1941-Plat
The Ion Channel-Kinase, TRPM7, is Required for Cardiac Automaticity
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Sick sinus syndrome and atrioventricular block are common clinical problems, often necessitating permanent pacemaker placement, yet the pathophysiology of these conditions remains poorly understood. Here we show that Transient Receptor Potential Melastatin 7 (TRPM7), a calcium-permeant channel-kinase highly expressed in heart, is required for cardiac automaticity, sinoatrial node (SAN) and atrioventricular node (AVN) function. We find larger TRPM7 currents in myocardial cells exhibiting automaticity such as embryonic ventricular myocytes (EVM) and SAN cells, as compared to quiescent adult ventricular myocytes. TRPM7 disruption in cultured EVM reduces spontaneous Ca^{2+} transient firing rates, impairing automaticity in vitro. Likewise, morpholino mediated TRPM7 knock-down in zebrafish embryo slows heart rate in vivo. Cardiac-targeted TRPM7 deletion in mouse (KO) eliminates TRPM7 current in SAN, inducing episodes of sinus pauses, AVN block and cardiomyopathy. Freshly isolated SAN from KO mice exhibit diminished Ca^{2+} transient firing rates and a blunted diastolic Ca^{2+} rise. Moreover, action potential firing rates are diminished in KO SAN due to slower diastolic depolarization. Accordingly, Hcn4 mRNA and the pacemaker current, If, are diminished in both SAN and AVN from KO mice. We conclude that TRPM7 both regulates Hcn4 expression and provides a novel, previously unrecognized diastolic Ca^{2+} current at hyperpolarized membrane potentials, each contributing to diastolic membrane depolarization and myocardial automaticity in SAN and AVN.

Platform: Protein-Ligand Interactions

1942-Plat
Ligand Binding on Intrinsic Disordered Proteins: Focus on Human Alpha-Synuclein
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Intrinsically disordered proteins (IDPs) are involved in a wide variety of human diseases (1). Hence, interfering with the function of IDP-disease-associated proteins offers a highly attractive objective for drug development (2). Unfortunately, rational approaches have been hampered so far because of variety of problems absent in traditional drug design protocols. These issues include the highly dynamic nature of IDPs (3), the presence of local and long-range conformational rearrangements (4), transient secondary structure, transient long-range tertiary structure (5, 6).
Here we present a combined NMR/molecular dynamics protocol that provides quantitative information on ligand poses to IDPs. The approach is based on a geometrical-based analysis of MD trajectory with a flexibility index able to detect conformational transition of residues' backbone (Caliandro, C, Rossetti, G, Carloni, P, *2012 JCTC in press*). The protocol is applied on dopamine in complex with the naturally unfolded protein human α-synuclein. The proposed protocol is very general and it could be used to investigate the pose of novel molecules binding to any IDP.
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1943-Plat
Targeting Protein Misfolding(Islet Amyloid Polypeptide) using Helical Mimetics
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Islet amyloid polypeptide (IAPP) is a 37-residue peptide hormone, which is co-secreted with insulin by the β-cells of the endocrine pancreas. IAPP belongs to a class of aggregation prone proteins, which includes Aβ from Alzheimer's, in which a wild-type protein precursor irreversibly forms/folds into β-sheet rich fibrillar amyloid plaques. The aggregation of IAPP results in β-cell dysfunction that leads to type 2 diabetes. Even though the mechanism behind the cell toxicity is poorly understood, recent reports suggest that the membrane bound intermediate oligomeric helical states mediate the cell toxicity and not the aggregated state.
The overall hypothesis pursued in this study is that small-molecule, structure based targeting of pre-amyloidogenic states will enable elucidation of the mechanism of IAPP induced cytotoxicity. These efforts provide novel descriptions of IAPP oligomerization at a molecular level, and provide a rational design path for the creation of lead compounds that ameliorate β-cell death. Importantly, we are targetting the α-helical intermediates of IAPP by synthesizing small molecules designed to mimic the surface presentation of one edge of an α-helix. Interaction studies between helical mimetic compounds and IAPP will be presented.

