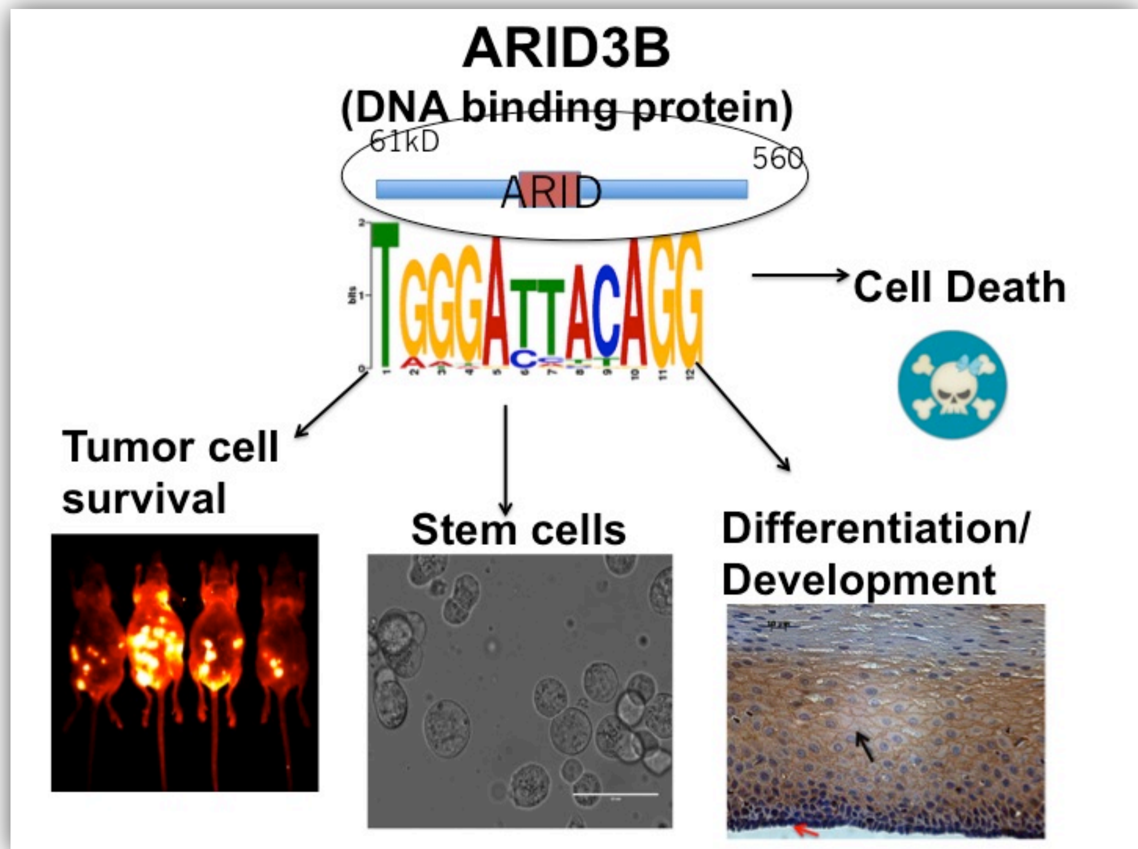
The O'Connor Papers

19th 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 11-12, 2014

♦ Organizer ♦

Anthony Serianni

♦ Retreat Sponsors ♦

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

University of Notre Dame

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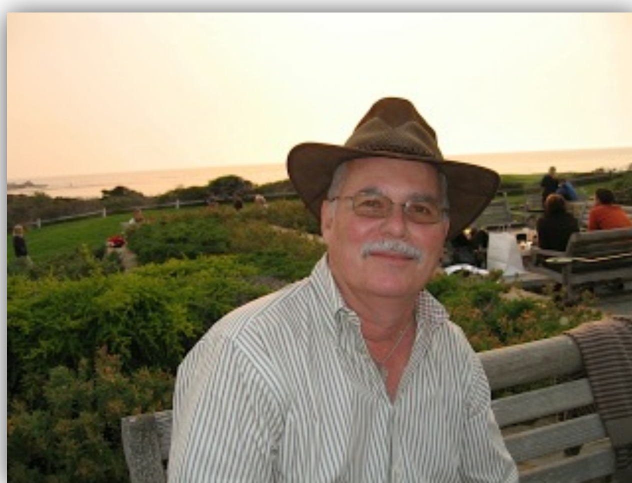
Cover Illustration

Courtesy of the Cowden-Dahl Lab

Cover Graphic: The DNA binding protein ARID3B (61kD, 560 amino acids) is critical in multiple biological processes. ARID3B (in blue) binds to specific DNA sequences (binding motif shown above/center) through its DNA binding domain called the ARID domain. Binding of ARID3B to its target sequences leads to the induction of genes involved in tumor growth, stem cell production, differentiation, and cell death. Overexpression of ARID3B is found in human ovarian cancer and promotes tumor growth in xenograft mouse models (left) as seen with *in vivo* fluorescence imaging. ARID3B expression is found in normal stem cell populations and promotes cancer stem cell production (middle). Loss of ARID3B is embryonic lethal and is critical for differentiation of many cell types (including epithelium, right panel). Additionally, ARID3B works in a dose-dependent manner. High levels of ARID3B promote cell death. The Cowden-Dahl lab is currently dissecting the molecular mechanism for how ARID3B regulates these processes using a variety of molecular and biochemical techniques.

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 recently, and the world is a poorer place as a result. His spouse, Barbara O'Connor, herself an accomplished scientist, has 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 post-doctoral 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.

Past 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 (Einstein)

2013: Adriaan Bax (NIH)

The John V. O'Connor Lectureship in Biochemistry

Enrique M. De La Cruz
Department of Molecular Biophysics and Biochemistry
Yale University

How Cells Use Chemistry and Physics To Break the Bones That Power Their Movement

The polymerization of the protein actin into helical filaments powers many eukaryotic cell movements and provides cells with mechanical strength and integrity. The actin regulatory protein, cofilin, promotes actin assembly dynamics by severing filaments and increasing the number of ends from which subunits add and dissociate. I will present results from biochemical and biophysical studies focused on defining in chemical and physical terms (thermodynamics, structure, mechanics and kinetics) how cofilin binds and fragments actin filaments. The experimental data are well described by a model in which discontinuities in filament topology and compliance promote fracture preferentially at junctions of bare and cofilin-decorated segments along filaments. Computational modeling and simulations indicate an intrinsic coupling between bending and twisting is an important component of filament elasticity.

Biography of Enrique M. De La Cruz

Enrique M. De La Cruz is a Professor in the Department of Molecular Biophysics and Biochemistry at Yale University. He is a first generation Cuban-American who was raised in Newark, NJ. Dr. De La Cruz received his undergraduate degree in biology from Rutgers University where he was inducted into the Phi Beta Kappa and Beta Beta Beta Honor Societies. He earned his PhD in Biochemistry, Cell & Molecular Biology (BCMB) with Dr. Thomas D. Pollard at Johns Hopkins University School of Medicine and received postdoctoral training in the laboratories of Dr. H. Lee Sweeney and E. Michael Ostap at the University of Pennsylvania School of Medicine. In 2009, he was a Visiting Scientist at Centre National de la Recherche Scientifique (CNRS), Commissariat à l'Énergie Atomique (CEA) & Université Joseph Fourier in Grenoble, France.

Prof. De La Cruz has published extensively in the areas of actin and myosin regulation, RNA helicases, and signaling enzymes, for which he has received a number of awards and honors. Among them are the American Heart Association Established Investigator Award, NSF CAREER award, Keith R. Porter Symposium Award from the Society for General Physiologists, the Anderson Award, and the Hellman Family Fellowship from Yale University. He was also the Abbott Distinguished Lecturer at Purdue University, a Plenary Lecturer at the Ibero-American Congress of Biophysics Meeting held in Brazil, and a Plenary Lecturer of the 44th Annual Meeting of the Biophysical Society of Japan.

Prof. De La Cruz is actively involved with various scientific societies, journals and peer review committees. He serves on the editorial boards of *Biophysical Journal* and *Biophysical Reviews*, the Publications Committee of the American Society for Biochemistry & Molecular Biology (ASBMB), and the Macromolecular Structure & Function C Study Section of the NIH. He has served on the Biophysical Society Council, chaired its Nominating Committee, and served on research panels for the NSF, American Heart Association, and funding agencies in England, Israel and France. In addition, he actively participates in a number of outreach activities focused on enhancing minority participation and career development in the sciences.

Program

Wednesday Afternoon
Session Chair: Holly Goodson

1:00-1:10 Welcome and Orientation – A. Serianni

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1:10-1:30 **In Appreciation: Barbara O'Connor - Endowment of the Biochemistry Research Retreat in Honor of Dr. John V. O'Connor ND'72**

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1:30-2:00 Benjamin Cressiot, Esther Braselmannn and Patricia L. Clark
Dynamics and Energy Contributions for Transport of Pertactin Through an Aerolysin Nanopore

2:00-2:30 Abigail Weaver, Sarah Halweg, Marya Lieberman and Holly Goodson
Paper-based Yeast Biosensors for Antibiotic Detection

2:30-3:00 Shailaja Kunda, Jaroslav Zajicek, John Cheriyan, Michael Hur, Rashna D. Balsara and Francis J. Castellino
A Structural and Functional Study of N-Methyl-D-Aspartate Receptor-specific Antagonistic Peptides from Conus Species of Marine Snails: Con-Pr1, 2, 3, and Con-RIB

3:00-3:30 Mid-Afternoon Break

3:30-4:00 **Guest Lecture**
Zachary T. Schafer
Department of Biological Sciences, University of Notre Dame
Carcinoma-associated Fibroblast-mediated Regulation of Anoiki in Breast Cancer Cells

4:00-4:30 Eve A. Granatosky, Jarred R. E. Pickering, D. Cole Stevens and Richard E. Taylor
Evaluation of the Polyketide GEX1A as a Potential Lead for Niemann-Pick Type C Disease

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4:30-6:00 Hotel Check-in; Free Time

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

Enrique M. De La Cruz
How Cells Use Chemistry and Physics To Break the Bones That Power Their Movement

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Wednesday Evening

7:15-8:30 **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 Kristen A. Johnson, Jordan L. Scott and Robert V. Stahelin
A New Mathematical Analysis Using MATLAB To Investigate Membrane Protein Dynamics in Live Cells

9:30-10:00 Marc A. Boudreau, Jennifer Fishovitz and Shahriar Mobashery
Potentiation of Oxacillin Activity Against Methicillin-Resistant Staphylococcus aureus (MRSA) by Kinase Inhibitors

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

11:00-11:30 **Guest Lecture**
John Duman
Department of Biological Sciences, University of Notre Dame
Antifreeze Proteins and Glycolipids: An Overview with Emphasis on Insects

11:30-12:00 Olivia F. Cox, Ning Wang and Paul W. Huber
The Effects of TiO_2 Nanoparticles on Xenopus laevis Development and Tight Junction Structure

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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 Nuno T. Antunes, Toni L. Lamoureaux, Marta Toth, Nichole K. Stewart, Hilary Frase and Sergei B. Vakulenko
The OXA-2 and OXA-10 β-Lactamases Behave as Carbapenemases in Acinetobacter baumannii

2:30-3:00 Blas Blázquez, Leticia I. Llarrull, Juan R. Luque-Ortega, Carlos Alfonso and Shahriar Mobashery
Regulation of the Expression of the β-Lactam Antibiotic-Resistance Determinants in Methicillin-Resistant Staphylococcus aureus (MRSA)

3:00-3:30 Mid-Afternoon Break

3:30-4:00 **Guest Lecture**
Basar Bilgicer
Department of Chemical and Biomolecular Engineering, University of Notre Dame
Engineering Multivalent Molecules for Binding Enhancement and Achieving Selectivity

4:00-4:30 Julia Beck, Victoria A. Ploplis and Francis J. Castellino
The Role of Lysine Binding Sites (LBS) within the Kringle Domains of Plasminogen in PAM Binding

4:30-4:40 Concluding Remarks (A. Serianni)

ABSTRACTS: GUEST LECTURES

Carcinoma-associated Fibroblast-mediated Regulation of Anoikis in Breast Cancer Cells

Zachary T. Schafer
Department of Biological Sciences, University of Notre Dame,
Notre Dame, IN 46556 USA

There is accumulating evidence that carcinoma-associated fibroblasts (CAFs) in the microenvironment are critical players in the promotion of tumor progression and metastasis. However, the precise mechanisms utilized by CAFs to enhance malignancy are only beginning to be understood. Given the fact that cancer cells lack normal extracellular matrix (ECM) attachment after breaking through the basement membrane, we hypothesized that CAFs may be actively involved in the inhibition of anoikis (ECM-detachment-induced cell death). To investigate this question, we utilized NIH-3T3 mouse fibroblasts lacking the caveolin-1 gene (Cav -/-), a cell line that has previously been demonstrated to share many similarities to human CAFs. Interestingly, the addition of media conditioned by Cav-/- and patient-derived human CAFs led to robust anoikis inhibition in detached MCF-10A cells, suggesting that factors secreted by CAFs can block anoikis. Moreover, the ability of CAF-conditioned media to block anoikis was not limited to MCF-10A cells as we obtained similar results in other non-tumorigenic and breast cancer cell lines. To examine if the observed differences in anoikis induction caused by secreted factors from CAFs manifest in a more physiologically relevant context, we employed a 3-dimensional (3D) cell culture model of mammary acinus development. MCF-10A acini exposed to secreted factors from CAFs were more prone to have filled lumen suggesting that factors secreted by CAFs can promote the survival of ECM-detached cells in the luminal space.

