Half 1 3:50PM — 4:50PM

In vivo and in vitro activities of auranofin against vancomycin-resistant enterococci

Nader S. Abutaleb[1], Marwa Alhashimi[1] , Ahmed Elkashif[1] , and Mohamed N. Seleem[1]

[1]Department of Comparative Pathobiology, Purdue University, WL, IN, 47905, US

Vancomycin-resistant enterococci (VRE) are the second-most common cause of nosocomial infections causing more than 5% of all deaths attributed to antibiotic-resistant infections in USA. VRE acquired resistance to all antibiotics used for treatment, severely limiting the number of available effective therapeutic options. Consequently, novel antimicrobials are urgently needed. However, new antimicrobials are becoming difficult to develop. Repurposing FDA-approved drugs, with well-characterized toxicology and pharmacology, to find new applications outside the scope of their original medical indication is a novel way to reduce time and cost associated with antimicrobial innovation. In an intensive search for antimicrobial activity among FDA approved drugs, we identified auranofin as a potent drug against VRE. It demonstrated a potent activity against 30 clinical drug-resistant VRE isolates with MIC90(1µg/mL) Additionally, no enterococci resistant mutants could be developed against auranofin after 14 passages. It also, inhibited highly resistant stationary phase cells, biofilm formation on urinary catheters, protease, lipase and haemagglutinins production at sub-inhibitory concentrations. The promising features and the potent antimicrobial activity of auranofin prompted us to evaluate its in vivo efficacy against drug-of-choice, linezolid, in our established lethal VRE septicemia mouse model. Both drugs protected 100% of mice against a lethal VRE dose. However, auranofin was superior to linezolid in reducing the bacterial load in internal organs (liver, kidney and spleen). Remarkably, auranofin cleared VRE efficiently from internal organs after 4 days of oral treatment using a clinically achievable dose. Accordingly, these results indicate that auranofin warrants further investigation as a frontline treatment for lethal VRE infections.

Poster #1

Development of Gateway clones for testing the Bordetella bronchiseptica Fic protein, BbFic, in bacterial, yeast and mammalian systems

Maha Ali [1], Ben Watson[1], Erica Zbornik [1], Seema Mattoo[1]

[1]Department of Biological Sciences, Purdue University, WL, IN, 47905, USA

Fic (filamentation induced by cAMP) proteins constitute a newly discovered enzyme family that plays critical roles in prokaryotic and eukaryotic signal transduction pathways. These proteins act predominantly as adenylyltransferases and are defined by a core HxFx(D/E)(G/A)N(G/K)RxxR motif, where the invariant His is required for activity. By catalyzing an adenylylation or AMPylation reaction, Fic proteins are able to hydrolyze ATP (adenosine triphosphate) to covalently add an AMP (adenosine monophosphate) to their target proteins. Fic proteins were originally identified as bacterial toxins that translocate into mammalian host cells, where they induce cytotoxicity by AMPylating and inactivating Rho GTPases, as a mechanism of evading the host immune response (Mattoo et. al, 2011). The respiratory pathogen, Bordetella bronchiseptica, encodes a single Fic protein (called BbFic) of unknown function. Our preliminary characterization of BbFic led us to hypothesize that it could be important for pathogenesis. We, therefore, opted to analyze BbFic biochemically, by expressing and purifying it from a bacterial protein expression system, and by using mammalian and yeast models to assess morphological phenotypes. BbFic clones for bacterial, yeast, and mammalian expression were generated using the Gateway Cloning Technology. Gateway Cloning Technology is a novel molecular biology technique that bypasses tedious DNA cutting and ligating methods that are traditionally used in cloning procedures. Instead, Gateway cloning relies on the use of a donor plasmid to transfer the same DNA insert into several vector backbones as a single event. With this technology, the wildtype BbFic gene was inserted into a bacterial expression vector containing an N-terminal His6 tag for protein purification by affinity chromatography. Additionally, mammalian and yeast expression constructs with GFP and V5 tags were also generated. Using site directed mutagenesis, I also created the constitutively active BbFic-E62G mutant and its catalytically inactive BbFic-E62G/H196A mutant. The creation of these clones and establishing the Gateway Cloning protocol in the lab has greatly facilitated not just further analysis of BbFic activity in various model systems, but has also allowed us to expand this technology to other Fic proteins being studied in the lab - in particular, Fic proteins from the genetically intractable gastric pathogen, Helicobacter pylori.

Half 1 3:50PM — 4:50PM

Using non-hydrolysable acyl-CoAs to study mechanism and substrate specificity within acyltransferases

Aaron Benjamin[1], Jeremy Lohman[1]

[1]Department of Biological Sciences, Purdue University, WL, IN, 47905, USA

Acyltransferases are a class of enzymes seen throughout metabolism. These enzymes are involved in fatty acid biosynthesis, polyketide synthesis, energy production, and metabolite storage. Understanding how these enzymes choose their substrates and how they function mechanistically is vital for effective treatment of diseases such as cancer, and in engineering of pathways to create antibiotics or antimicrobials. Many of these enzymes utilize metabolites tethered to Coenzyme A (CoAs) which can transfer metabolites due to the reactive thioester bond. Studying these enzymes in a crystallographic manner is often difficult due to this reactivity. As such, we have synthesized a variety of minimally- and non-hydrolysable substrate analogs for acetyl, malonyl, and longer chain acyl CoAs that are being used to study acyltransferases involved in antibiotic resistance, fatty acid biosynthesis, and polyketide synthase pathways. We synthesized these analogs by replacing the thioester sulfur with either an ester, and amide, or making the thioester into a ketone. Our lab is using these analogs to study acyltransferases through a combination of biophysical methodologies and X-ray crystallographically.

