cell cultures from mouse brain neurons and quantified dimerization of the BDNF receptor due to both direct and trans-activation via dopamine.

199-Pos

Why Is α4β2 Nachr More Sensitive to Volatile Anesthetics Than α7 Nachr?

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Two subtypes of neuronal nicotinic acetylcholine receptors (nAChRs) show different functional sensitivities to volatile anesthetics: the α4β2 nAChR is hypersensitive while the α7 nAChR is insensitive. To understand why these homologous proteins have different functional responses to volatile anesthetics, we performed multiple sets of 20-ns molecular dynamics (MD) simulations on the closed- and open-channel α7 in the absence and presence of halothane, and compared the results with those from a similar study on the α4β2 nAChR (Liu, et al. 2009). The details about construction of receptor structural models were published (Haddadian, et al. 2008). Initial halothane docking and subsequent MD simulations revealed several halothane-binding sites in α7. Consistent with observations from α4β2, free energy perturbation calculations showed that halothane had higher binding affinity in the closed- than the open-channel; α7 and α4β2 had a comparable number of high affinity sites. GNM analysis showed that halothane induced profound changes in correlated domain motion of the open-channel α4β2, especially between the Cys-loop and the TM2-TM3 linker, but had a negligible impact to the motion of the α7. Salt bridges between these loops in the β2 subunit may be responsible for the aforementioned observation. Flexibility of several key loops in the open-channel α4β2 changed considerably in the presence of halothane, but the same loops in the open-channel α7 showed little change. Taken together, our results suggest that halothane binding in nAChRs may be necessary, but not sufficient to produce essential dynamical changes that alter protein functions. Although α7 and α4β2 are homologous, specific residues in key loops may make α4β2 more susceptible to volatile anesthetics while α7 unaffected. Supported by NIH (R01GM66358, R01GM56257, and T32GM075770) and NCSA through the PSC.

200-Pos

Conformational Docking of Multiple Toxins Against Kv1-Channels Highlight Key Motifs For Selectivity

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Scorpions and other venomous predators are known to disable their prey via injection of peptides, which interfere with ion channels involved in neural signalling. These peptides are both highly specific blockers and potential scaffolds for toxin-based therapeutics of diseases such as multiple sclerosis, thus attracting continual research.

The key functional motif of toxins against Kv-channels is a dyad composed of a pore blocking lysine and a nearby aromatic residue. Several groups have also proposed the presence of 'basic ring' motifs to explain extreme selectivity for channel subtypes in several toxins. Since experimental validation of specificity is difficult due to numerous toxin-channel combinations, we sought to create a comprehensive database in-silico via the program HADDOCK. The Kv1.2 structure was used to construct its homologues Kv1.1 and Kv1.3, and submitted in blind-docking protocols versus ~30 toxins to isolate consensus binding modes. We find that all toxins share a small number of binding modes, classified by the identity of the residue inserted into the channel pore. HADDOCK outputs a near-native mode as the top-ranked pose in over 50% of runs, or ~90% if the top 5 is considered. Identification of other modes, e.g. associated with KCa2-channel binding, suggests that some toxins may bind to multiple targets. We also find peripheral residues have roles interacting with the channel S5P loop - confirming that the basic ring acts to discriminate Kv1 channels versus other potassium channels.

At the time of writing, we are in the process of completing the database with a hypothesis that Kv1-subtype selectivity arises from: (a) exact surface/hydrophobic matching, and (b) charge content and positioning. We ultimately aim to find an optimal scaffold for Kv1.3 and extension towards other K+-channel subtypes as more structures are published.

201-Pos

Theoretical Models of the Biological Catch-Bond

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The biological catch-bond is a fascinating and counterintuitive phenomenon, which was predicted theoretically 30 years ago. Recently, this predicted behavior has been observed in a number of protein receptor-ligand complexes. When an external force is applied to a catch-bond in an attempt to break it, either *in vivo* or *in vitro*, the bond resists breaking and becomes stronger instead. This is in contrast to ordinary slip-bonds which represent the vast majority of biological and chemical bonds and which dissociate faster when subjected to a force. This report focuses on the fundamental properties of catch-bonds and analyzes the simplest physical-chemical models to explain the experimental data. The simplicity of the theoretical treatment leads to analytic expressions for bond lifetime, concise universal representations of the experimental data, and explicit conditions required for catch-binding.

