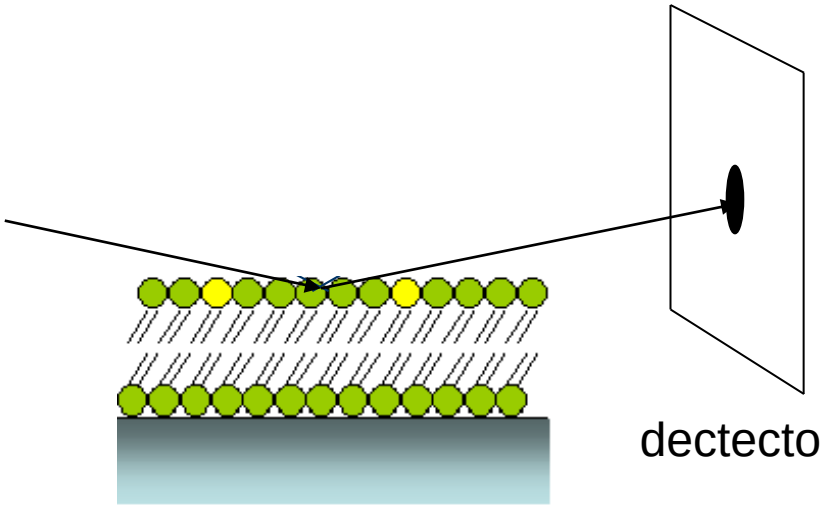
NEUTRON REFLECTIVITY IN BIOLOGY AND MEDICINE

DAVID SCOTT

NATIONAL CENTRE FOR MACROMOLECULAR HYDRODYNAMICS, SCHOOL OF BIOSCIENCES, UNIVERSITY OF NOTTINGHAM, UK.

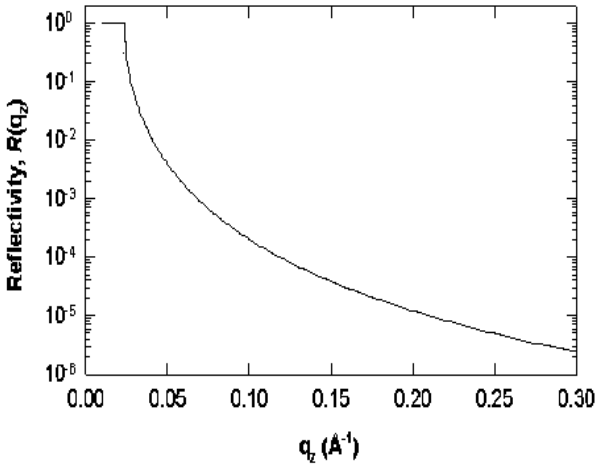
WHY NEUTRON REFLECTIVITY STUDIES?

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



dectector

specular reflection

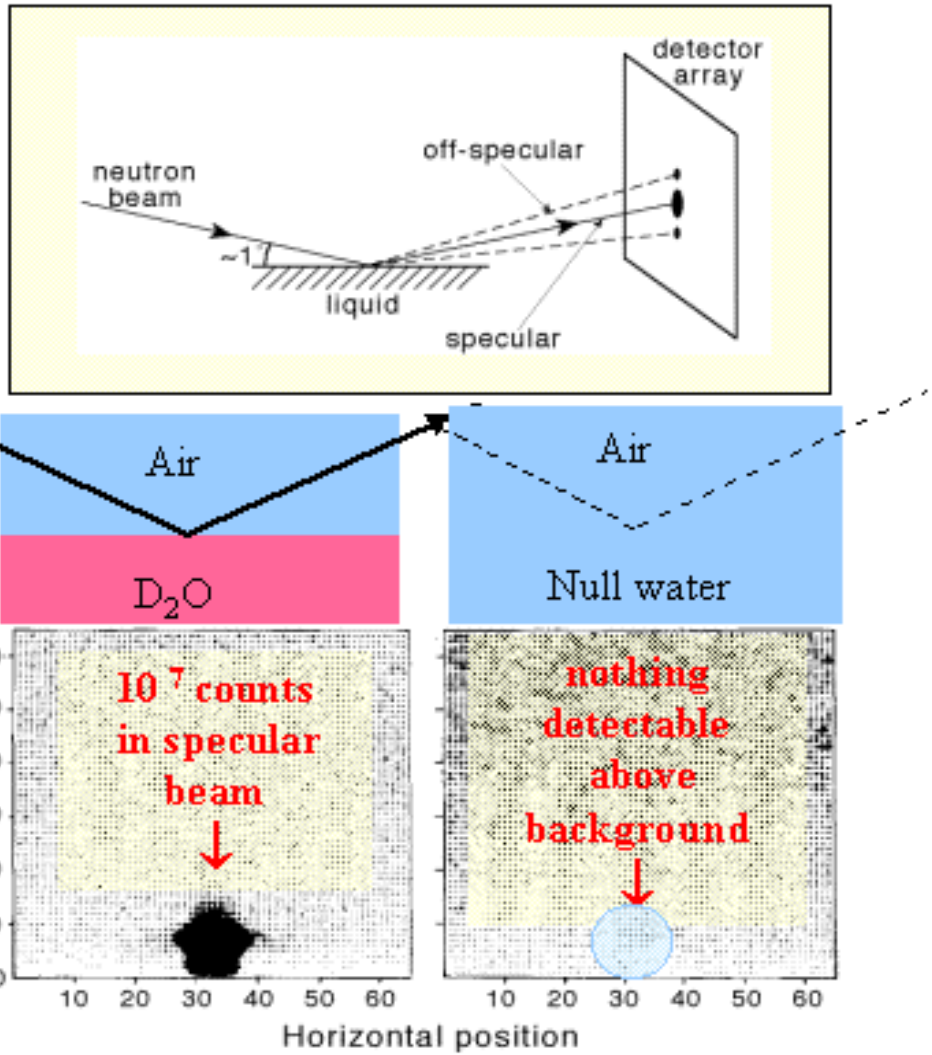


$R(\theta,\lambda) = I_R/I_0(\lambda)$ $Q = (4\pi/\lambda)\sin\theta$

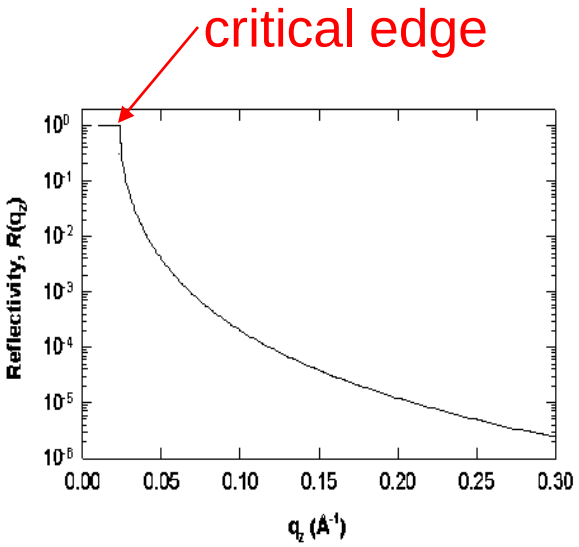
Solid-liquid data analysed using the 'optical maxtrix' method

At least three *different 'contrasts'* measured

WHAT DOES THE NEUTRON DATA LOOK LIKE?



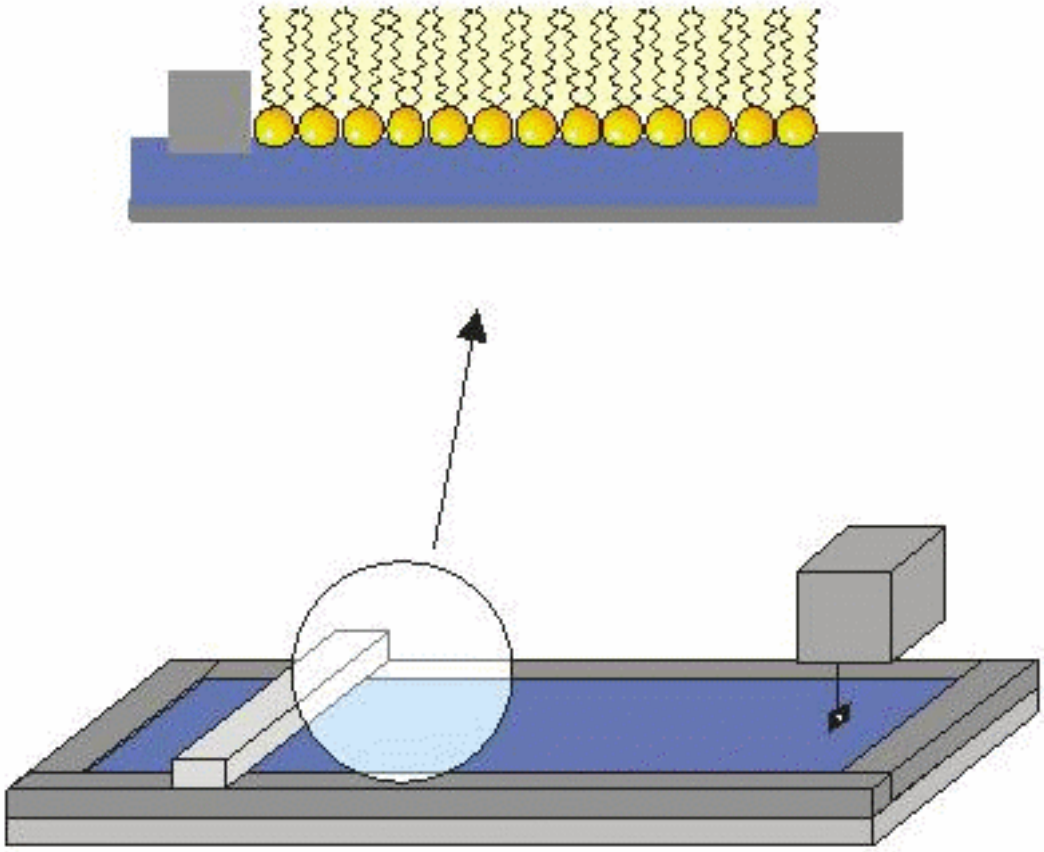
Reflection from a sharp D_2O/air interface



