suited for fast and efficient screening to study new transcription factor interactions. It is a prerequisite to unravel the molecular mechanisms of gene regulation.

934-Plat Predicting protein-ligand binding free energies

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We discuss recent work calculating accurate absolute and relative protein-ligand binding free energies in several different binding sites, in some cases predictively. These sites range from model binding sites to proteins which are targets of current drug discovery effort. We discuss insights gained into the contributions of multiple ligand orientations and protein conformational changes to protein-ligand binding. Conformational changes prove especially important: even small protein conformational changes at the amino acid side chain level in Interleukin 2 and a lysozyme model binding site can contribute several kcal/mol to binding free energies. Smaller conformational changes prove important, as well: When the protein is treated as rigid, RMS errors relative to experiment are extremely large; even a small amount of protein flexibility can substantially improve results.

935-Plat Agonist Binding and Allostery in NR3A Receptors

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N-methyl-D-aspartate (NMDA) receptors are heteromultimeric ligand-gated ion channels involved in cognitive processes such as learning, memory and, in dysfunctional cases, mental illness, stroke, and neurodegeneration. A less-characterized NMDA receptor subunit, NR3A, has been discovered to act as a dominant negative regulator of receptor activity that, upon binding of glycine, attenuates calcium permeability and magnesium sensitivity in the receptor.

Recent crystal structures of NR3A bound to glycine or the partial agonist ACPC show very few structural differences despite binding affinities that differ by four orders of magnitude. To elucidate the cause of this difference and investigate potential allosteric mechanisms, molecular dynamics and quantum chemical calculations were conducted.

In contrast to previous, conventional simulations of other NMDA receptor subunits, results from our replica exchange MD simulations showed increased order in the binding site residues of the NR3A-ACPC complex. In the NR3A-glycine complex an allosteric conformational change distant to the binding site was observed that was absent in the apo or ACPC-bound states. This change is transmitted through a hydrophobic cluster beginning with a tyrosine residue directly involved in ligand binding, and leads to 30 degree tilting of a helix in an NR3A-specific portion of the receptor. We hypothesize that this helix is involved in allosteric interactions with other domains of the NR3A receptor or other portions of the multimeric assembly.

Similar simulations of the apo state allowed the modeling of a hypothesized, but as yet not crystallographically resolved, "open" state in which the ligand binding site is fully exposed to solvent.

Free energy perturbation simulations exchanging glycine and ACPC in the binding site revealed the role of specific atomic interactions in binding affinities as well as allosteric coupling.

936-Plat Watching Insulin Dimers Dissociate using Ultrafast IR Spectroscopy and MD Simulation

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Insulin homodimer dissociation releases the monomers necessary to initiate glucose regulation by binding the insulin receptor. Understanding and controlling the interplay between association and internal coordinates in the monomer-dimer equilibrium would contribute to a paradigmatic protein-protein interaction with profound public health implications. We report the combination of two-dimensional infrared spectroscopy (2D IR) and molecular dynamics (MD) simulations as a new tool to study the con-formational changes associated with insulin dimer dissociation. By spreading a vibrational spectrum over two frequency axes, amide I 2D IR spectra can display cross-peak ridges between different secondary structure-sensitive protein vibrations. Spectral blueshift of the band center and cross-peak ridges formed only during close contacts of the intermolecular β-sheet are identified as markers for insulin dimer dissociation. During thermal dissociation, these markers are used to probe the depletion of interfacial structure. The 2D IR spectra are directly calculated from MD simulations of the dissociation reaction to provide an atomistic model of the changes.

Platform W: Voltage-Gated K Channels, K-Channel, Structure & Dynamics

937-Plat Ligand-dependent Conformational Dynamics in a Potassium Channel Ca^{2+}-binding Domain

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TvoK is a prokaryotic Ca^{2+}-activated K^+ channel whose ligand-dependent gating is regulated by its cytoplasmic RCK domain

