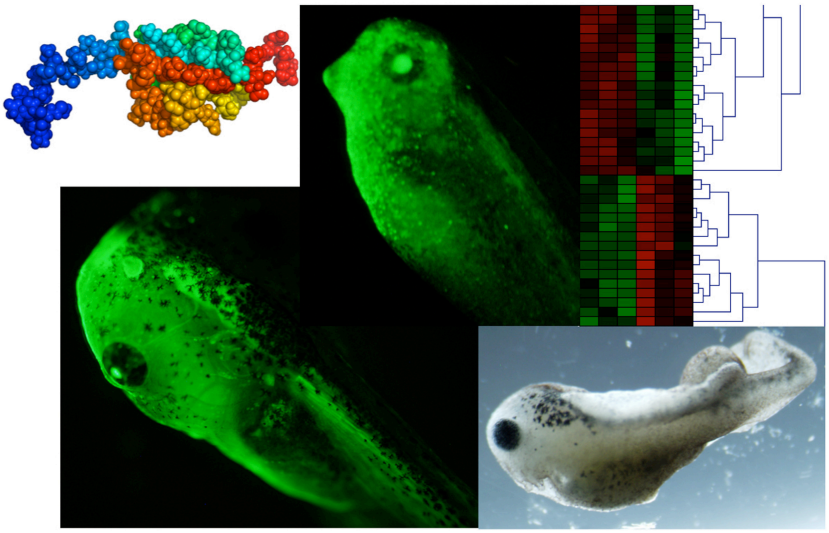
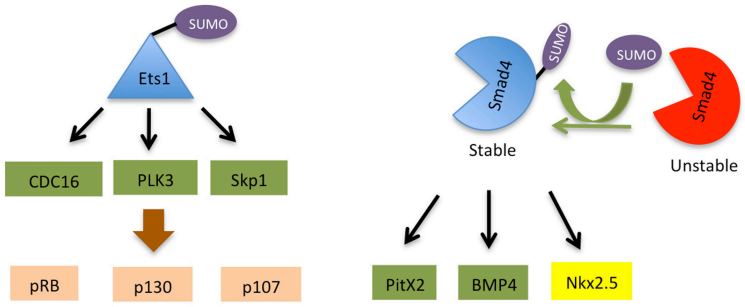
The Bretthauer Papers

18th ANNUAL BIOCHEMISTRY RESEARCH RETREAT

Department of Chemistry and Biochemistry
University of Notre Dame
Notre Dame, Indiana





Swan Lake Resort
Plymouth, Indiana
June 11-12, 2013

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Anthony Serianni

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Cover Illustration

Courtesy of the Huber Lab

Cover Legend. SUMO (upper left) is an 11-kDa protein that is conjugated through isopeptide linkages to lysine residues in target proteins. This process has been disabled by injection of mRNA encoding the viral protein, Gam1, that triggers proteolytic destruction of one of the enzymes required for attachment of SUMO. Co-injection of Gam1 and GFP mRNAs into one blastomere of a 4-cell embryo restricts the expression of Gam1 to a subset of progeny cells (GFP-positive). Disruption of SUMOlylation results in several phenotypes such as aberrant cardiovascular development (heart laterality and size) or incomplete closure of the neural tube (spina bifida), which is seen as a protrusion on the dorsal side of the tadpole (lower right). Microarray analysis of gene expression in these embryos (heat map, upper right) has revealed perturbations in pathways associated with cardiovascular development (lower panel). Images courtesy of Michelle Bertke and Olivia Cox (Huber lab).

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)

♦ 2013 Keynote Lecture ♦

Ad Bax
Chief, Section on Biophysical NMR Spectroscopy
Laboratory of Chemical Physics, NIDDK, NIH

Protein structure and dynamics viewed by weak alignment solution NMR

Simplicity of solution NMR spectra results from the fast Brownian rotational diffusion of molecules, which rapidly averages the strong dipolar interactions between nuclear spins to exactly zero. Much valuable structural information, contained in these dipolar interactions, is lost in this averaging process. It has long been known that alignment of solutes in a magnetically oriented liquid crystalline medium restores the dipolar interactions. By strongly limiting the degree of alignment, it is possible to measure the valuable structural information contained in the dipolar couplings even in macromolecules. This not only provides a much higher definition of protein structure by NMR spectroscopy, it also enables independent structure validation and is uniquely suited to probe domain motions. Examples will be shown for several proteins, ranging from calmodulin and hemoglobin to the fusion domain of hemagglutinin.

Biography of Adriaan Bax

Adriaan (Ad) Bax was born in The Netherlands and became a US citizen in 1999. He received his Ph.D. in 1981 from Delft University of Technology, The Netherlands, for work related to the development of two-dimensional nuclear magnetic resonance (NMR) techniques, which he carried out at Delft and Oxford Universities. His Ph.D. thesis was reprinted in book format and for many years served as a popular text, introducing students to the application of two-dimensional NMR in chemistry. Bax joined the NIH in 1983, where he has been working on the development and application of a wide variety of advanced multi-dimensional NMR techniques to problems of biochemical and biomedical interest. His group spearheaded the introduction of triple resonance NMR spectroscopy of $^{13}C/^{15}N$-enriched proteins, which has become the standard method for structure determination in solution. He also introduced the now standard, joint analysis of ^{15}N R_1, R_2, and NOE relaxation rates, providing a residue by residue view of protein backbone dynamics, and introduced the first methods for weakly aligning proteins in a magnetic field by the use of liquid crystals. Other advances originating in his laboratory include the use of perdeuteration in triple resonance NMR, which greatly extends the protein size limit; the quantitative use of chemical shifts in structural analysis which now allows rapid structure determination of small proteins; and direct use of SAXS data in NMR structure calculations. Bax's work has been recognized by numerous awards, including the Hans Neurath Prize from the Protein Society, and he is a member of the USA National Academy of Sciences.

Program

Tuesday Afternoon
Session Chair: Paul Huber

1:00-1:20 Welcome and Orientation – Anthony Serianni

1:20-1:40 Julie L. Chaney, Rory Carmichael, Aaron Steele, Scott Emrich and Patricia L. Clark
Co-occurrence of rare codons

1:40-2:00 Kamlesh K. Gupta, Deborah L. Donahue, Francis J. Castellino and Victoria A. Ploplis
Impaired binding of vitronectin to plasminogen activator inhibitor-1 increases activated protein C activity in endotoxemic mice

2:00-2:20 Jennifer Fishovitz, Matthew Dawley, Negin Taghizadeh, Juan Hermoso, Mayland Chang and Shahriar Mobashery
Generation and characterization of Staphylococcus aureus PBP2a mutants to probe active-site conformational changes

2:20-2:40 D. Cole Stevens, Ian M. Harrier, Andrew Gasparrini and Richard E. Taylor
Heterologous production of linear polyketide analogs from advanced synthetic substrates

2:40-3:10 Mid-Afternoon Break

3:10-3:40 **Guest Lecture**
Mark Schurr
Department of Anthropology, University of Notre Dame
Stable isotopes and prehistoric diet: Current accomplishments and uncertainties

3:40-4:00 Yueling Zhang, Zhong Liang, Jeffery Mayfield, Kristofor Glinton, Victoria A. Ploplis and Francis J. Castellino
Characterization of streptokinases SK1 and SK2b from Group A streptococci

4:00-4:20 Igor Drobnak and Patricia L. Clark
Autotransporter protein secretion across the bacterial outer membrane

4:20-4:40 Katherine E. Ward, James P. Ropa, Emmanuel Adu-Gyamfi and Robert V. Stahelin
Investigating the molecular basis of cPLA$_2\alpha$ membrane bending

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4:40-7:00 Hotel Check-in and Recreation
7:00-8:00 Dinner

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Tuesday Evening

8:00-9:30 **Poster Session**

9:30-12 **Social and Entertainment**
The Oblates of Blues

Wednesday Morning
Session Chair: Rob Stahelin

7:40-8:40 **Breakfast**

8:40-9:00 Sarbani Bhattacharya, Y. Zhong Y, Victoria A. Ploplis and Francis J. Castellino
Key residues within core-domain of Group A streptococcal plasminogen-binding M-like protein (PAM) downplays the role of dimerization in plasminogen binding

9:00-9:20 Edward Spink, Michelle Joyce, Malika Kumarasiri, William Bogess, Mayland Chang and Shahriar Mobashery
Identification of the target of a new class of antibiotics for methicilin-resistant Staphylococcus aureus using photoaffinity labeling and mass spectrometry methods

9:20-9:50 **Guest Lecture**
Laurie E. Littlepage
Department of Chemistry and Biochemistry, Harper Cancer Research Institute, University of Notre Dame
Fighting cancer, one experiment at a time: Using animal models to predict cancer progression and overcome therapy resistance

9:50-10:10 Michelle Bertke, Laura Cronin, Erliang Zeng and Paul Huber
SUMOylation knockdown in Xenopus laevis disrupts cell migration in early development

10:10-11:10 **Mid-Morning Break and Hotel Checkout**

11:10-11:30 Jill J. Bouchard, David A. Case and Jeffrey W. Peng
Investigating the functional long-range order of human Pin1

11:30-11:50 Garima Agrahari, Zhong Liang, Victoria A. Ploplis and Francis J. Castellino
Molecular mechanisms of antiphagocytic activity mediated by plasminogen-binding group A streptococcal M-like protein

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12:00-2:00 **Lunch and Presentation of Speaker Plaques**

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Wednesday Afternoon
Session Chair: Holly Goodson

2:00-2:20 Thomas E. Frederick and Jeffrey W. Peng
Exploring the molecular basis for BlaR1 signal transduction by nuclear magnetic resonance relaxation

2:20-2:50 **Guest Lecture**
Jing Zhao, Larry Zaino, Min Yu and Paul W. Bohn
Department of Chemical and Biomolecular Engineering and Department of Chemistry and Biochemistry
Single enzyme spectroelectrochemistry of redox-switchable flavoenzymes in zero-dimensional photonic devices

2:50-3:10 **Mid-Afternoon Break**

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3:10-4:10 **Keynote Lecture**
Ad Bax
Protein structure and dynamics viewed by weak alignment solution NMR

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4:10-5:00 **Keynote Lecture Reception and Departure**

ABSTRACTS: GUEST LECTURES

Stable Isotopes and Prehistoric Diet: Current Accomplishments and Uncertainties

Mark R. Schurr
Department of Anthropology
University of Notre Dame, Notre Dame, IN 46556 USA

Stable isotope analyses of preserved plant and animal remains (including human remains) have become an important tool for reconstructing past human diets and the ways that people used ancient ecosystems. For example, human stable isotopes of carbon and nitrogen have been extensively employed to study dietary variation between and amongst Late Prehistoric humans of the Ohio Valley. Intra- and inter-site variations in human and faunal isotope ratios can provide valuable clues about diverse topics such as subsistence practices, inter-community interactions, and climate. Human and faunal isotopes from Middle Mississippian and Ft. Ancient sites are used to illustrate the importance of currently neglected faunal isotope ecologies for understanding human ones. While such studies have been extremely useful in helping us understand some aspects of prehistoric subsistence such as maize consumption, they have not reached their full potential because human stable isotopes reflect the isotopic variation of the plants and animals consumed, and are also determined by biochemical fractionation pathways that are poorly understood (at least by archaeologists!).

Fighting Cancer, One Experiment at a Time: Using Animal Models to Predict Cancer Progression and Overcome Therapy Resistance

Laurie E. Littlepage
Department of Chemistry and Biochemistry, Harper Cancer Research Institute,
University of Notre Dame, Notre Dame, IN 46556-5670 USA

Tumors continually adapt to the changing microenvironment that surrounds the cancer epithelial cells in order to survive and spread outside of the primary tissue. In my lab, we use integrated biological approaches to understand the contributions of specific genes in vivo at multiple points in cancer progression, spanning from normal mammary development to tumor progression to metastasis to chemotherapy resistance. We both develop and use *in vivo* animal models, cell culture and organotypic cultures, and systems biology approaches to study biomarkers of epithelial plasticity and to determine how these genes drive aberrations in fundamental biological processes, *e.g.*, differentiation state, progenitor cell maintenance, metabolism, and genomic integrity. We also are identifying targeted therapies appropriate for personalized treatment of cancer patients based on these biomarkers. This research is relevant to both cancer prevention and to treatment of poor prognosis in cancer patients.

