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

Daniel Auguin[1,2], Yinshan Yang[1], Stephane Delbecq[3], Emilie Dumas[1], Virginie Molle[4], **Nathalie Saint[1]**.
[1]UMR 5048 CNRS,U554 INSERM, University of Montpellier, Montpellier, France, [2]UPRES EA 1207, INRA UCS 2030, University of Orleans, Orleans, France, [3]UMR 5235 CNRS, University of Montpellier, Montpellier, France, [4]UMR 5086 CNRS, University of Lyon, Lyon, France.

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

Florian Baumgart[1], Olga Lucía Ospina[1], Leticia Gómez-Gil[2], Ignacio Rodríguez-Crespo[1], Jesús Pérez-Gil[1].
[1]Universidad Complutense, Madrid, Spain, [2]Université Libre de Bruxelles, Brussels, Belgium.

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

Jeffrey L. Urbauer[1], Kathleen N. Nemec[2], **Suren A. Tatulian[2]**.
[1]University of Georgia, Athens, GA, USA, [2]University of Central Florida, Orlando, FL, USA.

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

Heather A. McMahon, Katherine N. Alfieri, Casey H. Londergan.
Haverford College, Haverford, PA, USA.

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

Masayoshi Sakakura, Arina Hadziselimovic, Charles R. Sanders.
Vanderbilt University School of Medicine, Nashville, TN, USA.

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

Disease (CMTD), the most common disorder of the PNS. Disease mutations induce misassembly of PMP22, resulting both in loss of its function and toxic accumulation of misfolded PMP22 in the cell. Here we present a structural comparison of the wild type and the L16P disease-linked mutant form of human PMP22 to obtain insight into the molecular basis of CMTD. Human PMP22 was expressed in *Escherichia coli*, and purified in the detergent tetradecylphosphocholine. The purified protein provided moderately well dispersed ^{1}H-^{15}N TROSY spectra. NMR resonance assignments for the wild type protein revealed that the 72 observed backbone amide peaks out of 157 expected originate exclusively from the N-terminal STREP tag, transmembrane region 1 (TM1), extracellular loop 1 (ECL1), and the extreme C-terminus. Chemical shift index analysis suggested the residues from TM1 (Met1 to Ile29) form an α-helix, while no secondary structure was predicted for ECL1 (Val30 to Pro58). The L16P mutant was analyzed in a similar manner. A significant finding for the mutant was that the resonances from Ile8 to Val17 located at the middle of TM1 were not observed due to line broadening. Moreover, chemical shift perturbations were observed for residues from Leu18 to Ile24 which are located at the C-terminal end of TM1. These observations suggest that the L16P mutation induced a global conformational change in TM1 that results in its recognition as being folding-defective by components of membrane protein folding quality control system of the endoplasmic reticulum.

Protein Aggregates III

3375-Pos

Possible Pathway between Alpha Helical and Beta Helical Structures of the C-terminal in the Mammalian Prion Protein

Shan Dai, Daniel L. Cox.
University of California, Davis, Davis, CA, USA.

The normal form of the prion protein(PrP^{C}) has mostly alpha-helical (AH) secondary structure in the C-terminal region (residues 166-230), while the infectious form (PrP^{Sc}) has been proposed to have a left-handed beta helical (LHBH) structure(1). The mechanism of conformational change from PrP^{C} to PrP^{Sc} is unknown, but recent electron microscope data(2) and computer modeling(3) of in vitro grown prion fibrils suggest a possible LHBH structure in the C-terminal region. We use high temperature (500K) AMBER molecular dynamics over 10 ns runtimes to study the unfolding transitions commencing from both LHBH and AH C-terminal starting structures. Using stability, contact map, and energetic analyses we find that both structures unfold to very similar AH-like conformations and discuss the potential implications of this result for normal prion cellular function and for prion disease.

References

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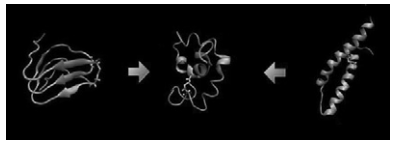
3) Kunes, K. C., et al. (2008) *Prion* **2,** 81-90.

The image below compares computational models of the initial LHBH structure(left), the initial AH structure (right), and the unfolded AH-like structure(middle).



3376-Pos

Molecular Mechanism of Inhibition of Amyloid Formation by Inositol

Grace Li[1,2], Régis Pomès[1,2].
[1]Hospital for sick children, Toronto, ON, Canada, [2]University of toronto, Toronto, ON, Canada.

Alzheimer's disease (AD) is a severe neurodegenerative disease with no cure. Currently, one method of targeting the underlying disease is to prevent or reverse the amyloid formation of Abeta1-42, a key pathological hallmark of AD. Scyllo-inositol is a promising small-molecule therapeutic that is found to exhibit stereochemistry dependent inhibition of formation of Abeta fibrils in vitro and is currently in phase II of clinical trials. However, the mechanism of action of scyllo-inositol at the molecular level is not known. We perform extensive atomistic molecular dynamics simulations of scyllo-inositol and its inactive stereoisomer, chiro-inositol, to systematically compare and characterize both the binding mode and the effect of inositol on the structure, morphology and aggregation equilibrium of the amyloidogenic fragment of Abeta42, KLVFFAE (Abeta16-22).