Poster #3

Half 1 3:50PM — 4:50PM

The use of dimeric substrates as prodrugs and inhibitors of the ATP binding cassette transporter, P-glycoprotein

Elias Beretta[1] , Christine Hrycyna[1], Jean Chmielewski[1]

[1]Department of Chemistry, Purdue University, WL, IN, 47905, USA

The Blood Brain Barrier serves as a protective barrier and poses a challenge for delivery of therapeutics to the brain. One component of the Blood Brain Barrier that affords protection is the ATP Binding Cassette (ABC) Transporter family, with P-glycoprotein (P-gp) being the most prevalent ABC transporter. Many chemotherapeutics are substrates of P-gp and accumulation of these drugs in the brain is limited by its expression. Here we describe the synthesis and in vitro characterization of heterodimeric prodrug inhibitors of P-gp. In addition to inhibiting P-gp, our dimers are also designed to break down in the reducing environment of the cytosol to release the therapeutics. Based on the glioblastoma multiforme chemotherapeutic, temozolomide, temozolomide quinine heterodimeric inhibitors were created. Temozolomide and quinine are substrates of P-gp, however, the temozolomide quinine heterodimers were found to be potent inhibitors of P-gp mediated efflux at low micromolar concentrations. Initial studies of heterodimer inhibition have shown to have an IC50 value of 8.4 μM in a cell line expressing P-gp. Our results demonstrate the potential of the temozolomide quinine heterodimers to act as inhibitors of P-gp, as well as serving as a prodrug for delivery of temozolomide to the brain. We anticipate that our dimers will improve the efficacy of temozolomide treatment of glioblastoma multiforme by increasing levels of the therapeutic within the brain.

Poster #4

High throughput Experimentation and Continuous Flow Synthesis of Sonogashira Coupling Reaction

Shruti Biyani[1], Prof. David H. Thompson[1], Tiago Sobreira[2], Larisa Avramova[3], David Logsdon[1]

[1] Department of Chemistry, Purdue University, WL, IN, USA, 47905
[2] Computational Life Sciences and Informatics, Purdue University, WL, IN, 47905, USA
[3] Discovery Park, Purdue University, WL, IN, USA, 47905

The aim is to find optimal conditions for the Sonogashira coupling reaction and translate it to continuous flow synthesis. High Throughput Experimentation (HTE) coupled with Mass Spectrometric techniques provide a rapid screen for a wide range of reaction conditions to identify the hotspot that can guide continuous flow synthesis. Flow chemistry enables safe and efficient scale-up due to superior heat and mass transfer. In these experiments, two discrete variables i.e., orders of mixing and catalysts are screened in 96 and 384 well plates using liquid handling robot. Design of Experiments (DoE) approach is used to study continuous variables, times, temperatures and stoichiometries in a 23 factorial design at two levels: 90 minutes, 240 minutes, 100°C, 150°C, 1:1, 1:2.5 with six different solvents, five base, and with/without ligand. 334 unique reactions are conducted with 4 replicates. The effect of individual variable and the interaction between the variables are evaluated against the product ion count. The datasets from HTE are converted into heat-maps and contour plots to highlight the conditions leading to the highest product intensity from Desorption Electrospray Ionization-Mass Spectrometry (DESI-MS) and (+) ESI-MS thereby directing the synthesis strategy for continuous flow. Chemtrix reactors equipped with SOR mixer is used to generate the product in flow at variable residence times at 100°C. Optimization and continuous flow synthesis of a potential drug target to treat Acute Myeloid Leukemia that utilizes Sonogashira coupling will be done in the future.

Half 1 3:50PM — 4:50PM

Elucidating the transcarboxylase mechanism of acetyl-CoA carboxylase

Trevor Boram[1], Lee Stunkard[1], Jeremy Lohman[1]

[1] Department of Biochemistry, Purdue University, WL, IN, 47905, USA

Acetyl CoA carboxylase (ACC) is an enzyme that catalyzes the first committed step in fatty acid synthesis; the formation of malonyl-CoA. ACC performs two half reactions; the ATP dependent carboxylation of biotin, and the transfer of a carboxyl group from carboxy-biotin to acetyl-CoA to form malonyl-CoA. Many have proposed ACC to be a drug target for the treatment of diabetes and obesity, but little is known about the mechanism of the carboxyl transferase reaction, as well as the conformational changes involved. Our lab has developed novel stable malonyl-CoA thioester and carboxylate analogs to probe this enzyme and gain insight into its reaction. These analogs are less susceptible to hydrolysis and decarboxylation, and have been able to reveal catalytic details of various other enzymes through x-ray crystallography. My goal is to use these malonyl-CoA analogs to probe the carboxyl transferase domain of ACC to gain insight into the mechanistic details of its catalysis.

Poster #6

Understanding neurotensin signaling through G protein association and C-terminal phosphorylation

Fabian Bumbak[1] , Joshua J. Ziarek[1]

[1] Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN, 47905, USA

G protein-coupled receptors (GPCRs) make up the largest family of membrane bound receptors in eukaryotic organisms. They exist as receptors for various hormones and neurotransmitters in the human body. Therefore, GPCRs are often involved in many immunological, endocrinological, cardiovascular and neurological diseases. This leads to GPCRs being important drug targets and important macromolecules to study. Agonist binding to GPCR's leads to a wide array of signaling pathways making them very versatile membrane proteins. GPCR's are an example of an allosteric protein in which agonist binding in the extracellular space leads to binding of a partner in the intracellular region. Two signaling pathways that result from ligand binding to a GPCR are G-protein signaling and arrestin signaling. Understanding of how these interactions occur and how signaling is regulated is vital to developing potential drugs to target these GPCRs. Advances in structural techniques and novel analytical methods have allowed probing of the structures of GPCRs and their binding partners. However, there is still much unknown about how signaling and recruitment of binding partners is achieved. Our lab is interested in using nuclear magnetic resonance (NMR) spectroscopy to answer questions about the structural dynamics of the neurotensin receptor, which is a GPCR found in the brain and gastrointestinal tract. Using NMR and targeted isotopic labelling, we hope to analyze how neurotensin receptor interacts with its agonists, antagonists, and binding partners in order to ascertain a clearer picture of GPCR signaling processes.

