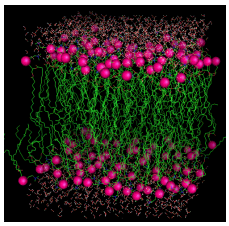
Measuring the Dynamics of Lipid Layer Assembly and Structure using Dual Polarisation Interferometry (DPI)

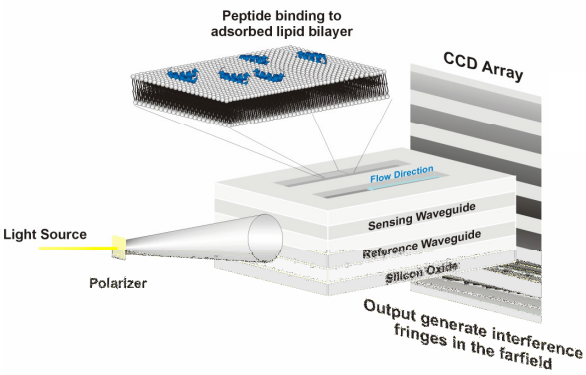
Lipid biology, both in the study of lipid chemistry and in the biophysical characterisation of the lipid bilayer, is currently a major research focus. It is a key requirement in determining the quality of the lipid layers being studied. Consequently, there is need for analytical techniques that can be used to study protein interactions occurring in a lipid environment.



The high level of sensitivity and precision behind the optical resolution achievable with Dual Polarisation Interferometry (DPI) makes it possible to dynamically measure the assembly and organisation of lipid layers forming on an ***Ana*Chip™** surface. It is therefore possible to investigate how proteins interact with such surfaces.

How DPI works

DPI is an interferometric technique which utilises two polarisation signals to derive dynamic information on the thickness, density, mass and hence conformational changes of the surface layer. DPI analysis of proteins uses the assumption that they form a uniform layer on the ***Ana*Chip™** surface. In analysing lipid layers this assumption is not valid because the layer is birefringent. Birefringence can now be directly measured with DPI using the new ***Resolver 4D*** software, and used to quantify the degree of order within the layer.



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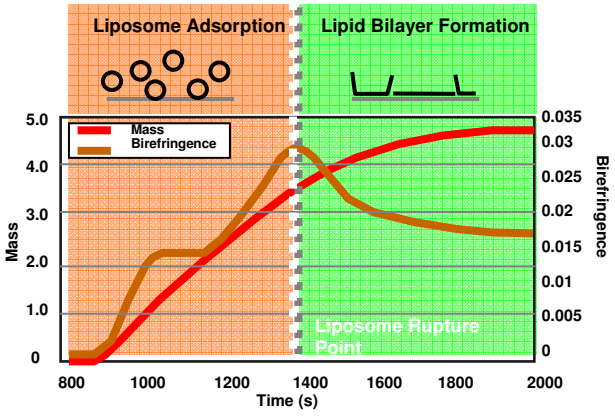
Measuring a bilayer



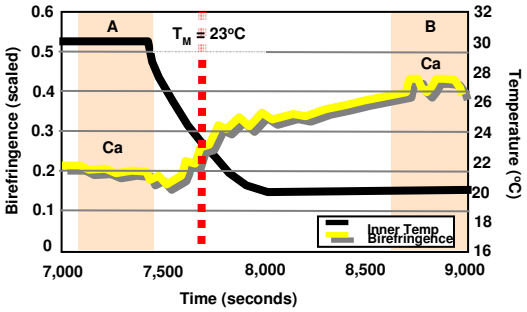
DPI can be used to study the dynamics of lipid layer deposition by using birefringence to probe how lipid layers are formed and to measure the degree of alignment of the polar lipid molecules as they form on the surface.

The ability to biophysically characterise the lipid layer is essential for any studies involving the behaviour of lipid layers. In addition, being able to determine the quality of the lipid surface is important for any subsequent analysis of proteins with the lipid layer.

The quantitative nature of DPI allows the calculation of the degree of coverage of the surface by the lipid and also the level of organisation within that lipid surface.

We gratefully acknowledge that this work was carried out by researchers at ETH, Zurich using Farfield's ***Ana*Light®** technology.

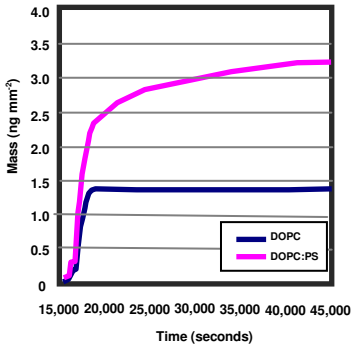
Analysis of the lipid layer



A DMPC bilayer is formed on the ***Ana*Chip™** surface. As the temperature is decreased the birefringence of the DMPC increases. Note that there is no effect on birefringence upon the addition of calcium ions above the T_M [A], but there is an increase when calcium ions are added below the T_M [B].

DPI enables the biophysical study of lipid layers in a dynamic way, with the ability to vary key parameters of lipid biochemistry, such as temperature, pH or ionic strength

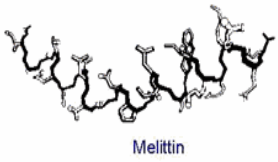
Association of prion (PrP) protein with lipid surfaces

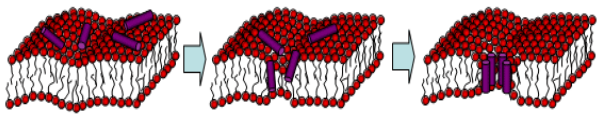


Prion proteins are membrane associated and are known to aggregate forming plaques which are associated with the pathology of nvCJD and other TSE's. Here PrP-α was injected over two lipid surfaces with different properties, namely DOPC and DOPC:PS, using a range of concentrations (50nM-2.5μM) after which the systems were left to incubate. Within seconds the mass association to the DOPC:PS increases, indicative of aggregation.

We gratefully acknowledge that this work was carried out by researchers at The University of Warwick using Farfield's ***Ana*Light®** technology.

Melittin – The journey of a peptide through a lipid environment





Melittin is a peptide found in European bee venom. It is a single transmembrane-spanning alpha-helix. It is an excellent model to illustrate the power of DPI in being able to study such complex processes. By accounting for the degree of birefringence of the lipid bilayer, it is possible to dissect various phases or transitions during the interaction of melittin with the lipid layer.

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