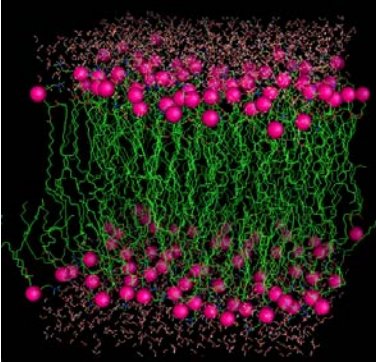


## Working with Lipid Surfaces using Dual Polarisation Interferometry (DPI)

### Pushing back the boundaries of biophysics



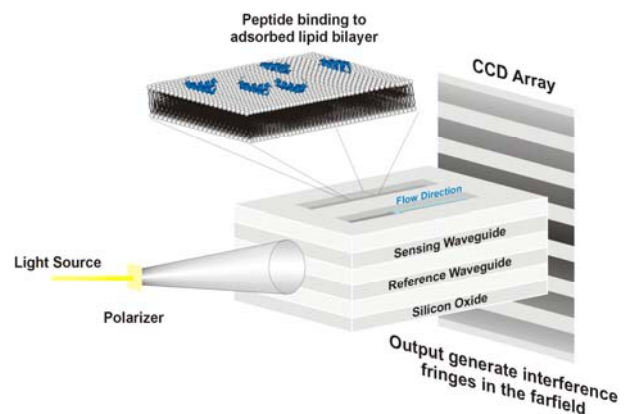
The advent of physicochemical and biotechnological methods that enable the study of supramolecular assemblies at the submolecular level has resulted in a particular focus on the cell membrane. In particular, techniques now exist which allow the study of the organisation and characterisation of lipid bilayers as biomimetic membranes. Increasingly, a diverse range of structural biophysical techniques, such as x-ray and neutron scattering techniques, are being used to study the nature of lipid bilayers. However, many of these techniques provide information regarding the steady-state situation of the lipid bilayer, rather than dynamic information about its formation.

### Dual Polarisation Interferometry – dynamic biophysical measurements

Dual Polarisation Interferometry (DPI) provides a dynamic biophysical measurement to follow the formation of a lipid bilayer on a surface. This provides a means of defining the lipid bilayer as well as allowing the study of subsequent interactions of peptides and proteins with that bilayer. In this way, DPI reveals a whole new area of lipid-mediated biomolecular interactions.

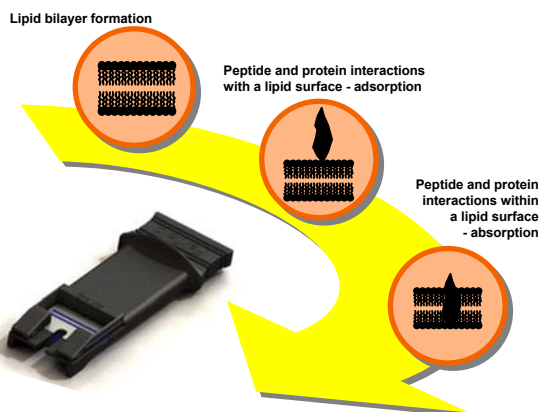
DPI is a technique which provides the capability to derive dynamic information on the thickness, density, mass and hence conformational changes of the surface adsorbed layer in real time.

DPI analysis of proteins utilises the assumption that they form a uniform isotropic layer on the **AnaChip™** surface. In analysing lipid layers however this assumption is not valid as the layer is birefringent. This birefringence can be directly measured with DPI and used to quantify the degree of order or alignment within the layer.



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### The key phases of lipid bilayer analysis



Studies with lipid surfaces follow a number of key steps:

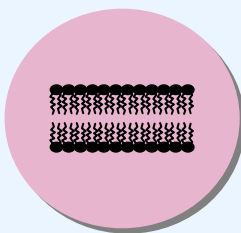
- the formation and characterisation of the lipid bilayer
- the use of the lipid bilayer as a surface with which to study the behaviour of peptides and proteins in a lipid environment
- the study of lipid-environment mediated biomolecular interactions.

