containing residues 227-236, which exhibits little sequence identity between the various PFKs. Currently Tt(222-242)/LbPFK is being characterized to determine its potential role in PEP binding. The only mutation to show enhancement in PEP binding in LbPFK is D12A (5-fold), which is located on the active site interface approximately 16 Å from the allosteric binding site. The role of D12A is LbPFK is currently under investigation. Funding provided by NIH grant GM33216 and Welch Foundation grant A1548.

204-Pos

Surface-Exposed Hydrophobic Residues on Small Ankyrin-1 Mediate Binding to Obscurin

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Small ankyrin-1 (sAnk1, Ank1.5) is a splice variant of the ANK1 gene that binds to the large modular protein, obscurin A, with nanomolar affinity, a reaction that may help to organize the sarcoplasmic reticulum in striated muscle. A subset of lysine and arginine residues in the 2 ankyrin repeats of sAnk1 interact specifically with 4 glutamate residues in a stretch of 30 amino acids of obscurin to mediate binding. Homology modeling and molecular dynamics simulations have revealed a ''hot spot'' of 4 hydrophobic residues exposed on the surface of the ankyrin repeat domain of sAnk1. We used site-directed mutagenesis of bacterially expressed fusion proteins, followed by blot overlays and surface plasmon resonance assays, to study the contribution of these 4 residues, V70, F71, I102 and I103, to binding to the 30-mer of obscurin. Alanine mutations of each of these four residues inhibited binding to residues 6316-6345 of obscurin ($Obsc_{6316-6345}$). In contrast, V70A and I102A mutations had no effect on binding to a second sAnk1 binding site on obscurin, located within residues 6231-6260 ($Obsc_{6231-6260}$). Using the same methods, we mutated the 5 hydrophobic residues present in $Obsc_{6316-6345}$ to alanine and identified V6328, I6332, and V6334 as critical for proper binding. Our results suggest that hydrophobic interactions as well as electrostatic interactions are important for the binding of sAnk1 to $Obsc_{6316-6345}$, consistent with studies of the complexes formed by other ankyrin repeat proteins with their ligands. Hydrophobic interactions are likely to contribute to the difference in affinity of sAnk1 for $Obsc_{6316-6345}$ and $Obsc_{6231-6260}$, and for the dominant role played by the more C-terminal sequence in binding.

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205-Pos

Clustering Method in QMMM Modeling of the HLADH Binding Site

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Some of the recent advances in quantum mechanical molecular mechanics (QMMM) related to work done on bio-molecular clusters are presented. The main framework of the discussion is related to the interface as made available in GROMACS, but also includes improvements of the same as well a newly developed clustering method and an interface code for the Massively Parallel Quantum Chemistry (MPQC) suite. The clustering method implemented provides an efficient means of studying systems undergoing large scale fragmentation processes where the Quantum Mechanical (QM) region is effectively split or large systems with several separate QM sites. Some aspects of the QMMM code will be presented as well as preliminary results from recent studies on the Horse Liver Alcohol Dehydrogenase (HLADH) binding site.

Keywords: QMMM, Clustering, ADH, GROMACS, MPQC.

206-Pos

Effects of KCL on Calmodulin Mutants Defective in Ion Channel Regulation

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Calmodulin (CaM) is an essential eukaryotic calcium sensor that regulates many ion channels and enzymes. CaM is comprised of two homologous domains (N and C), each with two calcium-binding sites. *Paramecium* mutants identified by a genetic screen to be defective in response to external stimuli showed that the two domains of CaM have different effects on ion channel regulation. Under-reactive mutants (changes in the N-domain of CaM) affect regulation of a calcium-dependent Na^+ current, while over-reactive mutants (changes within the C-domain) affect a calcium-induced K^+ current. Because CaM binds to the intracellular regions of these channels, it is subject to changing concentrations of Na^+ and K^+. This study explores the effects of potassium on the domain-specific conformation and calcium-binding energetics of under- and over-reactive mutants. Potassium-induced changes in altered thermal stability of apo (calcium-depleted) CaM explored effects on tertiary structure. Fluorescence-monitored calcium titrations over the range of 0 to 300 mM KCl showed that the total free energy of binding calcium to each domain became less favorable by about 2.5 kcal/mol. In thermal denaturation studies of apo PCaM, the melting temperature (T_m) increased by approximately 5°C and the enthalpy (ΔH) changed by 3.5 kcal/mol when [KCl] increased from 50 to 300 mM. The findings indicate that potassium ions increased tertiary constraints on apo CaM, making it less flexible. Linkage relationships resulted in lowering calcium-binding affinity. Thus, an influx of K^+ through an ion channel would shift the equilibrium of CaM towards the apo state. This effect would be exacerbated for over-reactive mutants that have intrinsically lower calcium affinity than wild-type CaM.

