INTRODUCTION TO NEUTRON REFLECTOMETRY

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The importance of interfaces

They are everywhere: our body, food we eat, drinks, plants, animals, soil, atmosphere, manufacturing, chemical factories....

In many cases interfaces have a significant effect in the behaviour of a system

Examples:

**Inner lining of lung:** surfactants prevent lung from collapsing at the end of expiration

**Nanotechnology:** solid surfaces are the places where the processes of interest take place

**Detergency**

**Biofouling**
**Why Neutron Reflectometry?**

- Probe relevant lengths (Å to µm)
- Sensitive to light elements (H, C, O, N)
- Buried systems and complex sample environment
- Possibility of **isotopic labelling**
- Non-destructive
Schematic view of elastic neutron scattering spectra

- Cavities; precipitates, clusters; pores and defects; polymers; organic macromolecules
- Small angle neutron scattering
- Residual Stress
- High resolution diffractometers
- Texture
- Four-circle diffractometer

Scattering cross-section $\frac{d^2F}{dQ^2}$

Sizes: $\sim 0.1 - 10 \, \mu m$
High resolution SANS

Sizes: $\sim 1 - 100 \, nm$
Conventional SANS

Scattering vector $Q \, [nm^{-1}]$
Specular $\theta_i = \theta_f$

- Thickness of layers at interfaces
- Roughness/interdiffusion
- Composition in the direction normal to the interface

Reflectivity measurements:

In-plane features (height fluctuations, domains, holes ...) can be probed by off-specular measurements: for thin films synchrotron radiation is more suitable.
$R(\theta, \lambda) = \frac{I_{\text{out}}(\theta, \lambda)}{I_{\text{in}}(\lambda)}$

Scattering length density profile extracted from data analysis

Film thickness

$q = \frac{4\pi}{\lambda} \sin \theta$

Liquid $\text{D}_2\text{O}$

Solid Si-SiO$_2$
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.

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.
For both kinds of radiation the refractive index is a function of the scattering length density and wavelength.

As with light, total reflection may occur when neutrons pass from a medium of higher refractive index to one of lower refractive index.
Optical Demonstrations

Reflection from a thin film
Newton’s Rings

Courtesy of R. K. Thomas
http://rkt.chem.ox.ac.uk/techniques/nrmain.html
Basic Principles of Neutron Reflection Theory
“Neutron man” personifies the neutron’s dual nature, exhibiting wave and particle properties. Here he enters a crystal lattice as a plane wave (blue), interacts with the crystal lattice (green), and becomes, through interference effects, an outgoing plane wave (red) with a direction dictated by Bragg’s law. His particle properties allow him to be absorbed by a He atom in a neutron detector, and his time of flight is measured.
Neutrons can be treated as a wave:

The Schroedinger equation is analogous to the wave equation for light and leads to neutrons showing characteristic optical behaviour such as total reflection and refraction.

The Schroedinger equation may be written as:

$$-\frac{\hbar^2}{8\pi^2m_n} \nabla^2 \Psi + V\Psi = E\Psi$$

Where $V$ is the potential to which the neutron is subject and $E$ its energy.
$V$ represents the net effect of the interactions between the neutron and the scatterers in the medium through which it moves.

$$V = \frac{h^2}{2\pi m_n} N_b$$

$N_b = \frac{\sum b_j n_j}{Vol}$

scattering length density
Coherent neutron scattering lengths [fm]

Average

-3.74  6.67  6.65  9.36  5.81  5.13  2.85

Spin up

10.82  9.4

Spin down

-18.3  3.8

Calculation of the scattering length density

\[ N_{b_{H_2O}} = \frac{2b_H + b_O}{V_{H_2O}} = \frac{(5.81 - 3.74 \cdot 2)}{30 \, \text{Å}^3} \]

\[ N_{b_{H_2O}} = -0.56 \cdot 10^{10} \, \text{cm}^{-2} \]

\[ V_{H_2O} = \frac{M_{H_2O}V_{H_2O}}{N_A} \]

Spin-dependent scattering lengths

neutrons deflected from hydrogen are 180° out of phase relative to those deflected by the other elements
Let us consider a beam approaching a surface with a bulk potential $V$, infinitely deep.

