268-Pos Board B48
Probing the Dependence of pH on Sugar Binding and Protein Structure in a Polysaccharide Lyase
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Polysaccharide lyases (PLs) are enzymes that our commonly used by microorganisms to degrade polyuronides. These enzymes are produced by bacteria to aid in reducing the high molecular weight of the host's extracellular matrix to enhance its virulence. PLs can also be used to degrade polysaccharides as a source of carbon for the pathogen. Our focus is on the alginate lyase of Stenotrophomonas maltophilia (Smlt1473), which has been shown recently to be active against alginate, hyaluronic acid (HA) and poly-D-glucuronic acid at varying pH values (J. Biol. Chem. 289: 312 (2014)). The active site appears to have regions specific to binding these three sugars and an active catalytic site. Our objective is to understand how pH influences binding of a HA and if this involves structural changes in the protein. Docking of HA to a homology-based structure of Smlt1473 (based on the crystal structure of alginate lyase A1-III) was used to provide initial starting conformations for our molecular dynamics (MD) simulations. ProPKa was used to determine the ionization state of residues at our three simulated pHs (5, 7 and 9). MD simulations at neutral pH confirm the binding residues seen in experiment. However, at the acidic and basic condtions, the homology-based model of the binding pocket opens. At ph=5, there is an increase in the exposure of HA-specific residues in the vicinity of W171 suggesting this exposure is the cause for Smlt1473 increased activity (J. Biol. Chem. 289: 312 (2014)). Therefore, Smlt1473's structure appears to strongly depend on pH and is used to control its activity toward different sugars.

269-Pos Board B49
Probing the Role of Conformational Entropy in Protein-Inhibitor Binding
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In silico drug discovery has long held the promise to revolutionize the way drugs are designed, but has largely failed to deliver. Historically inhibitors were designed based mainly on static structures that emphasized interaction energetics but ignored potential contributions from conformational entropy. This issue has been difficult to address experimentally. Recently, we have developed an NMR-based approach that employs measures of motion as a proxy for the conformational entropy. Using an empirical calibration, we have shown that the resulting ''entropy meter'' is both robust and general. We hypothesize that conformational entropy represents a critical ''missing piece'' in rational drug design. Using the ''entropy meter,'' we examine the effect of conformational entropy on protein-inhibitor interactions using the drug target and mitogen-activated protein kinase (MAPK) p38α as a model system. Given the relatively large size of this protein, all studies were performed in a perdeuterated background. Using standard methods, we have obtained nearly complete ILV methyl assignments of this protein. 15N and 13C NMR spin relaxation experiments were used to determine the sub-ns dynamics of the protein both in the apo state and bound to either a competitive and a non-competitive (allosteric) inhibitor. In both cases, we observe the a large unfavorable entropic effect of binding. We find that the conformational entropy change upon binding represents a significant contribution to the overall binding free energy. These results promote further studies to understand how classes of inhibitors may modulate the dynamics and corresponding internal entropy of protein targets, and how this information can be used to design better lead compounds.
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270-Pos Board B50
Ligand Discovery for the Alanine-Serine-Cysteine Transporter (ASCT2, SLC1A5) from Homology Modeling and Virtual Screening
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The Alanine-Serine-Cysteine transporter ASCT2 (SLC1A5) is a membrane protein that moves neutral amino acids into cells in exchange for outward movement of intracellular amino acid. ASCT2 is highly expressed in peripheral tissues such as the lung, kidney, intestines, and testis where it contributes to the homeostasis of intracellular concentrations of neutral amino acids. ASCT2 also plays an important role in the development of a variety of cancers such as melanoma, by transporting amino acid nutrients such as glutamine into the proliferating tumors. Therefore, ASCT2 is a key drug target with potentially great pharmacological importance. Here, we identify seven ASCT2 ligands by computational modeling and experimental testing. In particular, we construct homology models based on crystallographic structures of the aspartate transporter GltPh in two different conformations, and optimize the models' binding sites for protein-ligand complementarity. Virtual screening of 594,166 compounds including drugs, metabolites, and fragment-like molecules from the ZINC database, followed by experimental testing of 11 top hits with functional measurements using electrophysiological methods reveals seven ligands including five activators and two inhibitors. For example, aminooxetane-3-carboxylate is a more efficient activator than any other known ASCT2 natural or unnatural substrate. Furthermore, two of the hits inhibited ASCT2 mediated glutamine uptake and proliferation of a melanoma cancer cell line. Our results improve our understanding on how substrate specificity is determined in amino acid transporters as well as provide novel scaffolds for developing drugs targeting ASCT2, an emerging therapeutic target for cancer and neurological disorders.