At large Q – R varies as $1/q^4$

Below Q_{crit} R = 1

LIPID MONOLAYER STUDIES



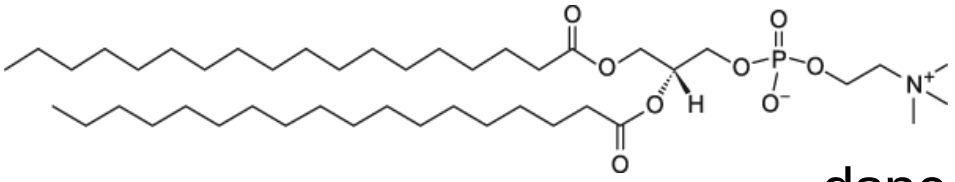
- relatively easy to prepare
- composition of monolayer well-defined
- hold monolayer at varying pressure
- good mimic of membrane
- number of advantages over bilayers deposited on a solid support

NEUTRON REFLECTIVITY

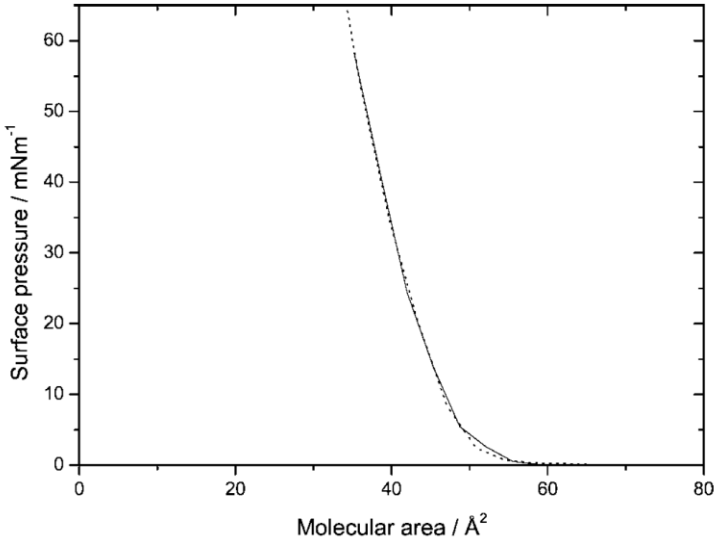
All monolayer studies performed at RAL on SURF (and CRISP) and at FIGARO (ILL)

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

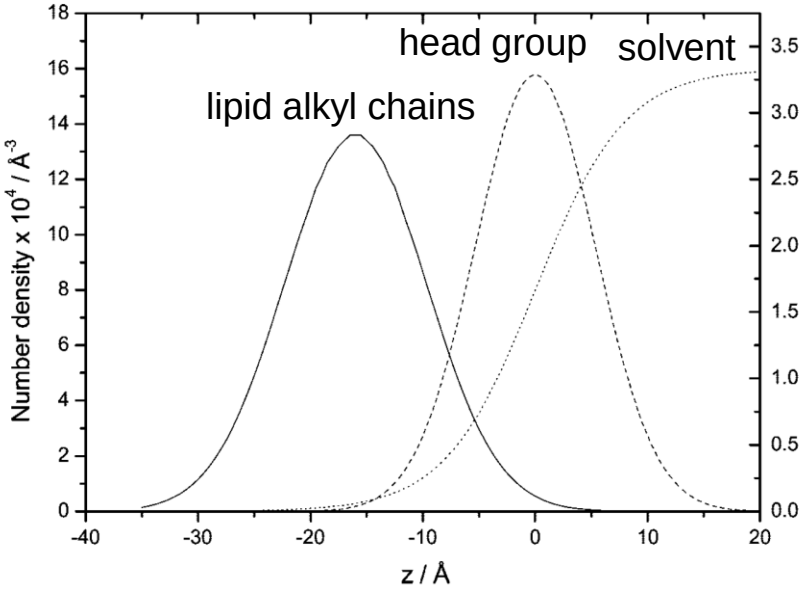
MONOLAYER OF DSPC



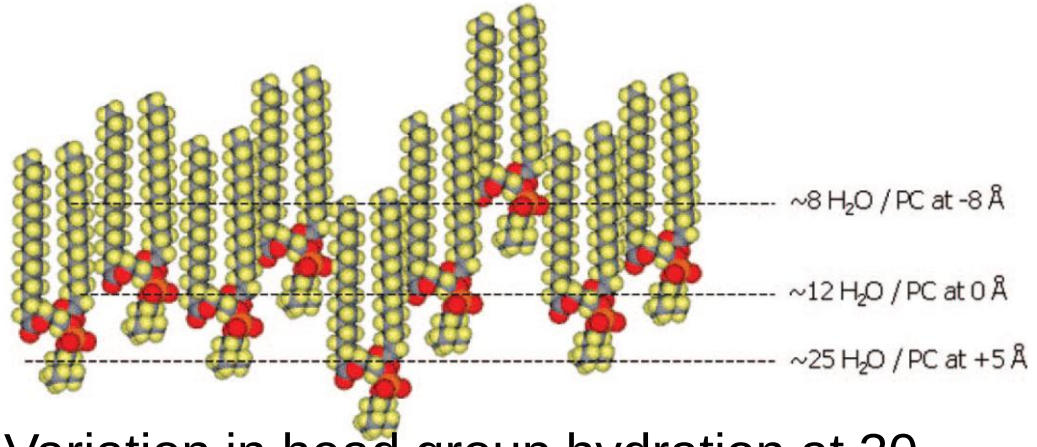
dspc



Isotherm of fully d-dspc and h-dspc



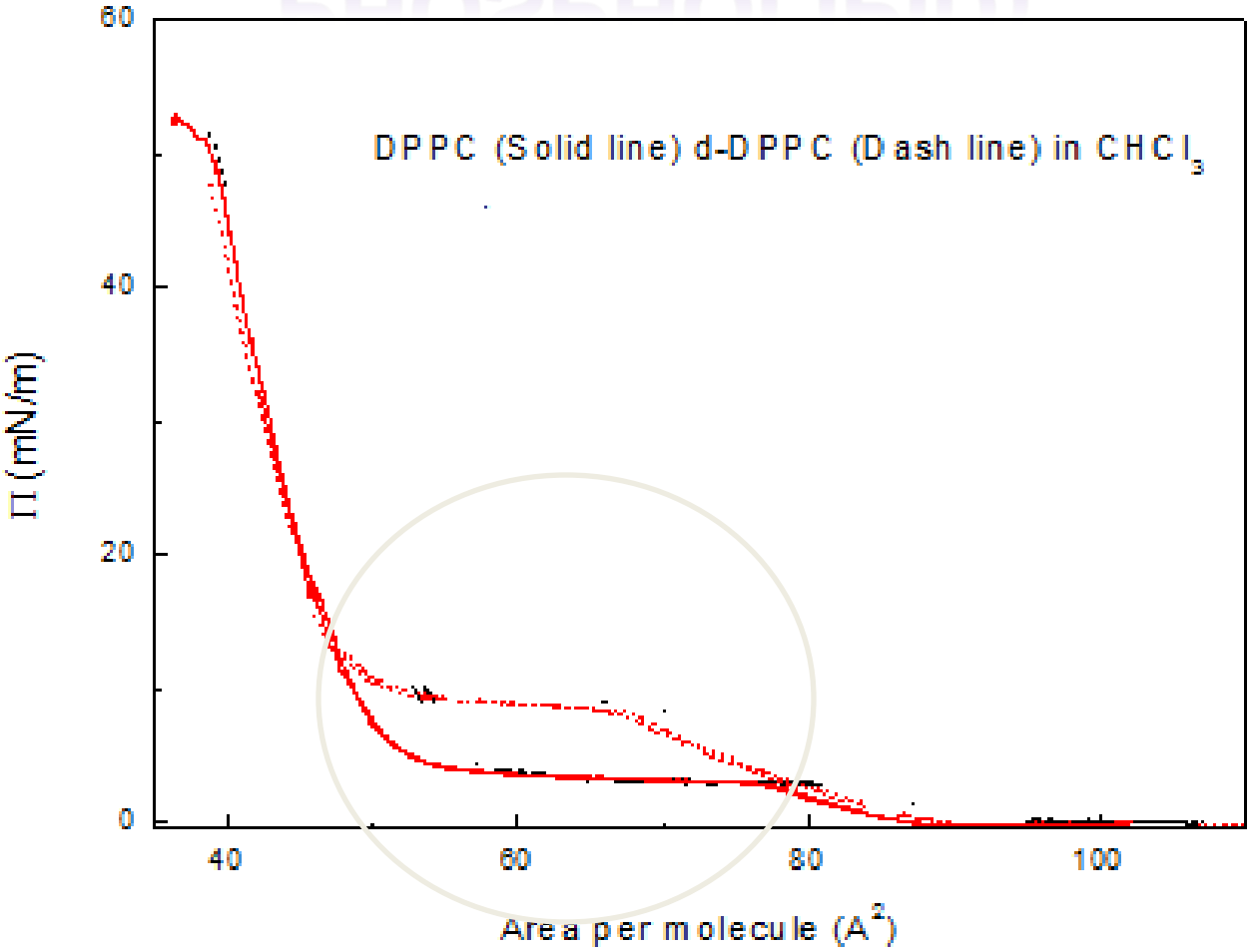
Number density profiles for the DSPC monolayer 30 mN/m



Variation in head group hydration at 30 mN/m

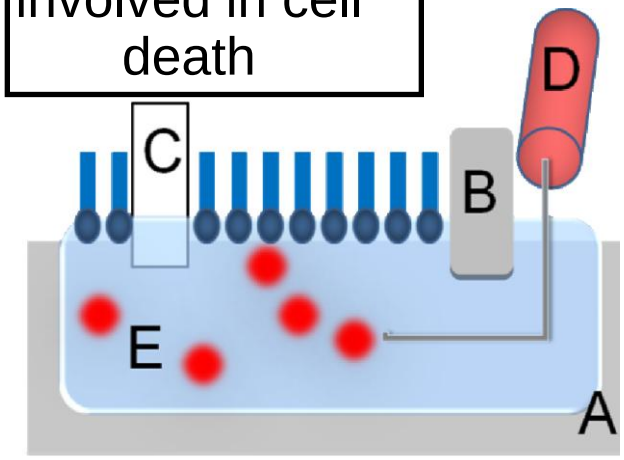
BARLOW ET AL LANGMUIR (2009) 25 4070

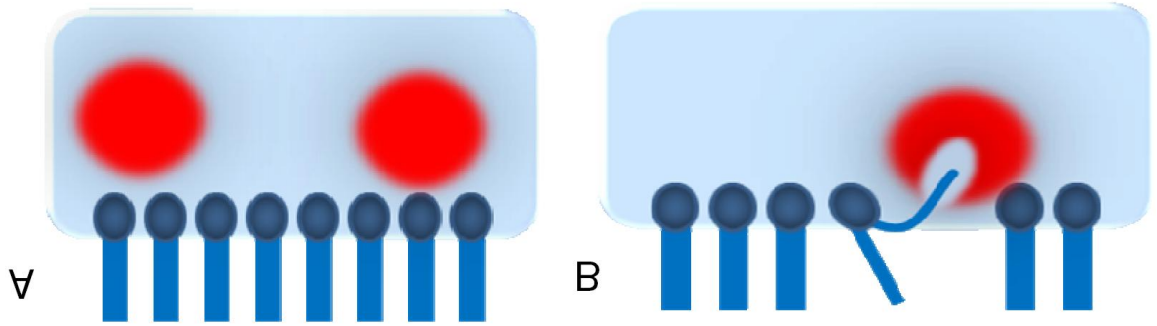
ISOTOPE EFFECT FOR DPPC (C_{16} PHOSPHOLIPID)



