with downstream effectors as a dimeric complex. Currently, crystal structure data of 2.34 Angstrom resolution is available for *A. Thaliana* Gα. The mechanism of activation for the plant heterotrimer is generally inferred by assuming analogy with the mammalian complex. We cloned and expressed *Arabidopsis Thaliana* α subunit (GPA1) using *Pichia Pastoris* and β(AGB1) and γ (AGG2) subunits using *E. coli*. Results show that the recombinant GPA1 forms higher oligomeric structures in solution which can be altered by exchange of bound GDP with GTP, by addition of GTPγS and by the presence of membrane mimetic compounds. We are able to express GPA1 in excessive amounts by using *P.Pastoris* and purify it by using FPLC system. However, since expression in yeast is more time and resource consuming compared with E. *Coli*, we have been trying to clone GPA1 sequence by using alternative methods. In this poster, details of these alternative cloning strategies for GPA1 such as using pETM41, pQE80 vectors and LIC system will be given. Additionally, results of structural characterizations of recombinant GPA1's produced using alternative vectors will be presented together with circular dichroism spectropolarimetry, dynamic light scattering and small angle X-ray scattering.

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Molecular Dynamics Study on Fluctuation Analysis of Mechano-Gating in the Bacterial Mechanosensitive Channel MscL

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One of mechanosensitive channels, MscL, is homopentamer of a subunit with transmembrane inner (TM1) and outer (TM2) α-helices and the closed form of 3D structure is solved. Neighboring TM1s cross and interact each other near the cytoplasmic side through hydrophobic interactions between Leu19-Val23 in one TM1 and Gly22 in the neighboring TM1, forming the most constricted part of the pore called gate. Among amino acids in TM2 facing the bilayer, Phe78 showed exceptionally strong interaction with lipids. Upon membrane stretch, Phe78 was dragged by lipids, leading to an opening of MscL. Thus Phe78 was concluded to be the major tension sensor. The major issue of MscL is to understand the detailed gating mechanism driven by tension in the membrane. To address this question, we have performed molecular dynamics (MD) simulations for the opening of MscL, however, it remains unclear the relationship between tension sensing and the following gate opening. In this study, in order to get insight into the coupling between a mechanosensor and the gate, we performed MD simulations to analyze thermal fluctuations of the whole protein using principal component analysis. We modeled wild type (WT), F78N, G22N and G22N/F78N mutant MscLs and firstly equilibrated for 40 ns to sample 5000 coordinate data sets. Then we challenged the channel opening under the condition of 150 dyn/cm membrane stretch to find differences of fluctuations between WT and mutant MscLs. As a result, we found that the fluctuations of mutants were different from the one of WT especially in the periplasmic loop connecting TM1 and TM2 helices. This result suggests that the substitution of Phe78 and/or Gly22 with Asn changes the dynamics of MscL itself and affects its opening behavior.

280-Pos Board B35

Molecular Dynamics Studies on Structural Changes in NK-Lysin and Saposins A, C, and D

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NK-lysin and the saposins A, C, and D are proteins belonging to the saposin-like family. They are small, nonenzymatic, heat-stable lysosomal proteins essential for the degradation of sphingolipids and lipid antigen presentation. For both the soluble and membrane-associated states of several saposin-like proteins, high-resolution structures have been determined which suggest the possibility of reversible transition between closed and open states. Such dynamic changes are, however, difficult to study experimentally. In this work we investigate structural changes in saposins A, C, and D, and NK-lysin using both atomistic and coarse-grained (CG) molecular dynamics simulations. Firstly we compare the outcome of two microsecond atomistic simulations using the AMBER99SB-ILDN and GROMOS45a3 force fields with respect to the stability of the proteins' ''saposin fold''. We also employ 100 microsecond CG simulations using the MARTINI force field, and compare the effect of using the standard and polarizable water models on conformational changes of the proteins.

281-Pos Board B36

Unraveling the Energy Transduction Mechanism of Resilin Elasticity-A Combination of Computational and Experimental Study

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Resilin, known as a ''super elastic rubber'', has drawn intensive research interests for its striking energy transduction and storage properties. However, due to lack of *atomic* structures of this protein and its constituent domains, no models with *atomic* details are available up to date to enable an in-depth understanding of the energy transductions and storage mechanism in resilin. In our current study, with model peptides, we explore the structures of the high-hydrophobicity sequence and the repeating unit of the Exon III domain of resilin *at the atomic level*. Bioinformatics method predicts interesting β-sheet and β-turn structures for the above respective sequences, which is consistent with earlier and present experimental acquisitions. Long-time molecular dynamics simulations with the explicit solvent model further confirm these structural features. The peptide secondary and tertiary structures, and peptide-water interactions are investigated in details with simulations, and the results are compared to experimental measurements from Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). The present study offers an atomistic view of resilin and elastomeric proteins in general where β-turn-related structures serve as fundamental units of the structure and elasticity.

282-Pos Board B37

Simple Models Characterize the Activation of G Protein-Coupled Receptors

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G Protein-Coupled Receptors, the largest family of proteins in the human genome, are integral membrane proteins responsible for transducing signals across the cell membrane. They play vital roles in vision, olfaction, taste and many other processes. They are major drug targets, so understanding their activation mechanism may lead to better drug design.

In this investigation, we studied the activation and deactivation of two class-A GPCRs, rhodopsin and β2 Adrenergic Receptor (B2AR), using computational methods. Specifically, we used structure-based potentials (Gō-models) to rapidly simulate the transition from the inactive state to the active state, as well as the reverse process. We monitored the transition using a biologically motivated quantity, as well as a novel tool based on principal component analysis of inter-residue contacts. The latter approach proved to be more informative, and helped us develop novel insights into the difference in activation pathway for structurally similar proteins.

283-Pos Board B38

Understanding Side-Chain Motions using Statistical Torsion Angle Potential

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To understand protein functions, an accurate protein structure is required. Because protein side-chain affects protein-protein interaction and protein-molecule interaction in tertiary protein structure, side-chain mobility is particularly important for understanding protein function. In general, determination of side-chain population is experimentally difficult, so we used computational method: Statistical Torsion Angle Potential (STAP) with Φ-χ1, Ψ-χ1 and χ1-χ2 combinations and simulated annealing protocol are used to predict side-chain mobility. Order parameters (S^2) of methyl carbon and their correlation coefficients are evaluated for accuracy check. The computational prediction of χ1, χ2 rotamer population is performed for 17 target proteins. Seven target proteins are used for training set to find optimal values of van der Waals and STAP force constant. Ten target proteins are used for test set. This study can be used to characterize the χ1, χ2 conformation of exposed residues and to understand side-chain motions.

284-Pos Board B39

Conformational and Dynamic Properties of Extracellular Domains of Cell Adhesion Molecules

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Due to their specific binding properties, cell adhesion molecules (CAMs), such as integrin, cadherin and the immunoglobulin superfamily CAMs are of