Dithiodimerised analogues of melittin and magainin:  
Pore kinetics and molecular dynamics simulations in membranes  
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C-terminally dimerised analogues of melittin and magainin are more active than the respective monomeric peptides in pore formation in phospholipid vesicles. Dimerisation of surface-bound melittin is rate-limiting for pore formation under some conditions [1]. These observations tend to rule out non-specific bilayer perturbation models for pore formation and support discrete-pore models.  

Definition of the pathway for the membrane insertion events that underlie pore-formation or membrane translocation requires knowledge of the pre-pore states, including the conformation and location of the peptide at the membrane interface. These have been defined for melittin in membranes using H/D exchange trapping to identify helical hydrogen bonds, and from membrane crystallography which has localized the peptide relative to the membrane normal in oriented phosphatidylcholine bilayers [2]. This has allowed construction of realistic starting structures for bilayer simulations in which the effects of the monomeric and dimeric helical peptides localized at the bilayer interface can be assessed.


TMAlpha: A software tool for analysis of transmembrane region alpha helices  
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Alpha helices make up approximately 90% of transmembrane (TM) regions in membrane proteins. The regularity of helical structure predisposes TM regions toward computational analysis and prediction. Nevertheless, where breakage of helical structure does occur, the consequences for TM region structure are significant. Occurrence of non-helical structure introduces irregularity in TM region length, tilt and orientation.

We have developed TMAlpha, an Internet-based tool for analysis of Brookhaven Protein Databank membrane protein structures. TM regions are defined using the TRANSMEM annotation of the Swiss-Prot sequence database. The software may be used for confirming alpha and beta structures, quantifying percentage alpha composition of individual TM regions and proteins, calculating 3D helix tilt, precisely locating points of helix breakage, and quantifying changes in helix tilt and orientation (kinking) that occur at given helix breaks. We are constructing a database of amino acid sequences involved in helix breaks, and the extent and nature of kinking brought about. The program is a valuable tool for analysis of TM region structure and the database allows development of software for prediction of helix breakage and kinking in TM regions directly from amino acid sequence.

Interaction of viscotoxin A3 with model membranes of acidic phospholipids  
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Viscotoxins are highly basic and cystein-rich proteins extracted from mistletoe, and related to the family of α- and β-thionins. They are known to display cytotoxicity activity against different types of tumour cells, but the mechanism by which they kill cells remains unclear.

As various thionins have been reported to have a wide array of effects on the viability of cells by acting directly on the phospholipids of the membrane, we studied the action of the viscotoxin A3, on monolayers and unilamellar vesicles.

Monolayer studies revealed a great affinity of the viscotoxin for acidic phospholipids and the interaction of the protein for such a monolayer can be described as an insertion. The role of electrostatic and hydrophobic forces is discussed. The orientation of the protein is analysed with the help of surface potential study.

The study of carboxyfluorescein release from vesicles made of acidic phospholipids shows that the extent of leakage is regulated by the viscotoxin/lipid ratio, and we found a threshold value for which the release mechanism can be described as an all-or-none or a gradual one.

Fluorescence polarization measurements agree well with these results and permitted us to go further in the interpretation of the mechanism of action.