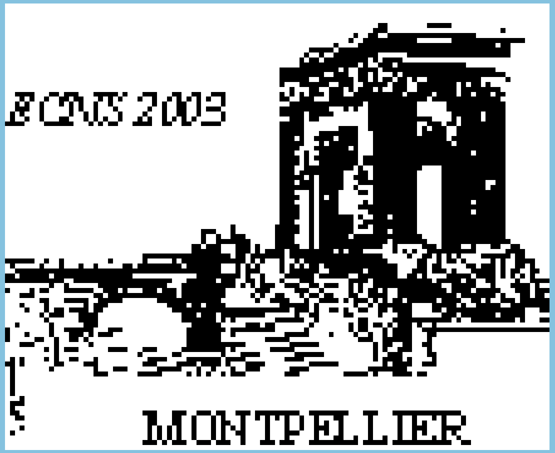
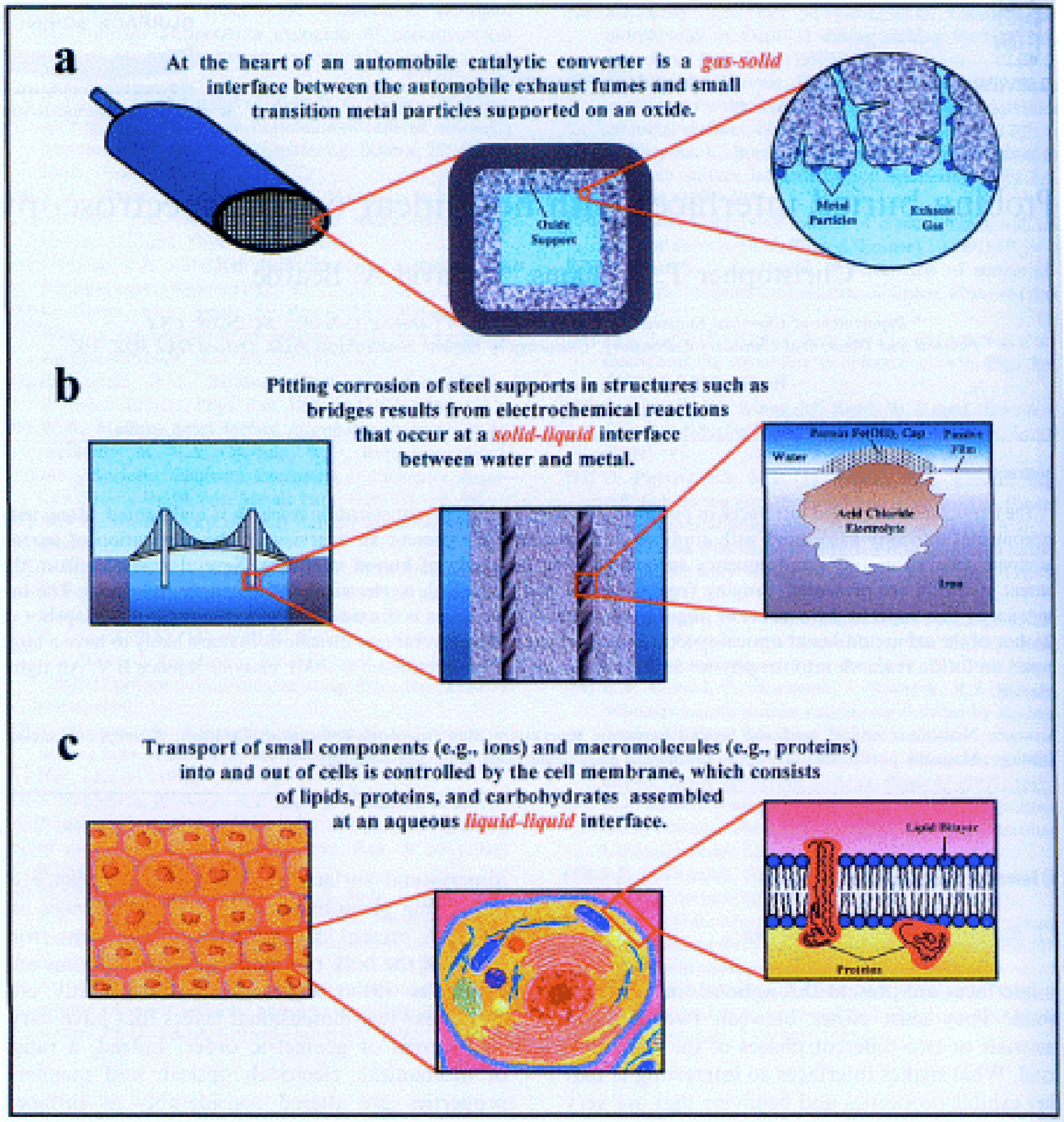
The use of neutrons to probe phenomena at interfaces



Introductory Course

Giovanna Fragneto 2 Sept 2003



At the heart of an automobile catalytic converter is a *gas-solid* interface between the automobile exhaust fumes and small transition metal particles supported on an oxide.

Pitting corrosion of steel supports in structures such as bridges results from electrochemical reactions that occur at a *solid-liquid* interface between water and metal.

Transport of small components (e.g., ions) and macromolecules (e.g., proteins) into and out of cells is controlled by the cell membrane, which consists of lipids, proteins, and carbohydrates assembled at an aqueous *liquid-liquid* interface.

Fig. 1. Buried interfaces everywhere.

Most reactions in biology occur at interfaces

Interfaces of biological importance include:
cell surface/ synthetic biomaterial; extracellular matrix/biomolecule or cell; hydrated tissue/air (lungs) and mineral/protein (bone)

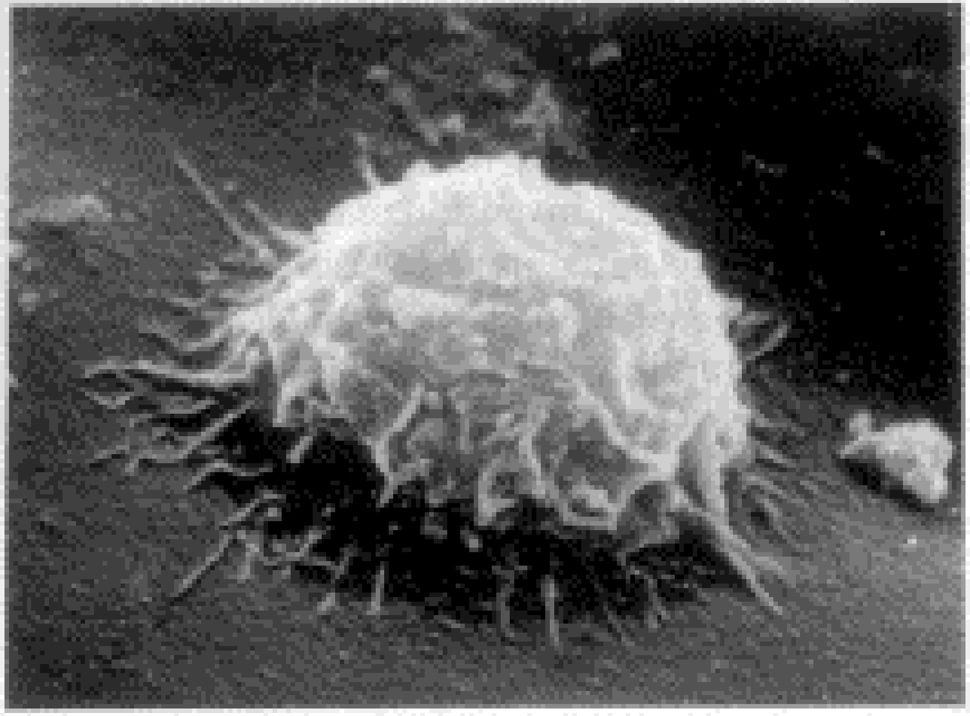
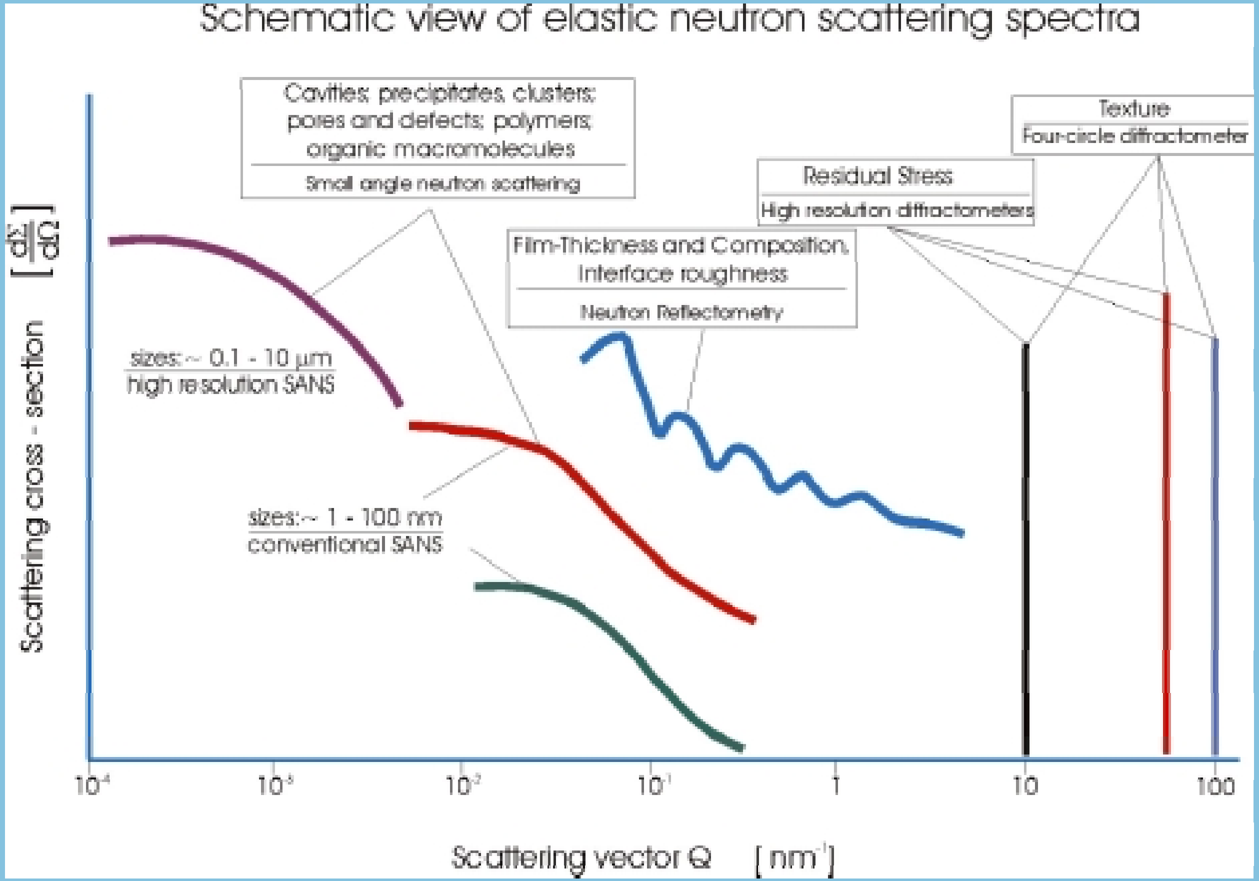
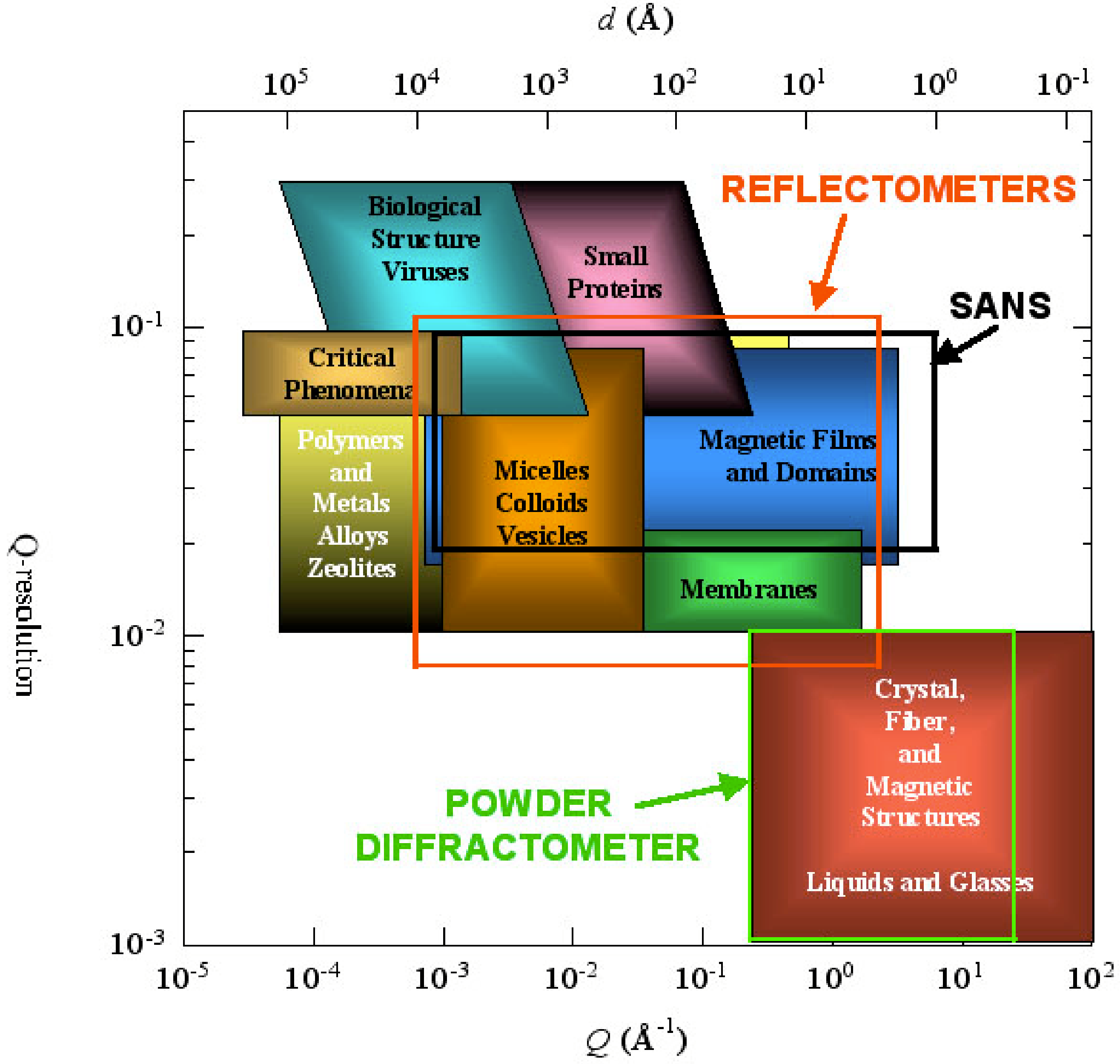


Fig. 1. A scanning electron micrograph of a myoblast cell interacting with a synthetic surface. The cell is approximately 10 μm in length.

Neutron reflection is an ideal tool to study buried interfaces because neutrons can penetrate solids (i.e. in solid/liquid systems), are not destructive, allow to gain information in the fraction of nanometer scale



Schematic view of elastic neutron scattering spectra



d (Å)
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
Q-resolution
10^{-1}
10^{-2}
10^{-3}
Biological Structure Viruses
Small Proteins
REFLECTOMETERS
SANS
Critical Phenomena
Polymers and Metals Alloys Zeolites
Micelles Colloids Vesicles
Magnetic Films and Domains
Membranes
Crystal, Fiber, and Magnetic Structures
Liquids and Glasses
POWDER DIFFRACTOMETER
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^0
10^1
10^2
Q (Å^{-1})

Soft matter systems:

weaker interactions than in hard materials that promote only partial order on length scales that are much larger than those of a single molecule.

Liquid crystals: *phases of anisotropic molecules that show orientational order but not necessarily positional (translational) order*

Colloidal dispersions: *consist of a molecular solvent and solid or liquid particles that are much larger than a molecular size*

Macromolecular solutions: *long-chain molecules in solution*

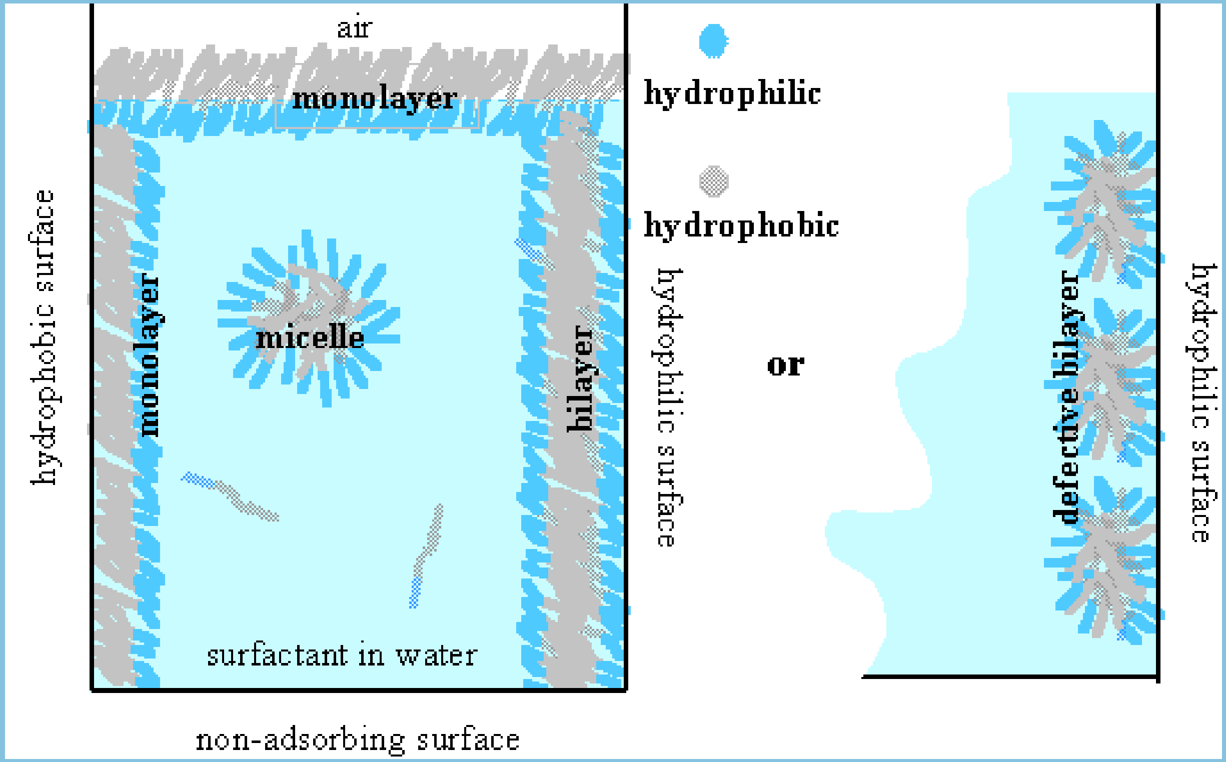
Gels: *long-chain molecules that are either chemically or physically interconnected by junctions*

Self-assembling amphiphilic systems: *molecules with both hydrophobic and hydrophilic parts that cause these molecules to be interfacially active* *http://physchem.ox.ac.uk/%7Erkt/*