(Parfenova *et al.*, *J. Biol. Chem. 282*:24302–24309, 2007). We have studied the structure of this RCK domain assembly in solution in the presence and nominal absence of Ca^{2+}, to gain insight toward mechanisms of ligand-dependent channel modulation. Results from analytical gel-filtration chromatography, native PAGE and glutaraldehyde cross-linking experiments demonstrate that the RCK domains assemble to form stable homo-octamers in solution. Small-angle X-ray scattering (SAXS) data show a robust and significant decrease in the radius of gyration of the assembly with added Ca^{2+}, consistent with a ligand-dependent conformational change. Electron density reconstructions constrained by SAXS data to an effective real-space resolution of 9.7 angstroms revealed the structure of the TvoK RCK assembly as a "gating ring" with four subdomains, in general agreement with crystal structures of the MthK RCK assembly (Ye *et al.*, *Cell 126*:1161–1173, 2006). Molecular surface reconstructions from data in the presence and nominal absence of Ca^{2+} reveal a Ca^{2+}-induced 'contraction' within each subdomain, such that a cleft that bisects the surface of each subdomain disappears in the presence of Ca^{2+}. This suggests the closing of a molecular interface within each subdomain upon Ca^{2+} binding. Consistent with this hypothesis, we find that the TvoK RCK assembly becomes more resistant to denaturation by urea in the presence of Ca^{2+}, as assessed by circular dichroism spectra and gel filtration chromatography. We are further analyzing these and other structural changes in the RCK domain to determine their relation to channel gating.

938-Plat Different Roles Attributed To The Hydrophobicity And The Aromaticity Of The Phenylalanine Residue Located At The Helix Bundle Crossing Region Of Girk Channels

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Functional and structural studies of potassium channels and related channels have implicated similar molecular motions of the channels, i.e., upon channel closure, the pore lining transmembrane helices come close toward each other to form a helix bundle crossing (HBC) near the cytosolic end of the ion permeation pathway. It is an open question, however, whether the HBC sterically restrict the ion flow at the closed states of the channels.

A phynelalanine (PHE) is located at the narrowest part of the HBC of all G protein activated inwardly rectifying K^+ (GIRK) channels. We have substituted the PHE residues with all other 19 possible residue types on GIRK2 wild type and its constitutively active pore helix mutant GIRK2 E152D, and on a homomerically functional mutant GIRK4 (GIRK4*). We have studied the effects of the substitutions on the whole-cell currents when the channels were expressed in *Xenopus* oocytes. We have also examined the ability of GIRK2 double mutants, which carry E152D as well as a mutation of the PHE residue, to rescue the growth of potassium transport-deficient yeast in low potassium media.

Our studies indicate that the hydrophobicity and aromaticity of the PHE residues of GIRK channels play key roles on the channel functions. The aromaticity of PHE may play an important role on the sensitivity of GIRK channels to $G\beta\gamma$ gating, while the hydrophobicity of this residue is crucial for its ability to remain non-conductive in the absence of $G\beta\gamma$. We are studying the effects of the substitutions on the single-channel properties of the channels to investigate how different amino acids at the position of the PHE residue affect the energetics of open and closed states.

939-Plat Structural Snapshots Of The Conformational Changes Involved In Kcsa Gating

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The pH gated KcsA potassium channel undergoes C-type inactivation similar to other biologically important K+ channels. After a transition to acidic pH, the lower gate at the inner-helical bundle opens and imparts conformational changes around the selectivity filter. This conformational wave leads to C-type inactivation. Based on these findings it is suggested that there are at least four states defining the gating behavior of KcsA potassium channel. Two states corresponding to the lower activation gate (closed and open) and the other two to the structural changes at selectivity filter (conductive and non-conductive). We have explored the conformational changes corresponding to these states using a combination of X-ray crystallography, electrophysiology and molecular dynamic simulations. The crystal structure of the closed state KcsA represents the closed-conductive conformation; the structure of the open state KcsA (open mutant) solved to 3.2Å resolution now provides us the open-inactivated conformation, with the inner helix bundle opened up to 30 Å in diameter and the selectivity filter in the non-conductive state. We also solved intermediate states with different degrees of opening at the bundle crossing and different extents of inactivation corresponding to multiple partially closed and open-inactivated states. Using electrophysiological studies we previously showed the existence of a partial closed inactivated state. Computational approaches provided a structural model bearing close resemblance to the low-K+ and M96V crystal structures. We have determined structure of the open mutant in Rb+ and 4-aminopyridine at 3.3Å resolution. Under these conditions the selectivity filter is stabilized in its conductive conformation even with the lower gate wide open. The solved structural snapshots provide us with wealth of information regarding the gating behavior in KcsA and the molecular basis of the interaction between ions, drugs, and the behavior of ion channel gates.

