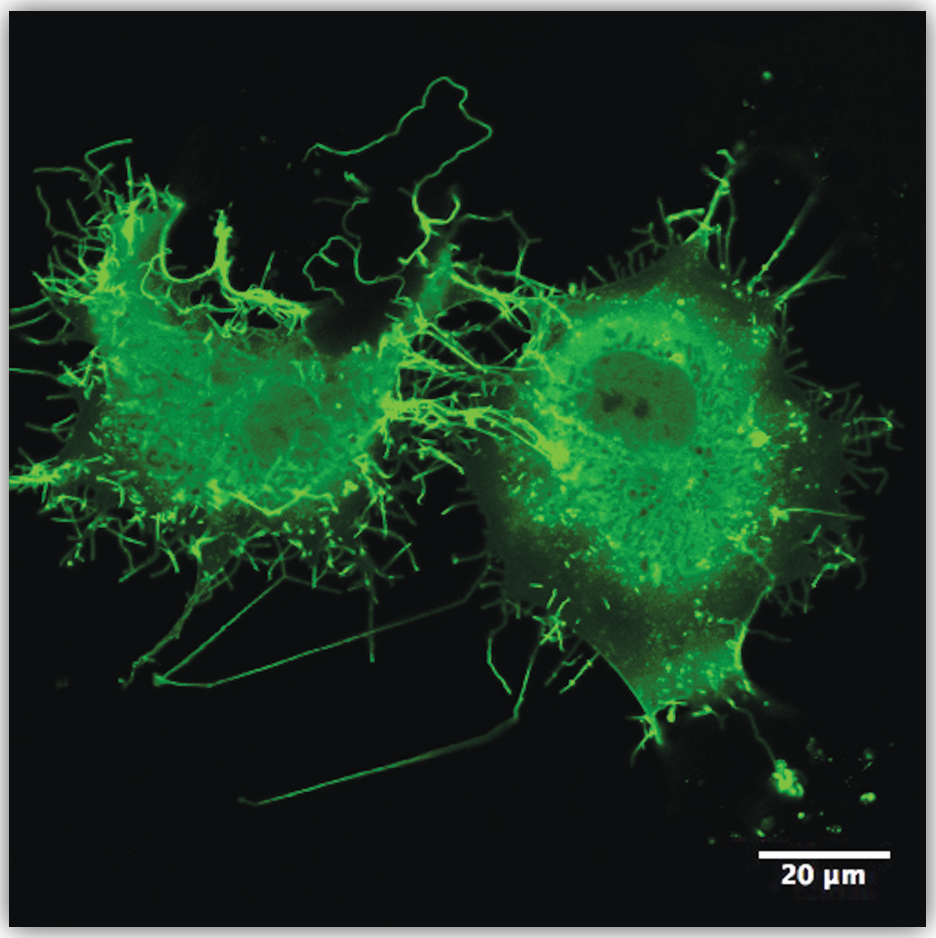
The O'Connor Papers

20th Annual John V. O'Connor Biochemistry Research and Education Conference

Department of Chemistry and Biochemistry
&
Integrated Biomedical Sciences Graduate Program
University of Notre Dame
Notre Dame, Indiana



Swan Lake Resort
Plymouth, Indiana
June 17-18, 2015

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Anthony S. Serianni

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In Memory of Dr. John V. O'Connor ND'72

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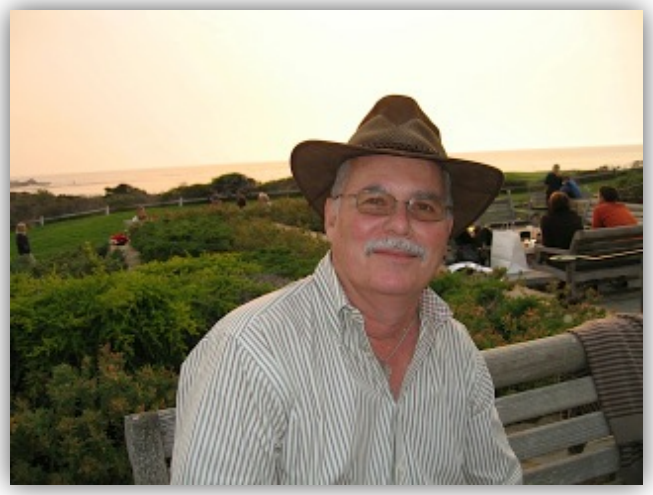
Cover Illustration

Courtesy of the Stahelin Lab

Cover Graphic: The Ebola virus matrix protein, VP40, forms virus-like particles and buds from the plasma membrane of live cells. VP40-EGFP 14 hours post transfection in COS-7 cells. Image taken on a Zeiss 710 laser scanning confocal microscope, Pln Apo 63X/1.4na.

Dr. John V. O'Connor ND'72
Science Leader and Friend of Notre Dame

For more than six years, we have been privileged to receive financial support for this retreat from Dr. John V. O'Connor, who befriended Anthony Serianni during their graduate studies in the Department of Biochemistry at Michigan State University some years ago. Sadly, John passed away in 2012, and the world is a poorer place as a result. His spouse, Barbara O'Connor, herself an accomplished scientist, recently endowed the retreat in John's honor, and we are greatly appreciative of this generous support of our graduate program. Below is a brief description of John's life, his professional accomplishments, and his legacy.



John V. O'Connor, PhD
March 3, 1951 – December 11, 2012

John received his Bachelor's Degree from the University of Notre Dame in 1972, and his PhD in Biochemistry from the University of Iowa in 1978. His PhD dissertation was entitled: "^{13}C and ^{1}H NMR Spectroscopy of Glycosyl Phosphates: Their Conformation and Stability." After postdoctoral training at The Hershey Medical School, Penn State University, John moved to California to join a fledgling Genentech, Inc. in 1981. During his successful thirty-year plus career there, he held a variety of positions where he developed many professional and personal relationships, both nationally and internationally. He was most recently the Head of Global Quality Inspection Management, working with scientists and regulators all over the world to ensure successful approval of both Genentech and Roche pharmaceuticals. While at Genentech, John received several patents and had many publications.

Previous Keynote Speakers

1996: Nicholas Paoni (Genentech)
1998: Les Kozak (Jackson Laboratory)
1999: Andy Mesecar (IU-Chicago)
2000: Thomas Kelly (Case Western)
2001: Kwok Yeung (Bayer)
2002: John Beals (Lilly)
2003: Dudley Strickland (Red Cross)
2004: Elizabeth Komives (UCSD)
2005: Erik Zuiderweg (Michigan)
2006: Lila Gierasch (U. Mass)
2007: Shelagh Ferguson-Miller (Michigan State)
2008: Christian R. H. Raetz (Duke)
2009: John L. Wang (Michigan State)
2010: Thomas A. Gerken (Case Western)
2011: John A. Gerlt (Illinois) (postponed)
2012: Vern L. Schramm (Albert Einstein)
2013: Adriaan Bax (NIH)
2014: Enrique M. De La Cruz (Yale)

The John V. O'Connor Lectureship in Biochemistry

C. Dale Poulter
Department of Chemistry
University of Utah

How Bacteria Synthesize Squalene. Clues from the Promiscuous Activities of Eukaryotic Squalene Synthase

Sterols are important components of eukaryotic plasma membranes, where they localize in specialized functional microdomains (lipid rafts). With only a few exceptions, bacteria do not have sterols, although there is evidence that their plasma membranes contain raft-like domains. It has been assumed that the hopanes, a family of structurally related triterpenes, serve as sterol surrogates in bacteria. Squalene is the first pathway-specific intermediate for biosynthesis of tetracyclic (sterols) and pentacyclic (hopanes, gammaceranes, lupanes, oleananes, and ursanes) triterpenes. In eukaryotes, squalene synthase catalyzes the NADPH-dependent coupling of two molecules of farnesyl diphosphate to produce squalene. However, in bacteria three genes in the hopanoid biosynthesis gene cluster have been implicated in squalene biosynthesis. I will present evidence for a novel three-step/three-enzyme pathway for biosynthesis of squalene from farnesyl diphosphate in bacteria, traces of which are seen in the promiscuous activity of eukaryotic squalene synthase that suggest a logical mechanism for evolution of the more efficient eukaryotic enzyme from a less sophisticated ancestor.

Biography: C. Dale Poulter

Dale Poulter is the John A. Widtsoe Distinguished Professor of Chemistry at the University of Utah. He was raised in southern Louisiana and received his BS degree in chemistry from Louisiana State University, where he was a member of the Phi Kappa Phi and Phi Lambda Upsilon honor societies. He earned his PhD at the University of California, Berkeley, working with Bill Dauben on the photochemistry of dienes, and moved to UCLA as an NIH Postdoctoral Fellow with Saul Winstein, working on direct observation of carbocations in superacid media by nuclear magnetic resonance spectroscopy. While an Assistant Professor, he received a Research Career Development Award from the National Institutes of Health that enabled him to shift the focus of his research program into mechanistic enzymology.

Professor Poulter's research incorporates organic chemistry, enzymology, and molecular biology to study enzymes that catalyze reactions in the isoprenoid biosynthetic pathway. He studies functional relationships among enzymes with similar core structures and how changes in protein structure leads to new functions.

Professor Poulter has received several awards, honors and lectureships, including an Alfred P. Sloan Fellowship (1975), the Ernest Guenther Award in the Chemistry of Natural Products (1991), the Cope Scholar Award (1998), the Rosenblatt Prize (1999), the Repligen Award in the Chemistry of Biological Processes (2002), the James Flack Norris Award in Physical Organic Chemistry (2004), and the Nakanishi Prize for the Study of Biological Phenomena (2011). He has served as an associate editor for the Journal of Organic Chemistry and Organic Letters, and is currently Editor-in-Chief of the Journal of Organic Chemistry. He is the founding scientist for Acacia Biosciences and Echelon Biosciences. He is a member of the American Academy of Arts and Sciences (2005) and the National Academy of Sciences (2009).

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Program

Wednesday Afternoon
Session Chair: Holly Goodson

1:15-1:30 Welcome and Orientation – A. Serianni

1:30-2:00 *Loss of SUMOyation alters cardiac development in Xenopus embryos*
Olivia Cox, Michelle Bertke and Paul W. Huber

2:00-2:30 *Measuring the effects of vectorial appearance of the polypeptide chain on pertactin refolding*
Micayla A. Bowman and Patricia L. Clark

2:30-3:00 *Investigating the role of cellular lipid recognition in viral particle formation by Ebola virus matrix protein VP40*
Kristen A. Johnson and Robert V. Stahelin

3:00-3:30 Mid-Afternoon Break

3:30-4:00 **Guest Lecture**
Utilizing ER stress inhibition to sensitize drug resistant pancreatic cancer to gemcitabine treatment
Reginald Hill
Department of Biological Sciences, Notre Dame

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4:00-6:00 Hotel Check-in; Free Time

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6:00-7:00 **The John V. O'Connor Lectureship in Biochemistry**

How Bacteria Synthesize Squalene. Clues from the Promiscuous Activities of Eukaryotic Squalene Synthase

C. Dale Poulter

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Wednesday Evening

7:30-8:30 **Dinner**
8:30-10:00 **Poster Session**

10:00-midnight **Social and Entertainment**
The Oblates of Blues

Thursday Morning
Session Chair: Brian Baker

9:00-9:30 *Identification and biostatistical analysis of conserved metabolic profiles in breast tumors from transgenic mouse models*
Chen Dai, Jun Li and Laurie E. Littlepage

9:30-10:00 *Developing realistic simulations of cotranslational protein folding*
Ian M. Walsh, Shuxiang Li, Adrian H. Elcock and Patricia L. Clark

10:00-11:00 Mid-Morning Break; Hotel Checkout

11:00-11:30 *Insights into β-lactam resistance in superbugs as revealed by protein dynamics*
Michael W. Staude, Thomas E. Frederick and Jeffrey W. Peng

11:30-12:00 **Guest Lecture**

Engineering approaches to investigate the regulation of epithelial growth and homeostasis

Jeremiah Zartman

Department of Chemical and Biomolecular Engineering, Notre Dame

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12:00-2:00 **Lunch; Presentation of Student Speaker Plaques; Open Time**

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Thursday Afternoon

Session Chair: Paul Huber

2:00-2:30 *The role of kringle domain lysine binding sites (LBS) of human plasminogen in interacting with group A streptococcal M-like protein (PAM)*

Julia Beck, Victoria A. Ploplis and Francis J. Castellino

2:30-3:00 *Understanding the recognition mechanisms of hnRNP complexes*

Brendan J. Mahoney and Jeffrey W. Peng

3:00-3:30 *A phylogenetic study of actin-related proteins (ARPs) and characterizing the structure-function relationships of S. cerevisiae Arp4p*

Benjamin A. Paulson and Holly V. Goodson

3:30-3:40 Concluding Remarks (A. Serianni)

ABSTRACTS: GUEST LECTURES

Utilizing ER Stress Inhibition To Sensitize Drug Resistant Pancreatic Cancer To Gemcitabine Treatment

Reginald Hill
Department of Biological Sciences, University of Notre Dame,
Notre Dame, IN 46556-5670 USA

Despite recent advances in multimodality therapy, pancreatic ductal adenocarcinoma (PDAC) remains the 4th leading cause of cancer-related deaths in the United States with a 5-year survival rate of 5%. Poor response to available therapies is a major factor contributing to this poor prognosis. A better understanding of the molecular mechanisms that contribute to chemoresistance would aid the development of more effective treatment strategies. GRP78 is an endoplasmic reticulum (ER) chaperone that facilitate the folding of proteins into their correct conformational form, prevent intermediates from aggregating, and target misfolded proteins for proteasome degradation to alleviate ER stress. This "pro-survival" protein can also act as a membrane receptor capable of transmitting signals that promote tumor proliferation, anti-apoptosis, survival, and resistance to routinely utilized therapeutic regimens. Gemcitabine, a nucleoside analog, is the standard chemotherapeutic agent for adjuvant therapy of resectable PDAC. Unfortunately, 74% of patients treated with gemcitabine after surgical resection show tumor recurrence. We hypothesize that increased GRP78 expression is the reason that chemotherapeutic regimens including gemcitabine fail to eradicate pancreatic tumors. We propose to increase the efficacy of gemcitabine in treating pancreatic cancer by reducing the expression of the "pro-survival", stress-induced molecular chaperone, GRP78. We have evaluated the effect of GRP78 inhibition on the chemoresistance of pancreatic cancer cells by inhibiting GRP78 expression using 1) shRNA directed against GRP78 and 2) heat shock protein 70 inhibitors that decrease GRP78 expression. By addressing these issues we will be able to develop a therapeutic strategy that targets the GRP78 pathway to inhibit chemoresistance and increase susceptibility to currently available chemotherapeutics. This will ultimately decrease the mortality rate of the people affected by this dismal disease.

