The use of neutrons to probe phenomena at interfaces
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)
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.
**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
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
Examples of surfactants

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

$$q = \frac{4\pi}{\lambda} \sin \theta$$
When $q \gg q_c$ this reduces to:

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

which is the reflectivity used in the Born approximation

$N_b$ is the scattering length density
Simulation of the specular neutron reflectivity as a function of $q$ from a silicon/air interface ($N_{b1}=2.07 \times 10^{-6} \text{Å}^{-2}$).
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 ($Nb_1=2.07\times10^{-6}\text{Å}^{-2}$, $Nb_2=9.04\times10^{-6}\text{Å}^{-2}$).

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 $\theta$ at constant $\lambda$
- or

- measuring the time-of-flight at constant $\theta$

For the same resolution TOF is less efficient
(flux at min and max $\lambda$ 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/cm²

**TOF**  
**Q:** 0.002 - 4 Å⁻¹  
**ΔQ/Q:** 0.1 - 20%  
**Min R:** <10⁻⁸

**MONO**  
**Q:** 0.005 - 1Å⁻¹  
**ΔQ/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

\[ q_{\text{max}} = 3 \text{nm}^{-1}, \quad d_{\text{min}} = \frac{\pi}{q} = 1 \text{nm}! \]

low resolution

With soft matter systems resolution may be greatly increased by using \textit{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; \( \text{D}_2\text{O} \), layer and air almost indistinguishable.

- **Deuterated** surfactant layer; \( \text{H}_2\text{O} : \text{D}_2\text{O} \) about 11:1 (null water), only layer observed.

**Hydrogen** and **deuterium** scatter neutrons with opposite phase. \( \text{H}_2\text{O} \) and \( \text{D}_2\text{O} \) 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.
Adsorption of Surfactants

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

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

3. **Thickness of whole surfactant layer** (a) or part of layer (b).
The method has been applied to the surfactant C\textsubscript{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.
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.
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.
Polymer/Surfactant Interactions

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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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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.
... at the solid/liquid interface

- Protonated surfactant layer, $\text{H}_2\text{O}$, layer and water almost indistinguishable
- Deuterated surfactant layer, $\text{D}_2\text{O}$, layer and water almost indistinguishable

- $\text{H}_2\text{O}:\text{D}_2\text{O}$ about 4:3, experiment specific to protonated layer
- $\text{H}_2\text{O}:\text{D}_2\text{O}$ about 4:3, experiment specific to deuterated layer
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$_2$O or H$_2$O and these give the stronger reflected signals. Note that the neutron beam enters and exits through the solid.
Hydrophobic solid/liquid interface

\[ \text{d-OTS} = \text{H}_2\text{C}_2\text{C}_{17}\text{H}_{35} \]

\[ \text{h-OTS} = \text{H}_2\text{C}_2\text{C}_{17}\text{H}_{35} \]
Hydrophobic solid/liquid interface

\[ d\text{-OTS} = \text{silicon} + \text{water}_2.1 + d\text{-OTS (27 Å)} + \text{water}_2.1 + \text{silicon} \]
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).
By suitable isotopic labelling neutron reflection can be made extremely sensitive to the presence of a water depletion layer.

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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.
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

NR at silicon/OTS/water interface

dense layer:
~23Å 61% protein

external layer
~35Å 12% protein

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

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

- Multilamellar vesicles
- Stacked bilayers
- Monolayers
- Unilamellar vesicles
MODEL MEMBRANES

- multilamellar vesicles
- stacked bilayers
- supported bilayer
- floating bilayer
- monolayers
- unilamellar vesicles
Lipids used are phosphocholines, abundant in membranes, with and without addition of negatively charged lipids and cholesterol.

Silicon substrates: transparent to neutrons, highly hydrophilic, smooth.
PENETRATIN: A 16 amino-acid polypeptide from the third helix of the DNA binding domain of the DROSOPHILA transcription factor Antennapedia translocates across the plasma membrane without disrupting it.
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.

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**HYDROGENATED LIPIDS**

**DEUTERATED LIPIDS**
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
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!!!
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 ... ...
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

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

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 solution conditions
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.
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