Starting from the crystal structure of beta2-adrenergic receptor (B2AR), we have used LITiCon computational method to predict the ligand stabilized receptor state with full (epinephrine and norepinephrine), partial (salbutamol and dopamine), and inverse agonists (carazolol) bound. We have calculated the minimum energy pathway going from the inactive to the ligand stabilized state for each of the ligand/receptor complex. The activation pathways derived for all the agonists studied here are in agreement with fluorescence lifetime measurements[1,2]. We have also studied the norepinephrine activation pathway for the mutant m23 avian beta1-adrenergic receptor (B1AR) that has been crystallized, and the wild type B1AR. We show that the activation barrier for activation of the mutant m23B1AR by norepinephrine, is larger than for the wild-type B1AR, which is in agreement with the previous experimental finding that m23B1AR requires higher concentration of norepinephrine for activation[3]. Virtual ligand screening with the salbutamol-stabilized B2AR conformation shows enrichment of non-catechol agonists over norepinephrine-stabilized conformation. Our computational method provides an unprecedented opportunity to understand activation mechanisms in GPCRs.

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2. Swaminath G, *et al.* (2005) , *J. Biol. Chem.* 280:22165-22171.
3. Warne T., et. Al., (2008), *Nature*, 454, 486-491.

3365-Pos

Functionnal Analysis of a Conserved Histidine Residue of the Extracellular Loop of Acid-Sensing-Ion-Channel-1a

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ASICs are non-voltage-gated neuronal sodium channels activated by protons. Activation of ASICs induces a membrane depolarization. ASICs are trimeric assemblies of homologous subunits. The structure of the chicken ASIC1 in its inactivated conformation is known. Each subunit has two transmembrane domains, a large extracellular loop (ECL) and two short intracellular termini. The ECL is the sensor of the extracellular acidity and the target of modulators. Zinc potentiates the acid activation of ASIC2a-containing channels. Two histidine residues are essential for this effect. Only the hASIC1a His163 is conserved in all ASICs and forms H-Bonds with the conserved Asn96 and Gln225 residues. To test their functional relevance, we mutated these and neighboring residues to cys. Most mutant channels showed poor expression in *Xenopus* oocytes suggesting that this part of the ECL is essential for the channel folding. The mutant channels were however functional and were unlike the wild type channel inhibited by Cd^{2+}. The Cd^{2+} IC_{50} values (N96C: $71 \pm 10\mu M$, Q225C: $73 \pm 53\mu M$, H163C: $331 \pm 36\mu M$) suggest together with the structural information that H163 participates in Cd^{2+} chelation in the mutant channels. Covalent modification by the charged sulfhydryl reagent MTSET was used to further investigate the structural and functional role of the His163 and its environment. After MTSET exposure the hASIC1a H163C channel current decreased to $4 \pm 1\%$ and the pH dependence of activation was shifted by -0.24 pH units, while the pH dependence of inactivation was not affected. The unitary conductance of the modified H163C mutant was not different from the conductance of unmodified H163C or wt ASIC1a. These observations suggest a role of this region in the gating, but likely not the pH-sensing of ASIC1a. Our study identifies a conserved region around His163 as critical determinant of ASIC1a expression and function.

3366-Pos

Structural Studies of the Integral Membrane Protein Human LTC4 Synthase by Electron Crystallography

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Human leukotriene C4 synthase (LTC4S), an 18kDa integral membrane protein and member of the MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) family, is critical to the biosynthesis of the cysteinyl leukotrienes. LTC4S catalyzes the conjugation of leukotriene A4 with glutathione to produce leukotriene C4. The cysteinyl leukotrienes, in particular leukotriene C4 and its metabolites, are important lipid mediators in inflammation and are central to acute and chronic inflammatory diseases of the respiratory system, in particular bronchial asthma. The structure of human LTC4S was studied by two-dimensional (2D) crystallization and electron crystallography.
When reconstituted into proteoliposomes under rigorously controlled parameters, human LTC4S forms large and well-ordered two-dimensional crystals that retain enzymatic activity. Despite two three-dimensional structure, many questions about the reaction mechanism of human LTC4S remain unanswered. To elucidate these structure/function questions by electron crystallography, we are crystallizing both the wild-type and mutant enzymes. Surprisingly, the number of subunits of human LTC4S is not limited to the trimers earlier observed by both electron and x-ray crystallography.

