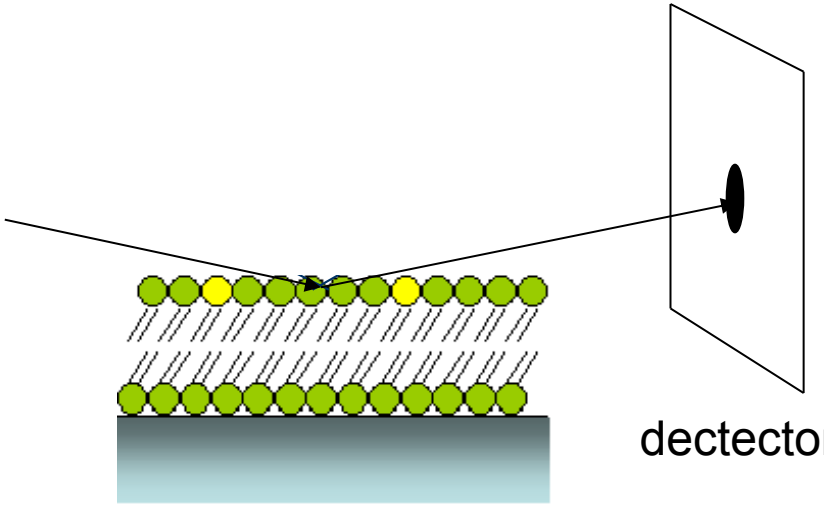
Neutron reflectivity in biology and medicine

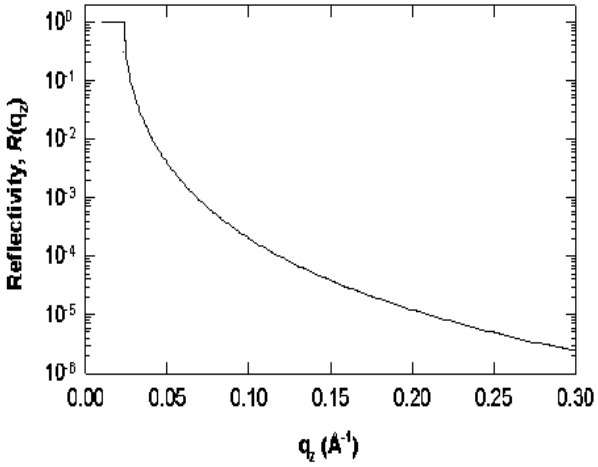
Jayne Lawrence

Why neutron reflectivity studies?

build up a *detailed* picture of the structure of a surface in the z direction



specular reflection

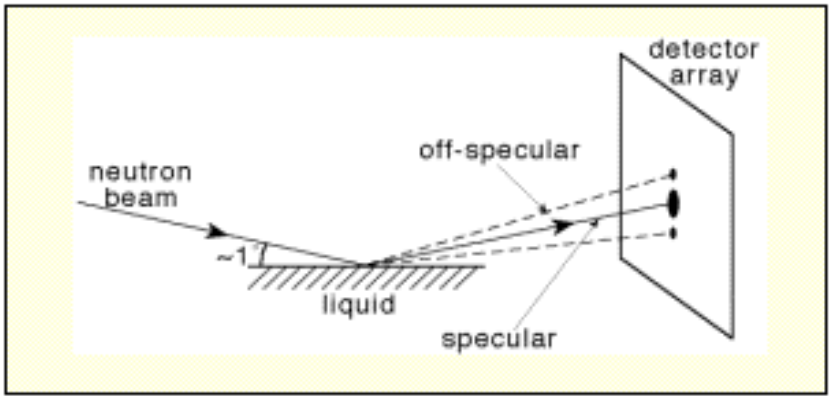


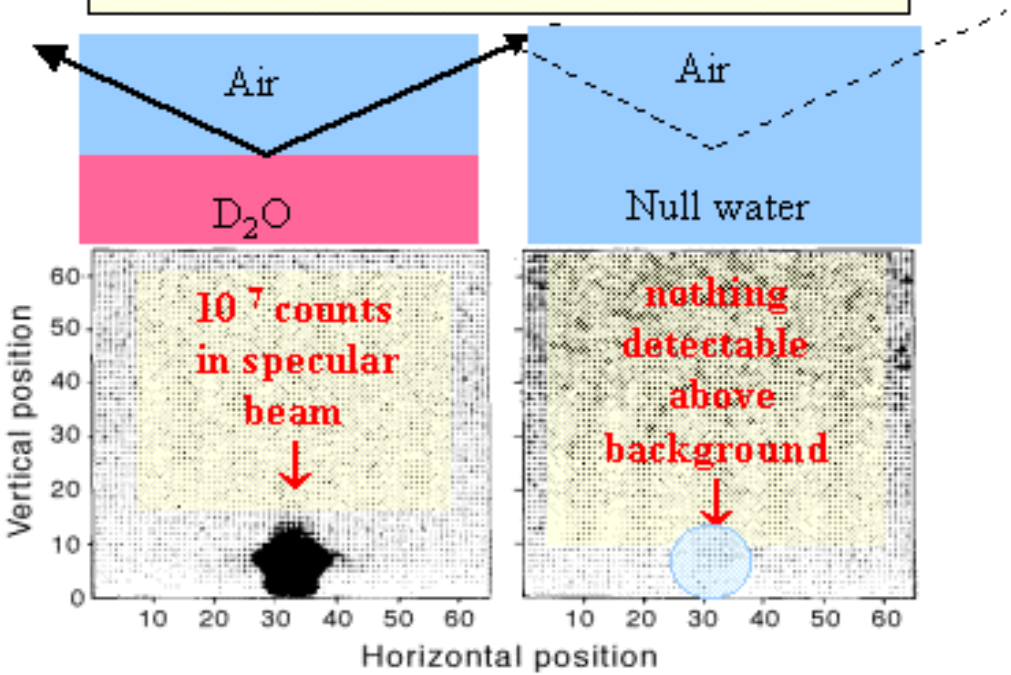
$R(\theta,\lambda) = I_R/I_0(\lambda)$ $Q = (4\pi/\lambda)\sin\theta$

Data typically analysed using the 'optical maxtrix' method

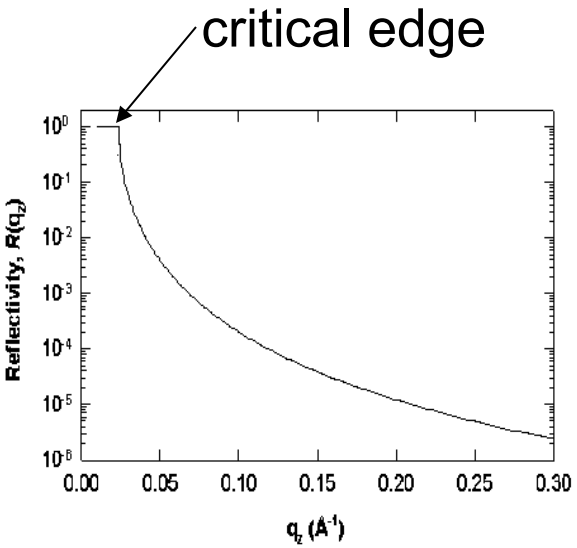
Frequently between 3-7 *different 'contrasts'* measured

What does neutron reflectivity data look like?





Reflection from a sharp D_2O/air interface

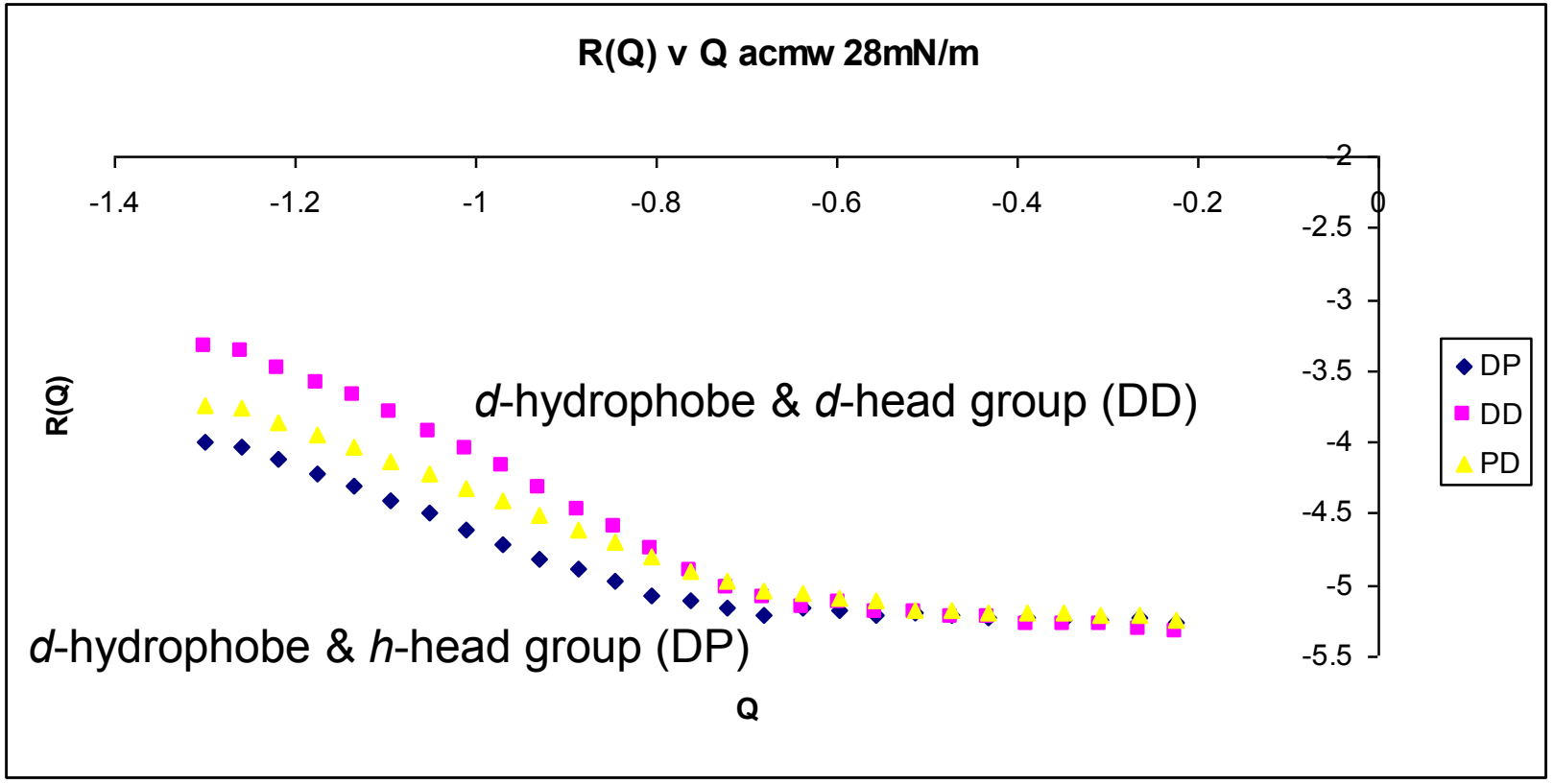


At large Q – R varies as $1/q^4$

Below Q_{crit} R = 1

What does neutron reflectivity data look like?

Various deuterated forms of a novel nonionic lipid on null water or air contrast matched water (amcw)

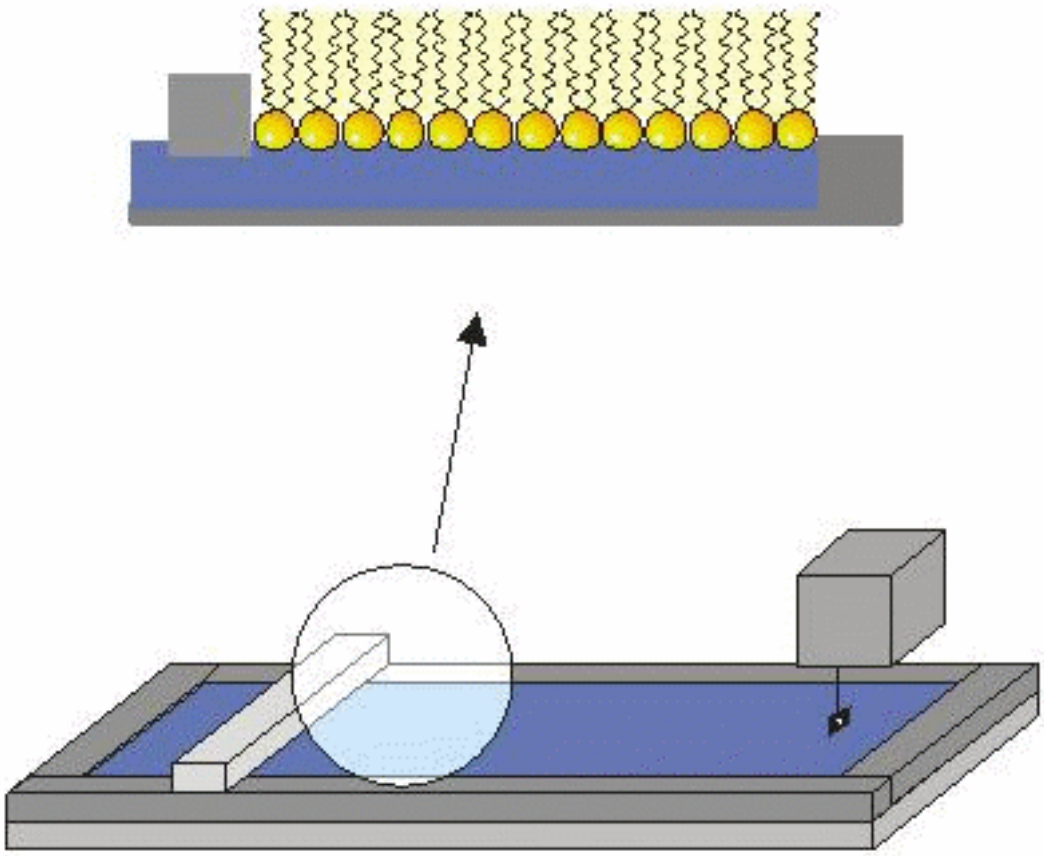


Neutron reflectivity

Monolayer studies typically performed at RAL on SURF (and CRISP)