In addition, when investigating the mechanism by which CAFs block anoikis, we discovered that the release of mitochondrial cytochrome *c* into the cytosol was robustly inhibited by CAF-conditioned media in MCF-10A cells. Additionally, we discovered that the degradation of Mcl-1 was strongly inhibited by CAF-conditioned media suggesting that Mcl-1 was not properly targeted to the proteasome. Stability of Mcl-1 is triggered by activation of ILK and p-ERK and inhibition of Gsk-3β. Furthermore, using proteomic analyses, we have identified proteins of the IGFBP family that are differentially secreted by CAFs in a fashion that is both necessary for anoikis inhibition by CAFs and sufficient to inhibit anoikis on their own. In aggregate, these data identify a novel mechanism by which CAFs contribute to tumorigenesis (the inhibition of anoikis) and suggest that targeting factors secreted from CAFs could be a novel therapeutic approach to eliminate ECM-detached cells through the selective induction of anoikis.

Antifreeze Proteins and Glycolipids: An Overview with Emphasis on Insects

John G. Duman
Department of Biological Sciences, University of Notre Dame,
Notre Dame, IN 46556 USA

Antifreeze glycoproteins (AFGPs) were first discovered by Art DeVries in the late 1960's in Antarctic marine teleost (bony) fishes. Additional antifreeze proteins (AFPs) lacking a carbohydrate component were subsequently identified in other polar fish, and today several structurally different fish AFPs are known to have evolved. The AF(G)Ps lower the freezing point of the body fluids of these hypo-osmotic fish by a non-colligative mechanism while not significantly affecting the melting point, thereby producing a difference between the freezing and melting points (thermal hysteresis) that is diagnostic of their presence. Additional structurally diverse AFPs have been identified in a range of organisms including terrestrial arthropods (insects, spiders, mites, centipedes), plants, fungi, and bacteria, but those of insects are the most active. Recently, antifreeze glycolipids (AFGLs) that produce thermal hysteresis were characterized from insects, frogs, and plants.

Organisms survive subzero temperatures by becoming either freeze tolerant (surviving freezing of their body fluids) or freeze avoiding (preventing freezing of the body fluids). AFPs and AFGLs are important components of the suite of subzero adaptations of many insects, both freeze tolerant and freeze avoiding species. Freeze avoiding larvae of the beetle *Dendroides canadensis* produce a family of over 30 AFPs (DAFPs). The DAFPs consist of 12- or 13-mer repeats with regularly spaced and highly conserved threonines involved in the ice binding site via hydrogen bonding, and every 6th residue is a cysteine. There is tissue specific expression with certain DAFPs present in hemolymph, gut, primary urine, and epidermis, suggesting variation in function specific to the site. Some DAFPs exhibit self-enhancement whereby activity is increased by certain other DAFPs, as well as by endogenous glycerol (~0.5M in hemolymph), a thaumatin-like protein, or AFGLs. Hemolymph and epidermal DAFPs inhibit inoculative freezing across the cuticle when larvae are in contact with ice. Hemolymph and gut DAFPs inhibit ice nucleator proteins and bacteria, thereby promoting supercooling. Likewise, DAFPs in the urine inhibit ice nucleating crystals commonly present in insect urine in winter. Similar AFPs in freeze avoiding larvae of the beetle *Cucujus clavipes* assist (along with high glycerol and cryoprotective dehydration) in the extreme supercooling ability of the Alaskan subspecies, whereby when in the mid-winter deep supercooling state they do not freeze even at -150°C, instead vitrifying at ~-60 to -70°C. AFPs that produce very low levels of thermal hysteresis are present in a number of freeze tolerant organisms, such as many plants. Similar low levels of thermal hysteresis are known in several freeze tolerant insects, but AFPs have not been identified from these sources. However, a xylomannan-AFGL was purified from a freeze tolerant Alaskan beetle, *Upis ceramboides*, and a few other freeze tolerant insects, plants, and frogs. AFPs and AFGLs inhibit potentially damaging recrystallization of ice and this likely a function in freeze tolerant species. However, most of the AFGL is present on cell membranes where it may function to inhibit the lethal inoculation of the cytoplasm by extracellular ice.

Engineering Multivalent Molecules for Binding Enhancement and Achieving Selectivity

Basar Bilgicer
Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN 46556 USA

Multivalency—the simultaneous binding of multiple ligands on one entity to multiple receptors on another—plays a pivotal role in biological systems. Primary effect of multivalency is the increase in binding affinity, however, multivalency can also be used to increase selectivity in targeting in various disease settings. This talk will convey multiple projects related to multivalent design: (1) heterobivalent ligands that competitively and selectively inhibit allergen binding to IgEs on mast cells to prevent allergic responses; (2) multivalent nanoparticles that target and overcome cell adhesion mediated drug resistance (CAM-DR) in multiple myeloma; (3) dual-receptor targeting strategy in nanoparticle design to provide synergistic enhancement in cellular uptake and achieve tumor cell selectivity; and (4) enhancement of antibody selectivity via bicyclic complex formation.

ABSTRACTS: ORAL PRESENTATIONS

Dynamics and Energy Contributions for Transport of Pertactin Through an Aerolysin Nanopore

Benjamin Cressiot, Esther Braselmannn and Patricia L. Clark
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Autotransporters are a large and diverse family of extracellular virulence proteins from Gram-negative bacteria. Despite their relative simplicity, many aspects of the autotransporter membrane secretion mechanism remain unclear. We are using pertactin, an autotransporter from *Bordetella pertussis*, as a model system for secretion studies. The final step of pertactin secretion is C-terminal to N-terminal threading of the central passenger domain through the outer membrane, mediated by a co-translated C-terminal translocator domain(*1*,*2*). The native structure is formed only after this final secretion step, which requires neither ATP nor a proton gradient. This folding mechanism has been proposed as the main driving force for pertactin secretion (*3*). For this reason, it is interesting to consider how autotransporters are secreted through their own translocator domain to the outer membrane.

As a first step, we are mimicking this transport using a simpler model consisting of well-known nanopores and patch-clamp techniques at the single molecule level. Transport of the pertactin passenger is detected using established electrophysiological techniques(*4*,*5*). We show that unfolded pertactin dynamics through a single aerolysin pore can be described using a model originally developed for another protein of different length and net charge. A Van't Hoff-Arrhenius law describes the frequency of blockades as a function of the applied voltage. The unfolded chains are dominated by an activation energy that has both an entropic component (mainly due to the confinement of the chain) and an enthalpic origin because of surface charges. We compare our experimental results to theory and show that the proteins cross the membrane by passing through the aerolysin nanopore. We have used these results to develop a general description of the comportment of an unfolded protein during its transport through a protein nanopore.

1. M. M. Junker *et al.*, Pertactin beta-helix folding mechanism suggests common themes for the secretion and folding of autotransporter proteins, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 4918–4923 (2006).
2. M. M. Junker, R. N. R. Besingi, P. L. P. Clark, Vectorial transport and folding of an autotransporter virulence protein during outer membrane secretion, *Molecular Microbiology* **71**, 1323–1332 (2009).
3. J. P. Renn, M. Junker, R. N. Besingi, E. Braselmann, P. L. Clark, ATP-independent control of autotransporter virulence protein transport via the folding properties of the secreted protein, *Chem Biol* **19**, 287–296 (2012).
4. C. Merstorf and B. Cressiot *et al.*, Wild Type, Mutant Protein Unfolding and Phase Transition Detected by Single-Nanopore Recording, *ACS Chem Biol* (2012).
5. B. Cressiot, A. Oukhaled, L. Bacri, J. Pelta, Focus on Protein Unfolding Through Nanopores, Springer, *BioNanoScience* (2014).

Paper-based Yeast Biosensors for Antibiotic Detection

Abigail Weaver[1], Sarah Halweg[2], Marya Lieberman[1] and Holly Goodson[1]
[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA; [2]Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN 46556 USA

Paper-based tests have the potential to serve as inexpensive tools to address analytical questions both inside and outside of the laboratory setting. Currently, paper-based devices containing a library of chemical tests can build a profile pharmaceuticals based on the presence of functional groups. To extend this paper-based technology to be more specific, *Saccharomyces cerevisciae* were used to construct the first example of a yeast whole-cell, paper-based biosensor device. This device is sensitive to antibiotics in the tetracycline family and could potentially address questions of pharmaceutical quality as well as antibiotic contamination in liquids. The biologically-based paper analytical device or "BioPAD" can qualitatively discriminate the presence/absence of doxycycline over a range of 30 – 10,000 μg/mL. Using a BioPAD, a doxycycline dosage form (tablet) commonly used for malaria prophylaxis, was confirmed to contain the antibiotic with 92% and 95% success, evaluated by eye and computer-assisted image analysis respectively, with no false positives by either method. Stored at 4ºC these tests were found to remain viable for greater than a year. This research demonstrates the utility of whole yeast cells in paper-based pharmaceutical testing, while highlighting the potential for the development of yeast-based BioPADs to address a range of qualitative analytical questions, especially in low resource settings.

A Structural and Functional Study of *N*-Methyl-D-Aspartate Receptor-specific Antagonistic Peptides from *Conus* Species of Marine Snails: Con-Pr1, 2, 3, and Con-RlB

Shailaja Kunda,[1,2] Zajicek J,[2] John Cheriyan,[1] Michael Hur,[1] Rashna D. Balsara,[1,2] and Francis J. Castellino[1,2]

[1]W. M. Keck Center for Transgene Research and [2]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

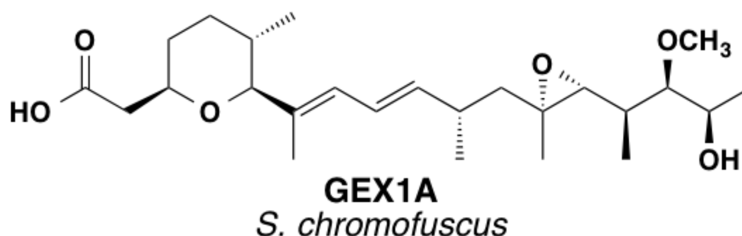
Conantokins are naturally occurring, gamma-carboxyglutamate (gla)-containing neuroactive peptides found in the venom of marine snails, which have been studied for their highly selective antagonistic activity towards N-Methyl-D-Aspartate Receptors (NMDAR). Here we report on the characterization of peptides from *Conus parius* and *Conus rolani* species of snail identified as Conus-parius1, 2, and 3 (Con-Pr1, 2, 3) and conantokin-RlB (Con-RlB), which uniquely differs from other conantokins by the presence of a 4-transhydroxyproline (Hyp 'O') residue. These conantokins have been studied for their structural α-helical conformation in the presence or absence of divalent ions, as well as their biological effects downstream of NMDAR activation. Con-Pr1 and Con-Pr2 showed an increase in α-helicity upon the addition of Mg^{2+} ions, whereas native-apo-Con-Pr3 is inherently helical. Con-RlB displays 62% α-helicity when compared to Con-G (100%), in the presence of Mg^{2+}. Further elucidation of the backbone structure of Mg^{2+}-Con-RlB by ^{1}H NMR and TALOSplus software has predicted a disruption in the helix due to the presence of Hyp at position 10. Robust inhibition of intracellular calcium influx [iCa^{2+}] and whole cell recordings was observed in GluN2A$^{-/-}$ mice cortical neurons, but not in GluN2B$^{-/-}$ neurons, revealing GluN2B subunit specific activity of Con-Pr peptides and Con-RlB. Synthesis of mutant peptides to assess the role of Hyp in the 10th position in Con-RlB was aimed at elaborating the molecular requisites of these peptides for their unique structural and functional attributes. Con-G[Insert 10 'O'] and Con-RlB[K8N,A9Q,ΔO] behave functionally and structurally similar to their respective parent peptides, Con-G and Con-RlB, calling to attention the role played by amino acids other than the second inter-gla fragment. In addition, Con-Pr1, 2, 3 and Con-RlB peptides mildly inhibited NMDA-mediated phosphorylation of CREB at Ser133, a transcription factor required for maintaining long term synaptic activity. Therefore, inhibitory properties displayed by the conantokins antagonize NMDAR-directed current and iCa^{2+} influx, while maintaining the receptor-coupled CREB signaling pathway. Similar experiments were performed with GluN2C$^{-/-}$ and GluN2D$^{-/-}$ neurons. A significant inhibition of iCa^{2+} influx was observed by Con-G and Con-RlB in DIV9 GluN2D$^{-/-}$ neurons, but not in DIV13-15 GluN2D$^{-/-}$ neurons. In conclusion, biophysical and cellular characterization in conjunction with genetic studies enables correlations of the structure-function relationships of conantokins as allosteric inhibitors of NMDAR.