MONOLAYER STUDIES AS A CRUDE MIMIC OF A BIOLOGICAL MEMBRANE

Cytochrome C anionic protein involved in cell death

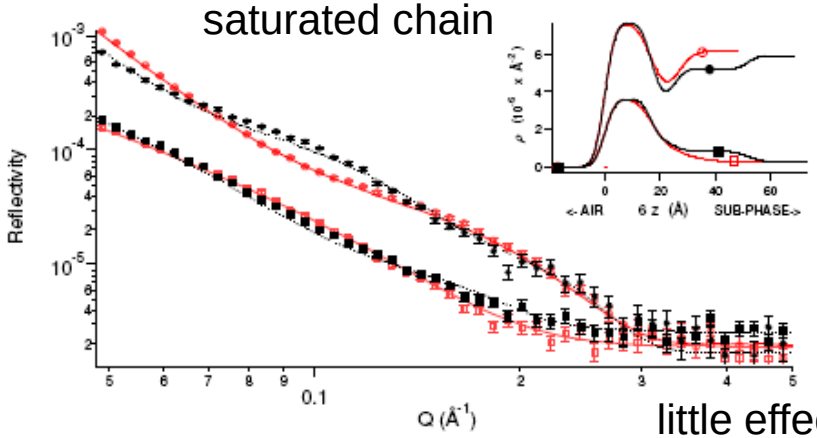




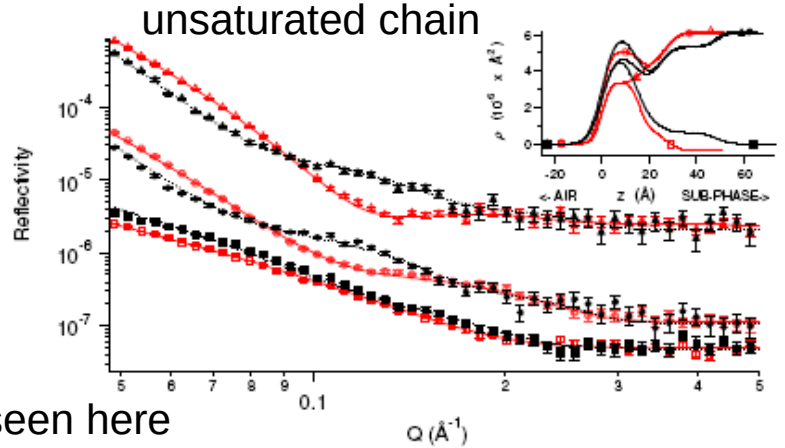
Lipid anchorage model of interaction of cytchrome c

Effect of head group and hydrophobic chain saturation

saturated chain



unsaturated chain

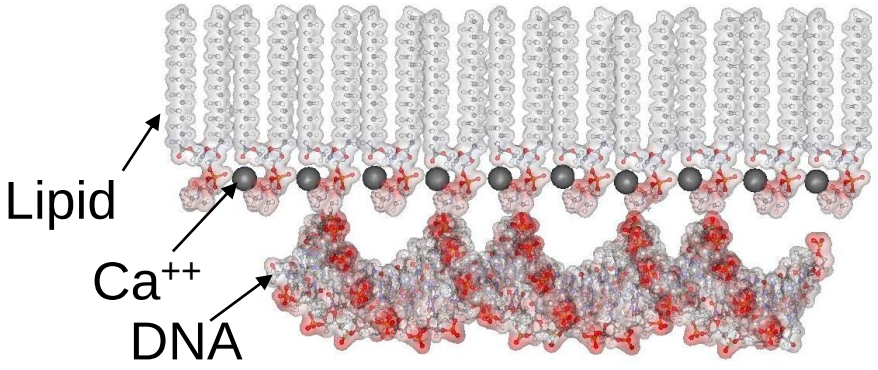


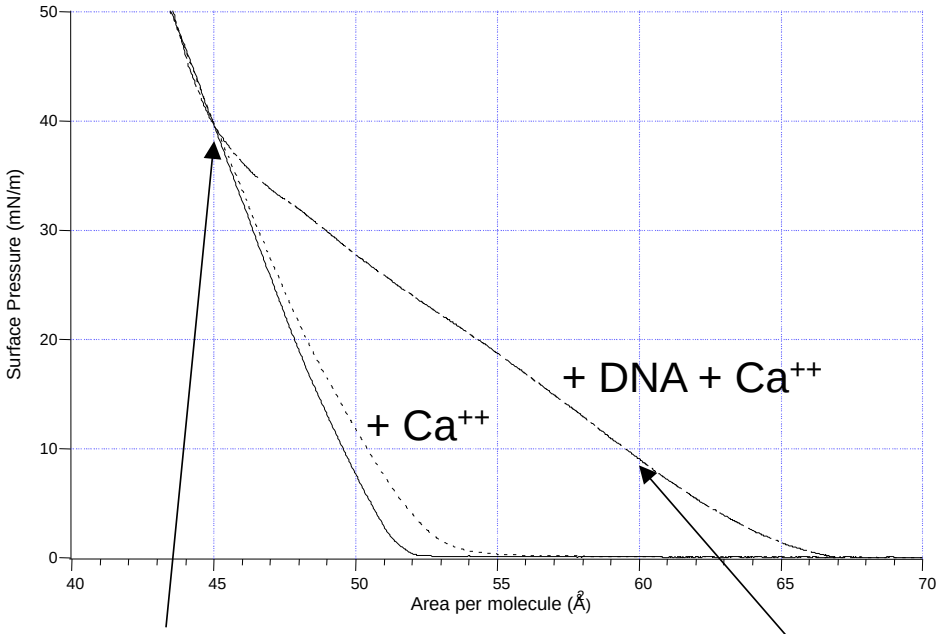
little effect seen here

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++}?

