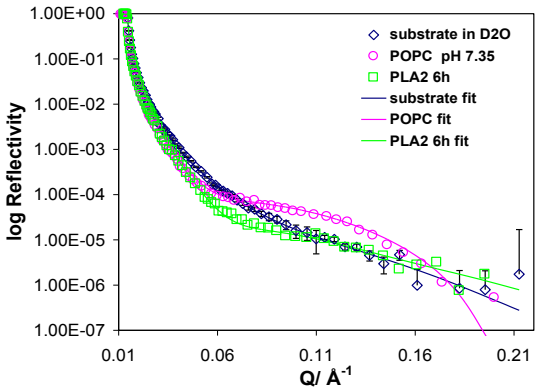
⇒ enzyme crystal structure is not enough to understand mechanism of PLA_2

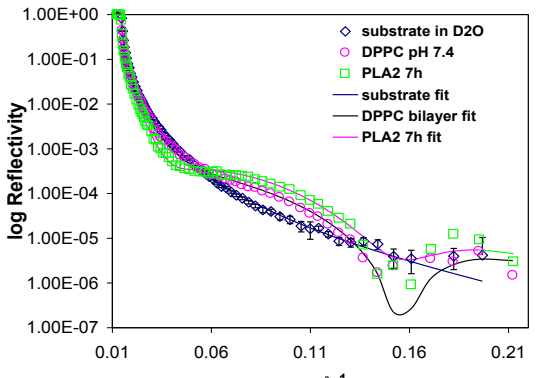
Insoluble lipid products change membrane composition ⇒ activate/inhibit PLA_2

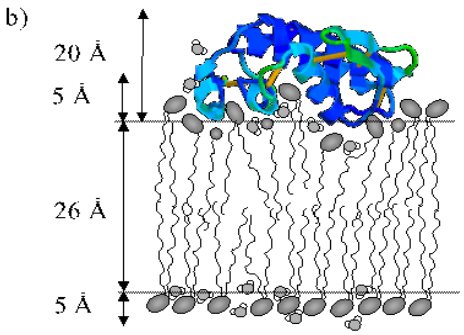
Location of enzyme during catalysis:

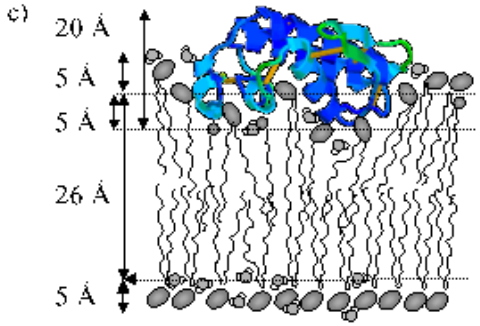
DOPC **POPC** **DPPC**

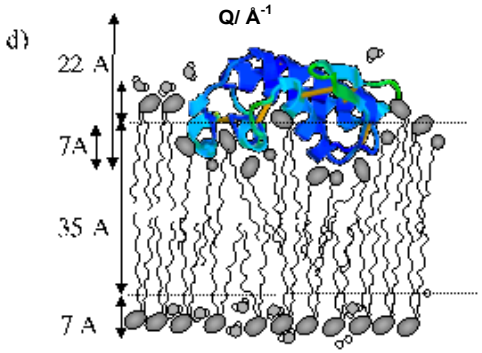








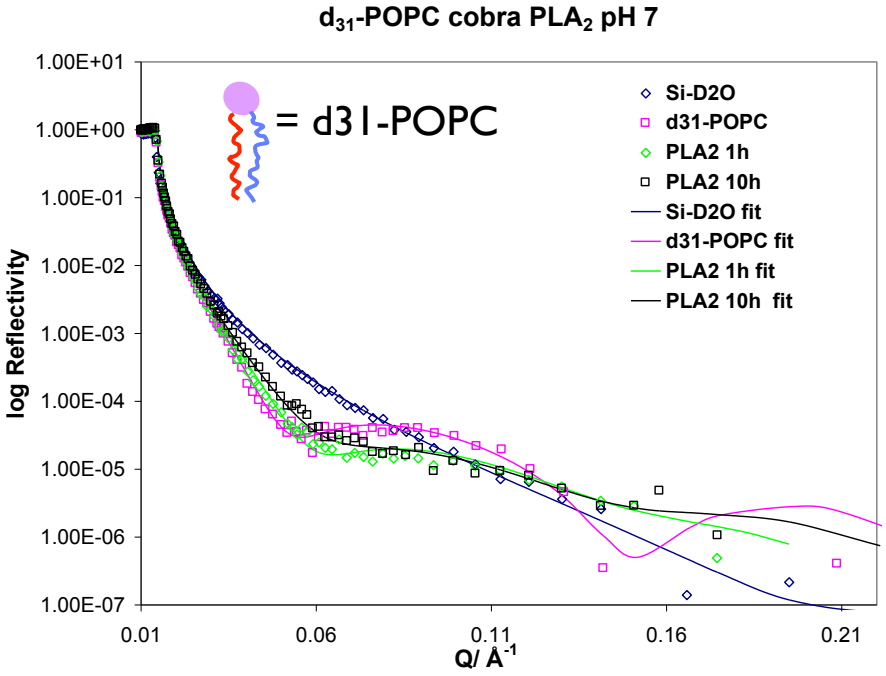


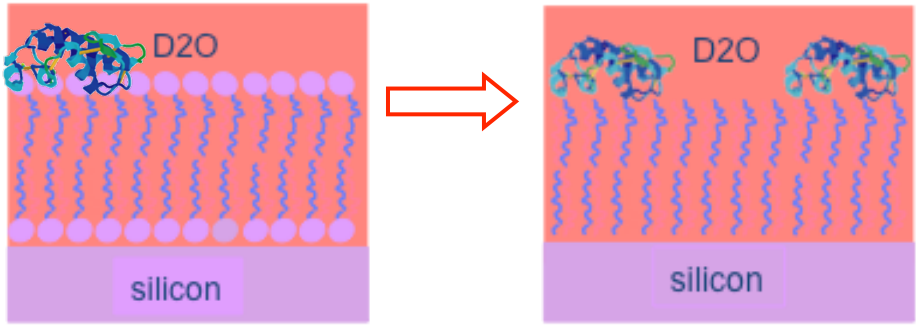


- The apparent penetration depth *increases* with lipid saturation (DOPC<POPC<DPPC)
- The rate and extent of membrane degradation *decrease* with increasing lipid saturation

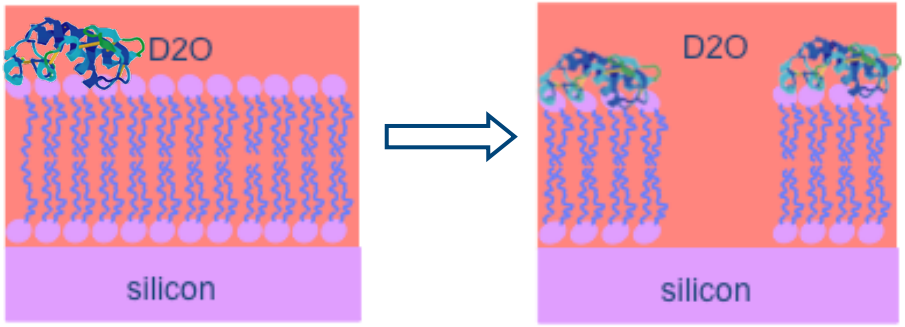
Vacklin et al. Biochemistry 44, 2811-2821 (2005)