SURF beam line at the Rutherford Appleton Laboratories, Didcot, UK

Monolayer studies



- relatively easy to prepare
- composition of monolayer well-defined
- hold monolayer at varying lateral pressure
- lipid monolayers are often a good mimic of membrane
- number of advantages over bilayers deposited on a solid support

Langmuir trough – known amount of lipid spread over a known area

Monolayers as models for bilayers

neutron reflectivity

lipid/surfactant monolayer	surface pressure (mN m^{-1})	surfactant distribution width σ_A(Å)
$2C_{18}E_{12}$[a]	34	24.0 ± 0.3
	40	27.0 ± 0.2
$2C_{18}E_{12}$:CHOL	25	25.9 ± 0.3
	34	31.3 ± 0.2
	40	32.9 ± 0.3
$2C_{18}E_{12}$:DSPC:CHOL	34	31.5 ± 0.1

small angle neutron scattering

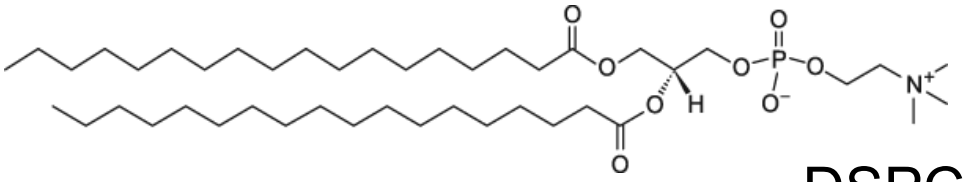
vesicle	layer L (Å)
$2C_{18}E_{12}$	51 ± 0.3
$2C_{18}E_{12}$:CHOL (1:1)	68 ± 0.3
$2C_{18}E_{12}$:DSPC:CHOL (1:1:2)	60 ± 0.4

Lateral pressure in membrane ~ 30-35 mN/m

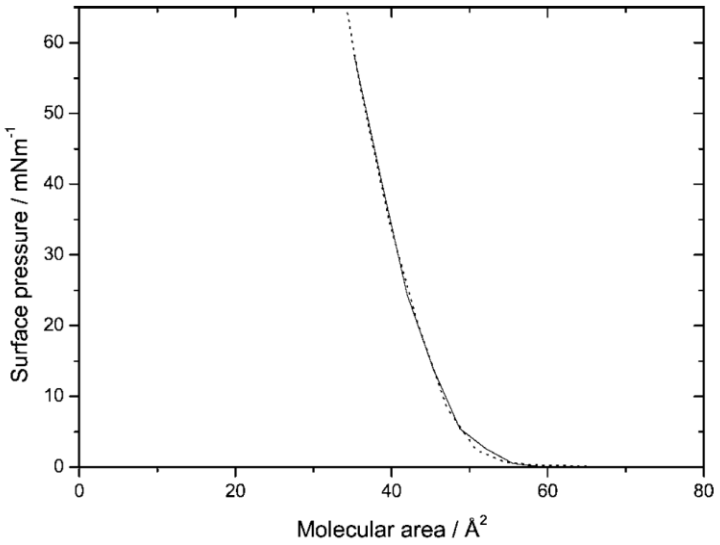
Bilayer thickness ~ x2 monolayer thickness

Holds if curvature of bilayer is low

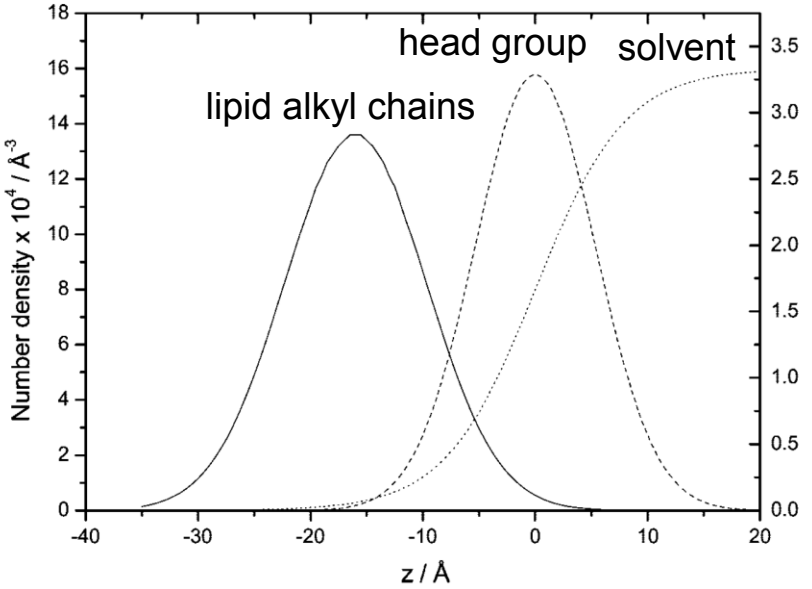
Monolayers of DSPC



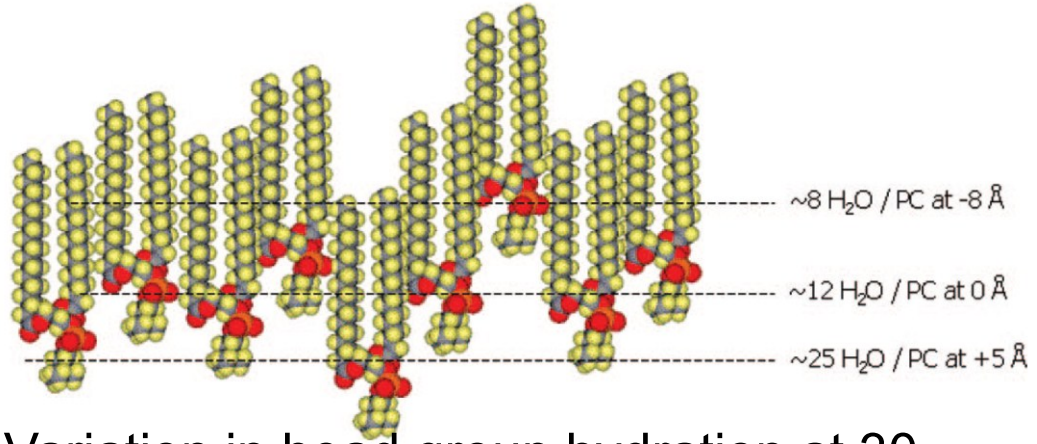
DSPC



Isotherm of fully d-DSPC and h-DSPC



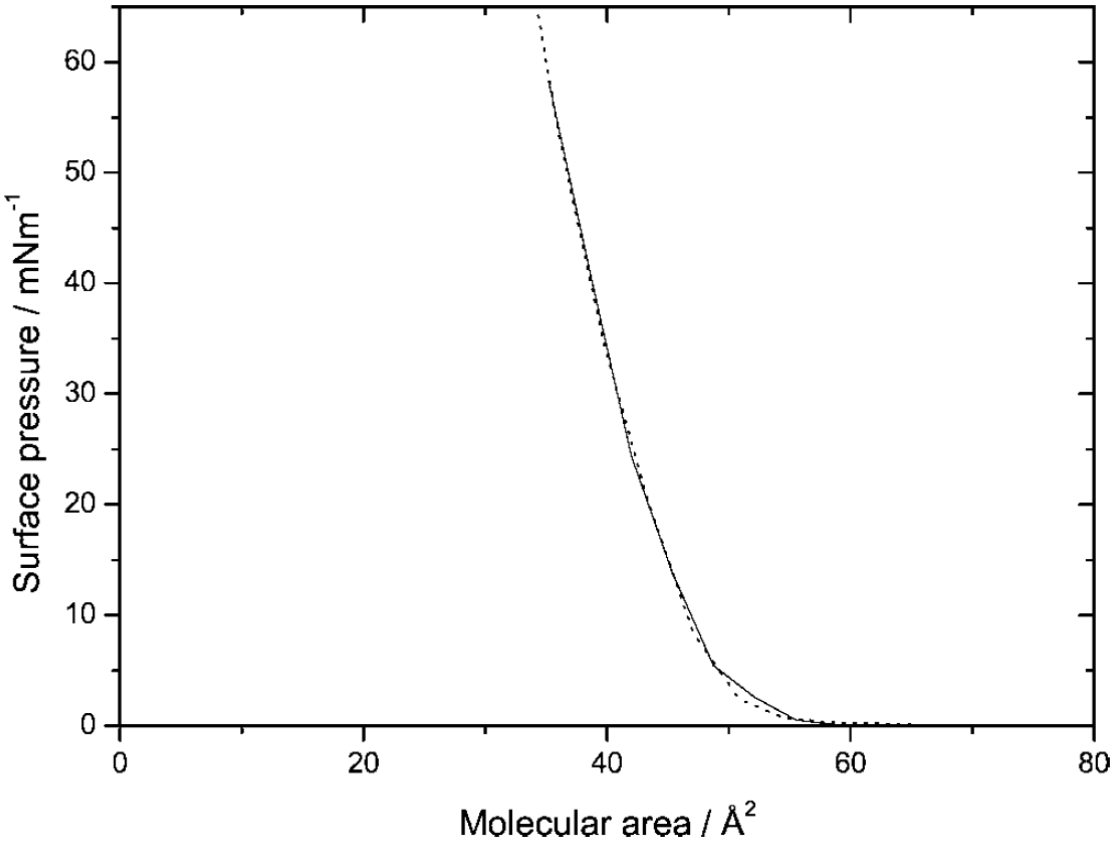
Number density profiles for the DSPC monolayer 30 mN/m



Variation in head group hydration at 30 mN/m

Barlow et al Langmuir (2009) 25 4070

Isotope effect for DSPC (C_{18} phospholipid)



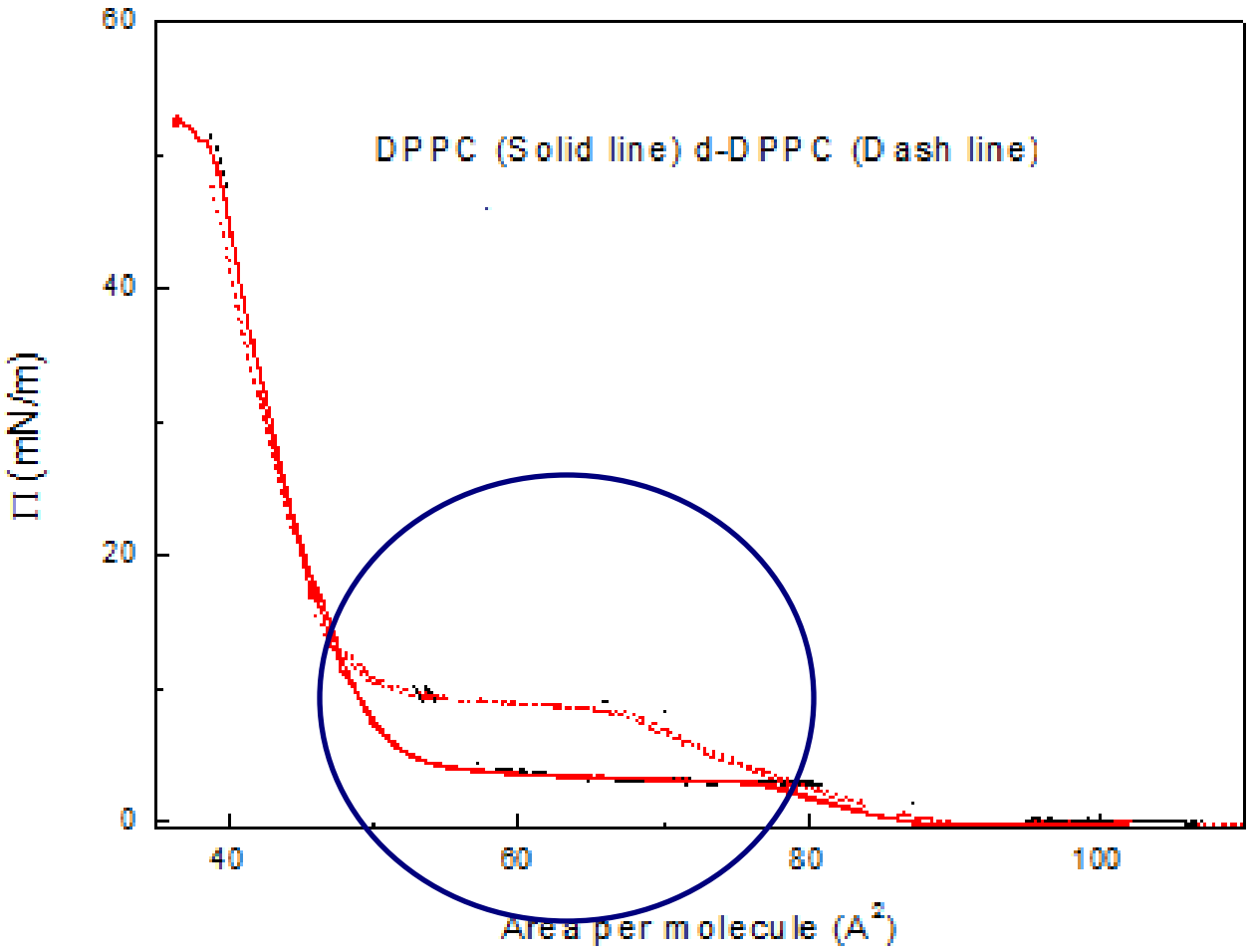
Little effect on lipid (or surfactant) phase behaviour noticed using:

D_2O instead of H_2O

d-head group instead of h-head group

d-hydrophobe instead of h-hydrophobe unless near a phase transition!