Evaluation of the Polyketide GEX1A as a Potential Lead for Niemann-Pick Type C Disease

Eve A. Granatosky, Jarred R.E. Pickering, D. Cole Stevens and Richard E. Taylor
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Niemann-Pick Type C (NPC) disease is a rare neurodegenerative disorder in which cholesterol accumulates in the lysosomes as a result of a mutation in either the *NPC1* or *NPC2* gene. Recent studies have shown that histone deacetylase (HDAC) inhibitors are effective in reversing cholesterol accumulation in human NPC1 mutant fibroblasts. Our efforts to identify a novel treatment option for NPC disease are focused on the polyketide natural product GEX1A, derived from *Streptomyces chromofuscus*, which increases gene expression similar to known HDAC inhibitor trichostatin A. We have observed that GEX1A is capable of facilitating cholesterol trafficking in NPC1 cells, however GEX1A does not affect histone acetylation and likely acts through a novel mechanism. Based on these findings, we have developed a multidisciplinary route to accessing GEX1A, as well as synthetic, semisynthetic, and bioengineered analogues, in order to evaluate their therapeutic potential as lead compounds for NPC disease. Screening of GEX1A analogues in NPC mutant fibroblasts will allow us to identify structural features important for bioactivity. Here we highlight our approach to accessing GEX1A, describe our recent work in developing a synthetic biology platform for the production of GEX1A analogues from *S. chromofuscus*, and present our current progress towards evaluating GEX1A in both NPC1 and NPC2 mutant fibroblasts.

This work is supported by CBBI Training Fellowship T32GM075762.



A New Mathematical Analysis Using MATLAB To Investigate Membrane Protein Dynamics In Live Cells

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

Fluorescence confocal microscopy is a powerful method used to investigate a vast range of biological, biochemical, and biophysical questions. While the qualitative aspects of fluorescence microscopy are interesting for understanding cellular localization of peripheral proteins, quantitative analysis of these images can produce powerful data garnering mechanistic information on biological processes. Currently, fluorescence microscopy data analysis workflows use instrument imaging software such as Zen or ImageJ. Unfortunately both of these workflows have limitations including expensive licenses or lengthy analysis times. MATLAB is a MathWorks computer programing language that has various numerical computing functions including plotting data, statistical analysis, matrix manipulation, and algorithm implementation. The MATLAB script can achieve rapid qualitative and quantitative analysis; it takes 10.3 seconds to calculate the average intensity of a fluorophore at the cells plasma membrane and cytosol. This can be used to rapidly quantify images before and after a chemical treatment. Here we demonstrate the analysis of two different data types with this script: protein dissociation from the plasma membrane induced by a lipid depletion system, the rapamycin inducible phosphoinositide lipid depletion system, and association of a protein to the plasma membrane after treatment with two different small molecules. In both cases quantification of data with the previous method was possible but very laborious. This MATLAB script has reproduced the results obtained by the more laborious methods in a fraction of the time. Additionally, this script has served as the foundation for a secondary script that quantifies virus matrix protein dissociation from the plasma membrane before and after the phosphoinositide lipid depletion system is activated. Due to the high intensity punctate structures induced by viral matrix proteins the approach for image quantitation has been modified. Currently Ebola virus matrix protein VP40 and HIV-GAG matrix protein have been investigated with the lipid depletion system in COS-7 cells and analyzed with the virus modified script.

Potentiation of Oxacillin Activity Against Methicillin-Resistant *Staphylococcus aureus* (MRSA) by Kinase Inhibitors

Marc A. Boudreau, Jennifer Fishovitz and Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556-5670 USA

Methicillin-resistant *Staphylococcus aureus* (MRSA) has evolved two mechanisms for resistance to β-lactam antibiotics: 1) expression of the PC-1 β-lactamase, and 2) production of penicillin-binding protein 2a (PBP2a). Expression of PC-1 is regulated by the β-lactam sensor/signal transducer protein BlaR1, an integral membrane protein that detects the presence of β-lactam antibiotics through acylation of a serine residue in its outer-membrane domain. This acylation activates signal transduction to the cytoplasmic zinc-dependent protease domain, which in turn degrades the gene repressor BlaI. This derepresses expression of PC-1 and of BlaR1 itself, resulting in the resistant phenotype. Recent results from our lab indicate that BlaR1 is phosphorylated on both tyrosine and serine residues in its cytoplasmic domain. We hypothesized that inhibition of this phosphorylation event with a kinase inhibitor might silence expression of the resistance determinant(s), which would manifest itself phenotypically as increased sensitization of the resistant organism to a β-lactam antibiotic. A library of known eukaryotic kinase inhibitors was initially screened in combination with β-lactam antibiotics against strains of MRSA, and several of these compounds reduced the MIC of the β-lactams. Of the active kinase inhibitors, a triarylimidazole was chosen for further optimization, and several analogues have been synthesized and tested for their ability to potentiate the activity of oxacillin against MRSA. We observe up to 128-fold reduction in the MIC of oxacillin in the presence of one of these analogues.

The Effects of TiO_2 Nanoparticles on *Xenopus laevis* Development and Tight Junction Structure

Olivia F. Cox, Ning Wang and Paul W. Huber
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Titanium dioxide nanoparticles are widely used in the production of consumer goods including food, medicines, and cosmetics. Although considered chemically inert, the biological activity of TiO_2 has not been established. Since humans have direct contact with this nanoparticle through topical exposure or ingestion, it is necessary to establish its safety and any molecular mechanisms associated with its toxicity. Direct microinjection of these nanoparticles into *Xenopus laevis* embryos has allowed the assessment of their effects on a developing embryo while circumventing the issue of bioavailability. We show that microinjection of TiO_2 into one- or two-cell embryos results in reproducible developmental defects in cell-cell contact and cell migration. These defects present as eye malformations, failure in blastopore closure, attenuated axis elongation, axis curvature, and edema. We have completed a microarray analysis in order to determine changes in gene expression in embryos injected with sub-lethal amounts of TiO_2 nanoparticles. These misregulated genes were enriched for processes associated with tight junction assembly (occludin, claudins 4 and 7, cadherin 26), calcium signaling that directs cell movement (calcipressin 1, rhoA, Gαi), and cell adhesion (ephrin A1, rhoA, Gαi). This has led us to examine the integrity of cell-cell contacts in embryos injected with TiO_2 nanoparticles. Western blot analysis confirms a decrease in levels of the tight junction protein, occludin, which could partially account for the observed phenotypes. Confocal imaging also reveals a change in levels of occludin throughout early gastrula stage embryos.

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The OXA-2 and OXA-10 β-Lactamases Behave as Carbapenemases in *Acinetobacter baumannii*

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Class D β-lactamases are active-site serine enzymes that have been identified in the chromosome and plasmids of various bacterial species. Recently their clinical importance has arisen as they have become major determinants of resistance to carbapenems, antibiotics of last resort for the treatment of various life-threatening infections. These carbapenem-hydrolyzing class D β-lactamases (CHDLs) are predominantly spread in *Acinetobacter baumannii* (OXA-23,-24/40, -58) and less frequently in *Enterobacteriaceae* (OXA-48). The OXA-2 and OXA-10 class D β-lactamases, found sporadically in *Pseudomonas aeruginosa*, are regarded as narrow-spectrum enzymes and thus considered less clinically important. Here we demonstrate that although OXA-2 and -10 produce a narrow-spectrum antibiotic pattern when expressed in *Escherichia coli*, they behave as extended-spectrum β-lactamases and confer resistance to carbapenem antibiotics when expressed in *A. baumannii*. Additionally, their steady-state kinetic parameters with carbapenems are similar to those of well-recognized class D carbapenemases of *Acinetobacter*. Our results demonstrate that contrary to the currently accepted classification, OXA-2 and OXA-10 β-lactamases are in fact CHDLs.

Regulation of the Expression of the β-Lactam Antibiotic-Resistance Determinants in Methicillin-Resistant Staphylococcus aureus (MRSA)

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β-Lactam antibiotics are becoming less effective as therapeutics in treatment of staphylococcal infections as resistance to them increases. Resistance is mediated by a β-lactamase (encoded by *blaZ*) that hydrolyzes penicillins and a penicillin-binding protein (PBP2a, encoded by *mecA*), which is not modified effectively by these antibiotics. The 14.8-kDa MecI protein represses transcription of *mecA* and its three-dimensional structure reveals a dimer of two intimately intertwining dimerization domains, held together by a hydrophobic core, and two independent winged-helix domains, each of which binds a palindromic DNA-operator half site. The *mec* operator consists of a single 30-bp palindrome with two 15-bp half-sites. We provide insight into the interactions of the MecI protein and the *mec* operator by a gel retardation assay. Equilibrium sedimentation studies provided the dissociation constant for the monomer-dimer equilibrium. Fluorescence anisotropy experiments have shown that binding of MecI to *mec* promoter is different, depending on protein concentration. The anisotropy data has been fit using equations derived for different binding models, taking into account the possibility of binding of MecI as a monomer and/or dimer, taking into account the *in vivo* MecI concentrations, which were evaluated.

The Role of Lysine Binding Sites (LBS) within the Kringle Domains of Plasminogen in PAM Binding

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Plasminogen (Pg) conversion to the serine protease plasmin via host and exogenous factors results in the activation of the fibrinolytic system and ultimately the degradation of fibrin and extracellular matrices. Group A *Streptococcus* (GAS), a bacterial pathogen responsible for infections including rheumatic heart disease, impetigo, and necrotizing fasciitis, activates Pg via its bacteria-derived streptokinase (SK). This process is enhanced when M or M-like proteins (PAM), virulence factors found on the surface of GAS, bind to Pg. It has previously been shown that PAM selectively binds to one kringle domain, K2, found on Pg. Pg has 5 kringle domains (K1-K5) which are homologous triple-disulfide-linked peptide regions of approximately 80 amino acids in length. These kringle domains, excluding K3, bind to ω-amino acids such as lysine. The lysine binding site (LBS) is formed by creating three loci within the kringle. Using K1 numbering, kringles 1, 4, 5 D54 and D56 amino acids form an anionic loci, while K2 forms this anionic loci with D54 and E56. This binding is further stabilized by the hydrophobic loci formed by aromatic amino acids at positions 61 and 71. For most kringles, a cationic loci is formed by the presence of a basic residue located one position before the aromatic residue at position 71. It is our hypothesis that if D56, or in the case of K2 D54, is mutated to N the LBS will be abolished. Native human Pg and mutants for kringles 1, 2, and 4 were expressed in S2-Drosophila cells and purified through a sepharose-lysine column. Binding analyses were performed using surface plasmon resonance. K1 and K4 mutants bound comparably to PAM as that of native hPg; however, no binding was detected for the K2 mutant. Additionally, activities of native human Pg and the mutants were determined. Similar to the binding studies, when PAM was required for activation, minimal activity for the K2 mutants was observed. These studies serve to further elucidate the role of LBS in PAM binding.

ABSTRACTS: POSTER PRESENTATIONS

1. Conserved residues in TCR CDR loops and their role in influencing flexibility and binding

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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.

2. A New Class of 1,2,4-triazolo[1,5-a]pyrimidine Antibiotics Against *Enterococcus faecium*

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Enterococci are important pathogens responsible for serious infections and are a major contributor to nosocomial infections in the United States. The increase in antibiotic resistance among *Enterococci*, specifically to vancomycin, has become a major clinical and epidemiological problem. As a result, novel antibiotic templates are urgently needed for treatment of this problematic organism. We used the x-ray structure of a penicillin-binding protein (PBP), an enzyme involved in bacterial cell-wall assembly, to screen *in silico* 1.2 million compounds. The 1,2,4-triazolo[1,5-*a*]pyrimidine template emerged from this screening with *in vitro* antibacterial activity. The syntheses and structure-activity relationships (SAR) of this series are described in this study. The target compounds were rapidly obtained using the Biginelli reaction, by treatment of 3-amino-5-(benzylthio)-1,2,4-triazole with various aromatic aldehydes and acetoacetamide. The SAR requirement has been established for optimal antibiotic activity. All compounds were screened for antibacterial activity against the ESKAPE panel of organisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella species, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*), as well as *Escherichia coli*, followed by investigation of their metabolic stability and plasma protein binding. These analyses identified compound 7-[4-(dimethylamino)phenyl]-1,7-dihydro-5-methyl-2-[(4-cyanophenylmethyl)thio]-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide, which exhibited good antibacterial activity, metabolic stability, and reduced plasma protein binding.