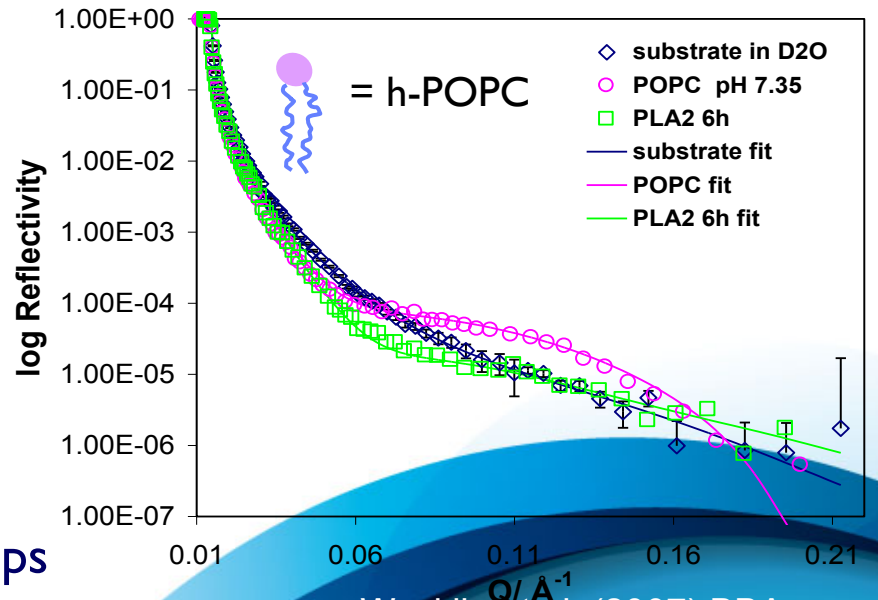
What happens to the products?