Isotope effect for DPPC (C_{16} phospholipid)



Little effect on lipid (or surfactant) phase behaviour noticed using:

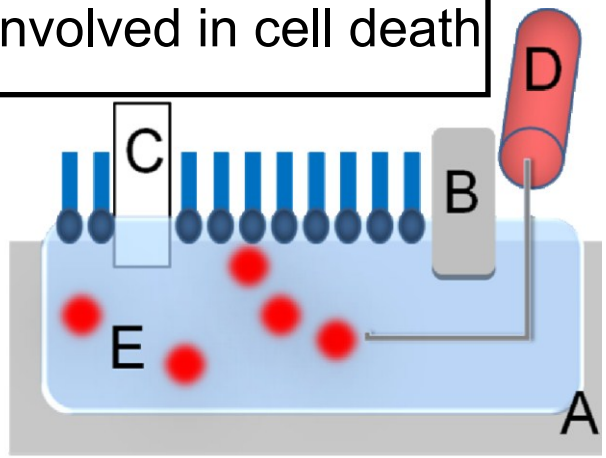
D_2O instead of H_2O

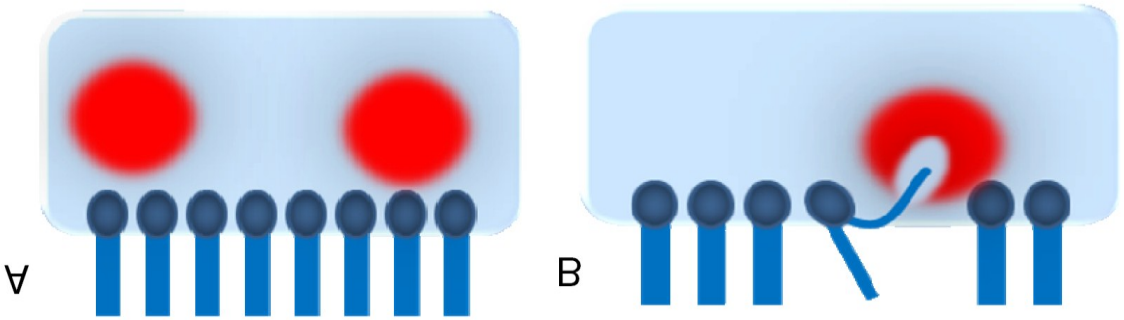
d-head group instead of h-head group

d-hydrophobe instead of h-hydrophobe unless near a phase transition!

Monolayer studies as a mimic of biological membranes

Cytochrome c – cationic protein involved in cell death

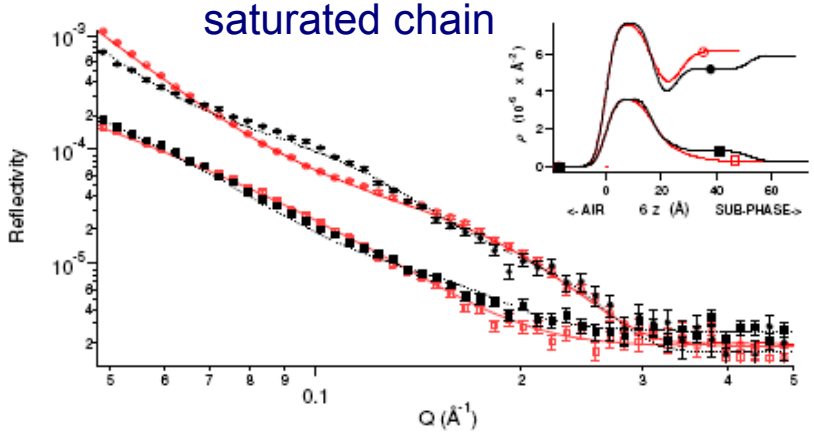




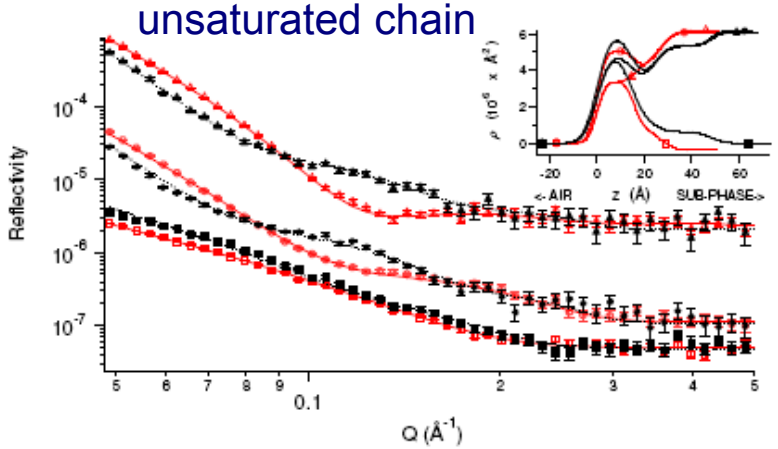
Lipid anchorage model of interaction of cytchrome

effect of head group and hydrophobic chain saturation

saturated chain



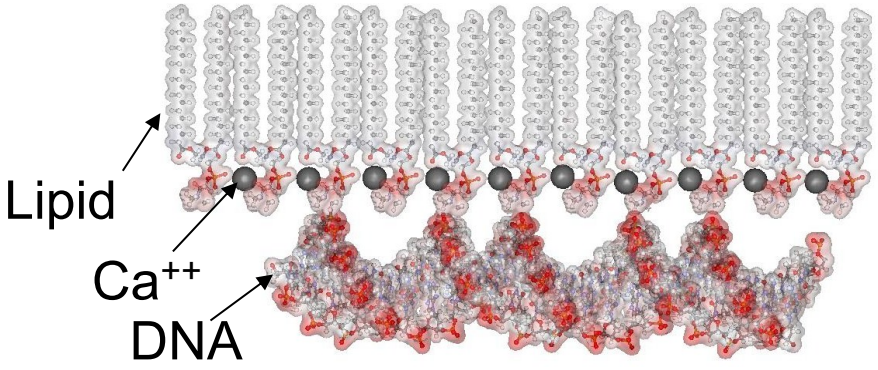
unsaturated chain

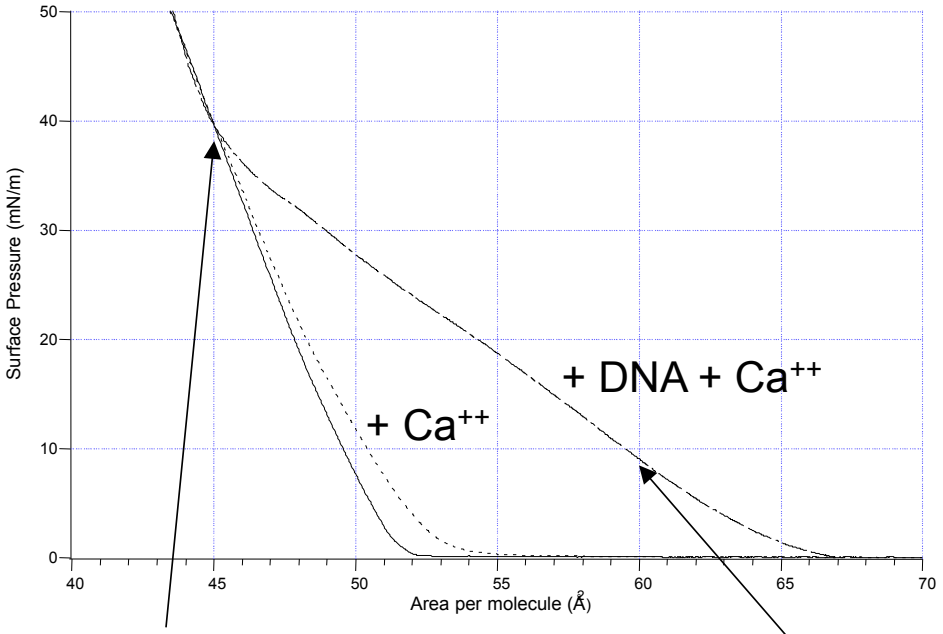


Monolayer studies to develop non-toxic gene delivery vehicles

Hypothesis

That it is possible to make a zwitterionic phospholipid act as a 'cationic' lipid and complex DNA by the addition of a divalent cation such as Ca^{2++}?



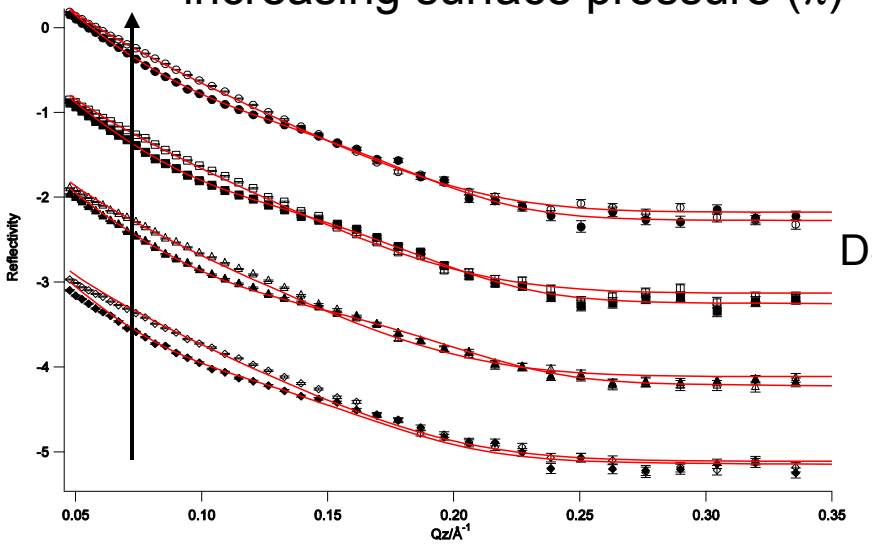


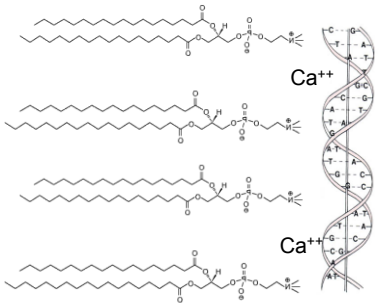
Isotherms overlay at low areas per molecule – DNA *excluded* from monolayer

DNA ➡ increase in area per molecule, DNA *associated with* the monolayer

Neutron reflectivity

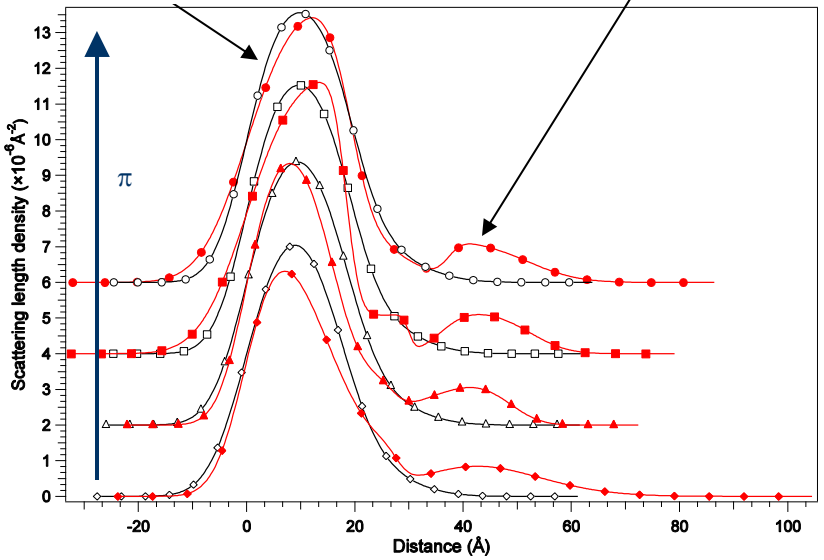
increasing surface pressure (π) - 10, 20, 30 and 40mN/m





DSPC chains

DNA

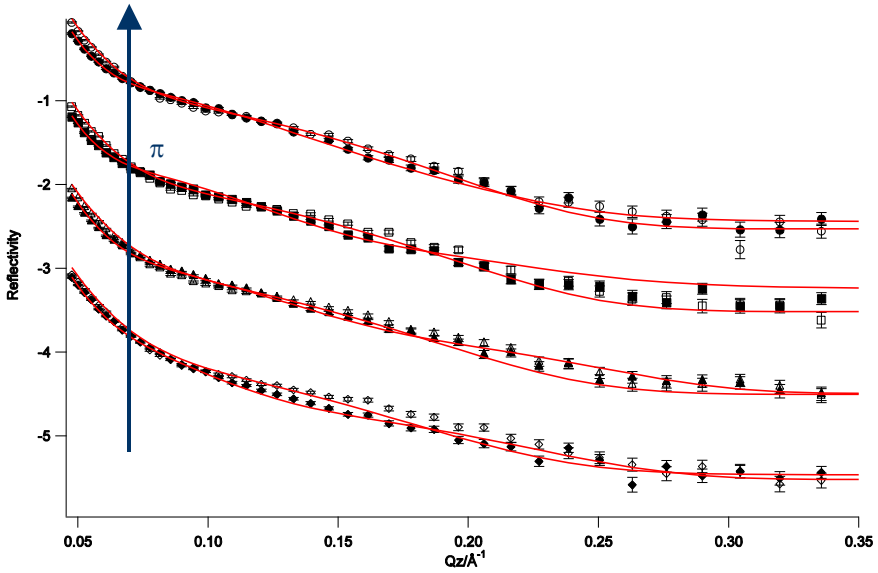


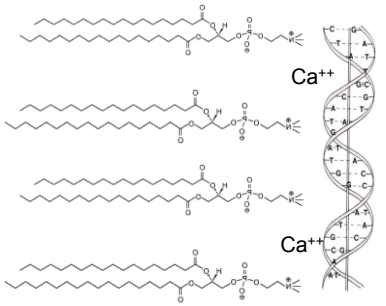
d_{70}-DSPC/acmw + 20mM Ca^{++} with (—) and without (—) DNA

