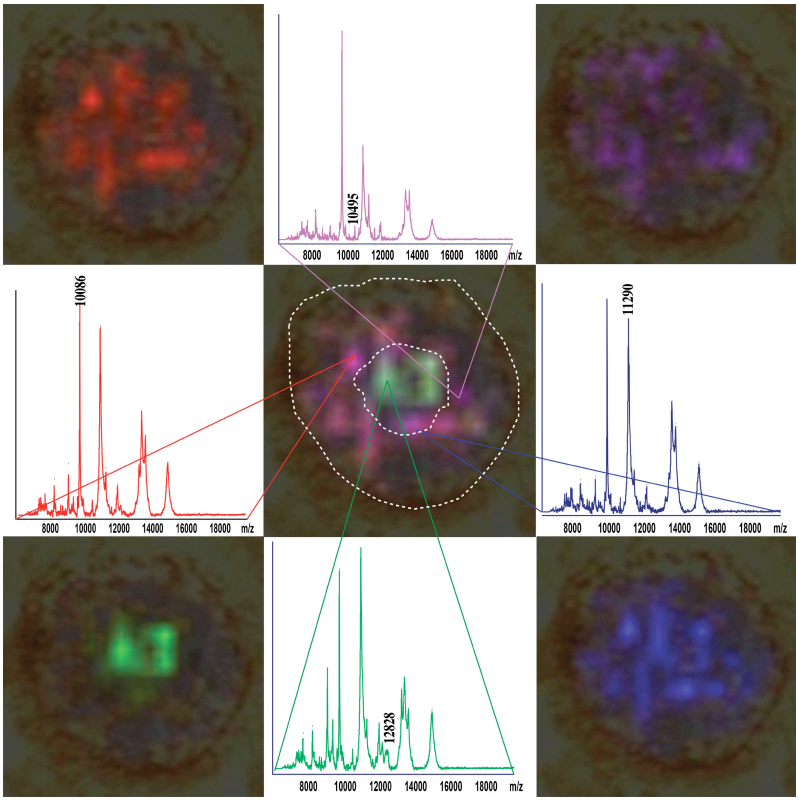
The Bretthauer Papers

17th ANNUAL BIOCHEMISTRY RESEARCH FORUM

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



Swan Lake Resort
Plymouth, Indiana
June 13, 2012

♦ Organizer ♦

Anthony Serianni

♦ Forum Sponsors ♦

University of Notre Dame
W. M. Keck Center for Transgene Research
Department of Chemistry and Biochemistry
Notre Dame Office of Research
Interdisciplinary Center for the Study of Biocomplexity
Notre Dame Integrated Imaging Facility

John V. O'Connor, PhD

Cambridge Isotope Laboratories, Inc.

Isotec, Inc.

Enzyme Research, Inc.

Omicron Biochemicals, Inc.

Cover Illustration

Courtesy of the Hummon Lab

Cover Legend. Spatial distribution of analytes in a single colon adenocarcinoma spheroid determined by matrix-assisted laser desorption/ionization mass spectrometry imaging. Ion intensity maps and mass spectra are displayed for four species. While *m/z* 12828 is located predominately in the central necrotic core, the distribution of other species is more widespread throughout the structure.

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)

♦ 2012 Keynote Lecture ♦

Vern L. Schramm

Professor and Ruth Merns Chair in Biochemistry
Albert Einstein College of Medicine
Yeshiva University

Drug Design from Transition State Analysis

Abstract

Transition state theory proposes that chemically stable mimics of the transition state will bind more tightly than substrates by the rate acceleration factor imposed by the enzyme, typically 10^{10} to 10^{15}. Transition state structures of enzymatic targets for cancer, autoimmunity, malaria and bacterial antibiotics have been explored by the systematic application of kinetic isotope effects and computational chemistry. Analogues of the transition states deduced by these methods bind up to 10^{8} times tighter than substrate. Four generations of transition state analogues have been produced for human purine nucleoside phosphorylase (PNP) and two of these are in clinical trials. Transition state analogues of human methylthioadenosine phosphorylase show promise as anticancer agents in mouse xenografts. Malaria parasites are purine auxotrophs and require host and parasite-specific salvage enzymes. Powerful transition state analogues of PNP cause purine starvation (purine-less death) in parasites cultured in human erythrocytes. Transition states of bacterial metylthioadenosine hydrolases have permitted the design of inhibitors of bacterial quorum sensing. Application of kinetic isotope effects and transition state theory have permitted synthesis of some of the most powerful enzymatic inhibitors. Insights into catalysis are provided by use of transition state analogues to probe enzymatic catalytic function. See: Enzymatic transition states, transition state analogs, dynamics, thermodynamics and lifetimes. *Annual Review of Biochemistry* (2011) 80:703-732.

Biography of Vern L. Schramm

A native of South Dakota, Schramm studied chemistry, microbiology, nutrition and enzymatic mechanisms at South Dakota State University, Harvard and the Australian National University. After postdoctoral studies at the NASA Ames Research Center he began his research in transition state analysis at the Departments of Biochemistry at Temple University School of Medicine and the Albert Einstein College of Medicine. He is the Ruth Merns Chair of Biochemistry. Schramm pioneered the use of kinetic isotope effects and computational chemistry to understand enzymatic transition states. Knowledge of the transition-state permits design of powerful inhibitors. Two of the inhibitors designed by the Schramm laboratory have entered clinical trials and others are in earlier stages of development.

Honors in recognition of his contributions to research and teaching include a Merit Award from the NIH, election as a Fellow of the American Association for the Advancement of Science, the Rudi Lemberg Award from the Australian Academy of Science, the George A Sowell Award for Excellence in Teaching from Temple University School of Medicine, and the Harry Eagle Award for Outstanding Basic Science Teaching from the Albert Einstein College of Medicine. Schramm received the Repligen Award from the Biological Chemistry Division of the American Chemical Society in 2006 and in 2007 he was elected to membership in the National Academy of Sciences.

Program

Wednesday Morning
Session Chair: Patricia Clark

8:30-9:00 Continental breakfast

9:00-9:10 Introduction and Orientation

****

9:10-9:30 Emily L. Ottenweller, Daniel F. Lyons, Ryan J. Murphy, Julia T. Philip, Aranda R. Slabbekoorn, John J. Correia and Holly V. Goodson
Does EB1 Bind to the Microtubule Seam or to the Lattice?

9:30-9:50 Michelle Bertke, Oliva Cox, Erliang Zeng and Paul Huber
The Role of SUMOylation in Early Development of Xenopus laevis

9:50-10:10 Esther Braselmann and Patricia L. Clark
Investigating Correlations Between In Vitro Autotransporter Folding Properties and In Vivo Secretion

10:10-10:40 Mid-Morning Break

10:40-11:10 **Guest Lecture**
M. Sharon Stack
Department of Chemistry and Biochemistry/Harper Cancer Research Institute, University of Notre Dame
Modeling Epithelial Ovarian Carcinoma Metastasis

11:10-11:30 Emmanuel Adu-Gyamfi and Robert V. Stahelin
Molecular Architecture of Ebola Virus Assembly

11:30-11:50 Kerry M. Bauer and Amanda B. Hummon
Interrogating the Effect of a miRNA Cluster on the Colon Cancer Transcriptome and Proteome

11:50-12:10 Kristofor E. Glinton, Victoria A. Ploplis and Francis J. Castellino
Host-Pathogen Interactions in Group A Streptococcus: Characterization of M1 Protein-Human Fibrinogen Binding

****

12:10-2:00 Lunch and Recreation

****

Wednesday Afternoon
Session Chair: Brian Baker

2:00-2:30 **Guest Lecture**
Allen G. Oliver
Department of Chemistry and Biochemistry, University of Notre Dame
Molecular Structure Facility
X-ray Crystallography: A Complementary Characterization Technique

2:30-2:50 Jill Bouchard, David A. Case and Jeffrey W. Peng
Investigating the Functional Long-Range Order of Human Pin1

2:50-3:10 Vishwanatha K. Chandrahas, Victoria A. Ploplis and Francis J. Castellino
Structure-Function Relationships Between Host Fibrinolytic System, M or M-like Proteins, and Streptokinase in GAS Virulence

3:10-3:30 Afternoon Break

3:30-4:00 **Guest Lecture**
Joshua D. Shrout
Department of Civil Engineering and Geological Sciences, University of Notre Dame
The Influence of Surface Environment upon Group Behavior and Motility of Bacteria

4:00-4:20 Ning Wang and Paul W. Huber
Mechanism of Titanium Dioxide Nanoparticle Toxicity in Developing Embryos of Xenopus laevis

****

4:20-5:00 Late Afternoon Break

****

Wednesday Evening

5:00-6:00 **Keynote Lecture**
Vern L. Schramm
Drug Design from Transition State Analysis

6:00-7:30 Poster Session and Reception

****

7:30-9:30 Dinner and Presentation of Plaques

9:30 Departure

ABSTRACTS: GUEST LECTURES

Modeling Epithelial Ovarian Carcinoma Metastasis

M. Sharon Stack
Department of Chemistry and Biochemistry, and Mike and Josie Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN 46556 USA

Metastasis of epithelial ovarian cancer (EOC) is initiated by shedding of single cells and E-cadherin-rich multi-cellular aggregates (MCAs) from the primary tumor into the peritoneal cavity, which often contains large volumes (0.5- 5.0 l) of ascites fluid. These free-floating cells and MCAs undergo subsequent integrin-mediated intra-peritoneal (ip) adhesion to interstitial collagens of the peritoneal cavity, promoting metastatic anchoring and growth of secondary lesions. As cells respond to changes in the physical properties of the microenvironment with altered signaling, gene expression, and behavior, a major focus of our group is to develop organotypic models that mimic key events in ip metastasis. Current studies are focused on understanding (1) the transition between suspended cells/MCAs and matrix anchored metastases and (2) the contribution of ascites fluid to altered ip mechanobiology. Using both 3-dimensional collagen cultures and microsphere-immobilized anti-integrin antibodies to evaluate the effect of integrin engagement on the functional integrity of cell-cell contacts, our results show a loss of junctional E-cadherin corresponding with nuclear translocation of β-catenin. Modulation of Wnt/β-catenin target gene expression is also observed, including a prominent downregulation of the Wnt inhibitor Dickkopf (Dkk) in response to integrin engagement. Loss of Dkk is correlated with enhanced expression of membrane type 1 matrix metalloproteinase (MT1-MMP) and increased expansive growth in and invasion of 3-dimensional collagen gels. As a first approximation to assess the contribution of ip fluid pressure on EOC cells, MCAs were cultivated in fluid filled sacs and subjected to compression (25 mm Hg) using an Instron. Compression differentially affected cell proliferation and gene expression. Interestingly, expression of the ligand Wnt5a was enhanced under compression, and addition of exogenous Wnt5a regulated cell-matrix adhesion. As activating mutations in Wnt/β-catenin signaling are rare in most histotypes of EOC, these data support a mechanism for matrix and mechanical activation of Wnt/β-catenin signaling in metastatic EOC cells.