3. The Tipper-Strominger Hypothesis and Triggering of Allostery in Penicillin-Binding Protein 2a of Methicillin-Resistant *Staphylococcus aureus*

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The Tipper-Strominger hypothesis stipulates that the active sites of bacterial transpeptidases (also known as penicillin-binding proteins, PBPs) have evolved to bind to the acyl-D-Ala-D-Ala termini of the stem peptides of the nascent peptidoglycan for their physiological roles in biosynthesis of cell wall. The hypothesis further stipulates that the mimicry by the backbone of β-lactam antibiotics of the acyl-D-Ala-D-Ala moiety accounts for inhibition of PBPs by these antibiotics, which has bactericidal consequences. We document that the same is true for the allosteric site of the PBP2a of methicillin-resistant *Staphylococcus aureus* (MRSA). Interactions of β-lactam antibiotics, as mimetics of the acyl-D-Ala-D-Ala moiety, at the allosteric site lead to conformational changes within the active site at a distance of 60 Å, which provides greater access to the active site for the antibiotics. This allosteric trigger lends itself to synergy among β-lactam antibiotics in killing MRSA.

4. Design and Evaluation of Novel Inhibitors of OXA-Carbapenemases Based on Benzimidazole-2-sulfonic Acids Scafoldings

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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 of OXA-23 carbapenemase, a class D carbapenemase, based on a 1-benzyl-1H-benzimidazole-2-sulfonic acid scafolding. A total of 1.2 million compounds were screened virtually against the OXA-23 structure. Subsequently, 100 of top scorers were tested *in vitro* for inhibition of class D carbapenemases enabling the identification of 1-benzyl-1H-benzimidazole-2-sulfonic acid as a promising lead compound with inhibitory constants in the 0.1 mM range. The application of this class of compounds in lead optimization resulted in a 100-fold improved inhibition of OXA-23 carbapenemase. Furthermore, the best candidate was successfully co-crystallized with the target β-lactamase, revealing the interaction between the active site of the enzyme and the inhibitor. Recently, this knowledge has been applied successfully in rational design of benzyl imidazole-2-sulfonic acid derivatives, resulting in new inhibitors showing activity against OXA-23 carbapenemase in the low micromolar range.

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5. Effects of Aging on the Peritoneum and Ovarian Cancer Metastasis

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Epithelial Ovarian Cancer (EOC) is the most fatal gynecological cancer. EOC, which often goes undetected until metastatic stages of the disease, follows a unique form of metastasis. Tumor cells are shed from the primary tumor into the peritoneal cavity. Metastasis progresses when EOC cells adhere to peritoneal tissue and invade through the surface layer of mesothelial cells into the submesothelial extracellular matrix, where they anchor and proliferate. The majority of women diagnosed with ovarian cancer are over 60 years of age and 90% are over 40. However, the role of aging in EOC metastasis has not been studied. Additionally, peritoneal tissues are understudied. Preliminary data on ultrastructure changes in the aged mouse peritoneum and the elevated incidence of EOC in the aged human population have led us to hypothesize that age-related changes in the peritoneum contribute to EOC progression. We aim to identify and characterize differences in peritoneal tissues of young, middle-aged and old mice and to elucidate mechanisms for how these age-of-host differences affect various stages of EOC peritoneal metastasis.

6. Selective Water-Soluble and Slow-Binding Matrix Metalloproteinase-2 and -9 Inhibitors that Cross the Blood-Brain Barrier

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Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that play important roles in physiological processes and pathological conditions. MMPs are generally present at low levels in healthy tissues, but their levels increase during disease, in particular the gelatinases (MMP-2 and MMP-9). SB-3CT, a selective and potent thiirane-based gelatinase inhibitor, has been shown to be effective in a number of animal models of disease, including cancer metastasis, stroke, traumatic brain injury, and diabetic wound healing. However, it is limited by its poor aqueous solubility (2.3 μg/mL) and extensive metablism. We addressed these issues by blocking the primary site of metabolism and introducing polar groups to achieve increased solubility. Among the inhibitors studied, *p*-aminomethyl-SB-3CT (referred to as ND-336) shows nanomolar potency and selectivity of inhibiting gelatinases and MMP-14, as well as excellent aqueous solubility (4.9 mg/mL). A pharmacokinetics (PK) and brain distribution study with ND-336 showed that the compound crossed the blood-brain barrier (BBB) and achieved therapeutic concentrations in the brain. The *p*-acetamidomethyl analog (ND-378) exhibits excellent selectivity in targeting MMP-2 and does not inhibit MMP-9 or MMP-14. A PK and brain distribution study indicated that ND-378 crossed the BBB and achieved therapeutic concentrations in the brain. As >98% of all known small-molecule drugs do not cross the BBB, both inhibitors are promising compounds in treatment of gelatinase-dependent neurological diseases.

7. 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. These remarkable viruses can vary in length from ~1 to 14 mm and are pleomorphic in shape. Mortality rates can be has high as 90% and to date there are no FDA approved vaccines or small molecules for treatment. EBOV harbors a genome of 7 proteins, the most abundantly expressed of which 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 (Scripps Research Institute) 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 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. Large Unilamillar Vesicle (LUV) assays have indicated that VP40 binds some, but not all, phosphatidylinositol (PIP) family members, indicating that the VP40-PIP interaction is not merely a charge-sensing interaction. ITC data on these PIP family members also suggests lipid headgroup specificity.

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8. NMR Spin-Couplings in the Solid State: New ssNMR Method to Investigate Saccharide Bound Conformation

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NMR Spin-couplings are commonly used to investigate the solution structures and conformations of molecules. This is especially true for *J*-couplings across three-bonds, so-called vicinal *J*-couplings, which have been shown to depend strongly on the torsion angle subtended by the coupled terminal atoms (Karplus relationships). One of the problems with *J*-coupling analyses in solution is that the molecule under scrutiny often assumes more than one conformation in the solution state, and thus the experimentally observed *J*-couplings represent a linear average of the *J*-couplings associated with specific conformers weighted according to their relative abundances in solution. In cases where complex conformational equilibria pertain, deconvolution of the experimental *J*-values into individual populations of conformers can be difficult if not impossible, although use of redundant *J*-values that report on the same torsional element can help. However, contrary to conventional wisdom, we show here that NMR *J*-couplings can be measured in the solid state, a new development that expands the applications of these NMR parameters substantially, including new opportunities to investigate ligand conformation bound to receptors. We describe herein our first efforts to demonstrate this possibility, using multiply-^{13}C-labeled saccharides as test compounds, and solid-state NMR, DFT and x-ray crystallography as experimental and theoretical tools to assist in data analysis. We then speculate on future developments of the technique.

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9. The Role of *Pseudomonas aeruginosa* AmpR in Beta-Lactamase Production

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Antibiotic resistance is a global crisis that renders many antibiotics obsolete. Bacteria have developed mechanisms of resistance capable of selectively degrading the β-lactam ring of a broad class of antibiotics. The AmpC β-lactamase, expressed in many Gram-negative bacteria including *Pseudomonas aeruginosa*, hydrolytically degrades the penicillins, cephems, and carbapenems and contributes to resistance. The transcription of the *ampC* gene is regulated by the repressor protein AmpR. This protein is comprised of a DNA-binding domain and an effector-binding domain. The DNA-binding domain of AmpR binds to the intercistronic region of DNA between the *ampR* and *ampC* genes, which have overlapping promoters that transcribe RNA divergently. The current hypothesis is that the transcription of *ampC* is dependent on the ligand bound to the effector-binding domain of AmpR. It has been hypothesized that several components of cell-wall recycling are able to bind to the effector-binding domain of AmpR. In the presence of β-lactam antibiotics there is a change in the effector ligand, leading to derepression of *ampC* transcription and production of b-lactamase. The mechanism of this process is currently unclear. We have cloned and purified both full-length and truncated versions of AmpR and these proteins will be used in ligand-binding assays to identify the activating ligand and elucidate the role of AmpR as a transcriptional regulator of AmpC.

10. Using Mutagenesis and Bioinformatics to Investigate Interactions Between EB1 and Actin

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Cytoskeletal components actin and microtubules (MTs) work in concert to achieve cellular organization, movement, and division. These processes require coordination and cross talk between MTs and actin. Although many proteins interact with each of these components separately, the process of coordination between MTs and actin is poorly understood. Previous lab data has indicated that EB1, a protein known to be a key regulator of the MT cytoskeleton, can also bind directly to actin *in vitro (Zhu et al, MBOC: 22, Abstract 207; 2011)*. We are using a combination of site directed mutagenesis and bioinformatic approaches to further characterize the EB1-actin interaction. More specifically, we have used EB1 sequence alignments to generate conservation maps of the CH domain of EB1 to identify conserved surface residues; we are generating mutations at these residues through site directed mutagenesis and testing their functional significance through a battery of assays. Study of these mutants *in vitro* and *in vivo* should provide insight into EB1-actin interaction and other aspects of EB1 function.

11. Determination of Structure-Activity Relationship for the Oxadiazole Class of Antibiotics and the Optimization of the Lead Compound

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A new class of antibacterials was recently discovered by our group from *in silico* screening against the penicillin-binding protein (PBP) 2a of methicillin-resistant *S. aureus* (MRSA). These compounds show excellent activity against Gram-positive organisms, including MRSA and vancomycin-resistant MRSA, and efficacy in the mouse peritonitis infection model. We report here the optimization of the lead compound to improve pharmacokinetic (PK) properties and *in vivo* efficacy. Over 370 derivatives of the lead structure were synthesized and evaluated against *S. aureus* to gain insight into the structure-activity relationship (SAR). These findings lead to a second generation of oxadiazoles with improved *in vivo* efficacy. A description of the oxadiazole library synthesis and their evaluation is presented here.

12. Tau Binding Laterally to the Microtubule Lattice Promotes Microtubule Stabilization

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Proper regulation of microtubule dynamics is essential for many cellular processes, such as polarized secretion and cell migration. Microtubules are very dynamic polymers that grow and shrink by addition and loss of tubulin dimers at the active plus end, respectively. Microtubule dynamics are intrinsically controlled by the nucleotide state of the tubulin dimers within the microtubule lattice. Specifically, GTP-tubulin dimers produce a microtubule structure that favors polymerization (growth), whereas GDP-tubulin dimers produce a microtubule structure that leads to rapid depolymerization (shortening). In vivo microtubule-associated proteins either promote microtubule stabilization or destabilization by regulating the intrinsic microtubule dynamic instability. Through a poorly understood mechanism the microtubule-associated protein, Tau, binds to microtubules and promotes microtubule stabilization. Here we used a combination of microtubule co-sedimentation assays, microscopy techniques and computational modeling to investigate the mechanism by which Tau stabilizes microtubules. Consistent with Tau binding along a single protofilament, we found that Tau binds with high affinity to Dolastatin-10 tubulin rings. Interestingly, we also found that Tau can laterally cross-link protofilaments to form stacked Dolastatin-10 tubulin rings. We also tested if Tau preferred to bind a specific microtubule structure by measuring Tau affinity for different tubulin nucleotide states, and found that that Tau prefers to bind GDP-microtubules (Taxol-stabilized) than GMPCPP-microtubules (GTP-like). Taken together these data suggest that Tau binding to the GDP-microtubule lattice promotes microtubule stabilization. To test this we computationally modeled Tau binding either laterally or longitudinally to the microtubule lattice, and analyzed microtubule dynamic instability. We found that Tau cross-linking adjacent protofilaments led to persistent microtubule growth by decreasing the number of catastrophe events, and increasing the number of rescue events. Tau binding along a single protofilament (longitudinally) did not significantly change the microtubule dynamic instability parameters. We speculate that other microtubule-associated proteins that promote microtubule stabilization may function through a mechanism of cross-linking protofilaments.

13. Proteomics of Single Three-Dimensional Colon Cancer Cultures: Characterizing a Model of Tumor Growth and Development

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Three-dimensional cell cultures (3DCC) are valuable model systems for cancer research. 3DCC are relatively inexpensive, high-throughput compared to animal models, while mimicking the growth, development, and characteristics of primary tumor samples. 3DCC grown with immortalized colon cancer cell lines, known as spheroids, retain chemical gradients of lactate and nutrients similar to primary colon cancer tumors, as well as pathophysiological gradients of proliferation, cell morphology, and cell death mechanisms. As the spheroids grow in a radial geometry, these gradients also develop radially from the center of the cell mass. The corresponding changes to the proteome are poorly understood and vary between cell lines. We are working to characterize colon cancer 3DCC model using mass spectrometry-based proteomics. Multiple pooled 3D colon spheroids have previously been examined via mass spectrometry, but analysis of single spheroids, or individual cell layers from single spheroids, has not been accomplished. Using an enzymatic treatment of trypsin, individual layers can be peeled from the spheroids and analyzed as sequential radial layers, thus examining distinct portions of the chemical and physiological gradients.