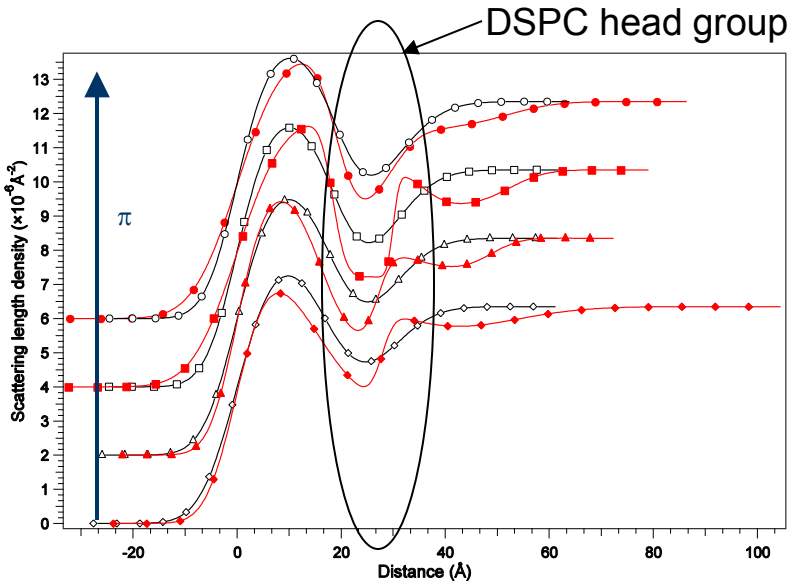
here 'seeing' mainly lipid chains and when present DNA

Neutron reflectivity

increasing surface pressure (π) - 10, 20, 30 and 40mN/m





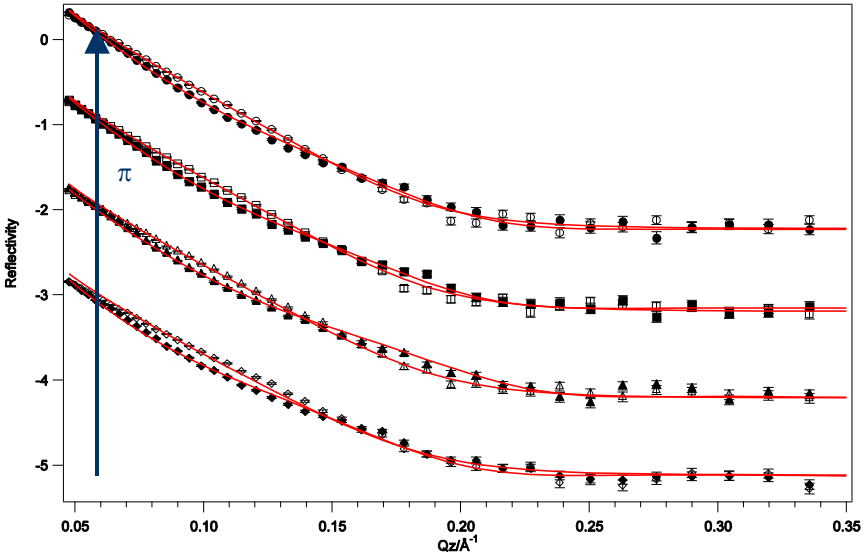


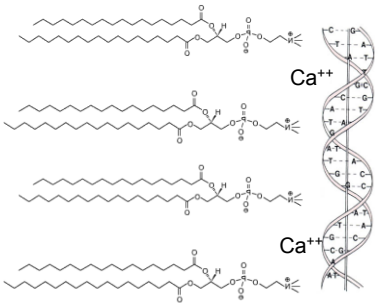
d_{70}-DSPC/D_2O + 20mM Ca^{++} with (—) and without (—) DNA

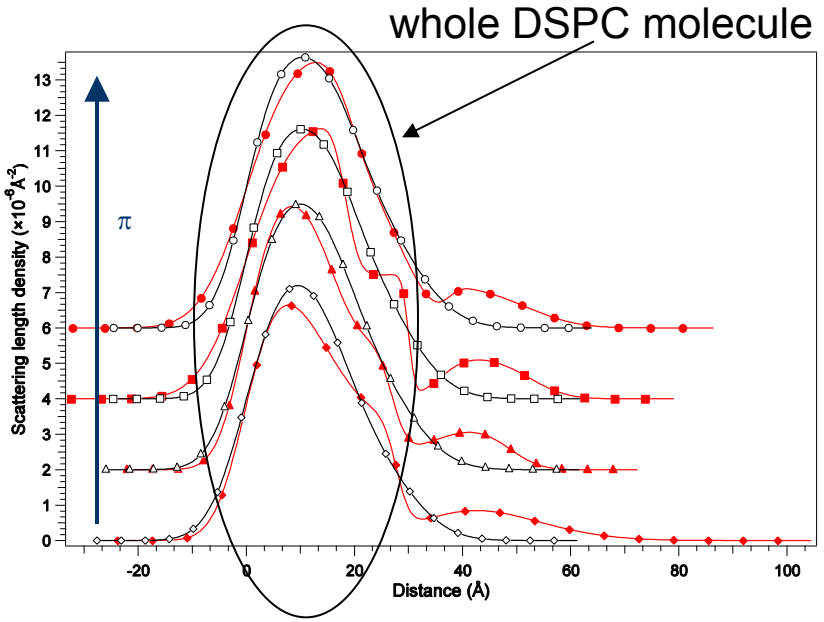
here 'seeing' mainly DSPC head group, and when present, DNA

Neutron reflectivity

increasing surface pressure (π) - 10, 20, 30 and 40mN/m







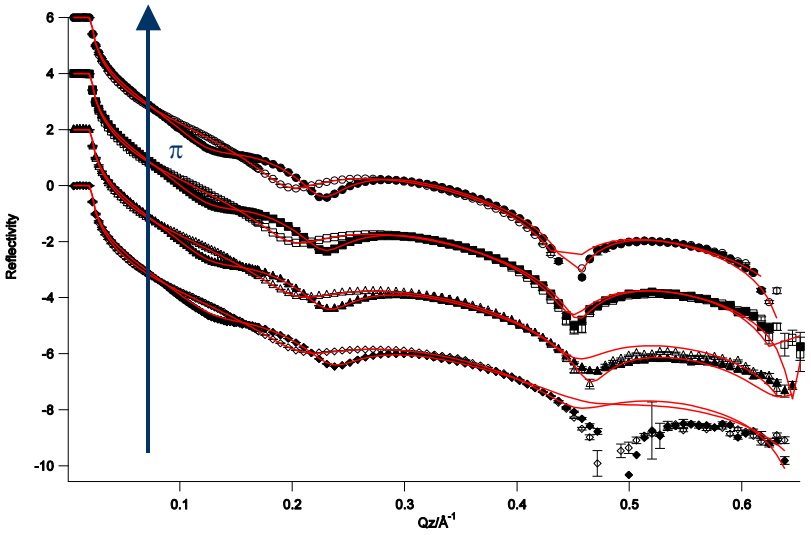
d_{83}-DSPC/acmw + 20mM Ca^{++} with (—) and without (—) DNA

here 'seeing' mainly whole DSPC molecule and, when present, DNA

X-ray reflectivity

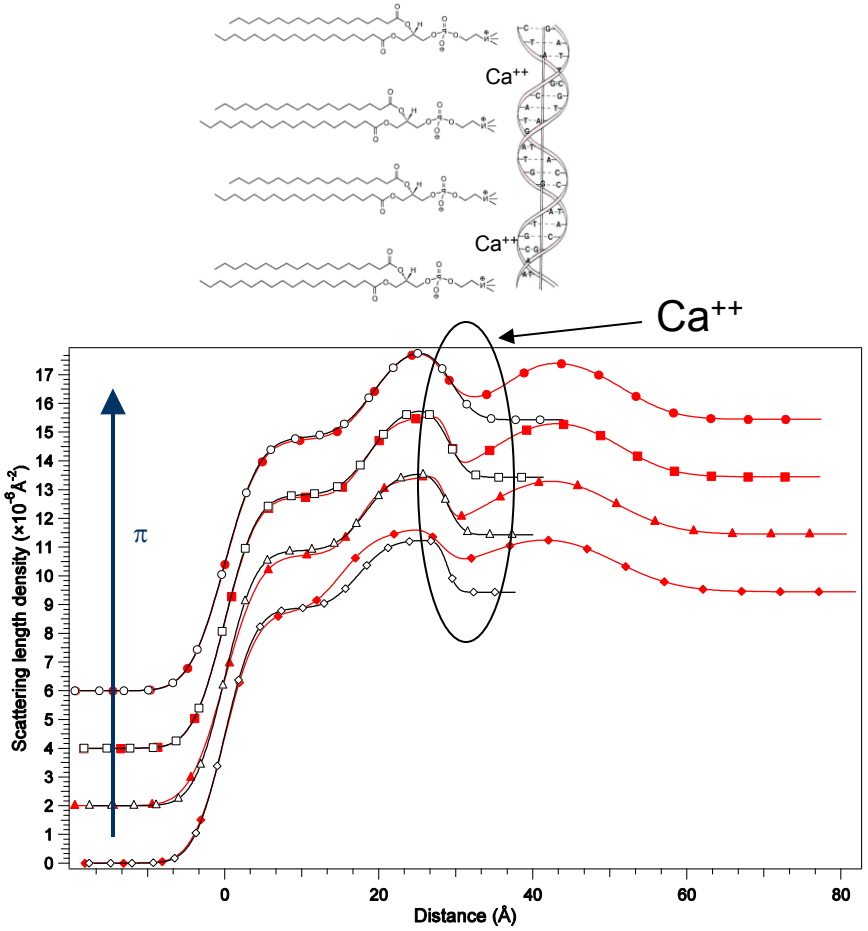
Performed on TROIKA-II beam line at the ESRF, Grenoble, France

X-ray reflectivity



DSPC/H_2O + 20mM Ca^{++} with (—) and without (—) DNA

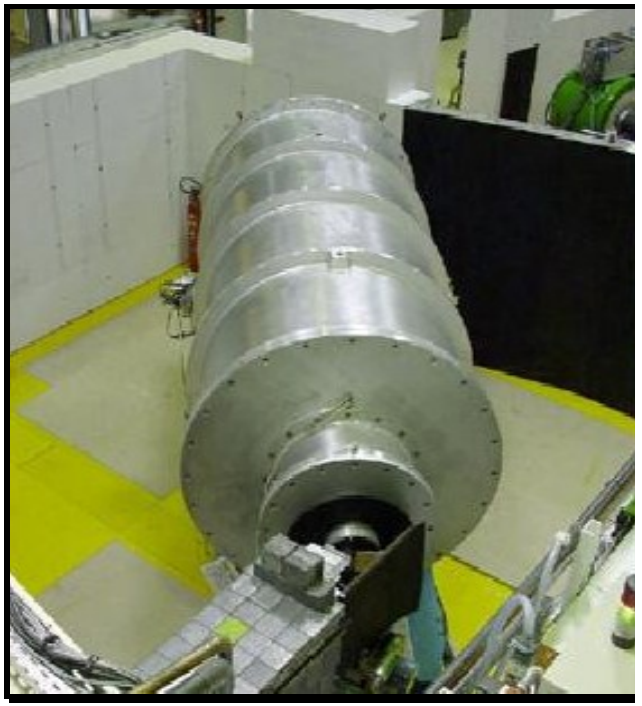
here 'seeing' Ca^{++} and, when present, DNA



Neutron reflectivity

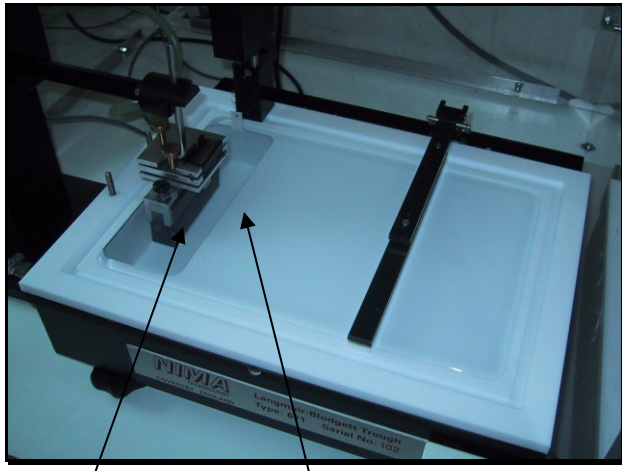
All studies at the solid-liquid interface performed at RAL and ILL

SURF beam line at the Rutherford Appleton Laboratories, Didcot, UK



D17 at the Institute Laue Langevin, Grenoble, France

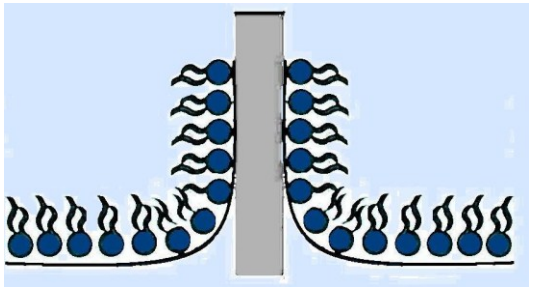
Preparation of supported lipid bilayers using a combination of Langmuir Blodgett & Shaeffer techniques