Engineering Approaches To Investigate the Regulation of Epithelial Growth and Homeostasis

Jeremiah Zartman
Department of Chemical and Biomolecular Engineering,
University of Notre Dame, Notre Dame, IN 46556-5670 USA

The revolution in molecular biology within the last few decades has led to the identification of multiple, diverse inputs into the mechanisms governing the measurement and regulation of organ size. In general, organ size and homeostasis is controlled by both intrinsic, genetic mechanisms as well as extrinsic, physiological factors. Examples of the former include the spatiotemporal regulation of organ size by morphogen gradients, and instances of the latter include the regulation of organ development by endocrine hormones, oxygen availability, nutritional status and the mechanics of the microenvironment. However, integrated model platforms, either of in vitro experimental systems amenable to high-resolution imaging or *in silico* computational models that incorporate both extrinsic and intrinsic mechanisms are lacking. Here, I will discuss collaborative efforts to bridge the gap between traditional assays employed in developmental biology and computational models through quantitative approaches. These interdisciplinary efforts are being applied to develop integrated models of epithelial growth and homeostasis in the larval wing imaginal disc, due to the wealth of previous genetic knowledge for the system. In particular, I will discuss our current understanding of how intrinsic and extrinsic factors impact the spatiotemporal dynamics and possible functional roles of Ca^{2+} signaling in development and homeostasis using a combination of microfluidics devices, organ culture and computational modeling of signal transduction. An integrated model of intrinsic and extrinsic growth control is expected to provide greater insight into how cells communicate to coordinate tissue-level responses.

ABSTRACTS: ORAL PRESENTATIONS

Loss of SUMOyation Alters Cardiac Development in *Xenopus* Embryos

Olivia Cox, Michelle Bertke and Paul W. Huber
Department of Chemistry and Biochemistry,
University of Notre Dame, Notre Dame, IN 46556-5670 USA

SUMOylation, a post-translational modification that occurs via an enzymatic cascade similar to ubiquination, effects a variety of biological processes including transcriptional regulation, intracellular localization, protein stability, protein-protein interactions, DNA binding, and cell-cycle progression. Transcription factors are a frequent target of SUMOylation, and this reversible modification plays a significant role in regulation of gene expression. Numerous transcription factors critical to cardiac development are SUMOylated (*e.g*. SRF, myocardin, GATA4, Nkx2.5, Mef2, YY1, and Prox1), and recent research shows that SUMOylation activity is necessary for normal heart formation. The role of SUMOylation during cardiogenesis is being studied in developing *Xenopus* embryos. Transgenic animals are being generated to study the consequences of reduced SUMOylation activity in cardiac progenitor cells at distinct stages of development. Expression of Gam1, an adenoviral protein that degrades the SUMO E1-activating enzyme, has been put under the control of cardiac specific promoters (Nkx2.5 and ANF), in order to specifically reduce SUMOylation in cardiac cells at distinct embryonic stages. Initial results demonstrate that reduction of SUMOylation activity results in a variety of cardiac malformations. Most notably, we observed ventricular noncompaction cardiomyopathy in the Gam1 embryos. This abnormality results when the sponge-like trabeculations of the fetal heart fail to compact. We also observed cardiac edema, reversal of cardiac laterality, and enlarged hearts. These transgenic animals should provide a useful model for studying congenital heart defects that are known to arise from perturbation in this post-translational modification.

Measuring the Effects of Vectorial Appearance of the Polypeptide Chain on Pertactin Refolding

Micayla A. Bowman and Patricia L. Clark
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556-5670 USA

Autotransporters (ATs) are the largest class of virulence proteins secreted from Gram-negative bacteria. They contain an N-terminal signal sequence, a central passenger, and a C-terminal translocator domain. The signal sequence initiates secretion across the inner membrane (IM) from N- to C-terminus to the periplasm, where the passenger adopts a non-native state. The C-terminal translocator inserts into the outer membrane (OM), and the passenger threads through the translocator from C- to N-terminus, beginning to form its structure extracellularly while its N-terminus still resides in the periplasm. Pertactin, an archetypical model AT from *B. pertussis,* refolds *in vitro* from chemical denaturant on the timescale of many hours. In contrast, *in vivo* folding occurs within an approximately 20 minute timescale. We hypothesize that pertactin might fold faster *in vivo* because its C-terminus is accessible for folding as soon as it is secreted across the OM, while the N-terminus still resides in the periplasm, and this vectorial folding scenario is not recapitulated during *in vitro* refolding. Further, secretion across the IM in the opposite direction (N→C-terminal) could contribute to pertactin's slow folding rate in the periplasm. Current protein folding techniques are unable to recapitulate the unique vectorial folding that occurs *in vivo*. We are developing novel *in vitro* methods to allow direction specific translocation of pertactin that will allow vectorial appearance and folding. This will allow for us to directly test the impact of vectorial folding during secretion, as well as the impact of secretion direction.

Investigating the Role of Cellular Lipid Recognition in Viral Particle Formation by Ebola Virus Matrix Protein VP40

Kristen A. Johnson[1] and Robert V. Stahelin[1,2]
[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670 USA; [2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine – South Bend, South Bend, IN 46617 USA

Virulent pathogens hijack host cell machinery during their infection scheme. Commonly, fundamental biochemical processes are discovered through investigation of pathogen infection, especially in the case of pathogen modulation of low abundance lipid species. Ebola virus (EBOV) is a highly virulent, filamentous, lipid-enveloped virus that causes hemorrhagic fever and a fatality rate of 50-90%. The limited understanding of viral replication in host cells proves difficult for understanding how it spreads. EBOV only has seven genes in its genome, one of which-VP40-is essential for viral egress. VP40 can form virus like particles (VLPs) without other viral proteins at the plasma membrane (PM) in live cells and is an excellent model for studying EBOV spread. We are implementing several assays in live COS-7 cells to decode the PM phospholipid specificity where viral particle formation occurs. Various methods including Confocal Laser Scanning Microscopy (CLSM), Raster image correlation spectroscopy (RICS), number and brightness analysis, VLP Collection are used to investigate VP40 VLP formation. We have observed that VP40 PM localization can be blocked by elimination of phospholipid substrates through various assays in live COS-7 cells. We have demonstrated that both phosphatidylserine (PS) and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) are important for VP40 localization and VLP formation at the PM in live cells. We have also observed that VLP budding is significantly decreased as the result of a decrease in PM localization. Finally, Pymol was used to compare the structures of Ebola, Sudan, and Marburg VP40 structures to each other and to confirmed $PI(4,5)P_2$ binding matrix proteins from HIV-1, HIV-2, and M-PMV. This study revealed a likely VP40 $PI(4,5)P_2$ binding site. Together, these findings reveal that phospholipid-focused assays in live cells combined with structural reveal VP40 lipid binding specificity at various cellular sites, other quantitative methods aid in decoding the dynamics and nature of these interactions.

Financial Support: NIH AI081077

Identification and Biostatistical Analysis of Conserved Metabolic Profiles in Breast Tumors from Transgenic Mouse Models

Chen Dai[1], Jun Li[2] and Laurie E. Littlepage[1]
[1]Department of Chemistry and Biochemistry, Harper Cancer Research Institute; [2]Department of Applied and Computational Mathematics and Statistics, University of Notre Dame, Notre Dame, IN 46556-5670 USA

Novel breast biomarkers with prognostic and therapeutic value include metabolites that are differentially expressed in breast tumors. The goal of this study is to identify global metabolic profiles of breast tumors derived from multiple established transgenic mouse models to gain insight into breast cancers of differing origin. We compared breast tumor samples to normal tissue and between cancer models from transgenic mouse breast cancer models overexpressing the following oncogenes: PyMT, PyMT-DB, Wnt1, Neu, and C3-TAg transgenic mice. Our breast tissue samples were analyzed on GC-MS and LC-MS/MS platforms, and analysis included 374 biochemical compounds of known identity.

Comparison of global metabolic profiles between the different mouse breast cancer and normal tissue revealed many metabolic differences between genetic models. The vast majority of metabolomic profiles demonstrated differential levels of biochemicals between normal and tumor tissues consistent with changes in metabolism that support rapid growth. Breast tumors had increased glucose metabolism, amino acid metabolism, catabolism, and levels of the TCA cycle intermediates, consistent with increased energy production and anaplerotic contributions from amino acid catabolism. We also saw increased phospholipid metabolism, cholesterol uptake, and nucleotide metabolites.

To eliminate the possible metabolic signatures due to difference in cell type (mainly adipocytes for normal mammary tissue, epithelial cells for tumors), we conducted metabolomics on these tissues separately and found many similarities and differences.

Combined with published gene expression data for these mouse models, we were able to conduct biostatistical analysis to search for correlation between gene expression and metabolite levels. We identified novel key metabolites and genes that correlate with the most other metabolites/genes; the list of these "hub" metabolites/genes could prove to be novel sites of regulation for breast cancer progression.

We identified novel metabolic profiles of breast cancer that will be foundational in identifying metabolites with clinical prognostic value. Currently we are using both 3D culture and preclinical animal models to examine the role of the differentially expressed metabolites in breast cancer progression and treatment.

Developing Realistic Simulations of Cotranslational Protein Folding

Ian M. Walsh[1], Shuxiang Li[2], Adrian H. Elcock[2] and Patricia L. Clark[1]
[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670 USA; [2]Carver College of Medicine, University of Iowa, Iowa City, IA 52242 USA

In order for a protein to exhibit its proper function, it must first fold correctly. Most of what we know about protein folding comes from *in vitro* studies of small, single-domain proteins. However, larger and more complex proteins often fail to fold efficiently *in vitro*, yet fold to high yield in cells. One striking difference between protein folding *in vitro* and *in vivo* is how folding begins. *In vitro*, protein folding begins from an enormous ensemble of random conformations of full length polypeptide chains, while *in vivo* a protein can begin to fold as it is synthesized by the ribosome. Vectorial appearance of the nascent protein chain during translation is a universal feature of every protein in the proteome, yet its effect on protein folding is poorly understood. Our lab has created YKB, a protein construct with two mutually exclusive native states (YK-B and Y-KB), to study the effects of translation on folding mechanisms. Synonymous mutations to YKB alter the ratio of the YK-B and Y-KB native structures, yet have identical protein sequences. From this we can infer that vectorial appearance affects YKB folding, but the ratios provide little molecular-level detail. We are currently developing a coarse-grain computational model to simulate YKB cotranslational folding. This model will be validated by recapitulating experimental results. These simulations provide us with details that cannot be learned through experiments such as folding trajectories and the effects of altering translation rate on the YKB folding mechanism. Integrating information gleaned from simulations with targeted experimental studies will enable unprecedented insight into *in vivo* folding mechanisms, particularly the effects of varying translation rate on cotranslational folding and overall folding efficiency.

Insights Into β-Lactam Resistance in Superbugs as Revealed by Protein Dynamics

Michael W. Staude, Thomas E. Frederick and Jeffrey W. Peng
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670 USA

Understanding the molecular origins of antibiotic resistance remains an unsolved problem. We have shown conformational dynamics play an important role in β-lactam resistance for Methicillin-resistant *Staphylococcus aureus* (MRSA), using NMR. Specifically, we have identified a dynamic β5/β6 hairpin in the protein $BlaR^S$ that enables signal transduction for antibiotic resistance. Interestingly, the same β5/β6 motif plays an important functional role in OXA-24, a structural homolog of $BlaR^S$, which causes β-lactam resistance via hydrolysis in *Acinetobacter baumannii*. Therefore, the β5/β6 motif provides a way to understand β-lactam resistance in both Gram-positive (MRSA) and Gram-negative (*A. baumannii*) pathogens. Similarities and differences between the results of NMR studies of $BlaR^S$ and OXA-24 will be discussed.

The Role of Kringle Domain Lysine Binding Sites (LBS) of Human Plasminogen in Interacting with Group A Streptococcal M-like Protein (PAM)

Julia Beck, Victoria A. Ploplis, Francis J. Castellino
W. M. Keck Center for Transgene Research and Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670 USA

Plasminogen (Pg) conversion to the serine protease plasmin via host and exogenous factors results in activation of the human fibrinolytic system and ultimately degradation of fibrin and extracellular matrices. Group A *Streptococcus* (GAS), a bacterial pathogen responsible for diseases including rheumatic heart disease, impetigo, and necrotizing fasciitis, activates Pg through the extracellular protein, streptokinase (SK). This process is enhanced when virulence factors M or M-like proteins, *i.e.,* PAM, found on the surface of GAS, bind to Pg. Pg has 5 kringle domains (K1-K5) which are homologous triple-disulfide-linked peptide regions of approximately 80 amino acids in length. It has previously been shown, by examining interactions of hPg and PAM fragments, that PAM selectively binds to the second kringle domain, K2, of Pg. Kringle domains, excluding K3, bind to ω-amino acids such as lysine and its analogues. The lysine binding site (LBS) is created by a minimum of five amino acids, two residues of Asp, one of Arg, and two of Trp/Tyr/Phe, within a defined binding pocket of the kringle. In this study, we have examined intact protein hPg/PAM interactions, by eliminating the LBS of each of the kringle regions in hPg. To accomplish this, mutations were constructed on one critical Asp residue of each kringle region (D->N), thus removing the anionic locus and abolishing the LBS. Native human Glu-Pg and mutants for kringles 1, 2, 4, 5, as well as a triple mutant of 1, 4, and 5, were expressed in S2-Drosophila cells and purified by Sepharose-lysine chromatography. Binding analyses of Pg mutants to recombinant PAM were performed using surface plasmon resonance. The mutants bound comparably to PAM to that of WT hPg. However, no binding was detected for the K2 mutant. Additionally, Pg activation assays using two SKs from cluster 1 and cluster 2b were performed with the Pg mutants in the presence and absence of PAM. In comparison to WT hPg, the mutants demonstrated a faster rate of activation in the absence of PAM. Upon the addition of PAM, native plasminogen showed increased activity. We have previously shown that the open conformation of hPg is more easily activated than the closed conformation. It is our hypothesis that the mutants also adopt this open conformation, and that PAM further shifts Glu-hPg into this open conformation. Analytical ultracentrifugation, in the absence and presence of LBS-directed ligands, has verified these changes in conformation. These studies serve to further elucidate the role of LBS in PAM binding and GAS virulence.

Understanding the Recognition Mechanisms of hnRNP Complexes

Brendan J. Mahoney and Jeffrey W. Peng
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556-5670 USA

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a family of protein:RNA complexes that act on pre-mRNA molecules. However, seemingly similar members of this family can perform distinct functions on target RNA molecules. For example, the proteins hnRNP A0 and hnRNP A1 share significant sequence similarity and structure, yet recognize different target sequences and act during different stages of RNA processing. Both proteins included two well-folded RNA-binding domains (RBDs) followed by an intrinsically disordered C-term tail. In the last year, our lab has begun to decipher the roles that each of these domains play in differential specificity and the effects of post-translational modification on domain-domain orientations. Solution-state nuclear magnetic resonance techniques combined with comparison with published structures of similar hnRNP proteins has revealed that amino acids at the RBD interface of A0 show significant chemical shift perturbations upon the introduction of a phosphomimetic mutation (S84E) at the domain linker. The degree of interaction between these domains and the impact of post-translational modifications is continuing to be investigated to explain the physiological impacts of these changes.