Amplification of human chromosome 20q13 occurs in many cancers, including ~25% of primary human breast cancers, and correlates with poor patient prognosis. The transcription factor ZNF217 is a candidate oncogene within this region. We studied the consequences of Znf217 overexpression during multiple stages of breast cancer progression by overexpressing Znf217 in normal and tumor mammary epithelium. Overexpression of Znf217 in primary mouse mammary epithelial cells in culture promotes progenitor cell self-renewal and represses an adult tissue stem cell gene expression signature that is also downregulated in cancers. We also developed both transgenic and transplant mouse models of Znf217 overexpression. Transgenic mice induced to express Znf217 in normal mammary epithelium have mammary glands with increased ductal branching, increased premalignant and malignant lesions, luminal expansion, increased progenitor cell markers, and increased self-renewal capacity. In addition, Znf217 overexpression in a breast cancer mouse transplant model increases the breast tumor burden and lung metastasis in vivo. The tumors are heterogeneous and express markers of both luminal and myoepithelial cells. We compared our mouse findings to clinical outcomes in breast cancer patients and found that patients expressing high ZNF217 in breast tumors have reduced survival, increased metastasis, and increased resistance to chemotherapy. Therefore, ZNF217 is both a prognostic indicator of survival and metastasis in breast cancer patients as well as a predictive indicator of response to treatment. Since ZNF217 acts in part through the AKT pathway, it may prove to be a novel drug target. Indeed, in a separate study, we identified the AKT inhibitor triciribine as an inhibitor of ZNF217 tumor burden and chemotherapy resistance. In summary, we find that Znf217 overexpression within neoplastic epithelium induces multiple phenotypes required for tumor progression and drives the differentiation state towards a progenitor cell-like status with increased self-renewal capacity, mesenchymal marker expression, invasion, metastasis, and resistance to cytotoxic drugs. Because ZNF217 is amplified in numerous cancers, this work also has implications for other cancers.

Single Enzyme Spectroelectrochemistry of Redox-switchable Flavoenzymes in Zero-Dimensional Photonic Devices

Jing Zhao, Larry Zaino, Min Yu and Paul W. Bohn
Department of Chemical and Biomolecular Engineering and
Department of Chemistry and Biochemistry
University of Notre Dame, Notre Dame, IN 46556 USA

Our interest in the behavior of biomolecules under conditions of crowding and confinement led us to develop new tools to study the dynamics of oxidoreductases. Zero-mode waveguides (ZMWs) strongly confine optical fields to zeptoliter volumes and can be coupled with fluorescence microscopy to study the dynamics of single enzyme molecules, due to their excellent optical confinement, precise positioning, and massive parallelism. The redox enzyme, monomeric sarcosine oxidase (MSOX) contains a covalently bound flavin adenine dinucleotide (FAD) cofactor which is highly fluorescent in the oxidized state and dark in the reduced state, thus producing a characteristic on-off fluorescence signal synchronous with transitions between oxidation states, thus allowing single enzyme turnover events to be monitored. For MSOX reactions involving both the nominal substrate (sarcosine) and an analogous substrate (proline), statistical analysis of single-molecule temporal trajectories reveal the static heterogeneity of single enzyme reaction rates, but no dynamic disorder. In addition, the single molecule data confirm the independence of reductive and oxidative reactions. These structures open the way for systematic studies of the effect of molecular crowding on enzyme dynamics. Furthermore, if redox species are immobilized on the metallic sidewalls of the ZMW, it is possible to observe the single molecule fluorescence signatures resulting from direct heterogeneous electron transfer from metallic electrodes to single redox-active molecules. Starting with a relatively simple construct based on the MSOX-FAD-$FADH_2$ system, we have isolated the FAD cofactor on the surface of the Au sidewalls of the ZMWs. A Au surface is derivatized with a self-assembled monolayer (SAM) presenting a terminal amine for derivatization with (1) pyrroloquinoline quinone, PQQ, and (2) *m*-aminophenylboronic acid (APBA), which then binds the sugar ring of FAD. We have prepared ZMW arrays with a significant number of single FAD chromophores bound to the Au sidewalls (see Figure). Experiments to illustrate potential control over electron transfer to single FAD molecules in ZMWs support the assertion that the transition rates between luminescent (oxidized, FAD^+) and dark (reduced, $FADH_2$) states can be modulated with electrochemical potential.

ABSTRACTS: ORAL PRESENTATIONS

Co-occurrence of Rare Codons

Julie L. Chaney[a], Rory Carmichael[b], Aaron Steele[b], Scott Emrich[b] and Patricia L. Clark[a]
[a]Department of Chemistry and Biochemistry and [b]Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, IN 46556 USA

The genetic code is degenerate, with most amino acids encoded by multiple synonymous codons. However, synonymous codons are not used with equal frequency, and rare codons are generally associated with slower translation and slightly lower translational accuracy. This has led to the conventional view that rare codons are deleterious, with selection favoring common codons and rare codons occurring by random mutational drift. However, this hypothesis was challenged by the observation that rare codons are not randomly distributed in genomes: rare codons form clusters in the open reading frames of most prokaryotes and eukaryotes analyzed at levels far greater than predicted by random chance. This observation suggested that the location of rare codons is shaped by selection and that rare codons might have a physiological function. Synonymous codon changes have been shown to alter the structure and function of certain proteins (MDR1, FRQ)[1,2]. One hypothesized function for rare codons is the modulation of translation rate to promote correct co-translational folding. If rare codons do have a function, this function could occur across homologs, and rare codons would be expected to occur in equivalent positions in the coding sequences of homologous proteins. To test this possibility, sequences from multiple species of eukaryotes, bacteria, and archaea were analyzed to determine if rare codon clusters co-occur in homologs. Results suggest that co-occurrence is widespread and is not explained by sequence GC content or amino acid conservation.

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2. Zhou *et al., Nature* 495, 111-115 (2013).

Impaired Binding of Vitronectin to Plasminogen Activator inhibitor-1 Increases Activated Protein C Activity in Endotoxemic Mice

Kamlesh K. Gupta[1], Deborah L. Donahue[1], Francis J. Castellino[1,2] and Victoria A. Ploplis[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

Activated protein C (APC) is a natural antiinflammatory, antithrombotic serine protease that plays a central role in physiological anticoagulation. APC provides beneficial effects via anticoagulant activity that involves inactivation of factors Va and VIIIa. During various pathophysiological conditions, such as sepsis/endotoxemia, the activity of APC was shown to be inhibited significantly, leading to reduced effective concentrations. However, the key factors involved during this process, and the mechanism by which inhibition of APC activity occurs during sepsis/endotoxemia is not well understood. A previous *in vitro* study demonstrated that plasminogen activator inhibitor-1 (PAI-1) can inhibit purified human APC activity which is enhanced in the presence of vitronection. In addition, using an LPS-induced mouse model of endotoxemia, we recently showed that mice either lacking PAI-1 ($PAI\text{-}1^{-/-}$) or expressing a PAI-1-mutant with diminished vitronectin binding ($PAI\text{-}1^{R101A/Q123K}$) have reduced mortality compared to wild-type (WT) mice. In the present study, we report that the reduced endotoxemia-induced mortality in the absence of functional PAI-1 is associated with an increase in APC activity in plasma. By using purified mouse recombinant APC, PAI-1, and vitronectin proteins, we validated the inhibition of mouse APC activity by mouse PAI-1 and vitronectin. We further demonstrated that these three proteins can form a tripartite complex *in vitro* and *ex vivo*. Taken together, our findings support a new physiological role for PAI-1 and provide insight into the mechanism of APC activity inhibition during sepsis/endotoxemia.

This study was funded, in part, by a grant from NIH (NHLBI) HL63682 to Victoria A. Ploplis.

References

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2. Rezaie AR. *J Biol Chem.,* 276:15567-15570 (2001).
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Generation and Characterization of *Staphylococcus aureus* PBP2a Mutants to Probe Active-site Conformational Changes

Jennifer Fishovitz, Matthew Dawley, Negin Taghizadeh, Juan Hermoso, Mayland Chang and Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA, and Department of Crystallography and Structural Biology,
Instituto Quimica-Fisica “Rocasolano", CSIC, Spain

One mechanism of resistance of *Staphylococcus aureus* is the presence of a gene that encodes for the penicillin-binding protein PBP2a. Like other PBPs, PBP2a is responsible for the biosynthesis of bacterial cell wall by catalyzing a transpeptidation reaction in its active site. In contrast to other PBPs, the transpeptidase activity of which can be inhibited by beta-lactam antibiotics by the irreversible acylation of the catalytic serine, PBP2a does not experience modification by beta-lactam antibiotics readily. Therefore, the presence of PBP2a is a key factor in resistance in *S. aureus*, as it is able to catalyze cell-wall biosynthesis despite the challenge by beta-lactam antibiotics. The crystal structure of PBP2a has been determined, identifying two mobile loops that cover the active site in a closed conformation and an allosteric site 60 Å away. Upon binding of substrate in the allosteric site, signal propagation takes place to cause the active site to open by movement of these two loops, allowing access for the transpeptidation reaction and ultimate biosynthesis of cell wall. In order to probe the conformational change around the active site, we have made substitutions of several amino acids throughout the protein, including around the active and allosteric sites, as well as along the backbone of the protein where signal is proposed to propogate. A combination of UV/Vis and fluorescent spectroscopy is used to investigate the binding of substrate and subsequent conformational change to allow active site opening and irreversible acylation of PBP2a.

Heterologous Production of Linear Polyketide Analogs from Advanced Synthetic Substrates

D. Cole Stevens, Ian M. Harrier, Andrew Gasparrini and Richard E. Taylor
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Our lab is interested in the pharmacophoric link between the myriaporones, tedanolides, and gephyronic acid. These structurally similar, geographically distinct natural products are potent eukaryotic-specific protein synthesis inhibitors. Significant effort in our lab has been applied to the syntheses, structural assignments, and biological investigations of both the myriaporones and gephyronic acid. Investigation of the biological impact of the distinct chemical diversity exhibited by these molecules has been limited due to poor access, both biologically and synthetically. To address these needs, a new heterologous platform for the production of linear polyketide homologs utilizing advanced synthetic substrates was developed. Through the use of our engineered strain of *Escherichia coli* we envisioned the production of gephyronic acid-myriaporone hybrids, as well as novel structural analogs. It is our hope that such analogs will provide insight into the pharmacophoric relationship between the two classes of molecules, as well as the potential evolutionary link between the two producing organisms. Initial trials utilizing advanced synthetic substrates were successful in producing a novel analog of gephyronic acid. Production of this analog has revealed a significant revision in the currently accepted biosynthesis of gephyronic acid, and provides evidence for an unreported protein-protein interaction involved in polyketide production.

Characterization of Streptokinases SK1 and SK2b from Group A Streptococci

Yueling Zhang, Zhong Liang, Jeffery Mayfield, Kristofor Glinton, Victoria A. Ploplis and Francis J. Castellino
W. M. Keck Center for Transgene Research and Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Group A *streptococcus* (GAS) strains secrete human plasminogen (hPg) activators, streptokinases (SK), which have been grouped into 2 clusters (SK1 and SK2), and one subcluster (SK2a and SK2b). SK1 and SK2b display significant evolutionary and functional differences, and attempts to relate these properties to GAS pathogenesis are of great interest. In our studies, using 4 purified SKs from each cluster, new relationships between Plasminogen-binding group A Streptococcal M protein (PAM) and SK2b have been revealed. All SK1s efficiently activate hPg, whereas all subclass SK2bs only weakly activate hPg in the absence of PAM. Surface plasmon resonance studies revealed that the lower affinity of SK2b to hPg serves as the basis for the attenuated activation of hPg by SK2b. Binding of hPg to either human fibrinogen (hFg) or PAM greatly enhanced activation of hPg by SK2b, but minimally influenced the already effective activation of hPg by SK1. Activation of hPg in the presence of GAS cells containing PAM demonstrated that PAM is the only factor on the surface of SK2b-expressing cells that enables the direct activation of hPg by SK2b. Since the binding of hPg to PAM is necessary for hPg activation by SK2b, this dependence explains the coinherant relationship between PAM and SK2b and the ability of these particular strains to generate proteolytic activity that disrupts the innate barriers that limit invasiveness. To study the extent to which the different domains of SK are responsible for the functional differences between SK1 and SK2b, we exchanged each of the three known SK domains (α, β, and γ) between SK1 from GAS strain NS931 ($SK1_{NS931}$) and SK2b from GAS strain NS88.2 ($SK2b_{NS88.2}$), and assessed the hPg solution activation and binding functions of the resulting mutants. Our results show that primary structural differences in the β-domains dictate these functional differences, while sequence differences in α- and γ-domains result in more minor and synergistic effects on the β-domain. This first report on the primary structure-functional relationships between naturally-occurring SK1 and SK2b sheds new light on the mechanism of hPg activation by SK, a critical virulence determinant in this species of human pathogenic bacteria.

Autotransporter Protein Secretion Across the Bacterial Outer Membrane

Igor Drobnak and Patricia L. Clark
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Notre Dame, IN 46556 USA

Autotransporters are a large and diverse class of monomeric virulence proteins secreted from Gram-negative bacterial pathogens. Secretion across the outer membrane is facilitated by the autotransporter C-terminal domain, which creates a pore in the outer membrane. However, there is no ATP in the periplasm nor an ion gradient across the outer membrane. It is therefore not clear where the energy comes from to drive transport of the N-terminal passenger (the functional, extracellular part of the protein) through the pore. Using kinetic modeling of limited experimental data on secretion kinetics, we show that the free energy of folding could be used as a driving force for secretion, provided that the passenger does not fold prematurely in the periplasm. We have tested this model experimentally by reversibly stalling secretion and probing the periplasmic conformation of the stalled protein. Our results show that the passenger remains unfolded in the periplasm, confirming that the major requirement for coupling folding to secretion is met. Further work is in progress to experimentally characterize the interplay between folding and secretion.

