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 a TM orientation. This is consistent with crystal structures of Kv channels and with experiments on translocon-mediated S4 helix insertion. Decomposition of the free energy profiles reveals the underlying physical basis for TM stability, whereby the preference of the hydrophobic residues of S4 to enter the bilayer dominates over the free energy penalty of inserting charged residues, accompanied by local distortion of the bilayer and penetration of waters. The unique combination of charged and hydrophobic residues in S4 allows it to insert stably in the membrane but yet fulfil its role as a voltage-sensing device.

3354-Pos

Structural Model of the Voltage Sensing Domain in Ci-VSP

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CiVSP is a voltage sensor-containing phosphatase whose N-terminal comprises a voltage sensing domain (VSD). Its lack of a conduction pore makes CiVSP an excellent model to study conformational changes in VSDs. Although the structure of CiVSP's VSD is unknown, it shares sequence homology with the VSDs of potassium channels with known crystal structures. We have taken advantage of this similarity to generate a model of CiVSP's VSD using the program ESyPred3D. The stability of the model was tested using the molecular dynamics simulation package, NAMD. After equilibration, we imposed an electric field to mimic a membrane potential of -200mV at 300K for a total simulation time of 20ns. One feature of the model is that residues R223 to R229 adopt a 3-10 helical structure with polar residues facing into the protein core. Meanwhile, water molecules in the interior of the helical bundle form an hourglass-shaped profile resembling those seen in Kv1.2 simulations. The water crevices are separated by a hydrophobic plug limited outside by R223 interacting with L155 and inside by R226 interacting with D159. These interactions serve as water barriers that focus the electric field. Experimentally, we have observed that the CiVSP mutant R217Q R223H produces a proton current at negative potentials. Simulations of the present model carrying these mutations effectively displayed a narrowing of the hydrophobic plug, allowing water molecules from the top and bottom crevices to come into close proximity, while R226 prevents the collapse of the hydrophobic plug. This effect might create the optimal conditions for proton conduction. We conclude that our model of the 'resting'state of CiVSP's VSD contains features that are consistent with experimental observations and is a good starting point to study conformational changes in VSDs. (Support: GM062342, GM030376)

3355-Pos

Computational Studies of Colicin Insertion into Membranes: the Closed State

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Colicins are water-soluble toxins that, upon interaction with membranes, undergo a conformational change and form pores in them. Pore formation activity is localized in one of the three domains common to all colicins; a 10 alpha-helical bundle termed the pore-forming domain (PFD). There is evidence that colicins attach to the membrane via a hydrophobic hairpin embedded in the core of the PFD. Two main models have been suggested for the inserted state of colicins: the penknife and the umbrella models, differing in regard to the orientation of the hydrophobic hairpin with respect to the membrane. To describe the arrangement of the other helices in the PFD, also two main descriptions have been used: a compact three-dimensional structure or a two-dimensional array of loosely interacting helices on the membrane surface. Using Molecular Dynamics simulations with an implicit membrane model, we have studied the stability and conformational characteristics of the different conformations possibly involved in the process. Our results give support to the idea that the two hydrophobic hairpin orientations (penknife and umbrella) are in equilibrium in the closed state of the channel. According to our results, this mechanism can be generalized within the different colicin groups. However, the details may differ from one colicin to another. Additional proposed physical events, such as helix elongation or membrane thinning, are also studied in this work. It is shown how these events relate to the stability of the closed state of the colicin pore.

3356-Pos

Molecular Dynamics Simulations of a Bacterial ABC Transporter

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ABC (ATP Binding Cassette) proteins are a large family of membrane transporters dependent on ATP hydrolysis. Members of this family are of interest due to their role in multidrug resistance by bacteria, chemotherapy resistance by tumors, cystic fibrosis, and adrenoleukodystrophy. Transporters consist of a nucleotide binding domain (NBD) and a transmembrane domain (TMD), and in the case of importers, a periplasmic binding protein that binds the substrate and delivers it to the periplasmic side of the transporter. In bacteria, transporters are often symmetric, existing as a homodimer containing two copies of the same polypeptide chain. While the substrates of ABC transporters are many and varied, the NBDs at which ATP hydrolysis occurs are highly conserved across all species. The structure of several members of the ABC family have been solved by X-ray crystallography, however the mechanism by which ATP hydrolysis is linked to the domain rearrangement that facilitates transport is unclear. Molecular dynamics simulations allow the dynamics of a representative ABC importer, the vitamin B12 uptake system (BTU) from E. coli, to be modeled at atomistic detail. The protein is composed of two copies each of BtuC, the TMD, and BtuD, the NBD, as well as the periplasmic binding protein, BtuF. Simulations of the protein in the presence and absence of ATP have been performed in order to observe the effects of ATP binding and hydrolysis on the arrangement of the protein. Simulations of the complete transporter, BtuC2D2F, demonstrate the influence of the binding protein on the structure of the complex.

3357-Pos

Structural Quality and MD Simulations of Homology Models of Major Facilitator Superfamily (MFS) Transporter Proteins

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Title: Structural Quality and MD Simulations of Homology Models of Major Facilitator Superfamily (MFS) Transporter Proteins

The MFS is a large group of transporter proteins that catalyze uniport; solute: cation symport; and/or solute:cation or solute:solute antiport. Currently, relatively few experimental structures are known for these membrane transport proteins. Homology modeling enables us to extrapolate from X-ray structures of (bacterial) MFS proteins. However, quality assessment of homology models is crucial, especially given the relatively low resolution of some of the template structures. Furthermore, although structurally conserved, the MFS transporters do not share high sequence identity (i.e. less than 30% identity is observed). In this work, homology models of MFS were constructed based on crystal structures of lactose permease (LacY, PDB ID 1PV7), GlpT (PDB ID 1PW4) and the multidrug transporter EmrD (PDB ID 2GFP). These MFS model structures, as well as MFS crystal structures, were subjected to atomistic and coarse grained (CG) MD simulations. Quality assessments and measures of conformational ''stability'' of the simulated structures were compared for homology models and X-ray structures. It was concluded that local stereochemical quality of MFS protein structures does not predict conformational ''stability'' of the protein in MD simulations. An extensive stereochemical analysis based on known membrane protein crystal structures and corresponding MD simulation data was used to assess the MFS model structures. It was shown that MD stereochemical profiles can be used to distinguish between models constructed based on high quality template structures, models constructed based on a low quality template structures, and 'decoys'(i.e. models constructed based on a incorrectly chosen templates).

3358-Pos

In Silico Investigations of Possible Routes of Assembly of 3a from SARS-Co Virus

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Most recently ORF 3a of SARS-CoV has been identified as a 274 amino acid membrane protein of human severe acute respiratory syndrome corona (SARS-Co) virus. When expressed in Xenopus oocytes the protein forms channels. Based on bioinformatics approaches the topology has been identified to include three transmembrane domains (TMDs).

Since structural models from experiments are still lacking, computational methods can be challenged to generate such models. In this study, we select a 'sequential approach'for the assembly protocol in which the individual TMDs are assembled one by one. This approach is seen to mimic in vivo expression of the protein. The role of different force fields on the assembly and the dielectric of the lipid bilayer are evaluated. Also the role of the loops between TMDs is investigated. Potential models and pore lining motifs will be shown. MD simulations for 20 ns are performed to assess the bundle stability in a lipid environment. The MD results show that the pore lining motif of the bundle is due to residues from TM2 which generate apore full of water molecules supporting the finding of 3a as an ion channel.