Half 1 3:50PM — 4:50PM

The evolving history of resolution at Purdue

Valorie Bowman[1]

[1]Department of Biological Sciences, Purdue University, WL, IN, 47905, USA

Cryo Electron Microscopy has been in use at Purdue University since the late 1980s to explore virus and protein structures . Early reconstructions were low resolution, relying on basic technologies, film, and good manual microscopy. As new and better instruments and detectors have become available, the "Resolution Revolution" has made it's way to Purdue with ever higher resolution structures of viruses and protein complexes. It is now possible to image protein complexes ever lower and lower in molecular weight, and these complexes can be resolved even without symmetry.

Poster #8

RNA helicase A enhances the efficiency of HIV reverse transcriptase

Samantha E. Brady[1], Zhenwei Song[1], Ioana Boeras[1], Kathleen Boris-Lawrie[1], and Xiao Heng[1]

[1]Department of Biochemistry, University of Missouri-Columbia, Columbia, MO, 65211, USA

DHX9/RNA helicase A (RHA) is a cellular RNA helicase that participates in many critical steps of the human immunodeficiency virus type 1 (HIV-1) life cycle. It co-assembles with the viral RNA genome into the capsid core, and virions deficient in RHA are less infectious as a result of reduced reverse transcription efficiency. Here, we report that the amount of HIV-1 reverse transcription products synthesized in infected cells depends on the RHA expression levels in the virus producer cells. Purified recombinant RHA promotes reverse transcription efficiency under in vitro conditions that mimic the strong stop cDNA synthesis and (-)cDNA synthesis after the first strand transfer. The RHA-dependent rate enhancement of DNA synthesis does not occur until after the incorporation of the first few nucleotides, suggesting that RHA participates primarily in the elongation phase of reverse transcription. Steady-state kinetic studies show that RHA does not affect the observed rate of nucleotide incorporation, but enhances the processivity of reverse transcriptase (RT) by reducing the dissociation rate of RT (koff) from the template-primer complex. These data demonstrate that RHA is necessary for the efficient catalysis of proviral DNA synthesis during HIV-1 infection.

Intra-surgical Detection of IDH Mutations in Human Glioma using a Miniature Mass Spectrometer

Hannah Marie Brown[1] , Fan Pu[1] , Clint Alfaro[1] ,Zheng Ouyang[1,2], Aaron A. Cohen-Gadol[3], Mahua Dey[3], Scott A. Shapiro[3], Graham R. Cooks[1]

[1] Department of Chemistry, Purdue University, WL, IN, 47905, USA
[2] Tsinghua University, Beijing, China
[3] Department of Neurological Surgery, Indiana University School of Medicine, Indianapolis, IN, 46202, USA

Isocitrate dehydrogenase (IDH) 1 mutations, prevalent in glioma, produce 2-hydroxyglutarate (2-HG), a possible biomarker of IDH mutation status. These mutations have potential prognostic value and knowledge of the mutation status could lead to improved tumor resection. While immunohistochemistry and genomic analysis remain the gold standards for detecting IDH mutations, such processes are time-intensive, often determining mutation status days after tumor resection. Here the intra-surgical analysis of 2-HG in human glioma is performed using a rapid extraction nanoelectrospray ionization (nESI) method coupled to a miniature mass spectrometer (Mini MS) that allows for near “real-time” determination of IDH mutation status. With its small size, low power consumption and noise, and fast, reliable IDH mutation status predictions, this system is a powerful tool that can be easily implemented in the current surgical workflow.

Half 1 3:50PM — 4:50PM

Characterization of a FIC Protein from the Respiratory Pathogen, Bordetella Bronchiseptica

Sherlene Brown[1] , Hee-jeong Kim, Sanjeev Kumar[2], Erica Zbornik[2], and Seema Mattoo[2]

[1] Department of Biochemistry, Purdue University, WL, IN, 47905, USA
[2] Department of Biological Sciences, Purdue University, WL, IN, 47905, USA

Bordetella sp. are respiratory pathogens, the most notorious being B. pertussis which causes whooping cough in humans. In contrast to the human-restricted B. pertussis, another species - B. bronchiseptica - has a broader host range, and causes various respiratory disease including kennel cough in dogs. Interestingly, while B. bronchiseptica infection in humans is rare, recent reports indicate an increase in the number of B. bronchiseptica infections in immunocompetent humans and suggest involvement of novel virulence factors that may not be under the control of the classical BvgAS two-component signal transduction system. We, therefore, sought to test whether the sole Fic protein in B. bronchiseptica, BbFic, could be involved in virulence. Fic proteins are a family of conserved adenylyltransferases that are implicated in several cellular processes including cytoskeletal rearrangement, protein trafficking and bacterial pathogenesis. Using molecular docking and kinetic analyses we elucidated a mechanism for Bbfic nucleotide specificity and show that Bbfic does not bind ATP but instead shows a 30-fold increased preferential usage for GTP.

Poster #11

Computational Model of Activity-dependent, Alpha/Beta-Actin Dynamics in the Postsynaptic Density (PSD)

Peter Brumm[1, 2],Matthew Pharris[2] , Tamara Kinzer-Ursem[2]

[1] Life Science - Interdisciplinary Studies, Purdue University, WL, IN, 47905, USA
[2] Department of Biomedical Engineering, Purdue University, WL, IN, 47905, USA

Activity-dependent modification of neural synapses provides one mechanistic explanation of how information is directed and stored as memories. The theory of synaptic plasticity poses that changes in synaptic strength depends on the molecular mechanisms of constituent signaling cascades. In the hippocampus, NMDAR-dependent signalling cascades in the dendritic spine have been shown to modulate the synaptic strength by: (1) activating ion-conducting AMPA receptor, and (2) increasing the dendritic spine size. Often associated with cellular structure, the activation-state of Calmodulin-dependent kinase II (CaMKII) deferentially regulates globular and filamentous actin dynamics. While many studies have begun to explain these modifications in relation to inter- and intra-cellular signaling cascades, spatio-temporal limitations of biological imaging techniques have frustrated a complete understanding. For this reason, computational models have been developed to mechanistically interpret synaptic plasticity. In this study, a spatio-kinetic model has been developed to explain activity-dependent structural modification through the differential activation of downstream signalling proteins.

