extracellular domain (ECD) of CaSR with various spectroscopic methods including the tryptophan fluorescence titration, circular dichroism (CD) and nuclear magnetic resonance (NMR) as well as molecular dynamic simulation. These results provide important implications for our understanding of how the CaSR integrates information about these two completely different classes of agonists–an inorganic divalent cation, and another hand, a nutrient– how the receptor senses these agonists in healthy and diseased states.

2069-Pos Board B88

Synthetic Demethylwedelolactones Derivatives Inhibit Invasive Growth of Mda-Mb-231 Breast Cancer Cells *In Vitro* and *In Vivo*

Yean-Jang Lee[1], Tsui-Hwa Tseng[2].
[1]Changhua University of Education, Changhua, Taiwan, [2]Chung Shan Medical University, Taichung, Taiwan.

Combretastatins, which are an important group of anticancer drugs,were isolated by Pettit et al. from the African tree Combretum caffrum in 1989. Additionally, Liang et al. have reported that ten coumestans were isolated fromthe roots of *Hedysarum multijugum*, which is a plant in *Hedysarum* Linn. of the family *Leguminosae* used as a folk herbal drug in northwest China. Coumestans comprise a class of naturally occurring products with a variety of biological activities including phytoestrogenic, antibacterial, antifungal, antimyotoxic, and phytoalexine effects. The anticancer properties of demethylwedelolactone (DWEL) and wedelolactone (WEL), which are naturally occurring coumestans, have not been well characterized. Due to their biological activities, the synthesis of DWEL is achieved in which the longest linear sequence is only eight steps in 38% overall yield from commercially available phloroglucinol. Furthmore, the molecular model was examined the interactions of proteins and ligands as well. Finally, in this study, we investigated the anti-invasive effects of synthetic WEL and DWEL on human MDA-MB-231 breast cancer cells. We found that WEL and DWEL inhibited the anchorage-independent growth and also suppressed cell motility and cell invasion of MDA-MB-231 cells. In addition, WEL and DWEL reduced the activity and expression of matrix metalloproteinases (MMPs) involved in blocking the IκB-α/NFκB and MEK/ERK signaling pathways in MDA-MB-231 cells. Furthermore, DWEL suppressed the metastasis and lung colonization of the tumor cells in the nude mice. Altogether, these data suggest that DWEL derivatives exert anti-invasive growth effect on breast cancer cells.

2070-Pos Board B89

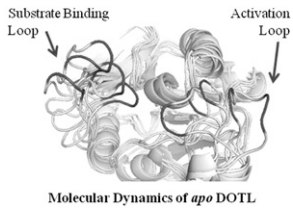
Molecular Dynamics of DOT1L and Modeling of EZH2

Javier Pineda[1], Jason Marineau[2], Guille Estiu[1], James Bradner[2].
[1]University of Notre Dame, Notre Dame, IN, USA, [2]Dana Farber Cancer Institute, Boston, MA, USA.

Histone methyltransferases are enzymes that modify histone proteins via methylation of lysine or arginine residues. These epigenetic modifiers, such as DOT1L and EZH2, have been found to play important roles in leukemogenic processes.

Crystallographic and docking methods studied interactions within the DOT1L binding site. Crystal structures of DOT1L also demonstrated variance in the binding mode of ligands, possibly due to rearrangement in the DOT1L binding site. We investigated this possibility by molecular dynamics (MD) simulations and confirmed significant rearrangement of the substrate binding and activation loops of DOT1L upon binding to competitive inhibitors. The druggability and volume fluctuations of the binding pocket over time were determined.

The EZH2 methyltransferase is of interest due to its aberrant activity in many cancers. Because there is no published crystal structure of EZH2, we used homology modeling with homologous proteins as templates. This model provides structural information regarding the binding modes of the S-adenosyl methionine (SAM) cofactor and potential inhibitors of EZH2. Through the synergistic combination of *in silico* drug design, organic synthesis and biochemical assays, our modeling efforts for DOT1L and EZH2 will guide the chemical syntheses of potent and selective inhibitors of these enzymes.



Molecular Dynamics of *apo* DOTL

2071-Pos Board B90

T Cell Receptor Specificity, Cross-Reactivity, and MHC Restriction are Inextricably Linked and Result from Cooperative Engagement of the Composite Peptide/MHC Surface

Brian M. Baker, Kurt H. Piepenbrink, Sydney J. Blevins.
University of Notre Dame, Notre Dame, IN, USA.