1944-Plat
Three-Pronged Computational Approach to Predict Regulatory Ligands of Cytochrome C Oxidase
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A crystallographically-defined bile acid binding site has been identified in the membrane domain of mammalian and *Rhodobacter sphaeroides* cytochrome *c* oxidase (*Rs*C*c*O). Previous studies indicate that several amphipathic molecules including detergents, fatty acids, and porphyrins also bind to this site and alter K-pathway dependent electron transfer between heme cofactors. Current studies are aimed at identifying physiological ligands specific for this site using several computational approaches, including: *ROCS* comparison of ligand shape and electrostatics, *SimSite3D* analysis of similarity to ligand binding sites in the Protein Data Bank, and *SLIDE* screening of small molecules by docking. Together, the results suggest several steroids, adenine and guanine deoxyribonucleotides, NAD^+, FAD, and phosphorylated isoprenes as top candidates for interacting at this site, along with bile acids and porphyrins. Deoxyriboside-containing ligands are all predicted to bind to this site by making key protein contacts with Pro315, His96, Ser98, Glu101, and Thr105, as seen in bile acid binding. *Invitro* oxygen consumption assays support some of these predicted interactions. In the wildtype *Rs*C*c*O, the steroidal antibiotic fusidic acid and T3 thyroid hormone inhibit the enzyme, while in the *Rs*C*c*O E101A mutant, fusidic acid has a stimulatory effect and an ATP analog and T3 thyroid hormone inhibit activity. Cytochrome *c* titration assays indicate that nucleotides also inhibit the E101A mutant enzyme at lower cytochrome *c* concentrations. The confirmed prediction of new ligands suggests that this three-pronged computational approach may be applied to identify native ligands in sites occupied by detergents or crystallographic additives. (supported by NIH GM26916 to SF-M)

1945-Plat
Reengineering the Oligosaccharide Specificity of CVN with BP_Dock
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Cyanovirin-N (CV-N) is a cyanobacterial lectin with potent anti-HIV activity, mediated by binding to Man(1,2)Man with high affinity and specificity. These sugar-binding sites are located in two quasi-symmetric domains (A and B) of the CV-N protein. We explore the sequence space of CV-N and reengineered CV-N mutants with different binding affinity for the oligo-mannosides, through integrated computational and experimental strategies. using the sequences of two engineered CV-N,[1,2] we model various CV-N mutants, and obtain these variants in complex with di-mannose sugar with a fast and flexible docking method called BP-Dock. BP-Dock can integrate both backbone and side chain conformational changes in a protein through a multi-scale approach. It mimics the nature of binding induced events by perturbation of the binding site residues through small Brownian kicks as a first order approximation of an approaching ligand. Then it computes the response fluctuation profile of the chains using the perturbation response scanning method. The response fluctuation profiles are then used to generate binding induced multiple receptor conformations of CV-N mutants for ensemble docking. The results from docking simulation are then validated with NMR experiments. Overall, this study helps us to explore the sugar binding properties of CV-N mutants and provides a better insight about the binding site of CV-N.
References:
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1946-Plat

Energetic Coupling between an Oxidizable Cysteine and the Phosphorylatable N-Terminus of Human Liver Pyruvate Kinase

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We previously provided data supporting that phosphorylation of Ser12 of human liver pyruvate kinase reduces the protein's affinity for its substrate, phosphoenolpyruvate (PEP), by disrupting an activating interaction between the N-terminus and the main body of the protein. The primary supportive data were a truncation series that demonstrated removal of the N-terminus results in the same response as phosphorylation. In addition, the isolated non-phosphorylated N-terminal peptide can be added to the phosphorylated protein, resulting in ''allosteric'' activation. Therefore, the N-terminal peptide may serve as a drug lead for the goal of activating liver pyruvate kinase to counteract hyperglycemia.

Unfortunately, currently available crystal structures do not reveal where/how the N-terminus interacts with the main body of the protein. This is primarily because the N-terminus is either not present or disordered in those structures. A 1.8Å crystallographic structure of human liver pyruvate kinase (L-PYK) provides evidence for a sulfenic acid derivatization of Cys436 in the vicinity of the N-terminus. The oxidized residue was further probed to demonstrate energetic coupling with PEP binding. Mutant cycles provide evidence that the mechanism for regulation by oxidation is similar/equivalent to that caused by phosphorylation, disruption of an activating interaction between the N-terminus with the main body of the protein. A second protein crystal of the C436M mutation indicates that the introduced mutation causes additional N-terminal residues to become orered in the structure. Finally, an alanine-scan across the N-terminus confirms that the residues that interact with methionine at the 436 position are important to N-terminal function rather than purly an artifact of the mutation. This study has not completely characterized all N-terminal/main body interactions, but has advanced our understanding considerably.

1947-Plat

Does Symmetry of Ligand Occupancy Matter to Conformational Transitions of Pentameric Ligand Gated Ion Channels?