A Phylogenetic Study of Actin-Related Proteins (ARPs) and Characterizing the Structure-Function Relationships of *S. cerevisiae* Arp4p

Benjamin A. Paulson and Holly V. Goodson
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556-5670 USA

Actin is a fundamental component of the cytoskeleton that has been characterized in critical roles for an ever increasing number of processes, ranging from mitosis to muscle contraction. While many of these processes have been studied in the context of the cytoplasm, it is now evident that actin is also present in the nucleus along with members from the family of actin-related proteins (ARPs). The ARPs represent a group of at least eight subfamilies evolutionarily related to conventional actin and have been named based on this similarity. Our research focuses on conducting phylogenetic studies of the fungal ARPs and particularly ARP4, the closest relative to be localized to the nucleus, and where it has been identified as a component of multiple chromatin remodeling complexes.

While ARP4 is clearly involved in chromatin remodeling, little is known about how it contributes to this process or what proteins it might bind to. In order to address these questions we initially hypothesized that ARP4 is a paralog of ancestral actin and that these two proteins still have some common ligands between them, which most likely bind at similar interfaces. This suggests that the surfaces which mediate protein-protein interactions are more likely to be conserved than surfaces that simply interact with the aqueous environment.

To identify these conserved surface residues the phylogenetic analysis allows us to identify a large set of fungal ARP proteins, which the conservation scores can then be mapped upon crystal structures or homology models. In the case of ARP4 a subset of potential ligands were identified from the side-by-side comparison of fungal ARP4 conservation to sites of known actin-ligand interactions including: actin, myosin, cofilin, profilin, gelsolin, CapZ, and the FH2 domain.

A workable model system has been assembled by supplementing the conditional null *S. cerevisiae* Tet-off-ARP4 strain with a plasmid based ARP4-TAP tagged construct, and site-directed mutations have been made on selected conserved surface residues. Phenotype screens are currently underway to test where these amino acids are functionally significant *in vivo*.

ABSTRACTS: POSTER PRESENTATIONS

1. Acceleration of Diabetic Wound Healing Using a Novel Protease-Anti-Protease Combination Therapy

Ming Gao[1], Trung T. Nguyen[1], Mark A. Suckow[2], William R. Wolter[2], Shahriar Mobashery[1] and Mayland Chang[1]
[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46656-5670 USA; [2]Freimann Life Sciences Center and Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556-5670 USA

Non-healing chronic wounds are major complications of diabetes, which result in >70,000 annual lower-limb amputations in the United States alone. The basis for why the diabetic wound is recalcitrant to healing is not fully understood and there are no therapeutic agents that could accelerate or facilitate its repair. We previously had identified two active matrix metalloproteinases (MMPs), MMP-8 and MMP-9, in the wounds of diabetic mice. We had argued that the former might play a role in the body's response to wound healing and the latter was the pathological consequence of the disease with detrimental effects. We demonstrate herein that the use of a novel highly selective inhibitor of MMP-9 (compound ND-336) accelerates diabetic wound healing by lowering inflammation, and by enhancing angiogenesis and re-epithelialization of the wound, hence reversing the pathological condition. The detrimental role of MMP-9 to the pathology of diabetic wounds was further confirmed by the study of diabetic MMP-9-knockout mice, which exhibited wounds more prone to healing. Furthermore, topical administration of active recombinant MMP-8 also accelerated diabetic wound healing as a consequence of complete re-epithelialization, diminished inflammation, and enhanced angiogenesis. The combined topical application of ND-336 (a small molecule) and the active recombinant MMP-8 (an enzyme) enhanced healing even more, in a strategy that holds considerable promise as a first-in-class therapeutic in healing of diabetic wounds.

This work was supported by the American Diabetes Association Pathway to Stop Diabetes grant 1-15-ACN-06 and Neilsen Foundation grant 282987.

2. Bridging the Gap: Combining Murine and 3D Breast Tumor Models To Understand ZNF217 Induced Chemoresistance

Christopher Suarez[1,2], Sunil S. Badve[1,3] and Laurie E. Littlepage[1,2]
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Despite most breast cancer patients being diagnosed with local or regional stage disease, most patient deaths result from metastasis as a result of recurrent disease after the development of therapeutic resistance. Developing a better understanding of the molecular mechanisms of therapeutic resistance is critical to help identify novel therapeutic strategies that overcome metastasis, resistance, and death.

We previously identified the transcription factor ZNF217 as a prognostic indicator for breast cancer patients. ZNF217 is overexpressed in breast cancer and this overexpression promotes reduced survival, increased metastasis, and reduced response to therapy. We found that Znf217 overexpression promotes an increase in self-renewal capacity, invasion, and metastasis as well as expansion of a progenitor cell population during both normal mammary development as well as during breast cancer progression. We next determined if Znf217 overexpression in vivo contributed to chemotherapy resistance. We treated mice overexpressing Znf217 with a combination therapy of microtubule inhibitor epothilone B, Adriamycin, and cyclophosphamide (EAC). Mice overexpressing Znf217 that were treated with EAC developed a significant increase in tumor volume over control mice within 21 days of EAC treatment. This confirmed that mice overexpressing Znf217 developed resistance to the EAC chemotherapy.

To overcome breast cancer chemoresistance caused by ZNF217 overexpression, we identified triciribine, a nucleoside analog and AKT inhibitor, as a drug that kills cells that overexpress ZNF217. We found that triciribine treatment inhibited tumor burden in vivo in tumors that overexpressed Znf217 and also had synergy with doxorubicin in human xenografts. In addition, we have used our preclinical animal models of Znf217 overexpression to elucidate the appropriate dosing for combination therapy of triciribine and the microtubule inhibitor paclitaxel to treat breast cancer and found that the order of treatment impacts the efficacy of the therapy. We are confirming these results using patient-derived tumor xenografts (PDX) to compare therapeutic response of human tumors with high versus low levels of ZNF217 expression.

We have also generated 3D organoids from murine breast tumors in an effort to bridge the gap between animal models and 2D cell culture. We monitor proliferation/invasion characteristics of the organoids using time-lapse microscopy. Because organoids remain in a 3D environment, this provides a more accurate model to examine the mechanistic details of treatment response and therapeutic resistance. Our future work aims to establish a drug-screening platform to identify combinations of chemo/targeted therapeutics aimed at overcoming resistance to therapy. Utilizing both PDX and 3D organoid models provides an ideal platform to gain mechanistic insight into therapeutic resistance. This work will lead to increased therapeutic efficacy by creating personalized treatment regimens for patients.

3. Matrix Metalloproteinase-3 Impact on Primary Tumor and Metastatic Burden in Aggressive Breast Cancers

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The second leading cause of death in women, breast cancer, claims the life of over 40,000 women each year in the United States alone. The majority of these deaths occur not as a result of the primary tumor burden, but rather metastatic tumor development at secondary sites. To better understand breast cancer progression we use mouse models to study tumors and metastases in their dynamic microenvironment. Within the epithelium, cell signaling, polarity, rigidity, and adhesion are regulated through extracellular matrix (ECM) protein interactions. Uncharacteristic interactions between ECM molecules can lead to disease, and excessive proteolysis has been shown to be involved in inflammation, tumorigenesis, and abnormal cell physiology. Matrix metalloproteinases (MMPs) are zinc dependent endopeptidases whose main responsibility is to degrade ECM proteins. MMP3 is produced by fibroblasts and encourages mammary epithelial branching morphogenesis. Up-regulation of MMP3 is common in human breast cancer where it is seen in both mammary epithelial and stromal cells. Overexpression of MMP3 induces epithelial-to-mesenchymal transitions (EMT) and promotes hyperplastic growth. To investigate the localization and role of MMP3 in primary tumor progression and lung metastatic growth, we injected MMP3 or vector overexpressing cancer cells (V0PyMT) into the mammary glands of MMP3 knockout or control mice, some of which with pre-cleared epithelial. These experiments allow us to differentiate between the role of the stromal and epithelial MMP3 and revealed that this proteinase is involved in the metastatic tumor extravasation in lungs. This study will help elucidate new mechanisms through which MMP3 changes the microenvironment and ultimately encourages cancer progression and poor patient prognosis. As we understand these mechanisms we can further demonstrate the relationship between stromal processes and cancer development and can begin developing novel treatment and diagnostic options.

4. ZNF217 Interacts With The Tumorigenic Isoform of Pyruvate Kinase PKM2

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The oncogene and transcription factor ZNF217 is overexpressed in 20-30% of breast cancers. Its overexpression correlates strongly with poor prognosis in patients and causes accelerated tumor progression, metastasis, and chemoresistance in vivo. While several studies have begun to examine downstream targets of ZNF217, no studies have looked at the regulation of this protein. To identify additional proteins that interact with and may regulate ZNF217, we used ZNF217 as bait in a two-hybrid screen and identified a panel of interacting proteins including pyruvate kinase isoform M2 (PKM2). PKM2 is the embryonic and tumorigenic form of pyruvate kinase. In tumors, it is one of the main drivers of the Warburg effect, the observed accumulation of lactate in tumors. The metabolically active PKM2 is a tetramer found in the cytoplasm, but in recent years, the PKM2 dimer has also been shown to also function as a histone kinase in the nucleus, highlighting a possible role for PKM2 in gene regulation. In this study, we investigate the interaction between and localization of ZNF217 and PKM2. Determining the cellular localization of the interaction between ZNF217 and PKM2 will help to uncover the significance of their interaction.

As determined by both immunohistochemistry and western analysis, we find that ZNF217 protein is predominantly nuclear but can be cytoplasmic in some breast cancer patient tumors and cell lines. Human tumor samples also express smaller isoforms of ZNF217 more than the full length. Interestingly, overexpressing p300, a histone acetyltransferase, increases the expression of the smaller ZNF217 isoforms. Our current efforts focus on further elucidating the underlying mechanisms of the interaction of ZNF217, PKM2, and p300 and in understanding the role of alternative ZNF217 isoforms in breast cancer progression. In future studies, we will investigate the effects on chromatin remodeling caused by this interaction. These mechanisms of regulation may be the basis of a biomarker assay used in patients for personalized treatment strategies. Identifying the mechanism and regulation of ZNF217 may bring about novel drug targets for tumors that overexpress ZNF217 and cause poor prognosis in patients.

5. A Novel Bone Bioreactor Used to Model Bone Metastasis *Ex Vivo*

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Metastatic breast cancer tumors, rather than the primary tumors themselves, contribute to patient death. At death, roughly 73% of women with breast cancer have bone metastases, which are incurable. Therefore, understanding what drives cancer to metastasize to bone and identifying treatments that eliminate bone metastasis are essential to improving the survival and quality of life of cancer patients with metastasis to bone. The current methods used to study bone metastasis are restricted to *in vitro* tissue culture models and to *in vivo* animal models, both of which have several limitations. The *in vitro* tissue cultures lack the 3-D environment of heterogeneous cell types of the bone and marrow, and *in vivo* animal models often are limited by the confounding primary tumor burden and also are not applicable to rapid screening aimed at targeting bone metastases. Both options generally are not applicable to rapid screening aimed at targeting bone metastases. In this interdisciplinary project, we use a novel bone bioreactor to culture mouse bone explants, study bone metastases, and develop therapies to help breast cancer patients that have developed bone metastases. The objective of this research is to develop an experimental system that preserves the 3-D environment and heterogeneous culture conditions (bone, marrow, and cancer cells) within the physiological context of an intact bone environment and apply the technology to develop faster screening techniques than the ones available in current animal models. In here we present our advances in the preservation of the bone microenvironment and the survival of our colonizing mammary epithelial cancer cells after 4 weeks in co-culture.

We propose to use this model to understand fundamental questions of bone metastases and to test therapies prior to use in patients. We will use this *ex vivo* bone culture bioreactor to identify the molecular factors that contribute to develop bone metastases and to aid in the screenings of new drugs aimed at targeting bone metastasis in breast cancer patients. We will validate the bioreactor as a means to understand the stages of metastatic tumor colonization, progression, and response to therapies. After validation in a murine model, our bioreactor will make it possible to study metastatic cancer progression temporally and independently from primary tumor growth. Later, we will use the bone bioreactor to study the effects on human bone coming from human orthopaedic surgical procedures. Because this system is amenable for investigating bone colonization by multiple cancer types, this study also has general application beyond breast cancer. Due the usage of bone explants and vibrational technology that is currently available to patients, this study has high translational value.

6. A Versatile Chemo-Enzymic Synthesis of Stable Isotopically Labeled Nucleosides Using a Common Inosine Precursor

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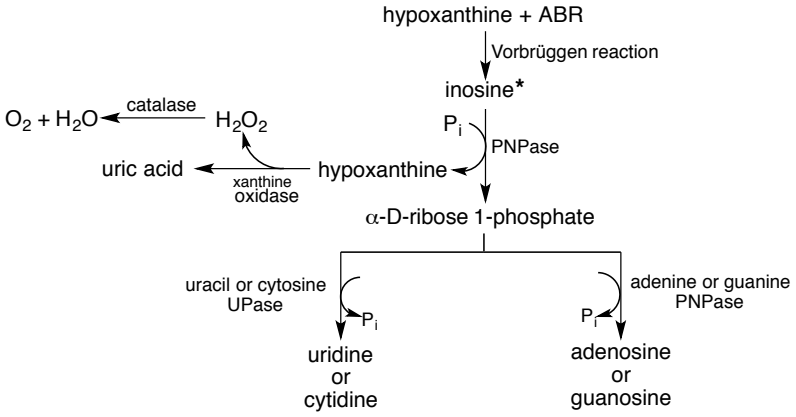
Adenosine (A), cytidine (C), guanosine (G) and uridine (U) are the monomers comprising the structure of ribonucleic acid (RNA). While it is possible to synthesize A, C and U chemically in high yield, G is often produced in yields ranging from 20–65 %. This low yield can be a problem when ribonucleosides need to be isotopically labeled and/or when production costs are high. To address these limitations, a new synthetic method is under investigation that exploits two common nucleoside precursors, inosine (I) and 2'-deoxyinosine (dI), from which all other biologically important ribonucleosides and 2'-deoxyribonucleosides, respectively, can be obtained enzymically. These two precursors are prepared chemically in high yield (*e.g.*, base-sugar condensation; Vorbrüggen reaction), and used in nitrogen base exchange reactions catalyzed by purine nucleoside phosphorylase (PNPase; EC 2.4.2.1) or uridine phosphorylase (UPase; EC 2.4.2.3) (see accompanying scheme). Specifically, inosine is first cleaved into α-D-ribose-1-phosphate and hypoxanthine by PNPase. This cleavage reaction is driven to completion by coupling it to the subsequent conversion of hypoxanthine to uric acid, catalyzed by xanthine oxidase (EC 1.17.3.2), with the by-product H_2O_2 converted to O_2 and H_2O by catalase (EC 1.11.1.6). The α-D-ribose-1-phosphate is not isolated, but used *in situ* to produce A, C, G and U by condensation with free purine or pyrimidine bases in the presence of PNPase (purine bases) or UPase (pyrimidines). A similar route can be used starting from 2'-deoxyinosine to give the corresponding 2'-deoxyribonucleosides. This process streamlines the synthesis of stable isotopically labeled nucleosides when the isotopes occur in the sugar constituent, and produces α-D-ribose-1-phosphate as a byproduct, which is difficult to prepare chemically and a valuable metabolite in studies of purine and pyrimidine nucleotide salvage pathways *in vivo*.