T cell receptors (TCRs) recognize peptides bound and presented by major histocompatibility complex (MHC) proteins using multiple complementarity determining region (CDR) loops. While numerous analyses have illuminated structural and biophysical aspects of TCR recognition, how the distribution of binding free energy within TCR-pMHC interfaces promotes unique TCR recognition features, including MHC restriction and the apparent dichotomy of specificity and cross-reactivity, remains unclear. Utilizing double mutant cycles, here we performed a comprehensive structural and thermodynamic deconstruction of the interaction between the A6 TCR and the Tax peptide presented by the class I MHC HLA-A2. In contrast with general expectations, we observed that the central regions of the peptide and its interactions with the hypervariable CDR3 loops contribute little to specificity, instead promoting by dynamic effects the cross-reactivity that is a hallmark of TCR recognition. We also observed that TCR restriction towards HLA-A2 results from not from conserved interactions with the germline loops, but instead from strong interactions with the hypervariable CDR3 loop of the α chain. Formation of these latter interactions, however, is dependent upon the unique structural properties of the peptide, highlighting that TCR specificity towards peptide and MHC can emerge from the need to engage a unique, composite peptide/MHC interface with tightly coupled structural properties.

2072-Pos Board B91

Dissecting Signal Control in the Multidrug Sensor, BMRR

Herschel Wade.
Johns Hopkins University SOM, Baltimore, MD, USA.

Multidrug (MD) (or xenobiotic) e□ux actively removes cytotoxic chemicals from the interiors of normal-functioning cells. However, high levels of e□ux can render drug-targeted cells resistant to a broad-range of therapeutic agents, including those to which cells were never exposed. Key multidrug resistance (MDR) contributors include allosteric e□ux pumps, gene regulators and other sensory systems that mediate the detection and extrusion of diverse drugs from cellular environments. To date, MDR functions remain only partially understood. Ligand-dependent allosteric control in BmrR has been quantitatively addressed using in vitro transcription experiments, dose-response curves and thermodynamic models that relate the observed transcriptional responses to ligand binding and changes in BmrR conformation. Preliminary results indicate that allosteric control in BmrR is sensitive to both energetic and structural aspects of ligand recognition. Importantly, increased cooperativity in signal control relative to recognition implicates a major allosteric role for the RNA polymerase.

2073-Pos Board B92

Fragment-Based Approach Identifies a Novel Inhibitory Site on DHPS

Dalia Hammoudeh, Mi-Kyung Yun, Ariele Viacava Follis, Ying Zhao, Brett Waddell, Richard E. Lee, Stephen White.
St. Jude Children's Research Hospital, Memphis, TN, USA.

Dihydropteroate synthase (DHPS) is an essential enzyme in the bacterial folate biosynthetic pathway. It catalyzes the condensation of 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPP) with p-aminobenzoic acid (pABA) to form the folate intermediate, 7,8-dihydropteroate. DHPS is the target of the sulfonamide class of antibiotics. Widespread resistance to sulfonamides has decreased their clinical use. The active site of DHPS is comprised of three subsites: the structured ''pterin'' site, the flexible pABA site, and the anion binding pocket. Most of the drug resistant mutations have been mapped to the pABA sub-site of DHPS. using an NMR ligand-based screening approach, a number of structurally unrelated fragment-like small molecules have been identified that inhibit the enzymatic activity of DHPS from Bacillus anthracis (Ba), Yersinia pestis (Yp), and Staphylococcus aureus (Sa). Fragment hits were shown to target the three sub-pockets of the active site and a novel site distinct from the active site. The latter site potentially inhibits via an allosteric mechanism and has been characterized by high resolution X-ray crystallography.

We screened the Maybridge® Fragment library of 1,100 fragments using water ligand observed gradient spectroscopy (waterLOGSY) as a primary screen which resulted in a hit rate of 6.7 %. Of the 74 hits, 25 were shown to inhibit DHPS activity using two independent enzyme activity assays. A total of eight compounds inhibited the activity of DHPS from three different species (Ba, Yp, and Sa). In addition to screening for inhibition, the fragment hits were validated using a number of biophysical techniques including 2D NMR, SPR, competition waterLOGSY, and X-ray crystallography. Herein, we focus on two fragment hits for which high-resolution x-ray crystal structures are available

2074-Pos Board B93

Interactomics of Blebbistatin

Miklós Képiró, Boglárka H. Várkuti, György Hegyi, Mihály Kovács, András Málnási-Csizmadia.
Eötvös University, Budapest, Hungary.