Investigating the Molecular Basis of cPLA$_2\alpha$ Membrane Bending

Katherine E. Ward[1], James P. Ropa[1], Emmanuel Adu-Gyamfi[1] and Robert V. Stahelin[1,2]

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Signal transduction mediates disease through key molecular targets that initiate signaling networks. As protein-lipid interactions have been examined in the literature, their role in cellular signaling has become more prevalent as lipid-binding proteins have become high impact drug targets in cancer, inflammation and viral egress. One such target, termed cytosolic phospholipase A_2 α (cPLA$_2\alpha$), has been shown to play a key role in the production of the inflammatory mediators prostaglandins and leukotrienes. A novel function of the protein was recently discovered in our lab showing cPLA$_2\alpha$ bends zwitterionic bilayers using model membranes, a process that is mediated by cPLA$_2\alpha$'s ability to deeply penetrate membranes. Others in the field have reported cPLA$_2\alpha$ to participate in Fc mediated phagocytosis, intra-Golgi trafficking and endosomal trafficking, further supporting cPLA$_2\alpha$'s ability to bend membranes in biological processes. In addition, direct evidence has been reported in the literature using siRNA showing that cPLA$_2\alpha$ C2 domain induced vesiculation in cells. These results translate into our cellular system as cells transfected with EGFP-cPLA$_2\alpha$ form cytoplasmic vesicular structures. We have preliminary evidence showing cPLA$_2\alpha$ membrane bending is mediated by curvature sensing and protein oligomerization. The origin of oligomerization is currently under further investigation using both *in vitro* and cellular techniques.

This work was supported by AHA Predoctoral Fellowship 11PRE7640028 and CBBI Training Fellowship T32GM075762.

Key Residues Within Core-domain of Group A Streptococcal Plasminogen-binding M-like Protein (PAM) Downplays the Role of Dimerization in Plasminogen Binding

Sarbani Bhattacharya[1], Y. Zhong[1], Victoria A. Ploplis[1, 2] and Francis J. Castellino[1, 2]
[1]W. M Keck Center for Transgene Research, University of Notre Dame, Notre Dame, IN 46556 USA; [2]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Group A streptococcus (GAS) is a highly specific human pathogen. Its efficient colonization and dissemination in the host leads to a broad spectrum of diseases ranging from simple and uncomplicated 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 their infection. Plasminogen binding M and M-like proteins (PAM) have long been recognized as major virulence factors of GAS. M-protein and PAM can bind to host plasminogen (Pg) directly or indirectly (*via* fibrinogen/fibrin) and enhance the activation of plasminogen to its serine protease, plasmin, thus assembling a host protease by cells[2, 3]. Of all known receptors of Pg, PAM is the greatest contributor to direct Pg binding by GAS and hence the target for vaccine development, as well as under scrutiny for structural studies. Pg-binding is attributed to two characteristic tandem repeat regions termed a1 and a2 located in the N-terminal variable region of PAM and five amino acid residues (R^{101}, H^{102}, E^{104}, R^{114}, H^{115}) are thought to be key binding determinants[4]. VEK30, a 30-residue peptide (residues 85-113) derived from the a1a2 domain and lacking $R^{114}$and H^{115} has been extensively used as a convenient surrogate of PAM for the structure/function studies[5]. However, VEK30 binds Pg with reduced affinity and activates Pg more weakly than full-length PAM[6]. It has been hypothesized that this is due to the lack of a dimeric coiled-coil structure of isolated VEK30, 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.

To define the minimal length of PAM required for the retention of dimeric coiled-coil motif and to examine the contribution of coiled-coil motif on Pg-binding properties, different constructs were generated where residues were added progressively to the a1a2 Pg-binding core. We determined the minimum number of residues required for dimerization which shows Pg binding efficiency at par with full-length PAM. However, a monomeric construct containing two residues in addition to VEK30 showed equally strong binding, therefore downplaying the role of dimerization in Pg binding. Also, mutagenesis of five key residues reduced the Pg binding by 1,000 fold which clearly established these residues as the key binding factor rather than PAM's dimerization ability. This study therefore highlights the relative contribution to Pg binding by residues within the a1a2 domain as well as identifies the minimum length which has the potential to be used as mini-PAM.

This work was supported by the National Institute of Health research grant HL013423.

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Identification of the Target of a New Class of Antibiotics for Methicilin-resistant *Staphylococcus aureus* Using Photoaffinity Labeling and Mass Spectrometry Methods

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A new class of antibacterial oxadiazoles has been developed that shows high potency against methicillin-resistant *Staphylococcus aureus* (MRSA) in *in vitro* (MIC 1 μg/mL) and *in vivo* models. A photoaffinity ligand of the lead oxadiazole was synthesized containing a diazirine moiety to crosslink with the target protein and following a trypsin digest step, the site of binding could then be identified by mass spectrometry. The photoaffinity ligand was first crosslinked with human serum albumin (HSA) to validate the efficiency of the photoaffinity probe and to identify the HSA-binding site from trypsin-digested peptide fragments. This knowledge has been used to help reduce the plasma protein binding of future molecules during the lead optimization process, improving the pharmacokinetic properties of the lead compound. The photoaffinity probe is currently being used in photolysis experiments with the whole proteome of *S. aureus.*

SUMOylation Knockdown in *Xenopus laevis* Disrupts Cell Migration in Early Development

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SUMOylation is a post-translational protein modification that occurs when the 11kDa protein, SUMO (small ubiquitin-related modifier), becomes covalently attached to a target protein in order to control the activity of that protein. In the case of transcription factors, DNA binding, subnuclear localization, and transcriptional activation activity can be affected. The goal of this work is to determine whether SUMOylation plays a significant role in the changing patterns of gene expression during early development. SUMOylation activity in *Xenopus* embryos was knocked down by expression of the adenovirus protein, Gam1, through the microinjection of its mRNA into one-cell embryos. Gam1 binds directly to the E1 SUMO-activating enzyme and triggers degradation of its SAE1 subunit, thereby inactivating the SUMOylation pathway. SUMO deficient embryos survive and display subtle, but reproducible, developmental defects, including incomplete closure of the blastopore or neural tube (spina bifida). Total mRNA was isolated from Gam1 or water injected embryos at three time points during embryogenesis (early gastrula, late gastrula, and early neurula) and quantified in microarray experiments. Lists of genes differentially expressed between water and Gam1 injected embryos show that SUMOylation is involved in the regulation of a far-ranging number of biological processes, including those that are critical during development. In an effort to link observed phenotypes with known pathways, differentially expressed genes were analyzed for pathway enrichment. Several of the genes identified (i.e., claudin1, claudin4, platelet derived growth factor-alpha, platelet derived growth factor receptor-alpha, ephrinA) regulate cell adhesion and epithelial to mesenchymal transition (EMT). Understanding how SUMOylation is involved in EMT pathways, which are necessary during early germ layer formation, would provide insight into the regulation of this important process. In order to monitor internal cell movements during development, Keller sandwich explants, from embryos injected with either water or Gam1 mRNA, were analyzed for proper cell migration. In water injected embryos, normal cell movements occur. However, when SUMOylation is knocked down in Gam1 injected embryos, cell migration is severely repressed and embryos no longer undergo convergence and extension. These observations provide evidence that SUMOylation plays a role in EMT, which is important for proper early patterning of the developing embryo.

Investigating the Functional Long-Range Order of Human Pin1

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Many proteins have multiple independently moving parts that carry out their functions. Residual dipolar couplings (RDCs), measured by Nuclear Magnetic Resonance (NMR) spectroscopy, report on both the average orientation of bond vectors (structure) as well as the ns-μs fluctuations about that average (dynamics), and can reveal long-range details about the domains in modular proteins. Coupling this NMR observable with molecular dynamics simulations can provide a powerful technique to quantify and visualize conformations and motions important for protein modularity.

We present a new application in the AMBER 12[1] molecular dynamics suite to perform rigid-body motion restrained by NMR RDC data. This implicit-solvent method combines four features: (i) Cartesian Restraints; (ii) NMR RDC Restraints; (iii) EMAP density map constraints;[2] and (iv) Self-guided Langevin Dynamics.[3] These simulations quickly hone in on conformations consistent with the two independent sets of ^{15}N-^{1}H NMR RDC data. Explicit-solvent simulations were also conducted to provide boundary conditions for the analysis.

This work applies the new AMBER method to the human peptidyl-prolyl isomerase Pin1. Pin1 has two domains: a WW domain that binds specific phosphorylated substrates, and a peptidyl-prolyl isomerase (PPIase) domain that catalyzes substrate isomerization. Our results show the range and frequency of conformations sampled by the WW domain with respect to the PPIase domain, providing insight into how the two domains communicate to function.

This work was supported by NIH grants R01GM083081 (to JWP) and NIH T32GM075762 (to JJB). The calculations reported in this work were performed at the BioMaPS High Performance Computing Center at Rutgers University, which is funded in part by the NIH shared instrumentation grant no. 1 S10 RR022375.

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Molecular Mechanisms of Antiphagocytic Activity Mediated by Plasminogen-Binding Group A Streptococcal M-like Protein

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Streptococcus pyogenes or Group A Streptococcus (GAS) is a spherical, gram-positive bacterium that is responsible for numerous diseases with diverse clinical manifestations specifically in humans such as impetigo, pharyngitis, scarlet fever and life-threatening diseases like necrotizing fasciitis, toxic shock, and rheumatic heart disease. The progression of GAS infection is a complex combination of host-pathogen interactions leading to favorable conditions for proliferation and dissemination of the pathogen. GAS is equipped with several surface-expressed extracellular virulence factors such as M or M-like proteins. Plasminogen-binding group A streptococcal M-like protein (PAM) is a surface-expressed virulence factor primarily involved in the bacterial cell surface activation of plasminogen (Pg) to plasmin (Pm) that facilitates GAS in adhesion, invasion, dissemination and evasion of innate and adaptive immunity. However, the antiphagocytic role of PAM and the mechanisms by which PAM-expressing GAS strains survive in the host bloodstream are not well known. In the present study, we showed that GAS strains lacking PAM expression have reduced survival in the presence of isolated neutrophils indicating a direct role of PAM in antiphagocytosis. We further found that lack of PAM strongly enhanced the deposition of complement factor C3b on the GAS surface indicating that expression of PAM prevents surface deposition of C3b, and therefore contributing to the ability of GAS strains to resist phagocytosis. By utilizing both *in vitro* and *in vivo* model systems, we are further exploring the mechanisms for the antiphagocytic activity of PAM. Our findings provide an insight into understanding the pathogenic interactions of PAM with the host system, and suggest that PAM could be a prominent target in the study of GAS virulence and the development of more effective vaccines for curing GAS-associated infections.

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Exploring the Molecular Basis for BlaR1 Signal Transduction by Nuclear Magnetic Resonance Relaxation

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BlaR1 is an integral-membrane β-lactam sensor protein involved with β-lactam resistance in bacteria such as methicillin-resistant Staphylococcus *aureus*. BlaR1 regulates the production of β-lactamase enzymes that destroy β-lactam (penicillin-like) antibiotics. Specifically, antibiotic binding to the extracellular BlaR1 sensor domain ($BlaR^S$) initiates a transmembrane signal that ultimately leads to β-lactamase production; the molecular details of signal transduction in BlaR1 are poorly understood. One hypothesis involves an essential interaction between an extracellular loop (L2) and $BlaR^S$, which is then mitigated by antibiotic binding. However, evidence of this interaction is conflicting and direct atomic-level evidence to support this interaction is lacking. Accordingly, we have used reduced spectral density mapping and paramagnetic relaxation enhancement (PRE) to characterize the interaction between L2short – a peptide mimic of L2 – and $BlaR^S$, and describe how that interaction responds to binding of a β-lactam antibiotic. Our results suggest that there is a transient L2short interaction with $BlaR^S$ in two locations: one close to the active site pocket and another distal from the active site. The significance of the two-site interaction is not fully understood. Addition of penicillin G – a β-lactam antibiotic – does not interrupt the L2short-$BlaR^S$ interaction. Together, these data support the hypothesis of an L2-$BlaR^S$ interaction; however, disruption of this interaction is not essential to the BlaR1 signal transduction mechanism driving β-lactam antibiotic resistance.

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ABSTRACTS: POSTER PRESENTATIONS

1. YKB: A Biosensor Used to Detect Changes in Translation Rate

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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. However, more recently it has been shown that many proteins begin to fold while still tethered to the ribosome. Co-translational folding can influence the pathway a protein takes to its final native state *in vivo*. Additionally, altering translation rate could further impact the final folded structure of a protein. Currently we have limited understanding of the cellular factors that control translation rate and how these might modulate co-translational folding. To observe the effects of translation rate on final protein structure, synonymous codon mutations can be introduced at the mRNA level. While most amino acids are encoded by multiple synonymous codons, these codons are not used with equal frequencies. Rare codons are generally translated more slowly than common codons and can cause pauses in translation. To measure changes in translation rate *in vivo* we developed the fluorescent protein biosensor YKB (Figure 1). YKB can assume one of two mutually exclusive folded states, yellow (YK) or blue (KB). Synthesis of YKB from N to C terminus will favor the yellow (YK) state, but the YK/KB ratio can be altered by synonymous codon substitutions that alter translation rate *in vivo*. Specifically, increases in the YK to KB ratio are generally observed as the rareness of the codon cluster in the B domain increases as well as during overexpression. These results demonstrate that the YKB biosensor provides a sensitive tool with which to develop a deeper understanding of how codon usage and growth conditions affect translation rate *in vivo*.