With no structure within the surface the only potential gradient and hence force is perpendicular to the surface.

Only the normal component of the incoming wave vector, $k_i$ is altered by the barrier potential and it is the normal component of the kinetic energy $E_{i\perp}$ which determines whether the neutron is totally reflected from the barrier or not.
If \( E_{i\perp} < V \) then there is total reflection and when \( E_{i\perp} = V = \frac{h^2}{2\pi m_n^2} N_b \):

\[
E_{i\perp} = \frac{(h k_i \sin \theta_i)^2}{8\pi^2 m_n}.
\]

Provided the sample is static, any off specular reflection must be a result of potential gradients within the xy plane of the surface.

If interaction is elastic then conservation of momentum and

\[
q_c = \sqrt{16\pi N_b} \quad \text{as} \quad q = 2k_i \sin \theta_i
\]

\[\theta_i = \theta_o \]

i.e. the reflection is specular
If $E_{i\perp} > V$, then the reflection is not total and the neutron can either be reflected or transmitted into the bulk of the material.

The transmitted beam, $k_t$ with its normal component of kinetic energy reduced by the potential must change direction i.e. it is refracted.

The change in the normal wave vector is

\[
k_{t\perp} = k_{i\perp} - 4\pi N_b
\]

\[
n^2 = \frac{k_t^2}{k_i^2} = \frac{k_{i\parallel}^2 + (k_{i\perp}^2 - 4\pi N_b)}{k_i^2} = 1 - \frac{4\pi N_b}{k_i^2} = 1 - \frac{\lambda^2 N_b}{\pi}
\]
Values of Refractive Index

- Small difference in refractive index mean that critical angles are small (less than 1 degree)
- As most $n < 1$, total external reflection is common. In optics $n > 1$
- Mixtures of isotopes can be used to match values of different materials
- $\beta$ absorption coefficient small with neutrons

$$n = 1 - \delta - i\beta$$

- **X-RAYS**
  $$\delta = \frac{\lambda^2}{2\pi} r_e \rho$$

- **NEUTRONS**
  $$\delta = \frac{\lambda^2}{2\pi} N_b$$
Quantum mechanical approach

The wavefunction describing the probability amplitude of a neutron near to the surface is:

\[ \frac{\partial^2 \Psi}{\partial z^2} + k^2 = 0 \quad \text{where} \quad k^2 = \frac{2m}{\hbar^2}(E_i - V) - k_{\parallel}^2 \]

Solutions for this above and below the surface are:

\[ \Psi_z = e^{ik_{\perp}z} + re^{-ik_{\perp}z} \quad \& \quad Y_z = te^{ik_{\perp}z} \]

where \( r \) and \( t \) are the probability amplitudes for reflection and transmission.
Continuity of the wavefunction and its derivative gives the expressions:

\[ 1 + r = t \]
\[ k_{i\perp} (1 - r) = t k_{t\perp} \]

where the second relation only holds for \( E_{i\perp} > V \);

this leads directly to the classical **Fresnel coefficients** found in optics:

\[ r = \frac{k_{i\perp} - k_{t\perp}}{k_{i\perp} + k_{t\perp}} \quad \& \quad t = \frac{2k_{i\perp}}{k_{i\perp} + k_{t\perp}} \]
Reflectivity is measured as a function of wavevector transfer or \( q \)

Note that what is measured is an intensity and thus is a function of the quantum mechanical probability amplitude squared.