Molecular Structure Facility
X-ray Crystallography: A Complementary Characterization Technique

Allen G. Oliver
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

The Molecular Structure Facility, located in 246 Nieuwland Science Hall, houses three start-of-the-art single crystal diffractometers and one powder diffractometer. This resource enables the study of the 3-D, solid state, composition of molecules. An overview of the facility and what it can offer researchers to enhance their science will be presented.

The Influence of Surface Environment upon Group Behavior and Motility of Bacteria

Joshua D. Shrout
Civil Engineering and Geological Sciences, University of Notre Dame,
Notre Dame, IN 46556 USA

Bacteria display several types of group behavior where their gene expression profiles and activity change with population size or density. Among the best-characterized examples of group behaviors for bacteria are: 1) growth of attached-growth biofilms and 2) intracellular quorum sensing signaling. The bacterium *Pseudomonas aeruginosa* is both a robust biofilm-forming organism and a quorum sensing organism. In fact, the acyl homoserine lactone (AHL)-based quorum sensing network of *P. aeruginosa* has been strongly linked with its biofilm development. In the Shrout laboratory, we have been studying how community behaviors such as quorum sensing influence motility, which can dictate how bacteria attach to surfaces and initiate biofilm formation.

Several environmental factors have been observed to influence bacterial motility, but few studies have linked environmental cues with bacterial mechanism. We have been studying a surface mode of motility called "swarming" and have used swarm phenotypes to identify that AHL quorum sensing is specifically inhibited when *P. aeruginosa* grows on harder surfaces. Quorum sensing is important to swarming because the quorum sensing regulon controls production of the *P. aeruginosa* surfactant rhamnolipid, which aids cell movement during swarming. Despite large populations of cells on these harder surfaces, cell populations near the advancing swarm edge were not induced for quorum sensing. This suggests that quorum sensing on surfaces is controlled in a manner that is not solely population dependent. We continue to investigate regulation of quorum sensing on surfaces as this may be one mechanism used by *P. aeruginosa* to discern between differing surfaces; understanding this process may allow improvements in biofilm control and treatment of many bacterial infections.

ABSTRACTS: ORAL PRESENTATIONS

Does EB1 Bind to the Microtubule Seam or to the Lattice?

Emily L. Ottenweller[a], Daniel F. Lyons[b], Ryan J. Murphy[a], Julia T. Philip[a], Aranda R. Slabbekoorn[a], John J. Correia[b], and Holly V. Goodson[a]*
[a]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA; [b]Department of Biochemistry, University of Mississippi Medical Center, Jackson, MS 39216 USA

EB1 is a highly conserved microtubule plus end tracking protein (+ TIP) involved in regulating microtubule dynamics, but the mechanisms of its +TIP behavior and MT stabilizing ability remain undefined. Microtubules consist of protofilaments associated in a cylindrical lattice that closes upon itself at a seam; this seam is generally believed to be weaker than the rest of the lattice. Previous electron microscopy has suggested that EB1 binds specifically to this seam, implying that EB1 promotes MT polymerization by stabilizing the seam (Sandblad *et al*., 2006). Microtubule binding experiments were interpreted as supporting the idea that EB1 binds strongly to the MT seam, providing further evidence for this model (Sandblad *et al*., 2006). However, close inspection of the data suggests that the reported MT binding experiments are equally consistent with weak binding along the microtubule body. Moreover, the published work was performed with S. pombe Mal3, leaving open the question of how mammalian EB1 behaves. To resolve the question of whether EB1 binds primarily to the MT seam, we are using a combination of MT binding assays and theoretical modeling with MTBindingSim. Our results argue against strong seam binding for EB1, and suggest instead that binding of EB1 to stabilized MTs is best explained by weak binding along the lattice, perhaps with parallel binding to the seam. These observations will help establish how EB1 interacts with microtubules, which in turn is essential for fully understanding how cells regulate the dynamics of the microtubule plus end.

The Role of SUMOylation in Early Development of *Xenopus laevis*

Michelle Bertke, Oliva Cox, Erliang Zeng and Paul Huber
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

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 neural tube (spina bifida) and cardiovascular malformations. Total mRNA was isolated from Gam1 or water injected embryos at three time points during embryogenesis (early gastrula, late gastrula, and early neurula) and quantitated in microarray experiments. Lists of differentially expressed genes 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. For example, a major pathway, controlled by the transcription factor ETS1, has been implicated in proper patterning of the early embryo and in heart development, both of which are disrupted in SUMO deficient *Xenopus* embryos. SUMOylation regulates ETS1 activity and the expression of several members of this pathway is affected in Gam1-injected embryos. Pathway analysis has also pointed to SMAD4 and TGF_-receptor 1, both of which are SUMOylated, to be major regulators of blastopore/neural tube closure and heart development that are controlled by this post-translation modification.

Investigating Correlations Between *In Vitro* Autotransporter Folding Properties and *In Vivo* Secretion

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

Autotransporter (AT) proteins are virulence factors in Gram-negative bacteria. They are synthesized with an N-terminal signal sequence, which is cleaved after inner membrane secretion, a central passenger domain (the mature protein), and a C-terminal outer membrane (OM) porin domain. The porin 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. Here, we are using a novel assay to probe the conformation of the pertactin passenger domain in the periplasm. We were able to confirm that pertactin adopts a soluble but unstable conformation in the periplasm, consistent with our proposed secretion mechanism. Presently, we are using the same assay to select random pertactin point mutants with improved ability to fold in the periplasm. We will investigate how these mutations affect secretion, and whether correlations exist between *in vitro* folding properties of the passenger domain, periplasmic folding, and secretion efficiency.

Molecular Architecture of Ebola Virus Assembly

Emmanuel Adu-Gyamfi[1], and Robert V. Stahelin[1,2]
[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame IN 46556 USA, and [2]Department of Biochemistry and Molecular Biology, IUSM-SB, South Bend, IN 46617 USA

Ebola belongs to the *filoviridae* family of viruses. Its pathogenesis 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 prescribed treatment, Ebola is classified as a bio-safety level IV agent with the potential to be used as a biological weapon. While mechanistic details of the virus assembly process are lacking, recent evidence suggest that the major matrix protein of the virus; VP40 is the main driving force for assembly from the plasma membrane. Generation of new virus involves a cascade of cellular events that recruit the viral genome, the matrix proteins and subsequent acquisition of the viral envelope from the host cell. The new virus or virus like particle (VLP) forms at a bud site at the inner leaflet of the plasma membrane and can serve as a primary therapeutic target for inhibiting Ebola virus replication. Preliminary results demonstrate that VP40 alone assembles in mammalian cells into VLPs independent of six other viral proteins by binding 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. The goal of this project is to elucidate the mechanistic details of VP40 assembly on the plasma membrane using an interdisciplinary approach. Specifically, we have employed in vitro lipid binding and curvature assays with cellular scanning and single molecule microscopy to investigate the basis of VP40 lipid binding, membrane bending and viral egress. Our results further demonstrate that VP40 oligomerizes on the plasma membrane in a PS-dependent manner and also remodels the 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 filoviruses.

Interrogating the Effect of a miRNA Cluster on the Colon Cancer Transcriptome and Proteome

Kerry M. Bauer and Amanda B. Hummon
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

MicroRNAs (miRNAs) are an abundant class of endogenously expressed non-coding RNA molecules. miRNAs function to activate the RNA interference (RNAi) pathway to sequester or degrade target mRNA, resulting in translational inhibition. They regulate genes in a sequence-specific manner involving complementary base pairing between the miRNA seed region and the target mRNA. Due to promiscuous base pairing, a single miRNA can potentially regulate hundreds of genes. While proper miRNA expression and function are critical for the health of normal tissues, recent evidence has shown that mutations and/or misexpression of miRNA correlate with various human cancers and have critical functions in cancer progression. With the loss of chromosomal region 5q32 in most colon cancers, the expression of the miRNA cluster comprised of miR-143 and miR-145 is reduced.

We have validated that miR-143 and miR-145 expression levels are significantly reduced in several human colon cancer cell lines and FFPE colon cancer tissue samples compared to normal colon mucosa. We have reintroduced the miR-143/miR-145 cluster of miRNAs commonly lost in colon cancer. Following transfection of these miRNAs into a human colon cancer cell line, we evaluated the effects of these molecules on the colon cancer transcriptome and the proteome using microarrays, quantitative mass spectrometry and qRT-PCR to interrogate the effect of miR-143 and miR-145 on the colon cancer transcriptome and the proteome. Global gene expression profiles following the reintroduction of miR-143 and/or miR-145 were assessed with Affymetrix GeneChip Human Exon 1.0 ST Arrays. The proteome was evaluated by high-resolution mass spectrometry using a Thermo Scientific LTQ Orbitrap-Velos. Stable Isotope Labeling of Amino Acids in Cell Culture (SILAC) was used to quantify the global protein expression levels. The microarray and SILAC mass spectrometry derived molecular expression ratios for potential miRNA targets were validated by qRT-PCR and selective reaction monitoring (SRM), respectively. Within our dataset, we aim to address a primary questions regarding miRNA function and gene regulation. Do you need to consider the entire cluster, or can you evaluate a single miRNA at a time, following current practice in miRNA research?