By analyzing the proteomes of single 3D spheroids and cell layers from single spheroids, we can obtain data on the particular growth and development mechanisms at work in the single culture, rather than a mean of conglomerates. Individualized data is useful for determining the effects of treatments, mutations, or other perturbations performed. Single-spheroid analysis lays the groundwork for deeper, more complete characterization of the 3D culture model, especially as it pertains to the chemical, biological, and morphological gradients found in both tumors and 3DCC. Using 3DCC, we can develop and test processes and sample work-up for analytical methods such as mass spectrometry-based proteomics for their effects on reproducibility, sensitivity, and robustness in a complex biological system, with speed, multiplicity, and relatively low cost.

14. A NMR-DFT Investigation of Exocyclic Acetate Ester Conformation in *O*-Acetylated Saccharides

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NMR spectroscopy is one the most important experimental tools for investigating the conformations of saccharides in solution. NMR yields a wide variety of observable parameters (*e.g.*, *J*-couplings, RDCs, spin-relaxation times) that can be correlated with structural properties through the use of mathematical models of the electronic state of the molecule. One of the computational tools available to produce these models is density functional theory (DFT). In the present work, NMR spectroscopic studies are supplemented by DFT calculations to determine the solution behaviors of C-O-C-H and C-O-C-C torsion angles involving exocyclic acetate ester substituents in *O*-acetylated saccharides. Through the use of redundant *J*-couplings that report on the same molecular torsion angle, and with use of a new software program, *MA'AT*, conformational populations can be identified in solution based solely on experimental input. This experimental strategy can be applied to any conformationally mobile element, including those found in protein side-chains and nucleic acids, provided that redundant *J*-couplings are available and measureable. The present work shows that the acetate ester group is most conformationally constrained when attached to C3 and less so at C2 and C6 of aldohexopyranosyl rings. This difference in behavior may be due to greater freedom of rotation at C6 (steric) and/or to non-covalent interactions involving the anomeric hydroxyl in proximity to C2. Interest in *O*-acetylated saccharides stems from the fact that several pathogenic bacteria display saccharide *O*-acetylation, and some of the enzymes involved in the *O*-acetylation of bacterial polysaccharides are considered potential drug targets. The design of inhibitors of these enzymes might benefit from a detailed understanding of exocyclic acetyl group conformational equilibria and dynamics.

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15. Electrophysiological Investigation of *N*-Methyl-D-Aspartate Subunit Specific Conantokins on the Intrinsic Properties of Cortical Neurons

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The *N*-Methyl-D-Aspartate Receptor (NMDAR) is expressed throughout the central nervous system and is involved in development, plasticity, learning, and memory. However, continued activation of the receptor leads to excitotoxicity, which is the hallmark of many neuropathologies, including ischemic stroke. The NMDAR exists as a heterotetrameric voltage and ligand-gated calcium channel, comprised of the glycine binding GluN1 subunit arising from splice variants (a-h), and a glutamate binding GluN2 subunit that is encoded by four separate genes (A-D) that varies in its expression both spatially and temporally. Antagonism of the NMDAR using subunit selective peptides naturally occurring in *Conus* snail venom, known as conantokins, have been studied through calcium imaging and electrophysiology of dissociated cultured neurons in wild type, GluN2A$^{-/-}$ and GluN2B$^{-/-}$. In addition to the Glu2A$^{-/-}$ and Glu2B$^{-/-}$ mice, Glu2C$^{-/-}$ and Glu2D$^{-/-}$ mice will also be utilized. Specifically, conantokin-G has been established as a highly selective antagonist for the GluN2B subunit. As the involvement of GluN2B subunit has been demonstrated in several neurological pathologies, the conantokins have been investigated for their highly selective subunit inhibition rather than high-affinity channel blockers. As a natural extension of the labs previous studies, my project will use a whole cell patch-clamp technique in brain slices to obtain electrophysiology recordings to further explore the effect and mechanism in which these peptides antagonize NMDAR. In addition to studying the peptides effect on the subunit knockout mice, another goal of the project will be to compare the intrinsic properties of two classes of neurons in the cortex of wild type, ischemic, and peptide-treated ischemic mice and rats. Since the core stroke region experiences irreversible damage, the outlying penumbra region will be studied to determine the extent to which damage may be mitigated after application of these antagonistic peptides. Whole cell patch-clamp recordings from live brain slices will further elucidate any neuroprotective effect of the conantokins after an ischemic insult.

16. Using Protein Biosensors to Detect Changes in Translation Rate in *E. coli*

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Anfinsen's principle asserts that all of the relevant information required for a protein to fold into its final native state is encoded by the primary amino acid sequence. More recently it has been shown that many proteins begin to fold while still tethered to the ribosome. This cotranslational folding can influence the pathway a protein takes to its final native state *in vivo*. Currently, however, there is limited understanding of the cellular factors that control translation rate and how these might modulate cotranslational folding. To observe the effects of translation rate, synonymous codon mutations can be introduced at the mRNA level. While most amino acids are encoded by multiple synonymous codons, they are not used with equal frequencies. Rare codons are generally translated more slowly than common codons and can cause pauses in translation.

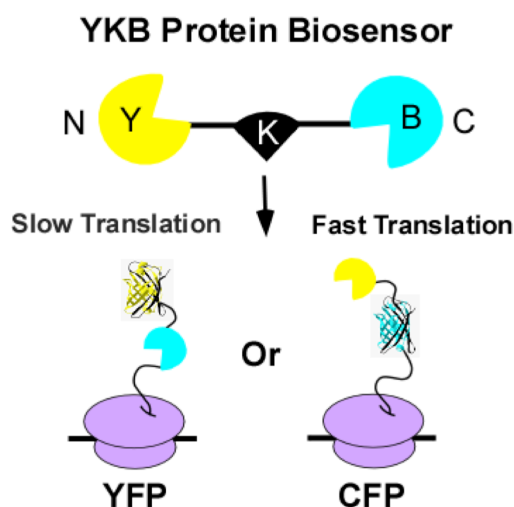

Figure 1 - YKB Protein Biosensor

The fluorescent protein biosensor, YKB, developed was designed to measure changes in translation rate *in vivo*. Synthesis of YKB from N to C terminus can produce one of two mutually exclusive folded states, YK or KB. The ratio of YK (yellow) to KB (cyan) fluorescence can be used to gauge changes in translation rate *in vivo* (Figure 1).

Current focus has been centered on determining how many single rare leucine codons are required to produce a detectable change in the YK to KB ratio. Leucine was chosen because its codon usage has the largest dynamic range. Several single leucine libraries have been constructed in the C terminus of YKB using site directed mutagenesis. These mutants were expressed in *E. coli* and their fluorescence emission spectra were measured in order to determine how the YK to KB ratio is changing. Future plans will focus on determining the extent to which growth conditions and other rare codon changes alter the YK to KB ratio.

17. Modified Zinc(II)-bis(dipicolylamine) Probes for Enhanced Molecular Imaging of Cell Death

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Zinc(II)-dipicolylamine (Zn-DPA) molecular imaging probes have been investigated for their ability to selectively recognize phosphatidylserine (PS), an anionic phospholipid that becomes exposed to the outer leaflet of the plasma membrane during cell death processes. A major weakness preventing further development of Zn-DPA affinity ligands as imaging agents is their relatively low membrane binding affinity. Through modification of the Zn-DPA scaffold, secondary non-covalent interactions that take place at the membrane surface and result in enhanced membrane binding affinity have been introduced. An iterative series of synthesis and screening processes has led to the identification of several lead compounds, one of which has been converted into a deep-red fluorescent probe for optical imaging. This probe was further evaluated in a series of comparative membrane binding assays, cell microscopy studies, and finally used for in vivo imaging of a rat prostrate tumor model, where it showed enhanced molecular imaging of cell death.

18. Interaction of Group A Streptococcal M or M-like Proteins and Host Fibrinolytic System in mediating GAS Virulence

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The virulence of the strict human pathogen, *Streptococcus pyogenes*, a Group A streptococcus (GAS), is determined by M or M-like (e.g., PAM) proteins and streptokinase (SK) in the bacterium, and an intact functional host fibrinolytic system (HFS). SK is a three domain protein with α, β and γ domains. While amino acid sequences of α and γ domains are conserved across SKs from different streptococcal strains, β domains exhibit sequence diversity. Phylogenetic analysis of amino acid sequences from the β-domain of SK from several strains of GAS, groups SK into cluster 1 and cluster 2. Cluster 2 is subdivided into 2a and 2b. GAS strains of cluster 2b are nearly always isolated from skin infections. They secrete SK subtype 2b and express PAM on their surface (e.g. AP53). Whereas cluster 2a strains are isolated from upper respiratory tract infections, secrete SK subtype 2a, and express M1 on their surface. PAM on cluster 2b strains binds to human Plasminogen (hPg) directly with high affinity *via* the a1a2 repeat domains at the N-terminus of PAM and B1B2 repeats, at the N terminus of M1 of cluster 2a strains, binds to human fibrinogen (Fg) which in turn binds to hPg. Subsequently, SK secreted by the corresponding GAS strain non-proteolytically activates hPg into a broad spectrum serine protease plasmin, which aids in further dissemination of GAS into deeper tissues. Due to the difference in the way the two strains interact with HFS and hence a different mode of hPg activation, we hypothesize that hPg binding and activation mechanisms of PAM and M1 containing GAS strains differ in their requirements for Fg and are coordinated with the SK subtype they secrete. Here we aim to study the consequences of altering M or M-like proteins on hPg binding and activation as well as their effect on virulence using a mouse model. In our experiments presented here, GAS strain AP53 a prototype of cluster 2b, was used to make changes in PAM or its domains. GAS strain SF370, belonging to cluster 2a, was used to obtain coding sequences for M1 or its domains. GAS strains AP53 (PAM→M1) where PAM in AP53 was replaced by M1 of SF370 and AP53 (PAM→M1 (B1B2→a1a2) which is AP53 (PAM→M1) strain but the B1B2 repeats of M1 were replaced by a1a2 repeats of PAM were developed. The ability of these new strains to bind and activate hPg was tested. We anticipate that our work will allow us to study different hPg binding modalities on GAS in their non-native background.

19. Chemical Synthesis of Nested Fragments of High-Mannose *N*-Glycans

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Cell-surface oligosaccharides appended to proteins and lipids are key binding epitopes in many critical biological processes, including bacterial infection, cell development and the immune response. Understanding these processes at the molecular level requires access to a wide range of oligosaccharides of known structure to support investigations of enzyme substrate binding and specificity, to screen for carbohydrate binding proteins, and to develop assays for enzymic activity. These oligosaccharides are currently unavailable in the commercial sector, thus impeding progress in this field. To address this deficiency, the proposed project, funded by a new initiative at NIH, focused on the synthesis of oligosaccharides derived from the parent high-mannose oligosaccharide (14-mer) that is transferred *en-bloc* to polypeptides during translation. Over thirty high-mannose oligosaccharides ranging in size from tri- to hexasaccharides were prepared. Chemical routes were developed to synthesize each oligosaccharide in mg or greater quantities and in high purity (>98%), as determined by NMR, high-resolution mass spectrometry, and high-pressure liquid chromatography. Samples will be used by the NIH/NCI to construct glycan arrays to screen for carbohydrate binding proteins, and for other biochemical or biomedical applications. Efforts are underway to expand this chemical space to all remaining nested fragments of the native 14-mer, including oligomers containing up to thirteen residues.

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20. The Role of the Human Fibrinolytic System in the Evolution of Virulence in *Streptococcus pyogenes*

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Group A Streptococcus (GAS) is a highly human-specific pathogen responsible for a plethora of clinical manifestations. GAS survival within its host is dependent on the interplay of a variety of virulence factors that act in adherence, inhibition of phagocytosis, dissemination, and the production of exotoxins. Highly virulent strains have been shown to interact directly with the host fibrinolytic system primarily through surface exposed M and M-like proteins and secreted streptokinase (SK). In such cases, human plasminogen (hPg) in complex with SK is accumulated on the cell surface directly through receptors or indirectly through fibrinogen binding, enabling the spread of the bacteria from the initial site of infection. The expression of M and M-like proteins and other virulence components are controlled through the stand-alone regulator, Mga, as well as the two component system CovR/S. Here we explore coordination of these regulatory systems at various stages of infection, particularly their effect on evading the host innate immune system and engaging with the fibrinolytic system. This study makes use of the highly invasive, skin tropic strain, AP53, which contains a naturally occurring CovS mutation. This mutation was shown to enhance virulence through down regulation of the secreted cysteine protease, streptococcal pyrogenic exotoxin B (SpeB), as well as through direct transcriptional influence on genes of the Mga regulon. This activity was shown to have important consequences on the cells ability to accumulate hPg as well as their ability to resist opsonization and phagocytosis. Alteration of this mutation to wild-type CovS significantly decreased the ability to bind host complement inhibitors, resulting in decreased survival of bacteria in neutrophils as well as overall reduction in lethality in the humanized mouse model.