Key to all of these is the ability to form a highly functional lipid bilayer and the capacity to be able to characterise that layer. DPI is able to provide a highly detailed visualisation of lipid deposition.

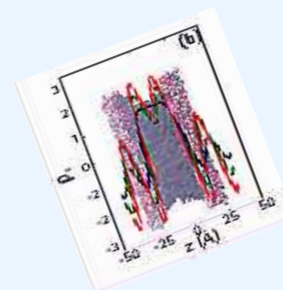
## The Biology of Lipid Surfaces

Lipid biology plays a significant role in maintaining cellular integrity, communication and recognition, as well as acting as both a barrier and translocator mechanism. While it is an ordered lamellar structure, it retains fluidity within its structure. This enables a vast array of complex interaction mechanisms to occur at both of its surfaces, as well as facilitating various transmembrane mediated mechanisms. Consequently, the analysis of the lipid bilayer requires a number of different strategies. Some of the key targets of lipid biology are summarised below.

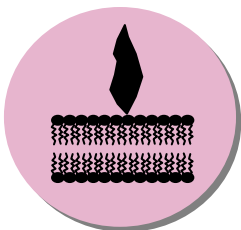
### Lipid bilayer formation



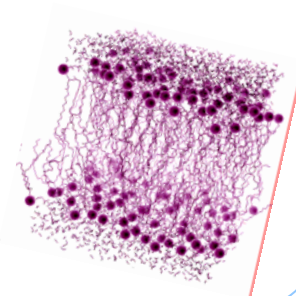
- Characterisation of lipid bilayer assembly and behaviour
- Environmental and compositional effects on order
- Corroboration of other measurement technologies, e.g. X-rays and neutrons



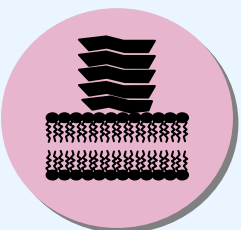
### Peptide and protein interactions on a lipid surface – adsorption



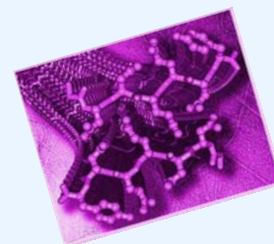
- Extracellular matrices
- Cell signalling
- Carbohydrate interactions



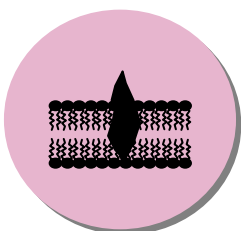
### Protein interaction and aggregation mediated by a lipid environment



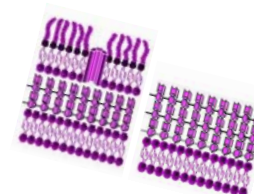
- Diseases such as  $\beta$ -amyloid (Alzheimer's)
- Prion interactions and aggregation (nvCJD)
- Alpha-synuclein (Parkinson's)
- Actin and other benign assembly processes



### Peptide and protein interactions within a lipid surface - absorption



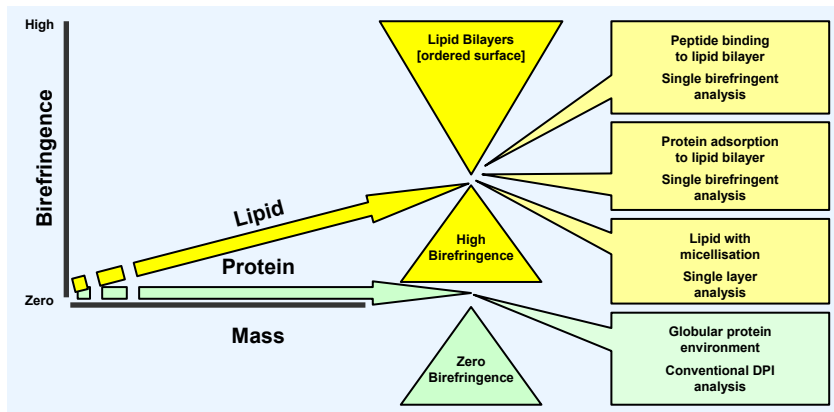
- Membrane penetration by toxins
- Antimicrobial action at the cell wall
- Viral protein characterisation
- Receptors