Chemical Cleavage and Mass Spectrometry Analysis of the Substrate Binding Site of Ste14

Ariana Cardillo[1], Amy Funk[2], Mark Distefano[3], Christine Hrycyna[1,4]

[1] Department of Chemistry, Purdue University, WL, IN, 47905, USA
[2] National Cancer Institute, ATRF, Bethesda, MD, 20892, USA
[3] Purdue Center for Cancer Research, Purdue University, WL, IN, 47905, USA
[4] University of Minnesota, Minneapolis, MN, 55455, USA

The Ras family of proteins are involved in cell signaling pathways including cell growth, differentiation and survival. K-Ras, a CAAX protein, is involved in 90% of all pancreatic cancers. Proper localization and function of these proteins requires three post-translational modifications. The integral membrane protein isoprenylcysteine carboxyl methyltransferase (Icmt), is involved in the third of these three post-translational modifications of CAAX proteins by methylating their C-terminal cysteine residue. However, the mechanism and residues associated with substrate binding to Icmt is still unknown. This study proposes a narrowed segment of focus of the yeast Icmt, Ste14 that is involved in substrate binding. By utilizing techniques like photoaffinity labeling and mass spectrometry, we hope to identify the specific amino acids involved in substrate binding. Continued research in the specific binding of Ste14 will aid in elucidating key drug targets for K-Ras mutated cancer.

Investigating host-pathogen interactions in EBV-associated cancers

Srishti Chakravorty[1] , Bingyu Yan[1] , Chong Wang[2], Joydeb Majumder[3], Luopin Wang[4], Alejandro Canaria[5], Matthew R. Olson5, Gaurav Chopra[2], Bo Zhao[2], Behdad Afzali[6], Majid Kazemian[1,4]

[1] Department of Biochemistry, Purdue University, WL, IN, 47905, USA
[2] Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[3] Department of Chemistry, Purdue University, WL, IN, 47905, USA
[4] Department of Computer Science, Purdue University, WL, IN, 47905, USA
[5] Department of Biological Science, Purdue University, WL, IN, 47905, USA
[6] Immunoregulation Section, Kidney Diseases Branch, NIDDKD, NIH, Bethesda, MD, 20892, USA

Epstein-Barr Virus (EBV) is a complex oncogenic virus and one of the leading causes of mortality. It is predicted that deaths due to EBV-associated cancers would increase drastically with rise in population and life-expectancy. EBV is implicated in malignancies of both lymphoid and epithelial origin such as Burkitt lymphoma (BL) and Stomach Adenocarcinoma (STAD) respectively. Till date, there is no cure or vaccines against EBV and the mechanism underlying EBV carcinogenesis remain elusive. One of the focus of our lab is to investigate the host-pathogen interactions in EBV-associated cancers. Using computational analyses of RNA sequencing data from different cancer patients, we have made several observations. First, a subset of EBV genes are preferentially expressed in EBV-associated cancers than normal cells immortalized by in vitro EBV infection. Select EBV transcripts that are inversely correlated with EBV RNA load are frequently mutated in cancer. A molecular signature characterized by type I interferon (IFN) response distinguish a subset of EBV+ tumors (e.g. STAD) from others, which results in elevated expression of several immune checkpoints including Programmed death-ligand 1 (PDL1) and Indoleamine-pyrrole 2,3-dioxygenase (IDO1). We have validated these findings in various cells lines at both mRNA and protein level. Based on our findings, we hypothesize that in some cancers (e.g. STAD), EBV re-activation upregulates the IFNγ-IRF1 axis which further upregulates the expression of PDL1 and IDO1, suggesting a mechanism for EBV immunoevasion. Our work also provides a rationale for the efficacy of checkpoint inhibitor treatment in a subset of EBV associated cancers.

Influence of the ubiquitin-like domain 2 on the papain-like protease 2 from human coronavirus, NL63

Mackenzie Chapman[1], Courtney Daczkowski[2], Andrew Mesecar[1,2,3]

[1] Department of Biological Sciences, Purdue University, WL, IN, 47905, USA
[2] Department of Biochemistry, Purdue University, WL, IN, 47905, USA
[3] Purdue Center for Cancer Research, Purdue University, WL, IN, 47905, USA

The NL63 virus is one of five human coronaviruses (HCoVs) that causes both upper and lower respiratory tract infections. Coronaviruses are positive-sense RNA viruses that encode a polyprotein that is processed by two or three viral proteases. One of these proteases, papain-like protease 2 (PLP2) is encoded in nonstructural protein (nsp3) of NL63 which contains multiple functional domains. Adjacent to the N-terminus of the PLP2 domain is a Ubiquitin-like domain 2 (Ubl2) that is thought to be involved in protein regulation as well as provide stability to the enzyme during infection. PLP2s are essential for viral replication and evasion of the host-innate immune system which results from PLP2s having deubiquitinating and deISGylating activity. Currently, it is not known whether the Ubl2 domain of NL63 nsp3 contributes to the catalytic activity, substrate specificity or thermostability of the PLP2 catalytic domain. In this study, it is demonstrated that the Ubl2 domain adjacent to PLP2 does not alter the steady-state kinetic properties of PLP2 hydrolysis of three of its substrates (Ub-AMC, ISG15-AMC and RLRGG-AMC). Currently, the influence of the Ubl2 domain on the thermostability and ubiquitin-peptide chain cleaving ability of PLP2 is being investigated. In addition, crystallization of PLP2 alone and in complex with free Ubiquitin and small molecule inhibitors is being pursued. These studies will further our understanding of the role of the Ubl2 domain on PLP2 catalysis and will help to evaluate potential inhibitors for drug design.