Self-assembling amphiphilic systems:

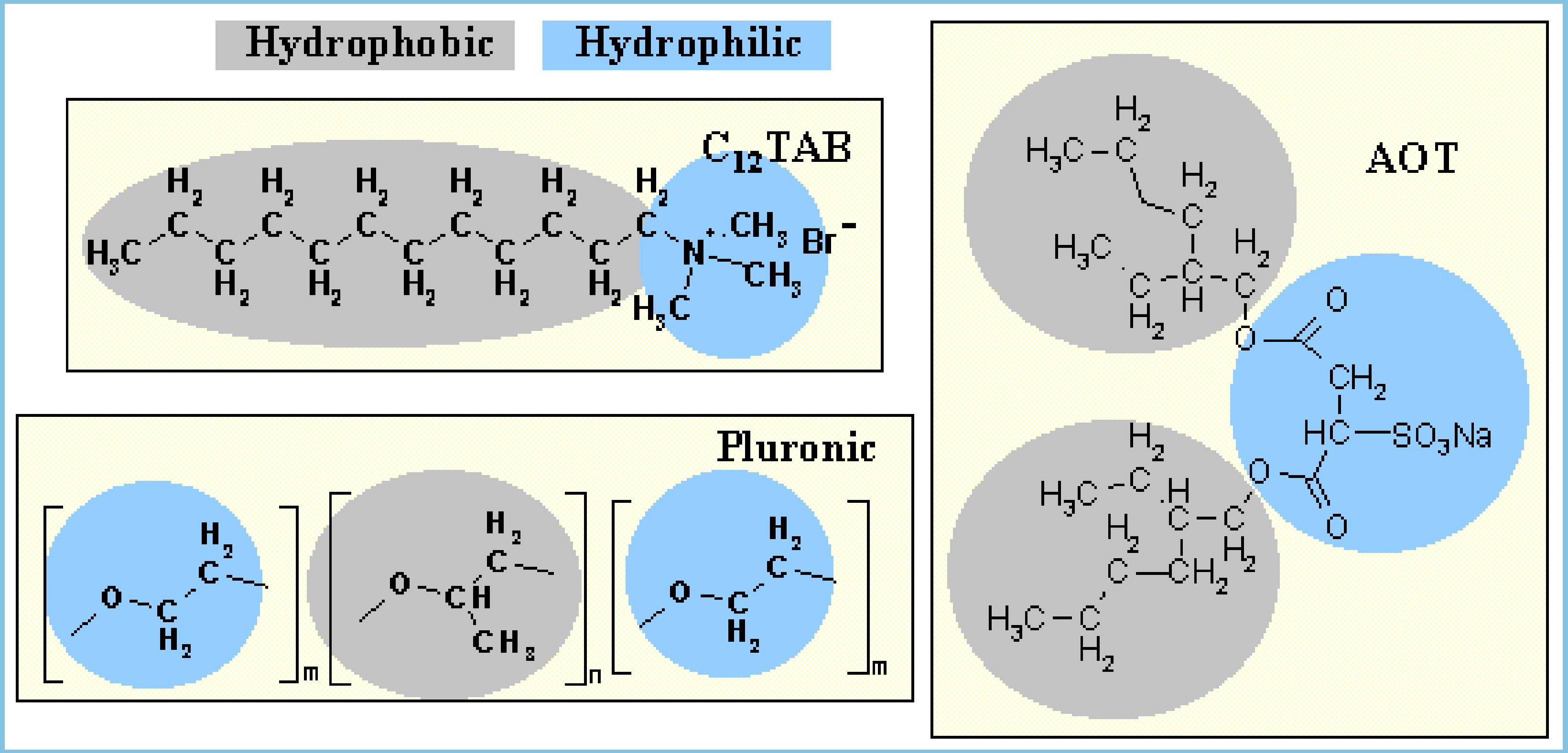
alcohols, surfactants or soap like molecules and lipids

reduce the surface tension of water or oil systems; in some cases allow stabilisation of phases with macroscopic amount of internal interface



Self-assembly is used in the chemical industry in dispersion, encapsulation, as oil-spill cleaning agents; in biotechnology industry in order to engineer smart drug encapsulation *http://physchem.ox.ac.uk/%7Erkt/*

Examples of surfactants



Crude understanding of how they work since systems are disordered at a molecular level

NEUTRONS FOR SCIENCE

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Surfactant products where the surfactants are the primary components

Products where surfactant is a secondary component in the material or the production.

Nature has found many uses for self-assembly one of the most important being the formation of membranes

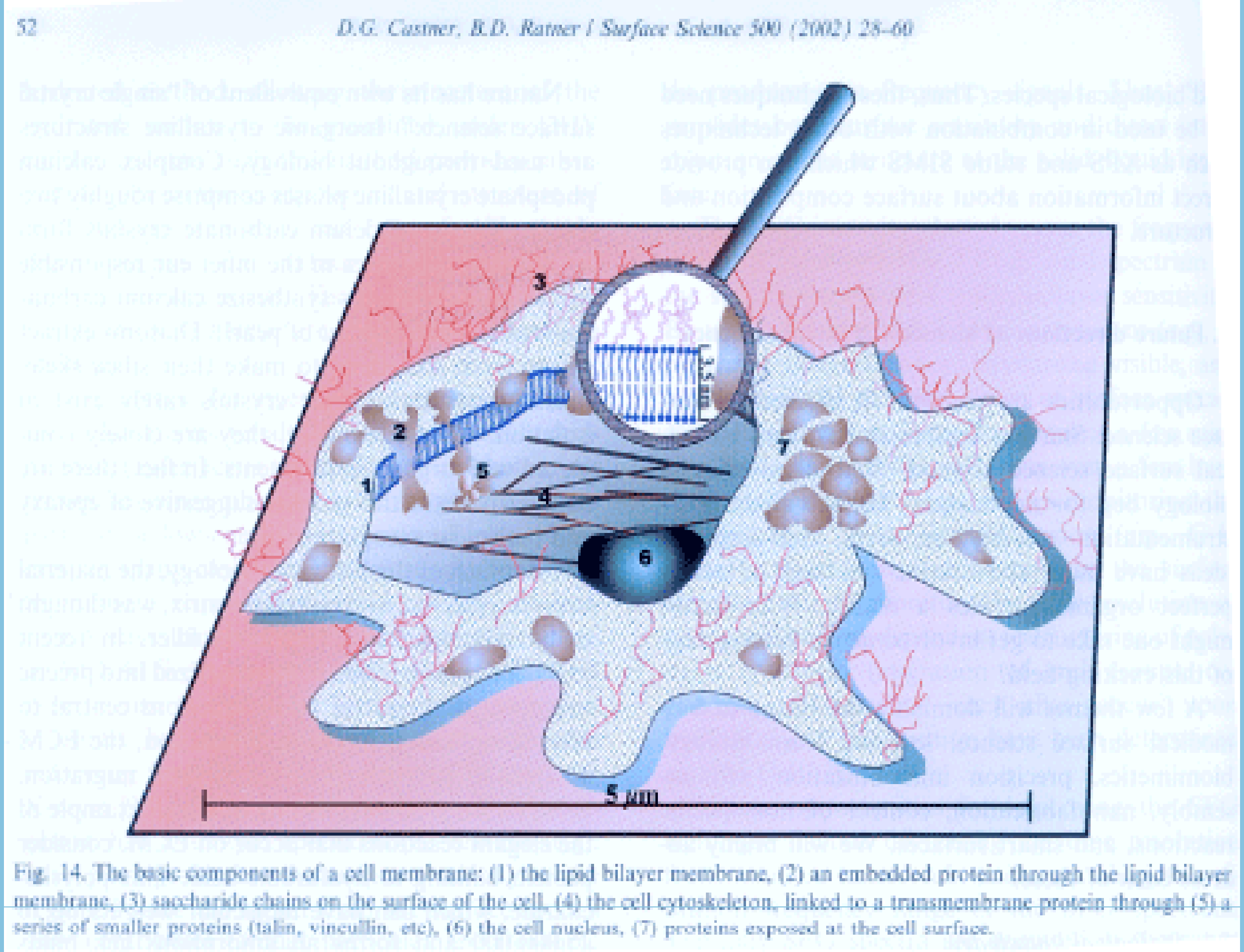
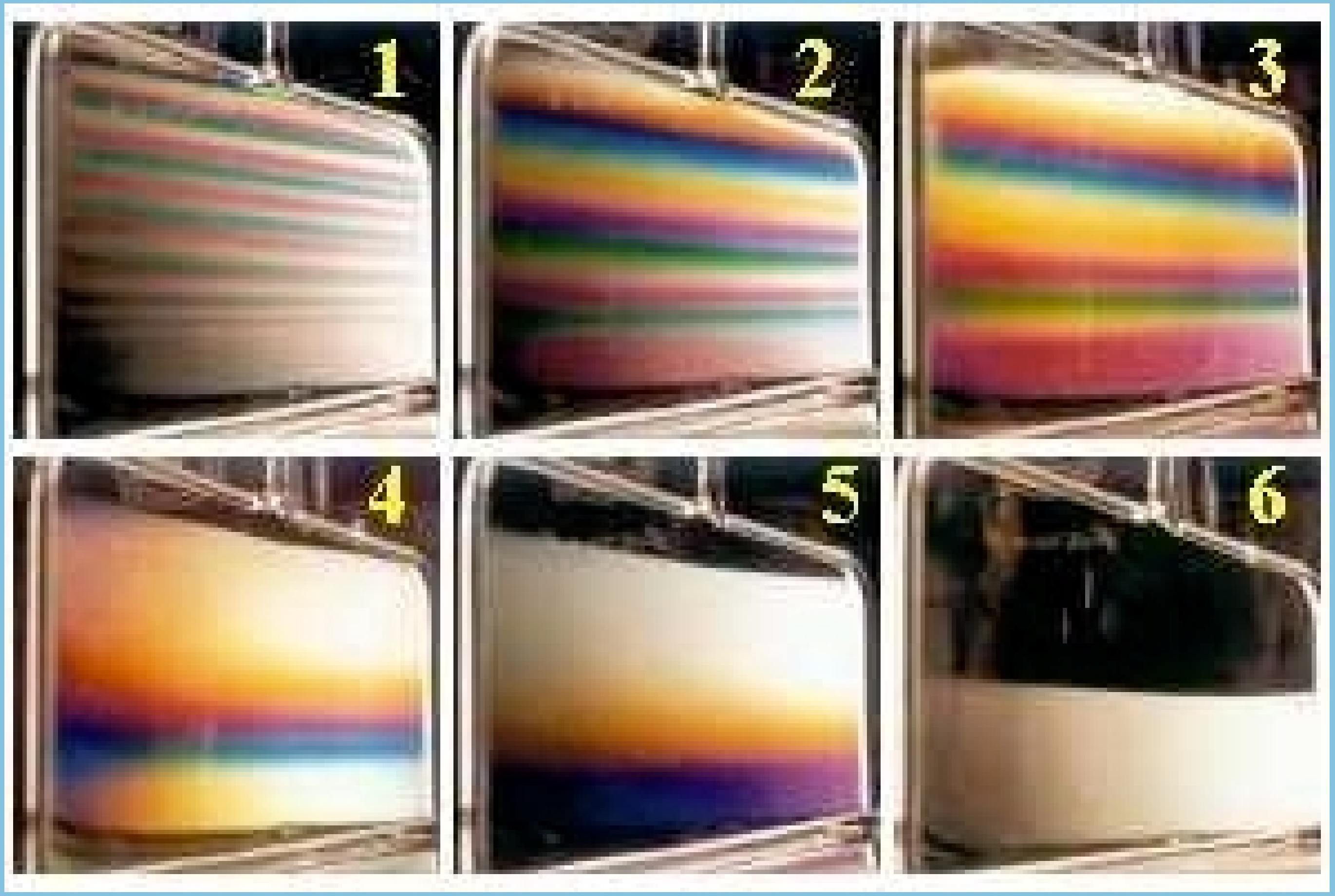


Fig. 14. The basic components of a cell membrane: (1) the lipid bilayer membrane, (2) an embedded protein through the lipid bilayer membrane, (3) saccharide chains on the surface of the cell, (4) the cell cytoskeleton, linked to a transmembrane protein through (5) a series of smaller proteins (talin, vincullin, etc), (6) the cell nucleus, (7) proteins exposed at the cell surface.

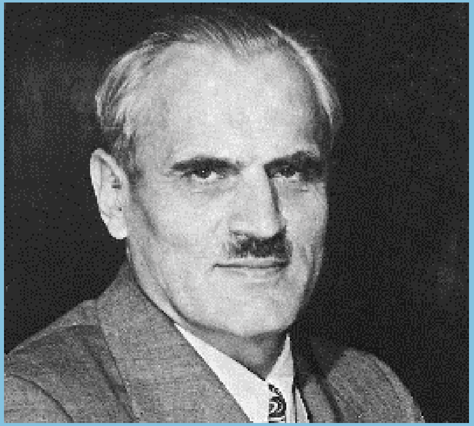
PRINCIPLES OF NEUTRON NEUTRON REFLECTION

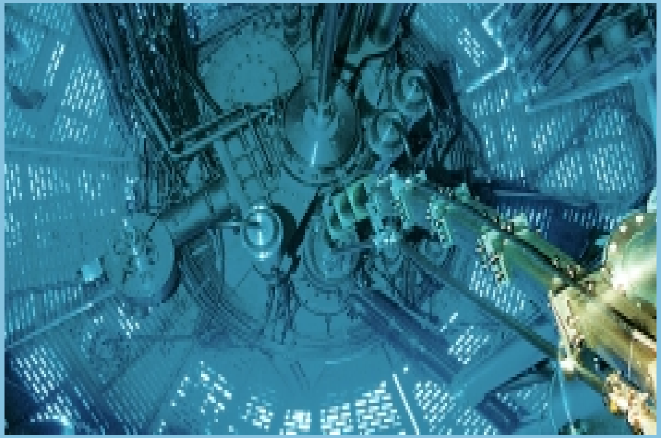
1675 - ***Newton*** realised that the colour of the light reflected by a thin film illuminated by a parallel beam of white light could be used to obtain a measure of the film thickness. Spectral colours develop as a result of interference between light reflected from the front and back surfaces of the film.



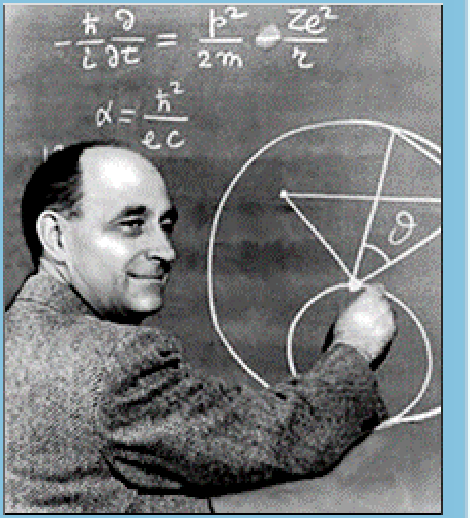
Draining Foam Film

1922 - ***Compton*** showed that x-ray reflection is governed by the same laws as reflection of light but with different refractive indices depending on the number of electrons per unit volume.



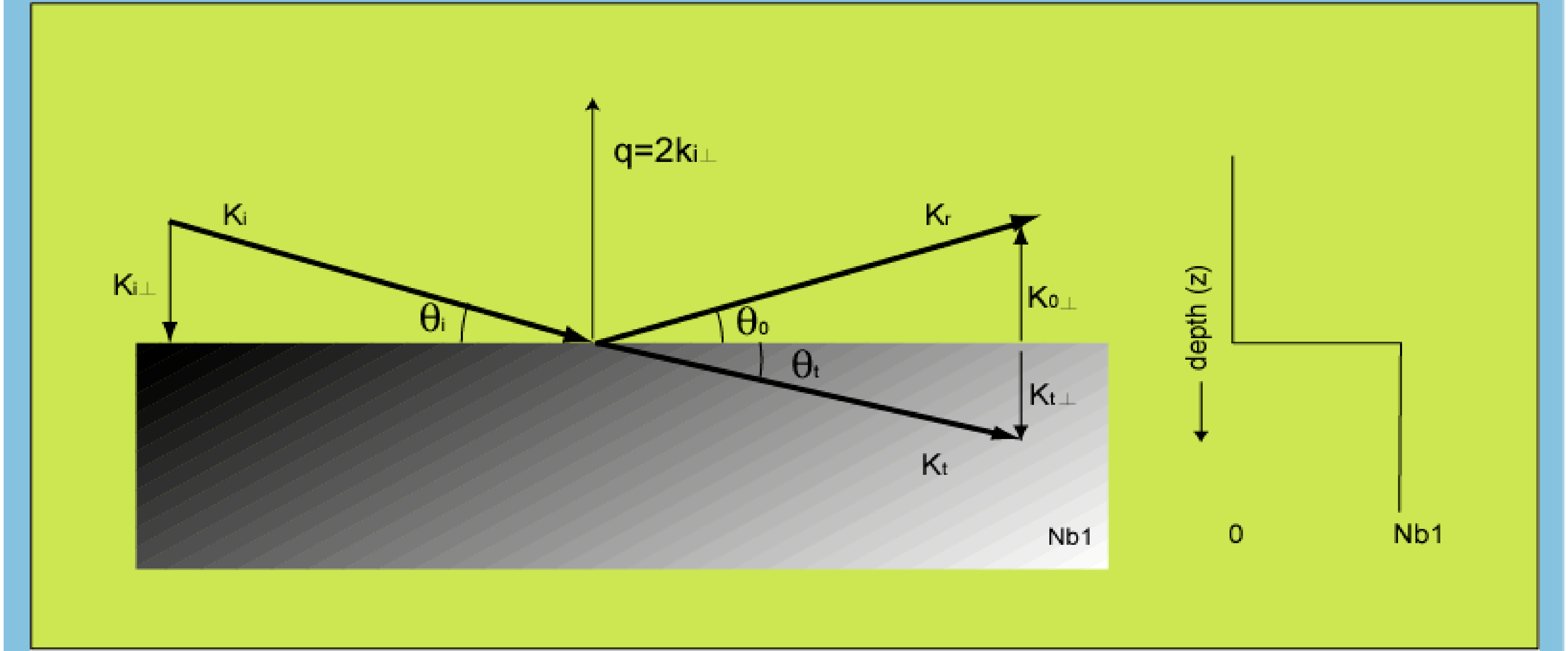


1944 - ***Fermi*** and ***Zinn*** first demonstrated the mirror reflection of neutrons. Again this follows the same fundamental equations as optical reflectivity but with different refractive indices.