## Stages in Data Analysis of Lipid Surfaces

### I: SURFACE CHARACTERISATION

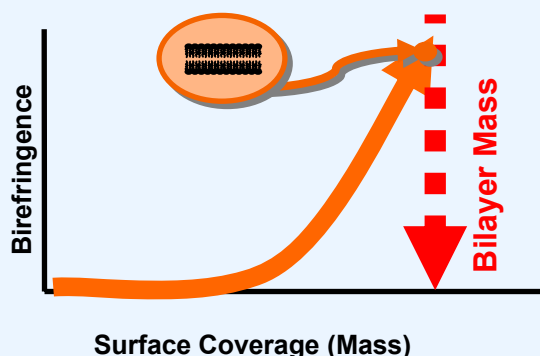
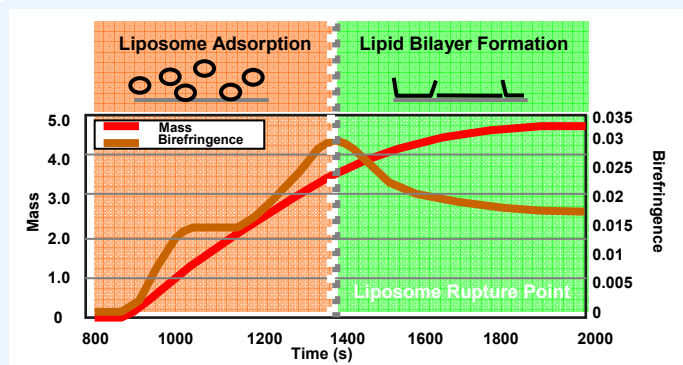
All DPI data analysis is derived from taking two real-time measurements of the optical properties of the surface adsorbed molecules. This then provides output on a number of different parameters, i.e. mass, molecular size, density, number of molecules, molecular footprint. With the latest **Resolver 4<sup>D</sup>** software, birefringence or order within the layer can also be measured. Additionally, rates of association and dissociation together with affinities can be derived.



In non-ordered surfaces where birefringence is zero, accurate thickness and density measurements can be made via conventional **Resolver<sup>TM</sup>** analysis. As the level of birefringence within the surface increases, analysis needs to account for the degree of birefringence. This is facilitated by the new **Resolver 4<sup>D</sup>** software.

When a specific surface loading of a lipid system is achieved, it is possible to use the characteristic birefringence value for the resultant lipid bilayer system as a constant within the **Resolver 4<sup>D</sup>** software. Various controls can be used to test the nature of the surface. With a well characterised surface layer, it is then possible to use the lipid bilayer environment to study protein interactions.