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

Fresnel reflectivity

\[
q = \frac{4\pi}{\lambda} \sin \theta
\]
**Born Approximation**

$q \gg q_c$

Ignored double scattering processes because these are usually very weak

$q_c = \sqrt{16\pi N_b}$

Scattering length density

\[ N'_b(q) = \int_{-\infty}^{+\infty} \exp(iqz) \frac{dN_b}{dz} dz \]

\[ R(q) = \frac{16\pi^2}{q^4} \left| N'_b(q) \right|^2 \]

\[ N_b = \sum_i n_i b_i \]

\[ N_b = \frac{i}{V} \]
\[ R = 1 \text{ below } q_{\text{crit}} \]
\[ \theta_c = \arccos\left(\frac{n_1}{n_2}\right) \]
Roughness and Interdiffusion

Both the rough and diffuse case the specular reflectivity is reduced by a factor very much like the Debye-Waller factor reduces scattered intensity from a crystal.

\[ R \approx \left( \frac{16\pi^2}{q^4} N_b^2 \right) e^{-q^2z\sigma^2} \]

\( \sigma \) is a characteristic length scale of the layer imperfection.
- Model calculation on smooth surface.
- Fringe spacing depends on thickness
- Fringe spacing $\sim 2\pi/d$

Model layer with $\rho = 5 \times 10^{-6} \, \text{Å}^2$ on Si ($2.07 \times 10^{-6} \, \text{Å}^{-2}$)
Blue 30 Å, Pink 100 Å. No roughness.

$$R_q^4 = [(N_{b2} - N_{b0})^2 + (N_{b1} - N_{b2})^2 + 2(N_{b2} - N_{b0})(N_{b1} - N_{b2}) \cos(qd)]$$
Reflectivity from m layers

layer number | Air | refractive index
---|---|---
0 | | \( n_0 \)
1 | \( d_1 \) | \( n_1 \)
2 | \( d_2 \) | \( n_2 \)
m-1 | \( d_{m-1} \) | \( n_{m-1} \)
m | \( d_m \) | \( n_m \)
m+1 | substrate | \( n_{m+1} \)
The reflection coefficient for the sample is calculated by firstly considering the coefficient between the substrate and the bottom layer, $r_{m,m+1}$, i.e. between the $(m+1)^{th}$ and $m^{th}$ layers

$$r_{j,j+1} = \frac{n_j \sin \theta - n_{j+1} \sin \theta_{j+1}}{n_j \sin \theta + n_{j+1} \sin \theta_{j+1}}$$
The reflectivity coefficient between the \((m-1)^{th}\) and \(m^{th}\) is then given by:

\[
r_{m-1,m}' = \frac{r_{m-1,m} - r_{m,m+1} \exp(2i\beta_m)}{r_{m-1,m} + r_{m,m+1} \exp(2i\beta_m)}
\]

A phase factor, \(\beta_m\), has also been introduced and represents an optical path length term for the \(m^{th}\) layer, such that

\[
\beta_m = \frac{2\pi}{\lambda} n_m d_m \sin \theta
\]

where \(n_m\) and \(d_m\) are the refractive index and thickness respectively of layer \(m\)
This approach of calculating reflectivity is exact but extending it to multilayers is cumbersome.

A more general solution widely used is the **OPTICAL MATRIX METHOD** (Abeles).

\[
c_m = \begin{bmatrix} \cos \beta_m & -(i / \kappa_m) \sin \beta_m \\ -i \kappa_m \sin \beta_m & \cos \beta_m \end{bmatrix}
\]

An overall sample matrix is then defined as the product of the individual matrices:

\[
M = \prod_{m=0}^{m} c_m = \begin{bmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{bmatrix}
\]
The reflectivity is simply related to the matrix elements from $M$ by:

$$R = \left| \frac{(M_{11} + M_{12}k_{m+1})k_0 - (M_{21} + M_{22})k_{m+1}}{(M_{11} + M_{12}k_{m+1})k_0 + (M_{21} + M_{22})k_{m+1}} \right|^2$$

where $m+1$ denotes the substrate and 0 the air.
Routine analysis of reflectivity data would ideally be solved by direct inversion of experimental data into either scattering length density, $Nb(z)$, or even volume fraction, $f(z)$, profiles.