3367-Pos

Metamorphic Response of CLIC1 Chloride Intracellular Ion Channel upon Interaction with the Membrane

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The Chloride Intracellular Channel (CLIC) family can be characterized as 'metamorphic'proteins; namely, capable of reversibly shifting between two or more different-folded conformations. The CLICs are expressed as soluble proteins but can also auto-insert into the membrane to form active ion channels. Such a conformational transition must involve large-scale structural rearrangement to confer favourable interactions with the membrane. To date, little is known about the process and cellular triggers for CLIC membrane insertion; although a unique feature of mammalian CLIC1 is its ability to undergo a dramatic structural rearrangement from a monomer to a dimer upon oxidation in solution. Whether this oxidation-induced metamorphosis in solution facilitates CLIC1 membrane insertion is unclear. We have sought to characterize the structural response of CLIC1 upon interaction with the membrane. A novel labeling scheme for CLIC1 was devised to enable site-directed-labeling of single native cysteines with either fluorophores or spin labels. Fluorescence resonance energy transfer (FRET) and electron paramagnetic resonance (EPR) revealed that the interaction of CLIC1 with the membrane results in a large-scale inter-domain movement. The two phases for membrane interaction, i.e. association followed by insertion, were also monitored by probing the local environment of an N-terminal transmembrane tryptophan residue (Trp35) using fluorescent quenching. A sucrose-loaded-vesicle sedimentation assay was also used to quantify membrane binding. Our results suggest oxidation of CLIC1 monomer in the presence of the membrane promotes CLIC1 binding and insertion. Our current model for the structural transitions and environmental triggers of CLIC1 membrane-induced metamorphosis will be discussed.

3368-Pos

Structural Analysis of Heptameric Alpha-Hemolysin under Extreme Conditions that Facilitate Nucleic Acid Translocation

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Protein nanopores are under intense investigation as sensors of various analytes, particularly for the rapid analysis of genomic material. In several important applications, notably ultrarapid sequencing, nucleic acids must be analyzed in unfolded single-stranded form. Therefore, conditions were examined that cause the denaturation of double stranded DNA (dsDNA), and single stranded DNA (ssDNA) and RNA with secondary structure. The behavior of the heptameric α-hemolysin (αHL) pore was investigated under alkaline pH conditions (8.0-13.0) and in varying concentrations of urea (0-8 M). The structural stability of the pore was examined by SDS-PAGE, intrinsic tryptophan emission fluorescence (ITFE) spectroscopy and circular dichroism (CD) spectroscopy. The pH studies revealed that the heptamer is resistant to structural change at up to pH 12.0. Electrical recordings revealed that DNA is capable of translocating through the pore at pH 11.7, a value at which its secondary structure is lost [1]. Studies conducted at various urea concentrations revealed that a substantial fraction of the heptamer remains stable in 8 M urea. At urea concentrations above 4.0 M, the secondary structure of single stranded DNA/RNA is denatured [2], permitting translocation.

