



Measuring protein interactions with lipid bilayers containing PIP2

INTRODUCTION

Dual Polarisation Interferometry (DPI) is an important enabling tool for cell biology, particularly the study of membranes and membrane proteins. In particular the behaviour and interactions of proteins and peptides with lipid bilayers can be studied. This application note describes the use of DPI for the real time, quantitative analysis of protein-lipid interactions. The molecular system under investigation incorporates phospholipids commonly found in cell membranes and known to be involved in the regulation of a range of membrane proteins, PtdIns(4,5)P₂ (D-myo-phosphatidylinositol 4,5-bisphosphate known as PIP2). See Fig 1.

The **AnaLight® 4D**, DPI has the capability not only to measure the dry mass of a lipid bilayer but also its' degree of birefringence (a direct measure of the order of packing of the bilayer). The birefringence is simply the difference in Refractive Index (RI) perpendicular and parallel to the bilayer. This provides a very rich perspective as compared to techniques which only measure the mass of interaction as changes within the bilayer structure can be measured at the same time as the interaction.

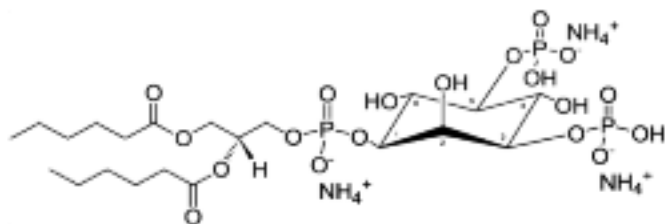


Figure 1

EXPERIMENTAL

Two bilayers composed of 5% PIP2: 95% POPC and 10% POPS: 90% POPC were laid down from extruded liposomes on a UV Ozone cleaned surface of an **AnaChip™** sensor. One bilayer was laid down on each separate channel of the sensor surface to create simultaneous experiment and control. The bilayers were then each challenged with a protein of interest (at 1uM concentration).

RESULTS AND DISCUSSION

Lipid bilayers were successfully deposited on the surface of the **AnaChip™** at a liposome loading of 0.1 mg/ml. The mass and birefringence values are given in the table below. The lipid bilayers were then challenged with 1uM of protein each.

5%PIP2: 95%POPC	Birefringence (RI units)	Mass
0.1mg/ml	0.019	4.23 ng/mm ²

The real time data in Figure 2 shows that mass information only provides limited data indicating that the protein is interacting with the PIP2 containing bilayer and to a much lesser extent with the bilayer containing POPS (i.e. the affinity for PIP2 is greater). The real time birefringence response shown in Figure 3 shows the birefringence decreasing with interaction in the case of the PIP2 containing bilayer with only a slight initial increase with POPS, i.e. the protein is disrupting the order in the bilayer containing PIP2 by an insertion process and is simply sitting on top of the bilayer in the case of the control.