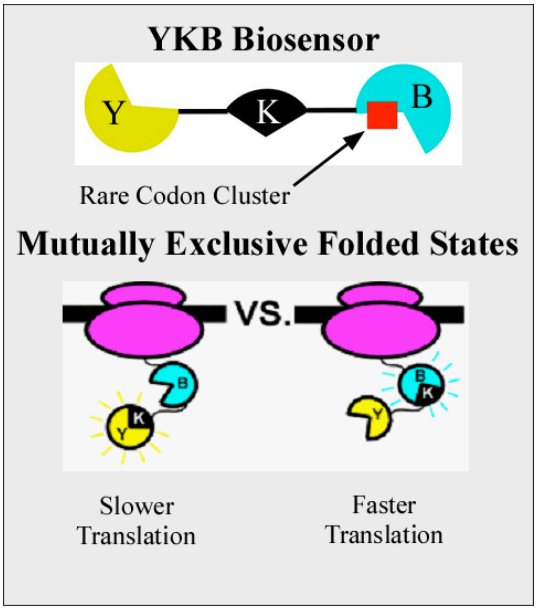

Figure 1 - YKB Biosensor

2. Crystal Structure of Cyclobutanone Analogues of β-Lactams-acylated Form of BlaR1 Sensor Domain of *Staphylococcus aureus*

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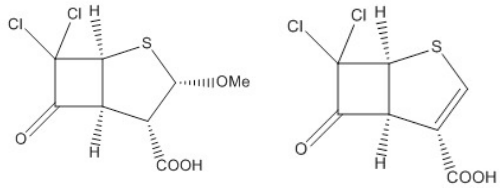
The BlaR1 protein of methicillin-resistant *Staphylococcus aureus* is an integral membrane protein which contains 3 parts: (i) the extracellular C-terminal β-lactam sensor domain, (ii) the N-terminal transmemebrane domian with 4 transmembrane helices, (iii) the intracellular Zn-dependent protease.[1] When the β-lactam antibiotics present in the living environment of the *S. aureus,* the sendor domain of BlaR1 protein would be covalently modified by the antibiotics. The intracellular Zn-depent protease would be activated and begin to hydrolyze the *bla* gene repressor-BlaRI once it received the binding signal. The *bla* gene can transcribe and express β-lactamase which has the ability to be secreted outside the cells and hydrolyse the antibitics in the media.

The active site for the β-lactamase is the β-lactam ring agent. Here, two new analogues of β-lactam antibiotics 1 and 2 were synthesized in Dr. Mobashery's lab. In the previous study, we already know that the classic β-lactam antibiotics, such as penicillin, oxicillin and CBAP, can acylated the Ser residue in the active site peptide sequence of Ser-X-X-Lys and there is carboxylation on the Lys's side chain.[2] These two modification also can be seen in the x-ray structure for the OXA-10 β-lactamase which is related to the BlaR1 sensor domian.[3] Also, we try to see what kind of effect will be brought by the new compunds on the BlaR sensor domain.



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3. Physical Basis of Recognition of Antigens by T Cell Receptors

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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 (MHC) protein presenting a peptide. The complete mechanism by which a TCR recognizes an antigen (peptide-bound MHC) and proceeds to initiate the signaling cascade to destroy infected cells is still unknown. Even less clear is how a particular T Cell Receptor distinguishes between foreign and self-peptides presented on an MHC. Much of our work aims to understand the properties that influence the recognition event between a TCR and a peptide-MHC. Three T Cell Receptors that all utilize the same Vα chain (TRAV 12-2*01) have been studied for their interactions with the peptide-MHC. Each TCR, while related by sequence, recognizes different peptides with varying affinities. The goal of this work is to determine which interactions are significant for binding each unique antigen, as well as gaining more insight about the properties that give rise to T Cell Receptor cross reactivity.

4. Understanding the Competition Between Folding and Aggregation for a Large β-Sheet-rich Protein

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As protein size and/or structural complexity increases, so does the propensity for misfolding and aggregation. Yet some large, complex proteins are able to refold reversibly, suggesting their folding mechanisms and intermediates possess folding features that help suppress aggregation. To gain an understanding of these features, we used a large (539 aa) all-β-sheet model protein, pertactin. Pertactin has a right-handed β-helical structure and folds extremely slowly, but reversibly, *in vitro* ($k_1 \sim 10^{-5}$ sec^{-1}). Native gel electrophoresis revealed the formation of transient insoluble oligomers during pertactin refolding, but it is not yet clear whether these oligomers serve merely to retard the folding rate or whether they prevent irreversible, off-pathway aggregation. Transient oligomer formation during refolding is sensitive to temperature, protein concentration, and residual denaturant concentration. This oligomerization could account for the extremely slow refolding kinetics. We are currently testing whether the transient oligomers are on-pathway for pertactin refolding and whether they play a role in refolding yield or rate.

5. Development and Biophysical Characterization of High Affinity T-Cell Receptors Recognizing Melanoma Antigen

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Our body's cytotoxic T-cells, also known as CD8+ T-cells, can recognize and target cancer cells through an interaction between its T-cell receptors (TCR) and antigen bound major histocompatibility complex (MHC complex) on cancer cells. This phenomenon has led to advent of adoptive immunotherapy for melanoma, where the T-cells of the patient are genetically engineered to express TCRs that respond specifically to melanoma associated antigens (MART-1_{26-35} & $_{27-35}$ epitopes), and then introduced back into the patients. The first set of clinical trials examining this approach in humans utilized DMF4 and DMF5 TCRs, and showed that DMF4 TCR led to a 13% rate of tumor regression, whereas DMF5 TCR led to a 30% rate of tumor regression.[i,ii] Soon after, using biophysical characterization techniques, our lab was able to show that the improvement in immunogenicity seen with DMF5 was due to its higher affinity towards the cognate melanoma antigen (MART-127-35).[iii] Hence, building upon our recent findings, we are currently working on developing and biophysically characterizing high affinity DMF5 TCR towards MART-1_{27-35} epitope.

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

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

7. Vectorial Folding Controls Secretion of a Virulence Protein Across the Bacterial Outer Membrane

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Autotransporter (AT) proteins are the largest family of virulence proteins secreted from Gram-negative bacterial pathogens. They are synthesized with an *N*-terminal signal sequence, a central passenger domain (the mature protein), and a *C*-terminal outer membrane (OM) translocator domain. The translocator domain is required for OM secretion of the central passenger domain. Transport of the passenger domain across the OM does not require ATP nor a proton gradient, and therefore the driving force for efficient secretion remains unknown. Previous studies suggest that the AT passenger domain is secreted from C- to N-terminus across the OM, and that vectorial folding on the outside of the cell could drive secretion. This mechanism implies that the AT passenger domain must remain in an unfolded, secretion-competent conformation in the periplasm, and that disrupting the vectorial folding process across the OM hinders secretion. To test this model, we are using pertactin, an AT from *Bordetella pertussis,* to study the conformation of the AT passenger domain as it crosses the periplasm. Using a novel assay to probe the conformation of the pertactin passenger domain in the periplasm, we have shown that pertactin adopts a soluble but unstable conformation in the periplasm, consistent with the proposed secretion mechanism. To develop a mechanistic understanding of the connection of AT folding and secretion, we are testing correlations between *in vitro* folding properties of the passenger domain, periplasmic folding, and secretion efficiency. We have used an *in vivo* screen to select random pertactin point mutants with improved ability to fold in the periplasm. We have characterized the *in vitro* folding behavior of these mutants and are currently investigating their effects on secretion *in vivo*. Preliminary results suggest that subtle changes in folding properties can affect pertactin biogenesis, and reduce OM secretion.

8. Ubiquitin Ligase Smurf1 C2 Domain May Employ Novel Phosphoinositide-binding Properties to Target Damaged Mitochondria to Autophagosomes in Mitophagy

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Smurf1 is an ubiquitin ligase in the Nedd4 family of E3 ligases. It has recently been shown that Smurf1 is essential for the proper targeting of damaged mitochondria to autophagosomes during mitophagy (Orvedahl et al., 2011, Nature). Mitophagy is a process essential to the maintenance of cellular homeostasis and has been shown to be dysregulated in several disease states including cancer and Parkinson's disease. Our data on the Smurf1 C2 domain indicates that it employs several positively charged residues to coordinate negatively charged lipids in the plasma membrane and at intracellular sites. Confocal microscopy has revealed that these intracellular sites include endosomes and preliminarily, autophagosomal membranes. We have employed surface plasmon resonance to determine the affinities of the Smurf1 C2 domain to the negatively charged phosphoinositides (PI) and phosphatidylserine (PS). Using site-directed mutagenesis and confocal microscopy, we have determined which residues are essential for phospholipid binding.

We hypothesize that the full-length Smurf1 may use phosphoinositide and phosphatidylserine lipid binding to target itself to various intracellular compartments, including autophagosomal membranes, where PIs are present. Our *in vitro* and cellular data show that a broad specificity of binding to PIs may enable the Smurf1 C2 domain to modulate its localization dependent on temporally regulated PI levels. We have employed a novel rapamycin-inducible phosphatase system to monitor the dissociation of the C2 domain from membranes upon PI depletion. It is known that other well-characterized C2 domains are involved in the fusion of adjacent lipid membranes, for example in the fusion of neurosecretory vesicles with the plasma membrane at synapses. It is possible that Smurf1 contributes to the fusion of depolarized mitochondrial membranes with autophagosomes in a similar fashion. We are currently working to decipher the exact mechanism of Smurf1 C2 domain lipid binding in the process of mitophagy.

9. The Subcellular Origin and Lipid Biochemistry of SPK1 Signaling in *Arabidopsis thaliana*

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Normal coordination of metabolism and cytoskeletal dynamics is known to be disrupted in many cancer cell types that rely heavily on glycolysis for ATP production and during neurodegeneration. Recently, strong evidence has emerged that DOCK family guanine nucleotide exchange factors (GEFs) positively regulate the conserved Rac-WRC-ARP2/3 pathway involved in cytoskeleton reorganization in both *Arabidopsis thaliana* and mammalian neurons, respectively.[1,2] In *Arabidopsis*, the DOCK family GEF, SPIKE1 (SPK1), activates the Rac-like small GTPase named Rho of Plants (ROP) by exchanging ROP-bound GDP with GTP, upon which active ROP signals through the WRC-ARP2/3 pathway leading to cell growth.[1] This research will specifically examine the epidermal cell growth pathway in *Arabidopsis* involving ROP and SPK1. Recent work has indicated that this signaling pathway originates at the endoplasmic reticulum (ER) at specific sub-domains called ER exit sites (ERES).[3] The proposed studies will investigate the lipid binding properties of SPK1 localization to the ERES and activation of ROP and therefore shed light on a crucial cellular growth pathway in plants, which may also revolutionize the current plasma membrane-centric view of SPK1 signaling. Our hypothesis is that SPK1 is a coincidence detector for two glycerophospholipids, phosphatidylserine (PS) and phosphatidylinositol-3-phosphate (PI3P), and that this interaction enables it to localize to the ER and activate ROP. This study will utilize an inducible lipid metabolizing system to analyze the effect of acute lipid depletion at the ERES on the localization of SPK1 *in vivo*.

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10. Virulence of Group A Streptococcus: Characterization of M Protein Interaction with Human Fibrinogen

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Streptococcus pyogenes, or Group A Streptococcus (GAS), is a highly human-specific pathogen responsible for an array of pathological manifestations, ranging from superficial skin and pharynx infections, to severe conditions, *e.g*., necrotizing fasciitis. During infection, GAS is able to exploit components of the host fibrinolytic system which may play a role in invasiveness. One such key interaction observed is between the surface exposed M1 protein of GAS strain SF370 and human fibrinogen (hFg). The resultant recruitment of human plasminogen (hPg) to its surface is proposed to occur indirectly through hFg. hPg is then activated to plasmin via GAS-secreted streptokinase (SK) or through host activators. Previous studies have shown the B1B2 region of M1 (residues W128-E192) is required for hFg binding. It is hypothesized that this region has the ability to bind hFg and in turn recruit the proteolytic SK-hPg complex independently of the complete M1 protein. However, the limits of this region and the specific residues involved are ill-defined. The aim of this study is to minimize and characterize the functional hFg binding unit of M1. To achieve this, systematic truncations of the protein (M1[G43-K276], M1[G43-E232], M1[D120-E201] and M1[D129-E194]) were expressed in *E.coli* and purified. The ability to of these fragments to bind hFg was evaluated using SPR. These studies revealed approximately 30-45 fold reduction in binding affinity for hFg in fragments M1[G43-E232] and M1[D120-E201] respectively compared to full M1 protein, while binding to M1[D129-E194] was undetectable. In fragment M1[G43-K276] binding affinity was reduced approximately four fold. This fragment contains a relatively well conserved sequence that has been indicated in protein self-association that will be further explored. Structural characterization was also carried out using CD spectrometry, and analytical ultracentrifugation to determine their structure-function relationship. A loss in helical content for M1[D129-E194] and M1[D120-E201] was noted, while M1[G43-E232] retained helical content comparable to full length M1. The role of this region in the recruitment and activation of hPg was also investigated via hPg activation assays, revealing a minor stimulatory effect. Such studies are beneficial in the elucidation of the mechanism of invasion that will aid in prevention and treatment GAS infections.