Generally, this cannot be achieved due to the loss of phase information, making this closely related to the phaseless Fourier problem.
Fitting data

Data (+errors) → Layers → Calculated reflectivity → Goodness of fit ($\chi^2$) → Best fit → Model

New parameters
Contrast variation

More than one model of $N_b(z)$ may give the same reflectivity profile – phase information is lost

Measurement with multiple ‘contrasts’ normally resolves ambiguity

Physical knowledge of system may define a unique model
Data modelling

Contrast variation
Multiple Contrasts

Courtesy of R. K. Thomas
http://rkt.chem.ox.ac.uk/techniques/nrmain.html
The Goal of Reflectivity Measurements Is to Infer a Density Profile Perpendicular to a Flat Interface

In general the results are not unique, but independent knowledge of the system often makes them very reliable

Frequently, layer models are used to fit the data

Advantages of neutrons include:
– Contrast variation (using H and D, for example)
– Low absorption – probe buried interfaces, solid/liquid interfaces etc
– Non-destructive
– Sensitive to magnetism
– Thickness length scale <5 Å – 5000 Å

• Issues include:
  – Generally no unique solution for the SLD profile (use prior knowledge)
  – Large samples (~10 cm$^2$) with good scattering contrast are needed
Some useful references:

• Russell T. P. 1990 Mat. Sci. Rep. 5 171
• Névot L and Croce P 1990 Rev. de Phys. Appl. 15 761

Web-sites:

http://www.mrl.ucsb.edu/~pynn/ (Roger Pynn)
http://www.pcl.ox.ac.uk/~rkt/ (Bob Thomas)

Basic Principles of Neutron Reflection Measurement
Reflected beam deflected: $2\,\theta$

Reflectivity

$$R(\theta, \lambda) = \frac{I_R}{I_0(\lambda)}$$

Momentum transfer

$$q = \frac{4\pi}{\lambda} \sin \theta$$
Measurement can be done by:

- varying $\theta$ at constant $\lambda$
- measuring the TOF ($\Rightarrow \lambda$) 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.
### Classes of Interface

<table>
<thead>
<tr>
<th>Interface</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air/Liquid</td>
<td>Samples can be limited by smoothness and by flatness</td>
</tr>
<tr>
<td></td>
<td>(capillary waves amplitude is 0.3 nm)</td>
</tr>
<tr>
<td>Air/Solid</td>
<td></td>
</tr>
<tr>
<td>Liquid/Solid</td>
<td>Constrained by passage through one phase. Signal can be limited by</td>
</tr>
<tr>
<td></td>
<td>absorption or scattering background</td>
</tr>
<tr>
<td>Solid/Solid</td>
<td></td>
</tr>
<tr>
<td>Liquid/Liquid</td>
<td></td>
</tr>
</tbody>
</table>

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.
Fate of a Neutron at an Interface

- Reflected
- Scattered/Diffracted from surface
- Absorbed
- Scattered from bulk (either side of surface)
- Other accidents
Practical Issues

- Reflectivity drops quickly with increasing Q (or angle). Signal is easily ‘lost’ in background.

- To observe fringes it will be necessary to measure over an appropriate range of Q and to have sufficient resolution ($\Delta Q$ small enough).

\[
\left(\frac{\Delta Q}{Q}\right)^2 = \left(\frac{\Delta \lambda}{\lambda}\right)^2 + \left(\frac{\Delta \theta}{\theta}\right)^2
\]

- Attenuation by reduced transmission (caused by scattering or absorption) may be significant
Sources of background:

Electronics (negligible)

Scattering from other parts of the instrument (can be efficiently shielded with B4C, Cd, etc.)