941-Plat Voltage Clamp Fluorimetry Reveals A Novel Outer Pore Instability In Kv1.5 Channels

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Fluorimetric approaches have provided significant insight concerning the structural dynamics of gating in *Shaker* voltage-gated potassium (Kv) channels. However, these techniques have not been applied to mammalian Kv1 channel homologues. Here, we use voltage clamp fluorimetry to directly observe the conformational changes associated with Kv1.5 channel gating by attaching a tetramethylrhodamine-5-maleimide (TMRM) fluorescent probe to substituted cysteine residues in the voltage sensor domain (M394C to V401C). We reveal that the fluorescence report of voltage sensor movement in Kv1.5 is uniquely different from that in the archetypical *Shaker* channel. Whereas the fluorescence report of voltage sensor movement (TMRM attached at A359C in the S3–S4 linker) in *Shaker* channels was mono-exponential and occurred with a similar time course to ionic current activation, the report of Kv1.5 voltage sensor movement reflected complex conformational changes. Upon depolarization, TMRM at M394C and A397C in the S3–S4 linker of Kv1.5 channels reported a transient fluorescence deflection that was followed by a prominent rapidly decaying component that, in A397C, represented 35±3% of the total signal and occurred with a τ of 3.5±0.6ms at +60mV (n=4). Using 4-aminopyridine and the ILT triple mutation (V407I/I410L/S414T) to dissociate channel opening from voltage sensor movement, we show that the unique decaying component of fluorescence is associated with channel opening. Using the W472F mutation, which prevents ion conduction by immobilizing the outer pore whilst sparing normal functioning of the intracellular pore gate, we show that the unique fluorescence component is associated with gating at the selectivity filter. Consistent with this, prevention of selectivity filter gate closure, by raising external K^+, abolished the decaying component of fluorescence. These data suggest that the unique rapidly decaying component of fluorescence from Kv1.5 reflects a novel rapid selectivity filter gate instability in these channels.

942-Plat Voltage Sensor Movements in *Shaker* K^+ Channels Utilizing Lanthanide Binding Tags

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Lanthanide based energy transfer (LRET) is a technique to measure intra- and intermolecular distances quite accurately. Here we describe distance measurements conducted in *Shaker* potassium channels expressed in *Xenopus lae*vis oocytes. We used the *Shaker* construct with the N-terminal inactivation removed, *Shaker* IR, and with 27 residues removed from the S3–S4 linker (remaining the *three amino acid S3–S4 linker* of Gonzalez *et al.*, 2001). A genetically encoded lanthanide binding tag (LBT), which is structured and is 17 residues long, was utilized as a donor probe (Nitz *et al.*, 2003) and inserted in the short S3–S4 linker. Energy transfer was done to an acceptor molecule (fluorescein maleimide, FM) attached to the potassium channel specific blocker Agitoxin 2 (AgTx2) in position 20. The transfer efficiency was measured from the decay of the sensitized emission of fluorescein upon Tb^{3+} excitation by a 266 nm pulse that excites the tryptophan antenna of the LBT. In these measurements, AgTx2 serves as an acceptor probe located close to a center of symmetry with respect to the four LBTs that are relocated as part of the backbone movement elicited by voltage clamp pulses. The main improvement of this study is the use of the LBT together with the use of the short linker construct. The rationale is that, as the LBT is part of the protein backbone, molecular modeling of the LBT position can be done more precisely using the known structures of Kv1.2 (Long et al, 2005) and LBT (Nitz *et al.*, 2004) and thus the distances measured from the Tb^{3+} to the FM can be used to estimate actual distances from the protein backbone to the toxin.

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943-Plat Structural Basis of Quaternary Ammonium Binding to Potassium Channels

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Tetraethylammonium (TEA) and related quaternary ammonium (QA) compounds are the best studied group of potassium channel blockers. TEA and its derivatives were first described by Armstrong during the 1960s and since that time TEA and QAs have been used to probe potassium channel structure, gating, and blockade (1,2). Yellen and colleagues, in particular, have demonstrated that the hydrophobicity of a QA blocker is a major determinant of both its affinity and its effect on C-type inactivation (2,3,4). Despite these findings, the precise nature of the hydrophobic interactions between channel and blocker remained unclear. Here we present the structure of KcsA in complex with two hydrophobic QAs. These structures demonstrate the existence of a hydrophobic binding pocket that binds the long alkyl chains of QA ions. The binding pocket is exposed following a slight conformational change in the S6 helix and is composed of residues from the S5, S6, and pore helices. We hypothesize that this pocket represents a general aspect of hydrophobic binding to potassium channels and underlies key features of cardiotoxic drug-binding and trapping. Finally, we also hypothesize that the S6 conformational change demonstrated herein also reveals an alternate pathway to the central cavity of potassium channels.