207-Pos

Redefining the Role of the Quaternary Shift in the Allosteric Inhibition of Bacillus Stearothermophilus Phosphofructokinase

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Bacillus stearothermophilus PFK (BsPFK) is a homotetramer that is allosterically inhibited by phosphoenolpyruvate (PEP), which binds along one dimer-dimer interface. The substrate, fructose-6-phosphate (Fru-6-P), binds along the other dimer-dimer interface. The inhibitor-bound structure compared to the substrate-bound structure of wild-type BsPFK exhibits a 7° rotation about the substrate binding interface, termed the quaternary shift. Evans, et. al. proposed that the quaternary shift is the mechanism for allosteric inhibition for BsPFK. However, the main role of the quaternary shift may be in ligand binding and not allosteric inhibition. The variant D12A BsPFK shows a 100-fold increase in the binding affinity for PEP, a 50-fold decrease in the binding affinity for Fru-6-P, and a coupling comparable to wild-type. Crystal structures of apo and PEP bound forms of D12A BsPFK both indicate a shifted structure similar to the inhibitor-bound structure of wild-type. Remarkably, D12 does not directly bind to either substrate or inhibitor, and is located along the substrate binding interface. A conserved hydrogen bond between D12 and T156 takes place across the substrate binding interface in the substrate-bound form of BsPFK. The variant T156A BsPFK, when compared to wild-type, shows a 30-fold increase in PEP binding affinity, a 17-fold decrease in Fru-6-P binding affinity, and an estimated coupling that is at least wild-type coupling. In addition, T156A BsPFK crystal structure exhibits a shifted structure similar to D12A BsPFK and the inhibitor-bound structure of wild-type. PEP still inhibits these variants of BsPFK despite the fact that the enzymes are in the quaternary shifted position prior to PEP binding. Therefore the quaternary shift of BsPFK primarily perturbs ligand binding but does not directly contribute to heterotropic allosteric inhibition. Supported by NIH Grant GM33216 and Welch Foundation Grant A1548.

208-Pos

Computational Studies of Evolutionary Selection Pressure on Rainbow Trout Estrogen Receptors

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Molecular dynamics simulations were used to determine the binding affinities between the hormone 17β-estradiol (E2) and different estrogen receptor (ER) isoforms in the rainbow trout (*Oncorhynchus mykiss*). Previous phylogenetic analysis demonstrated that a recent, unique gene duplication of the ERα subtype created two isoforms ERα1 and ERα2, and an early secondary split of ERβ produced two distinct isoforms ERβ1 and ERβ2. The objective of our computational studies is to provide insight into the underlying evolutionary selection pressure on the ER isoforms. For the α subtype our results show that E2 binds preferentially to ERα1 over ERα2. In addition, based on the phylogenetic analysis ERα2 should be free from selective pressure and accumulated a considerable amount of mutations. These results suggest that the presence of ERα2 in the genome and its lower binding affinity exhibits, at least, no deleterious effects to its host organism. For the β subtype, both isoforms bind competitively to E2. The strong binding affinity of ERβ2 suggests that the second isoform is likely on the verge of functional specialization and cannot be substituted by the first isoform.