Three different model of the biological catch-bond will be discussed, including the two pathway, deformation and allosteric models. Catch-binding is a consequence of a complex potential energy landscape in a biological receptor-ligand bond. Bond lifetime can increase with force, if this force prevents dissociation through a native pathway and instead drives the system over a higher energy barrier. The lifetime can also increase if the conformations of proteins in the complex are altered by the force in a way that strengthens receptor-ligand interaction. Such bond deformation can be associated with an allosteric effect, in which a conformational change at one end of the protein propagates to the binding site located at the other end. Both experiment and simulation indicate that catch-binding is accompanied by large-scale domain opening in the receptor protein. The models are used to describe catch-binding in P-selectin/PSGL-1, FimH/mannose, actin/myosin and integrin/fibronectin complexes.

O. V. Prezhdo, Y. V. Pereverzev, ''Theoretical aspects of the biological catch-bond'', *Acc. Chem. Res.*, **42**, 693 (2009).

202-Pos

Correlation Between Functionality and Biochemical Properties in Biotin Protein Ligases

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Biotin protein ligases are a family of enzymes that catalyze biotin linkage to biotin-dependent carboxylases. In microorganisms these enzymes are functionally divided into two classes including the monofunctional class that only catalyzes biotin addition and the bifunctional class that also binds to DNA to regulate transcription. Biochemical and biophysical studies of the bifunctional Escherichia coli ligase suggest that several properties of the enzyme have evolved to support its additional regulatory role. These properties include the order of binding of multiple substrates and linkage between oligomeric state and ligand binding.

In order to test the hypothesized relationship between bifunctionality and enzymatic properties in ligases, we have carried out studies of monofunctional ligase from Pyrococcus horikoshii. Sedimentation equilibrium measurements to determine the effect of ligand binding on oligomerization indicate that the enzyme exists as a dimer regardless of liganded state. Isothermal titration calorimetry and fluorescence spectroscopy measurements of substrate binding indicate that, unlike in the E. coli enzyme, substrate binding is not ordered. Finally, thermodynamic signatures of ligand binding to the monofunctional enzyme differ significantly from those measured for the bifunctional enzyme. Combined studies of the bifunctional and monofunctional biotin ligases indicate a link between the functionality of these enzymes and their detailed biochemical characteristics.

203-Pos

Remote Regions Involved in Phosphoenolpyruvate Binding to Lactobacillus Delbreuckii Phosphofructokinase

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Phosphofructokinase from Lactobacillus delbrueckii subspecies bulgaricus (LbPFK), unlike that from Thermus thermophilus (TtPFK) and Bacillus stearothermophilus (BsPFK), exhibits weak binding affinity for the allosteric inhibitor phospho(enol)pyruvate (PEP). LbPFK has 57% sequence identity, with 75% similarity, to BsPFK and 48% identity, with 65% similarity, to TtPFK. A comparison of crystal structures between apo-LbPFK and apo-BsPFK indicates an overall conservation in structure except for the allosteric binding site. The two regions within the allosteric binding site which differ between LbPFK and other PFKs include the end of an a-helix containing residues 55-59 and a loop containing residues 211-215. Individual mutations were made in both of these regions to the corresponding residues from either TtPFK or BsPFK, with no enhancement in PEP binding. Therefore, chimeric substitutions were introduced into LbPFK in which all the residues in these regions were replaced with those from TtPFK, since TtPFK binds PEP 18,000-fold tighter when compared to LbPFK. Tt(52-61)/LbPFK and Tt(206-218)/LbPFK resulted in no enhancement in PEP binding when compared to LbPFK. They were also combined to form Tt(52-61,206-218)/LbPFK, which again exhibits similar PEP binding to LbPFK. These results indicate that the weak PEP binding in LbPFK cannot be explained as due solely to the residues which directly interact with the ligand upon binding. Another region of interest is the a-helix

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.

Supported by grant R01- AR056330 from the NIH to RJB and training grants T32 GM08181 (to RJB) and T32 AR07592 (to M. Schneider).

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