INPHAZE High Resolution Characterisation

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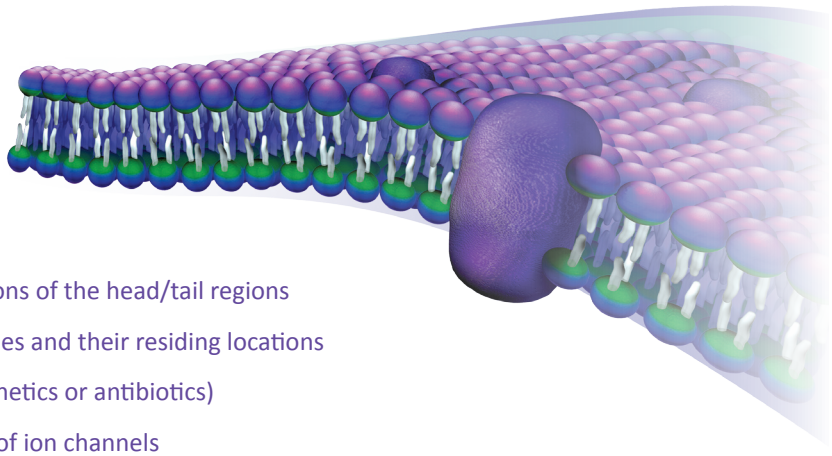
INPHAZE offers a powerful high-resolution system for characterising biological materials and processes at the nano scale. A wide range of precision chambers are available to suit specific applications. Along with user friendly software and dedicated support, talk to us today about using the INPHAZE system for your research.

Lipid Bilayers Applications

Benefits of high resolution characterisation

Synthetic lipid bilayers are important for studying and modeling biological membranes and emerging applications such as drug delivery, biosensors and biotechnology. The INPHAZE high resolution system has shown promising results in determining the substructural dimensions of pure and mixed lipid bilayers, and also for characterising the interactions and effects of macromolecule insertions and ion channels.



The INPHAZE system is ideal for:

- Determining the substructural dimensions of the head/tail regions
- Studying the insertion of macromolecules and their residing locations (such as cholesterol, hormones, anaesthetics or antibiotics)
- Studying the formation and properties of ion channels
- Optimising phospholipid-based biosensors
- Working with delicate lipid structures (very small measurement signals)

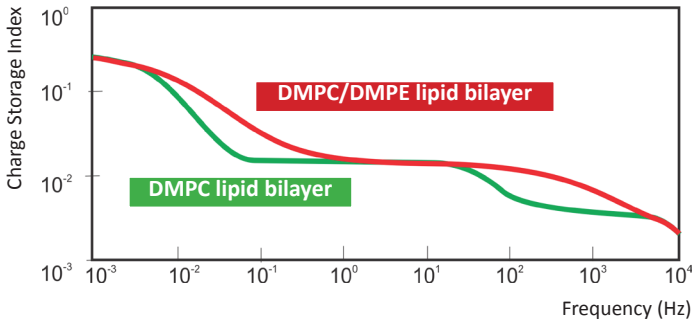
Pure & mixed lipid bilayers

Biological membranes are mixtures of many different lipid molecules, varying in their hydrophobic tail regions and hydrophilic head groups. In this study mixtures of two types of lipids (DMPC and DMPE) were investigated.