Half 1 3:50PM — 4:50PM

Discovery of Bisubstrate Inhibitors for Protein N-terminal Methyltransferase 1

Dongxing Chen[1,2,3],Guangping Dong[1,2,3], Nicholas Noinaj[4,5,6] , and Rong Huang[1,2,3]

[1] Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, WL, IN, 47905, USA
[2] Purdue Center for Cancer Research, Purdue University, WL, IN, 47905, USA
[3] Institute for Drug Discovery, Purdue University, WL, IN, 47905, USA
[4] Markey Center for Structural Biology, Purdue University, WL, IN, 47905, USA
[5] Department of Biological Sciences, Purdue University, WL, IN, 47905, USA
[6] Purdue Institute of Inflammation, Immunology, and Infectious Disease, Purdue University, WL, IN, 47905, USA

Protein N-terminal methyltransferase 1(NTMT1) was discovered several years ago and identified as the first eukaryotic methyltransferase which can methylate various proteins bearing an N-terminal X-P-K/R (X represents any amino acid other than D/E) consensus sequence such as regulator of chromatin condensation 1 (RCC1), the tumor suppressor retinoblastoma1(RB1), and damaged DNA-binding protein 2(DDB2). NTMT1 is upregulated in various cancers including colorectal cancer, melanoma and brain cancer, and knockdown of NTMT1 results in cell mitotic defects. Potent and selective NTMT1 inhibitors are valuable chemical tools for testing biological and therapeutic hypotheses. However, very few NTMT1 inhibitors have been reported. NTMT1 have two binding pockets: a protein substrate binding pocket and a methyl donor S-adenosylmethionine (SAM) binding site. In our previous studies, we have identified that NTMT1 followed a random sequential Bi-Bi mechanism and will form a ternary complex with both co-factor SAM and protein substrate. Guided by this mechanism, we designed and synthesized bisubstrate analogues that covalently link a SAM analogue with a peptide substrate moiety, thus potentially mimicking the ternary transition state of a bisubstrate enzymatic reaction. Bisubstrate analogues have three components: A S-adenosyl methionine (SAM) analog, a protein substrate fragment, and a carbon linker. The bisubstrate inhibitor NAM-C3-PPKRIA (4) is potent (IC50 = 0.15 ± 0.02 μM) and exhibits a competitive inhibition pattern for both SAM and peptide substrate. Furthermore, we confirmed its potent inhibition through an orthogonal MS-based assay, which displayed an IC50 of 35 ± 2 nM. Importantly, we obtained the first crystal structure of human NTMT1 in complex with the inhibitors. The structure of the NTMT1-4 complex has showed the compound 4 does occupy both cofactor and substrate binding sites. In summary, our study provides valuable chemical probes to interrogate the biological functions of NTMT1 and is valuable to develop the next generation of NTMT1 inhibitors with improved potency and selectivity.

Poster #16

Half 1 3:50PM — 4:50PM

Deciphering the role of N-terminal methylation in modulating yeast protein function including the multitasking stress response protein, Hsp31

Panyue Chen[1], Guangping Dong[1], Rong Huang[1] , and Tony R. Hazbun[1]

[1] Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, WL, IN, 47905, USA

As the second most common protein post-translational modification, protein methylation, especially a-N-methylation is largely underexplored compared to protein acetylation. It has been shown human N-terminal methyltransferases (NTMT1 and NTMT2) has important function in cell division by regulating RCC1 and centromere protein such as CENP-A and is implicated as a cancer target. In this study we are going to use yeast as an idea model to investigate the N-terminal methylome, taking advantage of its well observed phenotypes, fully sequenced genome and comparatively simplified N-terminal methyltransferase network.

The first and sole cytosolic N-terminal methyltransferase identified in yeast, Tae1, recognizes a protein N-terminal motif sequence M-X-P-K as its human homologues. Initial sequence based bioinformatic research indicates that 45 proteins might be the regulated by Tae1 through a-N-methylation including heat shock protein 31 (Hsp31) and its paralogues (Hsp32, Hsp33 and SNO4). The yeast chaperone, Hsp31, is a multitasking protein that adopts a homodimer conformation and has functional similarities to Parkinson's disease protein, DJ-1. Hsp31 is involved in multiple cellular functions including oxidative stress sensing, protein folding, proteasome degradation, and deglycase enzyme activity. Our lab have previously demonstrated that Hsp31 expression is induced by oxidative stress and aSyn mediated proteotoxic stress. However, it remains unclear how Hsp31 function is regulated post-transcriptionally. In this study, we are heading to identify this novel type of modification on Hsp31 and unravel its regulation mechanism through methylation.

We have verified the presence of mono- and di-methylation on Hsp31 using mass spectrometry and immune-detection while there is also evidence suggesting the rare presence on Hsp31 under normal condition and non-uniqueness of tae1 a-N-methyltransferase in yeast. In order to answering the question how the methylation regulates Hsp31 function, we made single amino acid mutations on the consensus motif of Hsp31 with varies methylation potency. Preliminary in vitro biochemical assays suggested that loss methylation might lead to the lower methylglyoxalase activity of Hsp31 and higher chaperone activity. Further study would be confirming the a-N-methylation level of each Hsp31 mutants and establishing the relevance between a-N-methylation level and different Hsp31 functions.

