polymerase and thus has a significantly larger radius of gyration. The combined data indicate that the folded state of the thermophilic polymerase is not more intrinsically stable; instead, its more globular denatured state has a considerably reduced entropic and structural barrier between the denatured and native states, resulting in a much more favorable ΔG of folding. The data also indicate that the stability-linked evolutionary differences between the two proteins are expressed primarily in the denatured state. This is the first direct structural demonstration of denatured state size-shape differences in a mesophile-thermophile protein pair. Despite their demonstrably different sizes and structures, both denatured state ensembles still fall within the range of random-coil behavior.

2059-Pos Board B78
Statistical Mechanical Model for the Transfer Free Energy of Amino Acids in the Context of Membrane Protein OmpLA
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Transfer free energy scale of amino acids is fundamental for understanding membrane protein folding and for predicting membrane protein structures. Several experimental studies have been carried out to measure the free energy of partitioning amino acids to artificial lipid bilayer or biological membranes in the context of model peptides. A recent study measured water-to-bilayer transfer free energy in the context of a native β-barrel transmembrane protein OmpLA. Here we report the development of a computational free energy scale based on an empirical potential energy function and statistical mechanical model. Our energy function incorporates the energy contribution of single-body burial, inter-strand interaction and sequential nearest neighbor contact interaction. Using a statistical mechanical model with a reduced state space, we computed the full partition function of OmpLA and the relative insertion free energy of amino acids replacing Ala. The computed relative free energy scale correlates well with experimental data (r² = 0.79 with water-to-bilayer scale [1] and r² = 0.88 with translocon scale [2]). In addition, depth dependency profiles of Arg and Leu are in excellent agreement with those measured by Moon and Flemming [1] and we also obtained depth dependency profiles of all 20 amino acids which provide insight to the folding and insertion of the toxin.

2060-Pos Board B79
Functional Expression of a β-Scorpion Toxin in the E.Coli Periplasm: A Tool for Exploring Sodium Channel/Ligand Interactions
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β-scorpion toxins are polypeptides of 60-76 residues that bind to site 4 of voltage-gated sodium channels and induce a hyper-polarising shift in the voltage-dependence of activation. The domain II voltage sensor is trapped in its outward, activated conformation through interactions with the toxin. β-scorpion toxins represent lead candidates for novel therapeutics and insecticides but their expression in bacterial systems is not straightforward; the reducing cytoplasmic environment hinders formation of four disulphide bridges that stabilise the toxin structure. Therefore reports to-date of recombinant β-toxin production include an oxidative-refolding step which follows initial purification of the mis-folded peptide. We report an expression strategy that produces correctly-folded β-toxin in E.coli. FPLC and HPLC analysis of β-toxin purified to homogeneity indicated that the protein adopts a single conformation. Crystallization and structure determination to 2.5 Å resolution confirmed that the β-toxin adopts the functionally-active form. Additionally, thermal denaturation studies using synchronous thermal circular dichromism (SRTCD) – the first application of this sensitive CD technique to this toxin class – demonstrated the stability of this cross-linked toxin. The methodology applied to produce this toxin may facilitate industrial scale-up or lab-based production of wild-type and mutant toxins for ligand interaction studies.

2061-Pos Board B80
Global Multi-Method Analysis of Affinities and Cooperativity in Complex Systems of Macromolecular Interactions
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Cooperativity, multi-site and multi-component interactions are hallmarks of biological systems of interacting macromolecules. Their thermodynamic characterization is often very challenging due to the notoriously low information content of binding isotherms. We introduce a strategy for the global multi-method analysis of data from multiple techniques (GMMA) that exploits enhanced information content emerging from the mutual constraints of the simultaneous modeling of orthogonal observables from calorimetric, spectroscopic, hydrodynamic, and biosensing experiments. We describe new approaches to address statistical problems that arise in the analysis of dissimilar data sets. The GMMA approach can significantly increase the complexity of interacting systems that can be accurately thermodynamically characterized.