Specular reflection, R, defined as the ratio between the reflected over the incoming intensities of a neutron beam, is measured as a function of the wave vector transfer, q, perpendicular to the reflecting surface:

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



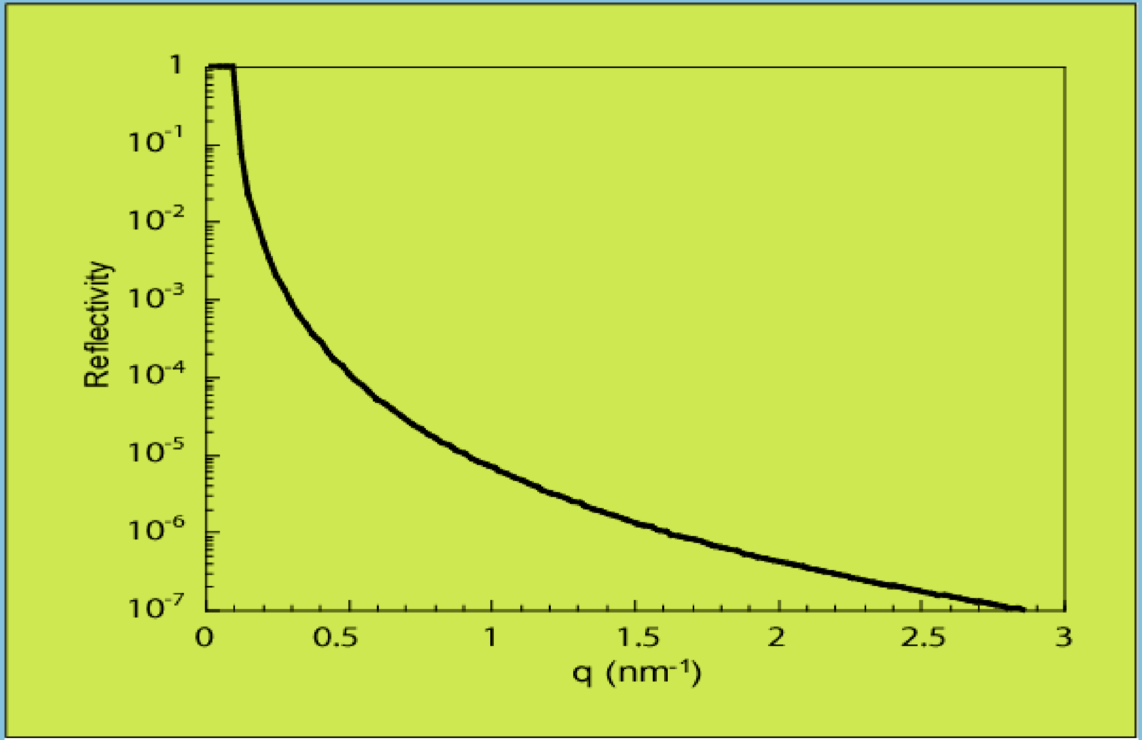
$$R = r^2 = \left[\frac{q - \left(q^2 - q_c^2\right)^{1/2}}{q + \left(\left(q^2 - q_c^2\right)^{1/2}\right)} \right]^2$$

When $q >> q_c$ this reduces to:

$$R \approx \frac{16\pi^2}{q^4} N_b^2$$

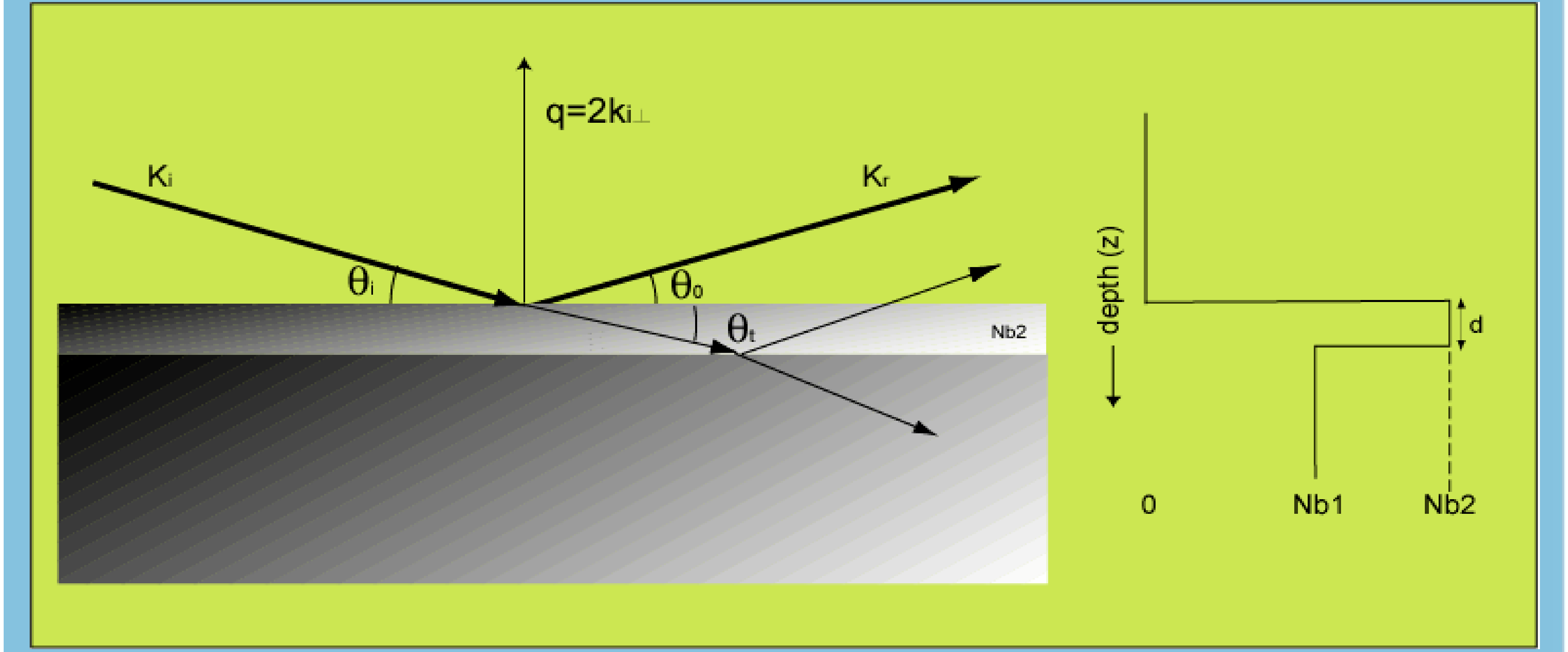
which is the reflectivity used in the Born approximation

Nb is the scattering length density



Simulation of the specular neutron reflectivity as a function of *q* from a silicon/air interface (Nb1=2.07x10^{-6}Å^{-2}).

Reflectivity from a layer at an interface



Calculating the reflectivity from such a system or one with many layers requires a general technique such as the optical matrix method.

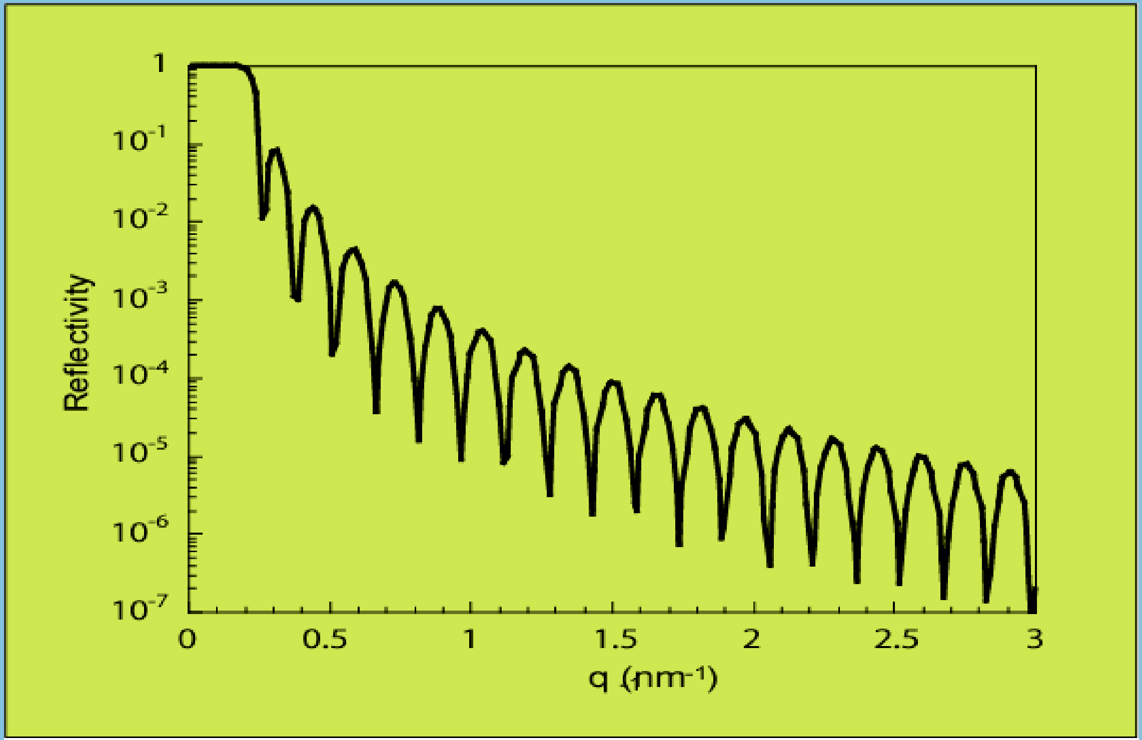
The transmission and reflection from one layer to the next can be described as a matrix multiplication product for each layer.

phase problem

The problem of inverting a reflectivity curve to extract N_b as a function of depth is complex and many profiles can produce the same reflectivity curve

Example of reflectivity profile associated with a single layer with d=400Å.

Simulation of the specular neutron reflectivity as a function of *q* from a 400 Å layer of nickel on a silicon substrate ($Nb1=2.07\times10^{-6}$Å^{-2}, $Nb2=9.04\times10^{-6}$Å^{-2}).



In the Born approximation these oscillations are simply the Fourier transform squared of N_b as a function of depth multiplied by the Fresnel reflectivity the period of which at $q \gg q_c$ is $2\pi/d$.

Basic features of a reflection experiment:

- **radiation source**
- **wavelength selector or choppers**
- **system of collimation**
- **sample**
- **system of detection**

Measurement can be done by:

varying the glancing angle of incidence θ at constant λ

or

measuring the time-of-flight at constant θ

For the same resolution TOF is less efficient (flux at min and max λ up to two orders of magnitude smaller than at peak flux)

but

better for kinetics

D17

Collimator angle: -2 - 25 deg

Detector angle: -2 - 50 deg

Detector distance: 1.1 - 3.5m

Detector size: 250 x 500mm

Max detector res.: 1.8 x 2.5 mm

Shutter closed background: 1n/s

Gamma detection efficiency: 10^{-7}

Wavelength range: 2.7 - 27Å

Q_z range (TOF): 10^{-4} - $4Å^{-1}$

Q_z range (Mono): 10^{-3} - $1Å^{-1}$

WHITE FLUX

10^{10} n/s/cm^2

TOF

Q: 0.002 - 4 $Å^{-1}$

$\Delta Q/Q$= 0.1 - 20%

Min R: $<10^{-8}$

MONO

Q: 0.005 - $1Å^{-1}$

$\Delta Q/Q$= 2 - 5%

Min R: $<10^{-8}$

POLARISED

Flip ratio: 20

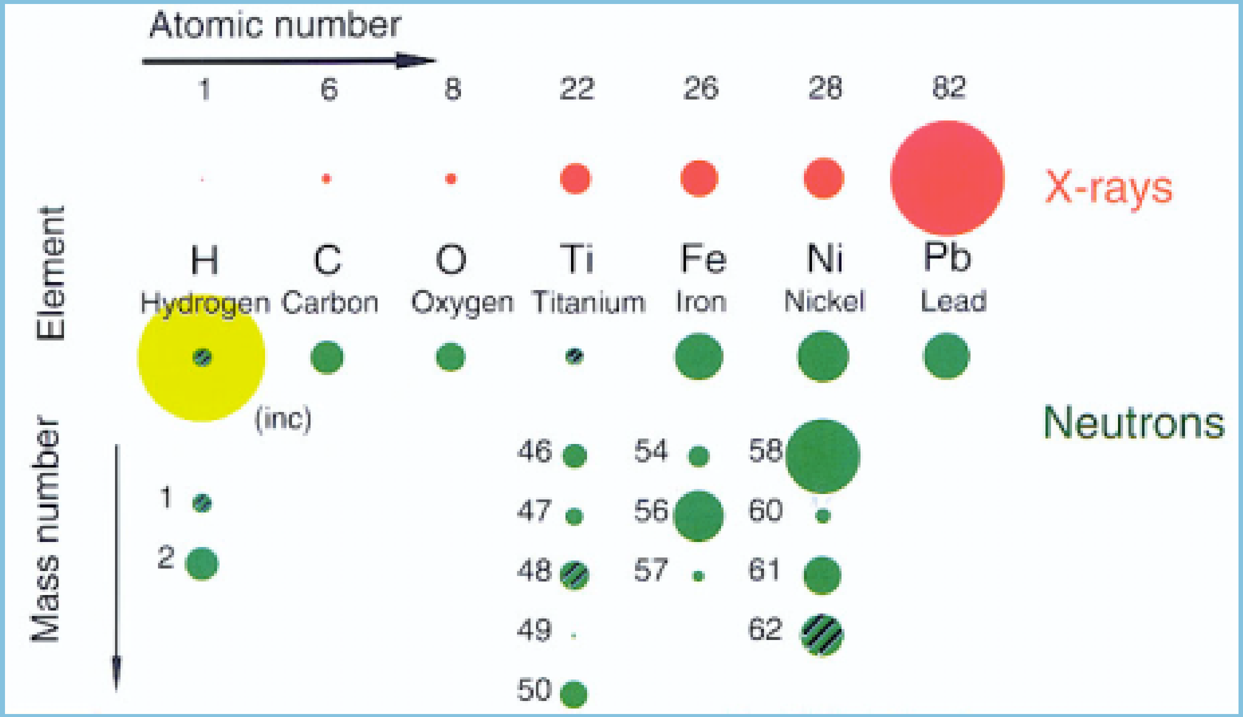
Low neutron flux (compared i.e. to x-rays) leads to small q range

low resolution

(q_{max}=3nm-1 d_{min}=π/q=1nm!)

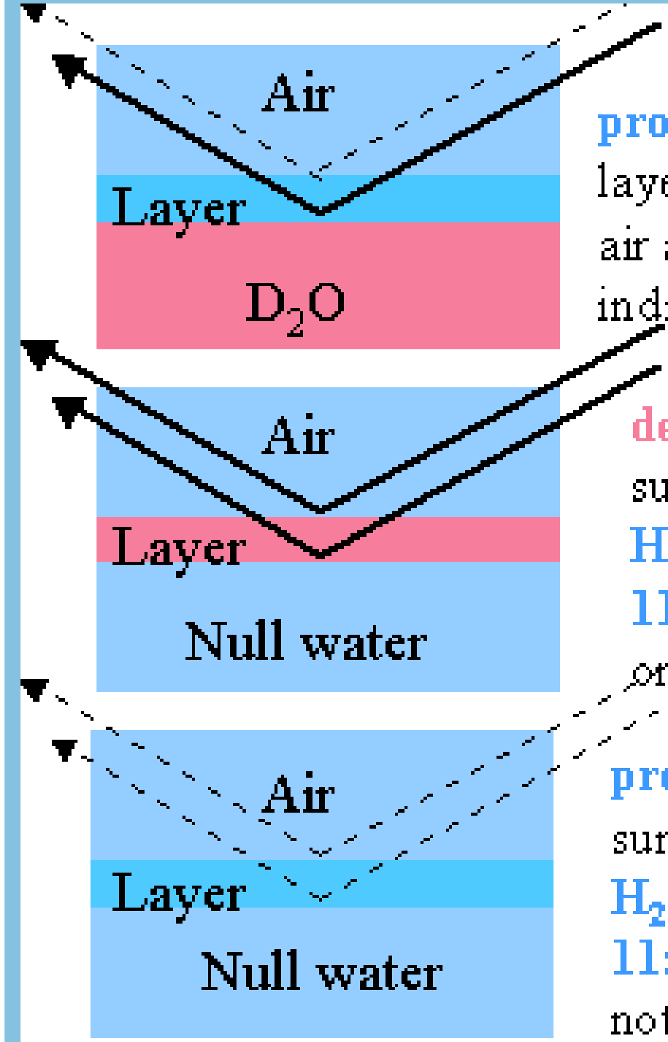
With soft matter systems resolution may be greatly increased by using contrast variation

Contrast variation relies on the fact that different nuclei scatter neutrons with different amplitude and, in the case of protons and deuterons, with opposite phase



By using a combination of deuterated and protonated materials the reflectivity profile of a system can be substantially changed while keeping the same chemical structure at the interface.

Contrast variation at the air/water interface

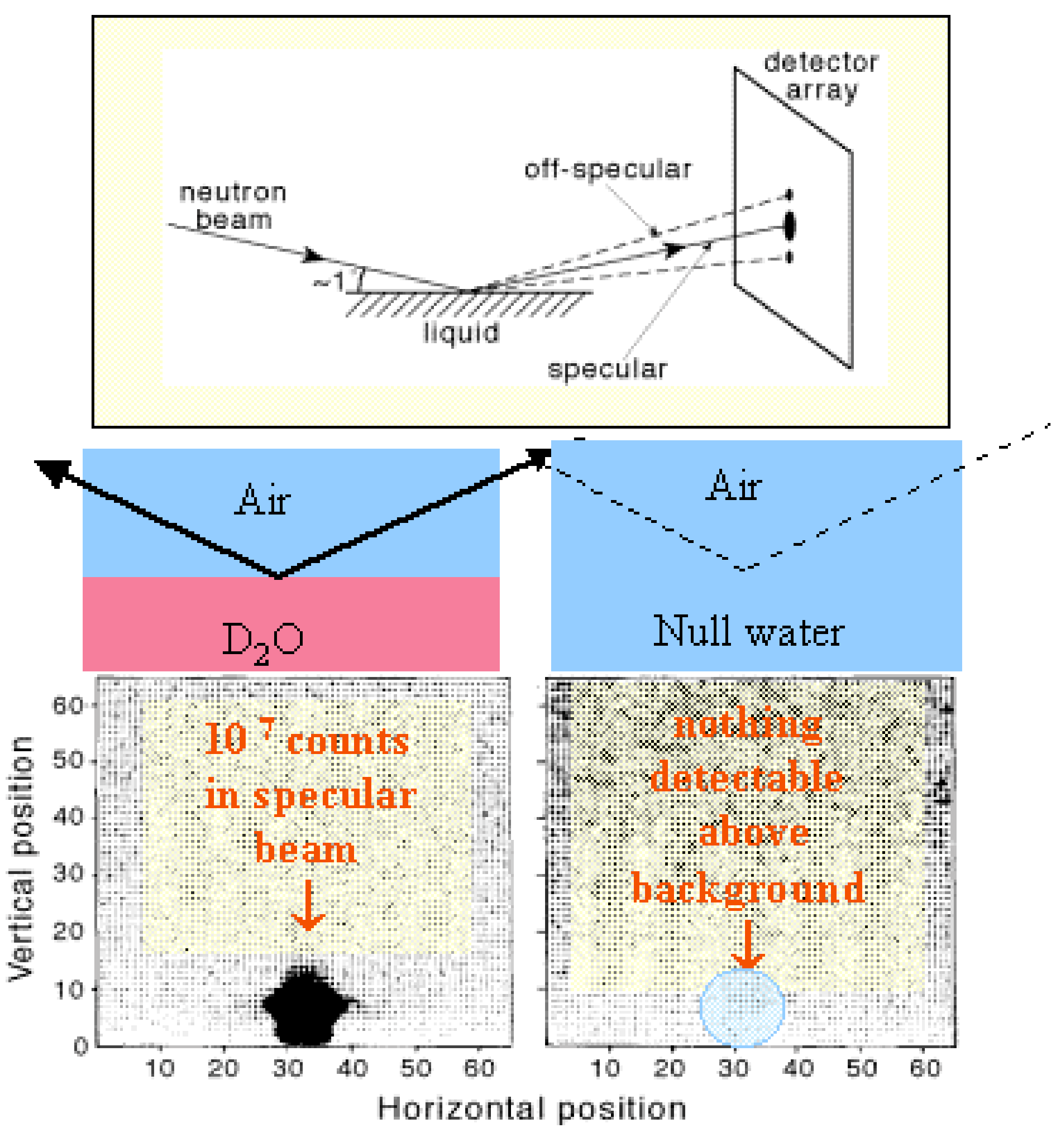


protonated surfactant layer; D_2O, layer and air almost indistinguishable

deuterated surfactant layer; **H_2O:D_2O about 11:1 (null water),** only layer observed

protonated surfactant layer; **H_2O:D_2O about 11:1 (null water),** nothing observed

Hydrogen and **deuterium** scatter neutrons with opposite phase. H_2O and D_2O respectively have scattering length densities smaller or greater than most solid materials. This allows the contrast between the various parts of the interface to be manipulated by varying the H/D content.