11. Platelet Dysfunction in a Rodent Model of Traumatic Brain Injury

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Traumatic brain injury (TBI), a major cause of death and disability for the very old and very young,[i] is reported to have approximately 1.7 million cases in the United States diagnosed annually.[ii] Among these cases, ~50,000 people die, ~230,000 are hospitalized and survive, and ~80-90,000 suffer long term disability.[iii] In many cases, these high mortality and morbidity rates are due to a coagulopathy, a disruption in the ability to properly regulate coagulation.[iv,v] Recent studies found a correlation between mortality and platelet dysfunction.[vi,vii] The goal of this study is to develop a rodent model that mimics the human condition following TBI. A closed-skull, constrained concussion model was performed on adult male Sprague-Dawley rats. Following injury, blood hemostasis and coagulation parameters were analyzed and no significant functional difference in fibrinogen levels, aPTT, or PT was found. There was a significant decrease in platelet activation *via* ADP for each time point post-injury. Additionally, at 15 min and 30 min post-injury, there was a decrease in platelet activation *via* arachidonic acid. A significant decrease in collagen-mediated platelet aggregation was found for all injured time points. We conclude that our model mimics the pharmacological responses and physiological changes that are found in human TBI patients.

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12. Antagonistic Effect of Conus parius Peptides on *N*-Methyl D-Aspartate Receptors in Primary Neurons

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The *N*-methyl-D-aspartate receptors (NMDAR) are ligand- and voltage-gated channels that require glutamate/NMDA and glycine as co-agonists. These receptors play an important role in neuronal development, synaptic plasticity, learning, and memory. Persistent activation of these receptors leads to excitotoxicity, which is implicated in acute conditions like brain ischemia and other neuropathologies. NMDA receptor antagonists hold promise in their use as therapeutic strategies to prevent excitotoxicity and thereby act as neuroprotective agents. Conantokins are a class of short peptides that can act as NMDA receptor antagonists and several of them are known to contain the unusual γ-carboxyglutamate residues. These peptides were identified from venoms of cone snails of genus *Conus*. Three peptides Conantokin Parius-1 (Con-Pr-1), Conantokin Parius-2 (Con Pr-2) and Conantokin Parius-3 (Con Pr-3) were studied for their inhibitory effect on NMDA-induced current in dissociated primary neurons. We present data obtained with whole cell NMDA current recordings in cultured primary neurons from WT, GluN2A$^{-/-}$ and GluN2B$^{-/-}$ mice cortices and rat hippocampi. It was observed that the overall inhibition rate by Con Pr peptides was slower compared to Conantokin-G, another member of the conantokin family. A perfusion protocol with Con Pr-1 resulted in 61 % inhibition while Con Pr-2 and Con Pr-3 elicited 50 % and 58 % inhibition of the NMDA-induced currents respectively, in rat hippocampal neurons. Inhibition of the steady state current after the application of conantokins, as well as the desensitization rates of the NMDA-induced current were analyzed and compared to neurons that were not exposed to the peptides. Additionally, real-time calcium imaging was performed to determine the effect of Con Pr peptides on NMDA-induced intracellular calcium influx in primary neurons. It was observed that Con-Pr-1, Con Pr-2 and Con Pr-3 effectively diminished NMDA-induced intracellular calcium influx in a dose-dependent manner. Thus, the *Conus parius* conantokins are effective NMDAR antagonists that decrease NMDA-mediated excitoxicity by decreasing current and intracellular calcium influx in primary neurons.

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13. *Streptococcus pyogenes* Strain A20: M1 or M23?

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Historically, bacteria have been classified epidemiologically based on a variety of factors, most recently the expression of surface antigens. *Streptococcus pyogenes* specifically has been categorized based on differentiation of the hypervariable *N*-terminal region of the surface M-protein[1]. Based on this, there are over 250 strains of *S. pyogenes*[2-5]. Of these, strain M1 is the most globally prevalent of the Group A *Streptococci*[1,4]. In particular, the M1T1 serotype, first discovered in the 1980's possesses a phage encoded DNAse, Sda1[1]. This variant is neutrophil resistant owing to the degradation of neutrophil extracellular traps by Sda1[1]. Neutrophil resistant M1T1 has led to a resurgence in severe hyperinvasive human infections[1]. We explore two unrelated strains of *S. pyogenes* which have randomly been given the same name, A20: one an M1[6,7], the other an M23 serotype[3,8,9]. In this work A20-M23 was found not to carry the gene for the streptodornase D, sda1, in contrast to A20-M1. Sequence alignment and preliminary data suggest that A20-M23 possess a streptokinase of subtype SK2a, while A20-M1 is also consistent with streptokinase from other M1 serotypes as being SK2a.

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14. Functional Studies of Ebolavirus 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 μm and are pleomorphic in shape (REF). 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 virus like particles (VLPs) from the plasma membrane of host cells. and is a viable target for pharmacological development. 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 will investigate 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.

This work is supported by NIH grant AI081077.

15. Structural and Binding Analysis of Group A Streptococcal M-like Proteins and Human Plasminogen

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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, streptokinase (SK) in the bacterium, and an intact functional host fibrinolytic system (HFS). Whereas, PAM binds to human Plasminogen (hPg) with high affinity through N-terminal a1a2 repeats, SK activates hPg to a broad spectrum serine protease plasmin and aids in further dissemination of GAS into deeper tissues. X-ray crystal structures of the complex VEK30, an internal binding determinant of PAM and Kringle 2 of hPg (K2Pg) have shown that side chains of R17, H18, and E20 of VEK30 form a pseudo lysine arrangement and inserts into the lysine binding site (LBS) of K2Pg; and targeted replacement of R113, H114, R126 and H127 by alanine residues in PAM of AP53 strain of GAS abolished its binding to hPg. In the present study we observed that unlike PAM-AP53, PAM-NS53 harbors an insertion of three additional residues *viz*. VHD (Val-His-Asp) next to H114 without significantly affecting its binding to hPg. Insertion of these residues adjacent to H114 of PAM-AP53 was carried out without any difference in its binding to hPg by altering the properties of PAM-AP53. We have also shown by selective replacement of R113, H114, E116, E117, R126 and H127 with alanine residues in PAM-AP53 in the presence of VHD that these 3 residue insertions do not contribute to/affect the binding of PAM to hPg. Therefore we hypothesized that both, PAM-NS53 and PAM-AP53 as well as other PAMs might bind to hPg in a similar manner and PAM might have evolved as a versatile protein with a flexible region between R113-H114 and R126-H127 to bind to hPg. Our future experiments involve extending the intervening region between R113-H114 and R126-H127 with alanine residues and analyze its binding to hPg. Additionally, we propose to develop an X-ray crystal structure of the complex VEK30+VHD and K2Pg. We anticipate that our work will shed light on the binding mechanisms of PAM to hPg in different strains of GAS.

16. *In Vivo* Fluorescence Imaging of Bone Using a Multivalent Molecular Probe Bearing Iminodiacetate Groups

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Molecular systems that target bone are needed for integrated imaging and therapeutic applications. Accurate, noninvasive techniques for monitoring changes in skeletal metabolism are required for sensitive screening of osteoporosis, arthritis, or tumor metastasis to bone. The most common approach is the use of bisphosphonates, but they have some drawbacks such as biological action on the bone and associated toxicity. Efforts to find alternative systems have looked into functional groups that presumably chelate with Ca^{2+} ions exposed on the bone surface. Therefore, in the present study we investigate the potential use of utilizing iminodiacetate groups rather than bisphosphonate groups to bind areas of bone turnover. We reasoned that dyes, specifically deep-red fluorescence emitting squaraine rotaxanes, decorated with a higher number of iminoacetates should exhibit increased affinity to target bone in a living animal.

We report the results of *in vitro* and *in vivo* imaging studies that compare the bone seeking capabilities of the divalent iminodiacetate **1** and the tetravalent iminodiacetate **2**. We also studied the tetravalent iminodipropionate **3** as a structurally related control probe that has negligible affinity for biological surfaces. We find that tetravalent probe **2** is an effective deep-red fluorescent bone imaging agent and likely to be useful for various bone imaging studies. From a broader perspective, our results suggest that molecules or nanoparticle probes bearing multivalent iminodiacetate groups have promise as bone seeking agents with tunable affinities for therapeutic and imaging applications.

17. Targeted Liposomes for Cell Death Imaging and Amplified Drug Delivery

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There is an ongoing need for targeted imaging agents that can rapidly assess the efficacy of anticancer therapies. Our goal is to develop liposomes that have selective affinity for dead and dying cells induced from cancer treatment. A subsequent application is to use the liposomes to deliver anticancer drugs to tumors and initiate cell death processes which will further amplify liposome targeting. The liposome surface is decorated with 2% Zn-DPA-PEG_{2000}-PE, an anchored phospholipid with an extended polyethylene chain that terminates with a zinc-dipicolylamine (Zn-DPA) targeting group. Previous studies with fluorescent Zn-DPA molecular probes have shown that they have high selective affinity for dead and dying cells. Specifically, they target the anionic phospholipid, phosphatidylserine (PS), a cell death biomarker that is exposed on the surface of dead and dying cells. Zn-DPA liposomes are composed of 2% Zn-DPA-PEG_{2000}-PE, 67% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 30% cholesterol and 1% near-infrared fluorophore (DiR). Untargeted liposomes are composed of 69% POPC, 30% cholesterol and 1% DiR. The ability of Zn-DPA liposomes to target a chemical model of the PS cell death biomarker was assessed using a fluorescence quenching assay. These studies revealed that the PEG chains do not inhibit the affinity of the Zn-DPA units.

In vitro fluorescence microscopy of several cancer model cell-lines (MDA-MB-213, Jurkat-J6) showed that the Zn-DPA liposomes strongly stain dead and dying cancer cells that were treated with the anticancer agent etoposide. Multicolor imaging of dead and dying cells using the small molecule DPA probe PSVue™480 demonstrates that Zn-DPA liposomes co-localize to the dead cell periphery, a definitive feature of surface targeting. A rat thymus atrophy model was used to access the *in vivo* cell death targeting capability of Zn-DPA liposomes. Rats were dosed with intraperitoneal dexamethasone to induce thymocyte cell death. Zn-DPA and untargeted liposomes were intravenously injected into separate cohorts (n = 3) and after 24 hours, the rats were euthanized and their organs were excised and imaged. The uptake of targeted liposomes into the dying tissue was more than 4-fold greater than the untargeted liposome system. In summary, liposomes coated with multiple copies of Zn-DPA targeting groups are effective cell death imaging agents for fluorescence microscopy and they can target dead and dying tissue in living animals. With further development, these targeted liposomes have potential utility for rapid clinical evaluation of anticancer drug efficacy in individual patients.

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18. Microtubules Promote Filament Formation from Unmodified Full-length Tau *in Vitro*

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Tau is a neuronal protein that stabilizes the microtubule network, but it also forms filaments associated with Alzheimer's disease. Understanding Tau–microtubule and Tau–Tau interactions would help to establish Tau function in health and disease. For many years, literature reports on Tau–microtubule binding behavior and affinity have remained surprisingly contradictory. Tau–Tau interactions have also been investigated, but whether microtubules might affect Tau filament formation is unknown. We have addressed these issues through binding assays and microscopy. We assessed Tau– microtubule interactions via cosedimentation and found that the measured affinity varies greatly depending on the experimental design and the protein concentrations used. To investigate this dependence, we used fluorescence microscopy to examine Tau–microtubule binding. Strikingly, we found microtubules promote Tau filament formation without characterized Tau filament inducers. We propose that these novel Tau filaments account for the incongruence in Tau–microtubule affinity measurements. Moreover, these filaments appear similar to the heparin-induced Alzheimer's model by electron microscopy. These observations suggest that the microtubule-induced Tau filaments provide a new model for Alzheimer's studies and support the possibility that microtubules play a role in the formation of Alzheimer's associated neurofibrillary tangles.

19. Probing Substrate Dependent Side-chain Dynamics of Human Pin1 with Deuterium Relaxation

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Pin1 is a highly conserved enzyme responsible for the isomerisation of phosphorylated Ser/Thr-Pro motif and thus regulates many cellular processes by affecting both the function and stability of its substrate proteins[1-2]. The phosphorylation-specific peptidyl-prolyl isomerasation represent a new signalling mechanism, whereby the enzyme regulates the conformation of substrate after their phosphorlyation. The main targets of Pin1 are responsible for cell-cycle progression, stress response, immune response, and neuronal functions thus the deregulation or upregulation of Pin1 has an important role in pathologycal deseases such as cancer, Alzheimer's deseas, asthma etc[3].