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944-Plat Crystal Structure of Full-length KcsA at 3.7 Å

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KcsA is a proton-activated potassium channel from *Streptomyces lividans* whose open probability is also modulated by transmem-

brane voltage. Several crystal structures of the C-terminal truncated transmembrane domain of KcsA have provided extensive information on ion selectivity and permeation, and have contributed to our understanding of the gating mechanism of the channel. Although spectroscopic evidence clearly shows that the 40-residue C-terminus forms four-helix bundle that projects to the cytoplasm, full-length (FL) KcsA has so far remained refractory to high-resolution crystallographic approaches. Here, we have generated novel nM affinity Fabs against FL-KcsA from a phage display library. These Fabs have been used as crystallographic chaperones to generate high quality crystals of FL-KcsA, diffracting to a resolution of 3.7 Å. The structure of the FL-KcsA-Fab complex reveals a well defined 2-fold symmetric four-helix bundle that projects ∼70 Å towards the cytoplasm. A second Fab, binding at a slightly different region of the C-terminus was used to determine the structure of the C-terminal domain of KcsA alone (Ser129 to Asn158) at 2.6 Å resolution. Binding of the Fab does not exert a major influence on KcsA single channel behavior, suggesting that the Fab epitope in the C-terminus does not undergo major conformational changes upon gating. Superposition of full-length and truncated KcsA main chains shows that the C-terminal deletion promotes a $\sim 15^{\circ}$ bending away from central axis of symmetry (residues Ala108 to His124), that the narrowest point along the inner bundle gate moves from residue Ala108 to V115 and that it contracts from ∼3.5 Å to < 2.5 Å in diameter. We suggest that this structure constitutes a better representation of the physiologically-relevant closed conformation of KcsA.

Platform X: Membrane Transporters & Exchangers

945-Plat Single-molecule FRET Study of Conformational Dynamics in Reconstituted LacY

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The N- and C-terminal six-helix bundles of lactose permease (LacY) form a large internal cavity open on the cytoplasmic side and closed on the periplasmic side with a single sugar-binding site at the apex of the cavity near the middle of the molecule. During sugar/H+ symport, an outward-facing cavity is thought to open with closing of the inward-facing cavity so that the sugar-binding site is alternately accessible to either face of the membrane. We use single-molecule fluorescence (Förster) resonance energy transfer (smFRET) to test this model with wild-type LacY and a conformationally restricted mutant. Pairs of Cys residues at the ends of two helices on the cytoplasmic or periplasmic sides of wild-type LacY and the mutant were labeled with appropriate donor and acceptor fluorophores, single-molecule fluorescence resonance energy transfer was determined in the absence and presence of sugar, and distance changes were calculated. SmFRET studies of LacY in detergent micelles revealed sugar-dependent conformational changes consistent with the alternating access model. However, upon reconstitution into liposomes, large distance changes in the molecule are detected even in the absence of sugar in diffusing liposomes; efforts are in progress to follow such dynamics on single, surface-tethered liposomes. The observed distance changes may reflect the effect of a true membrane environment on the dynamics of membrane proteins.

946-Plat Homodimer of the Mitochondrial Phosphate Transport Protein (PTP). In vitro Formation

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The concept of homodimeric mitochondrial transport proteins was first established with hydrodynamic studies of purified proteins. These elegant studies were difficult to carry out and quantitate due to the presence of large amounts of the detergent Triton X-100. Nevertheless the conclusion was reached that the ADP/ATP translocase, purified in a reconstitutively active form is a homodimer. Similarly, inhibitor titration studies of the ADP/ATP translocase, also somewhat difficult to analyze rigorously, lead to the conclusion that only one inhibitor molecule per two subunits of ADP/ATP carrier are required to block transport. To identify a homodimeric structure of mitochondrial transporters with transport function (1) we constructed two types of phosphate transport protein subunits that differed only in their affinity tag (His tag, FLAG tag). Such constructs permit the generation of PTP dimers with only one of each of these subunits. Reacting one of these subunit with N-ethylmaleimide (an inhibitor of phosphate transport) and combining it with the other subunit yields, as expected from a functional dimer, an inactive transporter. We have now characterized more carefully the interaction between PTP subunits, i.e. a wild type subunit and a subunit with a His tag bound to a Talon affinity column. We have demonstrated that homodimers do readily form and that the affinity between the subunits is significantly decreased when Cys28 is replaced with an Asp. This decrease in affinity between the subunits, predicted from disulfide formation results (2), is most likely due to both steric and charge repulsion effects.

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947-Plat Structure And Elastic Properties Of Tunneling Nanotubes

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Tunneling nanotubes (TNT's) are recently reported interconnection channels among cells, assumed to represent a novel mechanism for cell-cell interactions. They mediate actin-based transfer of vesicles and organelles and they allow signal transmission between cells. We describe, to our knowledge for the first time, the effects of lateral pulling with polystyrene beads trapped by optical tweezers on TNT's linking separate U87 MG human glioblastoma cells in culture. This cell line was chosen for handling ease and possible pathology implications of TNT persistence in communication