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 various concentrations. 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 biphosphatase aldolase (FBA) and triosephosphate isomerase (TIM) are the fourth and fifth enzymes in the glycolysis pathway and they are known to bind FBA. FBA cleaves the six-carbon fructose 1, 6-bisphosphate 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 active site mutations, allowing for elastic network model, we find the modes of motions that are functionally important for these proteins. We build models for the complex between these two proteins to 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. Importantly, this mechanism can be robustly encoded in the cell, and does not require that the regulation 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.

Relevant citation


2067-Pos Board B86

Allosteric Modulation of WT and H1047R Mutant PI3Kz Investigated by MD Simulations

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Kinasizes 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. We have reported several potential calcium-binding motifs for the complex between these two proteins to investigate their importance. The structural and functional properties of the PIK-108 placed in both catalytic and allosteric sites. Interestingly, PIK-108 remained stable in both sites in all three variants. While in both 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 WT human and murine proteins observed in the present study provide a rich basis for the design of mutant-specific PI3Kz 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 regulating 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...
A cofactor and potential inhibitors of EZH2.

2069-Pos Board B88

Synthetic Demethylwedelolactone Derivatives Inhibit Invasive Growth of Mda-Mb-231 Breast Cancer Cells In Vitro and In Vivo

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Combretastatins, which are an important group of anticancer drugs, were isolated by Pettit et al. from the African tree Combretum caffrum in 1989. Additionally, Liang et al. have reported that ten coumestans were isolated from the roots of Hedysarum multijugum, which is a plant in Hedysarum Linn. of the family Leguminosae used as a folk herbal drug in northwest China. Coumestans comprise a class of naturally occurring products with a variety of biological activities including phytoestrogenic, antibacterial, antifungal, antimyotoxic, and phytoalexine effects. The anticancer properties of demethylwedelolactone (DWEL) and wedelolacolactone (WEL), which are naturally occurring coumestans, have not been well characterized. Due to their biological activities, the synthesis of DWEL is achieved in which the longest linear sequence is only eight steps in 38% overall yield from commercially available phloroglucinol. Furthermore, the molecular model was examined the interactions of proteins and ligands to DWEL. Finally, they conduct the anti-invasive effects of synthetic WEL and DWEL on human MDA-MB-231 breast cancer cells. We found that WEL and DWEL inhibited the anchorage-independent growth and also suppressed cell motility and cell invasion of MDA-MB-231 cells. In addition, WEL and DWEL reduced the activity and expression of matrix metalloproteinases (MMPs) involved in blocking the IκB-α/NSF-B and MEK/ERK signaling pathways in MDA-MB-231 cells. Furthermore, DWEL suppressed the metastasis and lung colonization of the tumor cells in the nude mice. Altogether, these data suggest that DWEL derivatives exert anti-invasive growth effects on breast cancer cells.

2070-Pos Board B89

Molecular Dynamics of DOT1L and Modeling of EZH2

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Histone methyltransferases are enzymes that modify histone proteins via methylation of lysine or arginine residues. These epigenetic modifiers, such as DOT1L and EZH2, have been found to play important roles in leukemogenic processes. Crystallographic and docking methods studied interactions within the DOT1L binding site. Crystal structures of DOT1L also demonstrated variance in the binding mode of ligands, possibly due to rearrangement in the DOT1L binding site. We investigated this possibility by molecular dynamics (MD) simulations and confirmed significant rearrangement of the substrate binding and activation site. We investigated this possibility by molecular dynamics (MD) simulations and confirmed significant rearrangement of the substrate binding and activation site. Crystal structures of DOT1L also demonstrated variance in the binding mode of ligands, possibly due to rearrangement in the DOT1L binding site. Crystal structures of DOT1L also demonstrated variance in the binding mode of ligands, possibly due to rearrangement in the DOT1L binding site. Crystal structures of DOT1L also demonstrated variance in the binding mode of ligands, possibly due to rearrangement in the DOT1L binding site. Crystal structures of DOT1L also demonstrated variance in the binding mode of ligands, possibly due to rearrangement in the DOT1L binding site.