The relatively small Pin1 consists of two domains: the N-terminal WW domain which is responsible for substrate recognition and the C-terminal PPIase domain which catalyzes the cis-trans interconversion of pS/pT-P peptide bond. Here, we invesigate how a mutation at the catalytic site ($Met^{130} \rightarrow Ala^{130}$) affects the isomerase activity and the intinsic dynamics of Pin1. We measured the ps-ns time-scale side-chain dynamics of the $^{13}CH_2D$ methyl groups of ^{15}N, ^{13}C-labelled, fractionally deutarated Pin1 M130A both in apo and in substrate bound form by Nuclear Magnetic Resonance spectroscopy[4-5]. From the longitudinal, transversal and quadrupolar order relaxations of the methyl deuteron we could unambiguosly derive the spectral denisties values, $J(\omega)$ at $\omega = 0$, ω_D and $2\omega_D$ frequencies (where ω_D is the Larmor frequency of ^2H).

EXSY measurements clearly demonstrate that the M130A mutation deccelerates the isomerase activity of Pin1 by 100-fold while the overall structure of the enzyme remains unchanged. The mutation however significantly influences the dynamic conduit which connects the catalytic domain with the substrate recognition domain. Some side-chains gained flexibility while others rigidified. The mutation also altered the dynamic resoponse upon substrate binding. Similarly as seen for the I18A variant the small perturbation at one site of the protein affects the intrinsic dynamics of remote areas eg. at the domain-domain interface[6].

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20. Comparison of Hydrogen Exchange Rates between Apo and Antibiotic Bound BlaR^s

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The bacterial protein BlaR1 has been shown to signal for antibiotic degradation upon binding antibiotics via its extracellular sensor domain (BlaR^s). The mechanism responsible for the degradation signal is still not well understood. To investigate this mechanism we have performed Hydrogen Exchange (HX) experiments, to probe ms-s conformational dynamics and structural stability relevant to protein functions. We present HX results of BlaR^s in the presence and absence of benzylpenicillin (penG). Differences between the states may suggest a mechanism of signal transduction imperceptible to other biophysical techniques.

We have applied the WEX-III TROSY [1] method to BlaR^s in the presence and absence of penG. This method was selected for its ability to detect HX rates over a wide range. Infrared Radiation experiments on a homologous protein suggest BlaR^s amide protons exchange both rapidly and slowly [2]. It is also amenable to the relatively large size of BlaR^s due to the TROSY effect. Measurements of both states of BlaR^s were conducted at multiple pH values in order to separate physical HX rates from exchange due to the nuclear Overhauser effect.

Comparison of measured exchange rates for BlaR^s in the presence and absence of penG, and their implications for the BlaR signal transduction mechanism will be discussed.

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21. Increased Sensitivity of Methicillin-Resistant *Staphylococcus aureus* (MRSA) to β-Lactam Antibiotics in the Presence of Kinase Inhibitors

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has evolved two mechanisms that are responsible for its resistance to β-lactam antibiotics. One is expression of a β-lactamase (encoded by the *blaZ* gene), and the other is production of penicillin-binding protein 2a (PBP 2a, encoded by the *mecA* gene). Expression of BlaZ 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. The protease degrades the gene repressor BlaI, which derepresses expression of BlaZ as well as BlaR1 itself, resulting in a resistant phenotype. Recent unpublished 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 BlaZ, which would manifest itself phenotypically as increased sensitization of the resistant organism to a β-lactam antibiotic. As an initial screen, a library of known eukaryotic kinase inhibitors was evaluated in combination with β-lactam antibiotics against strains of MRSA. Indeed, several of these compounds reduced the MIC of the β-lactam antibiotics. 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.

22. Elucidating the Mechanism of Ebola Virus Assembly and Budding

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Ebola hemorrhagic fever is characterized by internal and external bleeding in primates due to coagulation abnormalities induced by the virus at the onset of the infection. With no vaccines or treatment, Ebola is classified as bio-safety level IV agent with the potential to be used as a biological weapon. Details of virus assembly process are poorly understood. Recent evidence suggest that the viral matrix protein VP40 is the main driving force for assembly and budding from the plasma membrane. The goal of this project is to elucidate the mechanistic details of VP40-mediated assembly on the plasma membrane using an interdisciplinary approach. Using vitro lipid binding and membrane curvature assays with cellular scanning and single molecule microscopy we have investigate the basis of VP40 lipid binding, membrane bending and viral egress. Results from our investigation demonstrate that VP40 alone assembles in mammalian cells into virus like particles (VLPs) independent of six other viral proteins. VP40 binds to membrane phosphatidylserine (PS) with nanomolar affinity and possesses the ability to modify membrane structure. VP40 induces membrane curvature changes, an important step for bud formation and egress of the newly formed virus. Our results further demonstrate that VP40 oligomerizes on the plasma membrane in a PS-dependent manner and also remodels actin network for assembly and maturation. Our results represent a key step to understanding the general principles governing the remodeling of membrane by matrix proteins from lipid enveloped viruses such as Ebola and HIV.

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23. Natural Inactivating Mutations in the CovS Component of the CovRS Regulatory Operon in *Streptococcal pyogenes* Strain Influences Virulence-associated Genes

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The Gram+ bacterium, Streptococcus pyogenes (or GAS), is the causative agent for a number of human infections. The natural inactivating mutations have been found in CovS in skin-invasive GAS strains, viz., AP53, A20 and NS88.2, each of which contains a natural inactivating mutation in the covS gene of the two-component CovRS regulatory system. The effects of this mutation in AP53 on specific GAS virulence determinants have been assessed, with emphasis on expression of the extracellular protease, streptococcal pyrogenic exotoxin B (SpeB), capsular hyaluronic acid, and proteins that allow host plasmin assembly on the bacterial surface, viz, a high affinity plasminogen (Pg)/plasmin receptor, Pg-binding group A streptococcal M protein (PAM), and the human Pg activator, streptokinase (SK). To further illuminate mechanisms of the functioning of CovRS in the virulence of AP53, two AP53 isogenic strains were generated, one in which the natural covSM gene was mutated to covS-WT (AP53/covSWT) and a strain that contained covR gene deietion (AP53/covR-/-). Two additional strains that do not contain PAM, viz. WT-NS931 and NS931/covSM, were also employed. SpeB was not measurably expressed in strains containing covRWT/covSM, whereas in strains with natural or engineered covRWT/covSWT, SpeB expression was highly upregulated. Alternatively, capsule synthesis via the hasABC operon was enhanced in strain AP53/covSM , whereas streptokinase expression was only slightly affected by the covS inactivation. PAM expression was not substantially influenced by the covS mutation, suggesting that covRS had minimal effects on the mga regulon that controls PAM expression. These results demonstrate that a covS inactivation results in virulence gene alterations and also suggest that the CovR phosphorylation needed for gene up- or down-regulation can occur by alternative pathways to CovS kinase. In conclusion , the mutation in CovS showed substantial effects on regulation of virulence genes . Expression of genes critical to GAS virulence can reveal reasons that specific strains of GAS are effective tissue specialists.

The Gram+ bacterium, Streptococcus pyogenes (or GAS), is the causative agent for a number of human infections. The natural inactivating mutations have been found in CovS in skin-invasive GAS strains, viz., AP53, A20 and NS88.2, each of which contains a natural inactivating mutation in the covS gene of the two-component CovRS regulatory system. The effects of this mutation in AP53 on specific GAS virulence determinants have been assessed, with emphasis on expression of the extracellular protease, streptococcal pyrogenic exotoxin B (SpeB), capsular hyaluronic acid, and proteins that allow host plasmin assembly on the bacterial surface, viz, a high affinity plasminogen (Pg)/plasmin receptor, Pg-binding group A streptococcal M protein (PAM), and the human Pg activator, streptokinase (SK). To further illuminate mechanisms of the functioning of CovRS in the virulence of AP53, two AP53 isogenic strains were generated, one in which the natural covSM gene was mutated to covS-WT (AP53/covSWT) and a strain that contained covR gene deietion (AP53/covR-/-). Two additional strains that do not contain PAM, viz. WT-NS931 and NS931/covSM, were also employed. SpeB was not measurably expressed in strains containing covRWT/covSM, whereas in strains with natural or engineered covRWT/covSWT, SpeB expression was highly upregulated.

24. Regulation of Cholesterol Homeostasis with GEX1A, a Potential Lead for Niemann-Pick Type C Disease

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Recent studies have shown histone deacetylase (HDAC) inhibitors can correct cholesterol storage defects in human NPC1 mutant cells and provide the potential basis for treatment options for NPC disease. The *Streptomyces*-derived polyketide GEX1A increases gene expression similar to trichostatin A, a known HDAC inhibitor. Recently we have observed GEX1A is also capable of facilitating cholesterol trafficking in human NPC1 mutant cell lines similar to HDAC inhibitors, however, GEX1A does not affect histone acetylation. Based on these findings, we developed a multidisciplinary approach to access GEX1A and GEX1A analogs to investigate their potential as treatments for NPC1 disease. Efforts to develop a synthetic route for GEX1A and analogs along with efforts to engineer mutant strains of *Streptomyces chromofuscus* capable of analog production and their evaluation in NPC cells will be presented.

25. Development of ^{13}C-^{1}H and ^{13}C-^{13}C NMR Spin-Couplings in Idohexopyranosyl Rings to Investigate Conformational Flexibility in Solution

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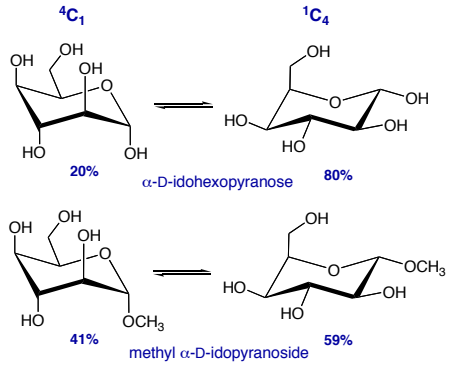
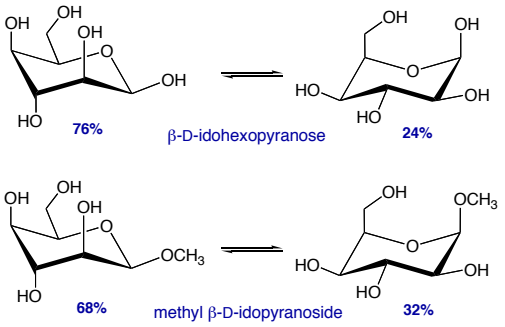
In contrast to other biologically important aldohexopyranosyl rings (*e.g.*, *gluco* and *galacto*), conformational equilibria and dynamics of the idohexopyranosyl ring in aqueous solution are complex, with substantial sampling of multiple conformers comprising the pyranosyl ring pseudorotational itinerary. This sampling imparts unique physical and biological properties to biopolymers that contain them, most notably the proteoglycan, heparin, and its derivatives. Conventional analysis of the limited number (4) of $^{3}J_{HH}$ values within the *ido* ring is insufficient to experimentally assign conformational behavior in solution. With ^{13}C-labeling at specific carbons, however, a wide range of J_{CH} and J_{CC} become accessible from inspection of ^{1}H and ^{13}C NMR spectra. Establishing quantitative correlations between their magnitudes and signs, and molecular structure, is one of the goals of this work, assisted by DFT calculations to predict specific *J*-couplings in defined conformers. Once established and properly parameterized, the full *J*-coupling ensemble encompassing >20 *J*-couplings can be analyzed collectively by our newly developed *MA'AT* program[1] to experimentally determine, *without introduction of MD bias*, the nature of the complex conformational equilibria. Preliminary NMR data analysis shows, unexpectedly, that simple methyl glycosidation of the *ido* ring in both anomeric configurations substantially shifts conformational equilibria between the $^{4}C_{1}$ and $^{1}C_{4}$ chair forms, as shown in the accompanying schemes. This finding is remarkable, and mimics behavior observed previously in this laboratory on aldopentofuranosyl rings.[2] This result has important implications for understanding the effects of ring substitution on pyranosyl ring conformational behavior, especially in rings that possess inherent flexibility, and for testing and validating recent conclusions drawn from extended (10 μs) MD simulations of *ido* ring systems.[3]





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26. Synthetic Conantokin Peptides Potently inhibit NMDA Receptor-mediated Currents of Retinal Ganglion Cells

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Conantokin-G (con-G) and conantokin-T (con-T) are small γ-carboxyglutamate (Gla)-containing gene products present in snails of the genus, *Conus*. Con-G has been widely studied due to its high selectivity for inhibition of NR2B-containing N-methyl-D-aspartate (NMDA) receptor, whereas con-T display broader NR2 activity, e.g., with NR2A and NR2B. It has been shown that these peptides have neruoprotection in a number of animal models of human pathologies including pain, convulsive disorders, stroke, and Parkinson's disease. However, it is unknown whether con-G and con-T have neuroprotective action on retinal neurodegenerative diseases such as glaucoma, a disease associated with retinal ganglion cell (RGC) death. Retinal ganglion cells are the sole output neurons of the retina and they express NMDA receptors and are susceptible to NMDA-induced damage. Here, we evaluated the effects of con-G and con-T on NMDA-mediated responses in mouse RGCs by using a patch-clamp whole-cell recording technique. We found that Mouse RGCs express NMDA receptors that are likely composed of NR2A and NR2B subunits. Con-T substantially suppresses NMDA receptor-mediated currents of RGCs through action on NR2A and NR2B subunits. Con-G partially inhibits NMDA-induced currents of RGCs through action on NR2B subunit. Inhibition by Con-T and Con-G is dose-dependent. Con-T and Con-G reduce maximum NMDA-induced currents of RGCs without affecting EC_{50}s. Con-T and Con-G have no effects on AMPA-type glutamate receptors of mouse RGCs. Taken together, our results demonstrate that both Con-G and Con-T non-competitively inhibit NMDA receptor-mediated currents of retinal ganglion cells in a dose-dependent manner. The results provide a basis for potentially developing effective neuroprotective agents to aid in the prevention of undesired glutamatergic excitotoxicity in neurodegenerative diseases of the retina.