Poster #17

Pharmacodynamics and Immune Cell Interactions during Immunomodulatory Treatment of HIV: a Mechanistic Modelling Approach

Jonathan Cody[1], Amy L. Ellis-Connell[2], Shelby L. O'Connor[2], Elsje Pienaar[1]

[1] Weldon School of Biomedical Engineering, Purdue University, WL, IN, 47907, USA
[2] Department of Pathology and Laboratory Medicine,U Wisconsin-Madison, Madison, WI, 53532, USA

Immunomodulatory drugs could be critical to a functional cure for human immunodeficiency virus (HIV). N-803 is an established superagonist for IL-15, an important cytokine that promotes maintenance and activation of cytotoxic immune cells. N-803 has been shown to expand cytotoxic T cell and natural killer (NK) cell populations and suppress the viral population in simian immunodeficiency virus (SIV), a widely-used animal model of HIV. However, the initially strong responses to the drug are attenuated with continued treatment, partially returning after a long treatment interruption. While biological evidence of IL-15 receptor downregulation and immune regulation could explain these dynamics, the potential contribution of such mechanisms has not been quantitatively assessed. We present an ordinary differential equation model of N-803 treatment of SIV-infected macaques. Our model includes separate cytotoxic T-cell and NK cell populations with N-803-dependent proliferation. We also incorporate mechanisms affecting cell cytotoxicity, such as drug stimulation, receptor downregulation, and immune regulation. Models with different combinations of these mechanisms are calibrated to viral and lymphocyte responses following weekly 0.1 mg/kg subcutaneous doses of N-803 given to chronically SIV-infected rhesus macaques who had all received prior vaccination. We compare these models based on their Akaike Information Criterion as well as their ability to qualitatively reproduce the experimentally observed data. The dynamics of the cytotoxic cells and the viral load are best represented by pharmacodynamic models that include both the fast action of immune regulators and the slower receptor downregulation. Results indicate that these regulatory mechanisms are important limiting factors in N-803 immunomodulatory treatment of SIV. These would need to be accounted for in regimen optimization and combination therapy for the treatment to be successfully applied to HIV. Our mechanistic model will inform such optimization and therapy design and help guide future studies.

Generating Properly Folded Neurotensin Receptor-1 Through Cell-Free Protein Synthesis

Austin Dixon[1], Prof. Joshua J. Ziarek[1,2,3]

[1] Department of Molecular and Cellular Biochemistry, Indiana University,Bloomington, IN, 47405, USA
[2] Department of Chemistry, Indiana University,Bloomington, IN, 47405, USA
[3] Department of Neuroscience, Indiana University,Bloomington, IN, 47405, USA

The United Nations Office on Drugs and Crime reported that in 2017 the most abused recreational drugs were cannabis, opioids and opiates, amphetamines, ecstasy, and cocaine. These compounds garnered over 312-million unique users resulting in an estimated loss of 28-million years of ‘healthy’ life. All of these drugs possess a striking similarity as they interact with G-protein-coupled receptors. GPCRs are the most prevalent and diverse group of eukaryotic membrane signaling proteins and are implicated in many diseases such as endocrinological, immunological, cardiovascular, and mental disorders. This has resulted in GPCRs being the target of over thirty-percent of current marketable drugs. Despite this enormous presence of drug abuse, drug targets and disease states there exist only 59 unique GPCR structures, out of 826 known receptors in humans. All of these full-length structures were solved via X-ray crystallography through static and inactivating fusion-protein constructs. Transmitting information of the outside environment to the inside of cells results in GPCRs being highly dynamic. In the absence of stimulating agonist these receptors possess basal signaling activity. It is therefore crucial that the structural and signaling dynamics of this class of proteins be elucidated as more efficacious drug targets will be found to treat drug addiction and disease. Currently, nuclear magnetic resonance spectroscopy is the premier technique for discerning global protein structural dynamics in solution. NMR signal detection requires purified and isotopically labeled protein, hindering previous attempts using conventional methods. We hypothesize that in vitro cell-free protein synthesis will generate properly folded Neurotensin Receptor-1 for NMR analysis, a canonical GPCR in the same class of receptors targeted in both drug design and abuse.

Half 1 3:50PM — 4:50PM

Synthetic Antibodies for Structural Biology Applications

Satchal Erramilli[1], Somnath Mukherjee[1], Przemyslaw Dutka[1], Piotr Tokarz[1], Kamil Nosol[1], Blazej Skrobek[1], Anthony Kossiakoff[1]

[1] Department of Biochemistry and Molecular Biology, UChicago, Chicago, IL, 60637, USA

We have developed strategies for selecting conformation specific synthetic binders for membrane proteins in native lipid environments. This approach can stabilize membrane protein conformations and aid structure determination efforts. We present multiple cases, including from our own lab, that make use of such reagents for cryo-EM. Additionally, we have developed approaches combining genetic fusion with BRIL, BRIL-specific binders, and synthetic Fab-specific reagents that potentially extend the range of cryo-EM for any target protein. These tools and approaches, when fully deployed, could ameliorate structural analyses by cryo-EM.

Poster #20

Structural Insights into Phospholipase C Epsilon Activity

Elisabeth E. Garland-Kuntz[1], Frank S. Vago[2], Ngango Y. Rugema[1], Satchal K. Erramilli[3], Monita Sieng[1],Michelle M. Van Camp[1], Srinivas Chakravarthy[4], Anthony A. Kossiakoff[3], Wen Jiang[2] , and Angeline M. Lyon[1,2]

[1] Department of Chemistry, Purdue University, WL, IN, 47905, USA
[2] Department of Biological Sciences, Purdue University, WL, IN, 47905, USA
[3] Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, 60637, USA
[4] Bio-CAT, Advanced Photon Source, Argonne National Laboratory, Lemont, IL, 60439, USA

Phospholipase C (PLC) enzymes hydrolyze phosphatidylinositol lipids to produce second messengers, including inositol-1,4,5-triphosphate (IP3) and diacylgycerol (DAG), which increase intracellular calcium and activate protein kinase C (PKC), respectively. PLC epsilon (PLCe) contributes to cardiac hypertrophy and contractility, as well as to oncogenic and inflammatory signaling pathways following activation of G protein-coupled receptors and receptor tyrosine kinases. PLCe shares a conserved core with the PLC superfamily, but the roles of individual domains in regulation of activity and membrane binding have not been established. We recently provided the first structural insights into domain organization of catalytically active PLCe using small-angle X-ray scattering (SAXS) and electron microscopy (EM) to reveal that the PH domain is conformationally heterogeneous in solution. Comparisons of the PLCe solution structure to that of the closely-related PLC beta enzyme demonstrate that the PLC beta PH domain is also mobile in solution, in contrast to previously reported crystal structures. We propose that the dynamic nature of the PLC PH domain and resulting conformational heterogeneity contributes to subfamily-specific differences in activity and regulation by G proteins. We are currently developing conformational antibodies against specific PLCe domains and are using these, together with crystallography and cryo-EM, to expand on these findings and obtain higher resolution structures.