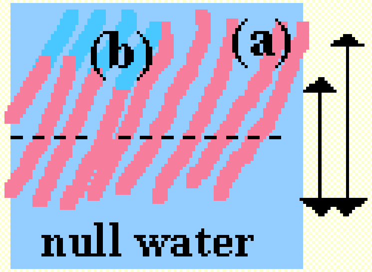
10^7 counts in specular beam

nothing detectable above background

Adsorption of Surfactants

Average number of surfactant molecules per unit area: lateral spacing not determined directly

Number of surfactant molecules per unit area in the presence of another component



Thickness of whole surfactant layer (a) or part of layer (b)

● deuterated

◍ protonated

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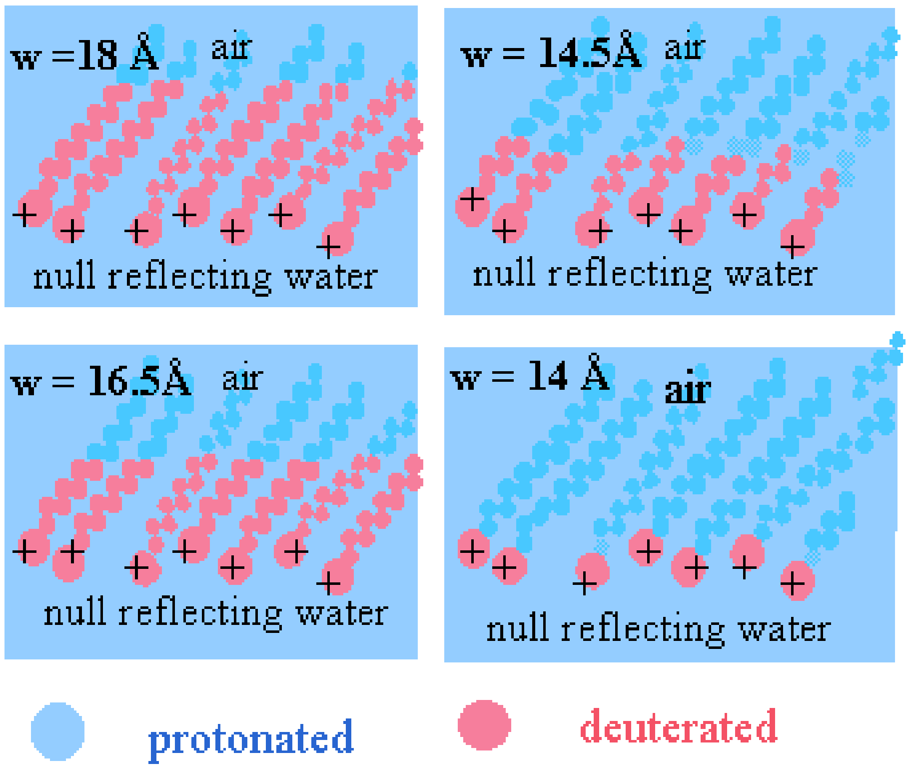
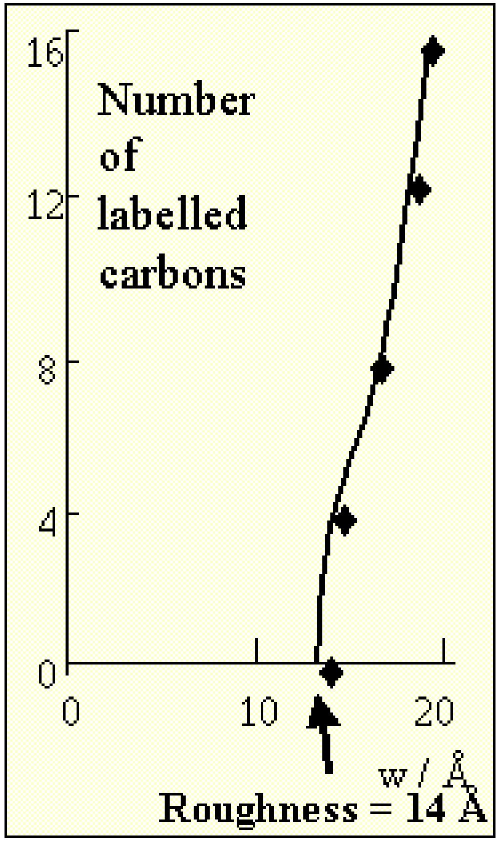
The method has been applied to the surfactant $C_{16}TAB$, whose hydrocarbon chain is 16 atoms long.

First it was labelled with deuterium to give a series of compounds with 4, 8, 12 and 16 CD groups.
The thicknesses of this series of compounds could then be used to show that there is a large residual roughness of the layer of 14 Å.

This is obtained by plotting the measured thickness of the layer against number of labelled carbons and extrapolating to zero length of label.





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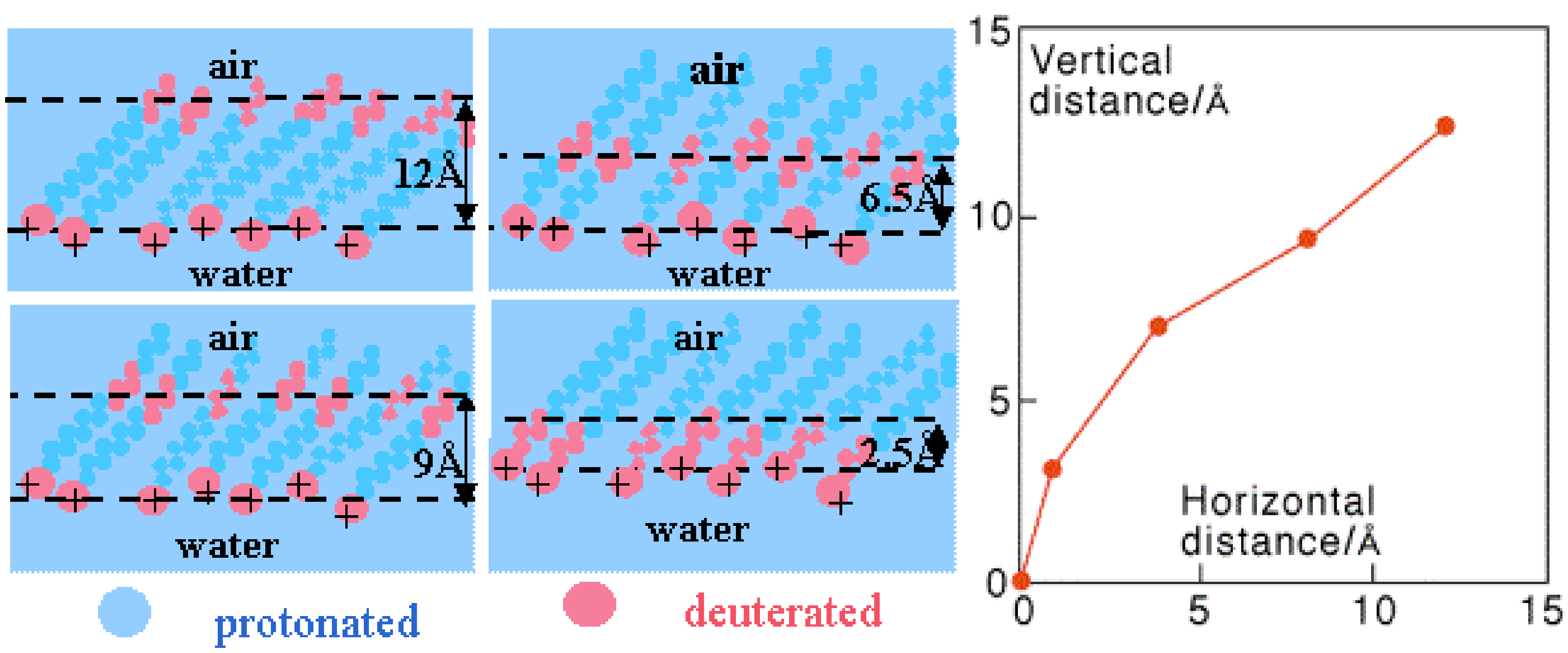
By making the same surfactant with different pairs of deuterium labelled butylene groups the mean vertical separation between the labels marked in red in the figure below was determined.

Making the further assumption that each four carbon fragment is on average fully extended, the mean conformation of the carbon chain may be deduced.



This series of measurements shows that the surfactant layer must be highly disordered, the roughness being comparable with the projection of the chain along the surface normal, a result that agrees with computer simulations.

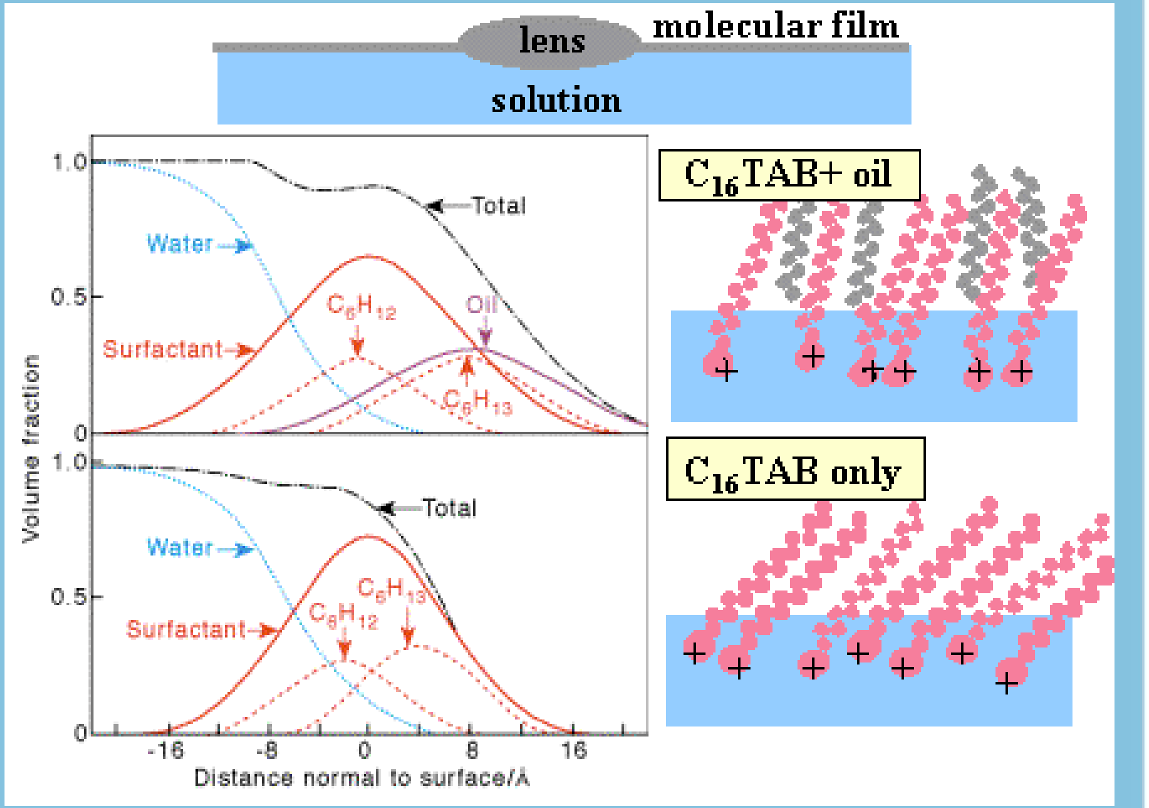
Lu, Thomas, Binks, Fletcher, Penfold, J. Phys. Chem., 99, 4113 (1995)
Lu, Li,Thomas, Binks, Fletcher, Penfold, J. Phys. Chem. B, 102, 5785-5793 (1998)

Oil/Surfactant Interactions

When an oil is spread on the surface of a surfactant solution it often forms a lens in equilibrium with a film of molecular thickness. The structure of the composite film of surfactant, oil and water may give clues about how oil and surfactant interact.

By labelling the oil and surfactant the relative positions of oil and surfactant to each other and to the water surface can be determined.

By labelling just the terminal hexyl fragment of the C_{16}TAB it could be shown that the centres of the oil (dodecane) and hexyl fragment distributions are located at the same position in the interface



The surfactant also changes its orientation when oil is added with the hexyl group of the surfactant moving outwards by 0.45 nm in the presence of the oil.

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Polymer/Surfactant Interactions

Water soluble polymers are often included in commercial surfactant formulations.
They modify both the surface behaviour and the rheology of the bulk solution.
These effects may become very large when the polymer/surfactant interaction is sufficiently strong.

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The behaviour of polymer/surfactant mixtures at interfaces has hardly been studied mainly because of a lack of experimental techniques.

One way of following the interaction of a polymer with surfactant in the bulk solution is to measure the surface tension of the mixture, keeping the polymer concentration constant and varying the surfactant concentration.

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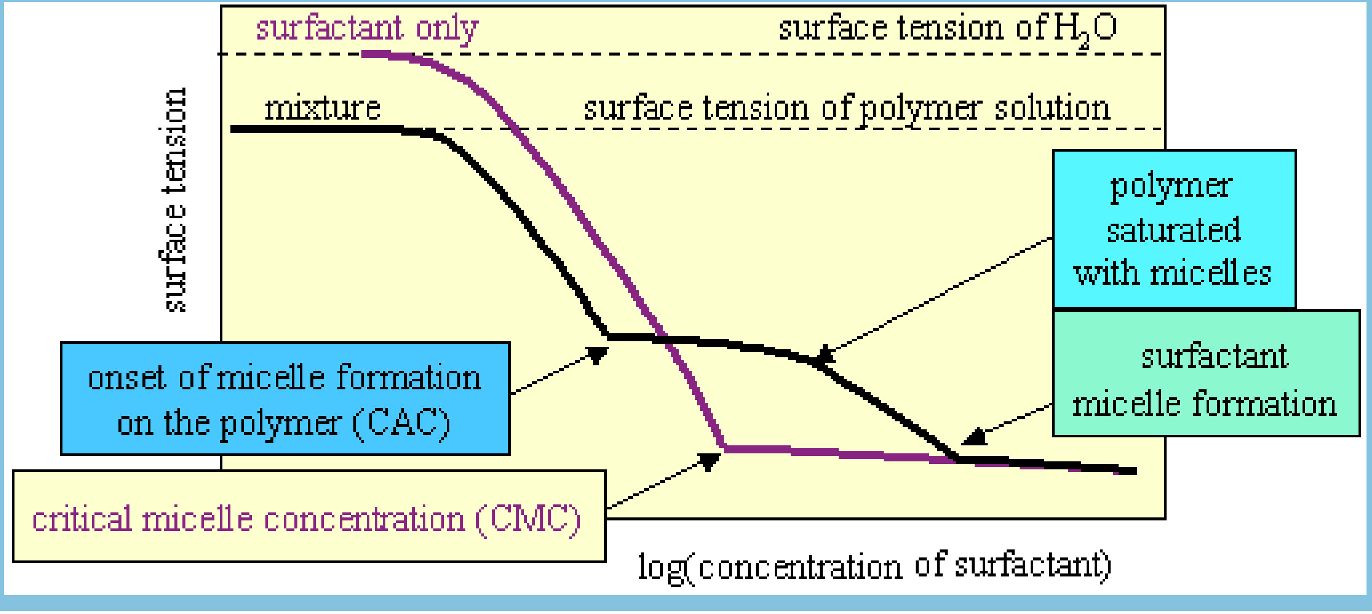

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Neutron reflection offers the opportunity to interpret the surface tension behaviour in terms of the composition of the surface. Separate deuterium labelling of polymer and surfactant can be used to determine the surface excess of each component.

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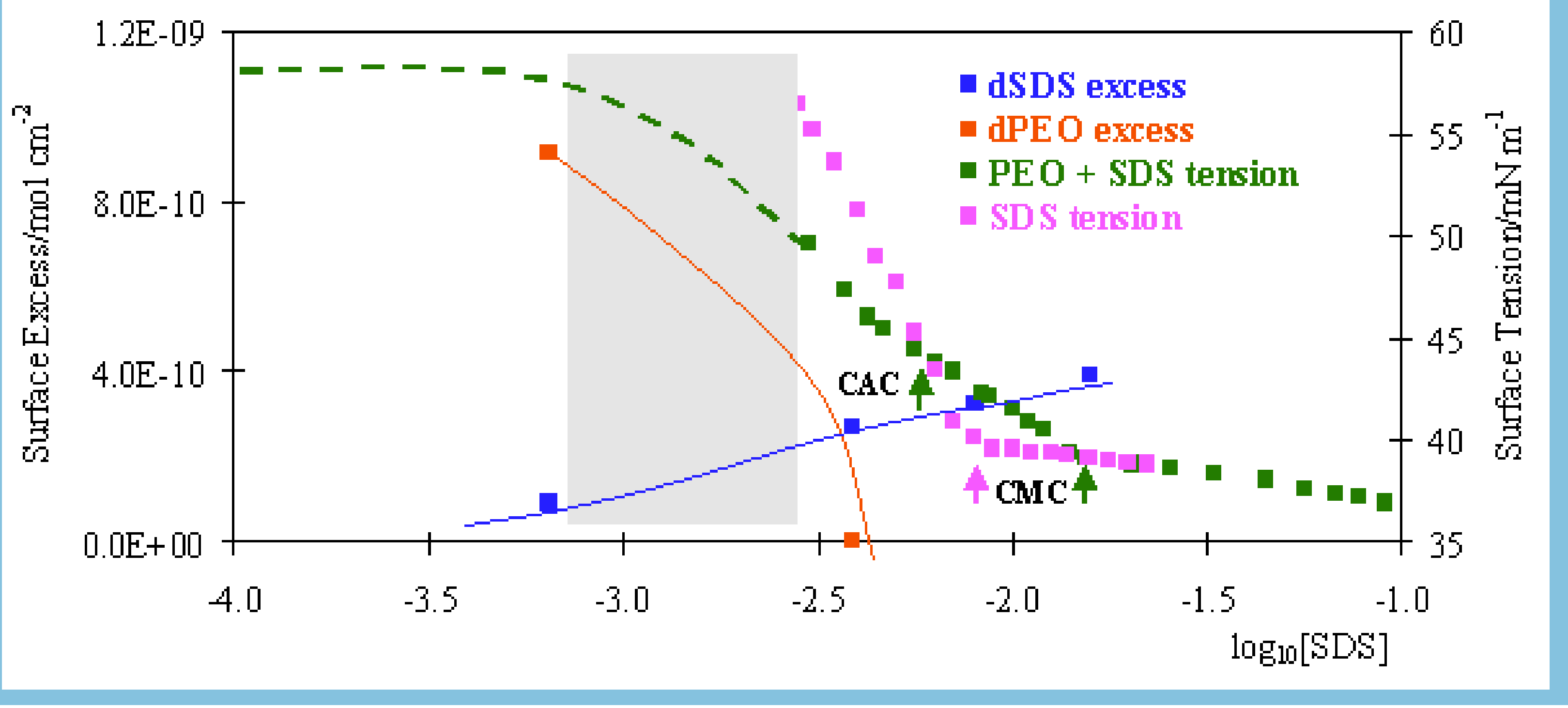
The surface excesses of each component vary most rapidly in the region shaded in grey, where the SDS displaces the PEO from the surface. At the CAC there is negligible PEO left on the surface.