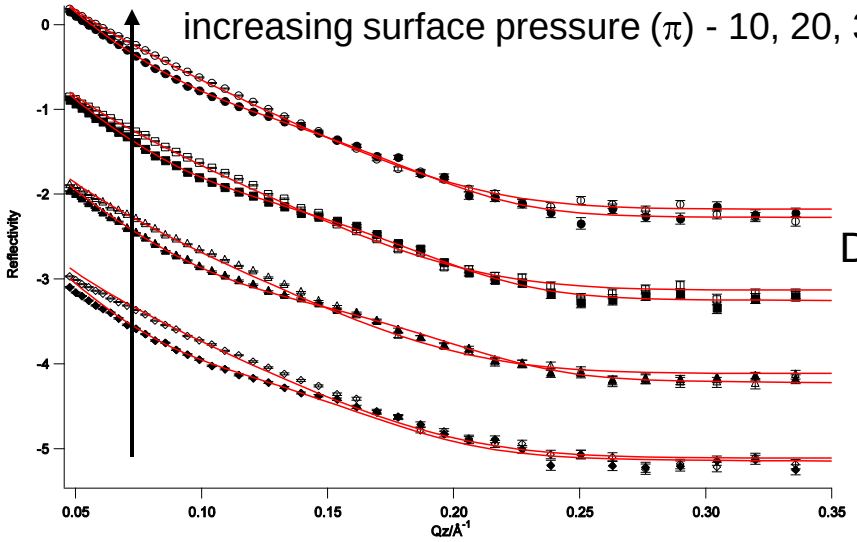


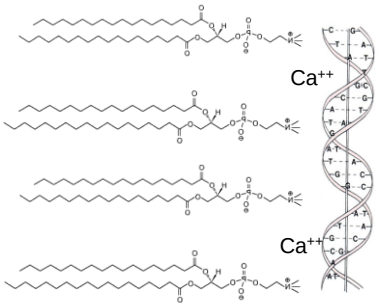


Isotherms overlay at low areas per molecule – DNA *excluded* from monolayer

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

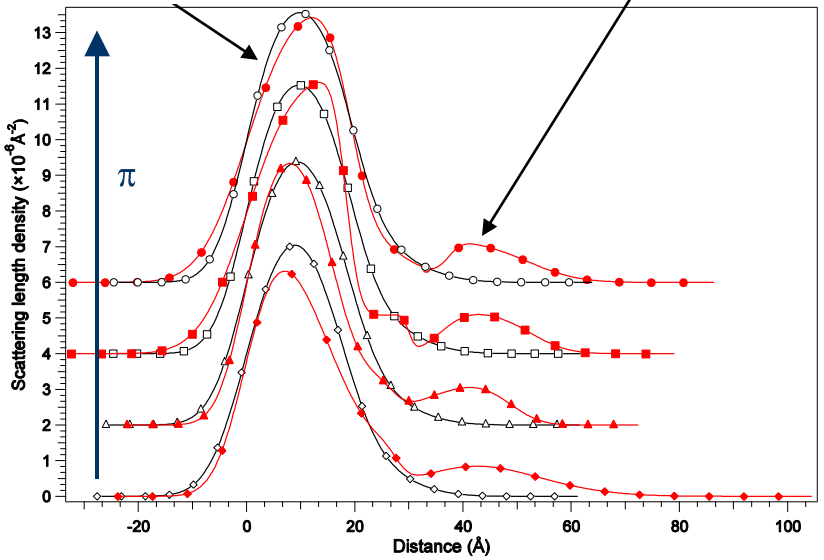
NEUTRON REFLECTIVITY





DSPC chains

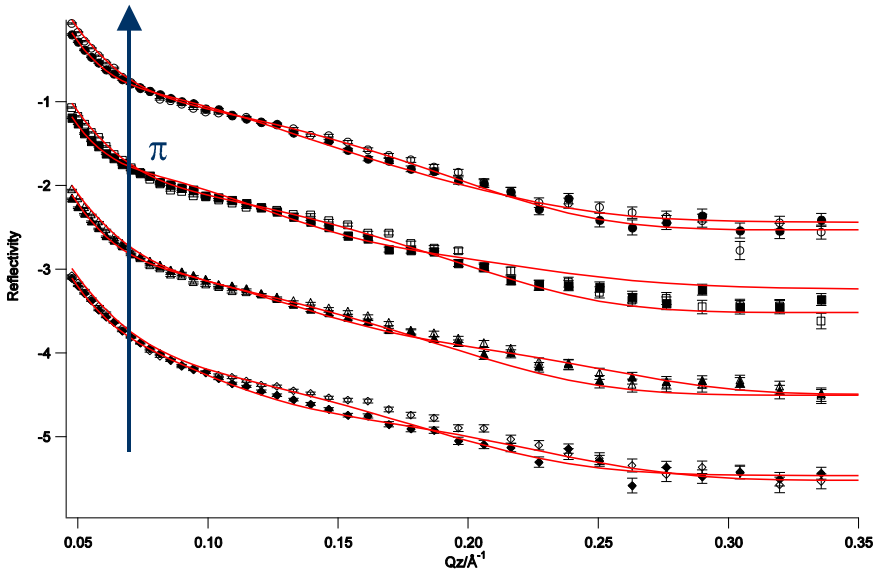
DNA

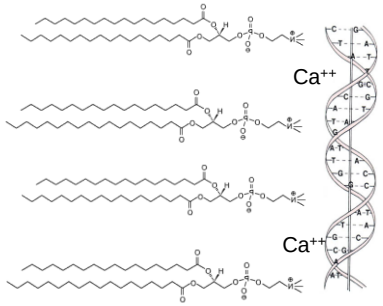


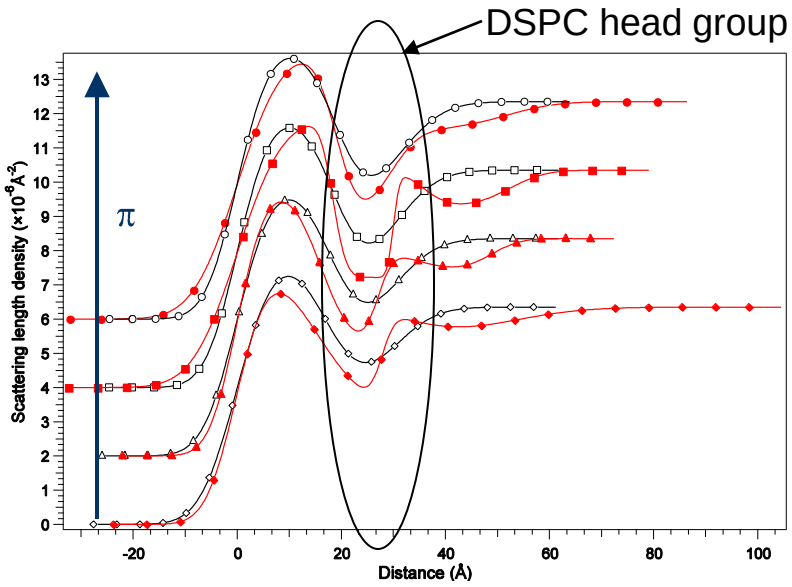
d_{70}-DSPC/acmw + 20mM Ca^{++}
with (—) and without (—) DNA

here 'seeing' mainly lipid chains
and, when present DNA

NEUTRON REFLECTIVITY



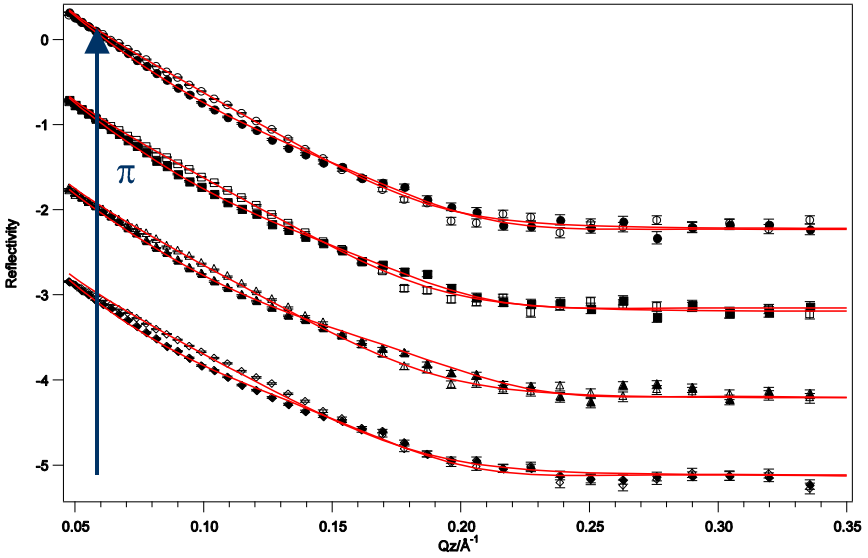


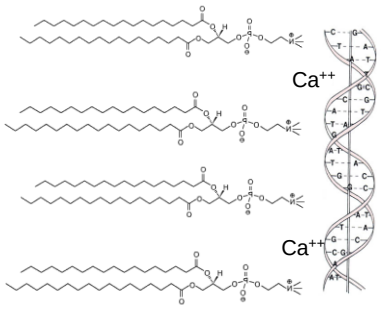


d_{70}-DSPC/D_2O + 20mM Ca^{++} with (—) and without (—) DNA

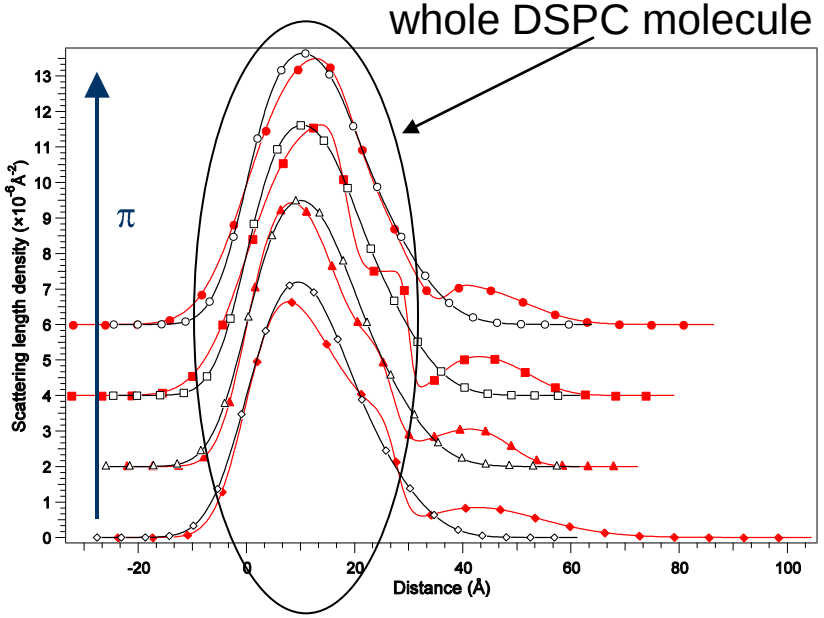
here ‘seeing’ mainly DSPC head group, and when present DNA

NEUTRON REFLECTIVITY





whole DSPC molecule



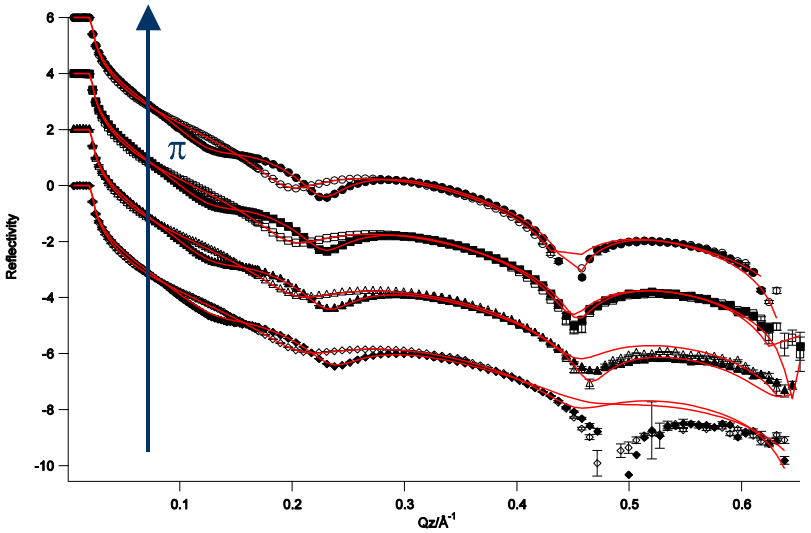
d_{83}-DSPC/acmw + 20mM Ca^{++} with (—) and without (—) DNA

here seeing mainly whole DSPC molecule and when present DNA

X-RAY REFLECTIVITY

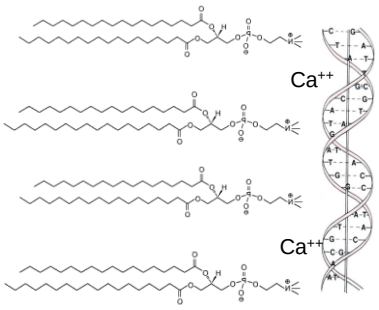
Performed on TROIKA-II beam line at the ESRF, Grenoble, France