*The same route can be used with 2'-deoxyinosine, but substituting thymidine phosphorylase for uridine phosphorylase in the protocol.

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7. Regulation of Cholesterol Homeostasis with the Polyketide GEX1A, A Potential Lead for Niemann-Pick Type C Disease

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Recent studies have shown that histone deacetylase (HDAC) inhibitors are effective in reversing the cholesterol storage defect characteristic of Niemann-Pick Type C (NPC) disease in human NPC1 mutant fibroblasts, likely through a global upregulation of gene expression. Our efforts to identify a novel small molecule treatment option for NPC disease are focused on the polyketide natural product GEX1A, derived from *Streptomyces chromofuscus*. GEX1A has been shown to have similar effects on gene expression as trichostatin A, a well-characterized HDAC inhibitor, however GEX1A does not impact histone acetylation. Recently, GEX1A has been shown to interact with splicing factor SAP155 and affect pre-mRNA splicing. Based on these findings, we have developed a multidisciplinary approach to accessing GEX1A, as well as synthetic and biosynthetic analogues, in order to investigate the molecule's potential to improve cholesterol homeostasis in NPC1 mutant fibroblasts. Here we present our efforts towards accessing significant quantities of GEX1A and analogues, and our studies of GEX1A's biological activity in the context of NPC disease.

8. Unique Genetic Variation in an Invasive Serotype M23ND Strain of *Streptococcus pyogenes* Influences Bacterium–Host Cell Interactions and Complement-Mediated Opsonization

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Streptococcus pyogenes, or Group A streptococcus (GAS), is a Gram-positive bacterium that is responsible for numerous diseases with diverse clinical manifestations in humans. The remarkable genetic variability present within the species results in the differences in the pathogenic potential between strains. Therefore, identification, sorting, and characterization of different GAS isolates have been central to pathogenic studies of GAS infections. Serotype M23 (emm23) strain (M23ND), isolated from an invasive human infection, showed unique genomic rearrangements that differ from those of previously sequenced GAS strains such as AP53. For example, a unique mutation in the sensor component of the cluster of virulence (cov) responder (R)/sensor (S) two-component gene regulatory system (covRS) has been identified in M23ND (M23ND/covR$^+$S$^-$). However, the influence of this genetic variation on virulence of M23ND is yet to be elucidated. To address this, an M23ND isogenic strain was generated in which the natural covS mutation was corrected to WT-covS (M23ND/covR$^+$S$^+$). Two additional AP53 strains - AP53/covR$^+$S$^-$ and AP53/covR$^+$S$^+$ were also employed for comparison. Unlike AP53/covR$^+$S$^-$, M23ND/covR$^+$S$^-$ cells displayed negligible binding of host complement inhibitors of C3 convertase, viz., Factor H (FH) and C4-binding protein (C4BP). However, despite minimal binding of FH and C4BP, M23ND/covR$^+$S$^-$ cells still show minimal C3b deposition, weak phagocytosis by human neutrophils, and a high mortality in mice after injection of these cells. Further, the mutation in M23ND/covR$^+$S$^-$ resulted in reduced adherence and internalization by human HaCaT cells. Together, these data support the hypothesis that the enhanced virulence in M23ND is due to the unique genetic variation in the CovRS system, which influences the bacteria-host interactions and opsonization process.

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9. Design, Synthesis and Evaluation of Novel Inhibitors of OXA-Carbapenemases

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β-Lactam antibiotics represent arguably the most important class of antibiotics and are widely used against infections by both Gram-negative and Gram–positive bacteria. The primary cause of resistance to these antibiotics in Gram-negative bacteria is production of β-lactamases, enzymes that hydrolytically inactivate these antibiotics. Carbapenems, a subgroup of β-lactam antibiotics, have largely escaped resistance, but this trend is reversing of late. Emergence of a new type of β-lactamase, the carbapenemases, in multidrug-resistant (MDR) bacteria threatens the clinical utility of carbapenems. Carbapenemases deactivate not only carbapenems, but also other β-lactam antibiotics. Therefore, β-lactamases are obvious targets for inhibition to combat resistant bacteria by a combination therapy of β-lactamase inhibitor and a β-lactam antibiotic. Here, we demonstrate a new class of inhibitors targeting class D of carbapenemases. The lead structure emerged from in silico search for inhibitors, followed by the process of iterative synthesis and evaluation in arriving at compounds that exhibit potent inhibition of these enzymes. The details of these efforts will be outlined in this presentation.

This work was supported by The Netherlands Organisation for Scientific Research (NOW).

10. Design and Synthesis of a non-Zinc-Chelating Inhibitor Selective for Matrix Metalloproteinase-13

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Osteoarthritis is a degenerative joint disease that affects 20 million individuals in the United States. Although current treatments for osteoarthritis relieve the pain, they do not halt the progression of the disease. Matrix metalloproteinase-13 (MMP-13) is found upregulated in patients with osteoarthritis. While potent inhibitors for MMP-13 have been reported, these compounds also inhibit other MMPs. A total of 23 MMPs are known for humans, which perform distinct functions in cell physiology and in the pathology of disease. As such, individual MMPs are targets of intervention by small molecule inhibitors. The issue of selectivity in targeting remains a profound problem in light of the disparate roles by various MMPs and the extreme similarity of the structures of the enzymes. We have designed and synthesized a library of potential benzoxazinone-based MMP inhibitors, which have been conceived for selective inhibition of MMP-13. This class of inhibitors induces a conformational change within the active site, which was used to exploit in achieving selectivity. We discovered a potent inhibitor of MMP-13, with over 200-fold selectivity over other MMPs.

This work was supported by the Craig H. Neilsen Foundation.

11. Synthesis and Biological Studies of a New Class of Antibiotic Potentiators for Methicillin-Resistant *Staphylococcus Aureus* (MRSA)

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterial global public health threat. The BlaR1 protein in MRSA is a β-lactam sensor/signal transducer protein. It senses the presence of the antibiotic and initiates a set of biochemical events that leads to transcriptional derepression that results in the antibiotic-resistance phenotype. Our lab has discovered that the BlaR1 protein experiences phosphorylation in response to the recognition of the antibiotic on the surface domain of the protein. We will describe a class of compounds that prevents this phosphorylation event, whereby the resistance phenotype is reversed.

This work was supported by a NIH grant 2021233503020000.

12. Functional Analysis of the Interaction Between the Microtubule Binding Protein CLIP170 and Actin

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Microtubules (MTs), intermediate filaments, and actin filaments are elements of the cytoskeleton. The interaction of microtubules and actin filaments is important for cell migration, cell shape, and cell division. However, little is known about the proteins that mediate this interaction. We hypothesize that a microtubule binding protein called Cytoplasmic Linker Protein-170 (CLIP170) facilitates the interaction between MTs and actin. To test this hypothesis, we examined the interaction between actin-CLIP170 binding by co-sedimentation assays. We found CLIP-170 binds directly to actin. However, our results indicate that actin-CLIP170 binding is a salt sensitive interaction, and is weak at physiological salt concentrations when experiments are performed in vitro. Since the concentration of actin in cells is much higher than in our in vitro experiments, we believe that the interaction between CLIP-170 and actin could still play a role in actin-microtubule coordination in vivo. More specifically, we suggest that the actin-CLIP170 binding may be significant at the leading edge of migrating cells, where the concentration of actin is more than 100μM. Further experiments include determining whether CLIP-170 alters actin polymerization, which part of CLIP-170 binds to actin, and whether CLIP-170 binds to actin simultaneously or competitively. Our work should help to elucidate how the actin and microtubule cytoskeletons are coordinated in processes such as cell division and migration.

This work was supported by National Science Fundation MCB-1244593.

13. Synthesis and Evaluation of 1,2,4-Triazolo[1,5-a]pyrimidines as Antibacterial Agents Against *Enterococcus faecium*

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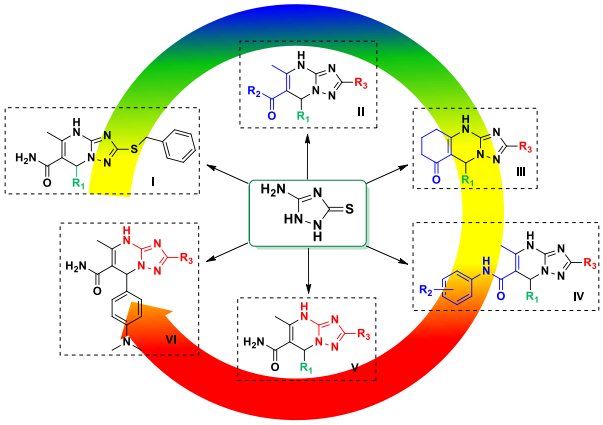
Rapid emergence of antibiotic resistance is one of the most challenging global public health concerns. In particular, vancomycin-resistant *Enterococcus faecium* infections have been increasing in frequency, representing 25% of enterococci infections in intensive care units. A novel class of 1,2,4-triazolo[1,5-*a*]pyrimidines active against *E. faecium* is reported herein. We used a three-component Biginelli-like heterocyclization reaction for the synthesis of a series of these derivatives based on reactions of aldehydes, β-dicarbonyl compounds and 3-alkylthio-5-amino-1,2,4-triazoles. The resulting compounds were assayed for antimicrobial activity against the ESKAPE panel of bacteria, followed by investigation of their *in vitro* activities. These analyses identified a subset of 1,2,4-Triazolo[1,5-*a*]pyrimidines that had good narrow-spectrum antibacterial activity against *E. faecium*, and exhibited metabolic stability with low intrinsic clearance. Macromolecular synthesis assays revealed cell-wall biosynthesis as the target of these antibiotics.



This work was supported by a Predoctoral Fellowship from the Eck Institute for Global Health, University of Notre Dame.

14. Discovery of Ceramide 1-Phosphate Binding Proteins

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Sphingolipids are a class of biomolecules that play key roles in cellular signaling and membrane trafficking, with main players including sphingosine, sphingosine-1-phosphate (S1P), ceramide, and ceramide-1-phosphate (C1P). C1P is an important metabolite that contains a phosphomonoester headgroup; it has been shown to regulate cell proliferation, apoptosis, phagocytosis, macrophage chemotaxis, and is a pro-inflammatory signal. However, more recently the role of C1P in cancer has come under the spot light as it was shown to be involved in rhabdomycarcoma, breast, and lung cancers. C1P is upregulated in tissues exposed to radio/chemotherapy creating a prometastatic environment allowing for egress and migration from the primary tumor to the bone marrow, liver or lungs. Further, ceramide kinase, the enzyme responsible for the production of C1P, was shown to be required in ER- and HER2+ breast cancer reoccurrences, and CPTP, the C1P transport protein, was observed at elevated levels in cancer patients, and has potential as a biomarker. New evidence is suggesting C1P plays a larger role in cancer, however little is known about the specific role of C1P and its binding mechanisms within these processes, and the need for further study is very evident.

My project aims to explore the proteins that specifically bind to C1P. Using an immunoprecipitation assay with lipid-coated beads followed by mass spectroscopy, we have obtained a list of C1P binding proteins and gene ontology (GO) domains, giving us insight on how C1P mediates many of its functions within the cell. We have also been exploring a fluorescently labeled C1P that behaves similarly to C1P in cells, and may be useful for investigating binding and activation of proteins, as well as membrane dynamics in the future. Elucidating the direct effectors of C1P will enable us to better understand the role of C1P in cancer metabolism.

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15. Functional Studies of Ebola Virus Matrix Protein VP40

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Filoviruses are filamentous viruses and include Ebola (EBOV) and Marburg (MARV), which are morphologically identical but antigenically distinct. Mortality rates can be has high as 90% and to date there are no FDA approved vaccines or small molecules for treatment outside the realm of emergency situations under the compassionate use clauses of FDA policy.

EBOV harbors a genome of 7 proteins, the most abundantly expressed in mature virions is Viral Protein 40 (VP40) also known as the matrix protein. VP40 is required for the assembly and budding of EBOV and alone VP40 can form virus like particles (VLPs) from the plasma membrane of host cells. Recent work by the Stahelin and Olmann-Saphire labs indicates that VP40 adopts two different structures to elicit different functions in the viral life cycle. Cellular data demonstrates that each structure adopts a specific function, one for budding form the plasma membrane of human cells and one for regulation of viral transcription. This work investigates how distinct VP40 structures assemble in the presence of synthetic lipid vesicles and at the inner leaflet of the plasma membrane in live cells. This project aims to determine lipid composition requirements for functionality of VP40 mutants *vs.* wild-type VP40, and to elucidate the function of VP40 oligomerization with site-specific mutants.

Preliminary data suggest that VP40 binds with nanomolar affinity to liposomes containing phosphatidylserine (PS), an anionic lipid found within the inner leaflet of the plasma membrane. Cellular data from the Stahelin lab provides precedent that VP40 requires PS in order to bud from the plasma membrane. PS-depleted cells show diminished plasma membrane budding that is subsequently rescued upon supplementation of PS. Probing other lipids with mutant forms of VP40 have helped identify regions of the protein that are integral in PS binding. *In vitro* assays using Giant Unilamellar Vesicles (GUVs) show that WT VP40 is capable of budding vesicles off of parent vesicles, while select mutants of VP40 appear to interact differently with these lipid vesicles.

This work was supported by NIH grant AI081077 and NIH grant T32GM075762.

16. Ischemia-induced Excitoxicity is Attenuated by GluN2B-specific Conantokin-G in a Rat Model of Focal Brain Ischemia

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Conantokin-G (ConG) is a GluN2B-specific potent N-methyl-D-aspartate receptor (NMDAR) antagonist, which displays neuroprotective activity on neurons that have extrasynaptically activated NMDAR. The neuroprotective effect of ConG was tested in an *in vivo* rat model of Middle Carotid Artery Occlusion (MCAO) with 2 μM ConG administered intrathecally 30 min post occlusion. The animals were evaluated at 4 hr and 26 hr after injury induction. A 30% reduction in edema volume and a 50% reduction in infarct size were observed at 4 hr post-MCAO. Though, reduction in edema and infarct size was not observed at 26 hr post-MCAO, neurological recovery was significant at this time point. ConG treated rats showed significant recovery of the brain cytoarchitecture and neuronal integrity in the penumbra region as demonstrated by hematoxylin and eosin staining and Microtubule Assisted Protein-2 immunostaining. This was accompanied by decreased number of degenerated neurons by con-G at both 4 and 26 hr in the ipsilateral penumbra. MCAO-induced loss of GluN1 and GluN2B localization around the soma and proximal dendrites was reinstituted by ConG administration at 4 and 26 hr, but ConG had no effect on restoring MCAO-induced loss of GluN2A localization, which showed increased perinuclear presence in the brains of ConG treated rats. These data provide evidence that ConG ameliorates the detrimental effects of ischemic stroke via the NMDARs by repairing certain neurological and neuroarchitectural deficits, as well as reconstituting neuronal localization of GluN1 and GluN2B subunits in the penumbra.