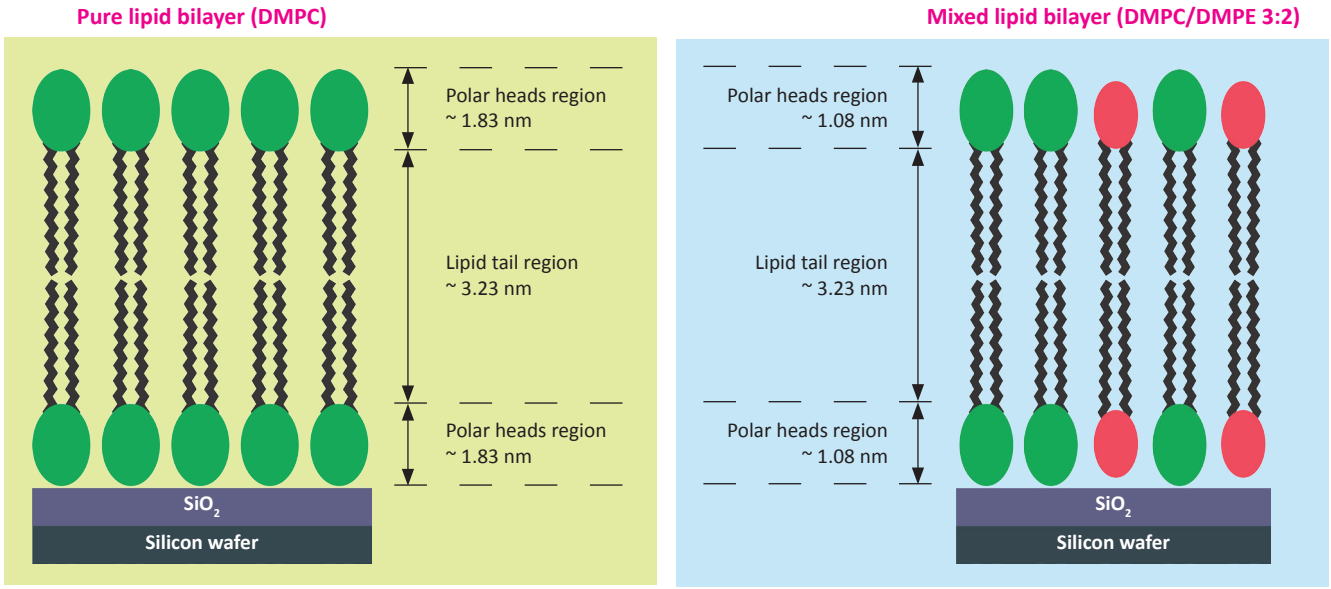
DMPC and DMPE are quite similar in chemical structure, differing only in regard to their hydrophilic head regions. The measurement and analysis using the INPHAZE system was able to detect the slight difference in the head regions.

The tail regions of DMPC and DMPE are known to be chemically identical. The INPHAZE system was able to verify that the hydrophobic regions of the pure and mixed lipid bilayers had the same thickness (~3.23 nm).

The head group of DMPE is slightly smaller than that of the DMPC and this was also verified in the results obtained using the INPHAZE system. The average thickness of the head region of the DMPC/DMPE mixture was smaller than that of the pure DMPC.



Characteristic spectra for pure DMPC and DMPC/DMPE mixtures



Dimensions of various regions in bilayers of pure DMPC and DMPC/DMPE mixtures as determined by the INPHAZE system

Reference:

- Christian Hendrich (2010) "Effects of compositional changes in lipid bilayers measured by broad range Electrical Impedance Spectroscopy", **MSc Thesis**, ANSTO and University of Erlangen-Nürenberg

Insertion of macromolecules

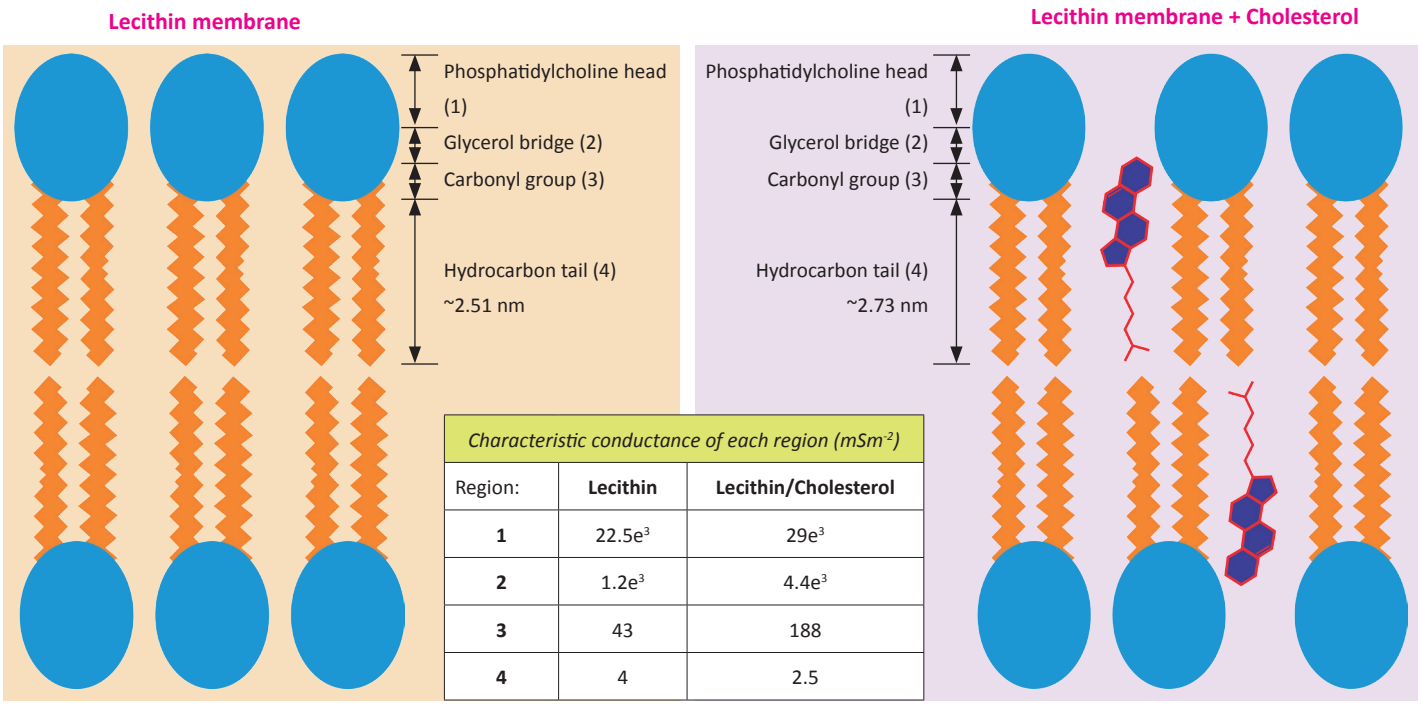
Lecithin is one of the phospholipids which make up mammalian cell membranes. Cholesterol is another component of cell membranes and amongst other things, contributes to the mechanical stability of the membranes. Like phospholipids, cholesterol also contains a hydrophilic and a hydrophobic part.