Host-Pathogen Interactions in Group A Streptococcus: Characterization of M1 Protein-Human Fibrinogen Binding

Kristofor E. Glinton, Victoria A. Ploplis and Francis J. Castellino
The W. M Keck Center for Transgene Research, and the Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, 46556 USA

Group A Streptococcus (GAS) is a human-specific pathogen responsible for producing a wide variety of pathological manifestations. These include mild superficial infections such as impetigo and strep throat, as well as more severe sequelae such as the recently publicized necrotizing fasciitis. During infection GAS is able to take advantage of the host fibrinolytic system to aid in adhesion, penetration and evasion of the host immune response. M and M-like proteins are surface exposed α-helical coiled-coil proteins that interact with a variety of host proteins. One such key interaction has been observed between M1 of GAS strain SF370 and human fibrinogen (hFg), resulting in multiple beneficial outcomes for the bacteria particularly the recruitment of human plasminogen (hPg) to its surface. It is proposed that hPg is bound to the surface indirectly through hFg and is activated non-enzymatically via the binding of GAS secreted streptokinase (SK) or through host activators such as tissue plasminogen activator and urokinase plasminogen activator. Previous studies have shown the B1B2 region of M1 to be necessary for hFg binding. This region consists of a repeated sequence of amino acids near the variable *N*-terminus of the protein. 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. In an effort to minimize the functional hFg binding unit of the protein M1(G43-E232), M1(D120-E201) and M1(D129-E194) were expressed and kinetic and structural studies were carried out using SPR and CD spectrometry respectively to determine their structure-function relationship. Presently however, these studies revealed reduced affinity compared to the full length structure. Such studies are beneficial in the elucidation of the mechanism of invasion and in the development of better vaccines to prevent GAS infections.

Investigating the Functional Long-Range Order of Human Pin1

Jill Bouchard[1], David A. Case[3] and Jeffrey W. Peng[1,2]
[1]Department of Chemistry and Biochemistry and [2]Department of Physics, University of Notre Dame, Notre Dame, IN 46556 USA, and [3]BioMaPS Institute and Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854 USA

Modular (multi-domain) proteins figure prominently in the interaction networks regulating the cell cycle. The human peptidyl-prolyl isomerase Pin1 is a modular protein well-recognized for mitotic regulation, and is of therapeutic interest for both cancer and Alzheimer's disease. We have investigated the inter-domain motions of Pin1 by measuring backbone (^{15}N-^{1}H) residual dipolar couplings (RDCs) by nuclear magnetic resonance (NMR) spectroscopy. To revel the atomic basis for these RDCs, we have developed a methodology in the molecular dynamics suite, AMBER, to perform rigid-body motion of the two domains of Pin1, enabling us to determine their relative orientations consistent with NMR RDC data. This work provides insights into how the inter-domain mobility of Pin1 assists the biochemical signaling regulating proper cell division.

Structure-Function Relationships Between Host Fibrinolytic System, M or M-like Proteins, and Streptokinase in GAS Virulence

Vishwanatha K. Chandrahas, Victoria A. Ploplis and Francis J. Castellino
The W. M Keck Center for Transgene Research, and the Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana, 46556 USA

The virulence of the Group A human pathogen, *Streptococcus pyogenes*, a Group A streptococcus (GAS), is in large part determined by M or M-like (*e.g.*, PAM) proteins and streptokinase (SK) in the bacterium, and an intact functional host fibrinolytic system (HFS). All GAS strains produce the human plasminogen (hPg) activator, streptokinase (SK), as well as M or M-like proteins, which interact with components of the HFS (fibrinogen/fibrin and hPg/hPm). M-like proteins (*e.g.*, PAM) of pattern D strains of GAS have at their N-terminus a1a2 repeats which interact with hPg via its kringle 2 module; whereas M1 proteins of pattern A-C strains (*e.g.*, SF370) interact via its B1B2 repeats with human Fg, which in-turn binds to hPg through kringle 1, kringle 4, and/or kringle 5. In addition to the differences in binding of M or M-like proteins to hPg, there is a difference in the SK secreted by these strains of GAS. However, the functional relevance of this association between secreted SK and M or M-like proteins is not known. To address this, we propose to study the structure-function relationship between domains of PAM and M1 and their *in vitro* function with SK as well as to determine the biological consequences of altering M or M-like proteins and SK. We hypothesize that the hPg binding and activation mechanism of PAM and M1 containing GAS strains differ in their requirements for Fg and is coordinated with the SK subtype. In this study we have cloned and purified PAM (a1a2→B1B2) and M1 (B1B2→a1a2) chimeric proteins and analyzed binding of these new proteins to hPg as well as their ability to stimulate SK-mediated activation of hPg. Our *in vitro* experiments suggest that the plasminogen binding ability of PAM was transferred to M1 with a1a2 repeats of PAM and the fibrinogen binding ability of M1 was transferred to PAM with B1B2 repeats of M1. These structure-function relationships are important in the rational design of vaccines to combat streptococcal infections.

Mechanism of Titanium Dioxide Nanoparticle Toxicity in Developing Embryos of *Xenopus laevis*

Ning Wang and Paul W. Huber
Department of Chemistry and Biochemistry,
University of Notre Dame, Notre Dame, Indiana, 46556 USA

The uncertainties of nanoparticle toxicity have spurred concerns over the safety of their wide application in manufacturing, medicine, and food production. Due to their low solubility and tendency to agglomerate, titanium dioxide nanoparticles have displayed a wide range of activities in different toxicity assays. Thus, a major challenge is to distinguish bioavailability from bioactivity. *Xenopus laevis* embryos offer a solution to these technical impediments by providing a means to compare environmental exposure (waterborne) with direct microinjection of known amounts of material into live cells. Comparably sized TiO_2 (140 nm) and ZnO (65 nm) nanoparticles exhibited similar dose-dependent toxicity when directly injected into embryos. However, in waterborne assays, the activities of these particles were distinctly different with TiO_2 having no detectable effect on embryo viability up to a concentration of 1 mg/ml. Smaller sized TiO_2 particles (24 nm) had a similar degree of toxicity upon injection, but also affected embryo viability in the waterborne assays, indicating that particle size and, thus, bioavailability can play a major role in determining the apparent toxicity of TiO_2 nanoparticles. Time-lapse images of developing embryos injected with a sub-lethal dose of TiO_2 particles reveal that cell migration (closure of the blastopore, exogastrulation) is compromised during the early stages of embryogenesis, leading to a wide range of abnormalities at later stages of development.

To address the underlying molecular mechanisms of the teratogenic effects of TiO_2 nanoparticles, total mRNA from embryos at early gastrula stage injected with water or TiO_2 nanoparticles were subjected to microarray (gene chip) analysis. A total of 180 genes showed statistically significant ($p < 0.05$) differential expression. In the initial analysis of these data, we have focused attention on genes within this list that can be correlated with the observed defects in cell migration (*i.e.,* blastopore closure). These include those encoding ephrin (regulation of cell migration), RhoA (signaling tight junction dissolution), occludin (component of tight junctions), FKBP12 (calcium homeostasis/TGF-β signaling). Our results indicate that TiO_2 nanoparticles have major disruptive impacts on embryo cell polarity, cell-cell contact, and calcium homeostasis.

ABSTRACTS: POSTERS

1. Evidence for a Dynamically Driven, Allosteric T-cell Signaling Mechanism

William F. Hawse[1], Mathew M. Champion[1], Michelle V. Joyce[1], Lance M. Hellman[1], Moushumi Hossain[1], Veronica Ryan[1], Brian G. Pierce[2], Zhiping Weng[2], and Brian M. Baker[1,3]

[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana, 46556 USA; [2]Program in Bioinformatics and Integrative Biology, University of Massachusetts Medical School, Worcester, Massachusetts, USA; [3]Harper Cancer Research Institute, University of Notre Dame, Notre Dame, Indiana, 46556 USA

T cells use the T cell receptor (TCR) to recognize peptide antigens presented by major histocompatibility complex proteins (pMHC) on the surface of antigen presenting cells. Upon formation of the TCR-pMHC complex, alterations in the TCR and its associated proteins trigger T cell signaling and subsequent activation. Changes in TCR oligomeric state and altered interactions with neighboring surface proteins are among the mechanisms proposed to contribute to TCR triggering [1]. These mechanisms could be facilitated by pMHC binding-induced changes in the TCR, but the nature and extent of any such alterations remain unclear. Here we demonstrate in multiple TCR-pMHC pairs that ligation globally rigidifies both the TCR and pMHC, which via entropic and packing effects will aid in the formation and impact the stability of both homo- and hetero-oligomeric complexes. A key region of the TCR implicated in signaling is particularly affected, which could influence specific inter-molecular associations. In the MHC protein, TCR binding impacts the dynamics of the coreceptor binding site. Computational modeling predicts a high degree of dynamic coupling between ligand binding and signaling domains of the TCR, suggesting that the general architecture of the TCR is poised to allosterically couple ligand binding to signal transduction. Altogether, these results support a dynamically driven allosteric mechanism for TCR triggering, where protein rigidification after TCR-pMHC complex formation triggers down-stream signaling events that promote T cell activation.

2. Imaging Mass Spectrometry and 3D Cell Culture

Eric Weaver and Amanda B. Hummon
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

3D cell cultures have increased complexity compared to simple monolayer and suspension cultures, recapitulating the cellular architecture in the body. For example, many colon cancer cell lines form rounded heterogeneous spheroid structures that mimic the pathophysiological properties of tumors when grown under appropriate cell culture conditions. Classical imaging methodologies, like immunohistochemistry, are commonly used to examine the distribution of specific species within the spheroids. However, there is a need for an unbiased discovery-based methodology that would allow examination of protein/peptide distributions in 3D culture systems, without a need for prior knowledge of the analytes. We have developed MALDI Imaging Mass Spectrometry (IMS) protocols to examine protein distributions in 3D cultures models. Using the HCT 116 colon carcinoma cell line, we have grown spheroids measuring 1 mm in diameter for MALDI IMS. We detect changes in the spatial distribution of proteins across the spheroids by IMS. While most proteins are distributed across the structures, some are localized to either the proliferative outer rim of the spheroids or to the central necrotic core. We are now expanding our studies to map the changes in protein distribution that accompany induction of the Epithelial to Mesenchymal Transition (EMT) in our spheroids. To do so, we are optimizing protocols that would enable the use of the MS/MS capabilities of our MALDI TOF-TOF to sequence peptides in a *de novo* fashion allowing us to use a label-free approach to examine protein changes during the EMT. Once we have established the most successful approach to determine protein identification in a 3D culture tissue slice, we will expand our studies to genetically manipulated spheroids to examine cancer-relevant changes to signal transduction pathways. The combination of 3D cell culture and MALDI IMS offer us a unique perspective on the biology driving EMT in these tumor mimics and the flexibility of the cell culture methods combined with the wealth of information provided by Imaging Mass Spectrometry promises to provide novel insight into the processes driving cancer progression.