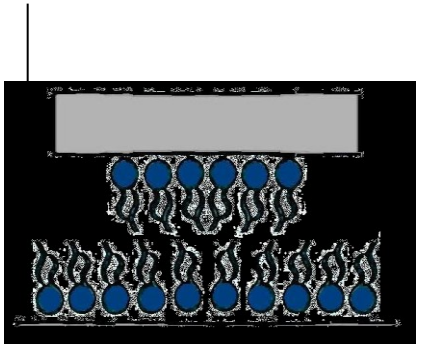
silicon block

phospholipid monolayer spread on surface of trough

lipid monolayer



Blodgett deposition



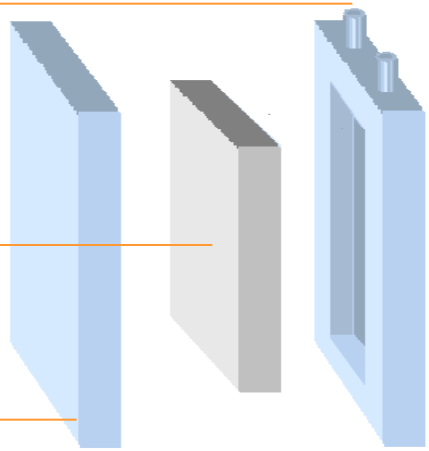
Shaeffer deposition

Preparation of supported lipid bilayers using a combination of Langmuir Blodgett & Shaeffer techniques

valves for solvent exchange

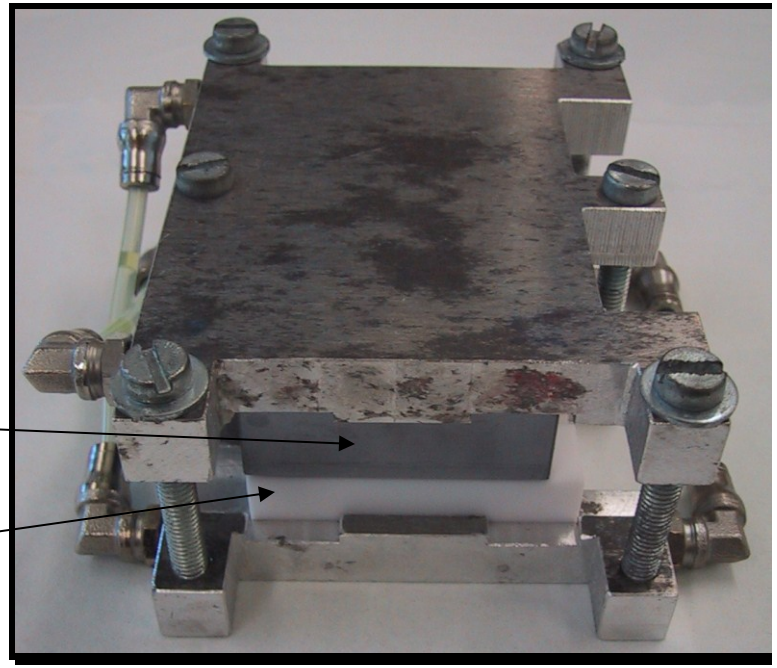
silicon block with bilayer

teflon cell

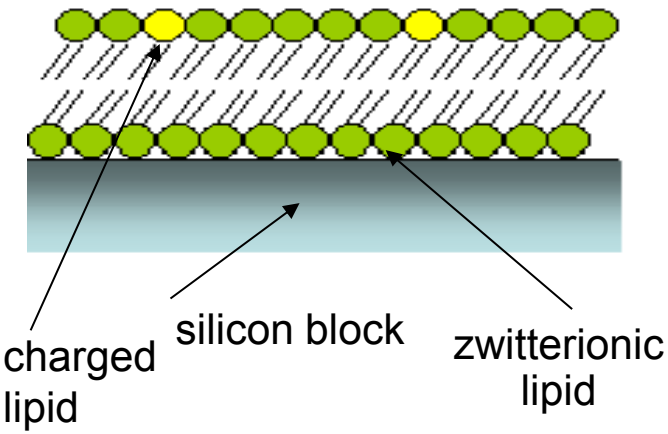


silicon block

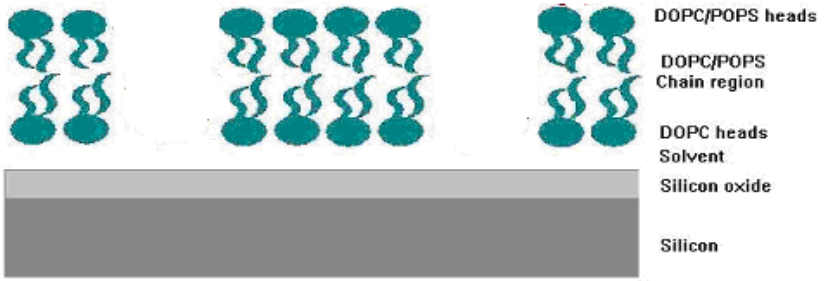
teflon trough containing sub-phase



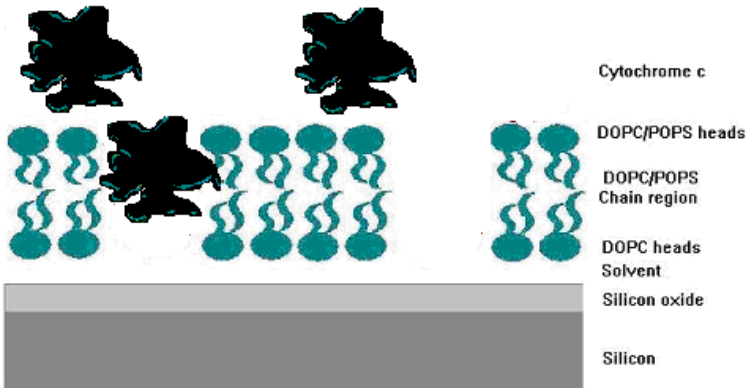
Binding of cytochrome c to bilayers



information obtained about overall thickness of bilayer, chain and head group, hydration of head group and % coverage of the silicon block by the bilayer



whether and where cytochrome c bound depended upon preparation method of silicon block and both anionic and neutral lipid



Ozone prepared block bound cytochrome c but a block prepared by RCA1 did not

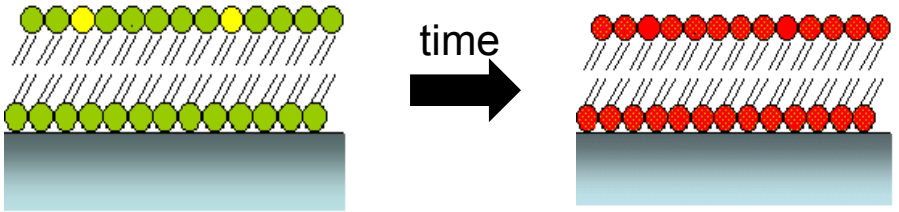
Unsaturated zwitterionic lipid increased cytochrome c binding to anionic lipid compared to partially saturated lipid

Nature of anionic lipid less important

Characterisation of artificial biomembranes before & after addition of cationic vesicles

The lipid dimyristylphosphatidylcholine (DMPC) *used to give a fluid bilayer*

Outer layer doped with 10 mol% negatively charged dipalmitoylphosphatidylserine (DPPS) *to mimic the biological membrane*



Bilayers exposed to 0.1mg/ml of DDAB vesicles for 6 hours

layer description	thickness (Å)	roughness (Å)	ρ ($\times 10^{-6}$ Å^{-2}) 9:1 *d*-DMPC:*p*-DPPS bilayer	ρ ($\times 10^{-6}$ Å^{-2}) 9:1 *d*-DMPC:*p*-DPPS bilayer after 6 h exposure to DDAB vesicles
SiO_2	13 ± 2	2 ± 1	3.4 ± 0.2	3.4 ± 0.2
solvent	3 ± 1	2 ± 1	6.4 ± 0.2	6.4 ± 0.2
headgroup	8 ± 1	3 ± 1	5.7 ± 0.2	6.1 ± 0.2
hydrocarbon	27 ± 2	3 ± 1	6.7 ± 0.2	6.7 ± 0.2
headgroup	8 ± 1	3 ± 1	5.7 ± 0.2	6.1 ± 0.2

Bilayers exposed to 0.1mg/ml of DDAB vesicles for 15 days

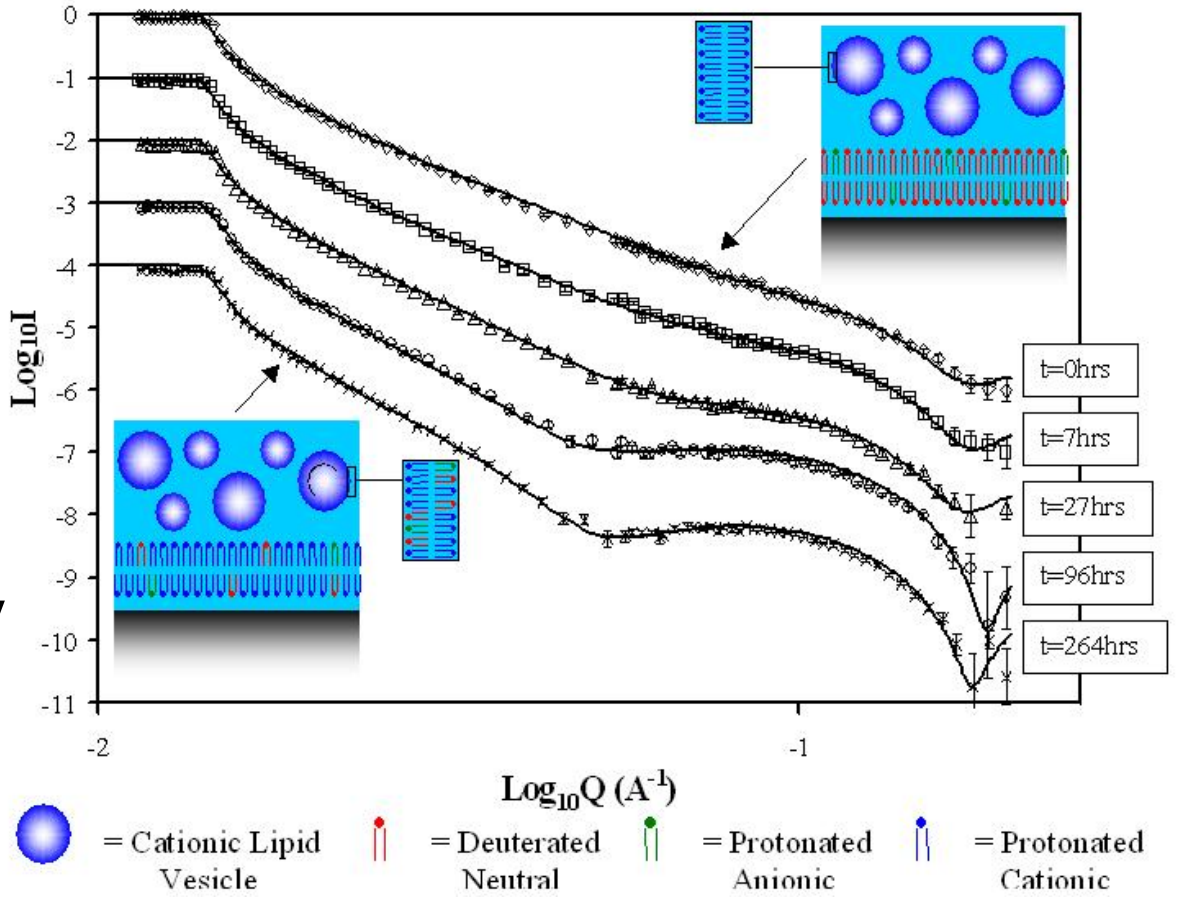
layer description	thickness (Å)	ρ ($\times 10^{-6}$ Å^{-2})	roughness (Å)	% solvent
SiO_2	13 ± 2	3.4 ± 0.2	2 ± 1	0
DDAB	37 ± 3	0.8 ± 0.2	2 ± 1	49 ± 5

Callow et al Langmuir 21 (2005) 7912

Interaction of cationic vesicles with artificial biomembranes

Change in neutron reflectivity curves for a 9:1 *d*-DMPC: *p*-DPPS bilayer in the presence of 0.1 mg/mL 1:1 DDAB:Chol vesicles (suspended in D_2O) over time

Interaction of vesicles very slow – over days rather than hours

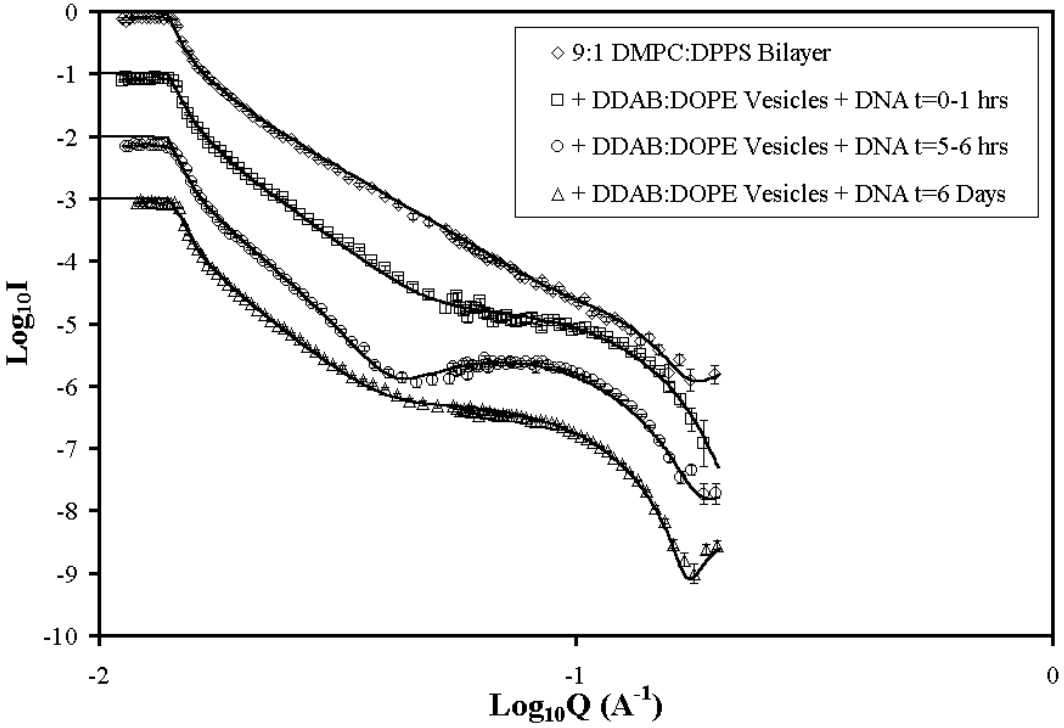


Callow et al Langmuir 21 (2005) 7912

Interaction of gene delivery vectors with artificial biomembranes

Interaction of gene delivery vehicle/lipoplex with artificial biomembrane occurs by lipid exchange

Regardless of vesicle composition the rate of lipid exchange is faster in the presence of DNA



Callow et al Langmuir (2009) 25, 4181

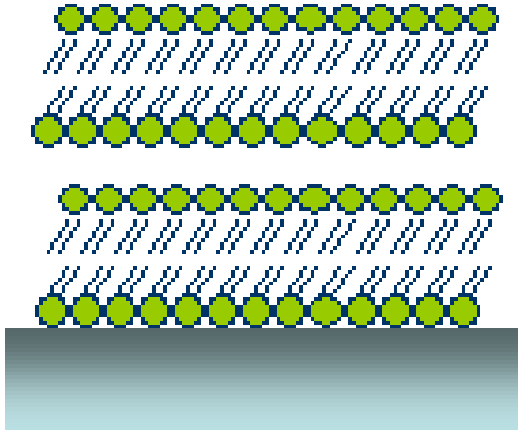
Improved artificial membranes?

