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.

1724-Pos Board B568

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

Barbara Perrone[1], Andrew J. Miles[2], Burkhard Bechinger[1], Soren Vronning Hoffman[3], B.A. Wallace[2].
[1]Université Louis Pasteur, Strasbourg, France, [2]Dept. of Crystallography, Birkbeck College, University of London, London, United Kingdom, [3]Institute for Storage Ring Facilities (ISA), Aarhus, Denmark.

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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1725-Pos Board B569

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

Roopa Kenoth[1], Ravi Kanth Kamlekar[1], Helen M. Pike[1], Franklyn G. Prendergast[2], Sergei Yu. Venyaminov[2], Rhoderick E. Brown[1].
[1]University of Minnesota, Austin, MN, USA, [2]Mayo Clinic College of Medicine, Rochester, MN, USA.

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]

1726-Pos Board B570

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

Jonathan M. Crane, A.S. Verkman.
UCSF, San Francisco, CA, USA.

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.

1727-Pos Board B571

Physical Properties of the Zona Pellucida

Massimiliano Papi, Giuseppe Arcovito, Marco De Spirito.
Université Cattolica del Sacro Cuore, Rome, Italy.

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).

1728-Pos Board B572

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

Kelly L. Virgil, Paul Sorgen, PhD.
Univ. Nebraska Med. Center, Omaha, NE, USA.

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,

we are investigating the interaction between Cx32 and ZO-1 to identify if a common mechanism of binding exists with hDlg. These data provide new insights into the regulation of MAGUK family scaffolding proteins and Cx32 interactions.

1729-Pos Board B573
Recombinant Expression Screening of P. aeruginosa Bacterial Inner Membrane Proteins
Constance Jeffery, Vidya Madhavan.
University of Illinois, Chicago, IL, USA.
Transmembrane proteins (TM proteins) make up 25% of all proteins and play key roles in many diseases and physiological processes. However, much less is known about their structures and molecular mechanisms than for soluble proteins. Problems in expression, solubilization, purification, and crystallization cause bottlenecks in the characterization of TM proteins. This project addressed the need for improved methods for obtaining sufficient amounts of TM proteins for determining their structures and molecular mechanisms.
We obtained plasmid clones encoding eighty-seven transmembrane proteins with varying physical characteristics, for example, the number of predicted transmembrane helices, molecular weight, and grand average hydrophobicity (GRAVY). All the target proteins were from P. aeruginosa, a gram negative bacterial opportunistic pathogen that causes serious lung infections in people with cystic fibrosis. We measured the relative expression levels of the transmembrane proteins under several culture growth conditions. The use of E. coli strains, a T7 promoter, and a 6-histidine C-terminal affinity tag resulted in the expression of 58 out of 87 test proteins (68%). In this study, factors related to overall hydrophobicity and the number of predicted transmembrane helices correlated with the relative expression levels of the target proteins.
Identifying physical characteristics that correlate with protein expression might aid in selecting the "low hanging fruit", or proteins that can be expressed to sufficient levels using this sort of expression system. The use of other expression strategies or host species might be needed for sufficient levels of expression of transmembrane proteins with other physical characteristics. Surveys like this one could aid in overcoming the technical bottlenecks in working with TM proteins and could potentially aid in increasing the rate of structure determination.

1730-Pos Board B574
Molecular Determinants of Neisserial Pathogenesis: Mapping the Interaction Between Opa I and a Human Binding Partner CEACAM1
Christopher Reyes, Daniel A. Fox, Kalyani Jambunathan, Thien Nguyen, Izabela Bielnicka, Linda Columbus.
University of Virginia, Charlottesville, VA, USA.
Neisserial Opa proteins mediate the internalization of the bacterial cell by host epithelial cells via an interaction between the extracellular loops of Opa proteins and the extracellular domains of the host binding partner present on the cell surface. The eleven Opa proteins can be subdivided into two classes on the basis of the human receptor target. The Opa_{HS} class is named for heparan sulfate proteoglycan (HSPG), while Opa_{CEA} proteins bind carcinoembryonic-antigen related cell adhesion molecules (CEACAMs), of which there are seven varieties. Significantly, each of the Opa_{CEA} proteins has a characteristic specificity for each CEACAM. Of the four extracellular loops of Opa proteins, binding specificity is attributed primarily to two, which correspond to hypervariable regions of the protein sequence. However, mutational and chimeric analyses have not revealed the sequence determinants of the hypervariable regions that are responsible for receptor target recognition. Furthermore, it has been shown that the binding requires a cooperative interaction between the two hypervariable domains, and that specificity is determined by specific pairing of the sequences. It is the goal of this study to determine at a molecular level how specificity is attained by studying the structure and dynamics of the Opa I - receptor interactions. To this end, OpaI, which binds to CEACAM1 receptors, has been cloned, expressed, purified, and refolded and the NMR backbone assignment is in progress. The progress towards structure determination will be presented. In addition, NMR data mapping the interactions between Opa I and the soluble receptor will be presented in order to characterize the functionally relevant structural interactions involved in bacterial pathogenesis.