3377-Pos

Side Chain Interactions can Impede Amyloid Fibril Growth:Replica Exchange Simulations of Abeta Peptide Mutant

Takako Takeda, Dmitri K. Klimov.
George Mason University, Manassas, VA, USA.

Aggregation of Aβ peptides is related to the onset of Alzheimer's disease, but the molecular mechanism of the Aβ fibril formation is still poorly understood. Recently, we have studied the thermodynamics and free energy landscape of Aβ fibril growth using a hexamer system of $A\beta_{10\text{-}40}$ peptides by replica exchange molecular dynamics simulations and atomistic implicit solvent model. The system consisted of four peptides forming a fibril fragment and two incoming peptides binding to the fibril edge. We have demonstrated that deposition of the peptides onto the fibril follows the ''dock-lock'' mechanism. In the docking stage, disordered peptides dock to the fibril without their incorporation into the ordered fibril structure. In the locking stage, the incoming peptides form parallel β-sheets with the fibril. In this presentation, we report the effect of D23Y mutation in $A\beta_{10\text{-}40}$ peptides focusing on the changes in the deposition free energy landscape and in the interactions between incoming peptides and the fibril. We found that although D23Y mutation has a weak impact on the docking stage, it induces strong stabilizing effect on the locking stage of fibril growth. We explain these findings by elimination of off-registry side-chain interactions formed by Asp23 in the wild-type Aβ sequence. We conclude that strong off-registry side chain interactions have a capacity to impede fibril growth.

3378-Pos

Elucidating the Association and Dissociation Mechanism of β-Amyloid Protein by Targeted Molecular Dynamics Simulations

Chewook Lee, Sihyun Ham.
Sookmyung Women's University, Seoul, Korea, Republic of.

The amyloid-β (Aβ) proteins are responsible for amyloid plaques in Alzheimer's disease and have been the most widely studied subject in the process of fibril growth. Although much progress has been made to elucidate amyloid fibril properties at a molecular level, the full identification and characterization of all the conformational states and oligomeric structures in the aggregation process and all the conformational changes that link between those different states are still needed to be revealed. Here, we present the results of targeted molecular dynamics (TMD) simulations with explicit water to investigate the structural and mechanistic aspects of the association and the dissociation of the Aβ42 dimer. We will discuss the reversibility and the driving forces of the Aβ42 dimerization process with several order parameters along the protein aggregation pathway.

3379-Pos

Charactering Amyloid-Beta Protein Misfolding from Molecular Dynamics Simulation with Explicit Water

Chewook Lee, Sihyun Ham.
Sookmyung Women's University, Seoul, Korea, Republic of.

Formation of amyloid-beta (Aß) protein aggregates is the primary cause of amyloid diseases including Alzheimer's disease (AD). Here, we present the state-of-the-art atomic-level characterization of the misfolded state of Aß42 and early misfolding events from helical structure to form aggregation-prone structure in water by using all-atom molecular dynamics (MD) simulations in explicit water environment. Our simulations reveal one of the most important yet unsolved structural mysteries in early misfolding steps that the aggregation-prone structure (APS) of Aß42 is characterized by the non-helical backbone H-bond formation between K16L17 and V39V40I41 associated with the expansion of the hydrophobic exposure. Characterizing the nature of the misfolded state (APS) of Aß42, we provide new insight into the experimentally observed different aggregation propensities of Aß42 compared to those of Aß40. Based on the structural features of APS, we also speculated the hypothetical aggregation mechanism from APS of Aß42 to form fibril accounting three mandatory steps. The structural and mechanistic observations based on these simulations agree with the recent NMR experiments and provide the driving force and structural origin for the Aß42 aggregation process to cause AD.

3380-Pos

Fiber Formation of Silk-Like Proteins

Marieke Schor, Peter G. Bolhuis.
University of Amsterdam, AMSTERDAM, Netherlands.

Beta-sheet forming proteins can fold and assemble into long fibers that play a structural role. While this phenomenon is most famous for its role in amyloidogenic diseases, such fibers also have high potential as biomaterials. In both cases, it is crucial to understand the entire formation process, but the fact that folding and assembly are often intertwined makes this very difficult.
The natural silk fibroin consists of several ''crystalline'' domains with a highly repetitive amino acid sequence, linked through hydrophilic, amorphous spacer sequences. Here, we focus on an artificial silk protein with a (EGAGAGA)x repeat for the crystalline domain (E is glutamate, G is glycine, A is alanine; x denotes the number of repeats) with hydrophilic flanking sequences. Experiments have shown that upon a change in pH, the EGAGAGA repeat will fold and