Identification of regions in proteins prone to structural changes from sequence data by deep learning

Ahmadreza Ghanbarpour[1] & Markus Lill[1]

[1] Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, WL, IN, 47905, USA

Protein flexibility is greatly important in accurate virtual screening and ligand docking. Many docking methods simply fail due to the lack of knowledge of correct structural changes of the proteins upon ligand binding, and due to experimental difficulties, many protein targets structures in complex with their corresponding ligands are not known. Some approaches have been developed to address this issue, such as induced-fit docking. However, they use of these methods is limited in tasks such as virtual screening due to high computational costs. Other methods, such as ensemble docking rely on most prominent protein conformations that are known or sampled during molecular dynamics (MD) simulations to improve the accuracy of screening by docking the molecules on different conformations of the target. However, sampling major structural changes in proteins by simulations is not straight forward in many cases and require very long simulations. To better predict different conformations of a protein, it is useful to first know which parts of a protein are prone to structural change. In the present work, we take the steps to predict such structural changes, more specifically, secondary structural changes, by only using the sequence data of a protein. We train a deep neural network using the “structural propensity database of proteins” and we show that the model is able to predict structural propensities of new sequences with sufficient accuracy and can identify the regions that are more likely to undergo structural change or exhibit different secondary structures in different conditions on the same protein. This study shows that using the structural propensity data, one can detect regions more likely to become disordered, although they may exhibit other forms of secondary structures such as alpha helix. We believe such information can aid strategies to predict different conformations of regions of the protein where limited structural information is available, and ultimately improve the virtual screening process in drug discovery.

Computational modeling of the calcium dependent competitive tuning of PDZ domains in the postsynaptic density

Patrick A. Giolando[1], Barrett Davis[1], Matthew C. Pharris[1], Tamara L. Kinzer-Ursem[1]

[1] Weldon School of Biomedical Engineering, Purdue University, WL, IN, 47905, USA

Approximately, 1 in every 60 children in the United States have been diagnosed with Autism Spectrum Disorder (ASD). Of the hundreds of genes associated with ASD, those most highly correlated with the disorder produce proteins involved in neuronal synapse function. Among these SYNGAP1 is a major constituent of the post-synapse and is a downstream target of excitatory neurotransmitter glutamate (via NMDA receptor activation). Equally highly expressed is the scaffolding protein PSD-95, that binds synGAP at all three of its PDZ domains, potentially restricting the binding of other proteins to these sites including TARPs and LRRTM proteins involved in regulating synaptic functions. To gain a full understanding on the competition for PDZ domains we developed a computational model that focuses on two events influencing the binding of synGAP to PDZ domains: the binding of Ca2+/Calmodulin (Ca2+/CaM) to synGAP, and phosphorylation of synGAP by Ca2+/calmodulin-dependent protein kinase II (CaMKII). These calcium dependent events act as a switch to free up PDZ domains and allow for the anchoring of receptors.

Reduced Accumulation of Fluorescent Substrates of P-glycoprotein through Mutation of a Conserved “Kink” Motif

Jason Goebel[1] & Christine Hrycyna[1]

[1] Department of Chemistry, Purdue University, WL, IN, 47905, USA

P-glycoprotein (P-gp) is an ATP Binding Cassette transporter and plays a major role in xenobiotic efflux at important blood tissue barriers. It is well known that many therapeutics cannot cross these barriers due to polyspecificity exhibited by P-gp. In order to achieve better delivery of therapeutics to their targets the structure and function of P-gp is being investigated. There are many conserved motifs that have been discovered in P-gp that are crucial to its activity. These include the Walker A, B, and ABC signature motifs for ATP binding and hydrolysis as well as intracellular loop motifs for protein crosstalk. Through investigation of primary sequences across multiple species a novel motif is being investigated in transmembrane domain (TMD) 12. Conserved residues 996-1001 PDYAKA confer an unordered region, “kink”, in the middle of TMD 12 that subsequently becomes ordered during efflux in crystal structures of hP-gp. Mutation of the “kink” reduces fluorescent substrate accumulation based on flow cytometry. Different fluorescent substrates accumulated differently potentially signifying reduced crosstalk between TMDs and nucleotide binding domains.

The miR-199-CDK2 axis modulates neutrophil migration and systemic inflammation

Alan Y. Hsu[1], Decheng Wang[1,2], Sheng Liu[3,4], Justice Lu[1], Ramizah Syahirah[1],David A. Bennin[5], Anna Huttenlocher[5,6] Jun Wan[3,4,7], Qing Deng[1,8,9]

[1] Department of Biological Sciences, Purdue University, WL, IN, 47907 USA
[2] Medical College of China Three Gorges University, Yichang, Hubei, 443002 PR China
[3] Department of Medical and Molecular Genetics, IU School of Medicine, Indianapolis, IN 46202 USA
[4] Collaborative Core for Cancer Bioinformatics, IU Simon Cancer Center, Indianapolis, IN 46202 USA
[5] Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, USA
[6] Department of Pediatrics, University of Wisconsin-Madison, Madison, WI, USA
[7] Center for Computational Biology and Bioinformatics,IU School of Medicine, Indianapolis, IN 46202 USA
[8] Purdue Institute for Inflammation, Immunology, & Infectious Disease, Purdue University, WL, IN, 47907 USA
[9] Purdue University Center for Cancer Research, Purdue University, WL, IN, 47907 USA