17. Insight into the Structure-Function Relationships of Conantokin Rl-B by NMR Spectroscopy

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Conantokins are naturally occurring, gamma-carboxyglutamate (gla)-containing neuroactive peptides found in the venom of marine snails, which have been studied for their highly selective antagonistic activity towards N-Methyl-D-Aspartate Receptors (NMDAR). Conantokin Rl-B (ConRl-B) is recently discovered from *Conus parius*and *Conus rolani* species of snail. ConRl-B uniquely differs from other conantokins by the presence of a 4-hydroxyproline (Hyp, 'O') residue, which causes disruption of the α-helical structure of conantokins. ConRl-B displays high inhibitory selectivity for subclasses of NMDARs that contain the functionally important GluN2B subunit. In our present study, two additional mutant peptides, Con-G[10▼O] and ConRl-B[ΔKAO, ▼NQ] have also been synthesized to establish the effect of the hydroproline-induced kink on the structure and activity of the conantokins. The solution structures of ConRl-B, Con-G[10▼O], and ConRl-B[ΔKAO, ▼NQ] are determined using 2D NMR. The cation binding sites of these three peptides were then characterized by the combined analysis using ITC, CD titration, molecular dynamic simulation, and solvent exchange experiments by NMR. ITC and CD titration experiments in the presence and absence of Mg^{2+} show that ConRl-B and the two above conantokin mutants behave quite differently with regard to the binding of magnesium ions. Upon addition of Mg^{2+} ions, ConRl-B and the mutant Con-G[10▼O] exhibit 62% and 100% increase in α-helicity, respectively, in comparison with ConRl-B[ΔKAO, ▼NQ] (72%) and Con-G (100%), which do not contain Hyp residue. The presented structure elucidation of these peptides furthers our understanding the relationship between primary sequences of the conantokins with their structures and the activities. The biophysical characterization in conjunction with genetic studies provides new insights into the structure-function relationships of conantokins as allosteric inhibitors of NMDAR.

The work was supported by grant HL019982 from the NIH.

18. Conserved Residues in TCR CDR Loops and Their Role In Influencing Antigen Recognition

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The adaptive immune system is the body's way of identifying and destroying foreign pathogens. This process involves a recognition event utilizing two major proteins: a T cell receptor (TCR) and a Major Histocompatibility Complex (MHC) protein presenting a peptide. The complete mechanism by which a TCR recognizes an antigen (peptide- MHC) and proceeds to initiate the signaling cascade to destroy infected cells is still unknown. Much of our work aims to better understand the properties that might influence how a T cell receptor recognizes a peptide-MHC. One area of interest is the role of conserved TCR residues and how this might influence TCR behavior. Preliminary bioinformatics work has shown that over 80% of TCRs have a conserved histidine residue in the CDR1β loop. Structural observation revealed that the histidine is contacting a surprisingly conserved serine or threonine residue in the CDR3β loop. To explore potential roles this interaction might have, two complementary approaches will be used to examine the effects of this interaction within T cell receptor CDR loops. Surface plasmon resonance will be used for the assessment of non-additivity between remote CDR loops and fluorescence anisotropy will be used to detect changes in CDR loop flexibility.

19. The Oxadiazole Class of Antibiotics Synergizes with β-Lactams Against Methicillin-Resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major human pathogen associated with serious community-acquired infections and is one of the leading causes of hospital-acquired infections in the United States and around the world. MRSA harbors the *mecA* gene that encodes penicillin-binding protein 2a (PBP2a), which confers resistance essentially to all the β-lactam antibiotics. Oxadiazoles have been identified as a new class of non-β-lactam antibiotics with excellent *in vitro* and *in vivo* activity against MRSA and other Gram-positive bacteria. A major concern is the emergence of drug resistance to any single antimicrobial agent used. In the current study, we investigated the synergism of the lead oxadiazole with the commonly used β-lactams and non-β-lactam antibiotics. The antibiotics included in the study were oxacillin, cefepime, piperacillin, imipenem, meropenem, vancomycin, linezolid, gentamicin, azithromycin and doxycycline. The synergy of these antibiotics with the lead oxadiazole was tested on four MRSA (NRS70, NRS123, NRS100 and MRSA 252) strains and one MSSA (NRS128) strain, using the checkerboard assay. The Fractional-Inhibitory Concentration (FIC) index was calculated for each combination. An FIC index of ≤ 0.5 was considered as synergistic. The synergistic combinations were further validated with time-kill assays. Oxacillin, piperacillin and cefepime displayed a high level of synergy with the lead oxadiazole in three out of four MRSA strains tested, while imipenem and meropenem showed synergy in two MRSA strains. No synergy was observed with non-β-lactam antibiotics such as linezolid or vancomycin. Developing newer antimicrobials to address the emergence of resistance is quite challenging and hence preservation of the existing antibiotics through the use of combination therapies may be more beneficial. Confirmation of the synergism of the lead oxadiazole with β lactams is a potential treatment strategy for MRSA infections.

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20. Engineering T-Cell Receptors to Optimize Anti-Tumor Immunity

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Recognition of malignantly transformed or virally infected cells by T-cells is mediated through the T-cell receptor (TCR). Malignant melanoma is one such malignancy that is immunosensitive. One of the melanoma antigens presented by the MHC is the MART-1_{27-35} (AAGIGILTV) nonameric peptide, which is recognized by the TCRs DMF4 and DMF5. Clinical trials involving adoptive cell therapy (ACT) of melanoma patients showed cancer regression of 13% and 30% for clonally expanded T-cells genetically engineered to express DMF4 and DMF5. Our work involves using structure-guided computational design to enhance the affinity of DMF5 towards the MART-1_{27-35} peptide, with the eventual goal of assessing the impact of enhanced affinity on anti-tumor immunity in mouse models of melanoma. Thus far, we have generated five higher affinity mutants of DMF5; αD26Y, αD26W, βL98W, and two double-mutants αD26Y/βL98W and αD26W/βL98W, and are optimizing a retroviral expression system to generate gene-modified human T cells.

21. Linkage Conformations in a Site-Specifically ^{13}C-Labeled βMan(1→4)βXyl(1→4)βMan(1→4)βXylOCH$_3$ Tetrasaccharide: Effects of Linkage Structure and Context

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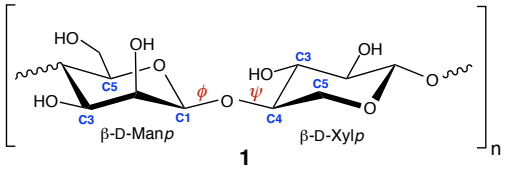
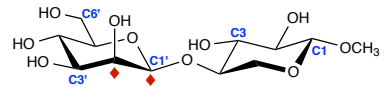
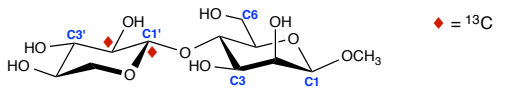
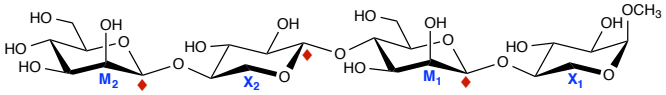
Recent studies by the Duman and Serianni laboratories have revealed the presence of a novel glycolipid in the Alaskan beetle, *Upis ceramboides*, that possesses potent thermal hysteresis properties (Walters *et al*., *Proc. Natl. Acad. Sci*. **2009**, *106*, 20210-20215). This antifreeze glycolipid (AFGL) is comprised of alternating βXyl*p* and βMan*p* residues connected by (1→4) *O*-glycosidic linkages **1**, although branching and covalent modification of this core structure may also occur. To study the preferred conformations about the ϕ and ψ torsion angles in the two different *O*-glycosidic linkages in **1**, four singly ^{13}C-labeled disaccharides (**2**$^{1'}$, **2**$^{2'}$, **3**$^{1'}$ and **3**$^{2'}$) and a triply ^{13}C-labeled tetrasaccharide **4** were prepared chemically. Multiple J_{CH} and J_{CC} values across the "isolated" linkages in **2** and **3**, and across the "in-context" *O*-glycosidic linkages in **4** were measured by ^{1}H and ^{13}C NMR spectroscopy, respectively. New parameterized equations relating specific J_{CH} and J_{CC} values to linkage torsion angles were derived by high-level density functional theory (DFT) calculations of molecular geometries in model structures and of specific NMR *J*-couplings associated with these geometries. Equipped with these new equations, the experimental trans-*O*-glycoside *J*-couplings (Bose-Basu *et al*., *J. Am. Chem. Soc.* **1998**, *120*, 11158-11173) were fit by a newly developed software algorithm, *MA'AT*, to deconvolute the redundant experimental *J*-couplings into conformational models for the ϕ and ψ torsion angles comprising each *O*-glycosidic linkage in **4**. The results show that (a) structural context plays a minimal role in determining linkage conformations in **4** (*i.e*., all conformational effects are local), and (b) the two types of β-(1→4) linkages present in **4** display conformationally distinct behaviors.




methyl β-D-[1-^{13}C]mannopyranosyl-(1→4)-β-D-xylopyranoside **2**$^{1'}$
methyl β-D-[2-^{13}C]mannopyranosyl-(1→4)-β-D-xylopyranoside **2**$^{2'}$



methyl β-D-[1-^{13}C]xylopyranosyl-(1→4)-β-D-mannopyranoside **3**$^{1'}$
methyl β-D-[2-^{13}C]xylopyranosyl-(1→4)-β-D-mannopyranoside **3**$^{2'}$


methyl β-D-[1-^{13}C]mannopyranosyl-(1→4)-β-D-[1-^{13}C]xylopyranosyl-(1→4)-β-D-[1-^{13}C]mannopyranosyl-(1→4)-β-D-xylopyranoside **4**

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22. Homology Modeling of Human Cancer Neoepitopes

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CD8+ T cell receptors are able to recognize antigenic peptides presented by the Class I major histocompatibility complex (MHC) exposed on the surface of all nucleated cells. If these peptides are non-self, the T cell signals the foreign cell for degradation, thus protecting the host from infection. The interaction between T cell receptors and the peptide-MHC complex is critical for this immune response. As with other nucleated cells, tumor cells present antigenic peptides on their surface. These new epitopes that result from random mutations are thought to be responsible for a T cell's ability to selectively target certain tumor cells. There are two major problems associated with this line of cancer immunotherapy. First, it has been predicted that there are tens to hundreds of neoepitopes presented on a single tumor. However, only a small fraction of these peptides are thought to be capable of eliciting an immune response. Second, the cancer genome is mutated-self rather than completely non-self. This poses a problem because the tumor peptides may mimic self-peptides, potentially leading to tolerance (suppression of an immune response). To better understand the role of the peptide-MHC complex in cancer immunogenicity, *in silico* modeling of the complex will be performed. Ultimately, the knowledge gained from these modeling experiments will be used to determine the best candidates for anti-cancer vaccines.

23. Study of biophysical properties of Hepatitis C virus TCR/HLA-HCV $NS3_{1406-1415}$ complex

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In the human body, the adaptive immune system provides a highly specialized pathway to eliminate and prevent pathogen growth. The glycoproteins encoded by the major histocompatibility complex (MHC) are located on the outer membrane of antigen presenting cells, or APCs. MHC proteins are used to display peptide antigens to T cells. In the case of MHC class I molecules, peptides are derived from cytosolic proteins, including virally produced proteins that are present in an infected cell. Previous work described the presence of HLA-A2-restricted; HCV-NS31406 specific T cells in an HLA-A2 negative transplant recipient who received an HLA-A2 positive HCV infected liver. This is a unique immunological opportunity, as the patient's T cells could not be positively selected on an HLA-A2 ligand. Such "allospecific" T cells are exceedingly rare, and by some theories of T cell development, should not even exist. We aim to characterize this unusual TCR-pMHC interaction, which may possess features distinct from more common interactions that have been used to develop the "rules" of TCR-pMHC engagement. Here, circular dichroism and differential scanning fluorimetry were utilized to study the stability of NS3/HLA-A2 ligands. Surface plasmon resonance was used to investigate TCR-pMHC binding, and preliminary work towards a structure of the complex is shown.

24. Structure-Activity and Selectivity Analysis of ADAM10 Inhibitors: Structural Insights for Novel Inhibitor Design

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A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) is a membrane-bound zinc metalloprotease that has been proposed as a detrimental enzyme in certain neurodegenerative disorders. Discovery of selective inhibitors of this enzyme that penetrate the blood-brain barrier will accelerate development of potential novel therapeutic agents for these diseases. Although several inhibitors for ADAM10 have been reported in the literature, chemical diversity of these compounds is poor. In addition, many of the inhibitors show cross-reactivity to other metalloproteases like ADAM17, as well as matrix metalloproteinases (MMPs). Identification of the molecular features that contribute to the potency and selectivity for these known inhibitors will pave the way for the design of novel inhibitors for this enzyme. Lack of X-ray crystal structures of the ADAM10 catalytic domain hinders the structure-based investigation of molecular recognition of these inhibitors. We have modeled the catalytic domain of ADAM10 through comparative homology modeling in concert with molecular-dynamics simulations. The model was used to comprehend the potential protein-ligand interactions of known inhibitors via molecular docking and scoring. Furthermore, ligand-based approach using 3D-QSAR analysis enhanced our understanding of the molecular-recognition features of ADAM10. This analysis shows that compounds that interacts with the S1 site of ADAM10 have an important role on potency and selectivity. The studies will assist the design and development of novel ADAM10 inhibitor scaffolds.

25. Tumor Imaging and Photothermal Therapy using Free Croconaine Dyes and Croconaine Dye-Doped Nanoparticles

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Effective cancer treatment continues to be a challenge, despite significant advances in cancer therapies, due to the diversity of cell types, progression and tumor size that varies from patient to patient. There is an ongoing need for improved strategies that can deliver and rapidly monitor the efficacy of cancer treatment. Recent efforts have focused on the development of individualized patient treatment regimens, which combine a therapeutic agent with a diagnostic agent into a single platform, giving rise to the term "theranostics". Presented here, is a theranostic nanoplatform that contain a novel near infrared (NIR)-absorbing, heat generating croconaine dye as a therapeutic agent for photothermal therapy and a deep-red fluorescent dye as a diagnostic agent. Using a single-step fabrication method, croconaine dye and/or fluorescent dye are loaded into a nanoparticle core of poly(DL-lactide-co-glycolide) (PLGA) and coated with 1,2-distearoyl-phosphatidyl ethanolamine-methyl-polyethyleneglycol (DSPE-MPEG-2000) to produce dye-doped nanoparticles (PEG-PLGA-Cr). The nanoparticle formulation has been optimized and tested in cell and small animal cancer models. Whole-body fluorescence imaging was used to monitor nanoparticle biodistribution, with subsequent photothermal treatment for tumor ablation. Free croconaine dye was also tested to observe the efficiency of the dye as a photothermal agent.