271-Pos Board B51
Thermodynamic Properties of ELP-Labelled Doxorubicin, a Drug Delivery System
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Macromolecular delivery by hyperthermia and thermal targeting of SynB1-Cys ELP-bound Doxorubicin (Dox) to solid tumors is investigated in animal studies as a means of circumventing multidrug resistance in cancer cells. The structural changes and hydrodynamic properties of ELP below and above its Transition Temperature TT make this construct a useful carrier for the delivery of Dox. At low temperature, the ELPs are soluble and consist of both random coils and β-turn structures. With increasing temperature ELPs undergo weak association, forming repeating type II β-turns and β-spirals. Above the TT, they undergo a cooperative phase transition involving μm size aggregates stabilized by desolvation. Sedimentation velocity (SV) experiments have shown that ELP is a nonideal monomer at low temperature, but with increase in temperature undergoes weak isodesmic self-association. Adding Cell Penetrating Peptides (SynB1) at the N-terminal domain of the ELP decreases its solubility and stabilizes the aggregated state. This study asks how the addition of Dox to SynB1-Cys ELP affects its biophysical and hydrodynamic behavior at physiological conditions, and how these changes influence its potential for aggregation and drug delivery. The preliminary SV results presented here show an increase in self-association of SynB1-Cys ELP-Dox at 20°C relative to the unlabeled construct. The goal of these experiments is to investigate the impact of different labeling efficiencies (10-90%) on self-association and TT. Preliminary turbidity results on unlabeled SynB1-Cys ELP in PBS show that TT decreases linearly with increase in the logarithm of the ELP concentration as solutions are heated from 20 to 60°C. The process is reversible as solutions are cooled to 20°C. The degree of conformational change associated with the TT will also be monitored using CD and FT-IR experiments.

272-Pos Board B52
Understanding the Function of a Pro-Angiogenic Polypeptide hFGF-1 with a Cancer Inhibitor Imatinib
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Fibroblast growth factors (FGFs) lack signal sequences, and are exported through endoplasmic reticulum (ER)-Golgi-independent non-classical routes. FGFs work as modulators of various cellular activities like mitosis, differentiation, survival etc. Among the FGF family, which comprises of 23 different heparin proteins, human FGF-1 (hFGF-1), a potent angiogenic factors are one of the targets in cancer inhibition, as they are involved in blood vessel formation in tissues. There has been intensive research directed at the development of drugs that could effectively inhibit angiogenesis. In this context, the purpose of this study is to fully understand the molecular principles essential to determine probability of inhibition of hFGF-1 signaling transduction by imatinib. Imatinib, a 2-phenyl amino pyrimidine derivative is a tyrosine kinase inhibitor with antineoplastic activity. Imatinib binds to the intracellular pocket located within tyrosine kinases and inhibit the downstream cell proliferation events, but the exact molecular mechanism is still elusive. In this study, expression of hFGF-1 in recombinant *E.coli* was carried out, and the expressed protein was purified using heparin affinity column chromatography. The structural interactions governing imatinib-hFGF-1 interaction was studied by monitoring its

stability, conformation and binding affinity by equilibrium unfolding using steady state fluorescence and proteolytic digestion assay. These data show that imatinib binds to hFGF-1 and enhances its thermal stability and solvent accessibility. In addition, Biacore analysis was carried out to determine the binding affinity of imatinib to hFGF-1. ^{1}H-^{15}N HSQC NMR was also performed in order to determine exact binding sites and stoichiometry of binding between imatinib and hFGF-1.