Sample:

off-specular from roughness, inhomogeneities (can be measured and removed)

incoherent scattering (liquids)
The **coherence length** is essentially the separation distance on the specimen from which neutrons or x-rays emerging will interfere coherently at the detector.

\[ \Delta k \Delta x = 2\pi \]

\[ \Delta x = 600 \text{ nm} \]

\[ \theta = 1 \]

coherence length \( \sim 30000 \text{ nm} \)

0.1 nm neutrons or x-rays
source divergence 0.005 deg
The coherence length will depend on factors including:
- wavelength of the incident radiation
- angle of incidence
- and beam divergence (instrument dependent)

Usually a slit defines the incident beam with good resolution in one dimension and poor normal to this
Inhomogeneous sample

lateral coherence length of wave $\gg$ dimensions of regions A and B

\[ \left| r \right|_{\text{observed}}^2 = \left| \frac{4\pi}{iq} \int_0^L \Psi(z) \left[ f_A N_{b_A}(z) + f_B N_{b_B}(z) \Psi_0 \right] dz \right|^2 \]
Inhomogeneous sample

lateral coherence length of wave << dimensions of regions A and B

\[ r_{\text{observed}}^2 = f_A r_A^2 + f_B r_B^2 \]
Rafts in membranes: can we see them with reflectometry?

Lateral coherence length of neutron beam ~10\text{th} microns

\[ \text{Domain size} \approx 100\text{th} \ \text{nanometers} \]

Signal will come from the averaged structure on the surface

\textit{Need to use GISANS}
Example of reflectometer TOF mode:

The reflectometer FIGARO at the ILL
Fluid Interfaces Grazing Angles Reflectometer

$\lambda = 2\text{--}30\text{Å}$

$\Delta \lambda / \lambda$ 1.2-10%

Beam strikes both sides of interfaces
Loose resolution allows high flux and measurements of thin films and liquid/liquid interfaces

\[
\frac{\delta t}{t} = \frac{D'}{D} + \frac{\phi}{2\pi q_{\text{min}}} 
\]

<table>
<thead>
<tr>
<th>(\Delta\lambda/\lambda)</th>
<th>Disc Numbers</th>
<th>Disc separation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 %</td>
<td>1 &amp; 4</td>
<td>800</td>
</tr>
<tr>
<td>8.8%</td>
<td>2 &amp; 4</td>
<td>700</td>
</tr>
<tr>
<td>4.2%</td>
<td>1 &amp; 3</td>
<td>350</td>
</tr>
<tr>
<td>3.0%</td>
<td>2 &amp; 3</td>
<td>250</td>
</tr>
<tr>
<td>5.4%</td>
<td>3 &amp; 4</td>
<td>450</td>
</tr>
</tbody>
</table>
Beam deflected upwards and downwards by $M=4$ supermirrors.
SAMPLE ENVIRONMENT

Adsorption troughs for adsorption from solution

Langmuir trough for insoluble monolayers
SAMPLE ENVIRONMENT

Solid/liquid cell for adsorption on surfaces

Humidity chamber

$\text{r.h.} = 100\frac{p(T_w)}{p(T_s)}$
**2-D DETECTOR**: simultaneous access to off-specular/GISANS *(the latter after optimisation of instrument settings)*

\[
q_x = \frac{2\pi}{\lambda} (\cos \theta_f \cos 2\theta_{in} - \cos \theta_i)
\]

\[
q_y = \frac{2\pi}{\lambda} (\cos \theta_f \sin 2\theta_{in})
\]

\[
q_z = \frac{2\pi}{\lambda} (\sin \theta_i + \sin \theta_f)
\]

\[10^{-5} \, \text{Å}^{-1} \leq q_x \leq 10^{-3} \, \text{Å}^{-1} \equiv 1 - 100 \, \mu m\]

\[10^{-3} \, \text{Å}^{-1} \leq q_y \leq 10 \, \text{Å}^{-1} \equiv 1 - 10000 \, \text{Å}\]

\[10^{-3} \, \text{Å}^{-1} \leq q_z \leq 1 \, \text{Å}^{-1} \equiv 10 - 10000 \, \text{Å}\]
Planning a Reflectivity Measurement

• Simulation of reflectivity profiles is essential
  – Can you see the effect you want to see?
  – What is the best substrate? Which materials should be deuterated?

• If your sample involves free liquid surface you will need to use a reflectometer with a vertical scattering plane

• If you want to follow a changes with time (kinetics) better to use a time-of-flight instrument.