In vitro laser-induced cell death studies in the presence of the PEG-PLGA-Cr nanoparticles, or free croconaine dye, indicates effective photothermal killing of cancer cells (808-nm diode laser, 1.0 or 2.0 W/cm^2 power density, 5 or 10 min duration). Photothermal tumor ablation using the PEG-PLGA-Cr nanoparticles was further examined in nude mice harboring EMT-6 mammary carcinoma tumors. Nanoparticles were intratumorally injected (n=4) and subsequently laser-irradiated (808-nm diode laser, 2 W/cm^2, 10 min duration). Tumor growth was monitored over time by measuring the tumor volume using a digital caliper. Laser-irradiated tumors were compared to a control cohort (n=4) that received an intratumoral nanoparticle injection but no laser irradiation. Results indicate: i) laser treatment using this PEG-PLGA-Cr nanoparticles for 10 minutes at 2 W/cm^2 suppresses tumor growth over 10 days and ii) maximum heating temperature plays an important role in achieving tumor ablation. *In vivo* fluorescence imaging of a rat model bearing prostate tumor (n=3) that had been dosed with fluorescently labeled PEG-PLGA-Cr nanoparticles showed accumulation in the tumor, presumably due to the enhanced permeation and retention effect. The ability to image the location of the nanoparticles will greatly facilitate ongoing studies to evaluate the efficacy of various photothermal regimens.

26. Regulation of the Oncogene ZNF217 by Cellular Localization During Breast Cancer Progression

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Poor prognosis in breast cancer patients occurs when malignant tumors adapt to environmental insults, become resistant to chemotherapy, evade immune surveillance, and metastasize to other tissues. Tumors are thought to arise from individual cells with multiple mutations, including amplification of genomic regions that provide a growth advantage. A region on human chromosome 20 called 20q13 is increased in ~25% of early stage human breast cancers and correlates with poor prognosis in patients. We have studied a novel oncogene, ZNF217, within this region. ZNF217 is not only a prognostic indicator of breast cancer progression in patients who have the worst prognosis, but also is itself a drug target and/or marker of patient response to therapy. We find that ZNF217 protein is expressed most strongly in a small subset of cells within normal mammary epithelium and localizes predominantly in the nucleus of mammary epithelial cells. In contrast, the localization of ZNF217 is heterogeneous in breast cancer cell lines and breast tumors, with localization in both the nucleus and cytoplasm. Moreover, truncated forms of the protein have been observed in the cytoplasm. We hypothesize that ZNF217 truncation and cytoplasmic localization affects ZNF217 function during cancer progression and can be used to predict poor prognosis in breast cancer patients. Thus, we aim to determine which regions of ZNF217 are required for oncogenesis.

27. Matrix Metalloproteinase-8 Accelerates Healing in Both Diabetic and Non-Diabetic Wounds

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Chronic wounds affect 6.5 million individuals in the United States, with the medical cost reaching $18.3 billion annually. The elevated glucose in diabetic patients leads to many complications, including impaired wound healing, which results in 73,000 annual amputations in the United States. Fifty percent of patients die within 5 years after amputation. In addition, acute wounds occur in patients after surgery or burns. Approximately 40 million inpatient and 31.5 million outpatient surgeries are performed in the United States every year. It has been recognized that matrix metalloproteinases (MMPs) play important roles in wound healing, since they are involved in the modification turnover and remodeling of the extracellular matrix.

We have identified activated MMP-8 in both the diabetic and non-diabetic wounds, whereas MMP-9 was upregulated in diabetic wounds only. In diabetic animals, we demonstrated that MMP-9 plays a detrimental role in wound healing and that MMP-8 is beneficial for wound healing by the use of selective pharmacological inhibitors. Here, we demonstrate that topical treatment with the protease MMP-8 accelerates wound healing and increases re-epithelialization in both diabetic and non-diabetic animals. Exogenous application of MMP-8 reduces inflammation cytokines and promotes angiogenesis in the mouse wound model. Topical application of MMP-8 holds promise in accelerating repair of both diabetic and non-diabetic wounds.

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28. A Proteomics Study of the miR-23a/27a/24-2 Cluster

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MicroRNAs (miRNAs) are ~21 nucleotide endogenous regulators of gene expression that have critical cellular functions. Due to their small seed sequences, miRNAs are thought to finely tune the expression of hundreds of target genes, and thus play a role in a wide variety of biological processes. Multiple miRNAs often exist in clusters that are transcribed as a single polycistron. Though clustered miRNAs often have similar seed sequences and target the same genes, many clusters without sequence homology are postulated to regulate different genes involved in the same pathway.

Previous studies in our lab of the miR-143/145 cluster indicate that clustered miRNAs can work in a synergistic fashion to negatively regulate protein targets. This study expands on these results through examination of the three-membered miR-23a/27a/24-2 cluster. miR-23a and miR-27a have similar seed sequences and overlapping predicted targets, indicating they may work to regulate a subset of the same genes. However, miR-24-2 is distinct from the other two members of the cluster. The miR-23a cluster has an added layer of complexity due to gene duplications forming a paralogous cluster in mammals, the miR-23b/27b/24-1 cluster. The miR-23 and miR-27 paralogs differ by one nucleotide but possess the same seed sequence, and the miR-24 paralogs are identical. Because of this, examination of protein targets of the miR-23a cluster will also uncover targets of the miR-23b cluster. Aberrant expression of the miR-23a/27a/24-2 cluster has been linked to various forms of cancer and other diseases.

To analyze the protein expression changes associated with the miR-23a/27a/24-2 cluster, we used quantitative liquid chromatography-electrospray tandem mass spectrometry. The 70Z/3 cell line was transduced with the Murine Stem Cell Virus (MSCV) plasmid containing the coding region for different permutations of the cluster. The proteome from each cell line was evaluated using a Q-Exactive mass spectrometer and quantified using Isobaric Tags for Relative and Absolute Quantification (iTRAQ). A list of putative target proteins was assembled for each miRNA, as well as the entire cluster together to discern targets regulated when the cluster works synergistically. Gene ontology analysis was performed to determine cellular functions and pathways regulated by the miRNAs.

29. Reconsidering the Concept of Critical Concentration As It Applies To Microtubules and Other Steady-State Polymers

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The concept of critical concentration (Cc) is a central idea in the understanding of steady-state biological polymers, such as actin and microtubules. Classically, the Cc is accepted to be a single discrete value with several equivalent definitions for equilibrium polymers: (1) that the Cc it the total subunit concentration needed to obtain any polymer; (2) that the Cc is the free subunit concentration once the concentration of polymer mass has reached a maximum; (3) that the Cc is the dissociation equilibrium constant for the subunit binding to the polymer ($K_d = k_{off}/k_{on}$); and (4) that the Cc is the minimum concentration of subunits required to elongate an existing polymer. Here we investigated if the classic definition of the Cc from equilibrium polymer theory applies to steady-state biological polymers using a computational model of a system of dynamic microtubules. We found that instead of the microtubule system having a single discrete Cc that is assigned by several equivalent definitions ($Cc1 \neq Cc2 \neq Cc3$). In particular, the models show that there is no well-defined value at which microtubules begins to appear, and that the experimental interpretations previously thought to yield the Cc1 for polymer formation actually yield the Cc2 for unbounded (persistent) polymer growth. Interestingly, we found that microtubule behavior is highly dependent on the Cc. Specifically, microtubules will undergo bounded growth between the Cc for elongation (Cc4) and the Cc for unbounded growth (Cc2) where dynamic instability limits polymer mass. At tubulin concentrations above Cc2, microtubules still exhibit dynamic instability but the high concentrations of free tubulin bias the microtubules to grow persistently. Using these observations, we revise the concept of critical concentration to include the idea that there are multiple different critical concentrations, each relevant to a different aspect of experimentally observable behavior. This revision has significant implications for the design and interpretation of experiments with microtubules and likely has relevance to any steady-state polymer.

30. Engineering T-Cell Receptors to Optimize Recognition of Tumor Antigens

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The identification of many tumor associated peptides recognized by T Cell Receptors (TCR) has bolstered the field of cancer immunotherapy, and manipulating the immune system has exhibited promising results in treating metastatic melanoma and other cancers[1]. When measured in solution, TCR affinity for a peptide bound to a Major Histocompatibility Complex (MHC) molecule is relatively weak. There is evidence to suggest that binding affinity correlates with *in vivo* potency, which has led to the generation of several high affinity TCR variants[1]. However, increases in affinity can result in cross-reactive, off target recognition. The potential negative consequences of TCR cross-reactivity has led to the suggestion that the most critical property for an engineered TCR is a high affinity, high *specific* interaction. Our research combines structural biology, computational mutagenesis, and conformational sampling to predict energetically favorable mutations at the TCR binding interface. Previous work with the A6 and DMF5 TCRs were able to identify several affinity-enhancing mutations with some correlation between predicted and measured changes in binding energy. This approach was refined and improved with dynamic information to accurately predict the effects of 9 point mutations in the B7 TCR. This method was then expanded to model variants of the Melanoma associated MART-1 peptide to detect differences in specificity between the wild type and high affinity variants of the DMF5 TCR. This iterative approach to design aims to optimize the "rules" for point mutations in a TCR/pMHC interface to better predict the impacts on affinity and specificity.

This work is supported by the CTSI Predoctoral Fellowship UL1 TR001108 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences.

31. Interdomain Communication in Pin1 Mediated by Conformational Dynamics

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Pin1 (peptidyl-prolyl cis-trans isomerase NIMA-interacting) is a modular, two-domain enzyme that targets pSer/Thr-Pro (pS/T-P) motifs in proteins regulating fundamental cell signaling pathways. Abnormal Pin1 activity has been linked to the onset of cancer and Alzheimer's disease, making Pin1 a potential therapeutic target. Pin1 consists of a substrate binding domain (WW domain) and a catalytic domain (PPIase domain) that accelerates cis-trans isomerization of the pS/T-P imide bond. While it is known that interdomain interactions can tune the PPIase domain catalytic activity, the underlying mechanism is not fully understood. Thus, we have used a combination of NMR spin relaxation, mutagenesis, and MD simulations to study how different pS/T-P phospho-peptide substrates affect Pin1 intra- and interdomain dynamics. Our results suggest that interdomain communication relies on substrate-induced changes in conformational dynamics, which can vary according to the amino acid residues flanking the pS/T-P motif. These results and their implications for the specificity of Pin1 interactions and the design of allosteric Pin1 inhibitors will be discussed.

Acknowledgement: NIH

32. The Fibrinogen-Dependent Interaction Between Group A Streptococcal M Protein and the Human Fibrinolytic System as a Virulence Indicator

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Subversion of the host fibrinolytic system has long been recognized as a powerful method employed by various bacteria to penetrate host tissue barriers. Highly virulent strains of Group A streptococcus (GAS) have been shown to acquire surface plasmin(ogen) (Pg) activity through multiple mechanisms; direct binding to Pg through Plasminogen binding Group A Streptococcal M-like protein (PAM) or indirect binding to fibrinogen (Fg), which requires Fg to directly bind various M proteins to mediate Pg binding. Although M protein-Fg binding has long been studied for its role in phagocytosis prevention in GAS, its role in Pg activation has not been extensively investigated. GAS also produces an endogenous Pg activator, streptokinase (SK). Our laboratory, as well as others, has shown that subclasses of SK exist which have different Pg activating potentials that are dependent on the mechanism of Pg binding. The goal of this study is to investigate the molecular mechanisms involved in the Fg-dependent acquisition of plasmin (Pm) activity. Various M proteins from strains thought to employ this mechanism, as well as their secreted SK's, designated subclass SK2a, were cloned and expressed in *E.coli*. Surface plasmon resonance (SPR) experiments were employed to the study binding affinity of M protein for Fg. The results obtained indicate that these M proteins have high affinities, not only for Fg, but for fibrinogen fragment D and D-dimer (<100nM), while Pg does not bind to these same M-proteins. The SKs isolated from these strains also show low activation potential with Pg alone, while the presence of Fg and M-proteins greatly enhances Pm generation. Whole cell assays also showed significant Pm surface activity only in the presence of Fg, while PAM-containing strains showed enhanced activity in the presence and absence of Fg. Future studies will seek to establish if these differences in Pg acquisition directly influence disease manifestations and tissue tropisms of GAS.

33. Can the Denaturated State of a Protein Differ From its Unfolded State?

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The structure of the unfolded state of a protein is unknown. This is in large due to conflicting data concerning the denaturant dependence of protein dimension. Small angle X-ray scattering (SAXS) data suggests that most, but not all, proteins *do not* undergo an initial collapse when transferred from high to low denaturant conditions. This is contrary to Förster resonance energy transfer (FRET) experiments that have consistently demonstrated polypeptide collapse after dilution from denaturant and before folding. In order to put to rest these discrepencies, it is necessary to identify a negative control that will not show collapse regardless of what technique is used. Here we describe a polypeptide candidate for that control. The passenger domain of the autotransporter protein pertactin is a 16-rung, right-handed β-helix. When the 334 amino acid N-terminal portion (PNt) is separated from the rest of the protein, it does not adopt any regular structure and behaves as a random coil. This has been shown using tryptophan emission fluorescence, far-UV circular dichroism, NMR, and SAXS. PNt gives us access to the unfolded state ensemble of a polypeptide in the absense of denaturant, allowing us to determine the source of descrepancy between SAXS and FRET data.

34. 3D Printed Diffusion-Based Dynamic Device Enables Generation of Pharmacokinetic Profiles and Facilitates Pharmacodynamic Studies on Bacteria and Multicellular Spheroids

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Of every 10, 000 drug candidates, only 1 will make it to market, and it is estimated that the cost of bringing one drug to market has surpassed 1.5 million dollars. Much of this cost is in part attributed to clinical testing in which animal models are used for candidate drug efficacy testing. *In vitro* dosing platforms, such as the hollow fiber chamber reactor (HFCR), have been developed in order to prescreen drug candidates prior to clinical trials, and they enable mimicry of observed *in vivo* dosing profiles on an *in vitro* platform. In an HFCR, the desired cell type is dynamically dosed with a candidate drug; however, the HFCR is a low throughput platform, and its use requires liters of media and relatively large amounts of drug. Although recovery of cells from the HFCR after dosing for pharmacodynamic analysis is possible, it is difficult, and the hollow fiber cartridges are not reusable. Here, we present a reusable, high throughput 3D printed device capable of generating dynamic dose profiles. The device contains 6 channels (2 mm x 0.5 mm) through which concentrated drug can be pumped. Interfacing the device with pumping equipment was accomplished through connection of threaded inlets of the device with syringe pumps using commercial fittings and silica capillary tubing (536 μm i.d.). Each channel contains a port that houses a commercial transwell polyester membrane insert (0.4 μm pore diameter), in which bacteria or spheroids can be loaded for dosing experiments. An aliquot of approximately 10^7 cells/mL of chemically competent, kanamycin-resistant *Escherichia coli* was loaded into each well insert, and a stock solution (119 μM in physiological salt solution, PSS) of the DNA gyrase/topoisomerase IV inhibitor levofloxacin (MW 361.4 g/mol) was pumped beneath the insert for 1 hour to load the insert with drug. At the 1 hour mark, the levofloxacin stock was replaced with buffer only, and drug was allowed to deplete from the well insert for 4 hours. Levofloxacin concentration was monitored by sampling 5 μL from each well insert every 30 minutes, and drug concentration was quantified using LC-MS/MS. Viability of the recovered bacterial samples was assessed using standard plating methods. It was observed that when exposed to a maximum concentration (C_{max}) of 21.0 ± 5.7 μM levofloxacin, viability of the bacteria significantly decreased (Log(CFU/mL) = 4.7 ± 1.0) relative to an undosed control sample (Log(CFU/mL) = 7.4 ± 0.1) but not significantly relative to a statically dosed sample (25 μM levofloxacin, Log(CFU/mL) = 1.0 ± 1.1). Viability of dynamically dosed bacteria samples exposed to a higher C_{max} (68.0 ± 7.1 μM) will be presented. Optimization of the 3D printed device for dosing multicellular spheroids using the anti-cancer drug irinotecan (MW 586.7 g/mol) will also be discussed. This application establishes the necessity for characterization of diffusion properties of compounds with a wide range of molecular weights in the 3D printed device.