3. The Phosphoproteome of a Colon Cancer Metastasis Model

Alissa Schunter and Amanda B. Hummon
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Ninety percent of cancer deaths are the result of metastasis, but the molecular events underlying this complex and multi-step process are not completely understood. A proteomic profile of the intracellular signaling events that contribute to disease progression, particularly phosphorylation events, would provide greater insight into this process. To this end, we are examining global phosphorylation in two syngenic colorectal cancer cell lines, one representing a primary tumor, the other a lymph node metastasis. Enriching phosphopeptides with immobilized metal affinity chromatography (IMAC) provides global coverage of S/T/Y phosphorylation, and stable isotope labeling in cell culture (SILAC) enables quantitation with LC-MSMS. We have optimized the IMAC chemistry to ensure maximal, reproducible recovery, and are now beginning the discovery and quantification phase of the project.

4. Mechanisms of Peripheral Protein PI(4)P Binding

Jordan L. Scott[1], Carl J. Mousley[3], Vytas A. Bankaitis[3]
and Robert V. Stahelin[1,2]
[1]Department.of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA; [2]Department of Biochemistry and
Molecular Biology, Indiana University School of Medicine, South Bend,
46617; [3]Department of Cell and Developmental Biology, University of
North Carolina School of Medicine, NC 27599 USA

Phosphatidylinositol-4-phosphate (PI(4)P) is a potent signaling lipid present in mammalian cells. This lipid is a ligand of several known peripheral proteins enabling them to dock reversibly at the Golgi membrane. In general, these protein effectors employ positively charged residues in addition to hydrophobic patches to specifically coordinate the negatively charged PI4P and to partially insert into the plasma membrane. Several of the known PI(4)P effectors are essential for cellular functions such as Golgi to plasma membrane trafficking, retrograde transport, lipid homeostasis and viral replication. PI(4)P levels at the Golgi are modulated by kinases (phosphorylating PI to PI(4)P) and phosphatases.

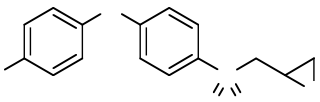
We are investigating the yeast protein Kes1, a known regulator of lipid sensing and trafficking at the plasma membrane. Kes1 has recently been shown by our collaborators to integrate lipid signaling with general amino acid synthesis acting as a nutrient sensor and signalling molecule. Kes1 is a regulator of Gcn4, the primary transcriptional activator of amino acid regulatory genes. A non-canonical PI(4)P binding structure, Kes1 has two PI(4)P binding sites and we have determined their binding affinities using SPR. This study highlights the essential nature of lipids in cellular homeostasis.

Additionally, in preliminary studies of several non-characterized lipid-binding domains, we believe that we have uncovered novel PI(4)P-binding proteins in the Nedd4 ubiquitin ligase family. This lipid binding is likely highly relevant to the localization and substrate targeting of these proteins *in vivo*. Members of this family are known to play a crucial role in several cancers and viral infections. These studies shed light on the importance of cellular lipid signaling and the need for a thorough structural and mechanistic understanding of lipid binding proteins.

5. Pharmacokinetics and Brain Penetration of ND-322 and ND-364, Potential Drugs for the Treatment of Traumatic Brain Injury

Wei Song, Valerie A. Schroeder, Mijoon Lee, Mark A. Suckow, Shahriar Mobashery and Mayland Chang
Department of Chemistry and Biochemistry, Freimann Life Sciences Center, and Department of Biological Sciences, University of Notre Dame, IN 46556 USA

ND-322 and ND-364 are potent and selective gelatinase inhibitors that show potential for the treatment of traumatic brain injury (TBI). Greater than 98% of small-molecule therapeutics does not cross the blood-brain barrier, representing a major challenge in the development of drugs for the treatment of neurological diseases. In order to better understand the potential of these compounds in the treatment of TBI, we investigated the pharmacokinetics and brain penetration in mice. C57BL/6 mice received single subcutaneous doses of ND-322 and ND-364. Blood and brain tissues were collected at specified time points after administration. Concentrations of ND-322 and ND-364 were determined by UPLC with multiple reaction monitoring. Maximum concentrations of ND-322 in plasma and brain were 7.29 pmole/mg and 0.685 pmole/mg, respectively, and were observed at 10 min (the earliest time point collected). Concentrations of ND-364 were 6.60 pmole/mg in plasma and 1.58 pmole/mg in brain. Concentrations of ND-322 in brain remained below the K_i for MMP-9 of 0.870 _M at all time points, while those of ND-364 were above the K_i for MMP-9 of 0.130 _M for 4 h. The half-lives of elimination in brain were 6.4 h for ND-322 and 1.0 h for ND-364. ND-322 and ND-364 are quickly absorbed and distribute rapidly to the brain. These compounds do not accumulate in the brain and are eliminated from the brain, indicating that these compounds would not cause neurotoxicity. The desirable pharmacokinetic properties of these compounds makes them promising in the treatment of traumatic brain injury.



6. Validation of Molecular Dynamics Calculations: Development of a Quantitative Treatment of NMR *J*-Coupling Ensembles in Saccharides

Luke A. Sernau and Anthony S. Serianni
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

A quantitative mathematical method has been developed to derive conformational models consistent with multiple (redundant) experimental NMR *J*-couplings that are sensitive to specific conformational elements in saccharides. This new modeling program is unbiased in that it assumes no specific model when treating the data (*e.g.*, one-, two- or three-state models), but instead uses an unlimited number of experimental *J*-coupling ensembles, along with pertinent Karplus or Karplus-like equations derived from density functional theory (DFT), to determine a best fit to a particular conformational model after a thorough search of multiple model options. Most importantly, this methodology allows validation of molecular dynamics (MD) predictions of conformation in saccharides, predictions that historically have been resistant to accurate testing via purely experimental means. This mathematical modeling technique has been tested on several conformational systems that display specific *J*-coupling trends (*O*-glycosidic linkages of several ^{13}C-labeled β-(1→4)-linked disaccharides), or have known conformational properties (*e.g.*, exocyclic hydroxymethyl (CH_2OH) conformation in aldohexopyranosyl rings). The method has also been applied to a more complex *O*-glycosidic linkage, namely, the α-Man-(1→2)-α-Man linkage commonly found in the high-mannose *N*-glycans of glycoproteins.

7. Effects of Coding Sequence on Protein Translation Rate

Julie Chaney and Patricia L. Clark
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

The rate of translation is not uniform across mRNA sequences. It has been hypothesized that translational pauses could modulate cotranslational protein folding by limiting which amino acids are outside the ribosome exit tunnel and available to interact, potentially separating the folding of protein structural units. Translation rate could also affect the time available for inter-molecular interactions during cotranslational folding, including multimerization, cotranslational modifications, chaperone interactions, and transport through the translocon. As a consequence of the degeneracy of the genetic code, there are many possible ways to encode the same protein sequence, so the coding sequence could potentially add an additional layer of information by encoding translation rate. Synonymous codons are not used with equal frequency, and rare codons are known to cause slower translation under certain circumstances. Translation rate is also affected by mRNA structure, and recent studies show that translational pausing can be mediated by specific nucleotide motifs[1]. Given the complexity of the problem, there is a need for computational tools to identify motifs associated with pausing, and experimental studies to determine the physiological effects of translational pauses. For example, analysis is underway to identify over- and under-represented nucleotide motifs in coding sequences and analyze their effects on codon usage and translation rate. A key question is whether specific codons or combinations of codons mediate translation rate, or whether translation rate is mostly determined by nucleotide motifs occurring in any reading frame.

1. Li GW, Oh E, Weissman JS. The anti-Shine-Dalgarno sequence drives translational pausing and codon choice in bacteria. *Nature*. 2012 Mar 28. doi: 10.1038/nature10965.

8. The Characterization and Identification of Ceramide-1-Phosphate Binding Proteins

Katherine E. Ward,[1] Nitin Bhardwaj,[3] Mohsin Vora,[2] Hui Lu,[3] and Robert V. Stahelin[1,2]
[1]Department.of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA; [2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, South Bend, IN 46617 USA; [3]Bioinformatics Program, Department of Bioinformatics, University of Illinois at Chicago, Chicago, IL 60607 USA

The activation of cytosolic phospholipase A_2 _ ($cPLA_2$_) has been implicated in atherosclerosis and cerebral ischemia in previous animal models. The sphingolipid metabolite ceramide-1-phosphate (C1P) is known to specifically regulate the activation of $cPLA_2$_ and has been shown to increase enzymatic activity 5-10 fold through increased membrane residence. We have recently determined the amino acids that are responsible for C1P binding to $cPLA_2$_ *in vitro* and in a cellular system. In addition to heart disease, the conversation of ceramide to C1P has been implicated in cancer and phagocytosis signaling, suggesting that there are unidentified proteins that are regulated by C1P binding in membranes. Using the novel $cPLA_2$_ consensus sequence to search the proteome, we have predicted and are currently investigating new C1P binding proteins. Through the characterization of the C1P binding site, we have constructed a novel model for C1P recognition in membranes that deviates from the traditional lipid binding mechanism. This molecular characterization provides great insight into the molecular basis of C1P recognition and selectivity in biological membranes and will significantly increase the molecular basis of C1P binding by proteins. The understanding of this binding site in particular would lay the groundwork for a promising therapy for heart disease.

9. Multivalent Squaraine Rotaxanes for Fluorescent Bone Imaging

Kara M. Harmatys, Erin L. Cole, Douglas R. Rice, and Bradley D. Smith
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Accurate, noninvasive techniques for monitoring changes in skeletal metabolism are required for sensitive screening of osteoporosis, arthritis, or tumor metastasis to bone. The development of near-infrared optical reporters of bone metabolism may be useful for preclinical biomedical studies of bone related diseases. Deep-red emitting fluorescent Squaraine Rotaxane probes are ideal bio-imaging agents since they exhibit strong photostability, strong chemical stability, and can be tailored for specific imaging applications. The Iminodiacetate Squaraine Rotaxane scaffold is found to be an effective general marker of skeletal features. The Iminodiacetic acid group can form complexes with hydroxyapatite exposed on the bone surface presumably through Ca^{2+} chelation. Various Squaraine Rotaxane scaffolds were compared in vivo; increased binding was observed comparing four Iminodiacetic acid groups to two groups. A control probe containing carboxylic acid groups showed no specific binding to bone in comparison to 4-Iminodiacetate Squaraine Rotaxane. Finally, studies co-injecting 4-Iminodiacetate Squaraine Rotaxane with commercially available bone imaging probe OsteoSense®750 verified colocalization to bone.

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

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

Methicillin-resistant *Staphylococcus aureus* (MRSA) has evolved two mechanisms that are largely 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 the β-lactamase 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 manifestation of the MRSA phenotype. Recent unpublished results from our lab indicate that BlaR1 signal transduction is also phosphorylation-dependent, with phosphorylation of a tyrosine and serine residue on its cytoplasmic domain being important for activity. 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. We have screened a library of known inhibitors of eukaryotic kinases against MRSA in the presence of β-lactam antibiotics. We report herein several instances of improved susceptibility of MRSA strains to either oxacillin or ampicillin in the presence of a kinase inhibitor. Future directions are discussed, including the development of analogues of the kinase inhibitors with increased potency, and identification of their target.