27. Structure-based Computational Investigation of MMP-14 Inhibitor Selectivity

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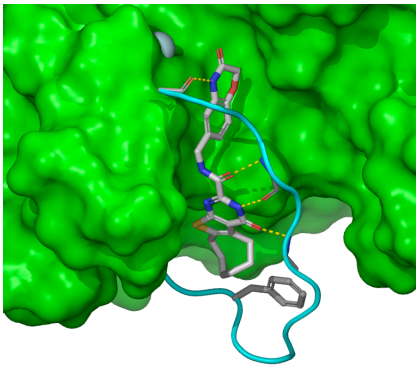
Matrix metalloproteinases (MMPs) are a family of zinc dependent endopeptidases that degrade extracellular matrix proteins. Recent studies shows that MMP-14, a membrane-type MMP, facilitates cancer cell transformation to invasive phenotype in lung and prostrate cancer. Therefore, inhibition of MMP-14 has been proposed as a strategy to abrogate tumor metastasis. Selective and potent inhibition of MMP-14 is crucial since cross reactivity to other members of the family could result in undesirable side-effects like musculoskeletal syndrome. In addition, discovery of a selective inhibitor would aid the investigation of this enzyme's role in tumor pathophysiology. To our knowledge, neither a selective MMP-14 inhibitor nor features of MMP-14 inhibitor selectivity has been reported in the literature.



Alignment of MMP crystal structures and structure-based sequence analysis show the highly conserved nature of the MMP catalytic site. Recent reports reveal that MMP-8 and MMP-13 selective inhibition could be achieved by targeting an adjacent site referred as S1' specificity pocket. We hypothesized that MMP-14 selective inhibition may also be achieved in a similar manner. Structural alignment of crystal structure of MMP-14 to these enzymes show that they have similar S1' specificity pocket loop length and adopt similar backbone conformation. Utilizing this information, we modeled a potential induced-fit loop conformation of MMP-14 based on MMP-8 inhibitor bound crystal structure. Through a structure-guided approach, we designed new analogs of this compound. Molecular dynamics simulations were applied to rationalize selectivity of these novel compounds. On this continuing effort to discover novel inhibitors of MMP-14, we attempt to uncover molecular features of MMP-14 inhibitor selectivity through an iterative process of computational modeling and small-molecule inhibitor synthesis coupled with experimental evaluation of their enzyme inhibition.

28. 3D-QSAR Analysis Toward The Improvement And Design of New 1,2,4-Oxadiazole Antibiotics

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Quantitative Structure-Activity Relationship (QSAR) relies on the principle that the molecular structure of the compound holds the chemical features that are responsible for its biological activity. 3D-QSAR analysis, known as Comparative Molecular Field Analysis (CoMFA), was performed on a series of 1,2,4-oxadiazoles synthesized in our lab, to gain insights into the relationship between the structure and the antibacterial activity of the compounds. Multiple CoMFA models were constructed based on different types of enery minimization and charge calculations. These included molecular-mechanics-based methods (Gasteiger-Hückel, MMFF94) and quantum-mechanical methods. The models proved to have good statistical results for a training set, verified by a cross-validated q^2 value and a correlation coefficient r^2, and therefore hold predictive ability. Models were further validated using an external test set of compounds, which gave the r^2_{pred} value. All the three models showed comparable statistical values. The steric and electrostatic contour maps of the models assisted in designing novel compounds with improved antibacterial activity.

29. The Use of Thromboelastography (TEG) for Analysis of Coagulant and Fibrinolytic Potential in Mice with Deficiencies of the Plasminogen System

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Thromboelastography (TEG; Haemoscope, Niles, IL, USA) is a whole blood coagulation assay that measures clot formation, strength, and stability. This tool has traditionally been used in the clinical setting to help manage acute bleeding in liver transplantation and cardiac surgery. Other clinical applications of the TEG have been in areas of trauma and obstetrics.

In this study, mice with genetic deficiencies of the plasminogen pathway were used to evaluate the time to initial clot formation (R time), the rate of clot development (K and angle), and the maximum clot strength (MA). Wild-type (WT) and mice with deficiencies in the plasminogen pathway that were used include plasminogen deficient ($PG^{-/-}$), urokinase-type plasminogen activator deficient ($UPA^{-/-}$), tissue plasminogen activator deficient ($TPA^{-/-}$), plasminogen activator inhibitor-1 deficient ($PAI\text{-}1^{-/-}$), expression of a mutant PAI-1 that can not bind to vitronectin ($PAI\text{-}1^{R101A/Q123K}$), anti-plasmin deficient ($AP^{-/-}$) and fibrinogen deficient ($FG^{-/-}$). Compared to WT mice $PG^{-/-}$, $UPA^{-/-}$, $PAI\text{-}1^{-/-}$, and $PAI\text{-}1^{R101A/Q123K}$ mice had increased R values. Additionally, $PAI\text{-}1^{-/-}$ and $UPA^{-/-}$ demonstrated increases in the K and angle. $PG^{-/-}$, $PAI\text{-}1^{-/-}$, $PAI\text{-}1^{R101A/Q123K}$, $UPA^{-/-}$, $TPA^{-/-}$, and $AP^{-/-}$, all showed lower MA values. $FG^{-/-}$ mice never clotted; therefore, a flat line was observed. In humans with a total deficiency in PAI-1, it has been reported that these patients have a tendency to bleed. This correlates with our findings on the TEG with the $PAI\text{-}1^{-/-}$ mice.

The coagulation and fibrinolytic profiles of these mice as measured by TEG can be used to facilitate the interpretation of TEG profiles in patients with imbalances in hemostasis.

30. Transposition Characterization of The Transcriptional Regulator MecI from *Staphylococcus aureus*

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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.

31. Hybrid QM/MM Studies of the Reaction Energetics of Lytic Transglycosilase E (MltE) of *Escherichia coli*

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Among the seven lytic transglycosilases of *Escherichia coli*, only MltE appears to be primarily endolytic in its cleavage of the β-1,4-glycosidic bond between the MurNAc and GlcNAc sugars of the cell-wall peptidoglycan. The reaction is thought to occur in four steps that involve higher-energy sugar conformations, leading to the formation of a 1,6-anhydro-N-acetyl-muramyl moiety in the peptidoglycan. With the recently constructed computational model of the peptidoglycan bound to MltE in hand, we have carried out hybrid quantum mechanical/molecular mechanical (QM/MM) studies to elucidate reaction energetics. We utilized the ONIOM method implemented in *Gaussian 09* with the density functional theory methods to calculate potential energy surfaces of each reaction step. A fully *ab initio* model system in the absence of the enzyme was studied first, followed by the QM/MM calculations of the full enzymatic system. The energy landscapes provided important insights of how the MurNAc conformational changes are brought about within the enzyme and their energetics. We observed that certain transformations that appear to be readily feasible in the model system would either prefer a slightly different path or would not happen at all within the enzyme environment.

32. NMR ^{13}C-^{1}H and ^{13}C-^{13}C Spin-couplings in Aldofuranosyl Rings: DFT Studies of Structural Correlations as a Foundation for Quantitative Analyses of *J*-Coupling Ensembles by *MA'AT*

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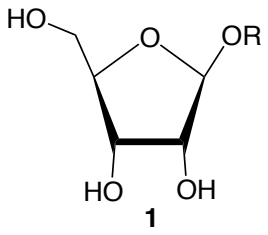
Conformational flexibility is a hallmark of the aldofuranose rings of nucleic acids, such as RNA and DNA, and other biologically relevant oligo- and polysaccharides. The conventional north/south (*N*/*S*; C3'-*endo*/C2'-*endo*) equilibrium commonly invoked to explain the behaviors of these rings is based largely on experimental observations made on model systems in the solid state (x-ray crystallography). Solution studies by NMR are largely confined to the observation of ^{1}H-^{1}H spin-couplings (*J*-couplings) and nuclear Overhauser effects (NOEs), and to a lesser degree, residual dipolar couplings (RDCs), which are used collectively to determine which regions of the pseudorotational itinerary are preferred and to establish conformational equilibria. For example, rings having the β-D-ribofuranose configuration **1** contain three $^{3}J_{HH}$ values ($^{3}J_{H1,H2}$, $^{3}J_{H2,H3}$, $^{3}J_{H3,H4}$) to describe an itinerary comprised of 20 conformers (*P* values), and considerably more conformers are potentially accessible if puckering amplitudes (τ values) are added to the mix. Clearly this system is severely under-determined. To address this limitation, we are investigating, by DFT,[1] NMR *J*-couplings in these rings involving carbon, specifically J_{CH} and J_{CC} values over 1–3 bonds that show strong sensitivities to ring conformation.[2] As shown recently for conformational analyses of *O*-glycosidic linkages in oligosaccharides using the computer program *MA'AT*,[3] we aim to interpret this ensemble2 of *J*-values quantitatively in order to place conformational assignments of aldofuranose rings in solution on firmer experimental ground and validate structural predictions made from MD simulations of these systems.



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33. Regulation of The Oncogene ZNF217 by Cellular Localization During Breast Cancer Progression

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Breast cancer is second only to lung cancer as the leading cause of cancer deaths in women. To reduce breast cancer mortality, additional research is necessary in order to identify marker genes that can be used to predict outcome in patients, to understand the nature of disease progression, and to identify effective therapies for these patients. 20q13.2 is a region in the human genome that may contain a gene with clinical prognostic or therapeutic value. This region is commonly amplified in breast cancer and correlates strongly with poor patient prognosis. A candidate driver gene in 20q13.2 is the transcription factor ZNF217. We previously identified ZNF217 as a biomarker of poor prognosis in breast cancer patients. We also developed mouse models that overexpress Znf217. Using these models, we found that ZNF217 supports cancer progression, promotes metastasis, and is a novel therapeutic target. We now want to understand how localization of ZNF217 influences cancer progression. We found that ZNF217 protein is expressed most strongly in a small subset of cells within normal mammary epithelium and localizes predominantly in the nucleus in normal tissues. However, the localization of ZNF217 is heterogeneous in breast tumors: ZNF217 localization can be either nuclear or cytoplasmic. Interestingly, we also identified a truncated cytoplasmic form of ZNF217 that is induced after growth factor stimulation. Based on these initial findings, we hypothesize that ZNF217 localization affects ZNF217 function during cancer progression. We currently are characterizing the regions required for cellular localization. We also are using a candidate approach to identify the protease required for post-translational cleavage of ZNF217. Once we identify mutants of ZNF217 with cytoplasmic localization patterns, we will determine if this localization is required for tumorigenicity in vivo. In parallel, to determine if ZNF217 has prognostic value in breast cancer patients, we also are comparing ZNF217 localization (e.g., nuclear, cytoplasmic, or both) in human breast tumors with the outcome in patients. This study will be used to validate ZNF217 as a prognostic indicator of breast cancer and to determine if ZNF217 localization is a reasonable marker in the clinical setting for the treatment of breast cancer patients.

34. Slow Conformational Dynamics of Side Chains in Substrate Binding: Application to Human Pin1

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Pin1 is a peptidyl prolyl cis-trans isomerase enzyme that regulates diverse cellular processes and relates to numerous diseases, including Alzheimer's disease and cancer. Pin1 contains a WW domain and a catalytic isomerase (PPIase) domain. Both domains recognize phospho-Ser/Thr-Pro (pS/T-P) motifs, such as the phosphorylated mitotic phosphatase Cdc25 peptide (pCDC25). WW domain has about ten-fold higher binding affinity for peptides than the PPIase domain *in vitro*. PPIase domain catalyzes the *cis-trans* isomerization of pS/T-P motifs. Previous work has explored slow (μs-ms) motions of backbone NHs and fast (sub-nanosecond) motions of side chain methyl groups. But, to date, there has been no investigation of the slow (μs-ms) motions of the side chains.

Accordingly, we have investigated the side chain μs-ms dynamics of Pin1 via relaxation dispersion measurements involving Carr-Purcell-Meiboom-Gill (CPMG) spin-locking. Our experiments probe the ε-N of Trp and Arg, and the ^{13}C of methyl group of Val, Leu, and Ile (Fig. 1). Residues Arg and Trp are interesting because they often take part in the ligand binding. Methyl groups usually compose the hydrophobic center of the protein, and have high quality NMR signal compared to other side chain. We specifically labeled nitrogen atoms with ^{15}N and carbon atoms of methyl group of Val, Leu, and Ile with ^{13}C (Fig.1).