• Preparing good (i.e. low surface roughness) samples is key
  – Beware of large islands

• Layer thicknesses between <10 Å and 5000 Å
  – But don’t mix extremes of thickness

For a list of neutron reflectometers and programs to analyze the data:
http://material.fysik.uu.se/Group_members/adrian/reflect.htm#Instruments
by Adrian Rennie (Uppsala University)
Examples:

❖ Ganglioside/cholesterol pair (V. Rondelli, L. Cantù, et al.)


❖ Neutron reflectometry and deuteriation to probe density profiles of proteins adsorbed onto polymer brushes (E. Schneck, A. Schollier, A. Halperin, M. Sferrazza, D-Lab)
Examples:

- Ganglioside/cholesterol pair  
  (V. Rondelli, L. Cantù, et al.)

- Interaction of antibiotic with natural membranes  
  (A. de Ghellinck, H. Wacklin, M. Sferrazza, J. Jouhet, D-Lab)

- Neutron reflectometry and deuteriation to probe density profiles of proteins adsorbed onto polymer brushes  
  (E. Schneck, A. Schollier, A. Halperin, M. Sferrazza, D-Lab)
Towards structural dynamics in complex biomimetic membranes

Valeria Rondelli, Laura Cantù, Elena Motta, Elena DelFavero, Paola Brocca, Sandro Sonnino

Università degli Studi di Milano
Langmuir Blodgett – Langmuir Schaefer technique

Precise control of number of layers
Homogeneous deposition over large areas
Multilayer structures with varying composition layer by layer
Lipids exchange

ANNEALING

$T=22^\circ\text{C}$

% volume exchanged

Rondelli et al., J. Phys. 2014
After annealing cholesterol was found to become **symmetrically** distributed in the hydrophobic region of the floating bilayer.
Effect of ganglioside: co-deposition

<table>
<thead>
<tr>
<th></th>
<th>bilayer thickness(Å)</th>
<th>%chol 1</th>
<th>%chol 2</th>
<th>solvent penetration (%vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 °C</td>
<td>57</td>
<td>74</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>51 °C</td>
<td>56</td>
<td>67</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>22 °C back</td>
<td>60</td>
<td>69</td>
<td>37</td>
<td>27.5</td>
</tr>
</tbody>
</table>

GM1 + 30% of the total amount of cholesterol

70% of the total amount of cholesterol
Effect of ganglioside: micelles in solution

GM1 enters the membrane

Cholesterol goes in the inner leaflet
The presence of GM1 forces asymmetry in cholesterol distribution, opposite to what happens for a GM1-free membrane where a full symmetrisation of cholesterol distribution is observed.

A preferential asymmetric distribution of GM1 and cholesterol is attained revealing that a true coupling between the two molecules occurs.
Effect of enzyme sialidase

SAXS measurements (ID02/ESRF) on gangliosides containing vesicles, after the addition of the enzyme sialidase

Courtesy L. Cantú, in preparation
Interaction with enzyme sialidase, detectable with synchrotron radiation but not with neutrons (biggest effect at q>0.3Å⁻¹)

Analysis of specular and off-specular data is in progress
Examples:

- Ganglioside/cholesterol pair
  (V. Rondelli, L. Cantù, et al.)

- Interaction of antibiotic with natural membranes
  (A. de Ghellinck, H. Wacklin, M. Sferrazza, J. Jouhet, M. Haertlein, …)

- Neutron reflectometry and deuteriation to probe density profiles of proteins adsorbed onto polymer brushes
  (E. Schneck, A. Schollier, A. Halperin, M. Sferrazza)
Production of natural deuterated lipids

Terpenoids
  ▶ Complex lipids
    ◀ Sphingolipids
    ◀ Glycerolipids
      ▶ Glycerophospholipids

Cholesterol
PC, PE, PS, PI, DAG, TAG

Unsaturation crucial for the fluidity of the membrane

Unsaturated deuterated lipids not commercially available

D-lab at the ILL uses yeast cells to produce d-proteins by biosynthesis

Yeast: a tool to produce lipids as well

D₂O d₆-glycerol

Time

d-proteins

d-lipids

d-biomass
Yeast cells are grown in H or D environment

Lipids are extracted according to the Folch method

Separation by 2D-TLC

Separated lipids are analysed by GC
Phospholipid composition non affected by deuterated growth culture
Fatty acid composition affected by deuterated growth culture
Deposition by vesicle fusion:
Optimisation by QCM-D (PSCM labs)
Structure of D-polar lipids