Substrate surface has prepared using ozone method has a negative charge

Nature of substrate influences how the cytochrome and vesicles/gene vectors interact with the artificial biomembrane – particularly if surface coverage is low

Need for better membrane models?

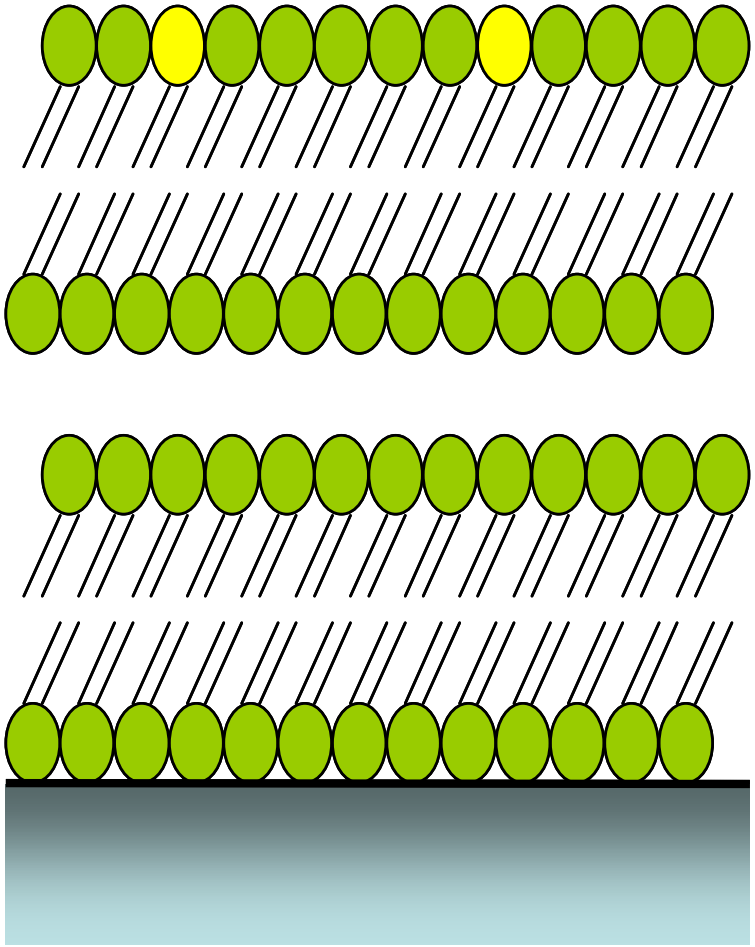
Would a supported (or double) floating bilayer be better?



double ‘floating’ bilayer

Fragneto et al Europhys Lett 53 (2001) 100

Supported Floating Bilayers (FSB)



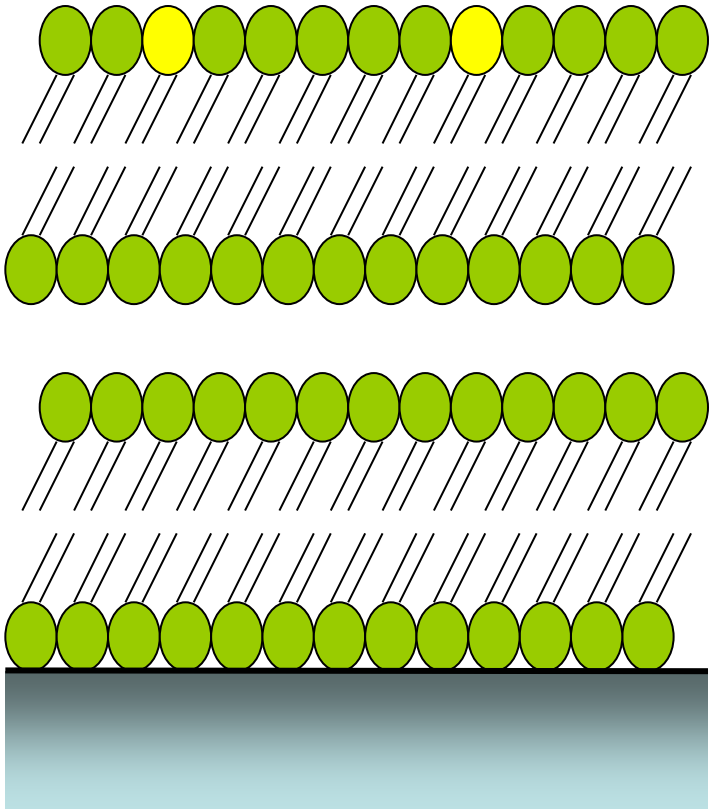
- The FSB is associated with the substrate
- Cushioned from the substrates constraining effects by a hydration layer 20 – 30 Å thick
- The separation from the substrate is the result of the balance between the electrostatic and Van der Waals interactions that hold the bilayer in place, and the repulsive, entropic ‘Helfrich’ force which prevents the membrane adhering directly to the support.
- The balance of forces mimics those present in multilammellar vesicles (MLVs)

Development of FSB

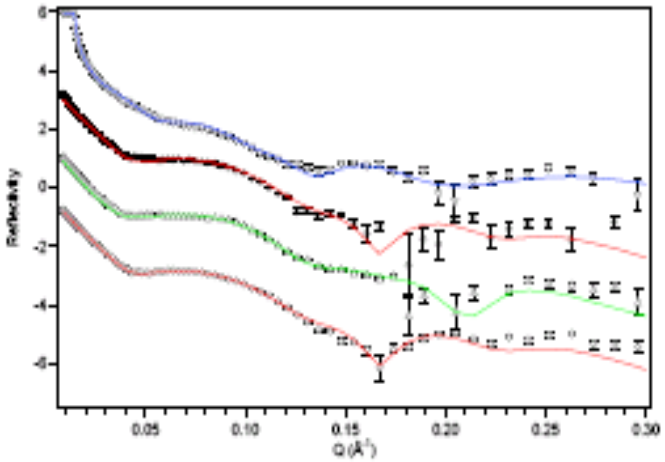
A cell membrane comprises of lots of different lipids generally in their ‘fluid’ liquid crystalline phase

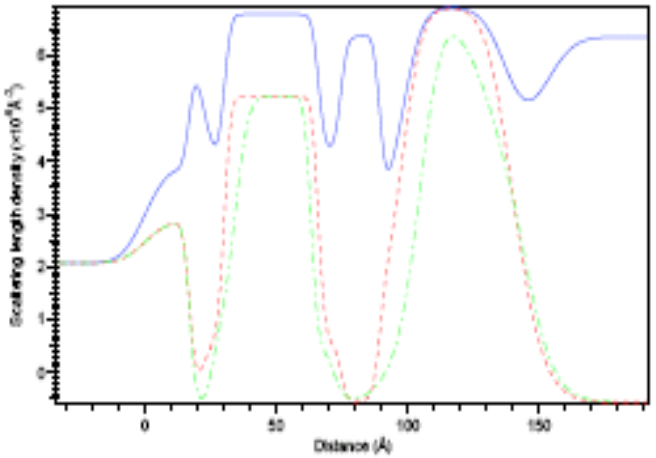
DMPC, a fluid phase lipid at 25 & 37°C, while a good model of the cell membrane, is not a good lipid for production of double bilayers

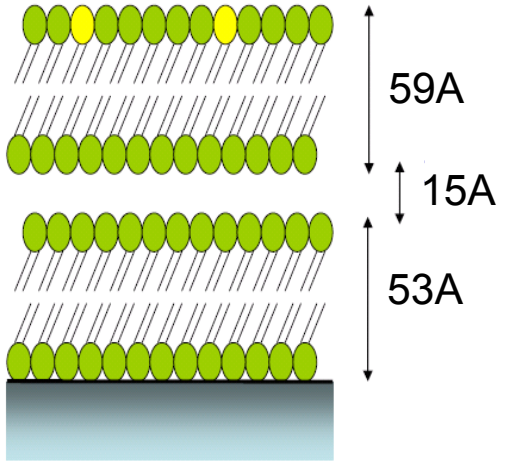
It is relatively easy to prepare double bilayers from DPPC or DSPC which are both the their gel phase at 25 & 37°C



d_{62}-DPPC FSB



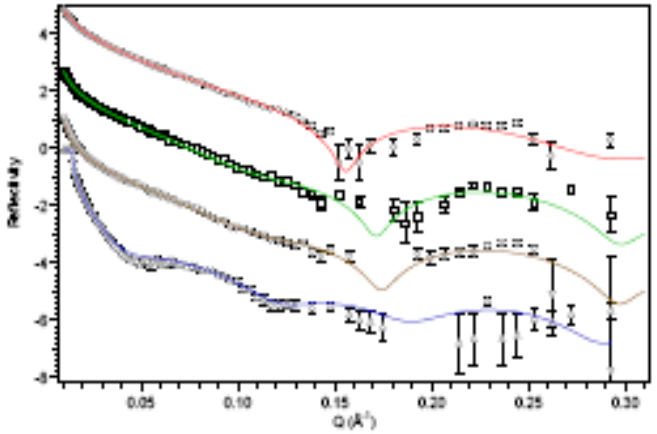


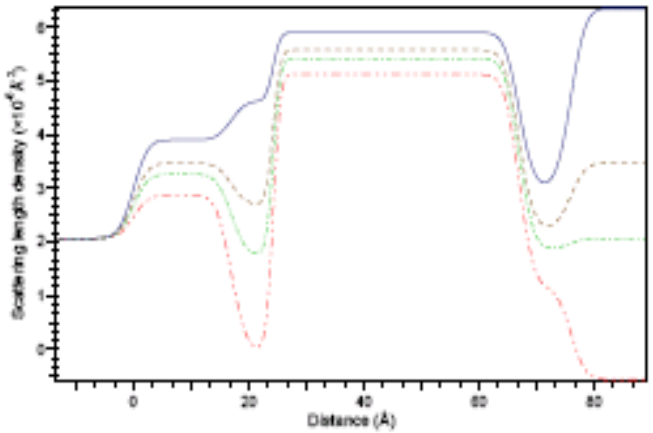


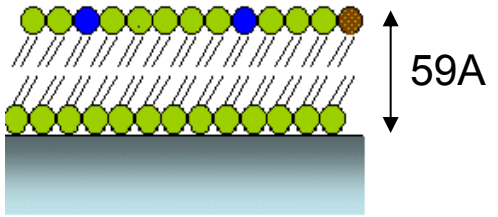
Reflectivity (a) and scattering length density (b) profiles of DDPC double bilayers in H_2O at 25^{o}C and 37^{o}C (dashed), at 45^{o}C (dot dashed) and in D_2O at 37^{o}C (solid)

Talbot et al Langmuir (2009) 25 4168

d_{62}-DPPC FSB – after incubation with DDAB-Chol lipoplexes







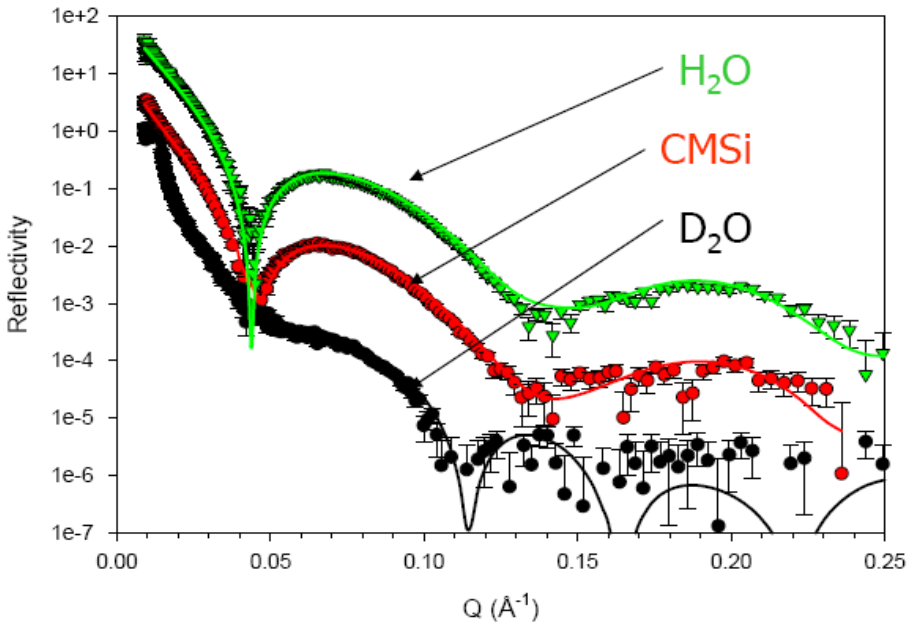
Neutron reflectivity profiles & fits (a) and SLD (b) from d_{62}-DPPC double bilayer at 25^{o}C after exposure to DDAB-Chol lipoplexes in D2O (up triangles), CMSiO2 (down triangles), CMSi (squares) and H2O (circles)