21. Direction-specific Translocation of Pertactin Through a Nanopore

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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* versus *in vitro* 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 a novel, single-molecule method to allow direction specific translocation of pertactin through a nanopore. This will allow for us to directly test the impact of vectorial folding during secretion, as well as the impact of secretion direction.

22. Structural Features for Optimal Human Plasminogen Binding and Plasminogen Activation by Group A Streptococcal Surface Receptor PAM

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Group A streptococcus (GAS) is a highly specific human pathogen responsible for a broad spectrum of diseases ranging from non-invasive pharyngitis and skin infections to life-threatening invasive illnesses like bacteremia, streptococcal toxic shock syndrome, necrotizing fasciitis, as well as infectious sequelae such as acute rheumatic fever[1].

GAS produces a variety of surface-bound and secreted virulence factors which are known to contribute to the severity of infection. Plasminogen binding M and M-like proteins (PAM) have long been recognized as major virulence factors of GAS. PAM, a dimeric helical coiled-coil surface receptor can capture host plasminogen (Pg) directly and enhance the activation of plasminogen to its serine protease, plasmin, which could potentially generate a proteolytic bacterial surface leading to GAS virulence[2, 3]. Pg binding is attributed to two characteristic tandem repeat regions termed a1 and a2 located in the N-terminal variable region of PAM. Since PAM is dimeric and it is possible that the ability of PAM as a virulence factor partly relies on its ability to dimerize and adopt a coiled-coil motif, we wanted to determine if dimerization of PAM is an absolute requirement for tight Pg binding.

Recently, we have determined minimum number of residues required for PAM dimerization. We also identified five amino acid residues (R^{101}, H^{102}, E^{104}, R^{114}, H^{115}) from the a1a2 domain of PAM which mediate high-affinity Pg binding and demonstrated that mutagenesis of these residues in the GAS genome resulted in reduced virulence, similar to PAM gene knockout. We, therefore, conclude that the presence of these five residues is more important for high-affinity Pg binding than its apparent oligomerization status. Although all monomeric and dimeric constructs bind to Pg with similar high affinity as PAM ($k_D \sim 1$nM), they differ in their Pg activation potential with dimeric constructs being more active. Using truncation variants of full-length PAM, we showed that there is a positive correlation between increasing length of coiled-coil in PAM and increased potential for Pg activation. We also identified the domain boundary which is required to form a fully functional active PAM.

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23. Measuring Local Translation Rates and their Correlation with Co-translational Folding

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Traditionally, protein folding is studied *in vitro* by serially diluting the denatured test protein out of the concentrated denaturant solution. However, inside cells, a nascent chain folds co-translationally, i.e., the N-terminal end of the peptide can start folding while the C-terminal end is still being translated. Co-translational folding is controlled by a variety of parameters that can modulate local translations rates. A few such parameters are relative occurrences of particular codons in the organism, abundance of tRNA, and mRNA and nascent chain sequences that stall and pause the ribosome. Currently, the interplay between these parameters, and their net effects on translation rate and co-translational folding, are poorly understood.

Yellow-Black-Blue (YKB) is a fusion protein of two fluorescent proteins: yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP). YKB was developed as a translation rate biosensor (*1*) . Even though the two domains, YFP and CFP, have same equilibrium Gibbs free energy ($\Delta G_f^{o\prime}$) *in vitro*; the protein folds vectorially *in vivo*, with the YFP domain (or the YK-part of YKB) folding first before the KB domain (*1*). The ratio of yellow to blue fluorescence (YK/KB) gives an indirect measure of the translation rates. We are modifying segments of YKB to measure the effects of the translation controlling parameters, on the YK/KB fluorescence ratio. We plan to apply the sensitivity of YK/KB to screen a random library at the 3'-end of YKB biosensor. This will further our studies in correlating local codon or amino acid sequences with translation rates. We will use fluorescence activated cell sorting to sort *E. coli* expressing the YKB tail end library depending on the varying YK/KB ratios.

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24. Streptolysin S Enhances Programmed Cell Death and Inflammatory Responses in Epithelial Keratinocytes During Group A Streptococcal Infection

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Pathogens use a variety of strategies to manipulate host cell death, survival, and inflammatory pathways to enhance their own replication and survival. Although the means by which *Streptococcus pyogenes* influence signal transduction in human cells has not been well characterized, an understanding of this interaction is attractive, as it may reveal new therapeutic options for treatment or prevention of infection. *S. pyogenes* is responsible for an estimated 18 million cases of severe disease each year, approximately 500,000 of which result in death. Despite several decades of research, therapeutic options for severe infection remain limited, and even the most effective vaccines developed to date can protect against less than 20% of the serotypes that have been identified for this pathogen. One of the primary weapons in the arsenal of *S. pyogenes* is Streptolysin S (SLS), which is encoded by the SLS associated gene cluster (*sag*). In addition to its role as a cytolysin, recent publications have indicated that SLS may also influence host cell signal transduction at various points during the infection process. We have utilized wild-type and SLS-deficient *S. pyogenes* to investigate the role of SLS in an *in vitro* human epithelial infection model. Using an antibody array-based approach, we identified numerous SLS-dependent changes in several host cell signaling pathways related to the regulation of cell death and inflammatory responses. Our results demonstrate that Streptolysin S manipulates the activity of several key cellular signaling proteins including AKT and NFκB to induce programmed cell death and drive the production of inflammatory signaling proteins in infected host cells. We anticipate that subsequent studies based on these data will allow us to develop a model detailing how SLS modulates programmed cell death and inflammatory responses in infected epithelial cells to enhance pathogenesis and progression to severe disease.

25. Quantitative Interpretation of Redundant J_{HH}, J_{CH} and J_{CC} in Aldopentofuranosyl Rings: Conformational Populations from *MA'AT* Analysis

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The inherent conformational flexibility of aldofuranosyl rings confers important functional properties to the nucleic acids that contain them. Previous experimental studies, particularly those involving x-ray crystallography, have suggested that these rings assume a north/south (*N*/*S*; C3'-*endo*/C2'-*endo*) two-state conformational equilibrium in solution. NMR ^{1}H-^{1}H spin-couplings (*J*-couplings) and nuclear Overhauser effects (NOEs), and to a lesser degree, residual dipolar couplings (RDCs), have been used to support these conclusions. However, the NMR *J*-couplings, J_{CH} and J_{CC}, show strong sensitivities to ring conformation and represent a largely untapped source of information to assign/test aldofuranosyl ring conformational assignments in solution, particularly when more complex equilibria are possible. To test this application, the ten envelope (non-planar) and planar forms of methyl 2-deoxy-β-D-*erythro*-ribofuranoside (A) and methyl β-D-ribofuranoside (B) were geometrically optimized using density functional theory (DFT) at the B3LYP/6-31G* level of theory under "solvated" conditions, and all available J_{CH} and J_{CC} values in these rings were calculated using a DFT basis set specifically designed for accurate (±5%) *J*-coupling calculations. *J*-Couplings that exhibited strong dependencies on ring conformation were chosen for further study using a new software program, *MA'AT*. As shown recently by the Serianni group in conformational analyses of *O*-glycosidic linkages in oligosaccharides, the ensemble of redundant *J*-values in aldofuranosyl rings could be treated quantitatively using *MA'AT* to yield conformational equilibria for these structures. These equilibria, which are exclusively based on experimental data, were compared to conformational equilibria based on solvated molecular dynamics (MD) simulations in efforts to validate the conformational predictions made from the latter. Similar studies of other biologically important aldofuranosyl ring configurations (*e.g.*, *arabino*, *xylo*) are underway.

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26. Optical Imaging of Brown Adipose Tissue in Living Mice

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Brown adipose tissue (BAT) plays a key role in energy expenditure and heat generation and is a very promising target for diagnosing and treating obesity, diabetes and related metabolism disorders. While several nuclear and magnetic resonance imaging methods are established for detecting human BAT, there are no convenient protocols for high throughput imaging of BAT in small animal models. Here we disclose a simple but highly effective method for non-invasive optical imaging of BAT in mice using a commercially available formulation that consists of lipophilic, deep-red fluorescent probe, SRFluor680, encapsulated within micelles comprised of 1,2-dipalmitoyl-*sn*-glycero-phosphatidylethanolamine-polyethyleneglycol-2000 ($DPPE-PEG_{2000}$). Whole-body fluorescence imaging of living mice shows extensive probe accumulation in the interscapular BAT. Ex vivo analysis of BAT and intraperitoneal white adipose tissue taken from the same animal confirm 5-fold selectivity for the BAT. Additional imaging studies indicate that SRFluor680 uptake is independent of BAT metabolic state. Multimodal PET/CT and planar fluorescence/X-ray imaging of the same living animal show co-localization of the BAT mass signal reported by the fluorescent probe and the BAT metabolism signal reported by the PET agent, ^{18}F-FDG. The results support a new dual probe molecular imaging paradigm that allows separate and independent non-invasive visualization of BAT mass and BAT metabolism in a living subject.

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27. Abrogation of Plasminogen Activator Inhibitor-1-vitronectin Interaction Ameliorates Acute Kidney Injury in Murine Endotoxemia

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Sepsis-induced acute kidney injury (AKI) contributes substantially to the high mortality and morbidity in patients. Although the pathogenesis of AKI occurring during the course of sepsis is poorly understood, it is well accepted that plasminogen activator inhibitor-1 (PAI-1) and the extracellular matrix protein, vitronectin, are involved in the development of AKI. However, the functional cooperation between PAI-1 and vitronectin in septic AKI has not been well studied. To address this issue, mice were utilized, lacking either PAI-1 (PAI-$1^{-/-}$), or expressing a PAI-1-mutant (PAI-$1^{R101A/Q123K}$) in which the interaction between PAI-1 and vitronectin is selectively abrogated, while other functions of PAI-1 are retained. It was found that both PAI-$1^{-/-}$ and PAI-$1^{R101A/Q123K}$ mice are associated with decreased renal dysfunction, apoptosis, inflammation, and ERK phosphorylation as compared to wild-type mice after LPS challenge. Also, PAI-$1^{-/-}$ mice showed attenuated fibrin deposition in the kidneys. Furthermore, a lack of PAI-1 or PAI-1-vitronectin interaction in these mice was found to be associated with an increase in activated Protein C (aPC) activity in plasma. Using purified mouse recombinant aPC, PAI-1, and vitronectin, the inhibition of mouse aPC activity by mouse PAI-1 and vitronectin was validated. Taken together, our findings demonstrate that PAI-1 through its interaction with vitronectin exerts multiple deleterious mechanisms to induce AKI. Therefore, selective targeting of the PAI-1-vitronectin interaction in kidney represents an appealing therapeutic strategy for the treatment of septic AKI by not only altering the fibrinolytic capacity but also regulating PC activity during endotoxemia.

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28. Identification of Conserved Metabolic Profiles in Breast Tumors from Transgenic Mouse Models

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Novel breast biomarkers with prognostic and therapeutic value include metabolites that are differentially expressed in breast tumors. The goal of this study was 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. The Wnt1 tumors had a significantly different metabolomic profile than did the other tumors analyzed. These differences may reflect changes in proliferation and lipid synthesis. In contrast, the PyMT, PyMT-DB, Her2/neu, and C3-TAg tumors did not segregate from each other.

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.