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3369-Pos

Mtb Membrane Protein Expression , Purification and, Structure Determination : Solid State NMR Approach

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We describe here the expression, purification, solid state NMR sample preparation, and initial structural and functional data for three membrane proteins from Mycobacterium tuberculosis (Mtb). The three proteins are FtsX, Rv0008c and Rv1861. Solid state NMR is uniquely able to characterize protein structure in a liquid crystalline lipid bilayer environment. We have used N terminal His tag for protein purification. Nickel-NTA chromatography was performed using a semi automated FPLC instrument. Purified 15N labeled proteins were eluted into 0.2% (Rv0008c and Rv1861) and 0.4% (FtsX) solution of dodecylphosphocholine (DPC) detergent. The approximate protein yield were 45mg/l (Rv0008c), 50mg/l (FtsX) and 25mg/l (Rv1861) respectively. Samples for solid state NMR were prepared by removing the detergent from the purified protein by exhaustive dialysis against 10mM Tris-HCl (pH-8.0) coincident with reconstitution into lipid bilayers. To prepare aligned samples, pelleted liposomes were layered on to thin glass slides and stacked. 30-35 glass slides were hydrated in a process called 'wet stacking' followed by sealing them into a rectangular glass cell. 400 and 600 MHz magnets were used to determine the 1D and 2D spectra of these aligned samples such that the bilayer is parallel to the applied magnetic field direction. FtsX is an ABC transporter containing 4 transmembrane helices (TMH) and its interaction with FtsZ participate in cell division. Rv1861 has 3 TMH and is known to hydrolyze ATP. It forms a stable octameric structure that is presumably facilitated by the GxxxG, GxxxA, and AxxxA sequences in the trasmembrane stretches. Rv0008c is a Mtb membrane protein and participates in cell division. It has been found previously in our laboratory that Rv0008c interacts with Rv0011c and this interaction along with other membrane proteins can facilitate the Mtb cell division process.

3370-Pos

First Structural Characterization of a Bon-Domain in a Protein from Mycobacterium Tuberculosis: OmpATb Tracks toward an Oligomerization Process to form a Cell Wall Pore

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The etiological agent of tuberculosis (TB), *Mycobacterium tuberculosis*, causing nearly two millions deaths per year is presently one of the greatest infectious agents of mortality worldwide. One of the major problems in TB therapy is the slow uptake of drugs across the thick mycobacterial cell wall made of unique lipid and glycolipid moities . Water-filled protein channels, called porins, are considered as the main pathway for hydrophilic drugs through the mycobacterial cell wall . In this study, we investigated the 3D structure of the porin-like protein OmpATb (326 residues) from *Mycobacterium tuberculosis* by NMR in solution. We have found that the N-terminal domain of OmpATb (73-204), sufficient to form channels in planar lipid bilayers, forms an α/βsandwich composed of 6 β-strands and 3 α-helices. It appears that a sub-domain of this structure is relied to the BON (bacterial OsmY and nodulation) domain fold which was initially identified in bacterial proteins as a conserved ~ 60 residues module supposed to associate with phospholipids. Thus our study gives rise for the first time to the 3D fold of a BON domain member. Other bacterial proteins belonging to macromolecular complexes of the type III secretion systems have BON-like domain structures and form multi-subunits membrane-associated rings at the basal body of the secretion machinery. By analogy with these superstructures, we have built a model of an oligomeric ring assembly of the OmpATb protein to support the formation of functional pores in the mycobacterial cell wall. The numbers of units involved in the pore structure is discussed regarding the biochemical and channel properties of OmpATb.

3371-Pos

SP-C Palmitoylation is Crucial for Stabilizing Cholesterol-Containing Surfactant Films during Continuous Compression/Expansion Cycling

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Cholesterol is critical to maintain a dynamic lateral structure in pulmonary surfactant membranes, including a defined fluid-ordered/fluid-disordered phase equilibrium and proper lateral sorting of surfactant proteins and lipids. However, an excess of cholesterol has been linked to impaired surface activity both in surfactant models and in surfactant from injured lungs. Surfactant protein C (SP-C), the smallest and most hydrophobic of all surfactant proteins, has been shown to interact with cholesterol and dual palmitoylation of its N-terminal segment has been shown to drive association with ordered phases in model membranes. Furthermore, it has been proposed that native palmitoylated SP-C can act in concert with surfactant protein B (SP-B) to permit cholesterol-containing surfactant films to reach very low surface tensions upon compression. In the present work, we report that palmitoylation of SP-C is important for its ability to counteract deleterious effects of cholesterol on surfactant film stability under continuous expansion/compression cycling, as evaluated in a captive bubble surfactometer (CBS) setup. Presence of 5% cholesterol impairs significantly the stability under quasi static and dynamic compression of films composed of DPPC/POPC/POPG/SP-B (50:25:15:1, w/w/w/w), which are able to reach tensions below 3 mN/m with only 20% compression and almost no hysteresis in the absence of cholesterol. Incorporation in the films of 2% native palmitoylated SP-C could alleviate these deleterious effects. However, recombinant non-palmitoylated SP-C was not able to reproduce the stabilizing effect of native SP-C, confirming that palmitoylation of SP-C at its N-terminal end is crucial for its potential function of stabilizing surfactant films during the respiratory cycles in the lung.

