NMR to indicate the degree of alignment. Based on these results, ssNMR spectrum will be obtained to show resonance patterns known as PISA wheel for the transmembrane domains of LspA.

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Oriented Synchrotron Radiation Circular Dichroism and Linear Dichroism Spectroscopy of Peptides in Model Membranes

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The orientation of membrane-associated alpha-helical peptides was investigated using novel methodologies of oriented Synchrotron Radiation Circular Dichroism (SRCD) and linear dichroism (SRLD) spectroscopies. Because of its enhanced signal-to-noise and a detector geometry that minimised optical artefacts associated with conventional CD studies of membrane suspensions, SRCD enabled the measurement of oriented CD spectra. To accomplish this a specially-designed sample cell holder was produced which would maintain constant humidity in hydrated film samples. Distinct spectra were obtained for peptides oriented parallel or normal to the direction of the beam, corresponding to the parallel and perpendicular pi to pi* and n to pi* electronic transitions.

To provide similar information for peptides associated with lipid vesicles, SRLD was used to examine suspensions of vesicles in a coquette flow-cell. SRCD studies of the samples in the same couette enabled interpretation of the information.

The model systems used in this study were peptides of the KALP family with a number of different phospholipids. In TFE solution and as well in lipid vesicle suspensions, KALP produced CD spectrum typical of an alpha helix in an isotropic solution, whilst in oriented samples different spectra associated with the different directional transitions of the peptide bonds were found for peptides oriented transmembrane or parallel to the membrane surface. The alignments of the peptides under the different conditions were compared with the results obtained by 15N solid state NMR of the peptide in oriented lipid multilayers. Thus, these new approaches to examining peptides in membranes can provide information that is complementary to the secondary structural information present in conventional CD spectra.

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Spectral Characterization Of Het-C2, A Glycolipid-transfer Protein

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Het-C2 is a small 23 kDa protein, isolated from the fungi Podospora anserina, homologous to mammalian GLTP, and capable of transferring glycospingolipids in vitro. The crystal structure of Het-C2 is unknown, but molecular models suggest conservation of the GLTP-fold. Here, the locations of the Trp residues in Het-C2 have been investigated to gain further insights into their function. Sequence homology shows one of Het-C2's two Trp residues aligned with GLTP Trp96 which resides in the sugar headgroup liganding site. The other Het-C2 Trp did not align with either of GLTP's other two Trp residues. The Trp fluorescence spectrum of native Het-C2 exhibited an emission maximum at 355nm which red shifted 2nm upon denaturation with 8M urea, indicating Trp localization to a more polar environment. Acrylamide and KI quenched >90% of the average Trp fluorescence confirming that Het-C2 Trp residues are not buried in hydrophobic environments but reside in exposed polar regions. The linearity of Stern-Volmer plots for native Het-C2 and urea-denatured (8M) Het-C2 suggested dynamic quenching at physiological pH and ionic strength. The Stern-Volmer constants were higher for native protein than denatured protein. Upon interaction with probe-sonicated POPC vesicles, the Trp emission maximum blue shifted (~2nm) and decreased in intensity (~13.5%). Including glycolipid in the vesicles slightly enhanced the blue shift (~3nm) and significantly decreased Trp intensity (~21%). Far-UV-CD of Het-C2 showed secondary structure dominated by alpha-helices and with a highly cooperative, thermally induced melting transition near 43°C. Near-UV-CD indicated the induced optical activity of Trp/Tyr residues was unaffected by interaction with vesicles lacking or containing glycolipid. The results are analyzed and discussed within the context of the known locations and functions of human GLTP's three Trp residues. [Support: NIH/NIGMS GM45928 & GM34847, NIH/NCI CA121493, The Hormel & Mayo Foundations]

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Aquaporin-4 Dynamics and Determinants of Assembly in Orthogonal Arrays Revealed by Single-Molecule Fluorescence Imaging