Photoreactive molecules like aryl azides play important role in life sciences, as practical tools to achieve precisely timed covalent cross-links between ligands and targets. Since the azido group is small, stable and biologically inert,

azido-modified aromatic drug molecules can keep their original pharmacological activity. By photoactivating of such modified molecules in vivo, both their strong and weak partners can be captured. Moreover, applying the azidated drug in a concentration range and utilizing proteomic tools the apparent binding constants of protein-ligand interactions can be determined.
We have developed a new and simple technique to azidate aromatic drug molecules in an easy two step reaction. Using our method we have synthesized azidoblebbistatin which is a new derivative of blebbistatin, the most widely used myosin inhibitor. In the absence of UV irradiation azidoblebbistatin and blebbistatin exhibits identical inhibitory properties. Using UV light azidoblebbistatin can be covalently crosslinked to myosin whereas the unbound inhibitor molecules become inactive. Using Dictyostelium discoideum cell lysate we performed interactomic investigation to identify the previously unknown targets of blebbistatin utilizing the self- fluorescence of azidoblebbistatin. Since the crosslinking was performed applying increasing concentration of azidoblebbistatin, densitometry of the fluorescent spots resulting from gelelectrophoresis revealed the apparent binding constant of azidoblebbistatin. In case of myosin II it was the exact value as measured in in vitro tests. With this technique the strongest interactant was found to be myosin II (EC50=5 μM) while eight weak partners (EC50>30 μM) were also detected including vacuolar H+-ATPase (EC50=50 ± 31 μM), malate dehydrogenase (EC50=55 ± 17 μM) and elongation factor 1α (EC50>100 μM).

2075-Pos Board B94

Purification Strategies and Assay Development for an Essential Plasmodial Protease

Lauren E. Boucher[1,2], Mike Martin[1], Jürgen Bosch[1,2].
[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA,
[2]Johns Hopkins Malaria Research Institute, Baltimore, MD, USA.

Plasmodium falciparum is the parasite responsible for the most deadly cases of malaria. Emerging drug resistance to current therapies requires research to develop new antimalarials. A potential target of new drugs is the proteolytic egress cascade, which has been identified as essential to the blood stage parasite. A key protein in the cascade is subtilisin-like protease 1 (SUB1). This protease undergoes two self-cleavage events to separate the N-terminal prodomain, necessary for correct folding, from the C-terminal, catalytic domain. To obtain protein suitable for crystallization, we have expressed SUB1 both in bacterial and insect cell systems. However, recovery is difficult and yield is often low. To improve the yield and purity of the protein, we employ a selective affinity purification scheme developed in the lab. Our SPR validation assays indicate specific binding of a protein originating from either insect cell or bacterial expression of recombinant SUB1. The possibility of producing active SUB1 in bacterial systems after undergoing limited proteolysis is also being explored and validated via SPR analysis. Determining dissociation conditions and residues important to the interaction via mutational studies will provide a more comprehensive understanding of this protease and aid in future structure based drug design.

2076-Pos Board B95

A Pharmacophore Approach for Novel Inhibitors of Pseudomonas Aeruginosa Exotoxin A

Miguel R. Lugo, Anthony Petrie, A Rod Merrill.
University of Guelph, Guelph, ON, Canada.

Pseudomonas aeruginosa (PA) is an opportunistic pathogen that causes lung infections in cystic fibrosis and other immune-compromised individuals. The most toxic factor secreted by PA is a 66 kDa protein, exotoxin A (ExoA), which belongs to a larger (mART) family of enzymes that catalyze the transfer of the ADP-ribosyl moiety from NAD+ to a protein target.
COMPUTATIONAL APPROACH: Here, we used the high resolution crystal structure of cholix as the structural model system for ExoA. We considered structures in complex with several ligands (NAD+ and various inhibitors: NAP, V30, etc.) in order to address the mode of ligand binding. All the analyses were performed with the Molecular Operative Environment suite (MOE.2011). For each residue in the pocket, the interaction energy was evaluated along with various other descriptors. Binding free energy was calculated according to the GBVI/WSA function. Based upon the consensus features of the cholix:ligand complexes, a pharmacophore model was developed and was used to dock these molecules as standards for the purpose of training, followed by the docking of a new inhibitor library (M-series) obtained from an in silico screening against another mART toxin, Iota toxin from C. perfringens.
EXPERIMENTAL APPROACH: A cell based assay was used for a coarse screen of the potency of the M-series inhibitors based on the viability of C38 cystic fibrosis human lung cells treated with ExoA. Compound M19 arose as the best inhibitor for protection of the cystic fibrosis human lung cell line with an EC50 of 2 μM. Future research will involve improvement of the M19 scaffold based on the information from computational approaches.
This work was funded by a Cystic Fibrosis Canada (CFC) summer studentship to AP, a CFC Canada-Kin postdoctoral fellowship to MRL, and CFC operating grant to ARM.