11. Co-translational Folding Monitored by Covalent Labeling

Giselle Jacobson and Patricia L. Clark
Department of Chemistry and Biochemistry
University of Notre Dame, Notre Dame, IN 46556 USA

Proteins fold co-translationally as they are synthesized by the ribosome, but little is known about the *in vivo* conformations these nascent polypeptide chains adopt while still attached to the ribosome or how these conformations affect subsequent folding steps. Current methods to study ribosome-bound nascent chains suffer from complications introduced by the ribosome's immense size and the complexity of *in vivo* samples. My project will develop a methodology, using covalent labeling coupled to mass spectrometry, to probe the conformations, flexibility, ribosomal interactions and extent of folding of nascent polypeptide chains while they are still attached to the ribosome. Covalent labeling will be used to probe the extent of polypeptide chain folding by identifying solvent exposed regions of nascent green fluorescent protein stalled on ribosomes. *S*-methylthioacetimidate (SMTA) is a thioimidate that covalently labels surface exposed primary amines in proteins via addition of an imine group. SMTA is membrane permeable and in *E. coli,* its *in vivo* labeling of ribosomal proteins has been shown to closely resemble its *in vitro* labeling. I plan to explore the conformations and extent of folding of nascent GFP by covalently labeling ribosome-nascent chain constructs of varying lengths using SMTA. Preliminary *in vitro* results using purified, natively folded GFP have shown that GFP can be amidinated using SMTA and this exchange can be viewed with LC-ESI Q-TOF MS. Furthermore, labeling GFP for longer periods of time results in a greater extent of amidination.

12. Investigating the Thermodynamic Contributions and Specificity of Proteins Involved in Cellular Immunity

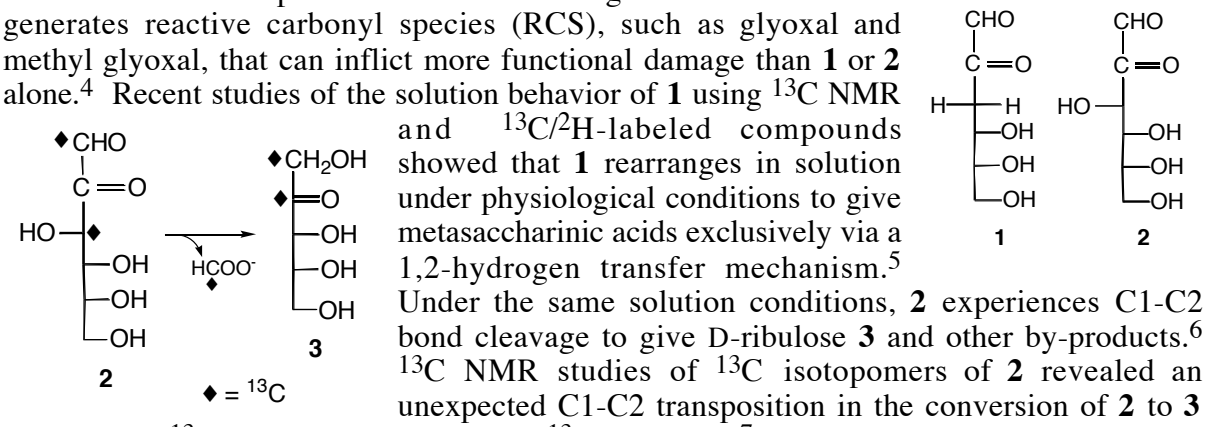
Sydney Blevins, Kurt Piepenbrink and Brian M. Baker
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

The cellular immune response 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 and a Major Histocompatibility Complex (MHC) protein presenting a peptide. The complete mechanism by which a TCR recognizes an antigen (MHC plus peptide) is still unknown. Several methods have been employed to determine more about the specificity of this interaction. The human TCR A6 has been used to extensively evaluate the strength and energetic significance of the interactions it makes with an MHC molecule. By creating double mutants of an interaction pair, a thermodynamic cycle can give the energetic contributions of individual interactions within a binding interface. Additionally, two other TCRs that share their V_ domain genes with A6 were evaluated using a double mutant cycle for interactions that contact MHC residues that have been hypothesized to be evolutionarily conserved and important for MHC recognition. Finally, it has been suggested that the presence of certain peptides can influence the dynamics of the entire MHC molecule. If this is true, do certain peptides influence the binding of not only TCRs to the MHC but also other accessory proteins such as the CD8 coreceptor? Isothermal calorimetry will be used to observe any changes in binding affinity or thermodynamic components that are directly related to the peptide being presented.

13. 1,2-Dicarbonyl Sugars: Phosphate-catalyzed Skeletal Rearrangement Involving C1-C2 Transposition

Wenhui Zhang and Anthony S. Serianni
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556-5670 USA

1,2-Dicarbonyl sugars such as 3-deoxy-D-*erythro*-hexos-2-ulose (3-deoxy-D-glucosone) (**1**) and D-*arabino*-hexos-2-ulose (D-glucosone) (**2**) have been implicated in the degradation of D-glucose *in vivo*, with **1** commonly detected as a metabolite in diabetic patients.[1] The contiguous electrophilic carbonyls in **1** and **2** render them susceptible to nucleophilic attack by protein side-chains (*e.g.*, arginine), leading to structural modification (glycation) and in some cases loss of protein function.[2,3] Degradation of **1** and **2** generates reactive carbonyl species (RCS), such as glyoxal and methyl glyoxal, that can inflict more functional damage than **1** or **2** alone.[4] Recent studies of the solution behavior of **1** using ^{13}C NMR and $^{13}C/^{2}H$-labeled compounds showed that **1** rearranges in solution under physiological conditions to give metasaccharinic acids exclusively via a 1,2-hydrogen transfer mechanism.[5] Under the same solution conditions, **2** experiences C1-C2 bond cleavage to give D-ribulose **3** and other by-products.[6] ^{13}C NMR studies of ^{13}C isotopomers of **2** revealed an unexpected C1-C2 transposition in the conversion of **2** to **3** (*e.g.*, D-[1,3-$^{13}C_2$]glucosone gives D-[1,2-$^{13}C_2$]ribulose).[7] This transformation is phosphate- and arsenate-catalyzed at pH 7.4, and probably involves an osone-phosphate (or osone-arsenate) adduct as an intermediate. These remarkable findings suggest a potential new role for inorganic phosphate in the degradation of 1,2-dicarbonyl compounds *in vivo*. (Work supported by the NIH NIDDK DK065138).



References

1. Wells-Knecht, K. J.; Lyons, T. J.; McCance, D. R.; Thorpe, S. R.; Feather, M. S.; Baynes, J. W. *Diabetes* **1994**, *43*, 1152-1156.
2. Biemel, K. M.; Friedl, D. A.; Lederer, M. O. *J. Biol. Chem*. **2002**, *277*, 24907-24915.
3. Chetyrkin, S. V.; Zhang, W.; Hudson, B. G.; Serianni, A. S.; Voziyan, P. A. *Biochemistry* **2008**, *47*, 997-1006.
4. Chetyrkin, S.; Mathis, M.; Pedchenko, V.; Sanchez, O. A.; McDonald, W. H.; Hachey, D. L.; Madu, H.; Stec, D.; Hudson, B.; Voziyan, P. *Biochemistry* **2011**, *50*, 6102-6112.
5. Zhang, W.; Carmichael, I.; Serianni, A. S. *J. Org. Chem*. **2011**, *76*, 8151-8158.
6. Wells-Knecht, K. J.; Zyzak, D. V.; Litchfield, J. E.; Thorpe, S. R.; Baynes, J. W. *Biochemistry* **1995**, *34*, 3702-3709.
7. Zhang, W.; Serianni, A. S. *J. Am. Chem. Soc*. **2012**, in press.

14. Dynamics of the MEL5 T Cell Receptor as Determined by Hydrogen-Deuterium Exchange and Mass Spectrometry

Veronica Ryan and Brian Baker
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Hydrogen-deuterium exchange monitored by mass spectrometry (HDX-MS) is a sensitive technique that probes protein conformation in solution and reveals dynamics on time scales longer than those commonly probed by techniques like NMR and fluorescence. Previous HDX-MS experiments done by the Baker lab have shown that A6 and DMF5, two different T-cell receptors (TCRs), globally rigidify upon ligation to the MHC/peptide complex with few differences between the changes in the two TCRs. This poster will present the results of similar experiments on an unrelated TCR, MEL5, to give more evidence as to whether this global change in stiffness seen in A6 and DMF5 is common to all TCRs. After letting free and MHC/peptide bound MEL5 exchange in a deuterated buffer for different periods of time and digesting the protein with pepsin, MALDI mass spectroscopy was used to determine the mass of the peptide fragments. The identity of the fragments was then determined from LC/MS/MS data and the percent deuteration was mapped onto the crystal structure of both the free and bound TCR for each different time point to determine the more dynamic parts of the TCR. Preliminary results from these experiments indicate that MEL5 dynamics are detectible by MALDI mass spectrometry.

15. Understanding the Local Backbone Dynamics of the PMHC and Kinetics of the TCR-PMHC Binding Interaction

Brian E. Gloor[1,3], Juana M. Corado[2], Kevin A. Kho[1] and Brian M. Baker[1,3]
[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA; [2]Henry Samueli School of Engineering, University of California – Irvine, Irvine, CA; [3]Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN 46556 USA

Cytotoxic T cells destroy virally infected cells and tumor cells and are also implicated in transplant rejection. These cells are also known as $CD8^+$ T cells, since they express the CD8 glycoprotein at their surface. The T cell receptor is a molecule found on the surface of T cells that is responsible for recognizing antigens presented by major histocompatability complex (MHC) proteins. MHC proteins are expressed on the surface of cells and display fragments of molecules from invading microbes or dysfunctional cells to the TCR.