Neutrophil migration is tightly regulated to defend against pathogens, resolve acute inflammation, and maintain homeostasis. MicroRNAs are attractive tools and targets for novel therapeutic approaches. However, the use of microRNAs in neutrophils has not been fully explored . Using an in vivo neutrophil-specific microRNA overexpression screen, we identified eight microRNAs as potent suppressors of neutrophil migration. Among those, miR-199 decreases motility and chemotaxis of both zebrafish neutrophils and human neutrophil-like cells. Intriguingly, in terminally differentiated neutrophils the pathways significantly altered by miR-199 are cell cycle-related. Cyclin dependent kinase 2 (cdk2) is identified as a direct target of miR-199. Pharmacological or genetic inhibition of Cdk2 disrupts cell polarity and chemotaxis of primary human neutrophils. Furthermore, miR-199 overexpression or Cdk2 inhibition significantly improves the outcome of lethal systemic inflammation challenge. Together, our results reveal previously unknown functions of miR-199 and Cdk2 in regulating neutrophil migration and provide new directions in alleviating systemic inflammation.

Insights into the Role of the Membrane on PLCb and Gaq-Mediated Activation and Adsorption

Brianna Hudson[1], Seok-Hee Hyun[1], David Thompson[1], and Angeline Lyon[1,2]

[1] Department of Chemistry, Purdue University, WL, IN, 47905, USA
[2] Department of Biological Sciences, Purdue University, WL, IN, 47905, USA

Phospholipase C beta (PLCb) enzymes are peripheral membrane proteins that are required for normal cardiovascular function and whose dysregulation results in cardiovascular disease. PLCb hydrolyzes phosphatidylinositiol-4,5-bisphosphate (PIP2) to produce the two potent second messengers inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG), which increase intracellular Ca2+ and activate protein kinase C. PLCb has low basal activity, but is activated downstream of G protein-coupled receptors (GPCRs) through direct interactions with the heterotrimeric G protein subunits Gaq and Gby. While G proteins stimulate activity, they are insufficient for full activation. This suggests that the membrane is required for full activation. However, the molecular basis for how the membrane and its properties contribute to PLCb adsorption, activity, and Gaq-dependent activation are not well understood. We seek to understand how membrane composition helps regulate adsorption of PLCb to the membrane and promote interfacial activation. We are also investigating how interfacial activation impacts known regulatory elements within PLCb and alters Gaq-dependent activation. Using a model membrane system, we are applying an innovative combination of atomic force microscopy, mass spectroscopy, and biochemical assays to begin understanding how the membrane itself, PLCb autoinhibitory elements, and Gaq regulate PLCb activation. These studies provide the first structure-based approach to understanding how the cell membrane regulates the activity of this essential effector enzyme.

Basis of UbE2N recognition by a bacterial ubiquitin deamidase/ligase

Shalini Iyer[1], Kedar Puvar[1], Kristos Negron[1], Sebastien Kenny[1], Chittaranjan Das[1]

[1] Department of Chemistry, Purdue University, WL, IN, 47905, USA

The intracellular pathogen Legionella pneumophila injects a variety of effectors into the host cell, several of which have been found to interfere with ubiquitin signaling. One recently discovered example is MavC, an enzyme found to catalyze ubiquitination of UbE2N via a new transglutaminase-based mechanism. Addition of ubiquitin (Ub) to key host proteins like UbE2N is central to the strategy employed by the bacteria to suppress the host immune response and ensure their survival. In this study, we investigated how MavC recognizes UbE2N and ubiquitin. An insertion domain, previously not observed in any other effector studied thus far, is required for UbE2N recognition as well as the enzyme's ubiquitinating activity. The overall mode of Ub and UbE2N recognition and specificity of MavC towards UbE2N over other structurally similar E2 proteins is presented. A clear structural explanation for the effect of ubiquitination on UbE2N activity is also provided. This work provides an important platform for further investigations into the process of ubiquitination by transglutamination.

Half 1 3:50PM — 4:50PM

Targeting Cancer via Inhibition of Folate-Utilizing Enzymes SHMT1 and SHMT2 in One-Carbon Metabolism

Jade M. Katinas[1], Justin Stiles[1], Adrianne Wallace-Povirk[2], Aamod S. Dekhne[2], Arpit Doshi[3], Aleem Gangjee[3], Larry H Matherly[3], and Charles Dann III[1]

[1] Department of Chemistry, Indiana University, Bloomington, IN, 47405, USA
[3] School of Medicine, Wayne State University, Detroit, MI 48201,USA
[3] Department of Chemistry, Duquesne University, Pittsburgh, PA 15282, USA

A vital step for cancer cells to develop into malignant tumors, cell proliferation must be increased through bypass of normal cell cycle control. One approach to ensure that the demands of increased cellular proliferation are met is to upregulate one-carbon (1C) metabolic pathways. Historically, antifolate inhibitors targeting cytosolic proteins in 1C metabolism have been used for cancer treatment, but these agents cause severe side effects due to action on healthy cells. Additionally these drugs, which act primarily on single enzymes in 1C pathways, can become ineffective due to selection during tumor progression leading to resistance. To address these issues, we have designed targeted cancer therapeutics to target diseased cells with potency against multiple critical enzymes in 1C metabolic pathways. Specifically, a robust assay to quantify the activity and evaluate the structure-activity relationship (SAR) of these molecules as inhibitors of cytosolic serine hydroxymethyltransferase (SHMT) 1 and mitochondrial SHMT2 has been developed. Our SAR studies have identified specific physical characteristics of the molecules that make them potent inhibitors of SHMT1/2, data that will be leveraged to improve design of future molecules.

Poster #28