2077-Pos Board B96

Repurposing of FDA-Approved Drugs for the Discovery of Inhibitors of Dengue Virus NS2B-NS3 Protease by Docking, Consensus Scoring, and Molecular Dynamics Simulations

Aldo Segura-Cabrera, Carlos A. García-Pérez, Xianwu Guo,
Mario A. Rodríguez-Pérez.
Centro de Biotecnología Genómica - IPN, Reynosa, Mexico.

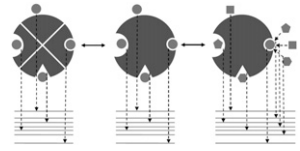
Dengue viruses (DENV) are transmitted by mosquitoes and infect ~50 million people annually with an additional 2.5 billion people at risk living in tropical areas. However, there are no approved vaccines or antiviral therapies to combat the disease. DENV genome is translates into a single polyprotein comprising 3 structural and 7 non-structural (NS) proteins. The polyprotein precursor is cleaved by both host proteases and the two-component virus protease NS2B-NS3. Thus, this protease is considered as a promising target for antiviral design. In order to identify novel inhibitors of the DENV NS2B-NS3 protease we focused our strategy on the allosteric inhibitors capable of targeting the NS2B-NS3 interaction rather than the NS3 active site. The computational protocol was performed for 7,240- FDA-approved drugs retrieved from the ZINC database. We firstly implement a structure-based virtual screening to identify a preliminary set of inhibitors against the catalytic domain in active form of DENV NS2B-NS3 by docking analysis. The preliminary set inhibitors was then used to perform a consensus scoring of docking poses, based on scoring functions from the DrugScore and Xscore packages. Six docked poses were ranked among the top 50 compounds according to consensus scoring and were used to molecular dynamics (MD) simulation and free-energy calculation. Three compounds belonging to the piperazine derivatives family were finally proposed as potential inhibitors for DENV NS2B-NS3. These compounds target the allosteric-binding site of protease so that compound binding produces a conformational change able to affect the interaction among the protease and peptide substrates. The computational drug discovery strategy employed in our study could be applied for the identification of inhibitors of other flaviviral proteases.

2078-Pos Board B97

Specificity Quantification of Biomolecular Recognition and its Implication for Drug Discovery

Zhiqiang Yan, Jin Wang.
Stony Brook University, Stony Brook, NY, USA.

Highly efficient and specific biomolecular recognition requires both affinity and specificity. The stability of the complex is determined by the affinity while the specificity is controlled by either partner binding to other competitive biomolecules discriminatively. Previous quantitative descriptions of biomolecular recognition were mostly driven by improving the affinity prediction, but lack of quantification of specificity. We developed a novel method SPA (SPecificity and Affinity) based on our funneled energy landscape theory. The strategy is to simultaneously optimize the quantified specificity of the ''native'' protein-ligand complex discriminating against ''non-native'' binding modes and the affinity prediction. The benchmark testing of SPA shows the best performance against 16 other popular scoring functions in industry and academia on both prediction of binding affinity and ''native'' binding pose. For the target COX-2 of nonsteroidal anti-inflammatory drugs, SPA successfully discriminates the drugs from the diversity set, and the selective drugs from non-selective drugs. The remarkable performance demonstrates that SPA has significant potential applications in identifying lead compounds for drug discovery.



2079-Pos Board B98

Computer-Aided Drug Design Utilizing Structure Templates Identified by Local Structure Alignment

Hui Sun Lee, Wonpil Im.
The University of Kansas, Lawrence, KS, USA.

With a rapid increase in the number of high-resolution protein-ligand structures, the known protein-ligand structures can be used to gain insights into how a ligand binds in a target protein. Based on the fact that the structurally similar binding sites share information about their ligands, we have developed a local structure alignment tool, G-LoSA (Graph-based Local Structure Alignment). Using G-LoSA, the known protein-ligand binding-site structure library