NeMPSIA: **Ne**utrons for **M**embrane **P**rotein **S**tructure, **I**nteractions, and **A**ssembly

Membrane proteins are a very important class of biomolecules, which have to date been underdetermined both structurally and dynamically. Thus far, only less than 250 unique membrane protein structures have been solved (ca. 1% of all non-redundant structures reported) primarily by X-ray crystallography, electron diffraction, and solution state NMR. These methods often require samples to be prepared using lipid mimetics. Solid state NMR is a viable alternative approach, since in many cases, it permits the study of membrane peptides and proteins in biologically relevant phospholipid environments and sample preparation is generally straightforward. For systems which form large peptide/lipid aggregates, such as antimicrobial peptides, solid state NMR is in fact the only method which can be used to obtain relevant structural information on the peptide. Another alternative approach involves the use of a neutron source. For example, small angle neutron scattering (SANS) can be used to obtain information on aggregate structures. Neutron diffraction can be used to obtain structural information of proteins in lipids.

The proposed projects made use of both solid state NMR and neutron methods to provide insight into membrane associated peptide and protein structure, the interaction of these molecules with lipids, as well as assembly of the proteins in the membrane. The projects can be subdivided into three general categories. The outcomes of these projects is also listed.

1) Antimicrobial peptide structure: The rise of highly resistant "superbugs" (e.g. MRSA) imposes significant health and economic costs, estimated at over billions of dollars worldwide. Over the last decade, the **Straus** group has been working towards developing new sustainable antibiotics, by investigating the mechanisms of action of different types of bactericidal agents, both as free compounds and tethered to solid supports (e.g. implants). Two classes of compounds which have been the focus of our attention are *cationic antimicrobial peptides and lipopeptides, since to date they display few resistance effects*. The work we proposed to do as part of this project will involve investigating the mechanism of action of the commercially available lipopeptides daptomycin, using a combination of NMR and neutron methodology.

OUTCOME: We have successfully characterized the aggregate structure of daptomycin in solution using SANS. We have also determined how daptomycin perturbs membranes in a concentration dependent manner, as a function of lipid composition, using a combination of the quartz crystal microbalance (QCM) technique and neutron reflectivity experiments. We are currently working towards complementing this data with the NMR structure of daptomycin in PG lipids. Once this work is complete, we will finalize the two manuscripts already in preparation (see section 2A).

2) Interaction of lipopeptides with membrane component lipid II: Bacterial cell wall (CW) biosynthesis was the first target in the development of antibiotics. Finding ways to perturb the pathway has lead to the development of such commonly known antibiotics as penicillin, nisin and vancomycin, to name but a few. As mentioned above, resistance is however becoming a major issue and therefore an important strategy in the development of anti-infective treatments is to try to find novel compounds which affect CW biosynthesis. At the heart of this is understanding how these new compounds interact with lipid II, a central building block in bacterial CW. Remarkably, there is only limited structural information on lipid II itself and on lipid II-peptide complexes. The aim of the proposed work is to obtain some structural information on labelled lipid II (to be obtained from H.G. **Sahl**) in a membrane on its own by neutron diffraction and also in the presence of binding partners such as lipopeptides.

OUTCOME: During the past year, our collaborators from the Sahl group have worked hard to produce labelled lipid II. Together, we have devised a protocol to produce sufficient quantities of deuterated lipid II for neutron reflectivity studies. Preliminary data on the insertion of lipid II in model membranes has

been collected. Future work will involve solid state NMR studies of lipid II in these membranes, as well as neutron reflectivity work.

3) Assembly of membrane proteins to form filamentous bacteriophage: The way in which biological macromolecules are inserted into membranes in order to form supramolecular assemblies and to carry out highly specific functions within the apolar environment of a membrane is very poorly understood. This is a major issue in structural biology given that almost every aspect of cell function is governed by the formation of multi-component macromolecular complexes. Our approach is to study a tractable well defined system, namely filamentous bacteriophage, and to study both the structure and dynamics of its assembly. In the last year, the **Straus** group has successfully expressed the major coat protein (also known as p8) associated with Gram-positive bacteria (B5 phage) and will conduct structural studies using solid state NMR. For the proposed work described here, we endeavoured to determine the arrangement of p8 in membrane using neutron diffraction methods, as well as to determine the structure of p8 in the filament.

OUTCOME: We have to date managed, with the help of our collaborators in Nantes, to produce sufficient quantities of B5 filamentous phage. We have collected EM images of the filaments and determined that the dimensions of the filament are in line with other filamentous bacteriophages from Gram-negative bacteria. We have also elaborated protocols to produce filaments of sufficient quality for X-ray fibre diffraction studies. As a continuation of the project, we plan to collect the X-ray fibre diffraction patterns and determine the p8 structure, as well as continue investigating the structure of p8 in membrane models, using solid state NMR.

In addition to these projects, we were involved with neutron diffraction work on fd filamentous fibres using neutron diffraction (see section 2A).