Here, through the utilization of fluorescence anisotropy, we investigate MHC flexibility when different peptides are bound. The peptides presented by class I MHC HLA-A2 utilized in this study are as follows: Tax 9 (LLFGYPVYV), Tel1p (MLWGYLQYV), ELA (ELAGIGILTV), gp100 T2M (IMDQVPFSV) and Flu M1 (GILGFVFTL). To assess the changes in flexibility, we engineered a series of cysteine mutants in the _1 and _2 helix of the MHC complex. The samples were refolded in the presence of peptide, purified and consequently labeled with Alexa-Fluor 488 (Alexa488) for fluorescence studies. Steady-state fluorescence anisotropy measurements were performed, and we were able to show that the overall flexibility on the _1 and _2 helix regions of the MHC varies dependent on the peptide presented on the MHC complex.
Measurements of TCR-pMHC binding equilibrium and kinetic parameters have been obtained through the utilization of surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). Unlike pure solution methods, SPR does not allow for detailed resolution of the kinetic mechanism for the TCR-pMHC interaction, which can be obtained from stopped-flow fluorescence anisotropy. The need for such an assay is essential to understanding the recognition mechanism of the TCR as well as enabling further investigation of TCR cross reactivity and specificity.

Fluorescence studies of the TCR-pMHC interaction were completed using a fluorescein derivative to label an MHC-free cysteine via maleimide chemistry. Current data with the A6 T cell receptor and the Tax 9 peptide presented by the class I MHC HLA-A2 shows that as the concentration of A6 increases, the pMHC is bound resulting in an increased anisotropy. The same trend is seen in various A6 constructs (A6 wild-type and A6 c134 zippered constructs, Cole/Sewell A6 wild-type and A6 c134) that have been studied. We have determined that upon ligation we see a change in anisotropy is observed using our fluorescein derivitized protein and determined the unique kinetic parameters and recognition mechanism of the A6/HLA-A2 binding interaction.

16. Investigating the *in vitro* Folding Efficiency and Secretion Mechanism of the YapV Autotransporter

Thomas J. Soderquist and Patricia L. Clark
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Autotransporter (AT) proteins are the most common class of secreted virulence factors found in Gram-negative bacteria. AT proteins are characterized by three main regions: an N-terminal signal sequence targeting secretion across the inner membrane, a functional passenger domain secreted across the outer membrane (OM), and a C-terminal _-porin domain that forms a _-barrel structure in the OM. With no ATP in the periplasm, nor a proton gradient across the OM, the driving force for secretion of the passenger across the OM is not yet known. Results from our lab on two well studied ATs, pertactin from *B. pertussis* and Pet from *E. coli*, suggest that extracellular folding of the passenger domain may drive OM secretion. However, unlike these proteins, YapV, an AT protein found in *Yersinia pestis* – the causative agent of the plague, does not seem to possess a stable C-terminal core in its passenger domain. Previous work in our lab on this particular protein has focused on the conformational differences in constructs of the passenger domain. My work will focus on finding more proficient methods to increase the folding and refolding efficiency of these YapV passenger domain constructs and to identify characteristics of the passenger domain that might influence OM secretion, if any exist. Work has already begun in this aspect through point mutations of two closely placed cysteine residues in the YapV45 passenger construct, which lacks the N-terminal signal sequence and C-terminal _-porin domain. Preliminary results, from SDS-PAGE and Native gel electrophoresis, suggest that the constructs containing a single or a double cysteine to serine mutation run similarly as wild-type YapV45. This might speak to a similarity in conformation between these mutated constructs and wild-type YapV45.

17. Controlling Protein Structure by Altering Local Translation Rate

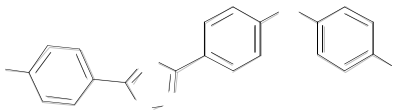
Jamie Luther, Ian M. Sander and Patricia L. Clark
Department of Chemistry and Biochemistry,
University of Notre Dame, Notre Dame, IN 46556 USA

Anfinsen's principle asserts that all of the relevant information required for a protein to fold into its final native state is encoded by the primary amino acid sequence. More recently it has been shown that many proteins begin to fold while still tethered to the ribosome. This cotranslational folding can influence the pathway a protein takes to its final native state *in vivo*. Additionally, altering the translation rate *in vivo* can further impact the cotranslational folding mechanism. 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, they are not used with equal frequencies. The most common codons are generally translated faster than rare codons which can cause pauses in translation. Here we have designed a model protein biosensor that can detect changes in translation rate *in vivo*. We have shown that this protein biosensor can assume mutually exclusive alternative folded states as a result of changes in both local and global translation rate.

18. A Comparison of the Pharmacokinetics of Oxadiazoles Antibacterials in Mice

Zhihong Peng, Valerie A. Schroeder, Peter O'Daniel, Sebastian Testero, Nuno Giao Antunes, Mark A. Suckow, Shahriar Mobashery and Mayland Chang
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

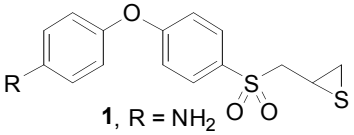
The oxadiazoles are a novel class of antibacterial agents that show potent antibacterial activity *in vitro* and some efficacy in animal model of infection. Compound **1** shows better *in vivo* activity than compound **2** after oral administration. Improvement of *in vivo* activity may be achieved by optimization of pharmacokinetic properties. The aim of this study was to evaluate the pharmacokinetics of oxadiazole analogs and correlate pharmacokinetic properties with biological activity. In this study, female mice received a single dose of oxadiazole analogs both after oral or intravenous administration. Blood samples were collected and analyzed by UPLC with multiple reaction monitoring. Pharmacokinetic parameters were estimated from the plasma concentration versus time curves by non-compartmental analysis. After oral administration, the compounds **1** and **2** were slowly absorbed, reaching maximum levels at 2 h. The oxadiazoles were slowly distributed to tissues, with half-lives of 6 h and 13 h for compounds **1** and **2**, respectively. The elimination half-lives ranged from 36 to 39 h. Oral bioavailability of the oxadiazoles was close to 100%. Systemic exposure and volume of distribution of compound **2** were 2- to 4- fold higher than those of compound **1**. Both oxadiazoles had excelled oral bioavailability. While compound **2** showed higher systemic exposure than compound **1**, the latter distributed to tissues faster, resulting in better efficacy in a mouse peritonitis infection model.



19. Evaluation of Selective Gelatinase Inhibitors in a Diabetic Mouse Model of Wound Healing

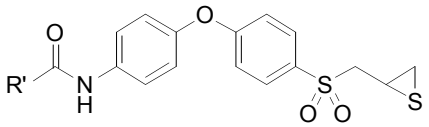
Major Gooyit[1], William R. Wolter[2], Mijoon Lee[1], Mark A. Suckow[2], Shahriar Mobashery[1] and Mayland Chang[1]
[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA and [2]Department of Biological Sciences and Freimann Life Sciences Center, University of Notre Dame, Notre Dame, IN 46556 USA

Chronic wounds (diabetic foot ulcers, pressure ulcers, and venous ulcers) are characterized by increased levels of inflammatory cells, giving rise to an elevated expression of matrix metalloproteinases (MMPs) and growth factors that are essential for healing. MMPs comprise a family of 26 zinc-dependent endopeptidases that are capable of degrading components of the extracellular matrix. Of the types of MMPs, the gelatinases (MMP-2 and MMP-9) are believed to be responsible for the increased destruction of the extracellular matrix observed in chronic wounds and thus could represent potential targets for inhibition to facilitate wound healing. We evaluated a selective gelatinase inhibitor **1** after topical administration in an excisional diabetic mouse wound healing model. On day 14, diabetic mice treated with **1** showed 92 ± 4% wound closure compared to 74 ± 12% with vehicle. *In situ* gelatin zymography indicated that compound **1** reduced gelatinolytic activity in wound tissues of diabetic mice compared to vehicle-treated controls. The same experiments were carried out in wild-type mice to ascertain the role of gelatinases in the disease. On day 14, wound closures were 96 ± 4% and 98 ± 1% for mice treated with **1** and vehicle respectively. No significant differences in wound closure were observed between wild-type mice treated with **1** and those treated with vehicle. These results suggest the involvement of gelatinases in impaired diabetic wound healing. Further studies to validate the role of gelatinases in diabetic wounds by MMP expression profiling of diabetic wound tissues are ongoing.

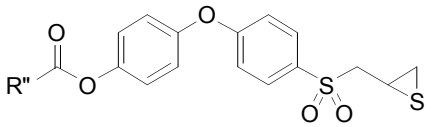


1, R = NH_2
2, R = OH

Second-generation lipophilic derivatives suitable for formulation as ointments, including *N*-phenyl carbamates **3**, amides **4**, *O*-phenyl carbamates **5** and esters **6** were synthesized. These compounds are currently being evaluated for gelatinase inhibition and for their ability to release **1** or **2** in a simulated wound fluid or plasma. Promising analogs will be evaluated in a diabetic mouse model of wound healing.



3, R' = OMe, OEt, OPr
4, R' = Et, *i*Pr



5, R" = NHEt, NHPr, NH*i*Pr, NME_2, NEt_2
6, R" = Et, Pr, *i*Pr, Bu, Hexyl

20. Selection For Resistance To Oxadiazole Antibiotics

Qiaobin Xiao, Mayland Chang and Shahriar Mobashery
The Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Bacterial resistance to antibiotics poses a serious threat to human health and is currently a global concern. *Staphylococcus aureus*, a Gram-positive bacterium frequently found on human skin, is responsible for many illnesses ranging from minor skin infections to life-threatening diseases such as meningitis, pneumonia and endocarditis. Methicillin-resistant *S. aureus* (MRSA) is resistant to most antibiotics currently available. Previous work from our laboratories led to the discovery of a novel oxadiazole class of antibiotics with both *in vitro* and *in vivo* activities against MRSA. However, the target of the oxadiazole class of antibiotics is currently unknown. To identify the target, we serially passaged *S. aureus* NRS100 (an MRSA strain) in LB medium supplemented with increasing levels of POD-71-01 (an oxadiazole antibiotic from our laboratories) and generated a new strain (designated *S. aureus* NRS100R) with elevated level of resistance to POD-71-01. *S. aureus* NRS100R exhibited MIC of >16 μg/mL (the limit of solubility of the compound) to POD-71-01, which is at least 8-fold higher than the value for the parental strain (MIC = 2 μg/mL). On the other hand, MIC measurements with the antibiotics ampicillin, ceftazidime, imipenem, linezolid and vancomycin showed no difference in susceptibility to these antibiotics between the two strains. Next, we explored if the involvement of an efflux pump accounted for this resistance. MIC measurements of the two strains with POD-71-01 in the presence of the known efflux pump inhibitors carbonyl cyanide m-chlorophenylhydrazine (CCCP), reserpine and sodium orthovanadate showed that *S. aureus* NRS100R maintained elevated level of resistance to POD-71-01. Together, these data indicate that the elevated level of resistance of *S. aureus* NRS100R is likely specific to the oxadiazole class of antibiotics. The resistance might arise from mutations of the antibiotic target, which may lead us to its identification in *S. aureus* by whole-genome sequencing.