273-Pos Board B53

A Shared Binding Site for Propofol and Thiopental in ELIC

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The intravenous general anesthetics propofol and thiopental target pentameric ligand-gated ion channels (pLGIC) and inhibit cation-conducting nAChRs. These drugs also inhibit ELIC, a prokaryotic pLGIC. However, the binding sites for these anesthetics are unknown for either nAChRs or ELIC. Here, using photoaffinity labeling, two-electrode voltage clamp electrophysiology and molecular docking, we identified a functionally relevant binding site for thiopental and propofol in ELIC. Molecular docking identified two binding pockets: an intrasubunit site near M265 of TM3, partially overlapped with the previously identified propofol binding site in GLIC; and an intersubunit site near W220 of TM1, which overlaps with the bromoform binding location identified previously. We generated two mutants, one targeted both predicted binding sites (W220F/W224F/M265C) and another targeted only the intrasubunit site (M265C). Functional measurements on Xenopus oocytes expressing the W220F/W224F/M265C mutant show a significant decrease of anesthetic inhibition, with a five-fold increase in the propofol IC50 and abolishment of thiopental inhibition. Interestingly, the M265C mutation alone could produce the same effect as the W220F/W224F/M265C mutant. Photoaffinity labeling experiments with a light-activated derivative of propofol (aziPm), in conjunction with mass spectrometry, confirmed the binding site at M265 for aziPm. Altogether, the results show that propofol and thiopental bind to a common functionally relevant site. This intrasubunit action site may also be shared by other intravenous anesthetics. Research supported by grants from the NIH.

274-Pos Board B54

Crystal View of Anesthetics and Alcohols Bound in the Pore of ELIC

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Cys-loop receptors, including the acetylcholine, glycine, 5-HT3 and GABA receptors, are molecular targets of general anesthetics and alcohols. Molecular mechanisms of anesthetics and alcohols interacting with Cys-loop receptors are still unclear. ELIC is a prokaryotic homolog of Cys-loop receptors and can be inhibited by general anesthetics and alcohols. Here, we report crystal structures (~3.1 Å) of ELIC bound with the volatile general anesthetic isoflurane, and bound with 2-bromoethanol. The crystal structures were obtained in the presence and absence of the agonist propylamine. Isoflurane was found inside the pore at two sites near T237(6′) and A244(13′), respectively, but 2-bromoethanol was only found near T237(6′). In addition, 2-bromoethanol also bound near Y102 and E150 in the extracellular domain. The presence of propylamine had no obvious effect on the binding sites for both isoflurane and 2-bromoethanol. This is the first time that an anesthetic or alcohol has been observed in the pore at an atomic resolution. The newly identified binding sites of isoflurane and 2-bromoethanol in ELIC are significantly different from previously reported anesthetic and alcohol binding sites. Neither isoflurane binding nor 2-bromoethanol binding introduced significant structural perturbation. The binding of isoflurane and 2-bromoethanol inside the pore suggests the possibility of channel occlusion as a mechanism for channel inhibition of Cys-loop receptors by general anesthetics and alcohols. Supported by grants from NIH.