2062-Pos Board B81
In Silico Investigations of Possible Therapeutic Peptides as Drugs for Anti-Inflammatory Responses
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The chemokine called interleukin-8 (IL-8 or CXCL8) plays a central role in human immune response by binding and activating the receptor CXCR1 that belongs to the G-protein coupled receptor (GPCR) family. Upon binding of CXCL8, CXCR1 undergoes conformational change resulting in signal transduction. The purpose of this study was to determine the role of the fragments of the CXCL8 binding sites of CXCR1 as potential therapeutic targets for developing novel drugs against inflammatory diseases. Homology modeling studies were firstly performed to construct the three-dimensional structure of CXCR1 by taking the crystal structure of bovine rhodopsin (PDB code: 1U19) as the template with the sequence similarity up to 41.8 %. By the following molecular docking programs several potential peptides were selected and synthesized to validate the anti-inflammatory efficiency through flow cytometry analysis. The preferred complexes of CXCL8 binding CXCR1 from docking predictions were then embedded into POPC lipid bilayers for 50 ns MD simulation to investigate the binding interaction. Simulation results show that electrostatic interaction dominates the binding of CXCL8 and CXCR1, consistent with the previous experiments. Small peptide drugs are novel therapeutic candidates for inflammatory diseases. High-throughput, structure-based virtual screening combining MD simulations is an effective computer-based drug design method for discovering anti-inflammatory drug candidates.

2063-Pos Board B82
Effects of Extracellular Calcium on the Modulation of Metabotropic Glutamate Receptor 1 Alpha (mGluR1α) by L-Quis, (S)-Mepg, Cpecoet and Ro 67-4853
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Metabotropic glutamate receptor 1α (mGluR1α), known as a member of the family C GPCRs, couples to Gq and modulates consequent PLC activity, IP3 accumulation and intracellular Ca2+ release from ER lumen. mGluR1α is abundantly expressed in the central nervous system and has been shown to be responsible for the slow phase of the action potential in post-synaptic neurons, and to be involved in chronic neuronal degenerative diseases, like Parkinson’s disease, Huntington’s disease and Alzheimer’s disease. We have predicted a potential Ca2+ binding site adjacent to the binding site previously reported for the endogenous agonist, glutamate, and receptor antagonists. In this study, we have applied single cell imaging and a radioactive binding assay to probe the effects of extracellular calcium in modulating various drugs modulating mGluR1α such as agonists, antagonists and allosteric modulators. We have shown that extracellular Ca2+ enhances the activation of the receptor by its agonists and...
positive allosteric modulators by interacting with the Ca\(^{2+}\) binding site. In addition, extracellular Ca\(^{2+}\) differentially reduces the inhibition of the receptor by antagonists and negative allosteric modulators. Our studies open a new avenue for modulating drug effects and developing novel drugs against neurodegenerative diseases.

2064-Pos Board B83
Improved Quantitative Modeling of Ligand-Activated Macromolecular Receptors using Conditional Binding
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Experimental investigations of ligand-activated receptors are often based on binding measurements that detect the total fraction of occupied binding sites at sites emanating from cells. We use calmodulin, an important calcium sensor protein, as a test system to document the uncertainties in model parameters estimated by fitting various models to total binding data. Using nonlinear least-squares methods, we obtain excellent fits (<1% RMS error) to synthetic total binding data with the same characteristics as the published binding data using parameter sets with binding affinities varying by over four orders of magnitude for each site. This result identifies a significant obstacle blocking progress toward the goal of developing accurate, quantitative models of receptor activation. The use of noiseless data in our analysis suggests that the large uncertainties in the estimated parameters are not a problem of data quality, but rather reflect an intrinsic limitation of total binding data. Using analytical matrix algebra techniques and numerical simulations, we discover a fundamental relationship between the mathematical structure of the equations describing various types of binding data and the number and type of parameters that may be determined accurately from regression analysis of that data. Ideas based on Boolean logical principles are used to design a new type of binding experiment that significantly improves upon total binding data in its power to constrain physically realistic models of receptor activation. These experiments, which we call conditional binding, report on the simultaneous occupancies of two different receptor binding sites. Our approach is general and the conclusions are applicable to the many macromolecular systems that are activated or modulated by ligand binding.

2065-Pos Board B84
Modeling Complex between FBA and TIM: Functional Motions of FBA and TIM are Preserved in their Complex
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Fructose bisphosphate aldolase (FBA) and triosephosphate isomerase (TIM) are the fourth and fifth enzymes in the glycolysis pathway and they are known to bind FBA cleaves the six-carbon fructose 1, 6-bisphosphate to two three-carbon components, dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP). TIM converts DHAP into GAP, a substrate for the subsequent synthetic step. These two alpha/beta barrel proteins have high structural similarity – with a core RMSD 4.8Å. These two enzymes have low activites in the monomeric form with the functionally active structures present in higher oligomeric states. By applying Elastic Network Model, we find the modes of motions that are functionally important for these proteins. We build models for these complexes and investigate their important motions in their complexes as well as for their different oligomeric states including those that are different in different species to learn their important modes of motions for different functionalities. For each protein, by multiple sequence alignment across the species, we predict the coevolving residues and cluster these residues along the structure. We build the information transfer pathways from the important interface residues to the catalytic residues. Change in these pathways in different oligomeric states may be related to the change of motions in the catalytic region in different oligomeric states. We use this knowledge about the changes in motions and the information transfer pathways within the structure of these interacting proteins as constraints for selecting the computational docked models of complexes between these two proteins to preserve their functional motions.