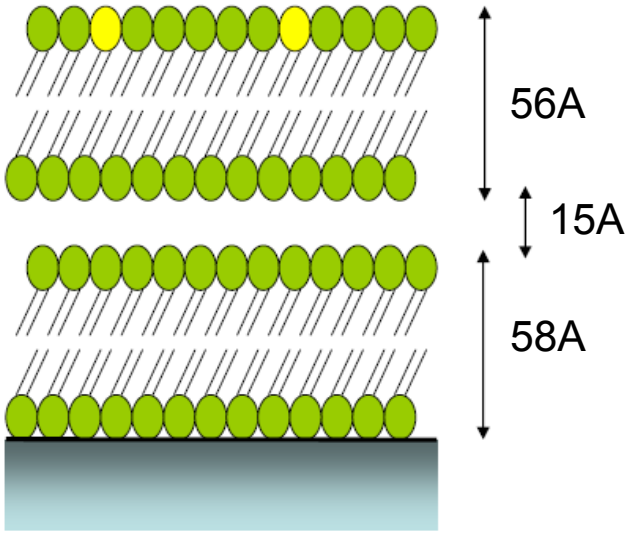
Destruction of upper bilayer, followed by lipid exchange in lower bilayer

Talbot et al Langmuir (2009) 25 4168

d_{83}-DSPC FSB

Reflectivity data from 3 solvent contrasts



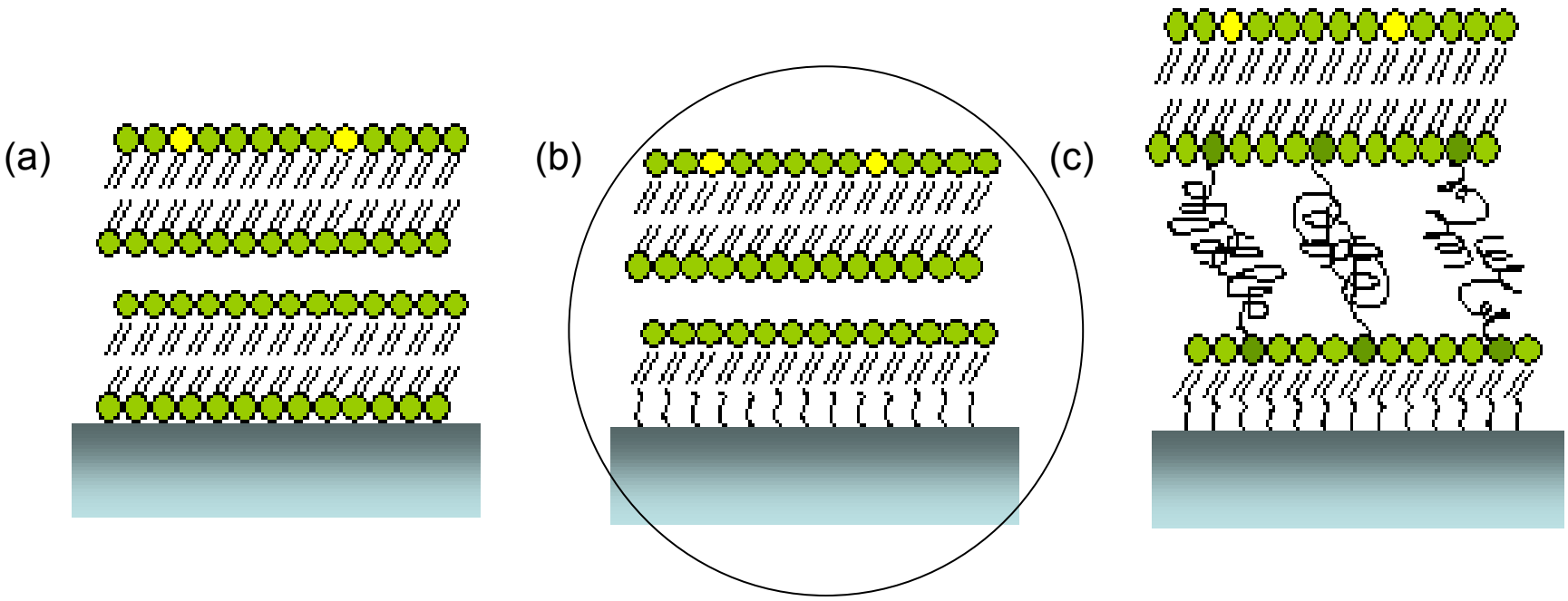


A lower vesicle concentration of 0.01mg/ml was used

Same effect occurred as was seen with DPPC after addition of lipoplex to DSPC FSB

Talbot et al Langmuir (2009) 25 4168

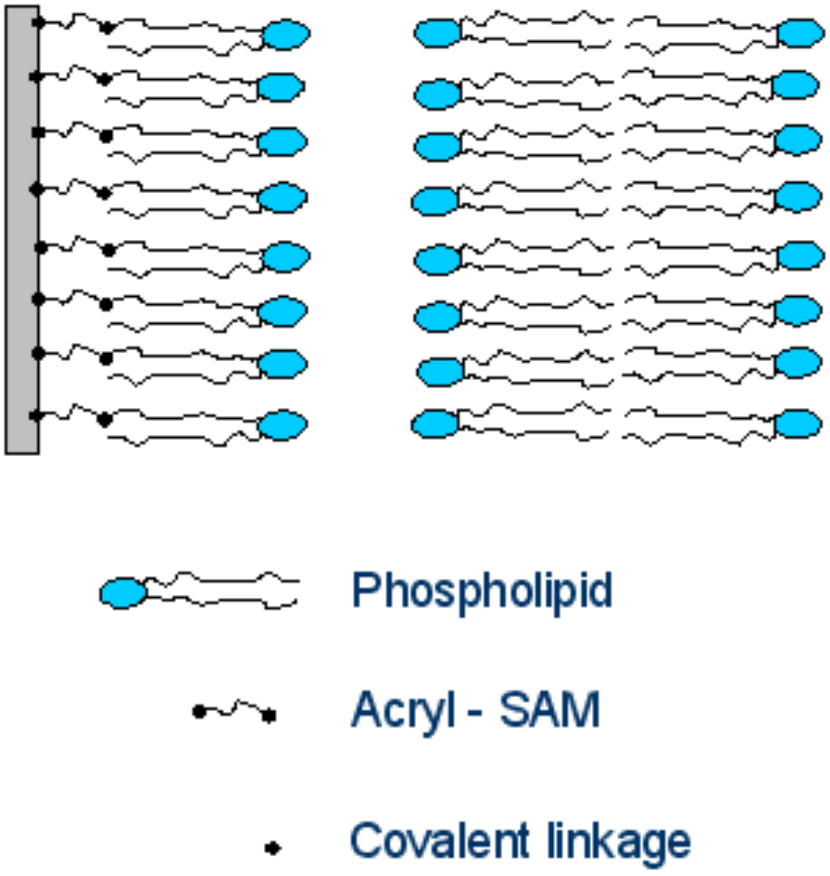
Improved artificial membranes?



the outer bilayer of systems (a) and (c) rapidly altered in the presence of gene delivery vehicles

surprisingly double bilayer (b) was unchanged over the 24 hour period of the experiment (result recently confirmed using DPPC double bilayers)

New, improved floating bilayer

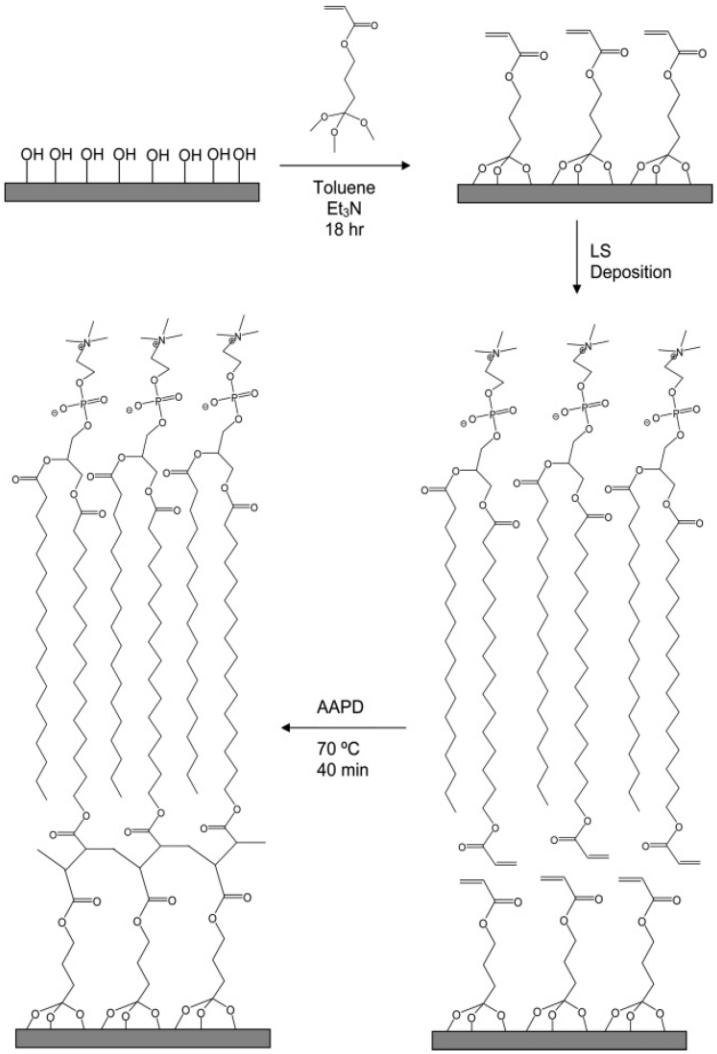


Advantages

- Reproducible
- SAM stable for long periods
- Can prepare using lipids with low phase transitions
- Allow the study of the transport of molecules across a bilayer

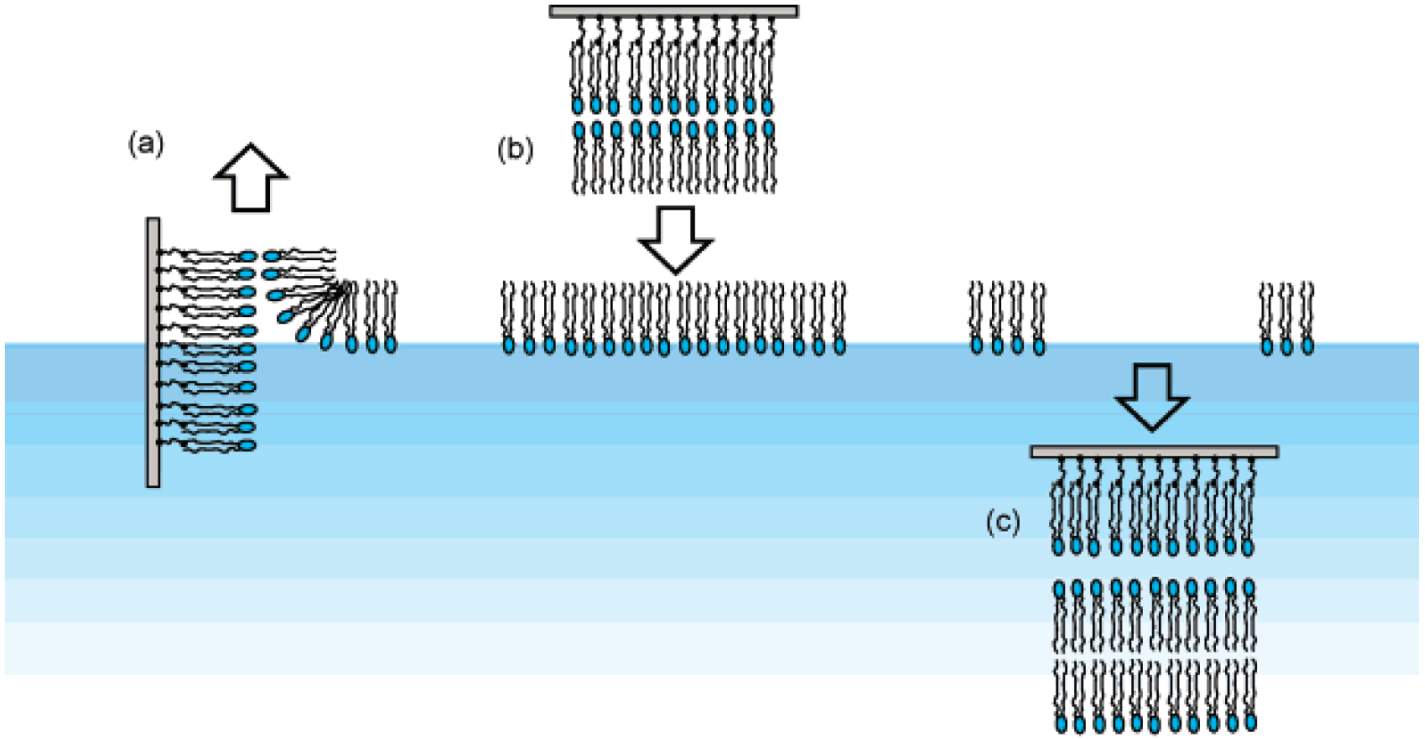
A. V. Hughes et al. Langmuir (2008) 24 1989-1999

Preparation of self-assembled monolayer



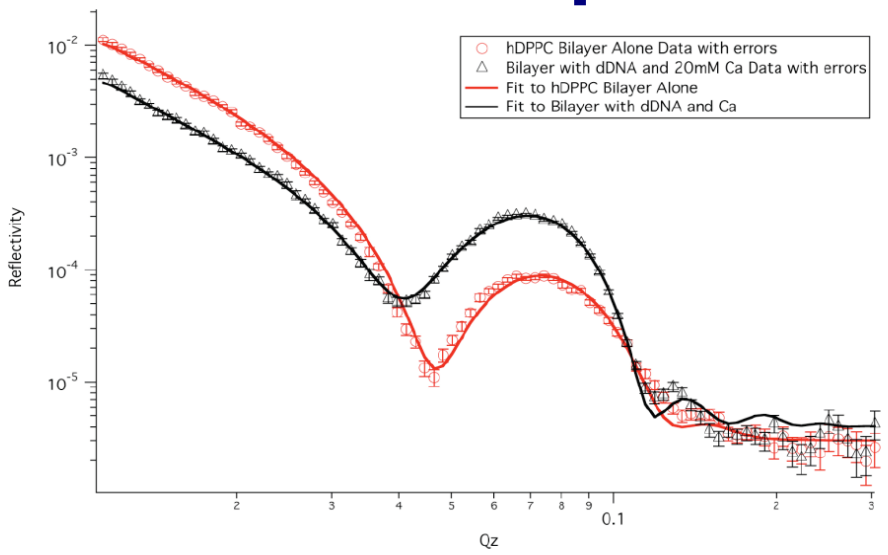
A. V. Hughes et al. Langmuir (2008) 24 1989-1999