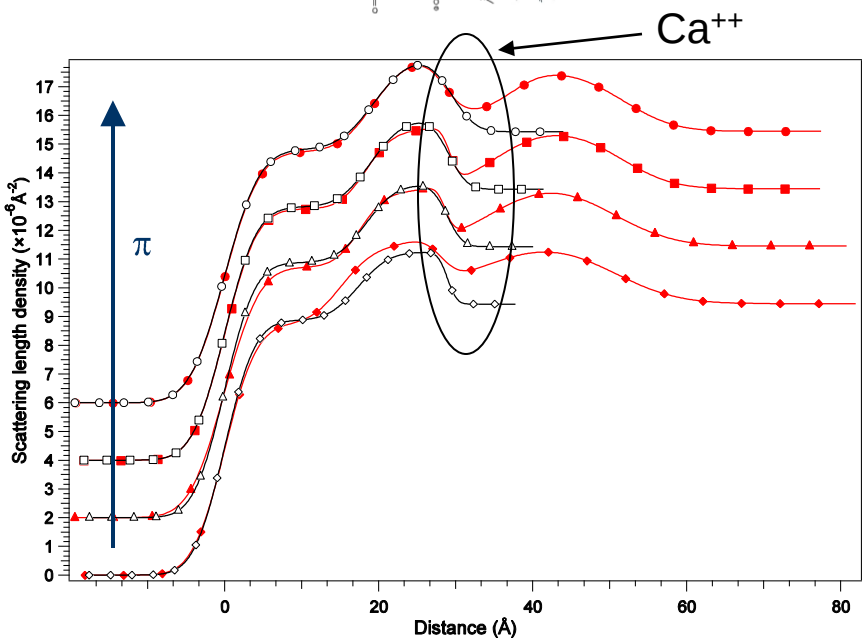
X-RAY REFLECTIVITY



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

here ‘see’ Ca^{++} and when present DNA





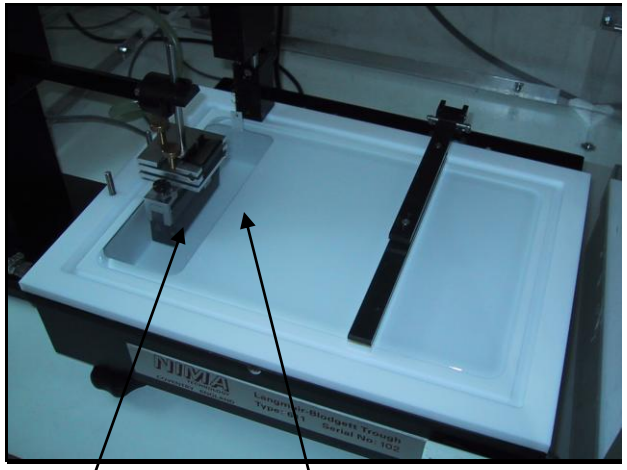
NEUTRON REFLECTIVITY

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

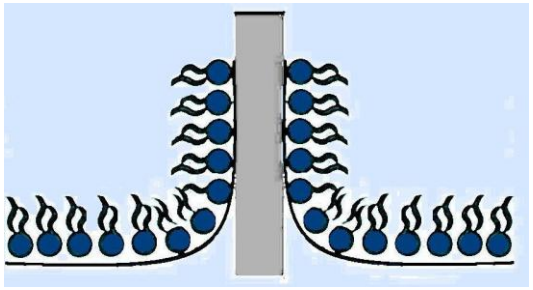
PREPARATION OF SUPPORTED LIPID BILAYERS USING A COMBINATION OF LANGMUIR BLODGETT & SHAEFFER TECHNIQUES



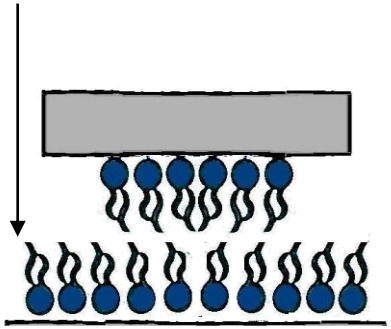
silicon block

phospholipid monolayer spread on surface of trough

lipid monolayer

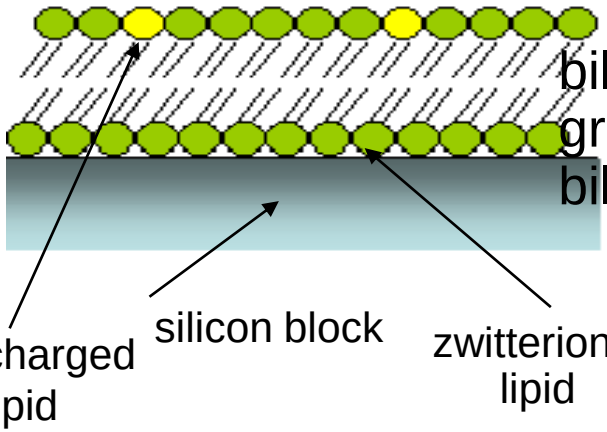


Blodgett deposition

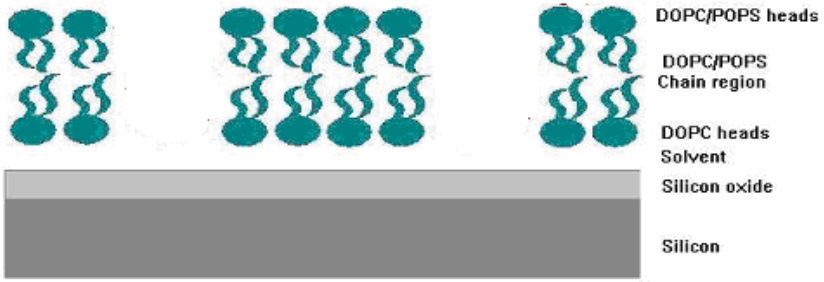


Shaeffer deposition

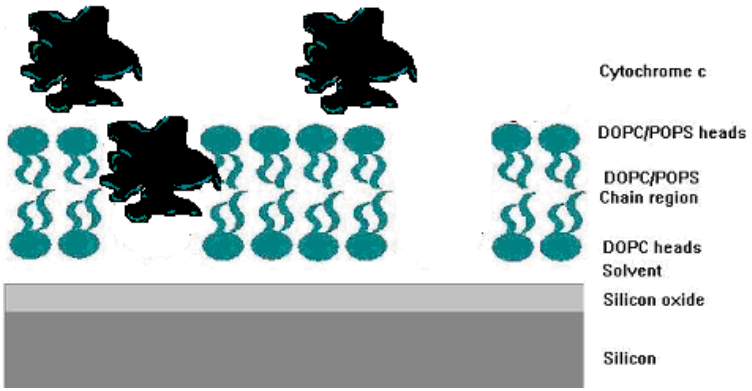
BINDING OF CYTOCHROME C TO BILAYERS



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

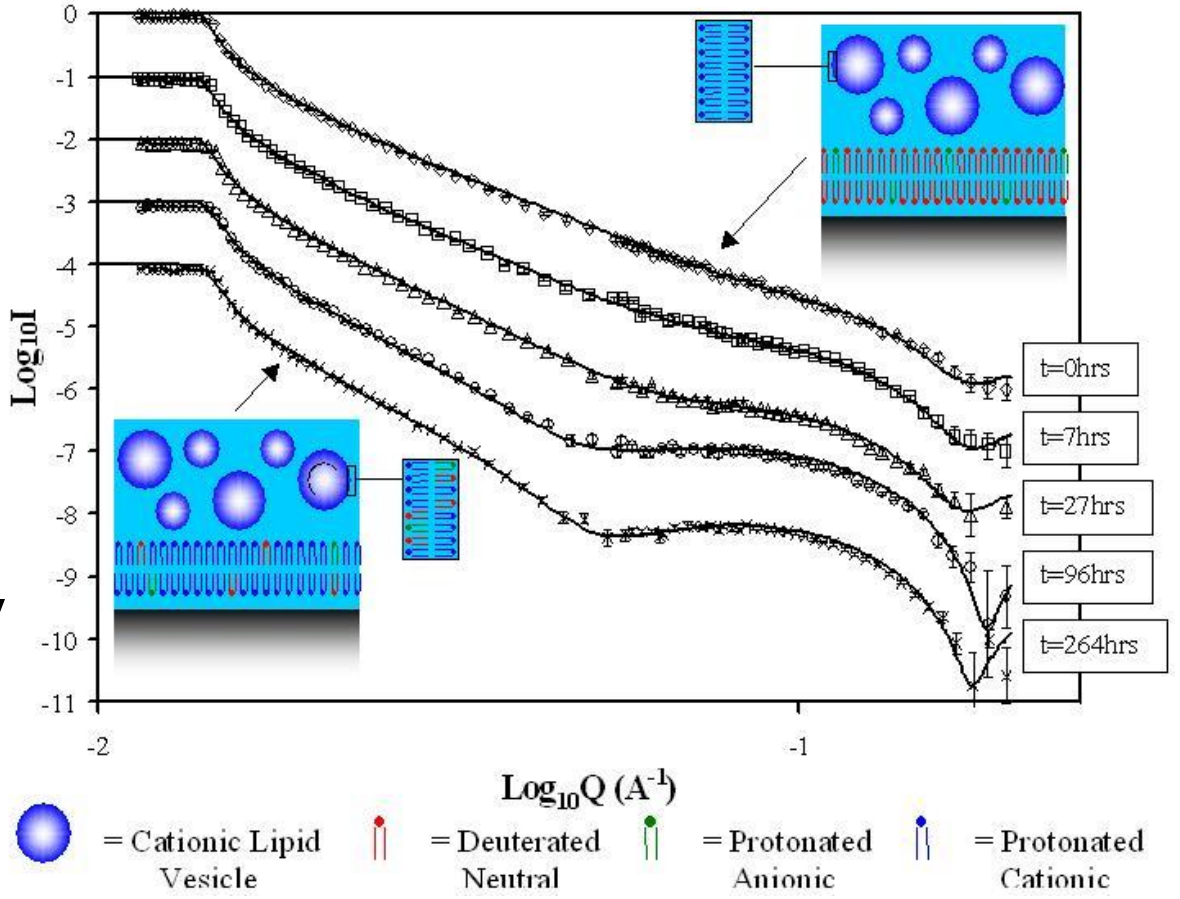
Unsaturated zwitterionic lipid increased cytochrome c binding to anionic lipid compared to partially saturated lipid