Full coverage
Full deuteriation
Structure similar to synthetic DOPC
Structure of H-polar lipids

- Full coverage
- Thinner than DOPC bilayer
- Rougher outer headgroup - tail interface

Neutron reflectivity from Figaro at the ILL
Structure of D-polar lipids + sterol

- Same thickness as D-polar lipids
- Increased roughness at the tail – headgroup interfaces

![Graphs showing molecular interactions and distributions](image)
Structure of H-polar lipids + sterol
Polyene antifungal drug used intravenously for systemic fungal/parasitic infections (AIDS & cancer patients)

AmB is well known for its severe and potentially lethal side effects

AmB binds with ergosterol, a component of fungal cell membranes, forming a transmembrane channel that leads to monovalent ion leakage, which is the primary effect leading to fungal cell death.

Oligomeric pore formation

Activity depends on aggregation state

Recently evidence was found that pore formation is not necessarily linked to cell death.

The actual mechanism of action may be more complex and multifaceted.
AmB effect on P. Pastoris yeast membranes: neutron reflectometry measurements

- AmB inserts in yeast membranes in the presence of ergosterol
- Amb also forms a dilute 30-40 Å layer on the top of the membrane
- Membrane thinning is more pronounced in H-lipids which are more polyunsaturated
- No water filled pores are observed
Amphotericin forms an extramembranous and fungicidal sterol sponge

- Paramagnetic Resonance enhancement of $^{13}$C-AmB by selectively spin-labelled lipids
- TEM + ultracentrifugation (cells)
A closer inspection of NR data ...

- sld of lipid chains (-0.14 10-6 Å-2) higher than for model bilayers due to PUFAs
- AmB exchanges protons with solvent
- Data allows for AmB insertion into bilayer, ergosterol extraction and incorporation into AmB layer and decoupling of lipid head groups from each other -> better global fit
A closer inspection of NR data ...

- sld of lipid chains (-0.14 10-6 Å-2) higher than for model bilayers due to PUFAs
- AmB exchanges protons with solvent

- only small amount of AmB inserts in the lipid bilayer (4v/v%)
- bilayer gets 4Å thinner
- 36Å thick Amb layer (83v/v% water) on top of bilayer much larger than AmB molecule

- h-yeast contains 14.5mol% ergosterol
- AmB insertion the same as above
- ergosterol all extracted into AmB layer above, which is 39Å thick (77v/v% water).
- Lipid bilayer gets 6Å thinner
A closer inspection of NR data ...

- sld of lipid chains (6.61 x 10^{-6} \text{ Å}^{-2}) slightly smaller than estimated from lipid composition.
- d-yeast contains mainly mono-unsaturated C18:1 lipids, and is thicker than h-yeast.
  - less AmB inserts in the bilayer (1.5v/v%)
  - bilayer does not get much thinner
  - 47Å thick Amb layer (91v/v% water)
  - d-yeast contains less ergosterol (6mol%)
  - 12 v%v AmB insertion
  - ergosterol all extracted into AmB layer above, which is 38Å thick (94% water).
  - Lipid bilayer gets 3Å thinner
Conclusions

- Data more **consistent with new model for AmB** mechanism than pore model

- **Future scope** for investigating ergosterol extraction and dependence on membrane composition, the kinetics, effect of AmB formulation etc.

- Experiments possible due to **set up for extracting, separating and purifying lipid components (PSCM)** from deuterated cells grown at ILL D-Lab

- **Analysis of lipid composition** (in collaboration with CEA Grenoble, UMIL Dept. Translational Medicine) enabled effect of polyunsaturation to be observed
Examples:

- Ganglioside/cholesterol pair (V. Rondelli, L. Cantù, et al.)