The INPHAZE system was used to characterise the electrical and geometrical properties of lipid membranes made of lecithin and of lecithin/cholesterol. In this analysis four regions could be distinguished in the lecithin membrane: (1) phosphatidylcholine heads, (2) glycerol bridges, (3) carbonyl groups and (4) hydrocarbon chain tails.

After cholesterol was inserted into the lecithin membrane, the characterisation results showed the properties of the phosphatidylcholine head region did not change noticeably. The conductance of the glycerol bridge and of the carbonyl group increased, while the conductance of the hydrocarbon tail decreased. This indicates that the hydrophobic part of cholesterol resides in the hydrocarbon tails of the bilayer.

Furthermore, with the insertion of cholesterol the most significant increase in conductance occurred for the carbonyl layer. This suggests that the hydroxyl groups of cholesterol align with the carbonyl region of the lecithin molecules.

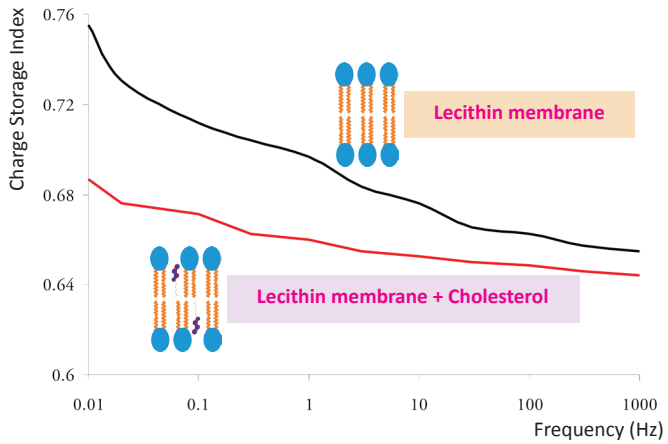
The dimensions of each region in the lipid membranes were also determined using the INPHAZE system. The thickness of the tail groups was ~2.51 nm in the lecithin membrane, and increased to ~2.73 nm after cholesterol insertion, which confirmed that cholesterol causes a swelling of the hydrophobic region of the lipid bilayers.



Characteristic conductance of each region (mSm^{-2})

Region:	Lecithin	Lecithin/Cholesterol
1	$22.5e^3$	$29e^3$
2	$1.2e^3$	$4.4e^3$
3	43	188
4	4	2.5

Dimensions and characteristic conductances of each substructural region in Lecithin and Lecithin/Cholesterol bilayers as determined by the INPHAZE system



Characteristic spectra for pure Lecithin and Lecithin/Cholesterol bilayers

References:

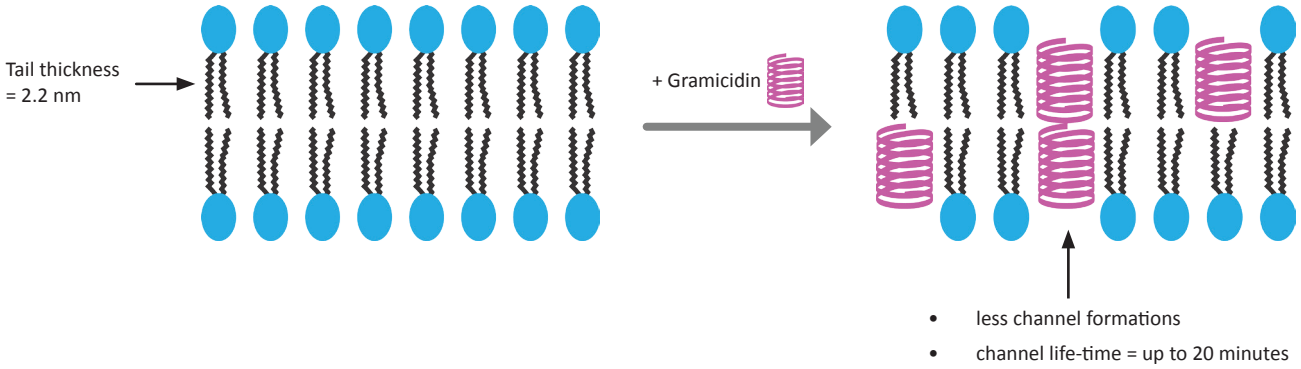
- Christopher Karolis, Hans Coster, Terry Chilcott and Kevin Barrow (1998) "Differential effects of cholesterol and oxidised-cholesterol in egg lecithin bilayers", **Biochimica et Biophysica Acta**, 1368: 247-255
- Hans Coster, Terry Chilcott and Adele Coster (1996) "Impedance spectroscopy of interfaces, membranes and ultrastructures", **Bioelectrochemistry and Bioenergetics**, 40: 79-98.

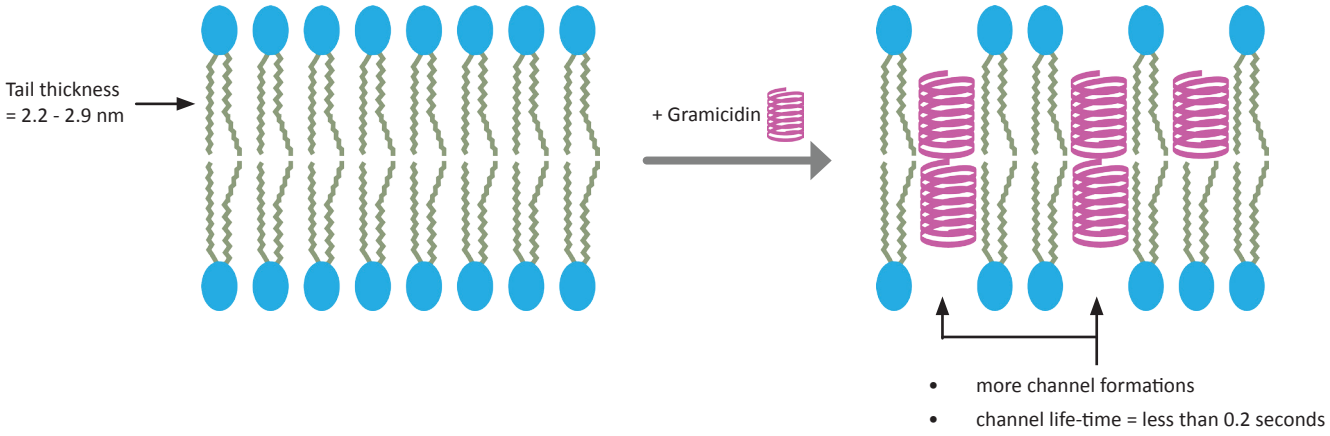
Formation of ion channels

Using compounds such as gramicidin is a good method for developing biosensors based on lipid bilayers. A gramicidin molecule can assemble inside the hydrophobic tail region of a lipid membrane and form a beta-helix. This gramicidin helix is not long enough to form an open channel through the lipid membrane. However, when two gramicidin helixes align and form a dimer, this can act as an open channel. The frequency and life-time of gramicidin channels are key factors in the development of lipid membrane sensors.

The relationship between the activity of gramicidin channels and the structure of lipid membranes has been studied using the INPHAZE system. Of particular importance was the thickness of the hydrocarbon region.

More gramicidin channels were formed in the lipid membranes when the hydrocarbon region was greater than the length of gramicidin (~2.2 nm). However the life-time of the channels was short, between 60 and 180 milliseconds. When the thickness of the hydrocarbon region was about the length of gramicidin, then fewer channels were formed, but the life-time could be as long as 20 minutes.





Characterising lipid bilayers to study the effect of lipid tail lengths on gramicidin channel formation and life-time

Reference:

- Masato Nishio, Atsushi Shoji and Masao Sugawara (2012) "Planar Lipid Bilayers Containing Gramicidin A as a Molecular Sensing System Based on an Integrated Current ", **Analytical Science**, 28: 661-667