3348-Pos
From in Silico Prediction to in Actu Characterization of a Novel Transmembrane β-Barrel
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Transmembrane β-barrels (TMBB) constitute a special structural class of proteins localized to the external membranes of Gram-negative bacteria, mitochondria, and chloroplasts. Because TMBBs are surface-accessible proteins that perform a variety of functions ranging from nutrient transport to cellular adhesion, they are tempting targets for vaccine or drug therapy development. Since it could be advantageous to identify TMBB-encoding genes and traditional experimental approaches such as crystallography have proven difficult, computational methods have been explored for identifying genes which encode members of this protein class. However, the cryptic nature of the proteins'sequence-structure relationship has made the computational prediction of TMBB-encoding genes a challenging task. The Freeman-Wimley algorithm, which was developed to predict TMBB-encoding genes from genomic databases, is a highly accurate prediction method based on the physicochemical properties of experimentally characterized TMBB structures. Predicted outer membrane protein L (OmpL) from *Salmonella typhimurium* LT2, was tested as a model for validating the prediction method. All of the physicochemical and spectroscopic properties exhibited by OmpL are consistent with other known TMBBs. Recombinant OmpL localizes to the outer membrane when expressed in *Escherichia coli*; OmpL has β-sheet-rich secondary structure with stable tertiary contacts in the presence of either detergent micelles or a lipid bilayer; when reconstituted into a synthetic lipid bilayer, OmpL forms a pore through which small non-electrolyte solutes can diffuse. Together, this data proves that OmpL is a true TMBB and thus, substantiates predictions made by the Freeman-Wimley algorithm.

3349-Pos
A Highly Accurate Statistical Approach for the Prediction of Transmembrane β-Barrels
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Transmembrane β-barrels (TMBB) belong to a special structural class of proteins predominately found in the outer membranes of Gram-negative bacteria, mitochondria, and chloroplasts. TMBBs are surface-exposed proteins that perform a variety of functions ranging from iron acquisition to osmotic regulation. These properties suggest that TMBBs have great potential for use in vaccine or drug therapy development. Membrane proteins, such as TMBBs, are notoriously difficult to identify and characterize using traditional experimental approaches due to a variety of technical limitations. However, in silico prediction methods have been considered for handling the task of identifying the enigmatic sequences which fold into TMBBs. A prediction method based on the physicochemical properties of experimentally characterized TMBB structures was developed to predict TMBB-encoding genes from genomic databases. The algorithm's prediction efficiency was tested using a non-redundant set of sequences from proteins of known structure. The algorithm was based on the work of Wimley (2002), but was greatly improved because of its disappointingly high false-positive prediction rate and thusly renamed the Freeman-Wimley algorithm. The improved prediction algorithm developed in this study was shown to be more accurate than previously published prediction methods. Its accuracy is 99% when using the most efficient prediction criteria, i.e. the threshold where the most known TMBBs are correctly predicted and the most non-TMBBs are correctly excluded. The Freeman-Wimley algorithm was used to make predictions in 611 bacterial chromosomes, where an average of 3% of the genes in a given genome encoded TMBBs.

3350-Pos
Accurate Ab Initio Prediction of Three Dimensional Structures of Beta-Barrel Membrane Proteins from Sequences
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Among the two classes of membrane proteins, beta-barrel membrane proteins are found in the outer membrane of Gram-negative bacteria, mitochondria, and chloroplasts. They carry out diverse biological functions, including pore formation, membrane anchoring, enzyme activity, and are often responsible for bacterial virulence. Although membrane proteins comprise approximately one third of all proteins encoded in a genome, they are sparsely represented in the protein structure databank, due to difficulties in experimental structural determination. We have developed a computational method to predict three dimensional structures of beta-barrel membrane proteins from transmembrane sequence segments. For this, we have derived an asymmetric potential function based on detailed combinatorial analysis. In addition, we have developed a model to account for interstrand loop entropy. In a set of 25 non-homologous proteins with known structures, we can successfully predict strand register at 76% accuracy. This is a significant improvement from previous results (44%) and from random chance (7%) [1]. Based on predicted strand registrations, we are now able to predict the three dimensional structure of the transmembrane region of beta-barrel membrane proteins. The average RMSD between predicted and native beta-barrel membrane protein structure is less than 3A°. Our method is general and can be applied to genome-wide structural prediction once sequences of beta-barrel membrane proteins have been identified.
[1] Ronald Jackups, Jr. and Jie Liang. Interstrand pairing patterns in beta-barrel membrane proteins: the positive-outside rule, aromatic rescue, and strand registration prediction *J Mol Biol.* 2005, 354:979-993.

3351-Pos
Sensitivity of Coarse Grain Models of Peptides to the Introduction of Charged Residues in Model Peptides and Bacterial Chemoreceptors
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Coarse grain (CG) techniques for molecular simulation have become increasingly popular over recent years, and have been shown to offer useful insights into a variety of different systems including protein conformational change, protein-membrane interaction and protein-protein interaction. Recently, the appropriateness of MARTINI based CG models for modelling introduction of arginine residues into the membrane has been called into question by explicit PMF calculations. We have used high throughput simulation techniques to probe the appropriateness of simulating arginine residues within the membrane core in the novel model peptide GWALP23 (GGALW(LA)6LWLAGA), alongside experimental evidence from solid state NMR techniques. We find close agreement between experimental and simulation results for two modifications of GWALP; introduction of arginine at position 14, which creates a stable TM helix with increased tilt and introduction of arginine at position 12, which creates an helix which adopts multiple positions (TM and interfacial). These results allow for the interpretation of mutational data from genetic screens of the bacterial chemoreceptor. In particular, the role of helix rotation in chemoreceptor signalling processes is clarified and the wild type behaviour understood.

3352-Pos
Transmembrane Helix Orientation and Dynamics Examined by Ensemble Dynamics with Solid-State NMR Observables and Potential of Mean Force Calculations
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Solid-state NMR (SSNMR) experiment is a very powerful technique to describe the orientation of membrane proteins and peptides in their native membrane bilayer environments. Such orientational description is invaluable since the function of membrane proteins and peptides involves a conformational change that often associates with their orientational changes. However, the present models used to interpret the SSNMR observables are rather static, and, in consequence, important motional information can be missing. In this work, we have investigated the orientation of single-pass transmembrane domain of virus protein ''u'' (Vpu) from HIV-1 by determining ensemble of structures using multiple conformer models. The resulting ensemble of structures showed significantly larger fluctuations in their orientation while averaged orientation maintained the compatible value with the static model. Further, we have compared the SSNMR ensemble dynamics results with the Vpu orientation from the molecular dynamics simulations and helix-tilt free energy calculations in explicit membranes.

3353-Pos
Membrane Insertion of a Voltage-Sensor Helix
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Membrane-spanning α-helices of membrane proteins are generally composed of a core of largely hydrophobic amino acids, with basic and aromatic amino acids at each end of the helix forming interactions with the lipid headgroups and water. In contrast, the S4 helix of ion channel voltage sensor domains contains 4 or 5 basic (largely Arg) sidechains along its length and yet adopts a transmembrane (TM) orientation. Multi-scale molecular dynamics (MD) simulations are used to explore how a charged TM S4 α-helix may be stabilized in a lipid bilayer. Free energy profiles for insertion of the S4 helix into a phospholipid bilayer suggest that it is thermodynamically favourable for S4 to insert from water to the centre of the membrane, where the helix adopts