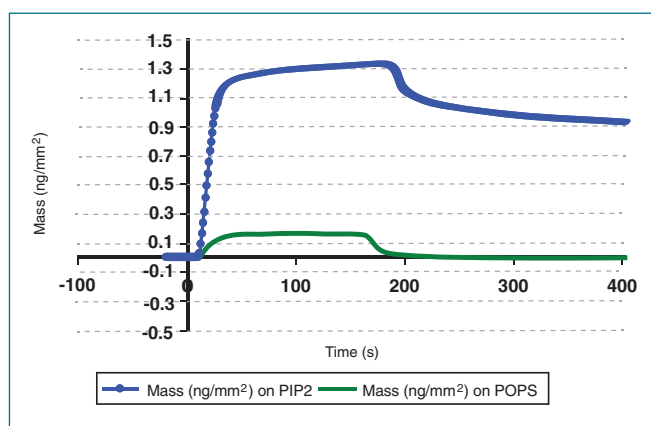


Figure 2: Mass versus time plot



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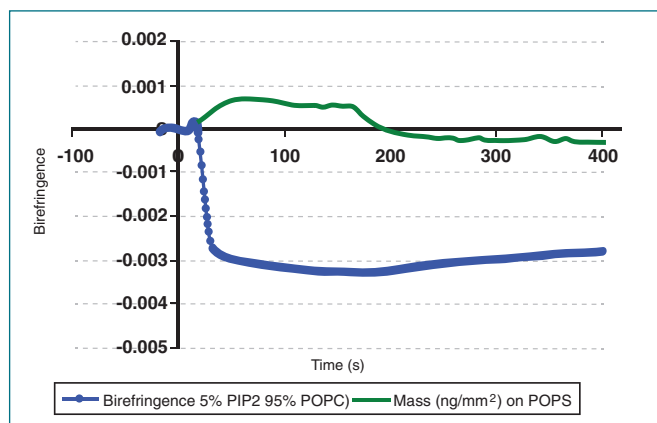


Figure 3: Birefringence versus time plot

More comprehensive information still is obtained by plotting mass versus birefringence for both bilayers and this is shown in Figure 4. Here it can be seen that the interaction with the PIP2 bilayer is a complex four stage process. Initially there is adsorption of protein on the bilayer surface to a mass value of 0.5 ng/mm² (no change in birefringence as mass increases). At this point the protein starts to insert into the bilayer with an increase in mass and a significant decrease in birefringence. Following a wash (return to buffer) there is protein removal from the bilayer surface (no change in birefringence but a loss in mass). Finally there follows an increase in birefringence (and decrease in mass) indicating that material is being removed from the bilayer and the bilayer order is returning. At the end of the experiment, a significant proportion of the protein remains associated with the bilayer and is clearly still inserted as the birefringence does not fully recover.

In contrast, in the POPS containing bilayer, we see a slight but reversible increase in birefringence and mass as protein simply adsorbs to the surface of the bilayer and then desorbs when washing.

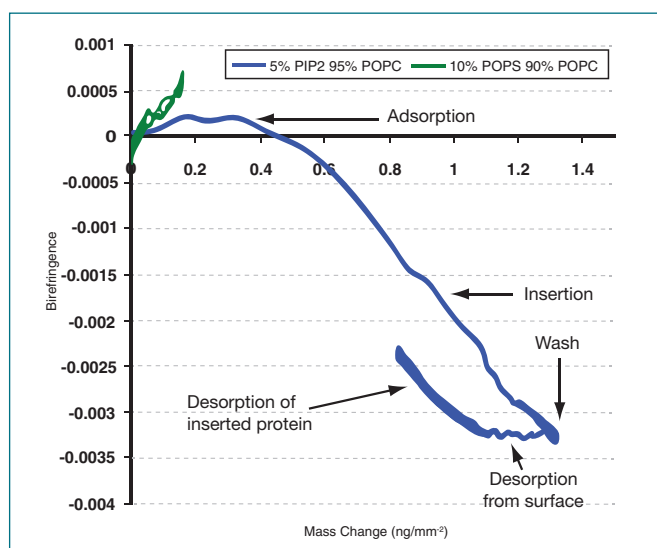


Figure 4: Birefringence versus Mass plot for 1µM protein interacting with the two lipid bilayers

CONCLUSIONS AND BENEFITS

These experiments demonstrate how a large amount of information can be obtained from a simple single real time experiment using the **AnaLight® 4D** Dual Polarisation Interferometer. The **AnaLight® 4D** instrument provides the researcher with a key tool with which to both quantify and interpret interactions of proteins and peptides with lipid bilayers.

- Rapidly and reproducibly lay down lipid bilayers and interrogate their quality.
- Utilise the measurement of birefringence to quantify the degree of order in the bilayers.
- Interrogate lipid bilayers challenged with proteins or peptides.
- Gain detailed insight into the mechanisms of interactions of proteins and peptides with lipid bilayers by utilising the birefringence versus mass plot.
- Differentiate and quantify absorption and adsorption processes in a lipid environment.

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