- Interaction of antibiotic with natural membranes (A. de Ghellinck, H. Wacklin, M. Sferrazza, J. Jouhet, M. Haertlein, …)

- Neutron reflectometry and deuteriation to probe density profiles of proteins adsorbed onto polymer brushes (E. Schneck, A. Schollier, A. Halperin, M. Sferrazza)
Density Profiles of Proteins in Polymer brushes

biocompatible surface functionalization

“brush failure” via protein adsorption

modes of protein adsorption:
primary, secondary, ternary

structural characterization for “rational design” of protein resistant functionalization (role of grafting density and polymer length)
Sample Preparation

**Preparation steps**

- Planar silicon substrates
- Hydrophobic functionalization
- Brushes at air/water interface (Langmuir trough) of PS-PEG diblock copolymers or PE-PEG lipid anchored polymers

**Resulting brush**

- defined grafting density, $\sigma$
- defined polymer length, $N$
- hydrophilic/hydrophobic grafting surface
for each parameter set:

- compute SLD profiles corresponding to all measurement conditions;
- discretized into 1Å slices;
- compute corresponding reflectivity curves (dynamical treatment: Fresnel reflection coefficients, Parrat formalism)
- parameters are varied to achieve best agreement between measured and modelled reflectivity curves
Data Analysis

Layers below grafting surface

- slabs with adjustable thickness, dry SLD, water content, interface roughness

PEG brush

- parabola (SCF theory) with adjustable brush length and density

After protein adsorption

- protein distribution that allows for primary, secondary, ternary adsorption (rough slabs+Gaussians)
- SLD of PEG and protein fixed
- dependence of protein SLD on water contrast (H/D exchange) taken into account

Schnick, Schollier et al., Langmuir 2013
Bare Brushes
Results consistent with SCF theory

- PEG 114<N<770 up to $\sigma \sim 2 \times 10^{17} \text{m}^{-1}$ (5nm$^2$ per chain)
- **parabolic brush model** gives density, $\Phi_0$ and length, $H_0$
Adsorption of deuterated myoglobin to PEG brushes grafted on hydrophobic polystyrene surfaces

- Significant adsorption for all brush parameters
- Only primary adsorption
Adsorption of deuterated myoglobin to PEG brushes grafted on hydrophobic polystyrene surfaces

- inner-layer: protein amount decreases with grafting density
- anchoring points obstacles adsorption
- outer protein layer depends on overall PEG amount and protein-protein interactions are altered by the presence of PEG
- Information only accessible with neutron reflection combined with protein perdeuteration
Specific adsorption: PEG antibodies

- Classically PEG purely repellent, in fact it is antigenic
- PEG antibodies produces in animals (0.1% - 25% in humans)
- Implications on brush functioning - failure?
- IgG AB bind specifically to end segments of PEG

Brushes grafted to hydrophilic phospholipid surface to prevent primary adsorption
Specific adsorption: PEG antibodies

Neutron reflectometry measurements

Brushes grafted to hydrophilic phospholipid surface to prevent primary adsorption
Specific adsorption: PEG antibodies

- Antibodies adsorbs at brush periphery
- No primary adsorption
- Amount increases with grafting density
- Saturation - molecular crowding

Antibodies become the dominant surface:
Brush no more functional
foreign-body reaction

(f_{PEG} \propto \sigma)
Many open questions regarding protein adsorption to polymer brushes

Neutron reflectometry (coupled to protein deuteriation) promising approach

Detailed structural insight

Unique tool to investigate the structure of biological interfaces and interfaces relevant for biotechnological applications
Conclusions

- Neutron reflectometry remains an essential tool for the study of structure at the nanometer level of soft self-assembled systems at interfaces.

- Complementary to x-ray and synchrotron radiation, advantages include high penetration, sensitivity to light elements (H, C, O, N, …) and isotopic labelling/contrast variation.

- Possibility to work in real (physiological) conditions

- Possibility for in-situ studies of systems under deformation.

- Need optimised sample preparation

- Perspectives in biology are very numerous.