35. New Insights Into How the C-Terminal Sequence of a Protein Controls Its Fragmentation *In Vivo*

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E. coli has a variety of proteases that degrade unfolded, misfolded and damaged proteins. Many protease substrates contain a specific amino acid sequence, also known as a degron, located at the N- or C-terminus that target the protein for degradation. For example, the SsrA tag (AANDENYALAA) is added to the C-terminus of proteins produced from an incomplete or otherwise anomalous mRNA. This tag is then recognized by a protease, usually ClpXP, and the tagged protein is degraded processively from C- to N-terminus. In contrast, other *E. coli* proteases, such as Tsp, have been shown to cleave a protein into large fragments rather than completely degrade them. Here, we show that the model protein YKB is cleaved *in vivo* into discrete fragments. YKB is a biosensor composed of three half-domains that folds into one of two alternative native structures (YK or KB), leaving the remaining half-domain unpaired. Constructs that lack an extra half-domain do not fragment, so presumably fragmentation is tied to the presence of a non-natively folded region. Rather than being non-specifically cleaved, we instead observe specific cleavage sites. Changing the C-terminal residues of YKB alters the amount of fragmentation observed. In general, YKB constructs with more polar C-terminal residues fragment less than those with non-polar residues. A construct susceptible to fragmentation can be protected by the addition of a single charged amino acid to the C-terminus. Oddly, a construct that increases ribosome stalling, but is not expected to fragment due to its polar terminus, does fragment suggesting translation rate might play a role in fragmentation.

36. Nuclear Magnetic Resonance as a Biochemistry Tool in the 21st Century

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In addition to solving protein structure, NMR is suited for characterizing dynamic processes in proteins over a range of time-scales including ps-ns fluctuations and μs-ms exchange dynamics. Previous challenges restricted NMR to small well-behaved proteins. Recent advances, however, have expanded the breadth of proteins amenable to NMR including high-molecular-weight proteins on the order of 100+ kDa. These advances include a combination of electronics/hardware, experimental methods, and isotope labeling strategies. Examples of these advances entail non-uniform sampling (NUS, significantly dicreases acquisition time), experiments such as relaxation dispersion and paramagnetic relaxation enhancement (characterize minor species), and ILV isotope labeling strategies that promote the characterization of large proteins. Here we present those advances that are currently available at the University of Notre Dame.

37. Large-Scale High-Yield Chemical Syntheses of Human Milk Oligosaccharides (HMOs)

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Human milk provides infants with a multitude of health benefits in additon to basic nutrition, including stimulation of the immune system and control of microbial contents in the gut. Human milk's functional constituents devive partly from human milk oligosaccharides (HMOs). Emerging analytical techniques and tools in glycobiology (*e.g.*, capillary electrophoresis; MS methods) allow the identification and structural characterization of many HMOs and the study of their biological properties. The ultimate biomedical goal of this work is to document the specific beneficial effects of HMOs for breast-fed babies, and quantify their impacts on the health of breast-feeding mothers. These investigations are heavily limited at present by the low availability of commercial HMO sources in quantities that allow *in vivo* study.

Synthetic methodologies to prepare kilogram amounts of a core HMO building block, *N*-acetyl-lactosamine, and seven HMOs on gram scales including two fucosyllactoses (2'FL and 3FL), lacto-*N*-tetraose (LNT), lacto-*N*-neotetraose (LNnT) and three lacto-*N*-fucopentaoses (LNFPs), under cGMP laboratory conditions are under systematic investigation. Enzyme-based methodologies to modify these oligosaccharides with *N*-acetyl-neuraminic acid using CMP-sialic acid and a sialyltransferase are under development. The longer range synthetic goal of this Phase I SBIR project is to chemically synthesize all of the core HMO building blocks on large scales and in high yields to support their use in human clinical trials, and to develop the capability of extending these core structures to larger oligosaccharides via regiospecific sialylation.

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38. Elucidating the Role of the MTSS1 Tumor Suppressor Gene in Pancreatic Ductal Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related deaths in the United States with a 5-year survival rate of 6%. This dismal outlook is largely due to the inability to diagnose the disease before metastasis occurs. 53% of patients afflicted with pancreatic cancer are diagnosed at the metastatic stage. These patients are offered treatment regimens that are unsuccessful or palliative care to ease their pain. PDAC deaths will continue to rise unless meaningful research is undertaken to both uncover new gene targets that suppress metastatic progression and to augment current treatment strategies that are being found to be ineffective in the clinic. One potential therapeutic target is the tumor suppressor gene, MTSS1. Recent work has found that MTSS1 is suppressed in a number of different cancers. Interestingly, though MTSS1 is expressed normally in early phases of cancer, it is lost in the metastatic stages of the disease. Despite the evidence showing that MTSS1 could be important for the suppression of tumor metastasis, the role of this gene in PDAC has not be studied. Our preliminary findings show a correlation between the MTSS1 protein and COX-2 driven inflammation, which is an early hallmark of PDAC initiation and progression. Treatment with non-steroidal anti-inflammatory drugs (NSAIDs), inhibitors of COX-2, has been shown to decrease the risk of pancreatic cancer in early stage patients. Surprisingly, COX-2 inhibition does not increase survival in patients who have already progressed to metastatic disease. However, no studies have explored why COX-2 inhibition is ineffective in these late-stage patients. Our hypothesis is that COX-2 expression causes eventual loss of MTSS1. Once MTSS1 is lost, currently approved therapeutic regimens are no longer effective. We propose to use *in vitro* models and genetically engineered mouse models to elucidate the molecular mechanism behind both the role and regulation of MTSS1 in PDAC in order to uncover not only a novel tumor suppressive pathway in pancreatic cancer, but also to unlock a potentially new biomarker of aggressive disease that could yield significant therapeutic advantages in late stage PDAC patients.

39. Biophysical Characterization of TCR Variants with Reengineered Specificity and Affinity

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Autologous cytotoxic T-cells, CD8+ T-cells, recognize and target certain cancer cells through an interaction between T-cell receptors (TCR) and antigen bound major histocompatibility complex (MHC complex) on the target cells. This phenomenon has led to advent of adoptive immunotherapy for melanoma. In this therapy, T-cells from the patient are genetically engineered to express TCRs that respond specifically to melanoma associated antigens (MART-1 epitopes) and then reintroduced into patients. The first set of clinical trials examining this approach in humans utilized DMF4 and DMF5 TCRs, and showed that DMF4 TCR led to a 13% rate of tumor regression, whereas DMF5 TCR led to a 30% rate of tumor regression. Using biophysical characterization techniques, it was shown that the improvement in immunogenicity seen with DMF5 was due to its higher affinity towards the cognate melanoma antigen (MART-1_{27-35}). This made higher affinity TCRs therapeutically relevant. Traditionally, higher affinity TCRs have been generated via *in vitro* selection techniques such as yeast or phage display. However, *in vitro* selection methods have inherent limitations. Most notably, gaining peptide independent TCR binding, which can lead to autoimmunity in patients. Structure guided design (SGD) is emerging as an alternative technique for developing therapeutically relevant TCRs. It is an *in silico* technique that combines structural and biophysical information in the modeling software to generate higher affinity variants of TCRs, allowing for highly controlled manipulation of affinity and specificity. While SGD of TCRs is promising, it is in early stages of development and requires further optimization. Structural and biophysical characterization of TCR variants that have high affinity or re-engineered specificity will help improve TCR SGD.

40. Molecular Dynamics Studies of the Role of Protein Flexibility in Immunological Molecular Recognition

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Activation of the cellular immune response involves the recognition of an antigenic peptide presented by either the class I or class II major histocompatibility complex (MHC) by T-cell receptors (TCRs). Because of the large population of potential antigenic peptides in comparison to the number of available TCRs, TCRs must be cross-reactive, yet must also maintain a degree of specificity to avoid indiscriminate T cell activation. A commonly implicated factor for TCR specificity and cross reactivity, as well as the current focus for this study, involves the flexibility of TCR complementarity-determining region (CDR) loops. This study focuses on the DMF5 TCR, which is specific for epitopes of the MART-1 protein, upregulated in the majority of melanomas. Beyond its potential utility in cancer therapy, this particular TCR is of interest because of the apparent rigidity of its CDR loops, which contrasts with what traditionally has been expected of TCR binding loops. Molecular Dynamics simulations of both the unligated and ligated DMF5 TCR were performed in order to assess its flexibility and to gain insight into the roles of mobility in DMF5 binding.

41. Characterization of Progression-Related Signaling Networks in a Colon Cancer Metastasis Model Using Phosphoproteomics

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Colorectal cancer (CRC) deaths overwhelmingly result from metastasis rather than from locally confined tumors. Cancer progression is associated with increasing invasive behavior, migration, angiogenesis, and resistance to apoptosis. Significant cross-talk among these pathways and others facilitates both the acquisition of increasingly aggressive traits and resistance to drug treatments over time. However, the relationships among these pathways and the molecular mechanisms of metastasis remain incompletely understood. One particularly valuable *in vitro* CRC model is the SW480/SW620 pair of cell lines. These patient-matched lines were derived from a primary adenocarcinoma and a lymph node metastasis, respectively. We performed comparative phosphoproteomic analysis of these cells by liquid chromatography-mass spectrometry (LC-MS) to assess the relative constitutive phosphorylation in the two cell lines. Pathway and gene ontology analyses of the sites significantly altered between the two cell lines revealed dysregulation in adhesion & migration, the cell cycle, and mRNA translational machinery. Among the latter group were sites on phosphoproteins with known roles in mRNA biogenesis and splicing, transport through the nuclear pores, initiating translation, as well as mRNA stability and degradation. Though alterations in these processes have been associated with oncogenic transformation, control of mRNA stability has typically not been associated with cancer progression.

Our current work focuses on three significantly changed but uncharacterized sites on proteins annotated as mRNA stability regulators. Serine 564 on 4E-T (eukaryotic translation initiation factor 4E transporter) was found more abundant in SW480. The protein is responsible for transporting and sequestering the mRNA-cap-binding protein eIF4E, along with its bound mRNA, into processing bodies (PBs). Within PBs, transcripts may be degraded, beginning with removal of the protective 5' cap by the decapping complex. DCP1a (decapping mRNA 1A) is a key component of this complex, and pThr401 and pSer525 were found significantly up-regulated in the metastatic cell line. Westerns indicate that 4ET and DCP1a are expressed at similar levels in the two cell lines, so the phosphopeptide abundance differences measured by MS are best explained by a change in site stoichiometry. IP followed by MRM will be used to confirm this difference and to determine the exact site stoichiometry.

42. The Role of Aging in Ovarian Cancer Metastasis to the Peritoneum

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Ovarian cancer is the most fatal gynecological cancer. Epithelial ovarian cancer (OvCa) is the most common subtype and often goes undetected until metastatic and often fatal stages of the disease. OvCa follows a unique form of metastasis, spreading through the peritoneal cavity and forming metastatic sites on the peritoneum. The peritoneum, a vast, serous membrane lining the abdominal cavity and organs, consists of a single layer of mesothelial cells (MCs) supported by a collagen-rich extracellular matrix (ECM). OvCa metastasis initiates when tumor cells or multicellular aggregates (MCAs) are shed from the primary tumor into the peritoneal cavity. OvCa cells or MCAs adhere to peritoneal surfaces, causing the MCs to retract. Metastasis progresses when OvCa cells penetrate through the mesothelium into the submesothelial ECM, where they anchor and proliferate. The vast majority of OvCa cases occur in women over 40 and the median age at diagnosis is 63. Despite age being a significant risk factor for the development of OvCa, very few studies have examined the role of aging in OvCa metastasis. Furthermore, there is a dearth of information on the aging peritoneum. Our preliminary data suggest that both the mesothelial and submesothelial compartments of the peritoneum accumulate changes as a function of age. Our data also suggests that the age of the host influences OvCa metastasis. Using a C57Bl/6 mouse model of aging and OvCa metastasis, we are testing the hypothesis that aging leads to alterations in peritoneal tissues and that the aging peritoneum impacts metastatic success of ovarian cancer. We found that the mesothelium of middle-aged mice (10-14 months) has a greater density of microvilli on the apical surface than does the mesothelium of young mice (3-6 months). The submesothelial collagen also appears to undergo age-related change. Using a peritoneal explant adhesion assay, we found that ovarian cancer cells attach more efficiently to the peritoneum of young mice than to the peritoneum of middle-aged or aged (20-23 months) mice.

43. Regulation of ARID3B and Its Direct Target Genes by EGFR Signaling

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Overexpression of epidermal growth factor receptor (EGFR) in ovarian cancer has been shown to correlate with poor disease outcome. Although evidence supports a link between EGFR signaling and resistance to apoptosis, the mechanism by which the EGFR signaling pathway inhibits apoptosis is not well understood. It has been previously shown that activation of EGFR signaling by increases expression of ARID3B, a member of the AT-rich interactive domain (ARID) family of DNA binding proteins. We further demonstrated that ARID3B can induce or activate genes in the TNFa and TRAIL signaling pathway in different contexts. We thus hypothesize that EGFR signaling pathway can induce resistance to TNFα signaling induced apoptosis by directly regulating ARID3B and its target gene expression. To investigate this, we first want to know whether genes that encode proteins involved in TNFα induced apoptosis are directly regulated by ARID3B. By using Chromatin immunoprecipitation (ChIP), we demonstrated that TNF and TRAIL are two direct targets for ARID3B. Next we want to know how EGFR signaling would affect TNFα/TRAIL signaling. We treated the OVCA429 ovarian cancer cells with EGF and found that the transcription of both TRAIL and TNF decreased after 4 hours treatment. This suggests that activation of EGFR leads to the inhibition TNFα/TRAIL induced apoptosis. After confirming that TNF and TRAIL are direct targets for ARID3B and the expression of both genes is inhibited by EGFR signaling, we next will investigate whether ARID3B act as a transcription activator or repressor for both genes. We will perform luciferase reporter assays to test if ARID3B transactivates or represses TNF and TRAIL promoters. We will also investigate whether ARID3B contribute for EGFR repression of TRAIL and TNF using ARID3B knockdown cell lines. Taking together, in this study we will determine the effects of EGFR activation on the regulation of ARID3B transcriptional activation or repression of its target genes that are involved in the TNF/TRAIL induced apoptotic signaling pathway.