21. Determination of the Structure of the Membrane Anchor of Penicillin-Binding Protein 4 of *Escherichia coli* by NMR

Edward Spink, Peter I. O'Daniel, Jaroslav Zajicek and Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556 USA

Penicillin-binding proteins (PBPs) are membrane-bound proteins that catalyze the synthesis of peptidoglycan, the major constituent of the bacterial cell wall. The importance of these proteins in the reproduction and proliferation of bacteria has made them a key target for development of new antibacterials. PBPs are attached to the cell membrane by anchor polypeptides and the nature of the membrane attachment is thought to have a significant bearing on the synthetic function of the PBP. PBP 4 of *Escherichia coli* is a bifunctional DD-carboxypeptidase/DD-endopeptidase that is anchored to the cytoplasmic membrane by a 24-residue polypeptide at the C-terminus of the protein. Using natural abundance NMR spectroscopy, the three-dimensional structure of the PBP 4 anchor peptide was determined within a dodecylphosphocholine (DPC) micelle. At 25 ^{o}C and pH 7.4, dynamic experiments indicated that the peptide was exchanging on and off the micelle in a transient manner. Therefore, further studies were undertaken at 5 ^{o}C and pH 3.5 to try and slow down the exchange to elucidate the nature of interactions between the peptide and the micelle. This study builds on the work previously done in our laboratory in which the structure of the PBP 5 membrane anchor was determined (*J. Am. Chem. Soc.* **2010**, *132*, 4110-4118). We wish to place the individual anchor structures in the wider context of the membrane environment as a whole in order to understand the relationship between the anchor domain structure and the positioning of PBPs for cell-wall synthesis.

22. On the Stability of D-Glucose in Aqueous Phosphate Buffer: A ^{13}C NMR Investigation with Singly ^{13}C-Labeled Isotopomers

Wenhui Zhang and Anthony S. Serianni
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556-5670 USA

D-Glucose **1** is the most thermodynamically stable aldohexose in nature, a property attributed to its cyclic pyranoses, especially the β-pyranose in which all ring-bound hydroxyl groups are equatorial in the preferred $^4C_1$ chair conformation. The high stability of the glucopyranoses is reflected in the very low abundances of acyclic forms in solution; the aldehyde and hydrate forms comprise ~0.01% of the total forms present.[1] The low percentage of aldehyde is considered beneficial to biological systems by reducing the possibility of spontaneous reactions between it and cellular nucleophiles (*e.g.*, lysine side-chains of proteins) which leads to potentially harmful chemical metabolites.

The solution stability of **1** has attracted attention in recently because of interest in the potential role of spontaneous glucose autoxidation in promoting protein modification *in vivo*, especially in diabetic patients. For example, aqueous solutions of **1** in phosphate buffer at pH 7.4, incubated at 37 °C and open to the atmosphere for several months, contain degradation products such as D-arabinose and glyoxal, both of which can cause protein covalent modification.[2] This behavior has important implications not only for studies of protein glycation, but also for biochemical studies where glucose solutions are part of the experimental protocol; aqueous solutions of **1**, incubated for moderately short periods of time at 37 °C, contain degradation products that could interfere with data interpretation. Solution stability is also critical for pharmaceutical preparations where **1** is involved either as an API or an excipient.

The solution stability of **1** was examined directly by ^{13}C NMR spectroscopy. To increase the sensitivity and selectivity of the analyses, the complete series of singly ^{13}C-labeled D-glucoses was used as substrates: D-[1-^{13}C]glucose; D-[2-^{13}C]glucose; D-[3-^{13}C]glucose; D-[4-^{13}C]glucose; D-[5-^{13}C]glucose; D-[6-^{13}C]glucose. This experimental strategy allows a determination of the specific fates of each carbon in **1** during degradation. We find that, contrary to prior work,[2] aqueous solutions of **1** contain mainly D-fructose after 2-3 months of incubation at 37 °C. The solutions also contain additional rearrangement and degradation products that have been characterized structurally; chemical mechanisms explaining the origins of these products have been proposed and additional labeling experiments with ^{13}C and ^2H are underway to test these mechanisms. (Work supported by the NIH NIDDK DK065138).

References

1. Zhu, Y.; Zajicek, J.; Serianni, A. S. *J. Org. Chem*. **2001**, *66*, 6244-6251.
2. Wells-Knecht, K. J.; Zyzak, D. V.; Litchfield, J. E.; Thorpe, S. R.; Baynes, J. W. *Biochemistry* **1995**, *34*, 3702-3709.

23. Antibacterial Activity and Synthesis of 2-Styryl-4(3H)-quinazolinones

Renee Bouley, Malika Kumarasiri, Nuno GiaoAntunes, Mark Suckow, Mayland Chang and Shahriar Mobashery
Department of Chemistry and Biochemistry, and Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556 USA

Methicillin-resistant *Staphylococcus aureus* (MRSA) confers resistance to β-lactam antibiotics by the acquisition of an additional penicillin-binding protein termed PBP2a. This protein has a low affinity for β-lactam antibiotics due to the presence of a loop that covers the active site. A potential way to inhibit PBP2a is through the use of noncovalent inhibitors that won't require the energetically costly acylation of the PBP2a active site serine. *In silico* docking and scoring of 1.2 million compounds was performed against the PBP2a active site, of which the top 90 were selected and purchased from the ChemDiv library. A 2-styryl-4(3H)-quinazolinone, one of these compounds, exhibited good antibacterial activity against *Staphylococcus aureus* when screened against the ESKAPE panel of living bacteria (a collection of several problematic strains). Subsequent derivatization of the initial hit led to an exceptionally active fluoro-2-sytyrl-4(3H)-quinazolinone with an MIC (minimal-inhibitory concentration) of 0.25 μg/mL for *S. aureus*, which is 8-fold improvement from the initial hit compound. This compound also displayed exceptional activity against two MRSA strains, MRSA252 and NRS70, with MIC's of 0.125 and 0.0625 μg/mL respectively. However, this compound exhibited minimal *in vivo* efficacy in mice infected with MRSA, displayed poor oral bioavailability, and was rapidly cleared from the system when given intravenously. Therefore, the structure-activity relationship must be further developed in order to improve the lead compound's pharmacological profile and *in vivo* efficacy.

24. Kinetic Characterizations of AmpD Amidases from *Pseudomonas aeruginosa*

Weilie Zhang, Dusan Hesek, Mijoon Lee and Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Pseudomonas aeruginosa is a Gram-negative bacterium that has been one of the top three causes of opportunistic human infections during the past century. A major factor may be a consequence of its resistance to the antibiotics. It has been predicted from genomics analyses that three AmpD genes for peptidoglycan amidases exist in *P. aeruginosa*. The three AmpD genes are designated AmpD, AmpDh2, and AmpDh3. These genes are believed to contribute to a stepwise regulation of ampC β-lactamase. The inactivation of AmpD in *P. aeruginosa* leads to moderately increase a level of basal ampC expression that is still further inducible, due to the presence of two additional AmpD proteins, AmpDh2 and AmpDh3, in this genus. During regular bacterial growth, muropeptides are processed by the AmpD. It has two major roles, recycling of peptidoglycan catabolism products and prevention of ampC induction. We recently synthesized a set of 1,6-anhydromuramyl peptides and muramyl peptides for characterization of *P. aeruginosa* AmpD enzymes. Results showed that AmpD is capable of turning over 1,6-anhydromuramyl species, but not other muramyl species, with the highest activity with tetrapeptide variants. AmpDh2 has the activity for both 1,6-anhydromuramyl species and muramyl species, but the activity for muramyl species was ten folds higher than 1,6-anhydromuramyl species. AmpDh3 has similar activity for both 1,6-anhydromuramyl species and muramyl species.

25. Quantifying the Effects of FLASH Knockdown on Regulation of Histone Synthesis by LC-MS/MS

Joshua A. Reavis , Kerry M. Bauer and Amanda B. Hummon
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Histone proteins are crucial for proper condensation of DNA in the nucleus. Transcription of histone genes is highly cell-cycle dependent, in order to avoid aggregation or non-specific binding of histones to structural RNA, mRNA, or accidental binding to DNA strands. One key component of the transcriptional processing machinery of histone genes is a protein termed FLASH (FLICE-Associated Huge), which is involved in 3'-end processing of all replication-dependent histone mRNA transcripts. We have previously identified that loss of FLASH from colorectal adenocarcinoma cells causes widespread alterations in the mRNA expression levels for multiple replication-dependent histones, as well as a tremendous decrease in the viability of colorectal cancer cells. This study aims to examine whether a corresponding change in histone protein abundance levels are also linked to a reduction in FLASH expression. FLASH knockdown was found to be detrimental to cell status during an assay of over 400 genes believed to be implicated in apoptosis. Upon knockdown of FLASH, a decrease in viability was seen, as well as a gross overabundance of histone transcripts. Not only does FLASH knockdown have a vital effect on histone transcription, but it also leads to an S-phase arrest and dysregulation of various proteins throughout other cellular pathways. Given the pronounced changes to histone transcript levels, the next step will be to analyze the protein abundance of replication-dependent histones within the cell. We are using LC-MS/MS methods to identify and quantify peptides from cell extracts, as well as a unique in-house quantification strategy to determine relative abundance of histone peptides.

26. Search for the Peptidoglycan Undecaprenyl-Releasing Enzyme

Chiranjit Chowdhury, Dusan Hesek, Mijoon Lee, William Boggess and Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Cell wall is critical for survival of bacteria. Assembly of peptidoglycan, the major constituent of the bacterial cell wall, is a multistep process starting in the cytoplasm and ending in the exterior of the cytoplasmic membrane. The first step in the biosynthesis of peptidoglycan starts at the cytoplasmic side of the plasma membrane with the transfer of UDP-activated peptidoglycan precursor from the cytoplasm to a lipid carrier, undecaprenyl phosphate, which results in the production of the Lipid II, the building unit of the peptidoglycan. Lipid II is translocated across the cytoplasmic membrane, before its polymerization could lead to the formation of the peptidoglycan, catalyzed by transglycosidases. In the course of the reaction of transglycosylases the undecaprenylpyrophosphate is the leaving group, however, one undecaprenylpyrophosphoryl moiety remains tethered to the nascent peptidoglycan, the one introduced by the final Lipid II molecule that gets incorporated into the polymeric structure. As undecaprenyl moiety is present in small quantities in bacteria, this final undecaprenylpyrophsporyl group has to be released from the peptidoglycan to meet the needs of the organism. Here, we have preliminary data indicating that an enzyme in periplasm is able to releases the terminal lipid (undecaprenyl moiety) from the peptidoglycan. The assay includes a mimetic of the polymeric substrate, which in the presence of periplasmic extract produces a cyclic 1,6-anhydroglucosamine that was detected by mass spectrometry. The reaction was also characterized by colorimetric determination of phosphate release from the pyrophosphoryl moiety.