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Aquaporin-4 (AQP4) water channels exist in two predominant isoforms in cell plasma membranes. The long N-terminus 'M1' form exists as dispersed tetramers, while the short N-terminus 'M23' form assembles in large supermolecular structures known as orthogonal arrays of particles (OAPs) that are visible in freeze-fracture electron microscopy. We investigated the determinants and dynamics of AQP4 assembly in OAPs by visualizing fluorescently labeled AQP4 isoforms and mutants in living cell membranes using quantum dot single particle tracking and total internal reflection fluorescence microscopy. In several transfected cell types, including primary astrocyte cultures, AQP4-M1 diffused freely with diffusion coefficient ~5 x 10^{-10} cm^2/s, covering ~5 μm in 5 min, while AQP4-M23 was relatively immobile, moving only ~0.4 μm in 5 min. Biophysical analysis of short-range AQP4-M23 diffusion within OAPs indicated a spring-like confining potential with a restoring force of ~6.5 pN/μm. Analysis of AQP4 deletion mutants revealed progressive prevention of OAP formation by addition of 3-7 residues at the AQP4-M23 N-terminus, with polyalanines as disruptive as native AQP4 fragments. OAPs disappeared upon downstream deletions of AQP4-M23, which, from analysis of point mutants, involves hydrophobic interactions at residues Val24, Ala25 and Phe26. OAP formation could also be prevented by disrupting secondary structure through the introduction of proline residues at sites downstream from the hydrophobic N-terminus. AQP1, an AQP4 homolog that does not form OAPs, was able to form OAPs upon replacement of its N-terminal domain with that of AQP4-M23. Our results indicate that OAP formation by AQP4-M23 is stabilized by a hydrophobic homo-tetrameric interaction involving a structured N-terminus domain near the cytoplasmic membrane interface. Absence of OAPs in AQP4-M1 results from non-selective blocking of this interaction by seven residues just upstream from Met23.

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Physical Properties of the Zona Pellucida

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The zona pellucida (ZP) is the extracellular coat that surrounds the mammalian oocyte. It forms a spherical shell of remarkably uniform thickness (5-10 μm in eutherian mammals) composed of three glycosylated proteins (ZP1, ZP2 and ZP3).

Penetration of this shell by spermatozoa plays a crucial role in mammalian fertilization and any inability of spermatozoa to penetrate the ZP inevitably leads to infertility. The purpose of this work is to shed light to the three-dimensional structure of the ZP, its construction and its properties as a polymer.

By means of the Atomic Force Microscopy (force-distance curves) we have determined for the first time several ZP structural properties (elasticity, plasticity, adhesion, etc.) during the ovulatory, periovulatory and fertilized phase.

Moreover information about single polymers interactions has been obtained by means detachment experiments (pull-off curves).

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Characterization of the Cx32/hDlg Complex and its Role in Tumor Suppression

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Gap junctions provide an intercellular pathway for the propagation of signals, which are necessary for cellular differentiation and development, metabolic homeostasis, and in excitable tissue, electrical coupling. This exchange of electrical and molecular signals allows individual cell events to synchronize into the functional response of an entire organ. Previous studies indicate that the disruption of gap junctional intercellular communication (GJIC) leads to a loss of growth control that contributes to the development of human cancer. The most direct evidence supporting the role of GJIC in transformation involved the introduction of the gap junction protein connexin32 (Cx32) into communication-deficient human tumors and tumor cell lines. Cx32 resulted in restoration of GJIC in liver epithelial and glioma cells leading to an inhibition of tumorigenicity. In this study, we are focusing on the intermolecular interactions that define the Cx32 structure when associated with the tumor suppressor protein human Discs Large (hDlg). Previously, we have shown that Cx32 interacts with the C-terminal half of hDlg, and in the absence of this interaction, hDlg aberrantly localizes to the nucleus. Using the yeast two-hybrid and HA Tag IP/Co-IP assays, we are defining the minimal region of hDlg required for the Cx32/hDlg interaction. Additionally, studies have reported that Cx32 can also interact with another MAGUK family member, Zonula Occludens-1 (ZO-1). ZO-1 interacts with other connexins via its PDZ2 domain; therefore,