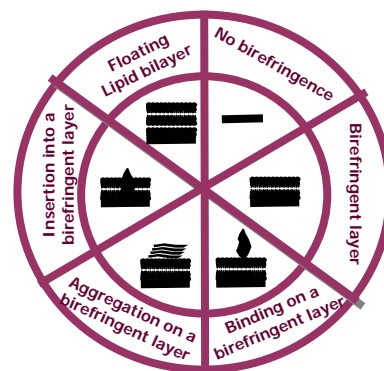
### Formation of Lipid Bilayer



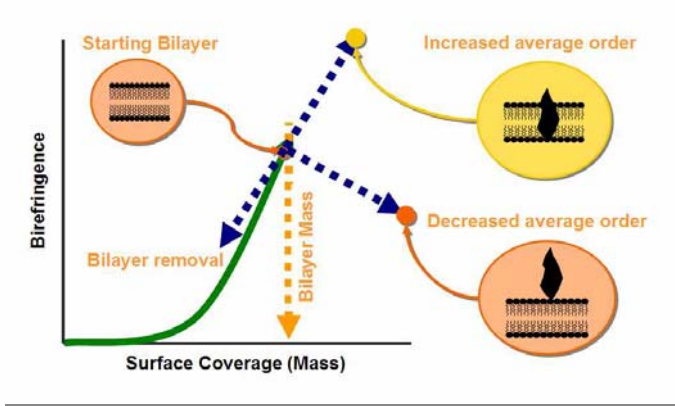
### II: SURFACE UTILISATION

Once the birefringence has been established for a particular lipid bilayer system, it is then possible to investigate the nature of protein or peptide binding to that surface.

With the birefringence value set for the lipid bilayer, the manner in which the protein or lipid 'adsorbs' **on to** the surface and any subsequent 'absorption' **into** the lipid bilayer can be monitored and determined.



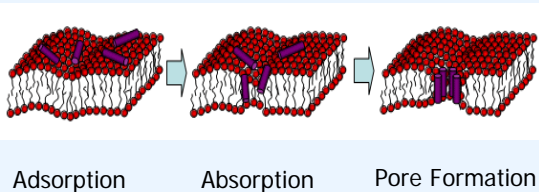
III: STRUCTURAL RELATIONSHIPS OF PROTEIN - LIPID-LAYER INTERACTIONS



In protein-lipid interactions that do not involve any removal of the lipid from the surface by micellisation, it is possible to conduct sequential protein injections over the lipid surface. In instances where the protein initiates lipid micellisation and removal of lipid from the bilayer, it will be necessary to account for this. There may possibly be a need to 'reload' the surface with more lipid prior to any further study.

Once a good lipid surface has been established, it is possible to look at different relationships between the various parameters of mass, thickness and density or any reordering within the bilayer itself. In this way, it is possible to establish data plots that can identify specific types of bio-molecular interaction. This can reveal the layering on, or the absorption of the protein into, the lipid layer and hence any changes in the level of order that occurs within the lipid bilayer itself.

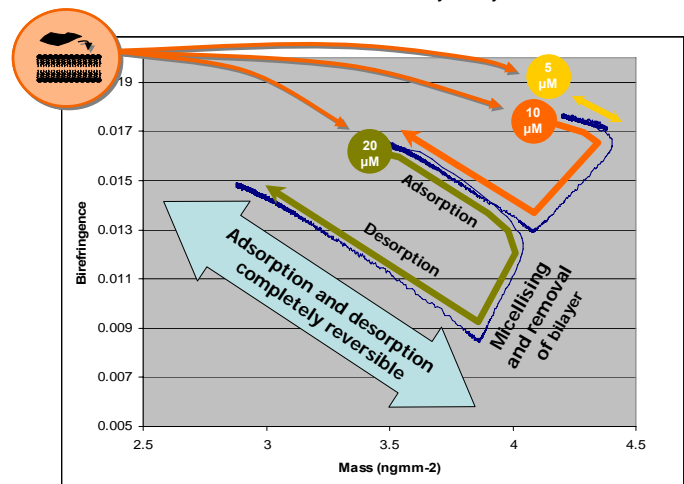
IV: ANTIMICROBIALS - THE JOURNEY OF A PEPTIDE THROUGH A LIPID ENVIRONMENT



AnaLight® 4D is an excellent tool with which to measure the degree of birefringence in complex systems such as lipid-peptide interactions. It is possible to dissect the various phases and transitions of the interaction of any antimicrobial candidate with the lipid layer as illustrated below.

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The example shown here (right) is an antimicrobial candidate binding to a DMPC/PG bilayer. Adsorption and desorption both to and from the layer are completely reversible events. With each injection there is loss of the underlying lipid surface, as observed by the decrease in the level of birefringence & mass. As higher concentrations are introduced, a greater mass is required to reach the critical concentration for micellisation. This mechanistic behaviour of the peptide correlates well with the model system and from the distinct structural phases correct association and dissociation constants can be measured.



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