Our results show that the binding of WW domain with pCDC25 promotes the binding of WW domain to the PPIase domain. The tumbling of WW domain and PPIase domain becomes coherent with the pCDC25 binding. From 13C dispersion experiments, we found that I78CD, V55CG1, L60CD1, V62CG1, V62CG2, and L160CD2 are very interesting and may be related to the biological functions.

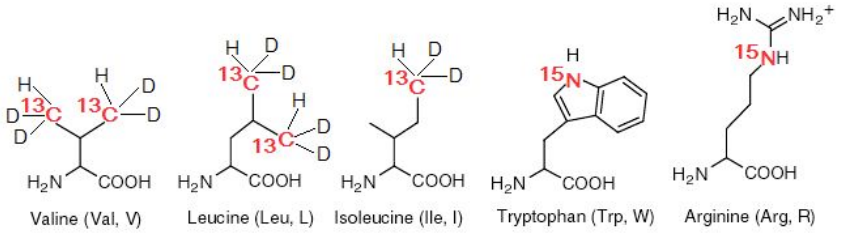

Fig. 1. The interested side chain atoms in red investigated by the CPMG dispersion experiments.

35. 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. We used the x-ray structure of penicillin-binding protein (PBP)-2a, an enzyme involved in bacterial cell wall assembly, to screen *in silico* over 1 million compounds. The 1,2,4-triazolo[1,5-*a*]pyrimidine template emerged from this screening with modest *in vitro* antibacterial activity. The syntheses and structure-activity relationships (SAR) of this series are described. 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. 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*. A clear SAR requirement has been established for optimal antibiotic activity.

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36. Thiirane-Based MMPs Inhibitors with Increased Water Solubility: Synthesis, Kinetic Characterization, Pharmacokinetics and Brain Distribution

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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).[1] SB-3CT (2-(4-phenoxyphenylsulfonylmethyl)thiirane), a selective and potent thiirane-based gelatinase inhibitor,[2] has been shown to be effective in a number of animal models, including cancer metastasis, stroke, traumatic brain injury, and diabetic wound healing;[3-5] however, it is limited by its poor aqueous solubility (2.3 μg/mL) and extensive metabolism[6-8]. We addressed these issues by blocking the primary site of metabolism and introducing polar groups to achieve increased solubility. Among the inhibitors studied, *p*-guanidino-SB-3CT (1-(4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy)phenyl)guanidine) shows very good potency of inhibiting MMPs (K_i: MMP-2: 21 nM; MMP-8: 350 nM; MMP-9: 93 nM; MMP-14: 40 nM) and good aqueous solubility. A pharmacokinetics and brain distribution study of this compound was conducted in mice after a single 5 mg/kg intravenous dose. *p*-Guanidino-SB-3CT distributed to the brain ($AUC_{brain/plasma}$ = 0.20 and Vd = 0.41 L/kg) and is highly cleared (CL = 110 mL/min/kg), with a plasma terminal half-life of 50 min. The terminal half-life in brain was 5.5 h. While *p*-guanidino-SB-3CT has increased water solubility and potency in inhibition of MMP-9 compared to SB-3CT, its spectrum of inhibition extends to MMP-8. Additional analogs with increased solubility were synthesized and evaluated. One of these analogs, *p*-aminomethyl-SB-3CT ((4-(4-((thiiran-2-ylmethyl)sulfonyl)phenoxy)phenyl)methanaminium), shows selectivity in targeting the gelatinases and MMP-14 (K_i: MMP-2: 85 nM; MMP-9: 150 nM; MMP-14: 120 nM) and is a promising compound in treatment of gelatinase-dependent diseases.

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37. Novel Preclinical Animal Models of Chemotherapy Resistance Using Patient-derived Breast Tumor Xenografts

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Breast cancer is the second leading cause of cancer related deaths behind lung cancer with an estimated 232,340 new cases and 39,620 deaths this year. Despite 93% of patients being diagnosed with local or regional stage of the disease (SEER database), most patient deaths result from metastasis as a result of recurrent disease by therapeutic resistance.

Current understanding of the molecular mechanisms of therapeutic resistance and metastasis has suffered because of the lack of appropriate model systems that accurately and reproducibly recapitulate human disease. It is critical to develop better models to test new therapeutic regimens aimed at overcoming resistance and decreasing metastatic disease. The proposed research will address these issues with the establishment of both *in vitro* and in *vivo* model systems that utilize patient-derived breast tumor tissues. These tumor grafts have previously been validated to reflect tumor pathology, growth, metastasis, and disease outcomes and will advance our understanding of the mechanisms involved in recurrence and metastasis.

Using *in vitro* organoid cultures we will characterize and quantify metastatic phenotypes and develop mechanistic insight into what promotes therapeutic resistance. Creating *in vivo* models will allow us to test novel treatment strategies using validated model systems of human breast cancer. Development and characterization of these model systems will provide new insight into the mechanistic understanding of therapeutic resistance and metastasis.

38. ADAM10/Kuzbanian is Upregulated During Neuroendocrine Prostate Carcinogenesis

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Prostate cancer (CaP) is the second leading cause of cancer death in men, behind only lung cancer. Advanced prostate tumors often contain neuroendocrine differentiation, which correlates with androgen-independent progression and consequently poor prognosis. Currently, androgen deprivation is the first line of therapy for metastatic prostate cancer. However, prostate cancer in patients often progresses to an androgen-independent bone-metastatic stage, where chemotherapy and radiotherapy are the primary therapeutic options. Despite recent advances in therapeutic strategies, many malignant cancers still develop resistance to radiation and targeted therapies. Thus, it is important to pursue new therapeutic factors that may have the potential to improve the lives and survival rates of patients.

A family of metalloproteinases known as the ADAMs is actively involved in these signaling pathways due to their role in releasing the extracellular domain of transmembrane proteins. The term "ADAM" stands for a disintegrin and metalloproteinase, which represents the two key structural domains in these molecules. ADAMs contain features of both adhesive proteins *and* proteinases. ADAM10 activates two important signaling systems controlling cell growth, invasion, and metastasis: the epidermal growth factor receptor (EFGR) and NOTCH signaling systems.

In this study, we looked at ADAM10 expression during neuroencocrine cancer progression in the mouse cryptdin-2 SV40-TAg (CR2-TAg) mouse model. These mice have a stereotypical pattern of tumorigenesis and metastasis. ADAM10 expression increased over time in these tumors. Matrix metalloproteinases (MMPs) are a family of enzymes that remodel the microenvironment and are associated with tumorigenesis and metastasis. Like ADAM molecules, they are capable of degrading many extracellular matrix proteins and cell surface receptors. Therefore, we also will determine if MMPs (MMP 2, 7 and 9) contribute to ADAM10 expression or localization by staining tissue sections from the CR2-TAg mice that are each missing expression of MMPs to see if that particular MMP contributes to ADAM10 expression or localization. In addition, we will quantify the expression levels and activity of ADAM10 over time using human tumor xenografts. We will treat these xenografts with inhibitors to ADAM10 to assess their ability to suppress prostate cancer progression.

39. ZNF217 interacts with the Tumorigenic Isoform of Pyruvate Kinase PKM2

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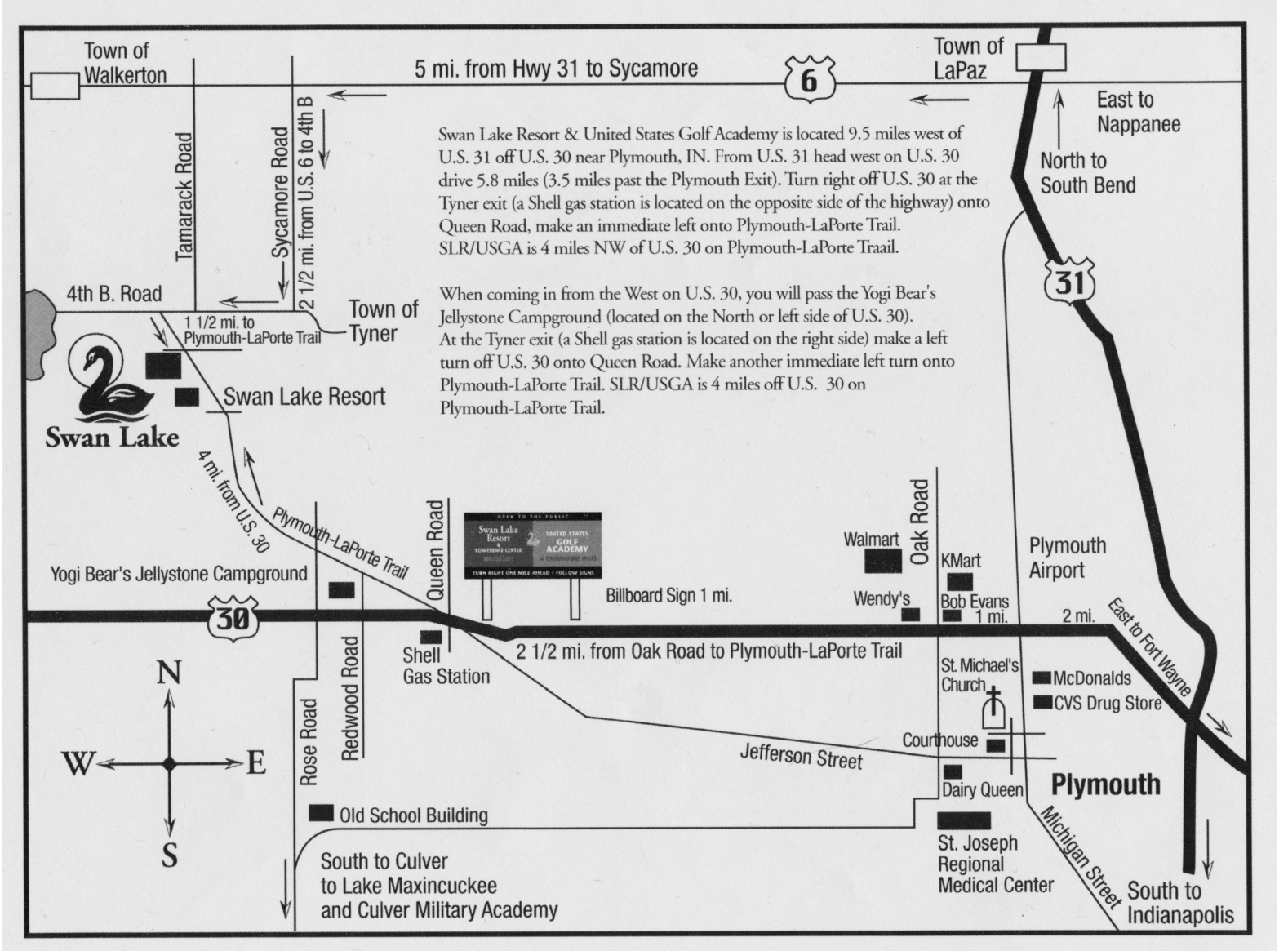
Aggressive tumor behavior and poor prognosis in many cancers, including breast, correlate with amplification of chromosome region 20q13.2. A candidate oncogene within this region is the transcription factor ZNF217, which is a protein that contains eight zinc finger binding domains and a proline-rich region. ZNF217 overexpression can immortalize human mammary epithelial cells and inhibit apoptotic signal pathways. We previously found that ZNF217 overexpression in breast cancer patients is prognostic of reduced survival, increased metastasis, and increased chemoresistance. We also found that Znf217 overexpression promotes cancer phenotypes in both tissue culture cells and mouse models. To better understand how ZNF217 promotes cancer, we next identified proteins that interact with ZNF217 by two-hybrid screen and co-localization studies. We identified the M2 isoform of pyruvate kinase (PKM2) as a protein that interacts with ZNF217. Pyruvate kinase is a key enzyme of glycolysis that catalyzes the production of pyruvate and ATP by dephosphorylation of phosphoenolpyruvate (PEP). Many tumor cells have elevated rates of glucose uptake but reduced rates of oxidative phosphorylation and high lactate production. These metabolic changes allow tumors to evolve and survive under oxygen starvation.

Most normal cells express the M1 isoform of pyruvate kinase. In contrast, the PKM2 isoform is overexpressed in all proliferating cells with high levels of nucleic acid synthesis, including embryonic cells, adult stem cells, and tumor cells. The PKM2 isoform promotes production of lactate, rather than oxidative phosphoryalation, and an accumulation of upstream glycolytic metabolites that can be used in nucleic acid, amino acid, and phospholipid synthesis. The PKM2 dimer is the predominant form found in tumors. We found that ZNF217 and PKM2 co-localize within the nucleus and cytoplasm, which is consistent with a direct interaction between these molecules. This finding also suggests a possible role for ZNF217 in tumor metabolism.

We currently are investigating the significance of the interaction between ZNF217 and PKM2 during breast cancer progression and during tumor metabolic pathways in order to elucidate the molecular mechanisms of ZNF217 in cancer progression. These studies could lead to potential early cancer detection and appropriate treatment in breast cancer patients.


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