Quantitative fitting of the behaviour of the surface tension and surface excess in the grey shaded area shows that there is an attractive interaction between polymer and surfactant of a magnitude similar to that between SDS and a non-ionic CnEm surfactant.



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The surface activity may be controlled in several different ways and it is not yet understood which is the best for a given purpose.

In many of the applications concerning surface behaviour a key component is the interaction of the polymer with surfactant.

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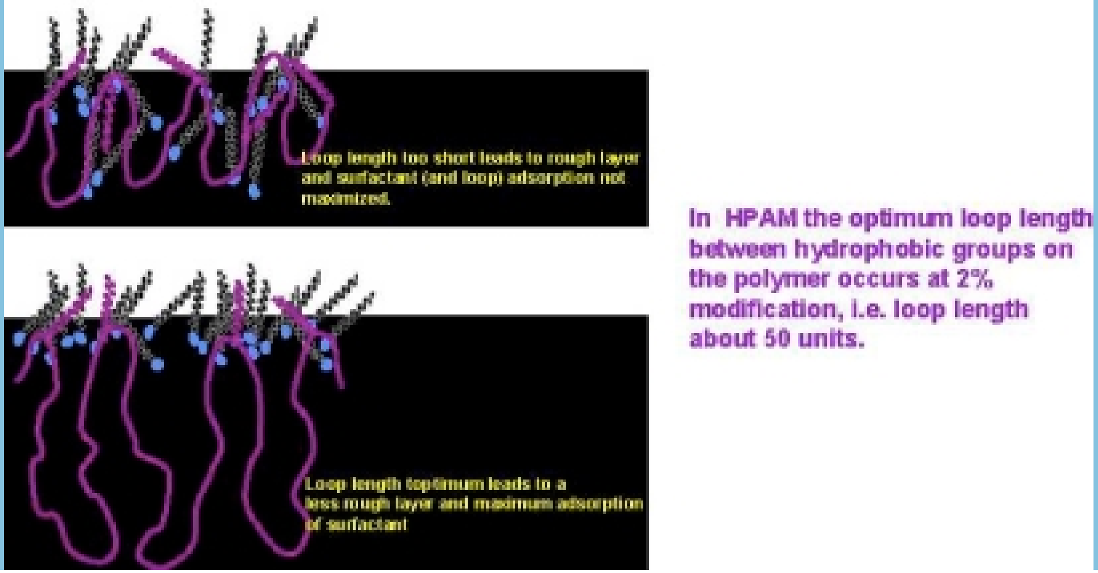
One of the ways to investigate the quality of the surface layer formed by the hydrophobic units dispersed along the backbone of the polymer is to label the hydrophobic units with deuterium and study the composition and thickness of the layer by neutron reflection.

The quality of the layer formed by interacting deuterium labelled surfactant and the more standard protonated polymer is also a good guide we to the optimization of loop length.

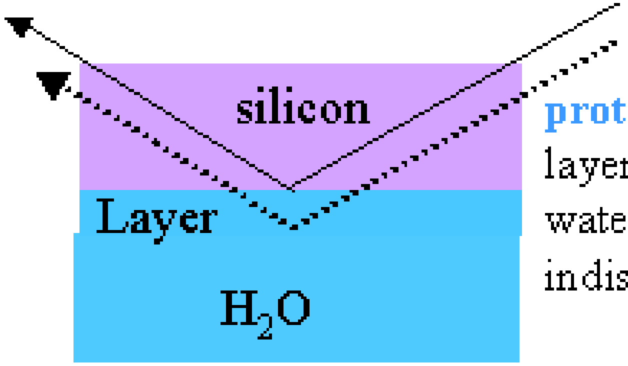


Loop length too short leads to rough layer and surfactant (and loop) adsorption not maximized.

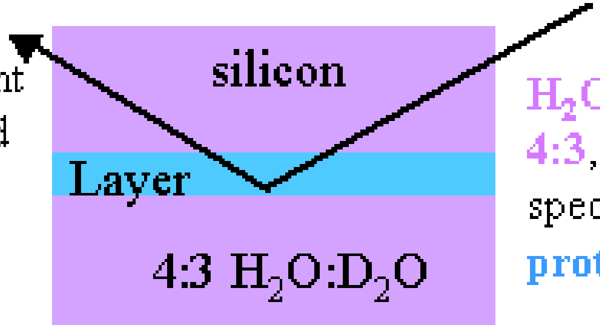
Loop length (optimum leads to a less rough layer and maximum adsorption of surfactant

In HPAM the optimum loop length between hydrophobic groups on the polymer occurs at 2% modification, i.e. loop length about 50 units.

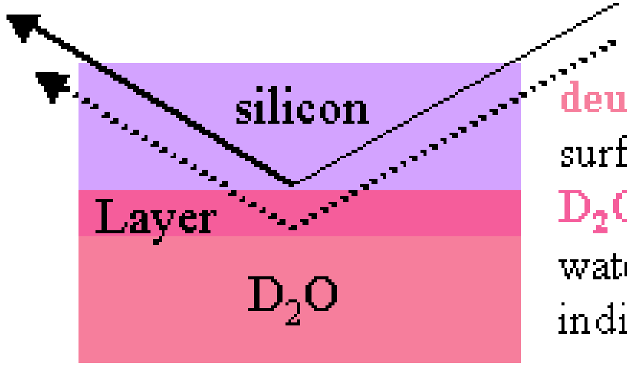
... at the solid/liquid interface



protonated surfactant layer; H_2O, layer and water almost indistinguishable



H_2O:D_2O **about** 4:3, experiment specific to **protonated** layer



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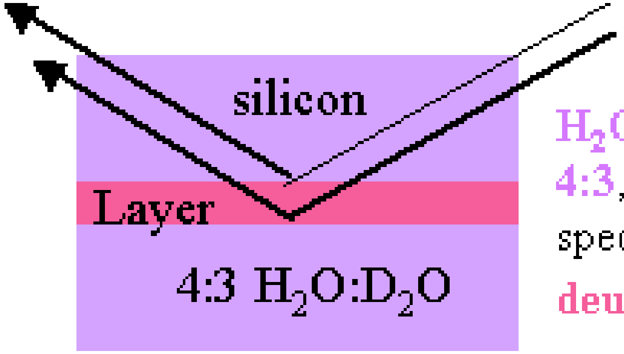

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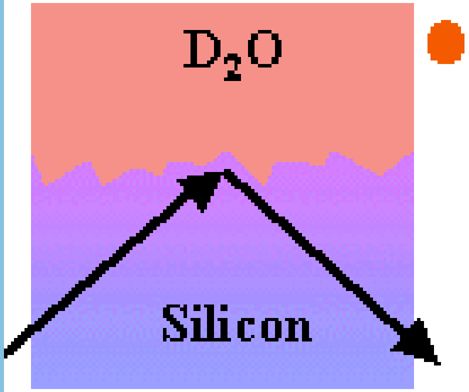
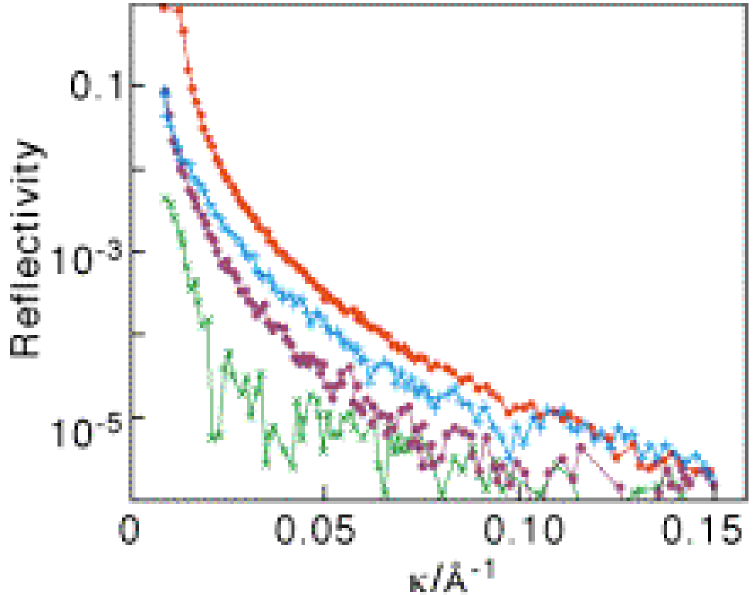
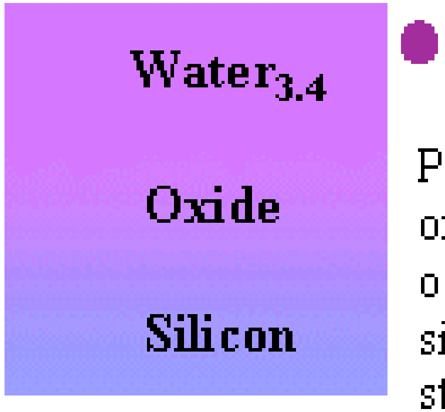
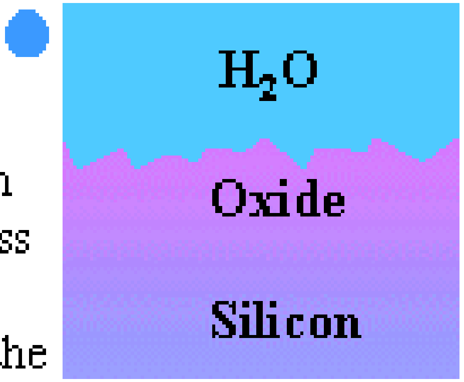


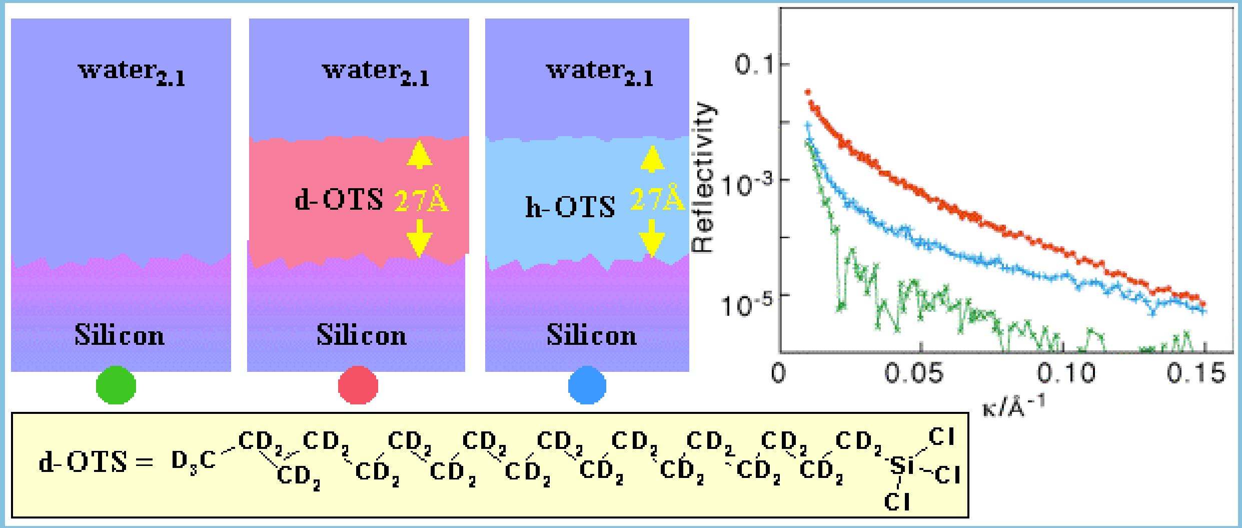




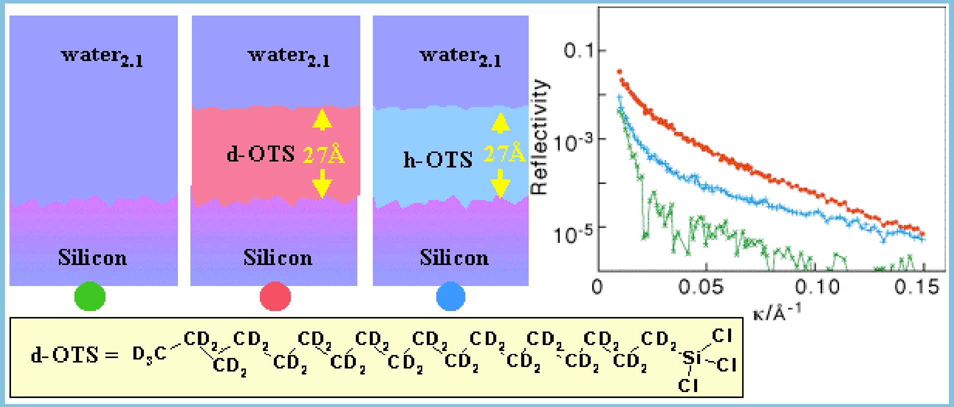


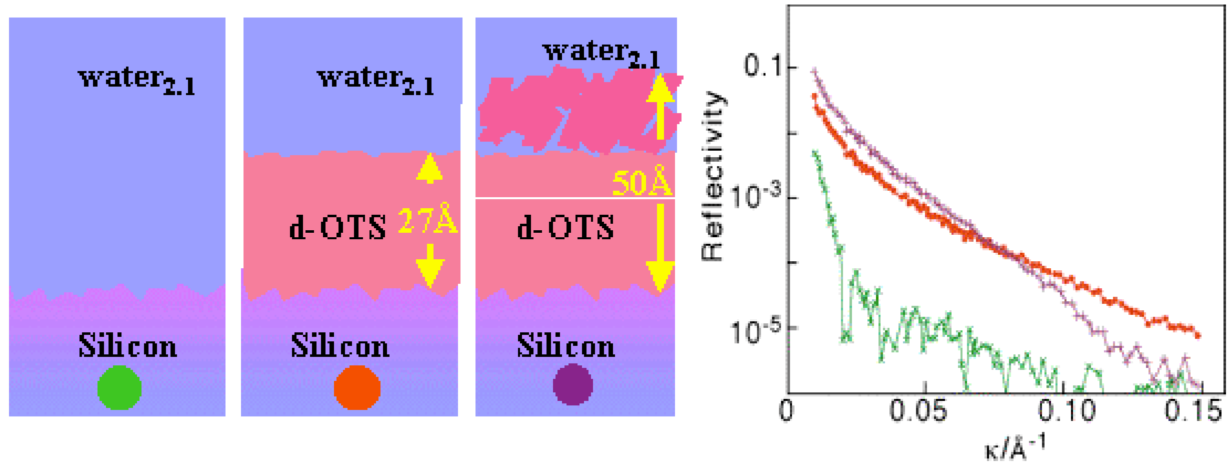
Polished silicon(111) normally has a thin 2 nm oxide layer and there is a measurable roughness of the surface. The largest contrast is between silicon and either D_2O or H_2O and these give the stronger reflected signals. Note that the neutron beam enters and exits through the solid

Hydrophobic solid/liquid interface

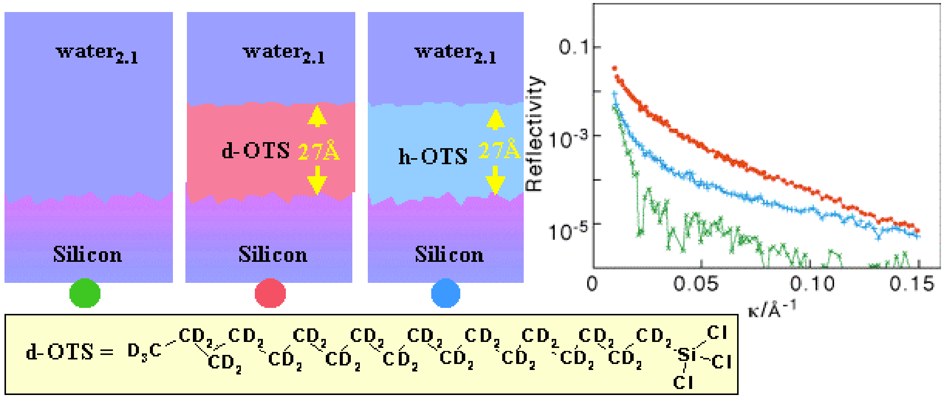


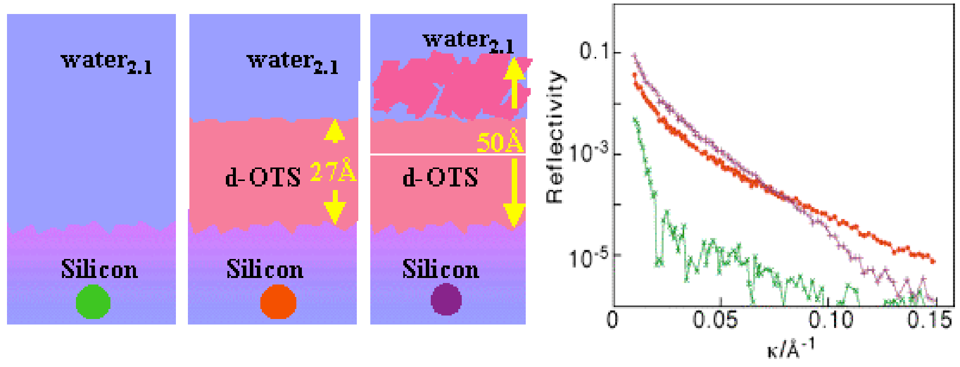
Hydrophobic solid/liquid interface

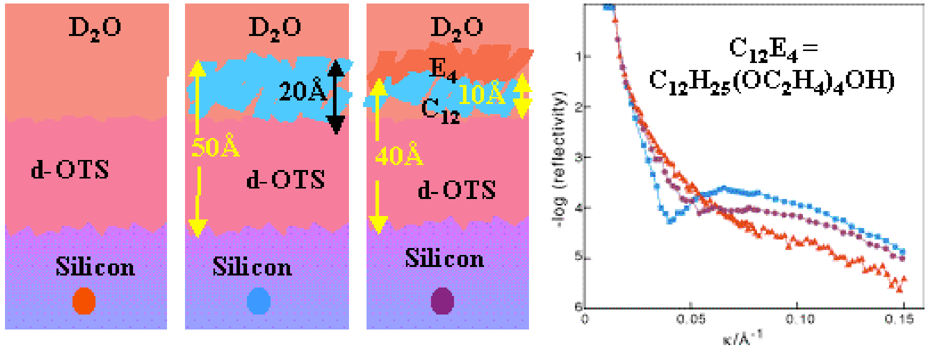




Hydrophobic solid/liquid interface







WATER DEPLETION LAYER

There has been a theoretical prediction that, at a flat and strongly hydrophobic surface in contact with pure water, a continuous air/vapour layer of about 1 nm thickness forms spontaneously (Lum, Chandler, Weeks, 1999).