29. Profiling of Lipid Metabolism in Colon Carcinoma 3-Dimensional Cell Culture with High-Resolution FT-ICR Imaging Mass Spectrometry

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Due to their increased complexity over monolayer culture, relative ease of manipulation and low cost compared to animal models, 3D cell culture is an ideal system for examination of disease states. Growing the human colorectal carcinoma (CRC) cell line HCT 116 in a 3D system, we have been able to obtain tumor mimics, also called spheroids, which share pathophysiological characteristics with tumors seen *in vivo*. Imaging Mass Spectrometry (IMS) can be used to probe the spatial distribution of proteins, peptides and lipids without the use of labels**. This system allows us to investigate changes that occur in the spheroids in response to drug treatment or other standard cell culture perturbations. In this study, HCT116 tumor mimics were treated with the anti-malarial drug Chloroquine, a lysosomal inhibitor and potential chemotherapeutic. Chloroquine interrupts normal lysosome function by raising the intralysosomal pH and inhibiting lysosome-mediated breakdown pathways. This mechanism of action could inhibit cancer cells from using alternative metabolic pathways to survive in otherwise hostile tumor microenvironments and render these drug-resistant cells susceptible to treatment with other common chemotherapeutics. IMS was performed on Chloroquine-treated spheroids at 6, 12 and 24 hours post-treatment with high-resolution FT-ICR mass spectrometry. With this method we were able to track both the distribution of Chloroquine as it was taken up by the cells in the spheroids, as well as changes in lipid profiles between control and treated spheroids as Cholorquine was metabolized. Using a novel isotopic pattern search algorithm, the IMS data was searched against the Human Metabolome database (HMDB) allowing for the putative identification of hundreds of lipids and small molecules present in the spheroids. With these search results we were able to detect distinct lipid expression and metabolism profiles between different microenvironments present in the spheroid models and monitor the response to Chloroquine treatment over time.

30. Pancreatic Stem Cell Function Through Matrix Metalloproteinase-3

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers and is known for having a poor survival rate. Pancreatic cancer only accounts for 3% of cancers in the US, but accounts for 7% of total deaths caused by cancer. While the five year survival rate has increased three-fold since the 1970s, the five year survival is still only a mere 5%. One of the hallmarks of PDAC is the expansion of the stromal cells in the microenvironment that surround the epithelium to form a dense stromal layer that accounts for ~90% of the tumor bulk. One function of the microenvironment during normal development is to release essential cues that control the behavior of epithelial cells, including stem and progenitor cells. We previously found that matrix metalloproteinase-3 (MMP3) is a regulator of Wnt signaling in mammary stem cells and is required for the mammary stem cell population. MMP3 inactivates Wnt5b, a noncanonical Wnt ligand that inhibits canonical Wnt signaling, in a nonproteolytic manner via its hemopexin (HPX) domain. Because of the important implications of this finding and the lack of good microenvironment markers of PDAC progression, we wanted to see if MMP3 also has an impact on stemness in the pancreas and in PDAC progression. We collected the pancreas from transgenic MMP3 knockout or heterozygous mice and analyzed the stem cell markers CD133+/CD44+ by flow cytometry. We are investigating if mice lacking MMP3 have a reduced pancreatic stem cell population when compared to mice heterozygous for MMP3. For future experiments, we have optimized the pancreasphere protocol and currently are investigating if MMP3 is required for pancreasphere formation and if MMP3 overexpression elevates pancreatic stem cell function, as is seen in mammary epithelial stem cells.

31. Negative Regulation of Pin1 Enzyme Activity via Interdomain Interactions

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Maintenance of the cell cycle relies on interactions between proteins containing Ser/Thr-Pro motifs. Phosphorylation of these Ser/Thr-Pro motifs alters strength of specific protein-protein interactions, thereby regulating the signaling networks of the cell cycle. Explaining how Ser/Thr-Pro phosphorylation leads to altered protein-protein interactions remains an ongoing challenge. One explanation involves conformational changes catalyzed by the two-domain peptidyl-prolyl isomerase (PPIase), Pin1. Pin1 specifically recognizes pSer/pThr-Pro motifs, and accelerates the cis-trans isomerization of the pSer/pThr-Pro imide linkage; in turn, this accelerates local conformational changes that alter signaling protein interaction properties. Pin1 targets include signaling proteins pertinent for cancer and Alzheimer's disease (e.g. p53, RNA Pol II, Cdc25C, tau), and revealing its mechanism is of great interest for inhibitor design.

A particularly vexing question has been: "How does Pin1 use its two domains for its function?" These include a WW domain for binding, and a larger catalytic PPIase domain. If and how their how their actions are coordinated is not yet understood. Accordingly, we have investigated these interdomain interactions via a variety of solution NMR studies (2-d exchange spectroscopy, spin-relaxation, and Residual Dipolar Couplings) and computational methods. We find that Pin1 interdomain interactions weaken upon substrate interaction, and that interdomain contact exerts negative control on the PPIase domain activity. This negative regulation via interdomain contact is reminiscent of other cell-cycle proteins consisting of flexibly linked modules. Negative regulation is a new perspective for Pin1 function; its implications for its signaling roles and protein interactions via pSer/pThr-Pro motifs will be discussed.

32. Connections Between Altered Translation Rate and Co-translational Folding in *E. coli*

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Translation rate in the cell is not uniform and can be altered by mRNA and protein sequence. Sequence motifs in mRNA and nascent peptides have been shown to cause pauses in translation. Sequences that can cause pauses in translation include the SecM stall sequence, and more recently characterized, polyproline and Shine-Dalgarno motifs. Translation rate can also be altered without changing the amino acid sequence, by altering synonymous codon usage. Clusters of rare codons can cause pauses in translation. YKB is a three-domain biosensor developed to sensitively probe changes in translation rate. It assumes alternative folded structures based on how fast the third domain is translated. It has been shown that synonymous mutations that modify codon usage alter the partitioning of YKB into its two alternative native structures. Here, non-synonymous mutations known to alter translation rate were inserted into the C-terminus of YKB and the folded state was biased to one alternative state. Additionally, we use YKB to investigate how different nutrient compositions in growth media affect translation rate and YKB folding in *E. coli*.

33. ZNF217 Interacts with the Tumorigenic Isoform of Pyruvate Kinase PKM2

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The zinc-finger transcription factor ZNF217 is found to be overexpressed in many tumors, and its overexpression correlates strongly with poor prognosis. Pyruvate kinase isoform M2 (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. In recent years, PKM2 has also been shown to also function as a histone kinase, highlighting a possible role in gene regulation.

Here we show that ZNF217 interacts with an acetylated form of PKM2, which has previously been shown to translocate to the nucleus as a dimer. Upon expression of the histone acetyltransferase p300, which acetylates PKM2, dimeric PKM2 is found in the nucleus. Only under these conditions is PKM2 binding to ZNF217 observed. Our current efforts focus on further elucidating the underlying mechanisms of this interaction, and in the future, we will investigate the effects on chromatin remodeling caused by this interaction.

34. Discovery of Ceramide 1-Phosphate Binding Mechanisms

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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, and macrophage chemotaxis. It also serves as a pro-inflammatory signal and has been implicated in tumor metastasis. However, little is known about the specific role of C1P and its binding mechanisms in these processes, and the need for further study is very evident. My project aims to explore the proteins that specifically bind to C1P and not other lipids, explore the difference between two RxRH recognition binding motifs (C2, PH and PX domains) and ultimately design and implement a biological sensor of C1P. This will provide cutting edge information for scientists globally, as lipid abnormalities contribute to a significant number of diseases worldwide. We have started immunoprecipitation assays with lipid-coated beads, which will be coupled with mass spectrometry to determine C1P specific binding proteins. We have also performed several confocal microscopy experiments using fluorescently labeled C1P. Our short-term goal is to show that the fluorescently labeled C1P behaves the same as non-labeled C1P in the cell, and to investigate C1P dynamics using cellular organelle markers, fluorescence recovery after photpbleaching (FRAP), and quenching experiments.

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

Ricardo Josué Romero Moreno

Bone metastases are incurable. 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. 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.

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. 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.

Due the limitations of current methodologies and lack of exploration to improve them, our unique interdisciplinary perspective to attack this problem will result in high impact publications in the fields of cancer and bone behavior.

36. Investigating Membrane Binding Properties of Ebola and Marburg Virus Matrix Protein- VP40

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The Ebola virus (EBOV) and Marburg virus (MARV) are from the family Filoviridae that cause severe hemorrhagic fever with high mortality rates in humans. These are filamentous lipid-enveloped viruses that extract their lipid coat during replication from the host cell they infect. Even though a significant amount of information on membrane association are available for EBOV VP40 almost nothing is known about MARV VP40 membrane interactions. Sequence comparison of EBOV VP40 (strain Zaire) and MARV VP40 reveals a similarity of 29%. It is highly possible that interaction between the host plasma membrane and the MARV VP40 is driven mainly through electrostatic interactions between certain cationic residues of MARV VP40 and anionic phospholipids in the inner leaflet of the plasma membrane similar to EBOV VP40. Marburg VP40, which is shorter in length by 23 residues compared to EBOV VP40 shows a notable difference at regions purported to mediate lipid binding of EBOV VP40. Based upon the hundreds of lipid-binding proteins studied to date, it is reasonable to suspect that this sequence dissimilarity may contribute to different membrane binding properties between the two proteins. Understanding of their lipid binding properties will be very useful to understand how these viruses differentially propagate and to better understand VP40 as a potential therapeutic target. We are currently using liposome co-flotation assays and MLV binding assays to discover the different membrane targeting properties of MARV VP40 and to identify the molecular basis of MARV VP40 lipid selectivity that regulates membrane association and subsequent budding from the host cell.

37. Molecular Profiling of Aggressive Breast Cancer in Kenyan Patients

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Breast cancer rates of incidence and mortality vary significantly between different nations and racial groups. African nations have the highest breast cancer mortality rates in the world, even though the incidence rates are below those of many other nations. In Kenya, breast cancer tumors are often highly aggressive at presentation and occur at a significantly earlier age (as early as the teens and 20s), relative to North American women. In the United States, non-Hispanic white women have the highest incidence of breast cancer, but African-American women have the highest mortality. These striking racial disparities are due not only to inequities in screening and treatment but also to variations in the rates of aggressive breast cancer. Differences in disease progression suggest that aggressive breast cancer tumors may harbor components of a unique molecular signature that result in racial disparities. We aim to identify molecular signatures with high prognostic value from tumor samples of patients with aggressive breast cancer with poor prognosis. We hypothesize that changes in the DNA, RNA, and post-translational protein regulation contribute to aggressive disease. We used samples from over 100 Kenyan breast tumor tissue samples to characterize the tumors from this patient population,. We prepared tissue microarray sections and stained for many clinical breast cancer markers including HER2, Estrogen and Progesterone Receptor, as well as some lymphocyte markers. To identify signatures of these aggressive breast cancer growth and metastasis, we analyzed gene expression, genome sequence, proteomics, and pathology by isolating DNA and RNA from the Kenyan tumor samples. Our data will be foundational in understanding how these lethal breast tumors from Kenyan patients differ from less aggressive tumors and will enhance our ability to diagnose and eliminate outcome disparities in breast cancer patients.

38. The Role of ZNF217 in the Development of Breast Cancer Chemoresistance

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Breast cancer is the second leading cause of cancer related deaths with an estimated 232,340 new cases and 39,620 deaths in 2013. Despite 93% of 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 has become an area of active interest. One protein known to play a role in the development of therapeutic resistance is the transcription factor ZNF217. This transcription factor is overexpressed in breast cancer and has the ability to increase self-renewal, invasion, metastasis and chemoresistance.
Because the majority of the patient population receives adjuvant therapy, it is critical to identify therapeutics that can overcome resistance, eventual metastasis, and death. One such drug that has shown promising results in human cancer patients is the microtubule inhibitor, epothilone B. Administering a combination therapy of epothilone B, adriamycin and cyclophosphamide (EAC) to mice overexpressing Znf217 decreased tumor volume and increased percent survival when compared to control mice. Although tumor volume was not significantly increased during the EAC treatment regimen in control mice, mice overexpressing Znf217 showed a significant increase in tumor volume after 21 days during EAC treatment. This confirmed that mice overexpressing Znf217 were more resistant to the effects of the EAC chemotherapy regimen. Previous work suggested the value of using ZNF217 as a prognostic indicator. Future work will compare therapeutic response in NOD/SCID mice using patient-derived tumor xenografts with high and low levels of ZNF217 expression. Utilizing animal models that overexpress ZNF217 will allow us to identify combination therapies that are able to overcome resistance. In addition, these studies will provide an ideal model system to search for mechanistic insight into the process of developing therapeutic resistance.

39. Exploring the Implications of Protein Flexibility in the Antibacterial Target, DNA Gyrase B

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Optimizing the selectivity of antibiotics is important for curbing antibiotic resistance. Current methods for optimizing selectivity are based on structure activity relationships that assume the protein antibacterial target is static. But many antibacterial targets bind substrate with non-conserved flexible loops, which offer new opportunities for inhibitor selectivity. Yet, our current understanding of how to target these flexible regions is lacking.

To address this broad issue, we are investigating a model system, DNA Gyrase. DNA Gyrase binds ATP substrate with flexible regions, and is a well-established antibacterial target. In particular, we have used NMR to compare how two antibiotics, Novobiocin and a new bithiazole, bind the 24 kDa DNA Gyrase B N-terminal subdomain from *S. aureus*. Novobiocin represents the old strategy by binding rigid regions of the ATP-binding pocket; by contrast, the bithiazole binding mimics that of the ATP substrate, which makes contact with the flexible non-conserved loop. Inhibitors which mimic the same intermolecular contacts as substrate, is a proven strategy to slow the onset of drug-resistant mutations. Our NMR data show that the bithiazole decreases the backbone flexibility of the ATP-binding loop at both conserved and non-conserved regions. Moreover the dynamic changes propagate beyond the immediate ATP-binding site, thus raising the possibility of future allosteric inhibitors, and thus enhanced selectivity.