lipid headgroups disappear, membrane becomes thinner

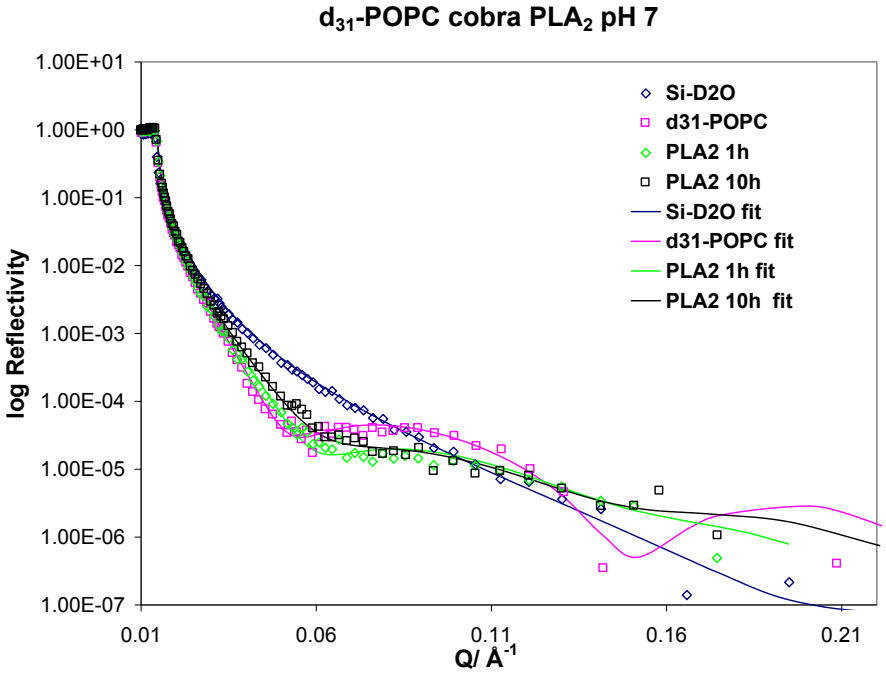


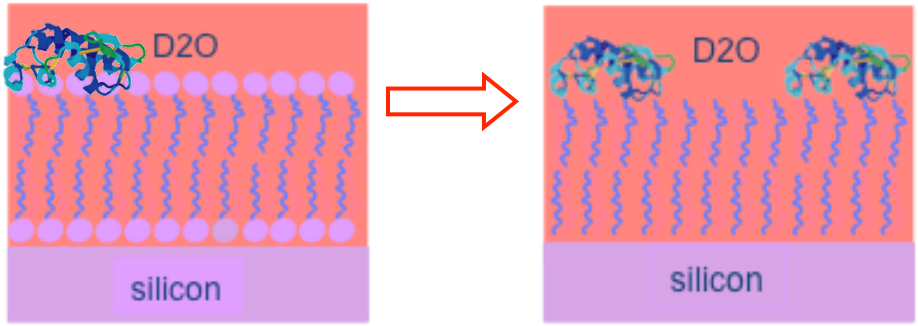


~50% of membrane solubilised, headgroups become very hydrated, membrane thins

Wacklin et al. (2007) BBA – Biomembranes 1768, 1036-49

What happens to the products?





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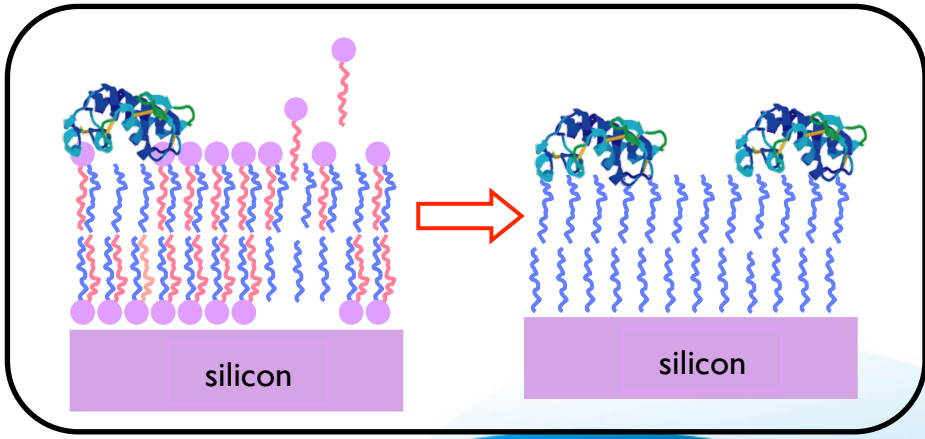