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For a homo-pentameric ligand gated ion channel, agonist occupancy of only two or three of the five possible binding sites has been found to produce the maximal channel response. Does asymmetric ligand binding also apply for channel antagonism? The anesthetic propofol inhibits the homo-pentameric GLIC, but the crystal structure of GLIC bound symmetrically with propofol to each of the five binding sites shows a virtually identical open channel as the one without drug binding. We hypothesize that symmetric ligand occupancy of all five binding sites may not occur under physiological conditions, and asymmetric ligand binding facilitates channel conformational changes? To test this hypothesis, we performed multiple simulations on multiple GLIC systems with propofol occupying 0, 1, 2, 3, or 5 of the potential sites. We found that systems with symmetric ligand occupancy (0 or 5 bound propofols) showed similar channel conformation and hydration statuses. However, systems breaking the five-fold symmetry (1, 2, or 3 bound propofols) showed accelerated channel dehydration, increased conformational entropy of the pore-lining TM2 helix, and shifted tilting angles of the TM2 helix towards the closed-channel conformation. The trajectory of the force generated by propofol within each subunit is ellipsoidal with the primary force component tangential to the pore. Asymmetric ligand occupancy induced an unbalanced force around the channel, perturbed global stability, and facilitated conformational change. Our study suggests that asymmetry induced by ligand binding plays an important role in eliciting conformational changes related to protein function. Supported by NIH (R01GM066358, R01GM056257, R37GM049202, and T32GM075770) as well as the NSF via TeraGrid resources.

1948-Plat

Enhancing Human Spermine Synthase Activity by Site Directed Mutations

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Spermine Synthase (SMS) is an enzyme converting spermidine into spermine, both of which are polyamines controlling normal cell growth and development. Several missense mutations in human SMS (*Hs*SMS) are known to cause Snyder-Robinson Syndrome (SRS) by either destabilizing the monomer/dimer conformation or directly affecting the hydrogen bond network in the active sites. Recently a comparison of protein sequence and crystal structure between the *Hs*SMS and its homologous protein *Thermotoga maritima* (*Tm*) spermidine synthase (*Tm*SRM) was performed. *Tm* is the only bacterium known to grow at a high temperature as well as 90°C, and the half-life of *Tm*SRM is longer than 25h under this temperature. In contrast, *Hs*SMS is much less stable than *Tm*SRM under the same temperature. Sequence alignment between *Hs*SMS and *Tm*SRM suggests that some key residues may be essential players for the elevate stability of *Tm*SRM. Such key residues were identified based on various biophysical and sequence criteria and four mutations (S165D, L175E, T178H and C206R) were selected for *Hs*SMS. Both *in silico* and *in vitro* experiments indicated that these four mutations strongly stabilize the monomer structure and dramatically improve the efficiency of SPM synthesis. The enhanced reaction rate in the mutant *Hs*SMS is attributed to the increase of the strength of negative electrostatic potential, calculated with DelPhi, in the dimer cleft between *Hs*SMS units, which presumably facilitates the substrate delivery to the active site.

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1949-Plat

Differential Fragment SPR (DF-SPR) for Antimalarial Drug Screening

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Malaria has been a human health concern for centuries, particularly in tropical and subtropical regions of the world. Nevertheless, our repertoire of medication to treat the disease has been very limited, and emerging resistance of the malaria parasite *Plasmodium* has further restricted the use of current medications. The most recent reports indicating artemisinin resistance in Cambodia are indeed alarming and underscore the critical importance of exploring novel pathways for interfering with the life cycle of the malaria parasite.

Surface plasmon resonance (SPR) has been a powerful tool to study protein ligand interactions. We utilize this technique for small molecule screening (~150 Da) to identify fragment molecules that are capable of binding near a specific protein site. We have developed a method, which we termed differential fragment SPR (DF-SPR). In the present study, we utilized our approach to identify two small molecule fragments capable of inhibiting the essential protein-protein interaction of the autophagic proteins Atg8 and Atg3 from the malaria parasite *Plasmodium falciparum*. These fragments provide a starting point for developing larger, drug-like molecules. Furthermore, we employed our protein-protein interaction inhibition assay to discover additional small molecule inhibitors of a site which we found specific to the *Plasmodium* parasite, but absent in the human homolog of Atg8. Small-molecules derived from our studies may represent useful tools for further dissecting and analyzing the autophagy pathway in *Plasmodium* and other apicomplexan species.

Platform: Molecular Mechanics & Force Spectroscopy

1950-Plat

Improved Peptide-Discrimination by Force-Induced Unbinding of T Cell Receptor from Peptide-MHC

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Cell surface receptors (CSRs) are transmembrane proteins that link the binding of an external stimulus to an intracellular signal. One example is the T cell receptor (TCR), which specifically binds to peptides presented by MHC (pMHC) on an antigen-presenting cell. Discrimination power of ''good'' versus ''poor'' ligands can be realized with kinetic proofreading, however, it fails when it comes to ligands with only marginal differences in their off-rates: even if the TCR response was a perfect step-function in time, stochastic ligand dissociation would ultimately limit the specificity. We show here that the specificity of antigen-recognition can be massively improved by putting the TCR-pMHC bond under load: while under no force the bond rupture probability decays exponential with time, force-induced bond rupture leads to much narrower probability distributions. We applied the theory originating from AFM pulling experiments on a variety of different pMHC, and find consistently that under a pulling rate the T cell is enabled with a means of improved ligand discrimination.