209-Pos

Use of Crystal MD Simulations to Speed Up Evaluation of Binding Free Energies of Dimannose Deoxy Analogs With M4-P51g-Cyanovirin-N

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Molecular Dynamics (MD) presents an advanced tool for scoring of the binding free energies (ΔG) between a target protein and a set of candidate substrates, narrowed by extensive virtual screening process. Molecular mechanics(MM)/continuum model approach for evaluation of ΔG includes calculation of (i) a critically important solvation energy electrostatic contribution by means of solving the Poisson-Boltzmann (PB) or generalized Born (GB) equation and (ii) nonpolar component estimated from the solvent accessible area (SA) of solutes. Both, MM/PBSA and MM/GBSA, methods imply averaging of ΔG over a set of snapshots generated through, preferably, explicit solvent MD

simulation which is the most time demanding step. In this work we demonstrated a possibility to reduce time of conformational sampling using crystal environment simulation. Amber10 program and FF99SB/GLYCAM06 force fields combination were employed for MD simulations and free energy calculations. Six 3, 4 and 3,4-deoxy dimannose analogs were studied as ligands of the m4-P51G-CVN mutant of the potent anti-HIV carbohydrate binding agent Cyanovirin-N (1). The use of crystal over solution simulations results in at least 8 times faster generation of the equivalent length trajectories. Binding free energy ΔA estimated from crystal NVT simulation trajectories shows 0.93 and 0.94 correlation with ΔG from solution NPT simulations for MM/PBSA and MM/GBSA approaches, respectively. We also evaluated performance of the relatively new GLYCAM06 carbohydrate force field and found reasonable agreement between calculated ΔG and experimental value. Results of this study further support our earlier hypothesis about importance (for CVN specificity) of the eight-component H-bond interactions of dimannose and protein main chain atoms that also was recently observed in experiment. [1] Vorontsov and Miyashita (2009) Biophys. J., 97.

210-Pos

Computational Prediction and Experimental Validation of a Novel Binding Site for Platelet Integrin AlphaIIb-Beta3

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Fibrinogen mimetic drugs and a γ-chain peptide bind to both the αIIb and β3 subunits of integrin αIIbβ3, providing the final coordination site for the metal ion-dependent adhesion site (MIDAS) metal ion within the β3 I domain, and inducing a less compact conformation of the protein. We investigated the binding of a novel human-selective αIIbβ3 small molecular inhibitor (RUC-1) that we recently identified by high throughput screening, and predicted to bind preferentially to a pocket within the αIIb β-propeller domain by flexible ligand/rigid protein molecular docking studies. We first carried out both standard and enhanced molecular dynamics (MD) simulations of the proposed RUC-1-bound form of human αIIbβ3 integrin. The results of these studies pointed to an energetically preferred conformation of RUC-1 into the αIIb β-propeller domain that had no contact with the MIDAS metal ion or other sites in the β3 I domain. This binding mode of RUC-1 appeared to be stabilized by interactions with specific human αIIb residues, such as D224 (already known to contribute to αIIbβ3 binding), Y190 (F in both mouse and rat), and (through two water molecules) D232 (H in rat). Well-tempered metadynamics simulations of Y190F and D232H mutants supported the contribution of normal residues to the stabilization of RUC-1 in a specific binding mode and location. Functional experiments on recombinant cell lines expressing Y190FαIIbβ3 or D232HαIIbβ3 validated this hypothesis by showing a ~80-95% reduction in RUC-1 affinity. X-ray crystallography confirmed the RUC-1 binding pose suggested by MD simulations, while gel filtration and dynamic light scattering experiments showed that RUC-1 favored a compact αIIbβ3 conformation, in sharp contrast to the effect of fibrinogen-mimetic drugs.

211-Pos

Membrane Binding and Lipid Extraction Studies of Gm2 Activator Protein (GM2AP)

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GM2AP is an accessory protein that solubilizes the ganglioside GM2 from intralysosomal vesicles for hydrolytic cleavage by HexA to form GM3. The precise molecular interactions and method of extraction of GM2 from lipid vesicles are unknown. GM2AP also functions as a lipid transfer protein. This non-enzymatic protein contains three tryptophan residues (W5, W63, W131) with two of these (W63, W131) located in putative membrane binding loops. In order to investigate the possible role of the tryptophan (TRP) residues in membrane binding and lipid extraction, gel filtration and resorcinol absorption assays were used to investigate the extraction efficiency of GM2 by GM2AP in a series of TRP to ALA substituted constructs. GM2AP is shown to have two distinct substrate binding modes, one for the GM2 ganglioside and another for phospholipids. Fluorescence experiments were used to determine the orientation of dansyl-DHPE in the binding pocket of GM2AP. Quenching results suggest that dansyl-DHPE is oriented such that the head group of the lipid is located in the hydrophobic pocket of the protein, consistent with the binding mode of other phospholipids which were previously studied. Dansyl-labeled lipids were used to monitor the changes in the rates of lipid extraction and transfer by GM2AP from liposomes as a function of both pH and the TRP to ALA substituted constructs. The ability of GM2AP to bind and/or extract dansyl-labeled lipids from liposomes was affected with increased pH of the lipid environment. Additionally, removal of TRP from the putative membrane binding loops resulted in slower lipid extraction rates, suggesting that these residues are relevant for membrane binding and/or extraction of GM2AP.