Nature of anionic lipid less important

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

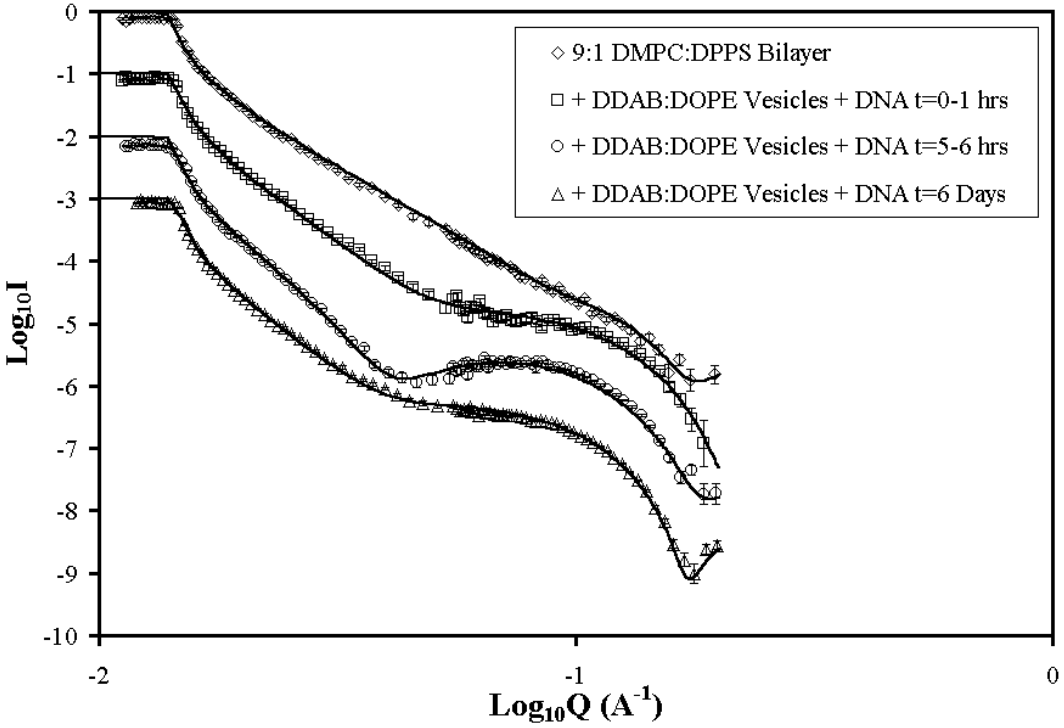


Callow et al Langmuir 21 (2005) 7912

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

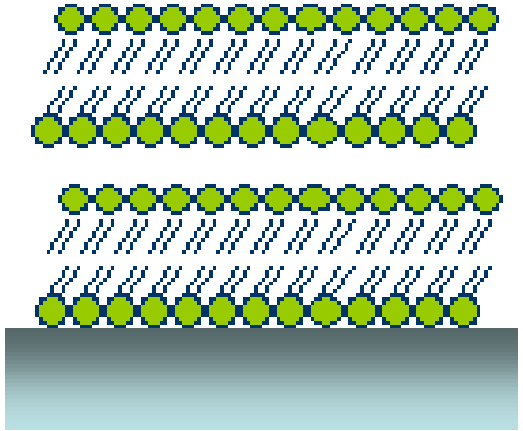
IMPROVED ARTIFICIAL MEMBRANES?

Substrate surface has prepared using ozone method has a negative charge

Does this influence how the vesicles/gene vectors interact with the artificial biomembrane – particularly if surface coverage is low

Need for better membrane models?

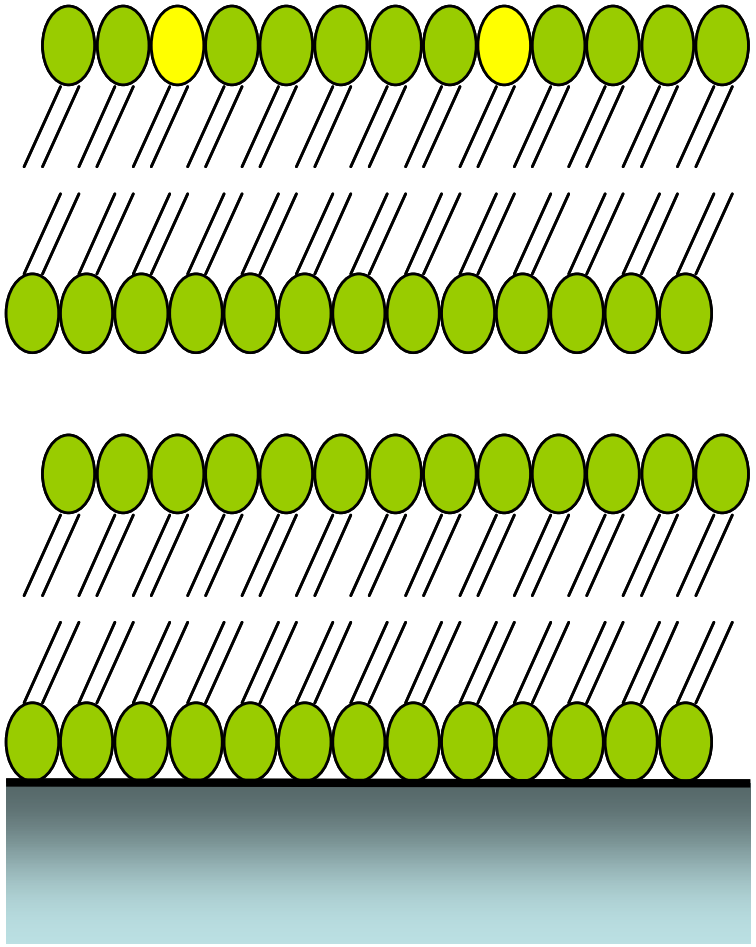
Would a supported (or double) floating bilayer be better?



double ‘floating’ bilayer

Fragneto et al Europhys Lett 53 (2001) 100

SUPPORTED FLOATING BILAYERS (FSB)



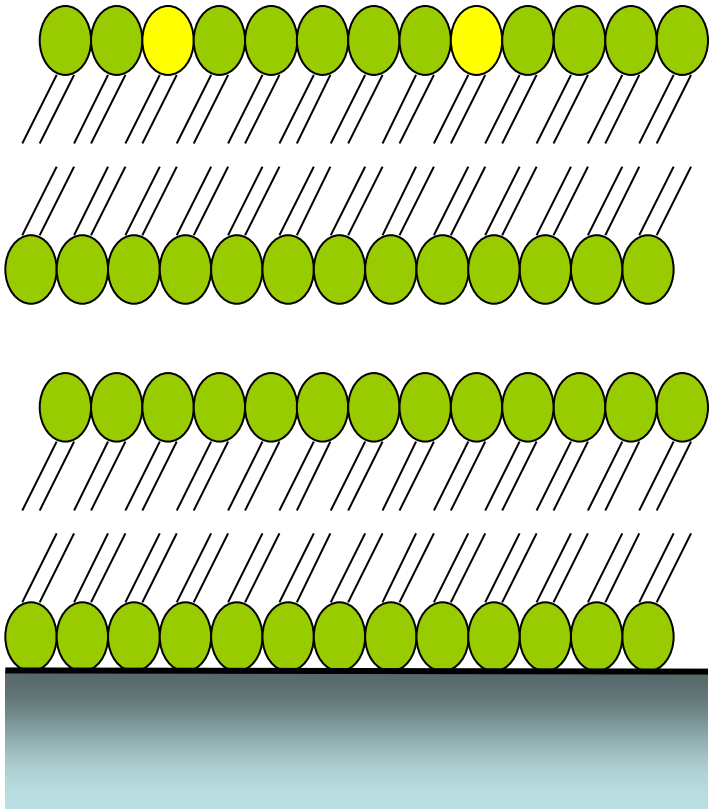
- The FSB is robustly associated with the substrate
- Cushioned from the substrates constraining effects by a hydration layer 20 – 30 Å thick
- The separation from the substrate is the result of the balance between the electrostatic and Van der Waals interactions that hold the bilayer in place, and the 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

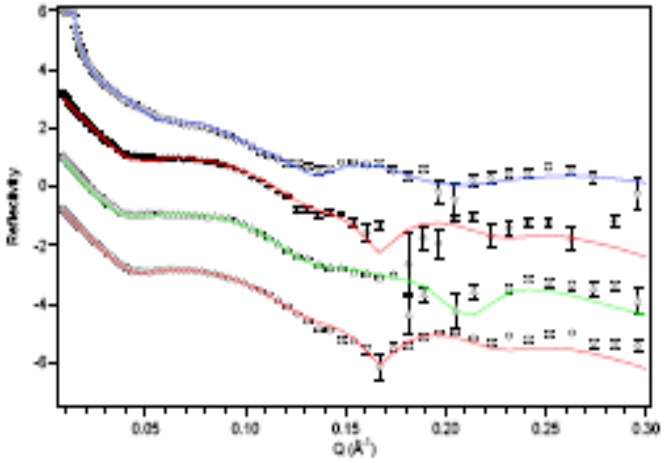
DMPC, a fluid phase lipid at 25 & 37°C, while a good model of the cell membrane, is not a good lipid for production of double bilayers

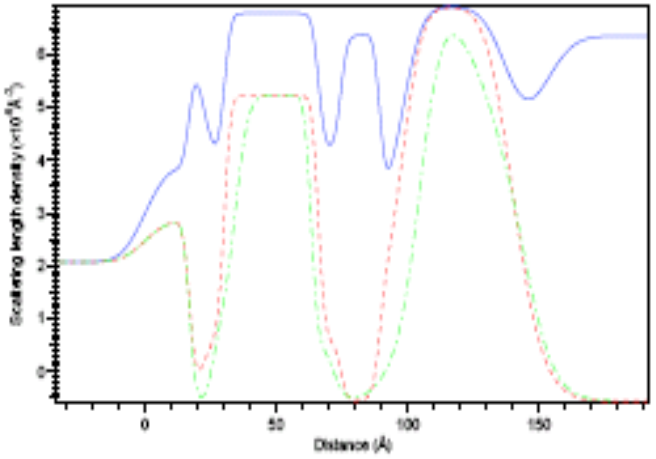
It is relatively easy to prepare double bilayers from DPPC or DSPC which are both the their gel phase at 25 & 37°C