2066-Pos Board B85
How Can a Ligand be a Positive and Negative Allosteric Effector for the Same Protein?
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For several transcription factors, the same ligand can act as a positive and negative allosteric effector in a context dependent manner. The structural and molecular bases of such effects are unknown. Here it is shown that modulation of conformational fluctuations within allosteric systems can be used as a mechanism to tune protein ensembles such that a given ligand can act as both a positive and negative allosteric effector. Importantly, this mechanism can be readily encoded in the cell, does not require that the interactions between the ligand and the protein differ when it is acting either as a positive or negative effector. Instead, the effect is due to the relative probabilities of states prior to the addition of the ligand and is encoded in the thermodynamic coupling architectures between protein domains. The ensemble view of allostery that is illuminated by these studies suggests that rather than being seen as switches with fixed responses to allosteric activation, ensembles can evolve to be “functionally pluripotent”, with the capacity to up or down regulate activity in response to a stimulus. This result not only helps to explain the prevalence of intrinsic disorder in transcription factors and other cell signaling proteins, it provides important insights about the energetic ground rules governing site-to-site communication in all allosteric systems.

2067-Pos Board B86
Allosteric Modulation of WT and H1047R Mutant PI3K\(_{\alpha}\) Investigated by MD Simulations
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Kinasas are one of the most intensively pursued drug targets investigated for the treatment of cancer. Kinase inhibitors usually target the ATP binding site; however, the similarity of this site across many kinases often results to non-selectivity. Therefore, allosteric modulation of kinases is of paramount importance as it may result in increased selectivity; many highly selective inhibitors have been reported to inhibit kinases by allosteric mechanisms. PI3K\(_{\alpha}\) is the most frequently mutated kinase in human cancers with one of its most common mutations being a histidine changed to arginine in exon 20 (H1047R). PIK-108, a known PI3K\(_{\alpha}\) inhibitor, was recently found to occupy an allosteric binding pocket in the wild type (WT) murine protein, close to the H1047R mutation. In order to assess the interactions, stability and allosteric effects of the inhibitor on PI3K\(_{\alpha}\), MD simulations in aqueous solution were performed for 130ns for the WT human, WT murine, and H1047R human mutant proteins with PIK-108 placed in both catalytic and allosteric sites. Interestingly, PIK-108 remained stable in both sites in all three variants. While in both the WT human and murine forms, the same ligand:protein interaction motifs are observed in the allosteric and catalytic pockets, these interactions are markedly different in the mutant form. In the mutant form, the allosteric pocket opens up and forms an altered hydrogen bond network with the ligand compared to the WT. Additionally, in the catalytic pocket, significant differences are evident in the interaction network formed between the inhibitor, P-loop, and the activation loop between the two protein forms. Overall, the ligand:protein interaction differences between the mutant and WT PI3K\(_{\alpha}\) proteins observed in the present study provide a rich basis for the design of mutant-specific PI3K\(_{\alpha}\) inhibitors.

2068-Pos Board B87
Hetero Interaction with an Amino Acid Globally Enhances Cooperative Activation of CaSR in Response to Extracellular Signaling
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Calcium sensing receptor (CaSR), along other members of the family C G protein-coupled receptors (GPCRs), play very important roles in responding to changes in the extracellular calcium concentrations and in circulating levels of amino acids and integrating these extracellular signals into alterations in intracellular signaling pathways. However, detailed structure properties of the CaSR which are necessary to characterize the mechanism of its physiological function are still unrevealed. We have reported several potential calcium-binding sites located within the CaSR’s extracellular domain using our developed computational algorithms. In the present study, we first report the differential effects of several disease-related mutations located at the predicted calcium binding sites on the inhibition and activation of intracellular calcium responses using single cell imaging. Mutating to different residues at two locations near the hinge region of the ECD could lead to either significantly lose of function of the receptor or gain of function (switch function mutations). Amino acid binding results in differential rescue effect in altering intracellular calcium responses, especially calcium oscillations. We have further probe the effect of mutation and amino acid binding on the correlation motion, cooperativity, and synergistic activation using mammalian expressed and purified.