Preparation of supported floating bilayer

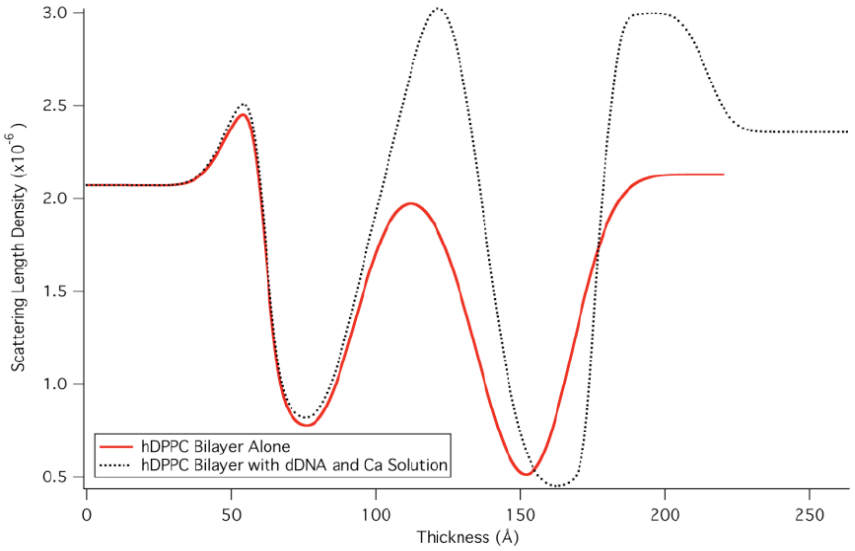


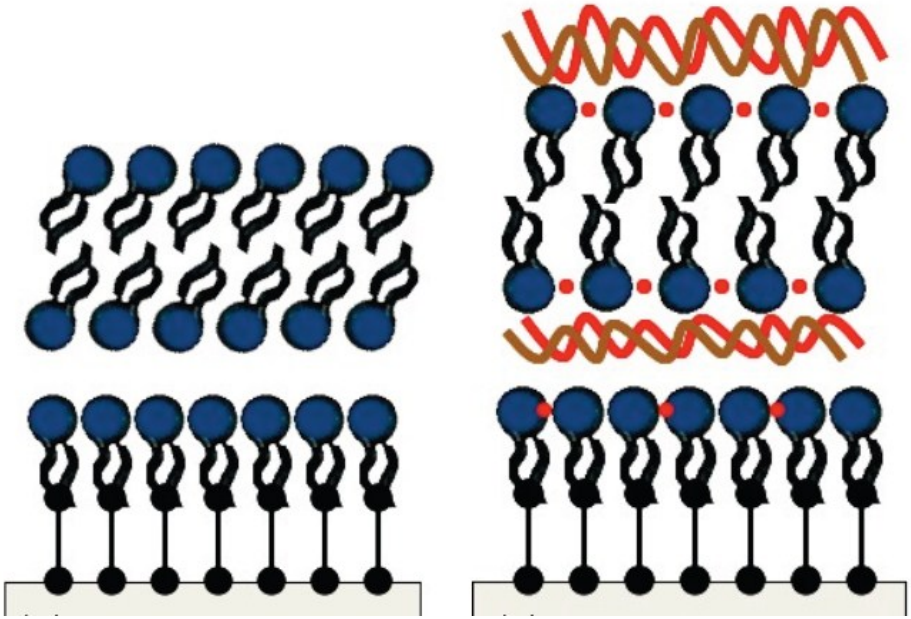
A. V. Hughes et al. Langmuir (2008) 24 1989-1999

Interaction of DNA with DPPC bilayers in the presence of Ca^{++}



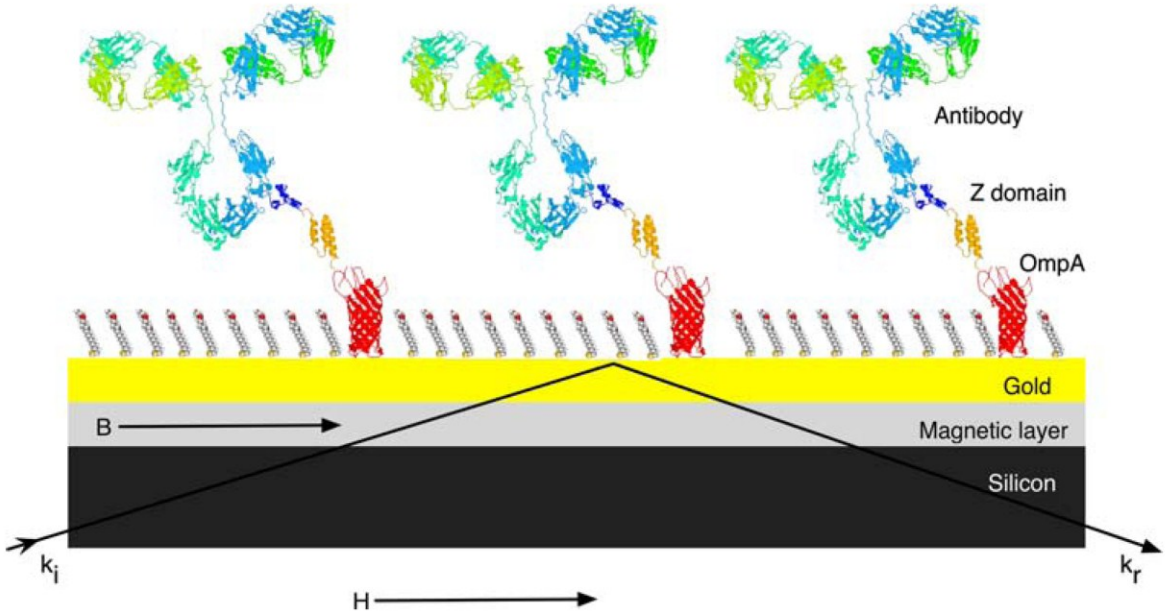
DNA coverage 33% on both inner and outer bilayer

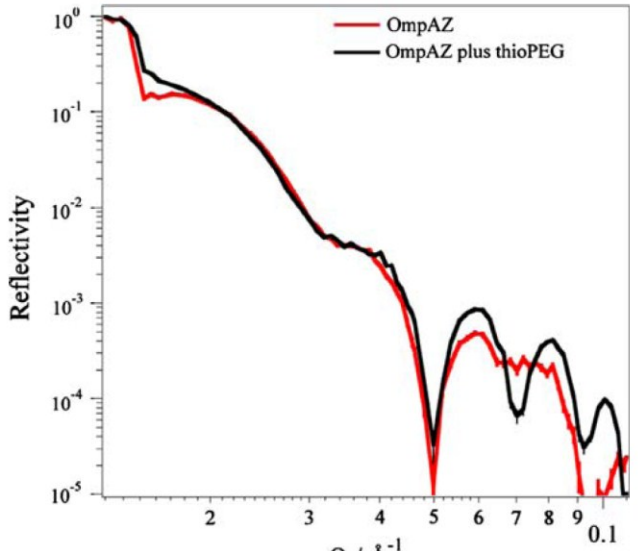


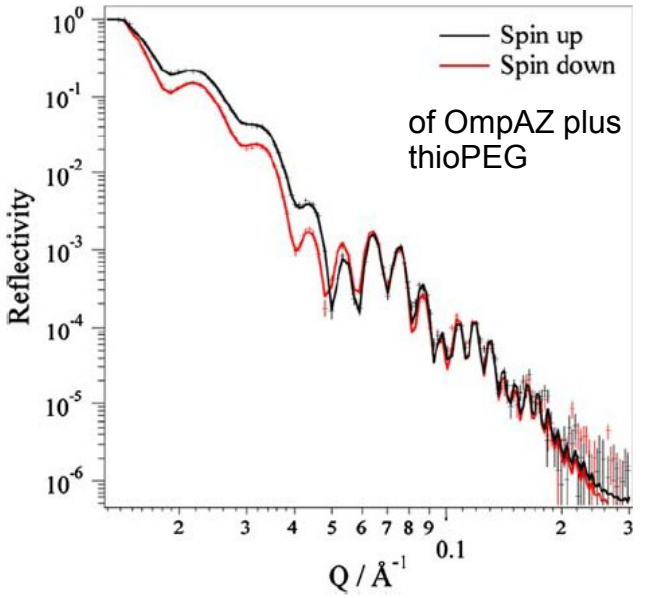


Cartoon of floating bilayer in the absence & presence of DNA & Ca^{++}

Use of polarised neutrons







Polarised neutron reflection used to probe the structu of an antibody on gold (separated by a thioPEG monolayer).

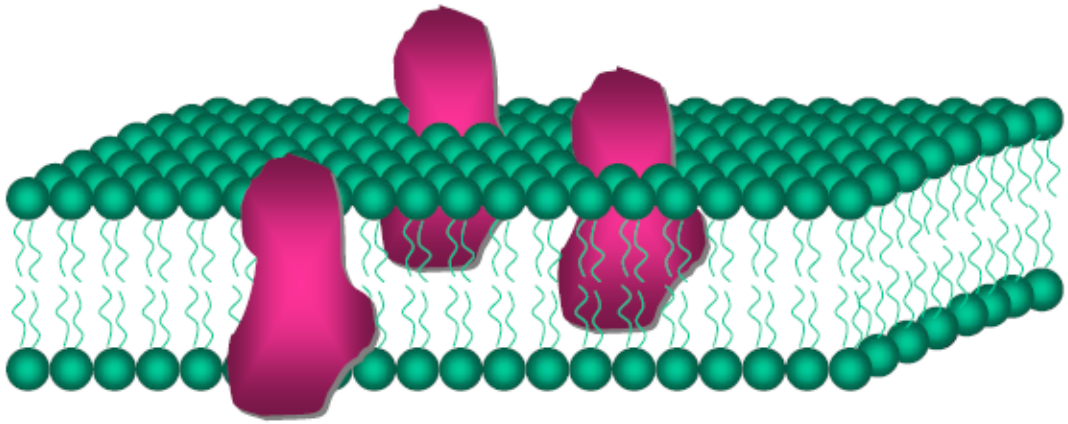
Polarised neutrons are used as this provides a mean of achieving extra contrast in samples having a magnetic metal layer (Fe or Ni) under the gold surfa

This contrast is attained without resorting to hydrogen/deuterium exchange in the biological layer

A.P. Le Brun et al Eur. Biophys. J. (2008) 37, 639

Phospholipid bilayers containing intergral membrane proteins

100% D_2O

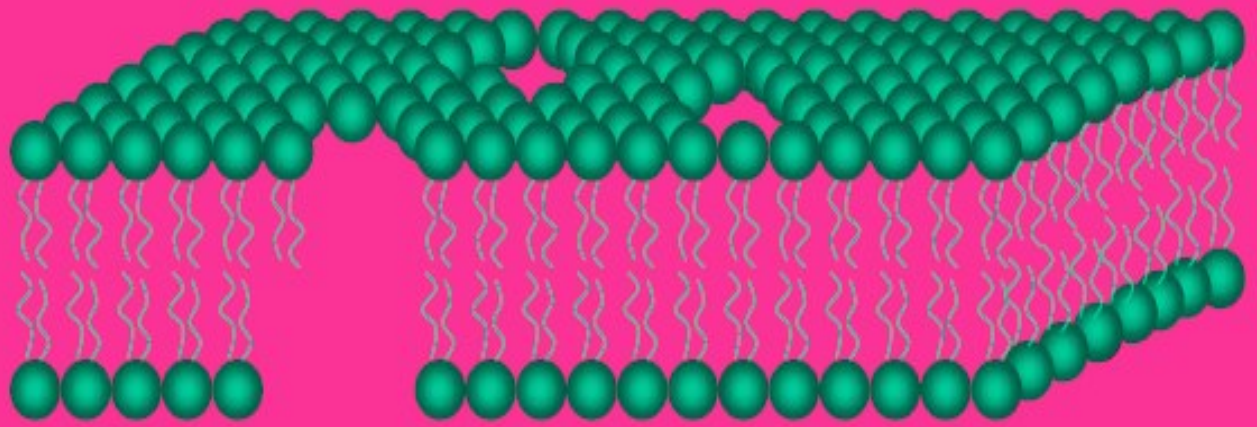


see whole membrane (if h-lipid used)

O.Byron (unpublished)

Phospholipid bilayers containing intergral membrane proteins

43% D_2O

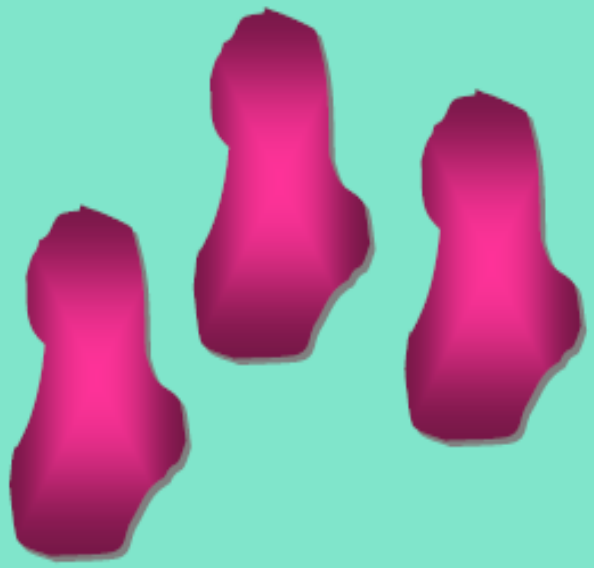


see only the lipid

O.Byron (unpublished)

Phospholipid bilayers containing intergral membrane proteins

13% D_2O



see only the proteins

O.Byron (unpublished)