To investigate these possible allosteric effects, we have begun investigating inhibitor binding to the larger 43 kDa DNA Gyrase B N-terminal domain, which dimerizes upon ATP binding (86 kDa). The large molecular weight of the bound complex is non-trivial, and calls for advanced isotope labeling and pulse sequences. Our results thus far, demonstrate the feasibility of detailed structural and binding studies. In particular, initial methyl-TROSY data map regions of substrate interaction to guide inhibitor design.

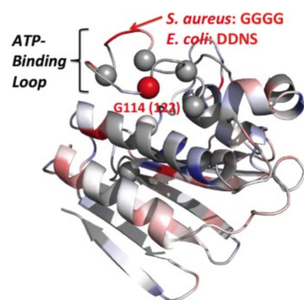

Fig. 1. Changes in *S. aureus* GyrB24 NH backbone dynamics caused by the bithiazole. Red/blue are decreased/increased mobility. Note decrease in conserved and non-conserved region of ATP-binding loop.

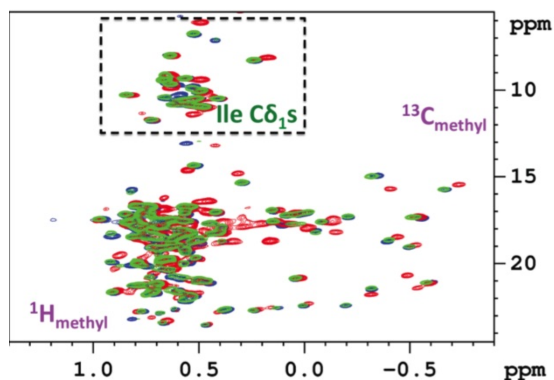

Fig. 2. $^{13}C_{methyl}$ $^{1}H_{methyl}$ spectra of GyrB43 in apo (blue); complexed with AMP-PNP (ATP mimic) (red) and complexed with ADP (green). The red (AMP-PNP) shift perturbations are most pronounced.

40. Investigating Phosphatidylserine Flipping of Ebola Virus VP40

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Ebola virus, a member of the *Filoviridae* family, causes hemorrhagic fever with up to a 90% fatality rate in humans. Key to the viral life cycle is the matrix protein, VP40. VP40 assembles at the plasma membrane where is creates high intensity punctate virus like particles (VLPs). VP40 is necessary for viral assembly and budding. Ebola virus had been shown to utilize Phosphatidylserine (PS) on the outer membrane of the viral envelope to facilitate entry into target cells through interactions with the TIM-1 receptor. Previous studies have demonstrated that VP40 VLPs alone are able to enter target cells in a PS-dependent manner. This suggests that PS exposure in VLPs may be due to VP40. The ratio of inner membrane PS to outer membrane PS is >24:1, which is maintained by ATP-dependent aminophospholipid flippases. We are investigating whether the low levels of PS on the outer cell membrane are sufficient for VLP entry to target cells or there is a mechanism employed by VP40 to enrich the PS at this membrane. We are investigating the effect of VP40 on PS transport pathways from the Golgi, through flippase activity, and through Calcium dependent scamblases.

41. Excited Conformational States of Proteins by Side-Chain NMR Relaxation Dispersion: An Application to *S. aureus* $BlaR^S$

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Microsecond-millisecond time-scale motions are under increasing scrutiny in proteins owing to their potential importance in protein function, ligand binding, and allostery. Recent advances in nuclear magnetic resonance have focused on methyl side-chain dynamics. Methyls have spectroscopic properties that are ideal for studying large protein systems >30 kDa for which standard backbone ^{15}NH methods are poorly suited. Furthermore, methyl side-chains can experience functional exchange dynamics that are not present in the protein backbone. Characterization of microsecond-millisecond exchange dynamics are achieved using CPMG relaxation dispersion. Here we demonstrate the recently established ILV $^{13}CH_3$ specific isotope labeling and $^{13}C/^{1}H$ dispersion experiments on the 29 kDa β-lactam sensor domain of BlaR1 ($BlaR^S$) which is a principal driver of the β-lactamase antibiotic resistance mechanism. These experiments highlight the methyl-bearing residues near the β-hairpin turn close to the active site as residues experiencing exchange dynamics. Interestingly, our previous studies highlighted this same region as making contact with the extracellular loop 2 which connects transmembrane helices two and three of BlaR1. Critically, this interaction is not discrupted by antibiotic binding. Combining those previous results with the new $^{13}CH_3$ dispersion data suggests a new basis for the BlaR1 signaling mechanism, in which sustained L2-$BlaR^S$ contact mediates transmembrane signaling stimulated by changes in $BlaR^S$ dynamics upon binding of b-lactam antibiotics.

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42. Study of Biophysical Properties of Hepatitis C Virus TCR/HLA-HCV $NS3_{1406\text{-}1415}$ 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. The pathogenic mechanism of hepatitis C virus (HCV) is not completely understood. Chronic HCV infection is becoming a worldwide problem because it is reported about 3% of world's population harbors this infection. HCV infection can increase the risk of liver cirrhosis and hepatocellular carcinoma occurrence in patients. HCV is known to be able to activate both B and T cell immunity in humans. So far, it is reported there are almost 50 known antigenic epitopes from HCV recognized by T cells. In spite of this, in most cases, HCV patients' immune systems fail to clear the virus. Previous studies showed the HCV T cell epitopes are able to mutate rapidly, leading to antigen escape variants. These factors make the development of vaccines difficult. In the previous study, circular dichroism and differential scanning fluorimetry were utilized to study the thermal stability of HLA- HCV $NS3_{1406\text{-}1415}$ wild type and escape variants. The results indicated the wild type is more stable than the escape mutants. To investigate the binding affinity of the pMHC molecule to HCV T lymphocytes receptor, the surface plasmon resonance technique is performed. The data indicated a weak binding for the wild type one. For some escape mutants, the binding even is hardly observed by SPR experiments. These data are promising for the future study to elucidate the escape mechanism of hepatitis C virus from T lymphocytes. In future investigation, the high-resolution crystal structure of TCR/pMHC complex becomes very important. By studying these escape variants, we will have a better understanding of the mechanism for escape and how to design an efficient treatment for patients infected with HCV.

43. Characterization of Progression-Related Signaling Network Differences in a Colorectal Cancer Metastasis Model using Phosphoproteomics.

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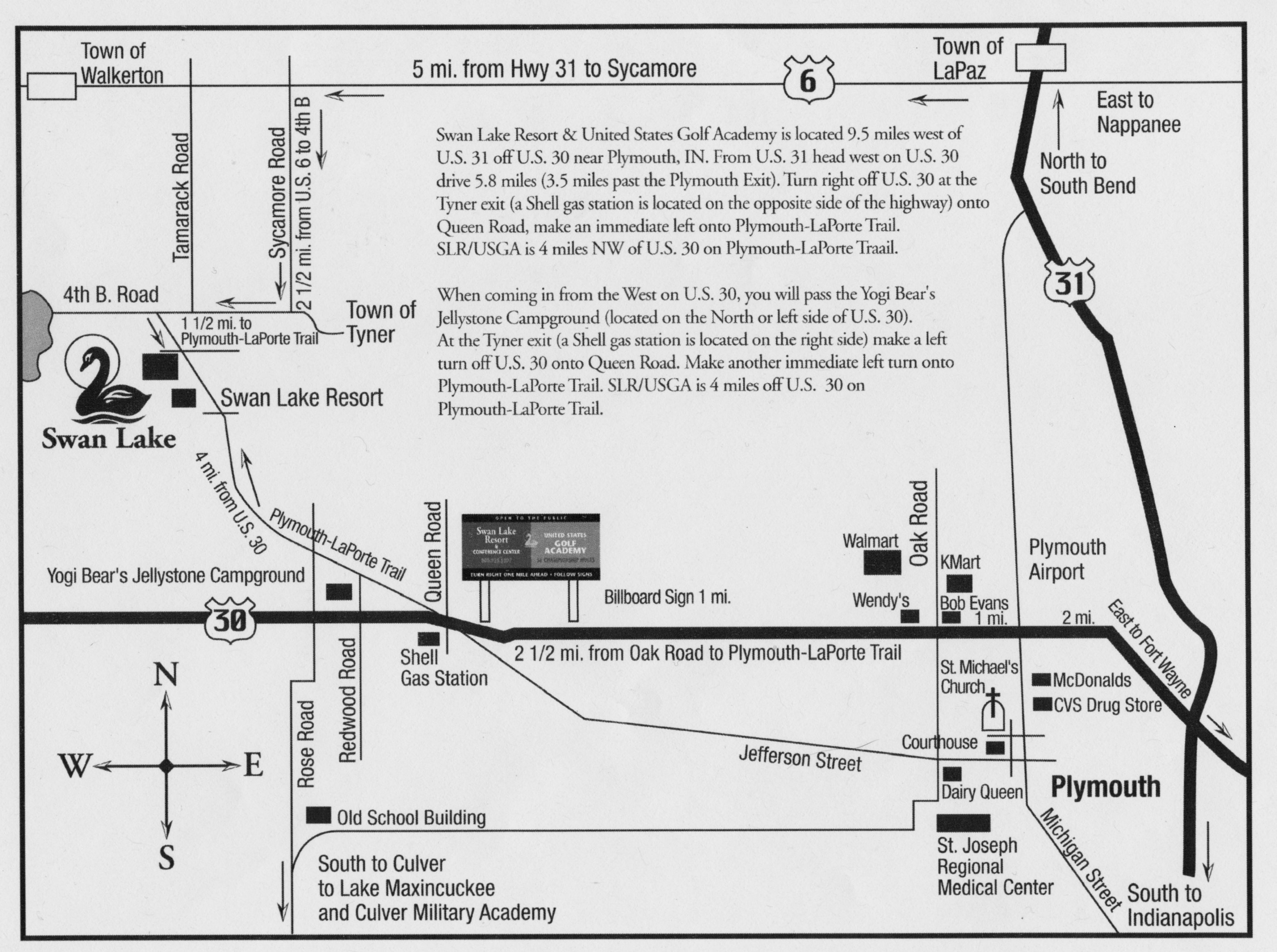
Phosphorylation-mediated signaling networks coordinate numerous intracellular processes, many of which become dysregulated in cancer. While many individual components of a particular pathway may be well characterized, the relationships between pathways remain poorly understood. In late-stage colorectal cancer (CRC), the highly interconnected nature of the signaling pathways allows tumor cells to rapidly adapt to changing conditions during treatment, which is a significant contributing factor in the development of drug and radiotherapy resistance. To gain insight into the intracellular signaling changes that accompany disease progression, relative protein phosphorylation levels in the CRC cell lines SW480 and SW620 were compared using phosphoproteomics. These patient-matched lines were derived from a primary adenocarcinoma and a lymph node metastasis, respectively, which makes them an important model of metastasis. Phosphopeptides were enriched from lysates of the two cell lines and prepared for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis using a phosphoproteomics workflow previously reported by our lab. Over 1800 phosphosites on greater than 1000 proteins were quantified in biological triplicate. Approximately 60% of the phosphopeptides were found to differ significantly in abundance between the SW480 and SW620 cell lines. Among the differentially regulated phosphoproteins are several proteins with known roles in the epithelial-to-mesenchymal transition (EMT) – a critical process for metastasis. Further pathway analysis will identify other significantly altered sites with potentially novel regulatory roles. Key findings from the discovery phase will be validated with a combination of targeted MS experiments and phenotypic assays.


Town of Walkerton
5 mi. from Hwy 31 to Sycamore
6
Town of LaPaz
East to Nappanee
North to South Bend
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
Swan Lake
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.
31
4 mi. from U.S. 30
Plymouth-LaPorte Trail
Queen Road
Swan Lake Resort
United States Golf Academy
Turn right one mile ahead • Follow signs
Billboard Sign 1 mi.
Yogi Bear's Jellystone Campground
30
Shell Gas Station
2 1/2 mi. from Oak Road to Plymouth-LaPorte Trail
Walmart
Oak Road
KMart
Wendy's
Bob Evans
1 mi.
Plymouth Airport
2 mi.
East to Fort Wayne
St. Michael's Church
McDonalds
CVS Drug Store
Courthouse
Jefferson Street
Dairy Queen
Plymouth
Rose Road
Redwood Road
Old School Building
South to Culver to Lake Maxincuckee and Culver Military Academy
St. Joseph Regional Medical Center
Michigan Street
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