R.K. Thomas et al.
http://www.physchem.ox.ac.uk/~rkt/

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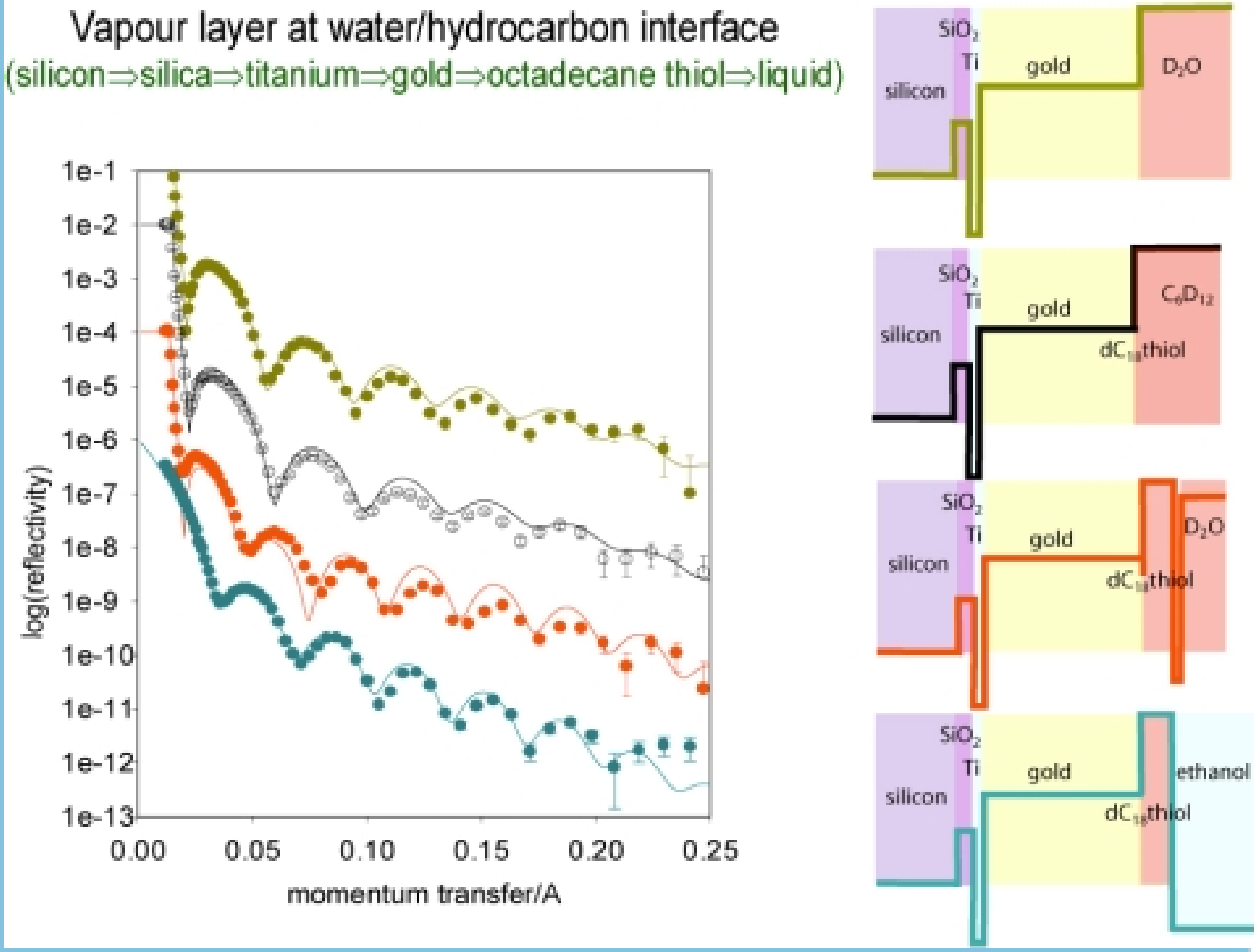
By suitable isotopic labelling neutron reflection can be made extremely sensitive to the presence of a water depletion layer.

Thomas, et al. have now observed what looks like a water depletion layer on three hydrophobic surfaces:
C18 thiol on gold;
C18 silane on silicon;
polystyrene on silicon.



R.K. Thomas et al.
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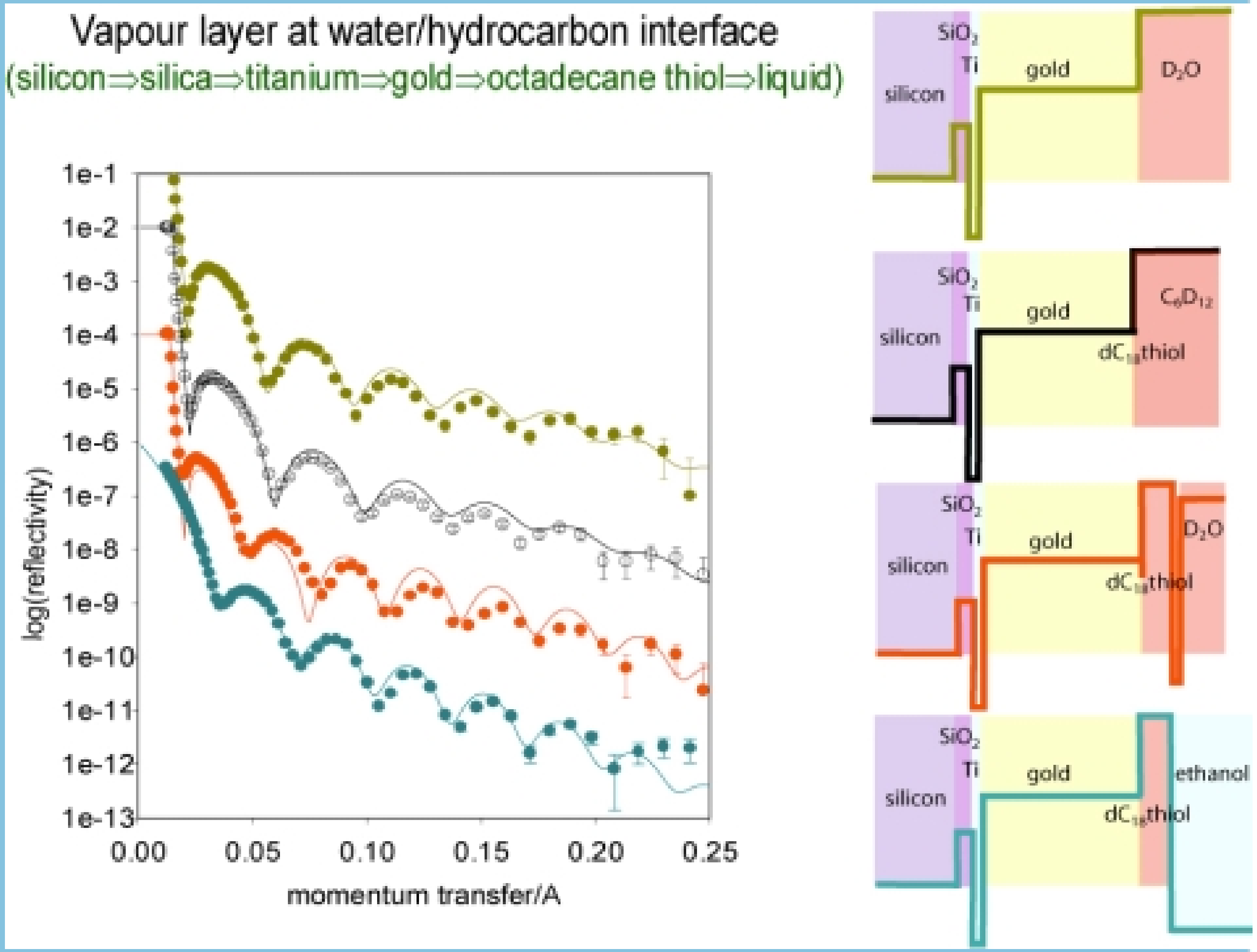


Although this is the first direct evidence for such a layer, much remains to be done.
We do not know whether the layer is air/vapour, as predicted, or a thicker less depleted layer.

Fragneto, Thomas, Rennie, Penfold, *Science*, 267, 657, 1995

ADSORPTION OF β-CASEIN AT HYDROPHOBIC INTERFACES

The use of a deuterated hydrophobic layer has provided enough contrast for determining the thickness and composition of the protein at the interface

209 residues
~24000 daltons
amphiphilic and
surface active

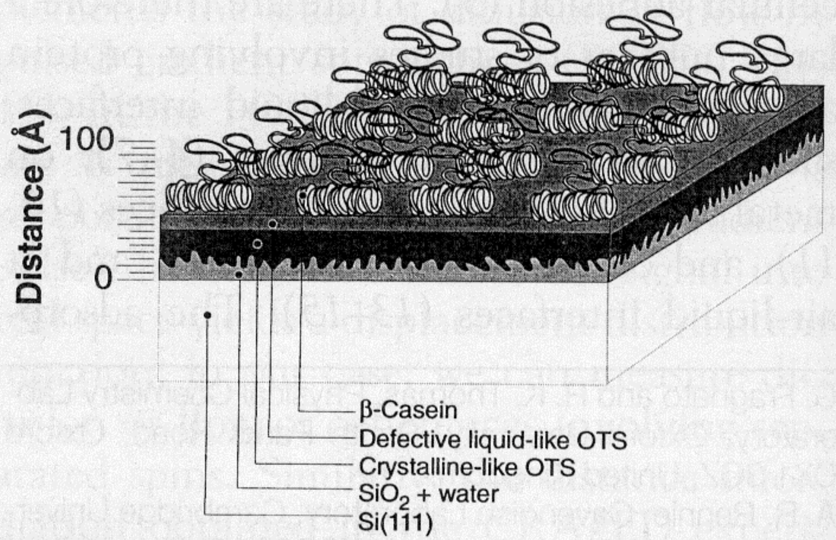


Fig. 2. Schematic representation of the adsorbed layer of β-casein at the interface between a modified hydrophobic silicon surface and an aqueous solution.

NR at
silicon/OTS/water
interface

dense layer:
~23Å 61% protein

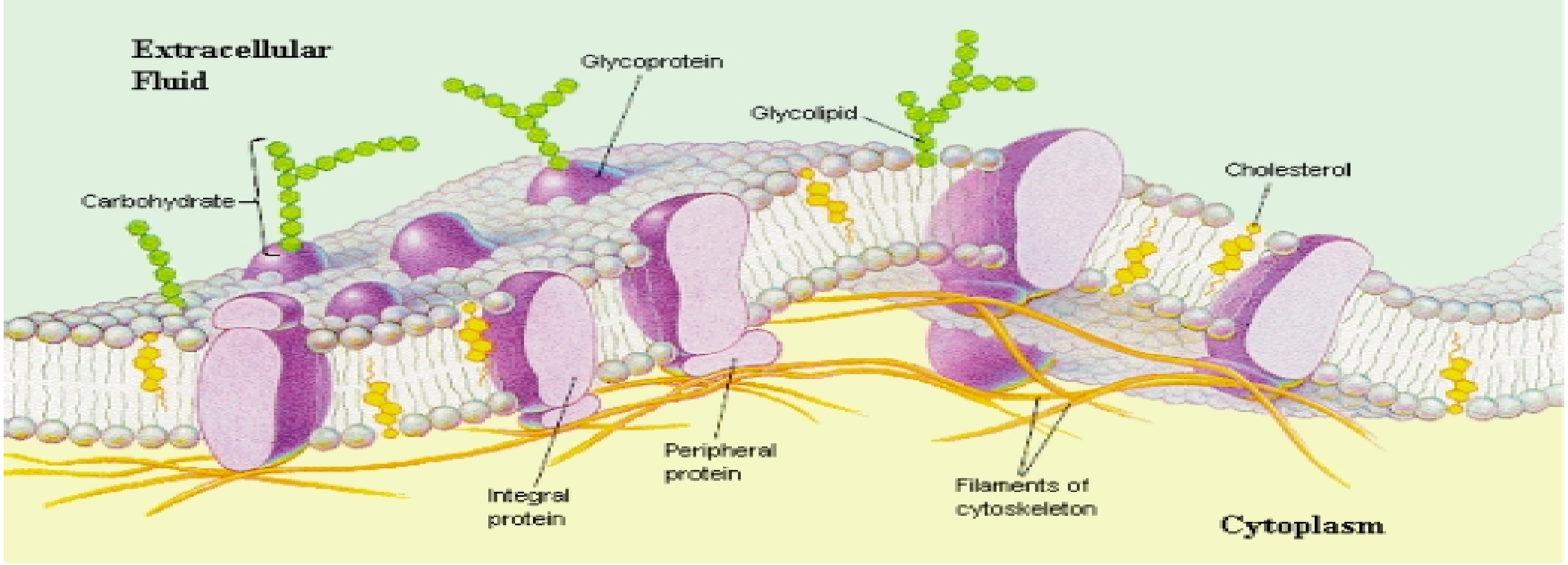
external layer
~35Å 12% protein

Biological role is to stabilise the colloidal form of calcium phosphate in milk and inhibit crystal growth in secretary cells

Stabilising ability of β-casein is the basis of its use as additive in food, paint, glue, ...

BIOLOGICAL MODEL MEMBRANES

Structure determination is an important pre-requisite to understand function

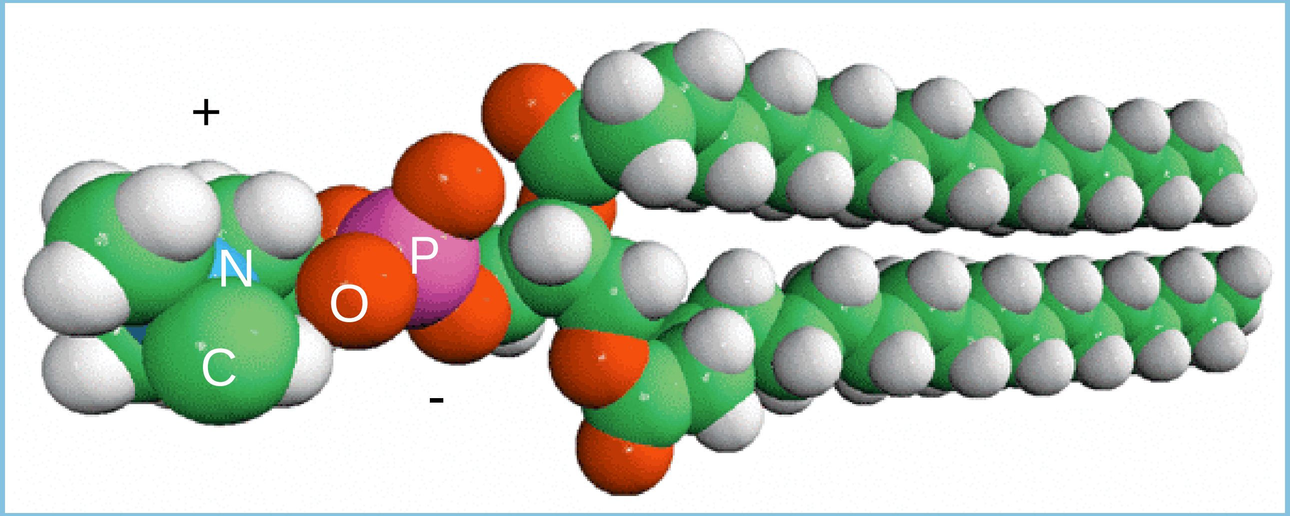


A biologically relevant model membrane should:

- have a composition that matches the content of (or at least the lipid part of) the real cell membrane under investigation
- be fully hydrated, i.e. lie in aqueous environment
- be able to interact with other biological entities (peptides, proteins, DNA, etc.)
- be free enough to allow for movements both out of plane
  (the bilayer position fluctuates)
  and in plane
  (the molecules freely diffuse within the bilayer ↪ fluid phase)

Simplest model: lipid bilayer

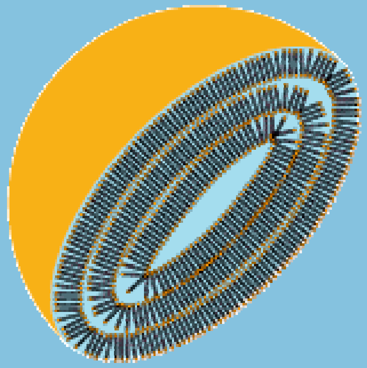
Simplest lipids: saturated phospholipids



DSPC
1,2-Distearoyl-*sn*-Glycero-3-Phosphocholine

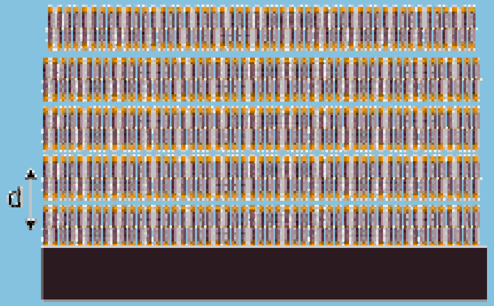
n=18

MODEL MEMBRANES

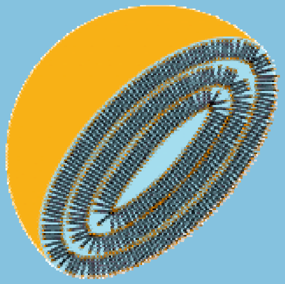


multilamellar vesicles

stacked bilayers



MODEL MEMBRANES



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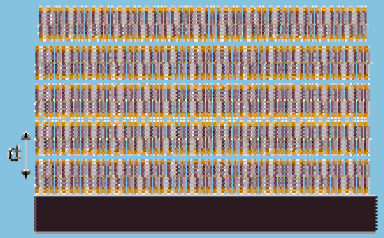
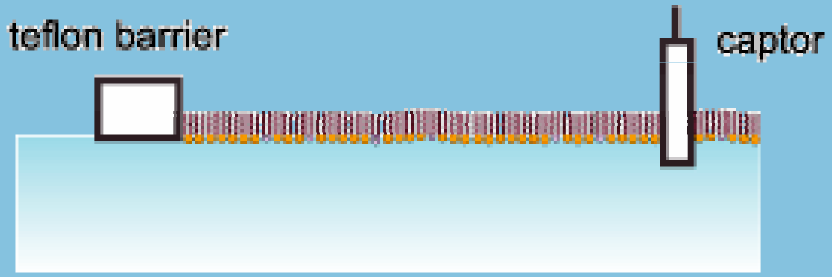
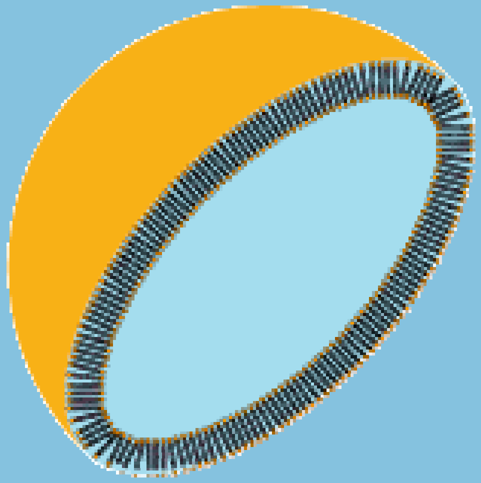