44. Investigating Protein-Protein and Protein-Lipid Interactions in *Ebolavirus* and *Plasmodium falciparum* Using Engineered Conformation-Specific Antibodies

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Protein-protein and protein-lipid interactions play a crucial role in the pathogenesis of a variety of diseases. The study of these interactions leads to a better understanding of membrane transport, pathogen replication, and cellular invasion. The *P. falciparum* Kelch domain and *Ebolavirus* protein VP40 undergo these interactions to perform their respective functions. We studied the effects of lipid binding and protein-protein interactions on protein conformational change/complex formation utilizing phage display. A phage library of humanized antibody Fab fragments was used to isolate Fabs that bind specifically to either wild-type or C580Y mutant *Plasmodium falciparum* Kelch protein. The C580Y mutation in the *pf*Kelch protein is responsible for artemesinin resistant malaria. For *Ebolavirus* VP40, phage display selection isolated Fabs specific to the monomer, dimer, or octamer conformations of the protein. Each VP40 conformation functions in important viral processes including transcription and viral egress. We believe that our Fabs can be utilized to disrupt these viral processes.

Several Fabs were specifically isolated for wild-type and C580Y Kelch, as well as the VP40 conformations. These Fabs bind to their respective target proteins with high specificity as shown by SPR. Work is ongoing to characterize the effect of Fabs on *pf*Kelch to better understand the mechanism of artemesinin resistance in malaria. The VP40 Fabs will be utilized to trap VP40 in specific conformations to inhibit *Ebolavirus* replication or viral budding and egress. Our preliminary data show that phage display selection can be used to explore and possibly inhibit protein-protein/lipid interactions in parasitic and viral infections.

45. Engineering V-ATPase Inhibitor Loaded Nanoparticles for the Treatment of Multiple Myeloma

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Vacuolar-ATPase (V-ATPase) is a proton pump located on the plasma membrane and membranes of internal organelles of tumor cells, primarily to maintain an extracellular environment optimal for invasion and metastasis. By definition, a diagnosis of multiple myeloma (MM) requires tumor growth that has spread to multiple bones. Therefore, V-ATPase is hypothesized to play an important role in this disease. Bafilomycin A1 and iejimalide B are small molecules shown to inhibit the activity of V-ATPase by preventing tumor growth through a variety of proposed mechanisms. Although both inhibitors are highly toxic, they have poor selectivity and solubility, preventing their use in clinics. These factors necessitate the use of a nanoparticle drug delivery vehicle which can address solubility and decrease systemic toxicity. In this study, we demonstrate the use of bafilomycin A1 and iejimalide B loaded liposomes for the treatment of MM. Drug loaded liposomes have IC_{50} values consistent with free drug *in vitro* for a variety of cancers with iejimalide B outperforming bafilomycin A1. Free iejimalide B was also evaluated *in vitro* in combination with other MM therapeutics to evaluate synergy. Slight to moderate synergy is observed with carfilzomib, doxorubicin, and ACY-1215. Mouse studies show increased survival for iejimalide B over bafilomycin A1 liposomes with slight tumor growth inhibition and minimal effect on body weight at low doses. Release studies indicate both V-ATPase inhibitors are released rapidly from the liposomes, likely the cause of mortality at higher doses *in vivo*. Taken together these data suggest that drug release must be addressed before continuing with V-ATPase inhibitor loaded nanoparticles *in vivo*. Additionally, iejimalide B should be evaluated in combination with other MM drugs in liposomal form to further elucidate synergistic effects.

46. Synthesis and Kinetics of MMP-13 Selective Inhibitors

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Matrix metalloproteinases (MMPs) are a family of 26 enzymes that remodel the extracellular matrix and play physiological roles, as well as are involved in the pathology of diseases. Of these, MMP-1, MMP-8, and MMP-13 are known as collagenases, which are capable of degrading bone and cartilage and have been found upregulated in various diseases. MMP-13 in particular has been shown to be overexpressed in various cancers, rheumatoid arthritis, and osteoarthritis. Due to the similarity in structure of the collagenases, a selective inhibitor of MMP-13 has not been reported. A series of 20 inhibitors were designed using computational chemistry, synthesized, and evaluated for enzyme kinetics. The inhibitors were screened initially at 10 μM, and seven compounds showed inhibition of MMP-13 above 50%. These compounds were further screened at several concentrations for inhibition to several MMPs. Two potent and selective MMP-13 inhibitors were identified. Compound MB050 inhibits MMP-13 with a K_i value of 36 ± 11 nM and has greater than 200-fold selectivity for MMP-13 over MMP-8. While compound MB071 is less selective (35-fold) than MB050, it is still a potent inhibitor of MMP-13. *In vitro* and *in vivo* studies with these inhibitors are ongoing to determine their potential as viable treatments for diseases where MMP-13 is overexpressed.

47. Synthesis of 5'-GMP and 3',5'-cGMP Containing Uniform ^{13}C-Enrichment in their β-D-Ribofuranosyl Rings

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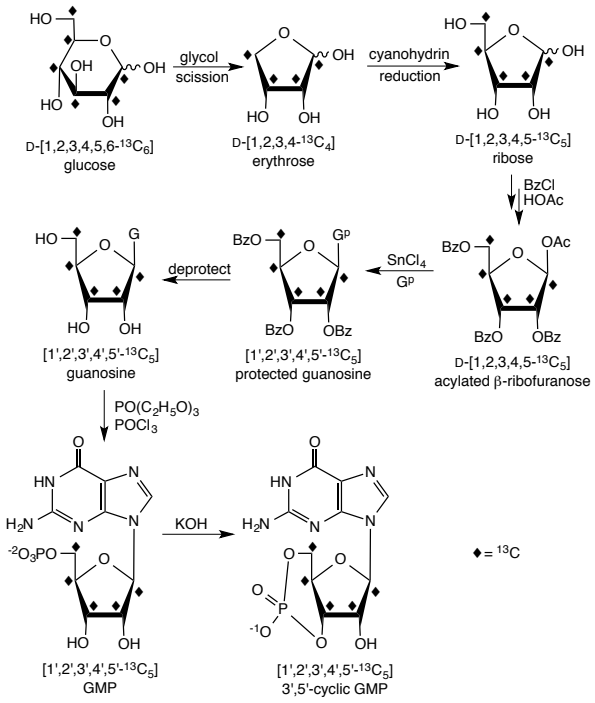
Nucleosides labeled with stable isotopes such as ^{13}C, ^{2}H, ^{15}N and/or ^{17,18}O are valuable biochemical reagents to elucidate the structures of biomacromolecules and determine their biological functions.[1-3] Studies of molecular metabolism, conformational analyses of oligoribo- and 2'-deoxyribonucleotides, investigations of oligonucleotide-drug interactions, and studies of nucleic acid-protein and nucleic acid-carbohydrate recognition are greatly assisted by stable isotopic labeling, especially when NMR is involved as the analytical method. In NMR studies, isotopically labeled nucleosides, nucleotides and oligonucleotides can be used to overcome inherent limitations of spectral sensitivity, resolution and/or selectivity.



Interest in tailored stable isotopic ^{13}C labeling within the sugar constituent of nucleosides/tides has increased over the past decade, especially for G-containing compounds. Both 5'-GMP and 3',5'-cGMP are important biomolecules used in the construction of isotopically labeled RNA and in studies of enzyme/metabolic control. To assist this work, chemical syntheses of 5'-GMP and 3',5'-cGMP were developed to allow uniform ^{13}C-enrichment (>99% at each carbon) in the β-D-ribofuranosyl ring (see scheme). The starting material for these syntheses is D-[1,2,3,4,5,6-$^{13}C_6$]glucose, which is obtained from the growth of algae in the presence of $^{13}CO_2$. The aldohexose is cleaved oxidatively with $Pb(OAc)_4$ to give the aldotetrose, D-[1,2,3,4-$^{13}C_4$]erythrose. The latter is then chain-extended by cyanohydrin reduction ($K^{13}CN$; hydrogenolysis) to give, after chromatography, D-[1,2,3,4,5-$^{13}C_5$]ribose. The labeled pentose is acylated, and the acylated ribose derivative condensed with a protected guanine base to give, after deprotection, [1',2',3',4',5'-$^{13}C_5$]guanosine. Phosphorylation at C5' with phosphoryl chloride gives 5'-GMP in good yield. The latter monophosphate can be converted into the 3',5'-cyclic phosphate, cGMP, upon treatment with KOH at low temperature. Efforts are underway to convert labeled 5'-GMP into labeled GTP, and to apply similar chemical methodologies to prepare isotopically labeled 5'-AMP, 3',5'-cAMP and ATP.

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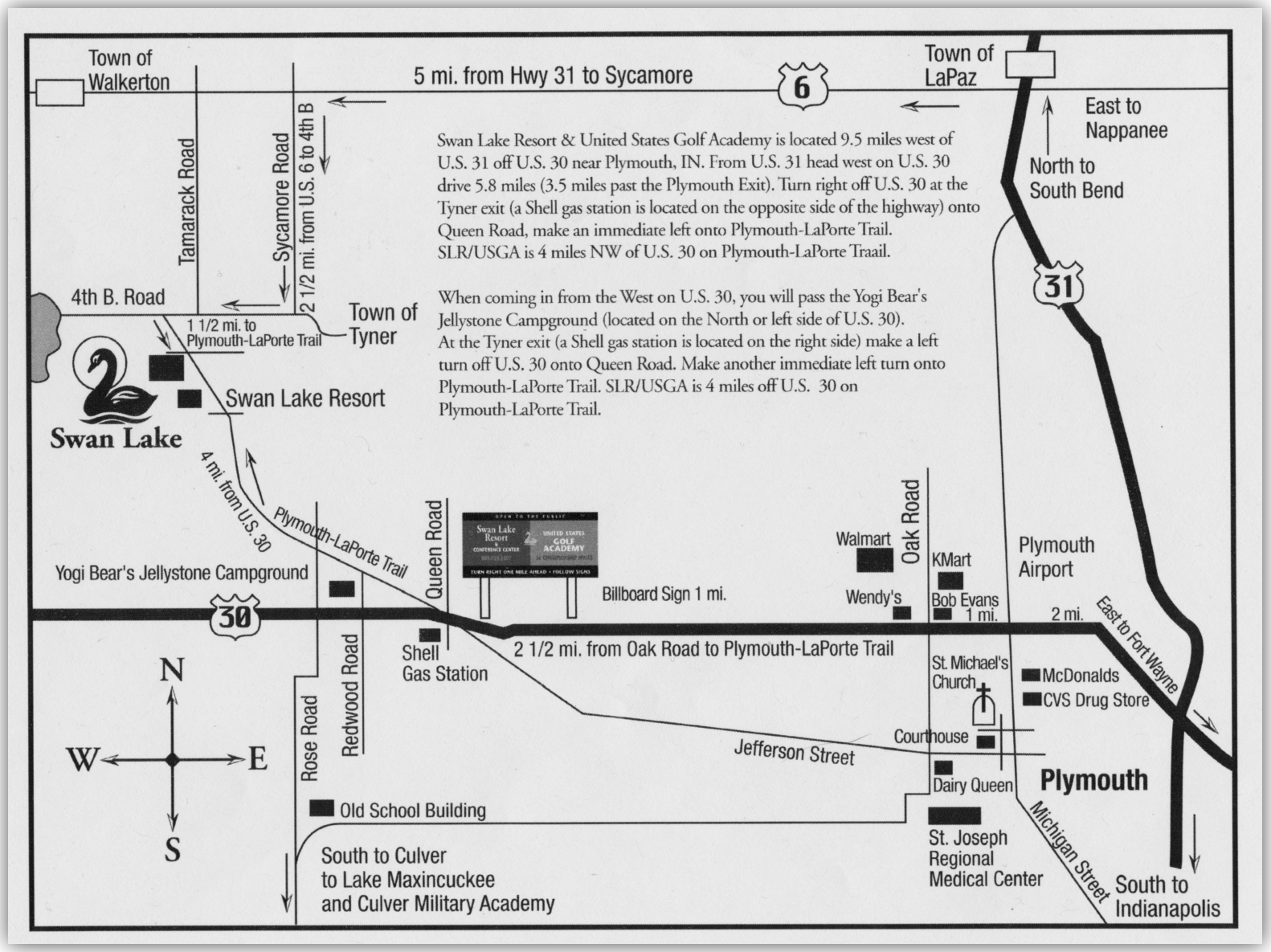
2. Etsuko, K., Kazuo, K. *Mini Rev. Org. Chem*. **2004**, *1*, 309-332.
3. Batey, R. T., Inada, M., Kujawinski, E., Puglisi, J. D., Williamson, J. R. *Nucl. Acids Res*. **1992**, *20*, 4515-4523.


Town of Walkerton
5 mi. from Hwy 31 to Sycamore
6
Town of LaPaz
East to Nappanee
North to South Bend
31
Tamarack Road
Sycamore Road
2 1/2 mi. from U.S. 6 to 4th B
4th B. Road
1 1/2 mi. to Plymouth-LaPorte Trail
Town of Tyner
Swan Lake Resort & United States Golf Academy is located 9.5 miles west of U.S. 31 off U.S. 30 near Plymouth, IN. From U.S. 31 head west on U.S. 30 drive 5.8 miles (3.5 miles past the Plymouth Exit). Turn right off U.S. 30 at the Tyner exit (a Shell gas station is located on the opposite side of the highway) onto Queen Road, make an immediate left onto Plymouth-LaPorte Trail. SLR/USGA is 4 miles NW of U.S. 30 on Plymouth-LaPorte Traail.
When coming in from the West on U.S. 30, you will pass the Yogi Bear's Jellystone Campground (located on the North or left side of U.S. 30). At the Tyner exit (a Shell gas station is located on the right side) make a left turn off U.S. 30 onto Queen Road. Make another immediate left turn onto Plymouth-LaPorte Trail. SLR/USGA is 4 miles off U.S. 30 on Plymouth-LaPorte Trail.
Swan Lake Resort
Swan Lake
4 mi. from U.S. 30
Plymouth-LaPorte Trail
Queen Road
Yogi Bear's Jellystone Campground
30
Billboard Sign 1 mi.
Walmart
Oak Road
KMart
Wendy's
Bob Evans 1 mi.
Plymouth Airport
2 mi.
East to Fort Wayne
2 1/2 mi. from Oak Road to Plymouth-LaPorte Trail
Shell Gas Station
Redwood Road
Rose Road
N
W
E
S
St. Michael's Church
McDonalds
CVS Drug Store
Courthouse
Jefferson Street
Dairy Queen
Plymouth
Old School Building
St. Joseph Regional Medical Center
Michigan Street
South to Culver to Lake Maxincuckee and Culver Military Academy
South to Indianapolis