3372-Pos

New Insights into the Interfacial Activation of Secreted Phospholipase A2

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Despite numerous studies towards elucidation of the structural basis of activation of secreted PLA2s upon membrane binding (interfacial activation), no consistent or clear picture has emerged thus far. Previously we have reported significant changes in the secondary and dynamic structures of human group IB and IIA PLA2s, as well as changes in their mode of membrane binding during activation. Here we have conducted atomic resolution NMR studies on free and phospholipid micelle-bound human group IIA PLA2 (hIIAPLA2) to detect more detailed molecular events underlying interfacial activation. Two-dimensional 1H,15N-HSQC spectra have been obtained at 600 MHz on Ca2+-free and Ca2+-loaded hIIAPLA2 in the presence of dodecylphosphocholine (DPC) micelles. Upon complex formation with the micelles, signals from arginine side chain -NH2 groups of Ca2+-loaded hIIAPLA2 are observed, whereas for Ca2+-free PLA2 these signals are absent because of fast H/D exchange with the solvent. This suggests that the Ca2+-loaded hIIAPLA2 tightly binds to the micelles so these groups are sequestered at the PLA2-micellar interface and shielded from the solvent, or that they are otherwise stabilized by strong hydrogen bonding in the micelle-bound state. TROSY experiments (900 MHz) on Ca2+-loaded, 15N,13C-labeled hIIAPLA2 in the absence and presence of DPC micelles (1:600 protein-to-DPC molar ratio) identify substantial conformational changes in PLA2 upon binding to the micelles. Based on the assigned chemical shifts, important structural changes occur throughout the protein. The molecular mechanism of the strong increase in activity of hIIAPLA2 upon phospholipid surface binding is likely to involve a widening of the substrate binding pocket, mediated by a rigid-body movement of the N-terminal helix via interactions of the cationic residues (e.g., Arg7) with lipid phosphate groups. This mechanism will be tested in further studies and may be shared by other secreted PLA2 isoforms.

3373-Pos

Peptide-Membrane and Peptide-Peptide Interactions between Myelin Basic Protein and Myelin-Like Lipids Revealed by Covalently Attached Vibrational Labels

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Cyanylation of cysteine reveals the local solvent environment of the modified side chain and the ps-time scale dynamics of membrane-protein interactions via the infrared absorbance of the covalently attached CN probe vibration. Single-cysteine mutants of the primary membrane binding sequence from the myelin basic protein, were synthesized and cyanylated. A combination of transmission and horizontal attenuated total reflectance (HATR) infrared spectra are used to investigate the dynamics of the membrane-peptide interactions. The SCN-labeled side chains report on the geometry of the peptide-membrane binding interface. They also provide information about the reversible aggregation of the peptide, which occurs at high concentration in solution and when in contact with a myelin-like membrane system.

3374-Pos

Structural Characterization of Human Peripheral Myelin Protein 22 Using NMR

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Peripheral myelin protein 22 (PMP22) is a 160-residue integral membrane protein with four putative transmembrane spans. PMP22 is a major protein of peripheral nervous system (PNS) myelin, where its importance is underscored by the fact that heritable mutations in this protein result in Charcot-Marie-Tooth