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High Resolution Impedance Spectroscopy Studies of Supported DMPC and DMPC/PE Bilayers

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Introduction: Cellular membranes are complex mixtures of lipids and proteins, and physical interactions between these components are thought to affect a range of cellular functions by altering the activity of membrane-associated proteins and regulating various signalling pathways[1]. The complexity of cell membranes and their fluid nature makes them difficult to study directly[2,3]. For this reason, work on cellular membranes is often performed in more simplistic model systems, such as free bilayers, substrate-supported bilayers or tethered bilayers. Electrochemical impedance spectroscopy (EIS) is a non-invasive, non-contact method to provide structural information about such fluid materials[4]. This technique has been used to characterise bulk electrical and structural properties of membranes formed from a variety of lipid compositions[4-8]. The superior resolution provided by new ultra-high resolution impedance instruments has the potential to characterise the sub-molecular structural detail of these model membranes, and to further advance the understanding of lipid-lipid interactions.

Methods: This study presents structural characterisations of free dimyristoylphosphocholine (DMPC) and dimyristoylphosphocholine/dimyrsitoylphosphoethanolamine (DMPC/PE) model membranes using INPHAZE high resolution EIS and atomic force microscopy (AFM). Supported DMPC and DMPC/PE lipid bilayers were formed on highly doped silicon wafers by vesicle fusion (see Figure 1). EIS measurements of the supported bilayers were performed over the range 0.1 Hz to 1 MHz. The INPHAZE Analyser Software, based on the least-squares-error method, was used to find the equivalent electrical circuit for each sample, and to estimate the thickness of the layers.

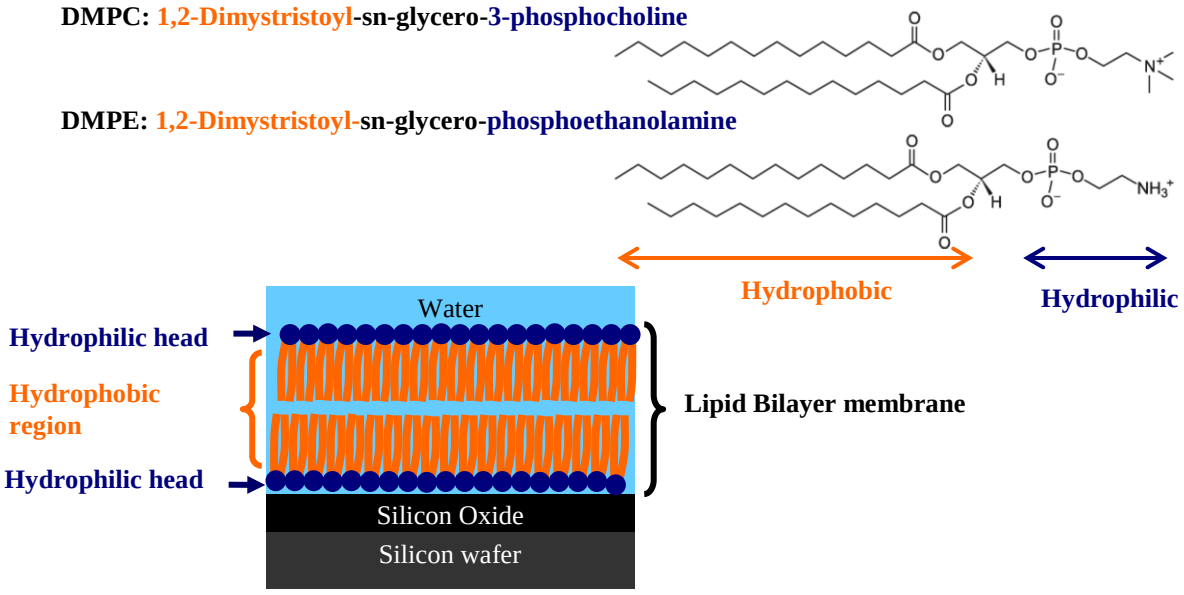

Figure 1: Formation of lipid bilayer membranes

Results: Impedance spectroscopy measurements revealed that the presence of DMPE in the bilayer reduced the average head group thickness and increased the conductance of the DMPC bilayer (see

Figure 2). This suggests that the presence of the DMPE disrupts the packing of the bilayer and influences the interaction between the lipid bilayer and the surrounding aqueous environment[9].

Domains detected in the DMPC/PE bilayer using atomic force microscopy supported these observations and indicate inhomogeneous lateral association of the two lipids. These results suggest that the addition of phosphoethanolamine to phosphocholine bilayers alters the lateral forces and lipid packing of the DMPC bilayer, resulting in changes the bilayer thickness.

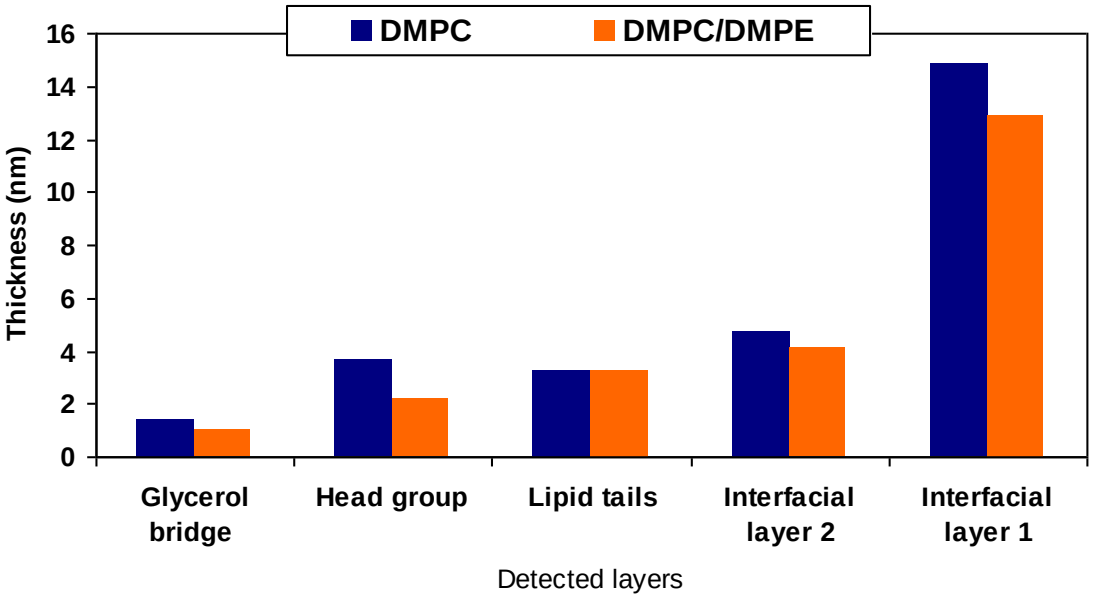

Figure 2: Thickness of the regions estimated by analysing high resolution impedance spectroscopy results

Conclusions: This study demonstrates the feasibility of using high resolution impedance spectroscopy to provide structural information about different regions of free-standing and supported lipid bilayers. Further validation of these results using complementary techniques will lead to an improved understanding of lipid-lipid interactions and advance our knowledge of the complex mechanisms by which cellular membranes regulate cellular function.

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