275-Pos Board B55

Analysis of Antifolate Drugs with Disease Tissue Specificity

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Antifolates, analogues of the essential vitamin folic acid, are used in the clinic to treat cancers and inflammatory diseases. Antifolates are primarily transported into cells via the endogenously expressed reduced folate carrier (RFC). Conversely, our collaborators in Aleem Gangjee's group at Duquesne University have synthesized antifolates (AG antifolates) that transport poorly by the RFC, but are efficiently transported by the folate receptor (hFR). The GPI-anchored hFR is lowly expressed on the apical surface in a subset of normal epithelial lineages, but is highly expressed in many cancers of epithelial origin and on activated macrophages in inflammatory disease. Therefore, AG antifolate molecules have specificity for transport into disease cells over healthy cells. These newly developed AG antifolates cause cell death via inhibition of an enzyme involved in *de novo* purine synthesis, glycinamide ribonucleotide (GAR) transformylase.
We analyzed a series of AG antifolates using biophysical and biochemical techniques to understand both the specificity for transport by the folate receptor as well as the inhibition of the GAR transformylase in order to drive informed, hypothesis-based drug design. Our data, including pH-dependent binding profiles, enzyme inhibition data, and crystallographic models of protein in complex with AG molecules will be presented in the context of drug design and development.

276-Pos Board B56

PKA-Dependent Potentiation Mechanisms of Human CFTR Activity

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Both curcumin and VX-770 potentiate the channel activity of human CFTR (hCFTR) and two most common cystic fibrosis (CF) mutants G551D and F508delta in an ATP-independent but PKA-dependent manner. The underlying molecular mechanisms are unclear. Herein, HEK-293T cells cultured in a Fe^{3+}-containing medium were transiently transfected with hCFTR constructs and curcumin with well-known chemical nature was employed as a template to explore PKA-dependent potentiation mechanisms of hCFTR activity. The results showed that curcumin potentiation of Fe^{3+}-sensitive hCFTR activity was partially weakened by Fe^{3+}-insensitive mutations at the interface of the R domain and intracellular loop (ICL) 3 and completely suppressed by sufficient Fe^{3+}. Thus, release of the inhibitory Fe^{3+}-bound R domain from ICL3 by curcumin may be critical for curcumin potentiation. Further study indicated that curcumin potentiation was significantly prohibited by a missense alanine mutation of F157, Y161 or K166 from ICL1, or R1066, F1074 or F1078 from ICL4, or S795 or S813 from the R domain with or without the involvement of nucleotide-binding domain 2 (NBD2). More importantly, curcumin potentiation was also suppressed by the R811A/S813D or Y808A/S813D mutation and disulfide crosslinking of K162C to S795C enhanced channel opening. Therefore, the phosphorylated R domain may function as a length- and gating-regulatory cross-linker between two transmembrane domains (TMD1 and TMD2). Curcumin may potentiate hCFTR activity by stabilizing the stimulatory ICL1/ICL4-R interactions that promote channel opening by pulling all ICLs together and thus triggering a gating inward-to-outward reorientation of TMDs. Possible chemical interactions may involve cation-π interactions, π-π interactions and hydrogen bonding. Taken together, both release of the R domain from ICL3 and the stimulatory R-ICL1/ICL4 interactions may be necessary for PKA-dependent hCFTR activation and potentiation. These findings may help optimize the potentiators for treating those CF mutants with an ATP-dependent gating defect.

277-Pos Board B57

Kinase Structural Dynamics Enables Tight and Selective Binding of Inhibitors

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Protein kinases are obvious drug targets against cancer due to their central role in cellular regulation. With oncologic diseases being the second leading cause of death in the US kinases rapidly gain attention and are likely to become the number one drug target. Using NMR and fast kinetics, we establish a novel model that solves a longstanding question of high selectivity of clinically relevant drug Gleevec that effectively inhibits Abl tyrosine kinase while closely related Src family of kinases is affected much less. Our study of an entirely different family of Ser/Thr Aurora kinases and its specific inhibitors suggests that an energy landscape that provides tight affinity via an induced-fit and binding plasticity via a conformational selection mechanism is likely to be general for many inhibitors.

278-Pos Board B58

Glutathione Reductase of *Plasmodium Falciparum* as an Antimalarial Drug Target of Methylene Blue

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Plasmodium falciparum is the cause of human malaria and is one of two malaria parasites known to have drug resistance. Since there are no preventative