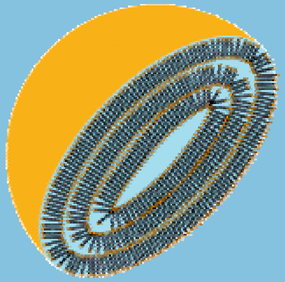


monolayers



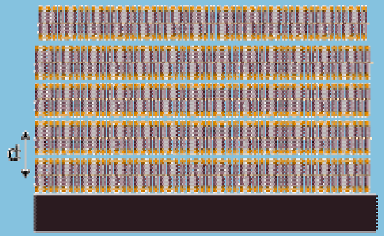
unilamellar vesicles

MODEL MEMBRANES

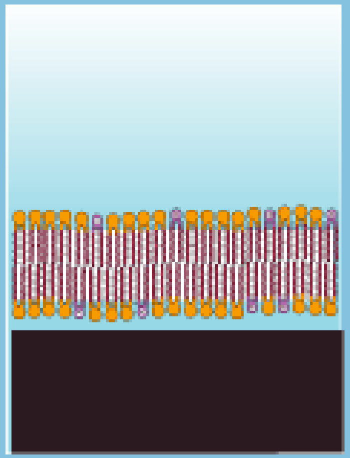


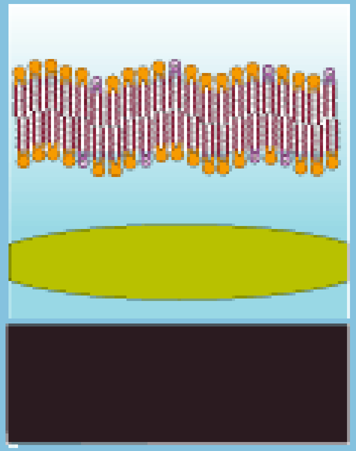
multilamellar vesicles

stacked bilayers

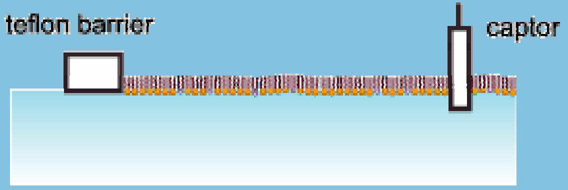


supported bilayer



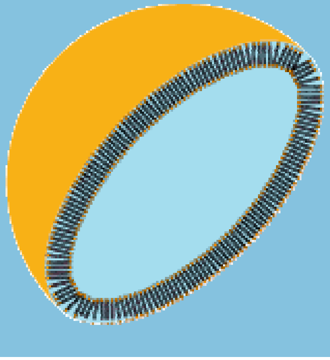


floating bilayer



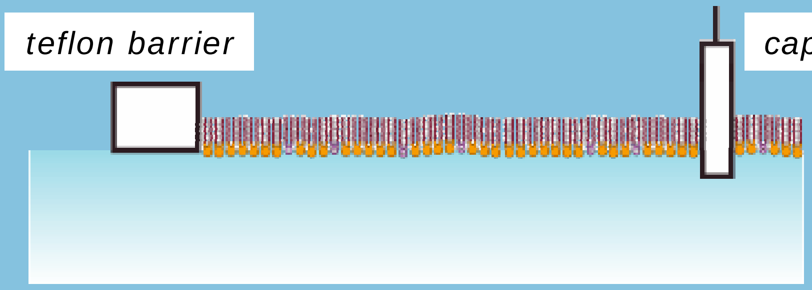
monolayers

unilamellar vesicles



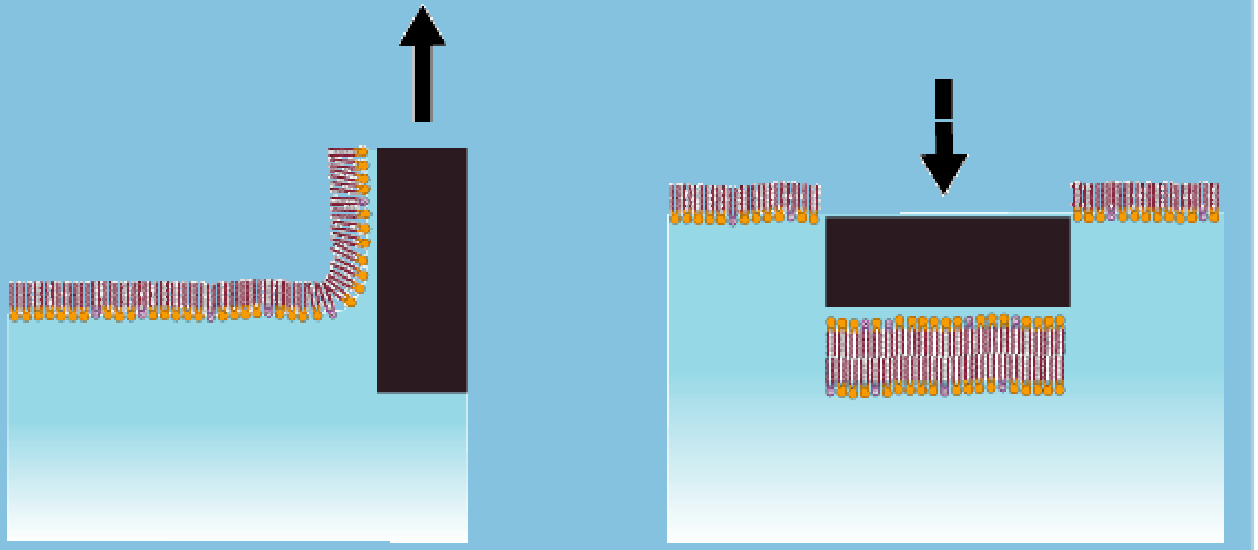
LANGMUIR-SCHAEFER DEPOSITION

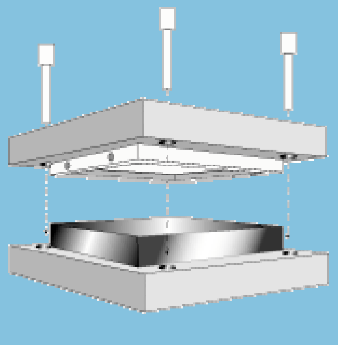
Lipids used are phosphocholines, abundant in membranes, with and without addition of negatively charged lipids and cholesterol



P=30mN/m

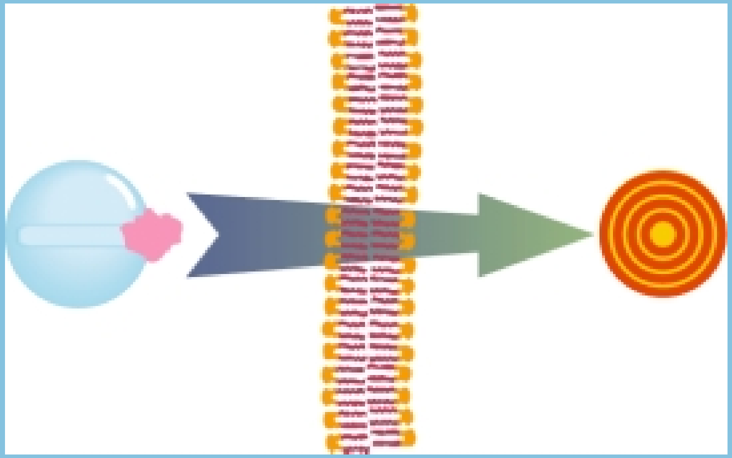
SILICON SUBSTRATES:
TRANSPARENT TO NEUTRONS
HIGHLY HYDROPHILIC
SMOOTH





PENETRATIN

16 amino-acid polypeptide from the third helix of the DNA binding domain of the DROSPHILA transcription factor Antennapedia



IT TRANSLOCATES ACROSS THE PLASMA MEMBRANE WITHOUT DISRUPTING IT

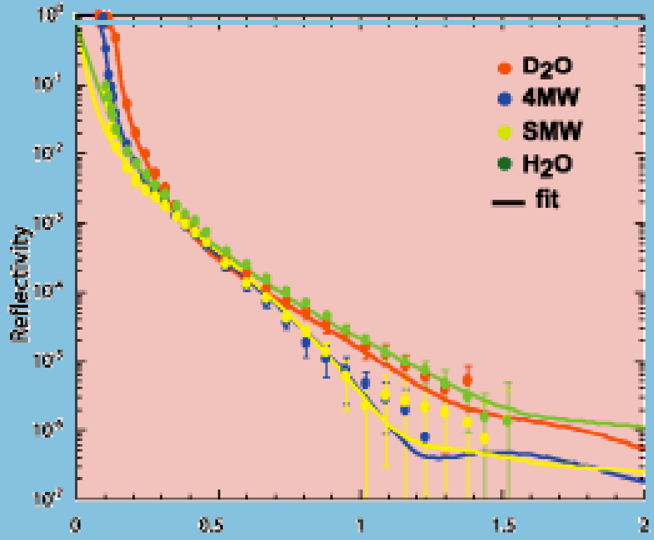
Fragneto, Charitat, Dubos, Bellet-Amalric, Graner, *Langmuir*, 16, 4581, 2000

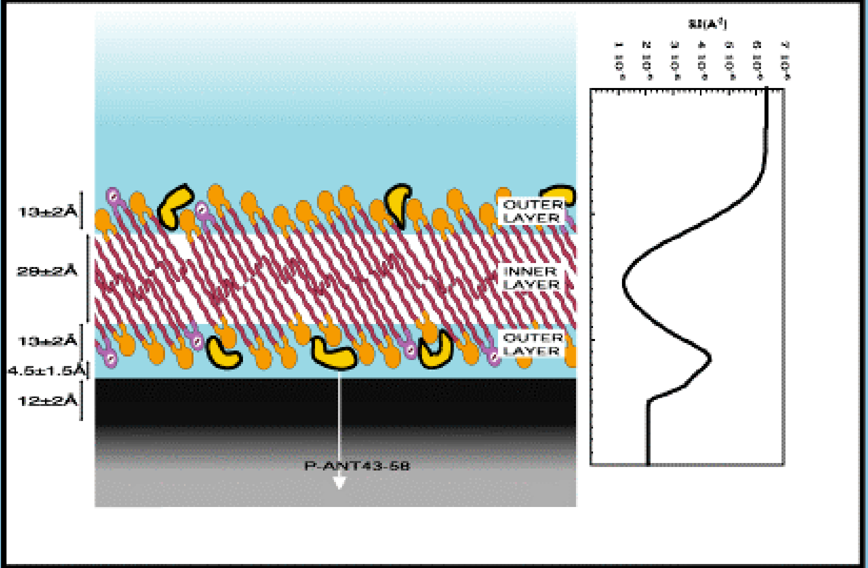
In the presence of negative charges the peptide adsorbs in the lipid headgroups

System characterised at a resolution of 0.1 nm thanks to deuterium labelling and contrast variation

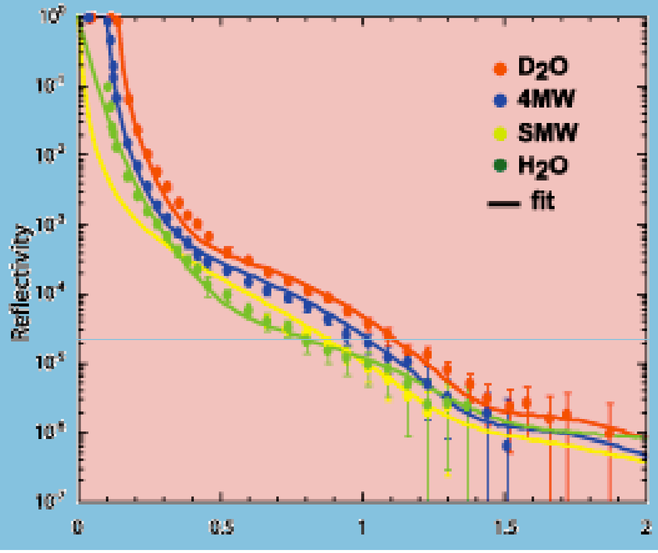
PROBLEM
because of the interaction of the lipids with the substrate the membrane is not fluid and free to fluctuate

HYDROGENATED LIPIDS



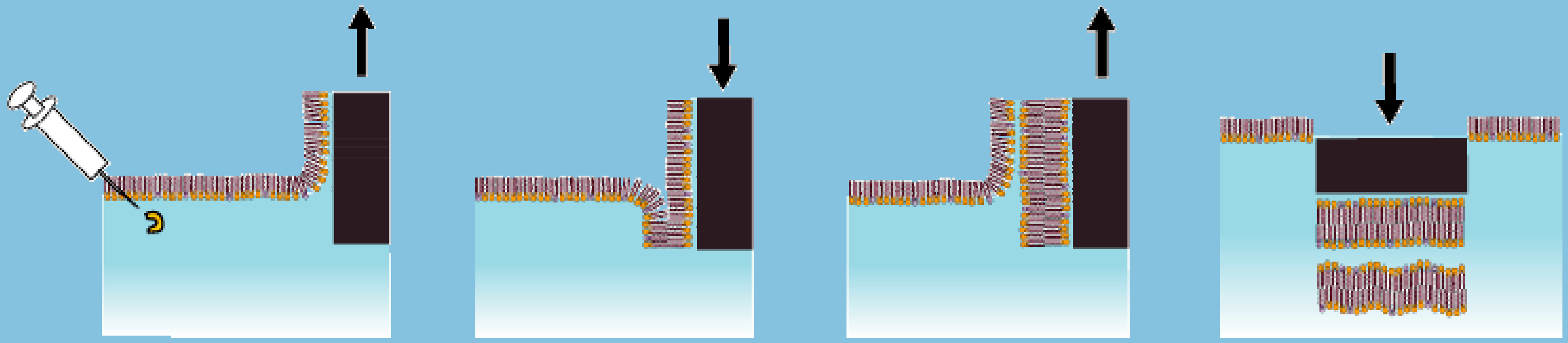


DEUTERATED LIPIDS



Charitat, Bellet-Amalric, Fragneto, Graner, *Eur. Phys. J. B*, 8, 583, 1999

FLOATING BILAYERS



Bilayers prepared in the gel phase and heated to overcome gel/fluid phase transition

STABLE IN FLUID PHASE

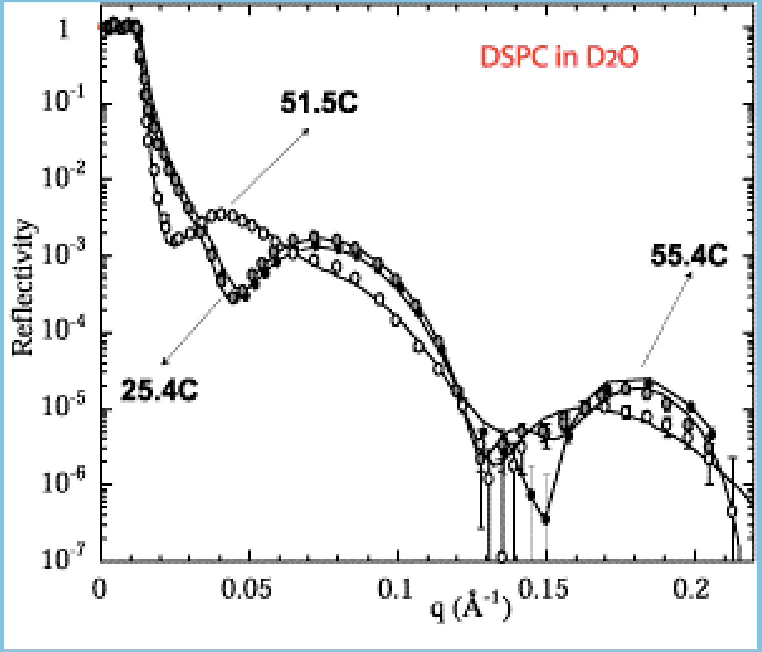
VARIABLE COMPOSITION

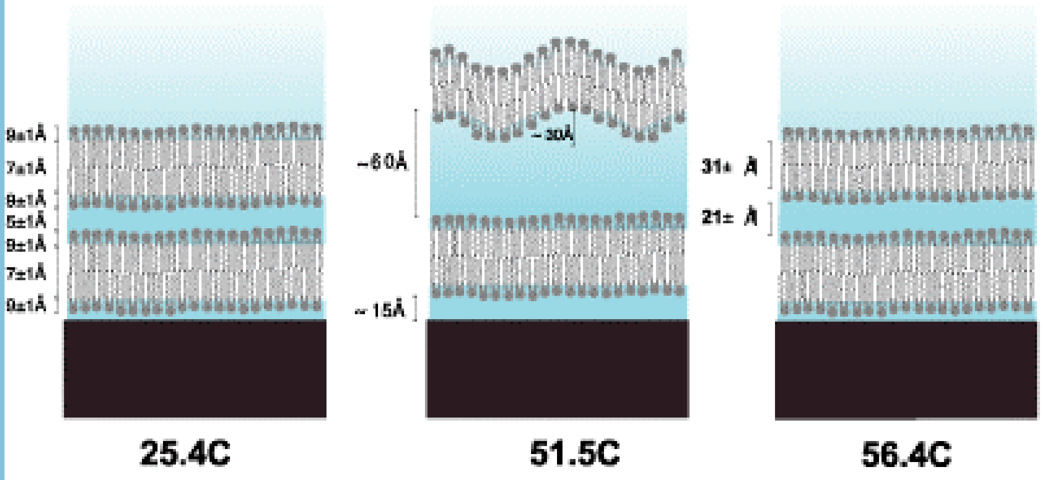
STABLE IN BULK WATER

FLUCTUATING

NO AVERAGED INFORMATION

Fragneto *et al.*, *Europhys. Lett.*, 53, 100, 2001





Determination of water thickness between bilayers

Estimation of membrane bending modulus for the same sample in both phases

ADDITION OF 10% CHOLESTEROL LEADS TO A STABLE FLUID FLOATING BILAYER

BUT

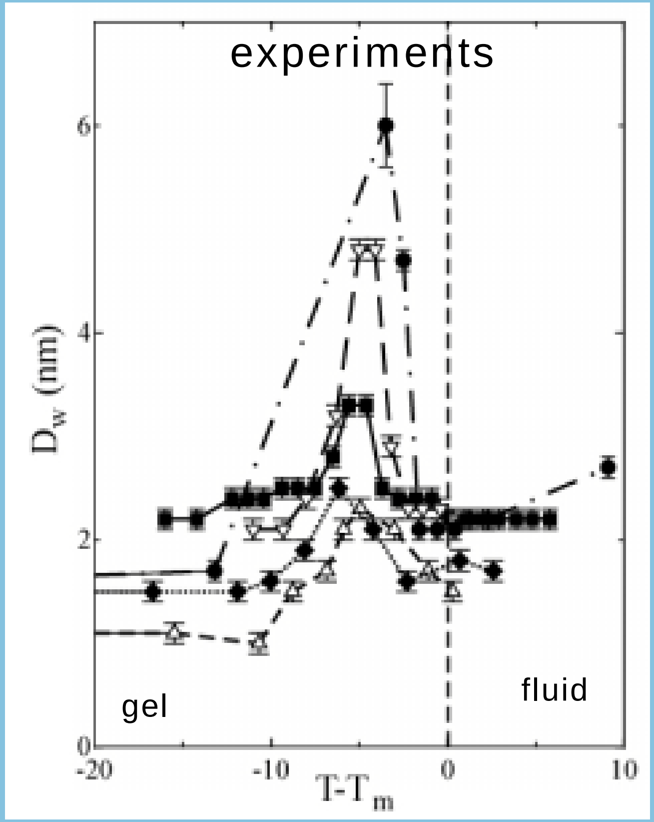
NO SWELLING IS OBSERVED AROUND THE TRANSITION!!!!