2071-Pos Board B90

T Cell Receptor Specificity, Cross-Reactivity, and MHC Restriction are Inextricably Linked as Result from Cooperative Engagement of the Composite Peptide/MHC Surface

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T cell receptors (TCRs) recognize peptides bound and presented by major histocompatibility complex (MHC) proteins using multiple complementarity determining region (CDR) loops. While numerous analyses have illuminated structural and biophysical aspects of TCR recognition, how the distribution of binding free energy within TCR-pMHC interfaces promotes unique TCR recognition features, including MHC restriction and the apparent dichotomy of specificity and cross-reactivity, remains unclear. Utilizing double mutant cycles, here we performed a comprehensive structural and thermodynamic deconstruction of the interaction between the A6 TCR and the Tax peptide presented by the class I MHC HLA-A2. In contrast with general expectations, we observed that the central regions of the peptide and its interactions with the hyper-variable CDR3 loops contribute little to specificity, instead promoting by dynamic effects the cross-reactivity that is a hallmark of TCR recognition. We also observed that TCR restriction towards HLA-A2 results from not conserved interactions with the germline loops, but instead from strong interactions with the hypervariable CDR3 loop of the z chain. Formation of these latter interactions, however, is dependent upon the unique structural properties of the peptide, highlighting that TCR specificity towards peptide and MHC can emerge from the need to engage a unique, composite peptide/MHC interface with tightly coupled structural properties.

2072-Pos Board B91

Dissecting Signal Control in the Multidrug Sensor, BMRR

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Multidrug (MD) (or xenobiotic) cacyx actively removes cytotoxic chemicals from the interiors of normal-functioning cells. However, high levels of cacyx can render drug-targeted cells resistant to a broad-range of therapeutic agents, including those to which cells were never exposed. Key multidrug resistance (MDR) contributors include allosteric cacyx pumps, gene regulators and regulatory systems that mediate the detection and extrusion of diverse drugs from cellular environments. To date, MDR functions remain only partially understood. Ligand-dependent allosteric control in BmrR has been qualitatively addressed using in vitro transcription experiments, dose-response curves and thermodynamic models that relate the observed transcriptional responses to ligand binding and changes in BmrR conformation. Preliminary results indicate that allosteric control in BmrR is sensitive to both energetic and structural aspects of ligand recognition. Importantly, increased cooperativity in signal control relative to recognition implicates a major allosteric role for the RNA polymerase.

2073-Pos Board B92

Fragment-Based Approach Identifies a Novel Inhibitory Site on DHPS

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Dihydropteroate synthase (DHPS) is an essential enzyme in the bacterial folate biosynthetic pathway. It catalyzes the condensation of 6-hydroxymethyl-7,8-dihydropterin (DH) with p-aminobenzoic acid (pABA) to form the folate intermediate, 7,8-dihydropterdoate. DHPS is the target of the sulfonamide class of antibiotics. Widespread resistance to sulfonamides has decreased their clinical use. The active site of DHPS is comprised of three sub-sites: the structured “pterin” site, the flexible pABA site, and the anion binding pocket. Most of the drug resistant mutations have been mapped to the pABA site. Using an NMR ligand-based screening approach, a number of allosterically unrelated fragment-like small molecules have been identified that inhibit the enzymatic activity of DHPS from Bacillus anthracis (Ba), Yersinia pestis (Yp), and Staphylococcus aureus (Sa). Fragment hits were shown to target the three sub-pockets of the active site and a novel site distinct from the active site. The latter site potentially inhibits via an allosteric mechanism and has been characterized by high resolution X-ray crystallography. We screened the Maybridge fragment library of 1,100 fragments using water ligand observed gradient spectroscopy (waterLOGSY) as a primary screen which resulted in a hit rate of 6.7 %. Of the 74 hits, 25 were shown to inhibit DHPS activity using two independent enzyme activity assays. A total of eight compounds inhibited the activity of DHPS from three different species (Ba, Yp, and Sa). In addition to screening for inhibition, the fragment hits were validated using a number of biophysical techniques including 2D NMR, SPR, competition waterLOGSY, and X-ray crystallography. Herein, we focus on two fragment hits for which high-resolution x-ray crystal structures are available.