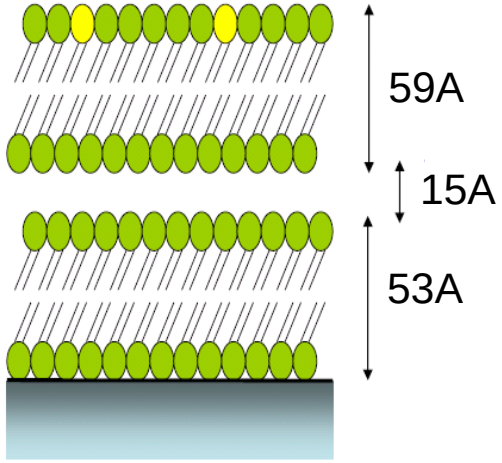


NB all double bilayers contained 10% PS in their outer leaflet

d_{62}-DPPC FSB





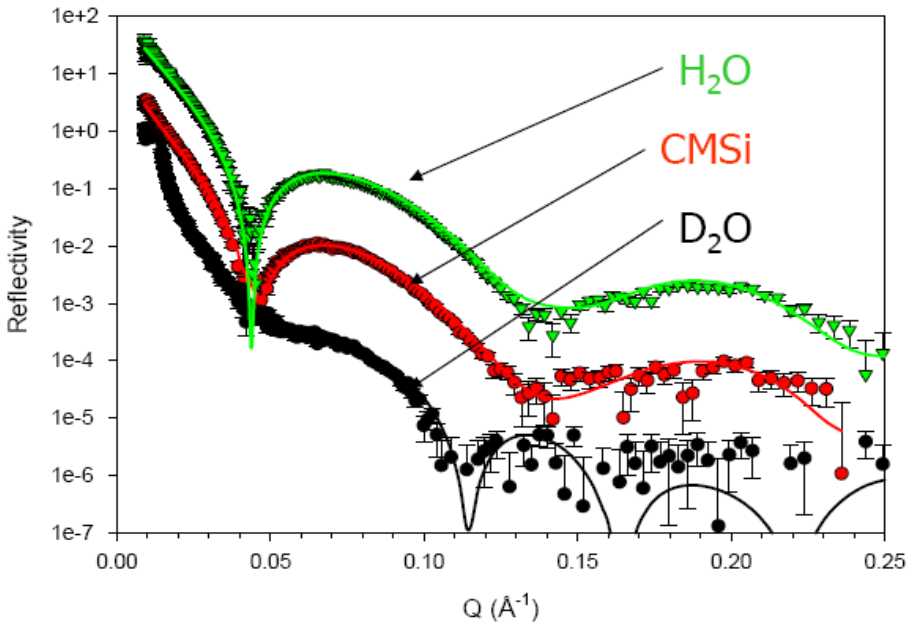


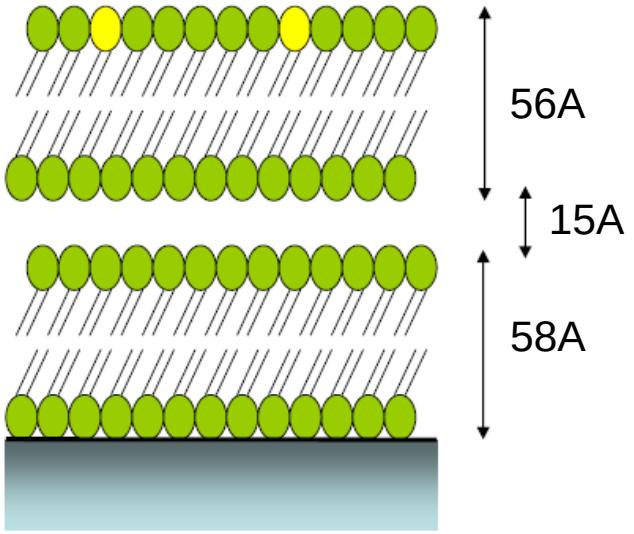
Reflectivity (a) and scattering length density (b) profiles of DDPC double bilayers in H_2O at 25^{o}C and 37^{o}C (dashed), at 45^{o}C (dot dashed) and in D_2O at 37^{o}C (solid)

Talbot et al Langmuir (2009) 25 4168

Double bilayers – d_{83}-DSPC

Reflectivity data from 3 solvent contrasts



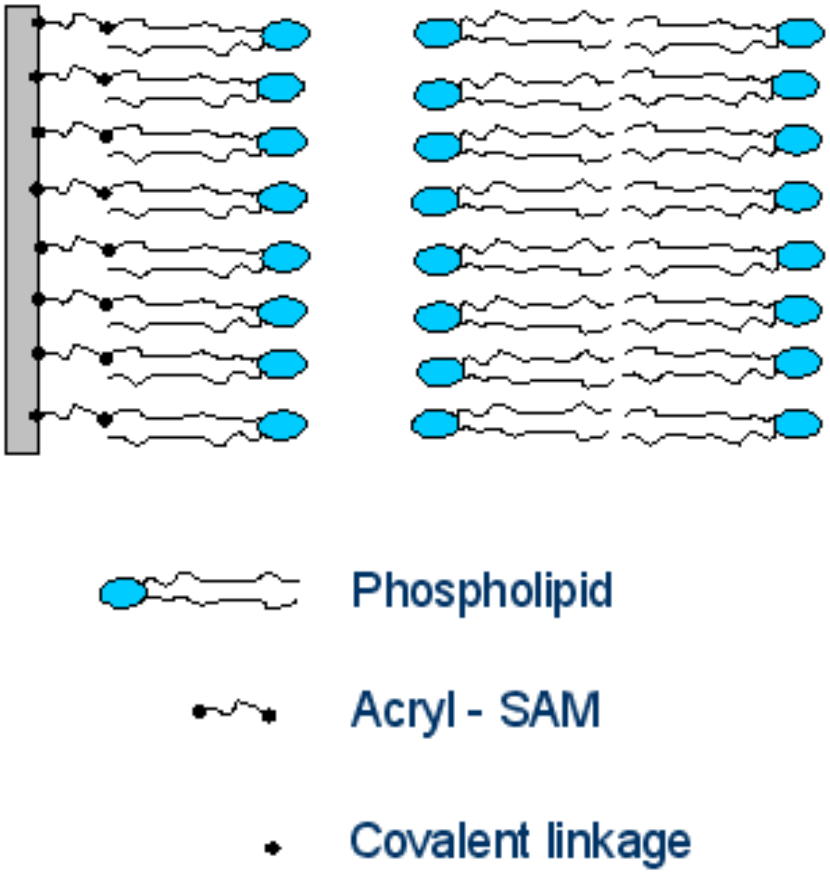


A lower vesicle concentration of 0.01mg/ml was used

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

Talbot et al Langmuir (2009) 25 4168

NEW, IMPROVED FLOATING BILAYER

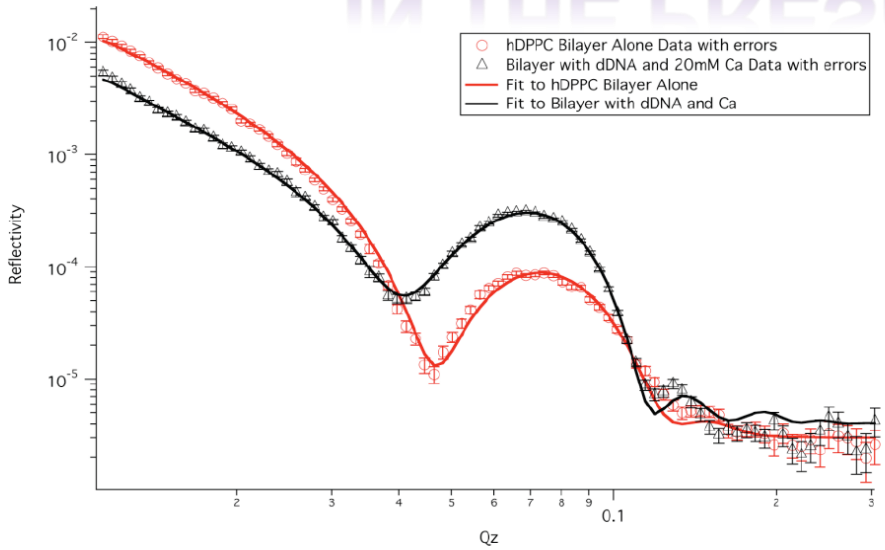


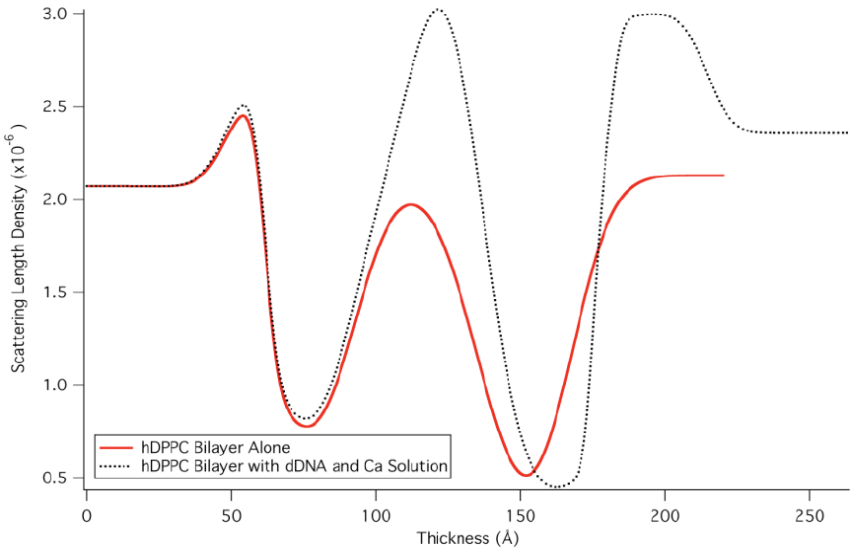
Advantages

- 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

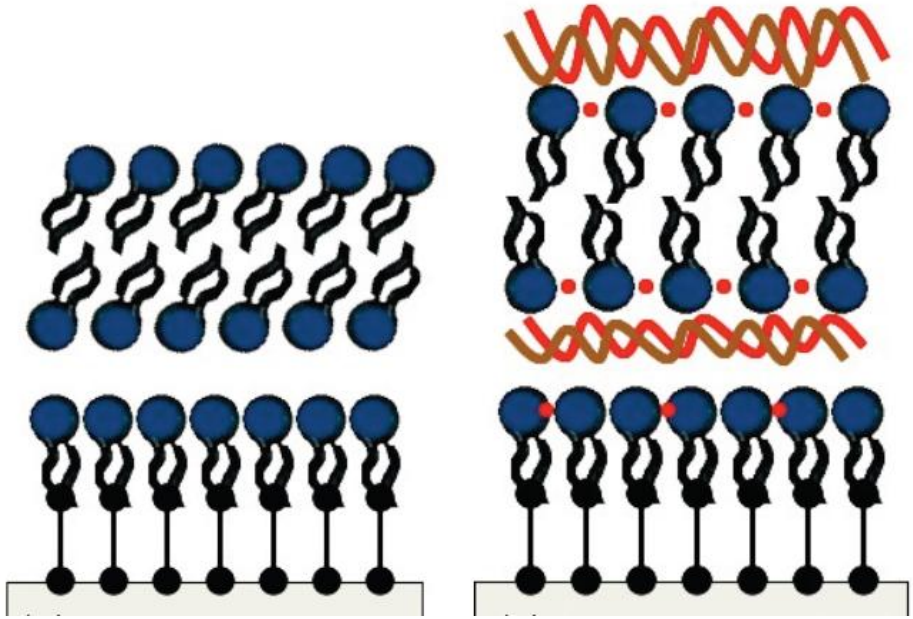
A. V. Hughes et al. Langmuir (2008) 24 1989-1999