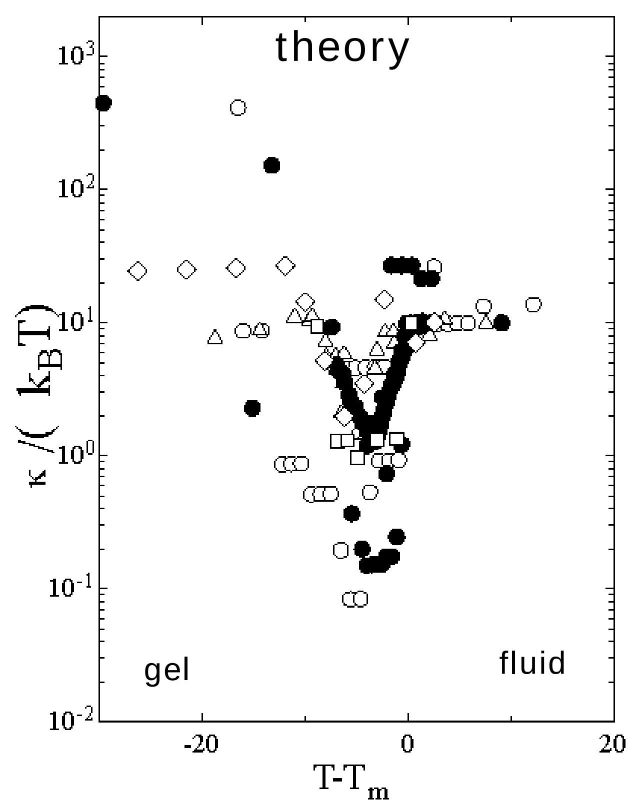
Fragneto, Charitat, Bellet-Amalric, Graner, Mecke, Langmuir (ASAP) 2003

Mecke, Charitat, Graner, Langmuir, 2003, 19, 2080

SWELLING OF FLOATING BILAYERS: THE EFFECT OF CHAIN LENGTH

di-Cn-PC n=16,17,18,19,20





Minimum of the bending modulus around the phase transition
…swelling occurs at T<Tm … …

Hughes, Roser, Stidder, Cubitt, Fragneto, 2002

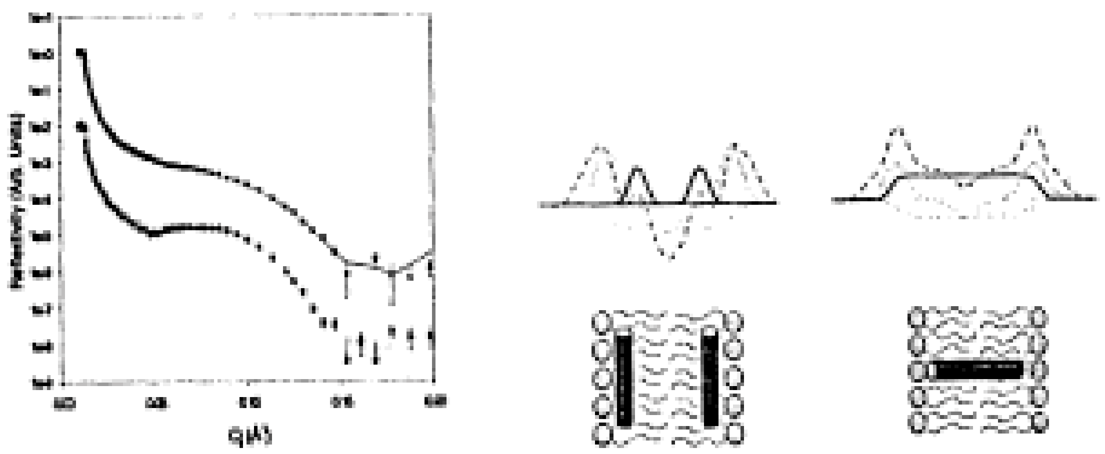
Minimalist peptide: repeating sequence of leucine (hydrophobic) and serine (polar)

Cation selective channels are formed

Interesting for understanding the behaviour of antimicrobial peptides which show voltage-gated ion-channel activity (they do not interact with specific cellular proteins like conventional antibiotics)

Application of a transmembrane potential causes a proportion of the peptide to switch from lying along the bilayer surface to a transmembrane orientation

HYDROGENATED DPPC
DEUTERATED PEPTIDE



Reflectivity from a DPPC floating bilayer without an applied potential (upper) and with a transbilayer potential (lower). The Quasimolecular models shown indicate the difference in peptide orientation (bold lines).

ENZYME ATTACK ON PHOSPHOLIPID BILAYERS

MANY SOLUBLE ENZYMES PERFORM THEIR CHEMISTRY AT THE AQUEOUS MEMBRANE INTERFACE

Phospholipases play a key role in lipid digestion, host defence, signal transduction

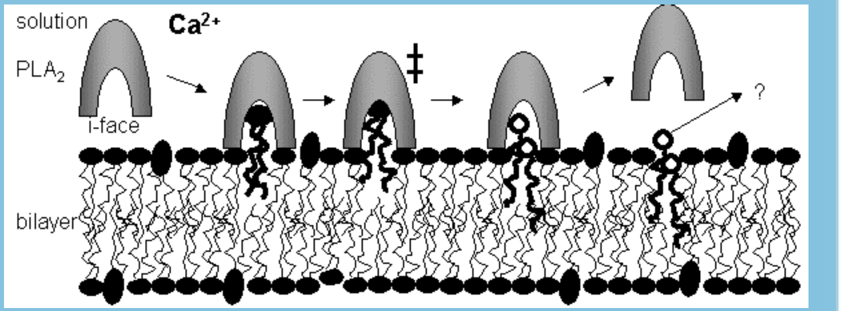
PLA2 occurs in mammalian pancreatic juice, snake and bee venom

Role of PLA2 in inflammation makes them a potential target for drug development

Structure known by x-rays

Schematic model of the attack by PLA2 on a phospholipid bilayer based on conclusions from kinetic data on vesicles

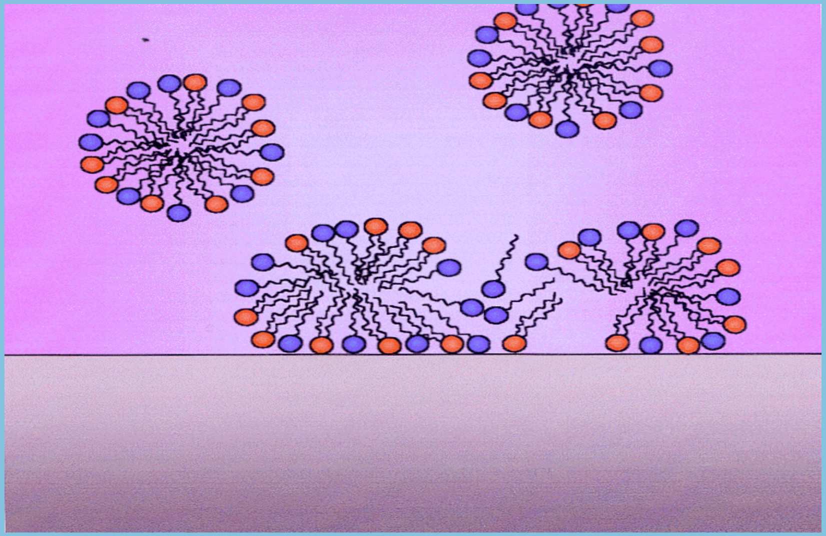
The PLA2 enzyme hydrolyses the acid in the two positions from the phospholipid and generates a soluble lysophospholipid and an insoluble fatty acid



Neutron Reflectivity will help understanding:

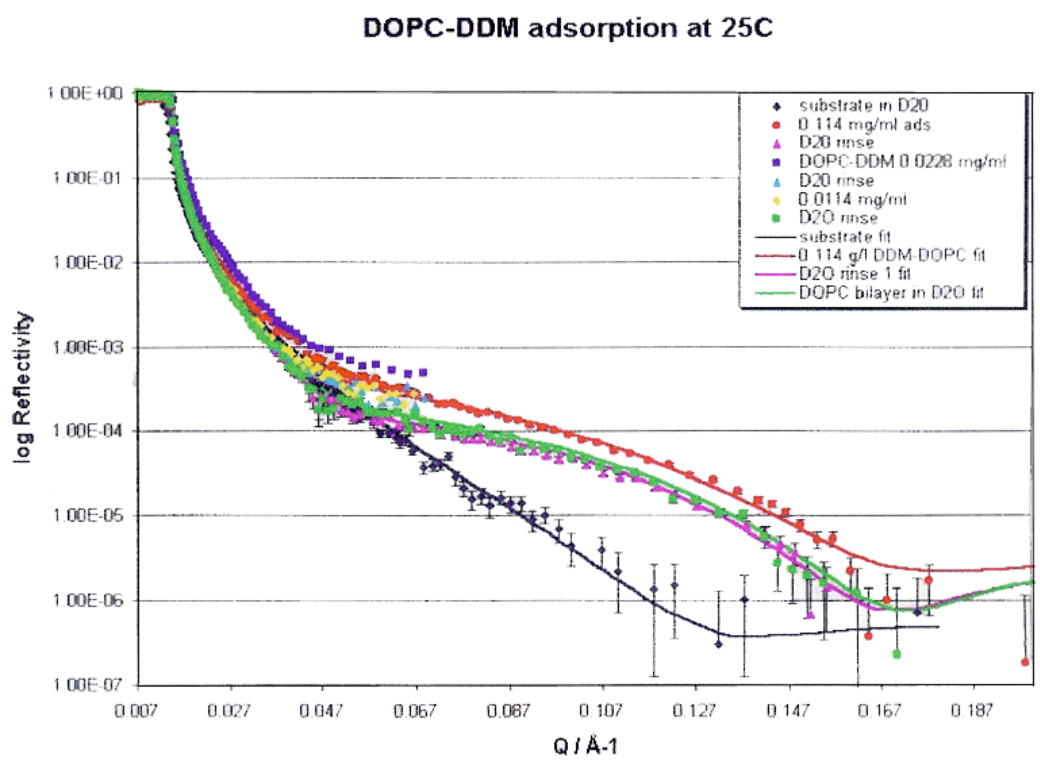
- Penetration of PLA2 into the surface
- Structural rearrangement of the enzyme
- Affinity of PLA2 for a range of PL surfaces
- Affinity for the PL surface under a wide range of of solution conditions

Tiberg et al., *Eur. Biophys. J*, 29, 196, 2000



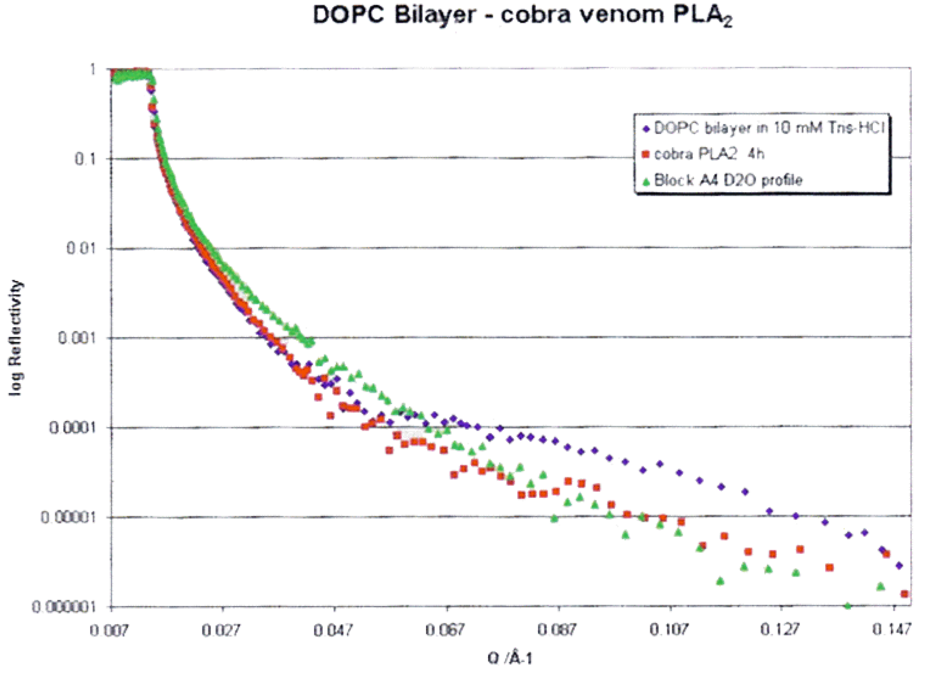
Bilayer formed via co-adsorption of phospholipids (DOPC, POPC, ...) with the non-ionic sugar-surfactant β-D-dodecyl-maltoside

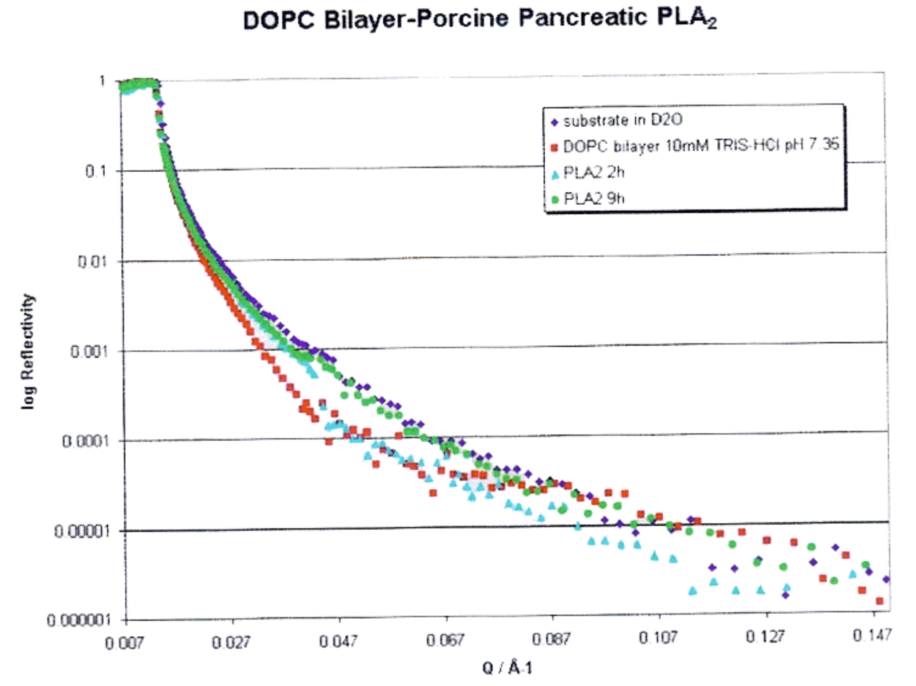
The surfactant is removed by rinsing with water and a phospholipid bilayer 4.0 nm thick covers 95% of the surface



Vacklin, Thomas, Tiberg, Fragneto 2002

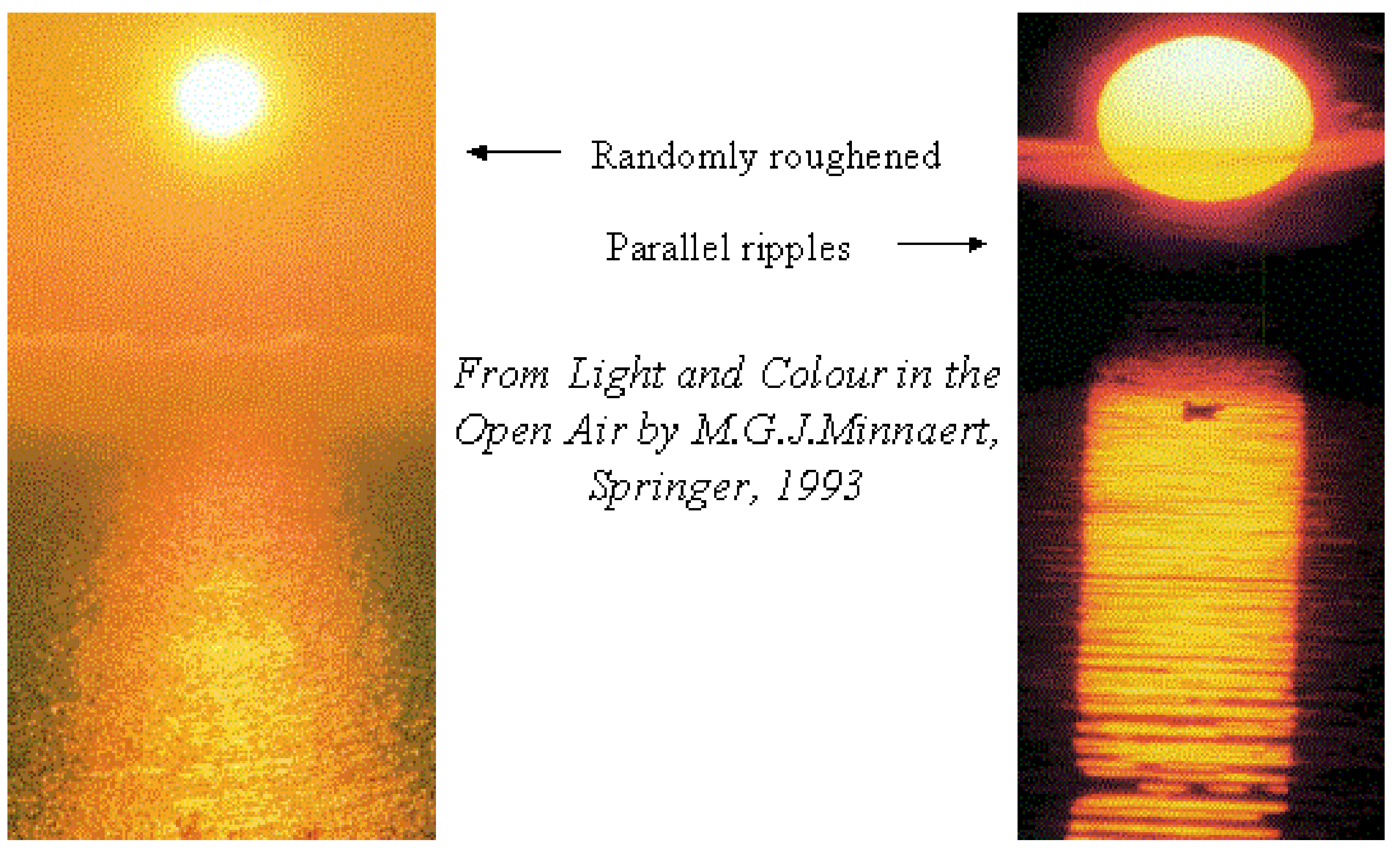
After a few hours of contact with a solution of PLA2 either from cobra venom or porcine pancreatic source the DOPC bilayer is removed from the surface





Off-specular measurements allow the determination of the structure parallel to the interface (fluctuations)

Not fully exploited for thin soft-matter layers because of low intensity of neutron sources



From Light and Colour in the Open Air by M.G.J.Minnaert, Springer, 1993

NEUTRONS
FOR SCIENCE