212-Pos

Extracellular pH and Regulation of Integrin-Ligand Interactions

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In the tumor and wound microenvironments, the extracellular matrix often exhibits acidic extracellular pH. It is well known that acidic pH can strongly affect processes such as cell adhesion and migration. However, the molecular mechanisms governing these effects have not been established. Here, we consider the hypothesis that acidic extracellular pH directly alters the interactions between cell surface integrin receptors and ECM ligands, which are critical to cell adhesion and migration. We employed Multi-Conformation Continuum Electrostatics to predict amino acid pKa values in the integrin avb3 headpiece, and conducted molecular dynamics simulations at acidic and physiological pH to examine the effect of pH on integrin conformational states. Our results suggest that acidic pH promotes opening of the avb3 headpiece, an important step in activation that can enable more effective ligand-receptor association interactions. This has important implications for downstream cell processes in the cancer and wound environments. We also conducted molecular-level experimental approaches, including flow cytometry and atomic force microscope-enabled force spectroscopy, to further examine the role of pH in regulating integrin-ligand interactions. These molecular-level results are connected to cell-level measurements of adhesion and migration at different pH levels, providing a detailed, multi-scale understanding of how acidic extracellular pH affects cell behavior.

213-Pos

Effect of Molecular Sway on the Recognition of Peptide/MHC Complex by T Cells

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CD4+ T cell responses require the recognition of specific peptide-MHC complexes displayed by APC. It is important to determine how antigen presentations affect the ensuing T cell response. Immunizations of B10.BR mice with naturally processed peptide 48-61 of Hen egg lysozyme elicit two different types of T cell responses. First type of T cell (type A termed by Unanue et. al) respond to APC pulsed with either peptide or whole HEL protein. Second type of T cell (termed type B) respond to APC incubated with peptide but showed no response to APC pulsed with whole protein. Reactivity of the type A T cell clones correlated well with the affinity of the peptide to the MHC molecules. However, some type B T cell clone exhibit better response to the low affinity truncated (52-61) peptide than to high affinity peptide (48-61). Since weak MHC binding peptides form unstable complex, we hypothesize that type B T cells respond to the transitional conformations generated by unstable peptide/MHC complex. To test this hypothesis, we analyzed the movement of peptide/MHC complex at the single molecular level by using diffracted X-ray tracking (DXT) method. It was found that movement of the low affinity peptide/MHC complexes was different from that of high affinity peptide/MHC complexes. Moreover, comparison of the movement of a series of low affinity peptide/MHC complexes revealed clear correlation between magnitude of twisting movement of peptide and T cell recognition. Thus, our results clearly demonstrated that movement of peptide in MHC strongly affects to T cell recognition and some but not all T cells recognize a transitional conformation generated by weak binding peptides.

214-Pos

A Force Spectroscopy-Based Protein-Ligand Interaction Assay

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Binding of small molecules are crucial to the function and folding of many protein machineries inside cell. Thus it is of fundamental importance to measure the binding affinity of small molecule ligands to proteins and reveal the binding mechanism. Here we report a force spectroscopy based single-molecule binding assay that is capable of determining the binding affinity as well as the binding mechanism of ligands to proteins at the single-molecule level. This assay is based on the difference in the mechanical stability of the given protein upon ligand binding. As a proof-of-principle, we use the binding of metal ions, Ni2+, to an engineered metal binding protein, G6-53, as a model system to establish this method. The apo-G6-53 and Ni2+-bound G6-53 exhibit distinct mechanical stability: apo-G6-53 unfolds at around 120 pN while Ni2+-bound