27. Protonation States of the Active-Site Lysines of *Escherichia coli* Penicillin-binding Protein 6 and Their Mechanical Implications

Malika Kumarasiri, Weilie Zhang, Jed Fisher and Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Among the 12 penicillin-binding proteins (PBPs) in *Escherichia coli*, PBP 6 is a DD-carboxypeptidase that is implicated in maturation of the bacterial cell wall. PBP 6 is acylated by β-lactam antibiotics at the serine (Ser66) of the conserved Ser-X-X-Lys sequence motif. It has been proposed that the lysine (Lys69) of the said motif acts as the general base during the acylation process. However, the presence of another lysine (Lys235) in the active site necessitates an investigation to establish that Lys69 is better poised to act as the base in the catalytic event. Here we estimate the pK_a of both lysines to investigate their mechanistic involvement. Our k_{cat}/K_m and k_{cat} profiles are consistent with an active-site residue having a pK_a of 8.5. We additionally calculate the pK_a of both lysines computationally, using a thermodynamic cycle and free-energy simulations. The computationally estimated pK_a of 8.3 for Lys69, agrees well with the experimentally determined value, while Lys235 produced a pK_a of 9.6. These pK_a values confirm that Lys69 initiates catalysis in its free-base form, while Lys235 remains in the protonated form at optimal pH for catalysis. Additionally, we observe that only a minimal structural change is required at the active site to realize protonation/deprotonation of Lys69 although the event alters the hydrogen-bonding network of the active site. Based on our observations, a reaction sequence for the chemistry of PBP 6 is proposed, wherein protonated Lys235 serves as the electrostatic substrate anchor and Lys69 as the conduit for protons in the course of acylation and deacylation steps.

28. An Amino Acid Position at Crossroads of Evolution of Protein Function: Antibiotic Sensor Domain of BlaR1 Protein from *Staphylococcus aureus* versus Class D β-Lactamases

Malika Kumarasiri, Leticia Llarrull, Oleg Borbulevych, Jennifer Fishovitz, Elena Lastochkin, Brian Baker and Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

The BlaR1 protein has been implicated in the mechanism of β-lactam antibiotic resistance of *Staphylococcus aureus*. It is a membrane protein that upon acylation in its sensor domain, initiates a signal transduction to a cytoplasmic domain, a metalloprotease. Upon activation, this domain of BlaR1 is responsible for the degradation and subsequent removal of the BlaI repressor off the bla operon, leading to transcription of the blaZ gene and production of β-lactamase, culminating in antibiotic resistance within minutes of exposure to antibiotics. The x-ray structure of the sensor domain of this protein exhibits similarity to those of the class D β-lactamases. The two are clearly related to each other from an evolutionary point of view, however, the high resolution x-ray structures for both by themselves do not reveal why the former is a receptor/sensor and the latter an enzyme. It is documented herein that a single amino acid change at position 439 of the BlaR1 protein is sufficient to endow the receptor/sensor protein with modest turnover ability for cephalosporins as substrates. The x-ray structure for this mutant protein and the dynamics simulations revealed how a hydrolytic water molecule may sequester itself in the antibiotic-binding site to enable hydrolysis of the acylated species. These studies document how the nature of the residue at position 439 is critical for the fate of the protein in imparting unique functions on the same molecular template, to result in one as a receptor and in another as a catalyst.

29. Chemiluminescent and Fluorescent Nanoparticles: A New Paradigm for Optical Imaging

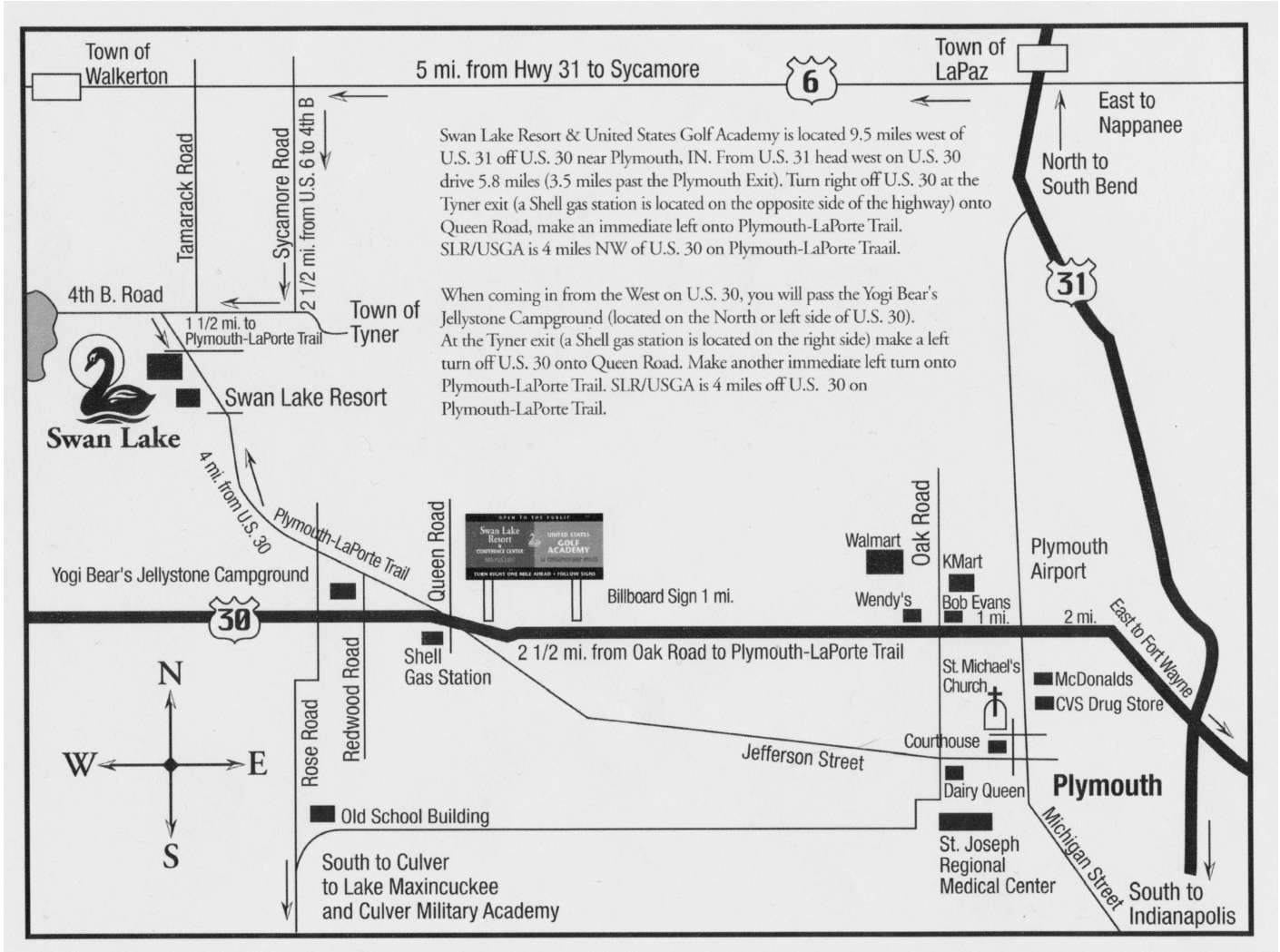
Douglas R. Rice, Jung Jae-Lee, Alexander G. White, Serhan Turkyilmaz, Reuben Chacko, Sankaran Thayumanavan and Bradley D. Smith
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA, and Department of Chemistry, University of Massachusetts at Amherst, Amherst, MA 01003 USA

Optical imaging is a powerful, non-invasive and non-ionizing technology for *in vivo* diagnosis. The current optical imaging paradigm uses passive or actively targeted particles doped with near-infrared (NIR) fluorophores to detect and image a variety of disease states. While useful, these particles suffer from low signal contrast due to high background signal from the required excitation laser. We have discovered a new set of NIR rotaxane dyes that are both fluorescent and chemiluminescent and we have used them to fabricate dye-doped nanoparticles for dual modality optical imaging. The chemiluminescence is thermally-activated (that is, no chemical or electrical stimulus is needed) which means that the probes can be stored at low temperature and they only become chemiluminescent when warmed to body temperature. Self-illuminating, chemiluminescent systems are well suited for optical imaging since they have inherently high signal contrast due to the lack of background signal. A new paradigm for optical imaging is proposed using glowing rotaxanes. They can be used in high contrast chemiluminescence mode to locate relatively deep anatomical locations in living patients and subsequently employed in fluorescent mode to identify the microscopic targets within thin histopathology sections taken from the same specimen. This new imaging paradigm has been illustrated through recent mouse imaging experiments that track the biodistribution of polymeric nanoparticles after intravenous injection. This study shows that chemiluminescent nanoparticles are revolutionary optical imaging probes.

30. Molecular Dynamics Studies of the Free DMF5 TCR and DMF5 HLA-A2 ELA pMHC Complex

Cory Ayres, Daniel M. Scott, Brian M. Baker and Steven A. Corcelli
The Computational Molecular Science and Engineering Laboratory
and the Department of Chemistry and Biochemistry,
University of Notre Dame, Notre Dame, IN 46556 USA

One method of activation of the adaptive 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 available TCRs, TCRs must be cross-reactive, yet must also maintain a degree of specificity to avoid indiscriminate activation of the immune response. 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. It was found in previous MD studies that for the A6 TCR, the two most flexible CDR loops are CDR3_ and CDR3_. CDR3_ moves slowly and samples both its free and bound conformations, while CDR3_ moves rapidly and samples a variety of conformations. This current study focuses on the DMF5 TCR, both unligated and bound to the HLA-A2 ELA pMHC complex. This particular TCR was chosen because of the conformational differences between it and the previously studied A6 TCR: while A6 shows significant differences between its free and bound state, DMF5 shows little, if any, conformational differences. Analysis of DMF5 TCR MD trajectories will be performed to gain insight into the flexibility and dynamics of the CDRs and the roles of mobility in DMF5 binding.


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