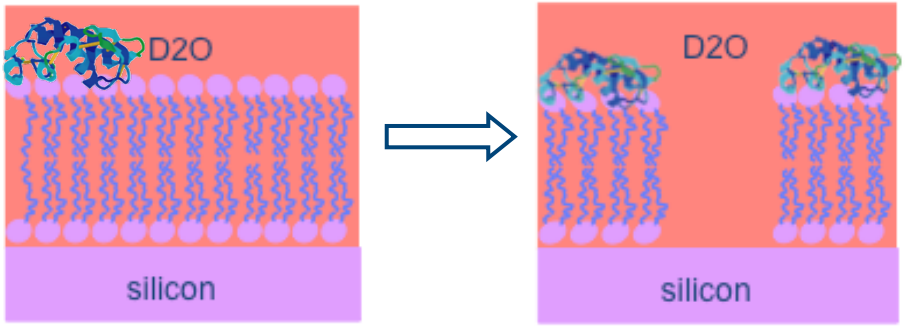

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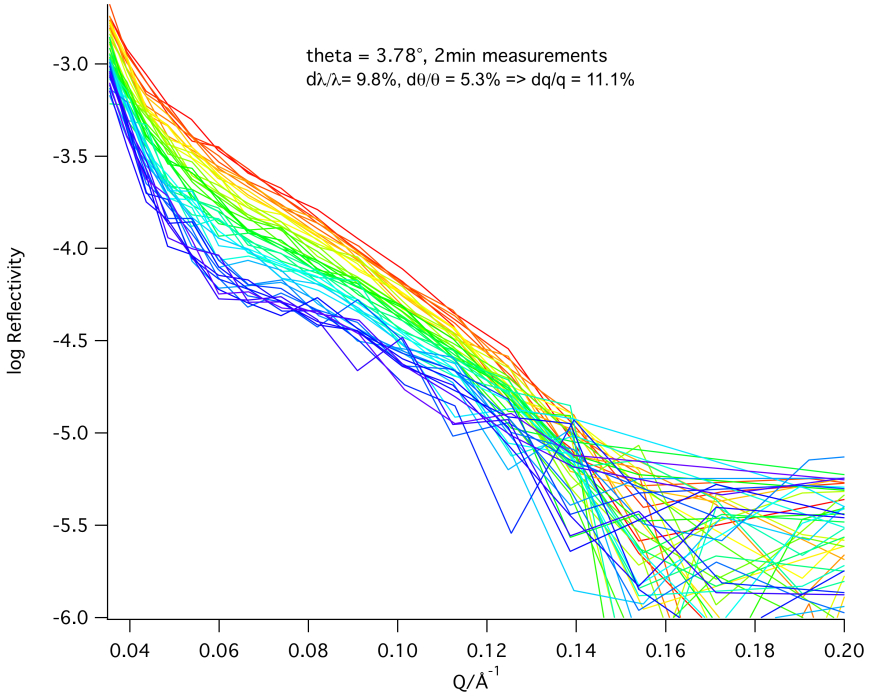
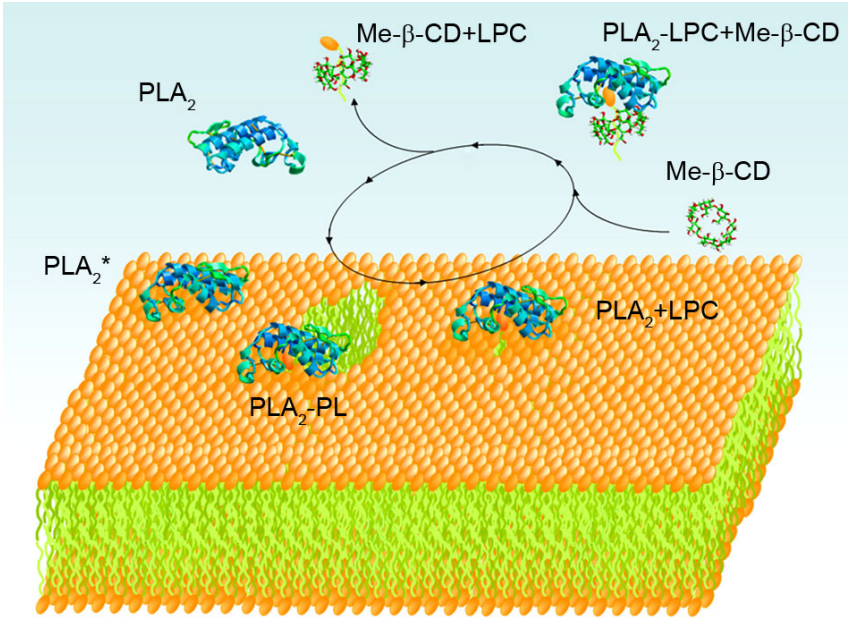
Kinetics and Interfacial mechanism



TODAY: membrane structure and composition every 2 min.

At ESS we will be able to go up to 100 times faster



Avoiding isotope effects: in D2O enzym functions 10 times more slowly than in H2O - at ESS we can do the measurements in H2O (too fast today)

Summary

- Lipid bilayer structure

=> inter and intra-layer structure + lipid composition

- Insertion peptides in membranes

=> location and amount as function of concentration

- Protein conformation at membrane surfaces

=> extension from membrane/conformational changes

- Enzyme catalysis in a membrane

=> location/amount of enzyme, changes in membrane structure and composition during reaction

Thank you for your attention!