INTERACTION OF DNA WITH DPPC BILAYERS IN THE PRESENCE OF CA^{++}



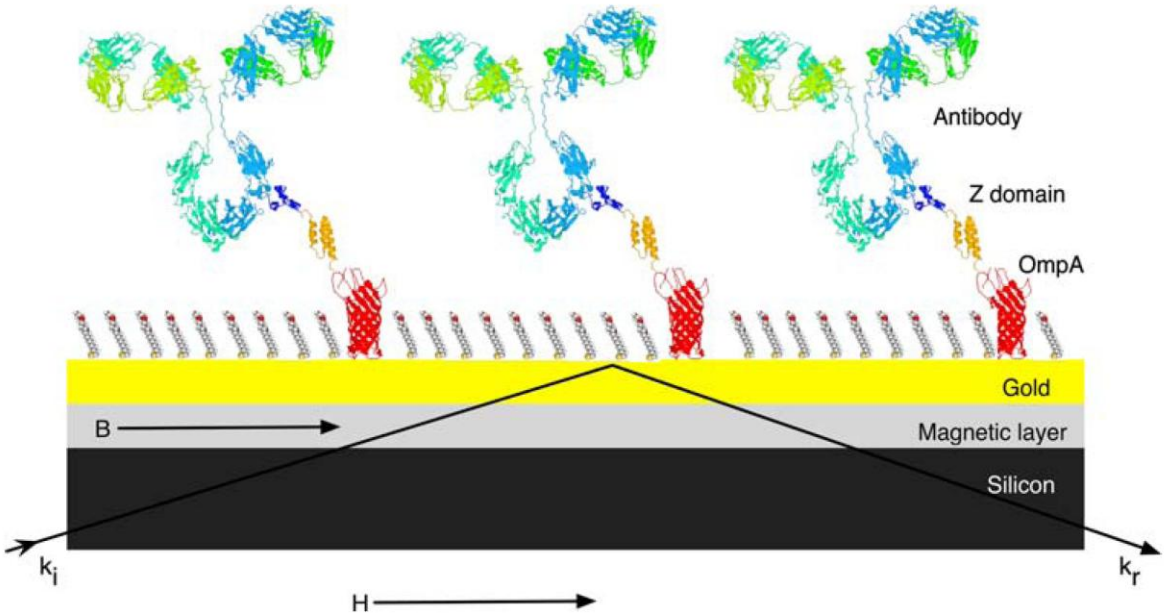


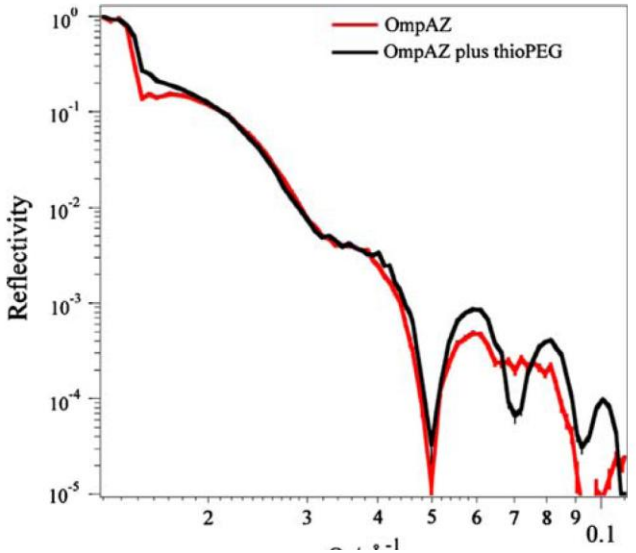
DNA coverage 33% on both inner and outer bilayer

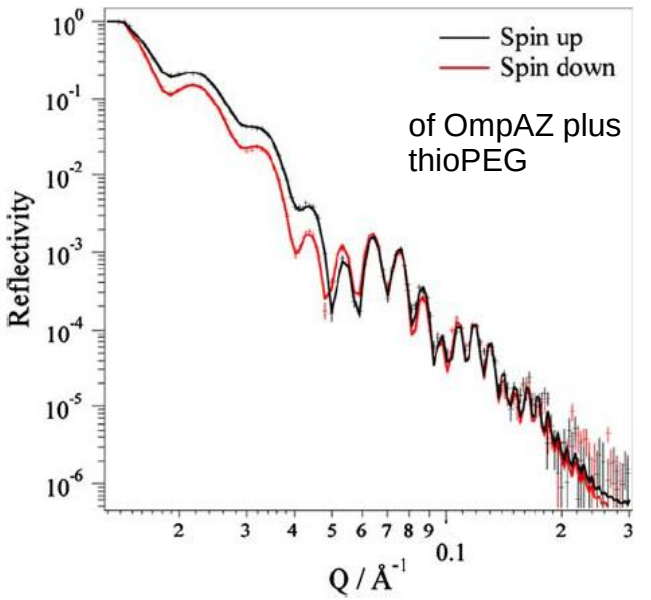


Cartoon of floating bilayer in the absence & presence of DNA & Ca^{++}

USE OF POLARISED NEUTRONS







of OmpAZ plus thioPEG

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

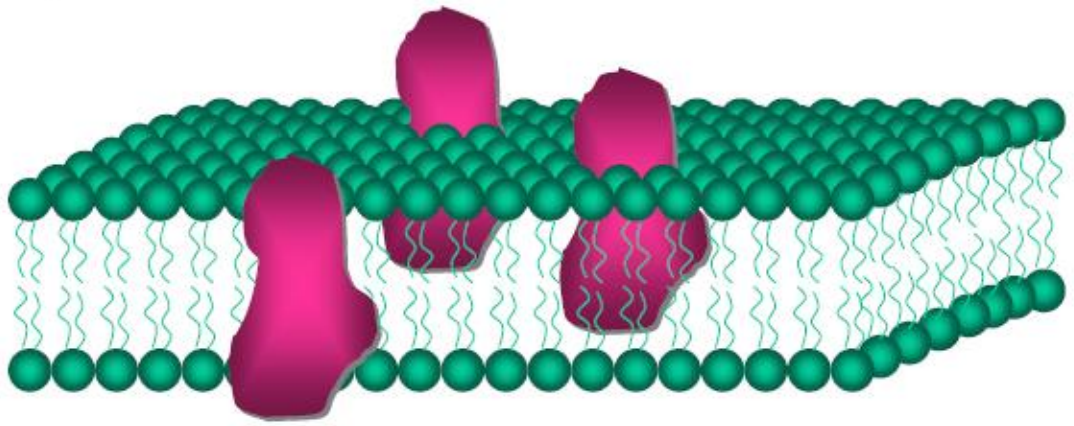
Polarised neutrons are used as this provides a means of achieving extra contrast in samples having a magnetic metal layer (Fe or Ni) under the gold surface.

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

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

PHOSPHOLIPID BILAYERS CONTAINING INTERGRAL MEMBRANE PROTEINS

100% D_2O

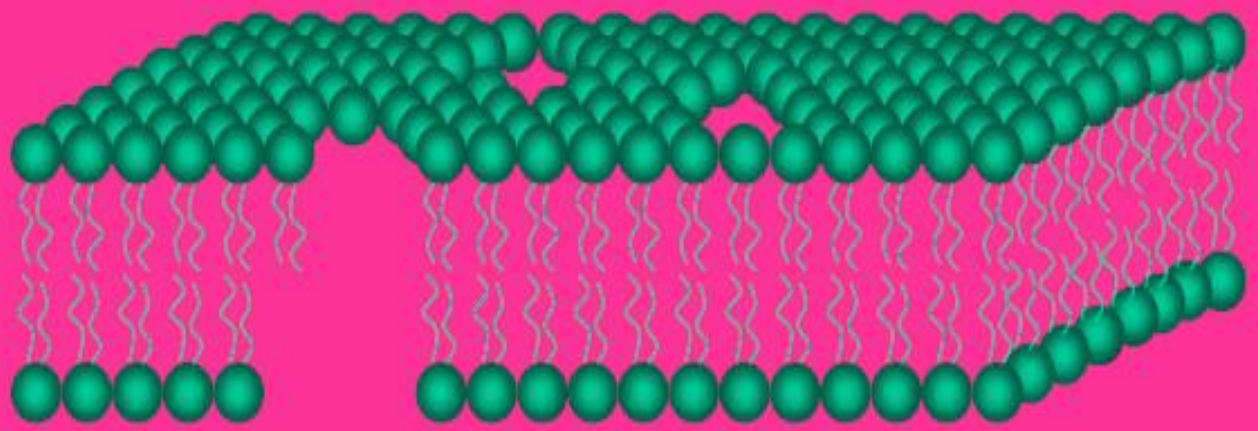


see whole membrane (if h-lipid used)

O.Byron (unpublished)

PHOSPHOLIPID BILAYERS CONTAINING INTERGRAL MEMBRANE PROTEINS

43% D_2O

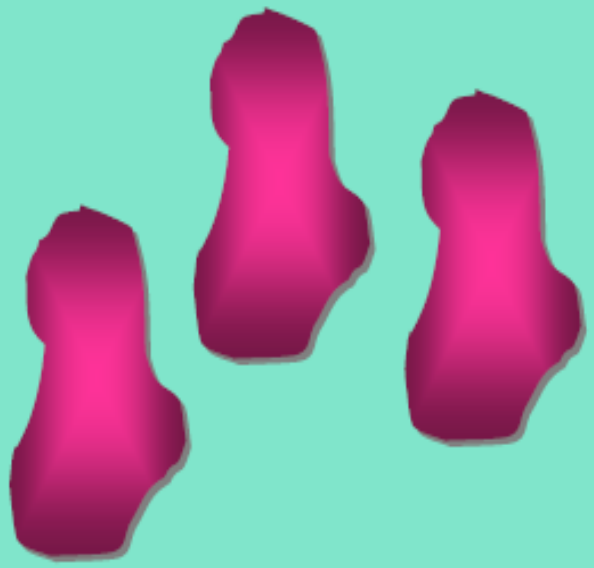


see only the lipid

O.Byron (unpublished)

PHOSPHOLIPID BILAYERS CONTAINING INTERGRAL MEMBRANE PROTEINS

13% D_2O



see only the proteins

O.Byron (unpublished)