1731-Pos Board B575
Associate Professor of Chemistry
Garth J. Simpson.
Purdue University, West Lafayette, IN, USA.
Second-order nonlinear optical imaging of chiral crystals (SONICC) enables sensitive and selective detection of sub-diffraction limited protein microcrystals with negligible contributions from solvated proteins or amorphous protein aggregates. Under low magnificaiton (large field of view) applications, SONICC can detect individual crystals as small as 100 nm in diameter, which is conservatively 6 orders of magnitude lower that achievalbe using current methods for crystal detection in commercial screening platforms. In studies of microcrystallites of green fluorescent protein (GFP) prepared in 500 pL droplets using a crystallization micro-array, the SHG intensities rivaled those of fluorescence but with superb selectivity for crystalline regions. Furthermore, SONICC is directly compatibility with virtually all common protein crystallization platforms without modification.

1732-Pos Board B576
Expression, Reconstitution and Biophysical Studies of Neuronal Uncoupling Proteins: UCP4 and UCP5
Marina V. Ivanova[1], Matthew D. Smith[2], Masoud Jelokhani-Niaraki[1].
[1]Department of Chemistry, Wilfrid Laurier University, Waterloo, ON, Canada, [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada.
Uncoupling proteins (UCPs), located in the inner membrane of the mitochondria, uncouple ATP-synthesis from the respiratory chain by transporting protons across the inner membrane into the matrix, hence dissipating the proton-motive force and releasing heat. The neuronal UCPs (nUCPs), UCP4 and UCP5, were discovered recently (in 1998) and little is known about their structure and function. To gain further insight into the potential importance of these two proteins in the neuroprotection and neuromodulation of neurodegenerative diseases, this study will focus on the structure, function and interaction of the nUCPs with nucleotides (inhibitors) and fatty acids (activators). A recombinant version of the proteins, utilizing a hexa-histidine tag and a TEV protease site (for subsequent His-tag cleavage) has been designed, expressed as insoluble inclusion bodies, and isolated and purified using immobilized metal affinity chromatography. Subsequent reconstitution of the proteins in mild detergent (DDM and digitonin) allowed for biophysical studies by circular dichroism and fluorescence spectroscopy. Circular dichroism spectroscopy has shown that, similar to the recombinant UCP1, nUCPs possess dominantly helical structures in digitonin and DDM [1]. Furthermore, detergent-mediated reconstitution of the proteins into preformed liposomes can give more physiologically relevant structural and functional information. Comparison of the structure and function of human UCP1 (thermogenin) to nUCPs, in lipid membranes and membrane-like environments, will eventually show whether these proteins have any similarity in conformation and functional behaviour.
[1] Jelokhani-Niaraki, M., Ivanova, M.V., McIntyre, B.L., Newman, C.L., McSorley, F.R., Young, E.K. and Smith, M.D. (2008) *Biochem. J.*, **411**, 593-603.

1733-Pos Board B577
Helix-Helix Interactions in Membrane Proteins: Structural Analysis and Free Energy Calculations of Polyleucine-based Dimers
Anirban Mudi, D. Peter Tieleman.
University of Calgary, Calgary, AB, Canada.
According to Popot and Engelman's 'two stage' model, lateral interaction of helices in the membrane is a key step towards the folding of helical membrane proteins. In order to understand, how protein self-association regulates biological functions and how mutations may be involved in diseases, it is important to understand the constituent free energies. In this study, we investigated the effect of a wide range of mutations, at the 'a' or 'd' position of the heptad in the amino acid sequence, on the helix-helix interactions in a polyleucine dimer in lipid (DOPC) environment. We used molecular dynamics simulations and the thermodynamic integration method to calculate the free energies for various mutations. Our free energy calculations show that highly polar residues like Asn, Asp, Gln, Glu, Cys, and His enhance the stability of the polyleucine dimers much more than that by smaller polar residues like Ser and Thr, which agrees with previous experimental studies on similar peptides [1,2]. A past study showed that Ser, Thr, and less polar amino acids occur at a higher frequency, as compared to highly polar residues (Asn, Asp, Gln, Glu, Cys, His), in membrane proteins [2]. Recent experiments suggest that there is a high occurrence of weak H-bonds in membrane proteins [3]. Our findings and other studies suggest that membrane proteins prefer a wide range of moderately stabilizing interactions instead of strong ones, which lends them a greater degree of flexibility in terms of conformation and stability [3,4].
References :
1. Zhou, F.X. et al. *PNAS* **98**, 2250-2255 (2001).
2. Gratkowski, H. et al. *PNAS* **98**, 880- 885 (2001).
3. Joh, N.H. et al. *Nature* **453**, 1266-1270 (2008).
4. Grigoryan, G. et al. *Nat. Chem. Bio*. **4**, 393-394 (2008).

1734-Pos Board B578
Assembling Within The Lipid Membrane: Viral Membrane Proteins
Wolfgang B. Fischer.
National Yang-Ming University, Taipei, Taiwan.
Viruses encode a number of membrane proteins which are interacting with lipid membranes. One class of these proteins are known to form homo-oligomeric