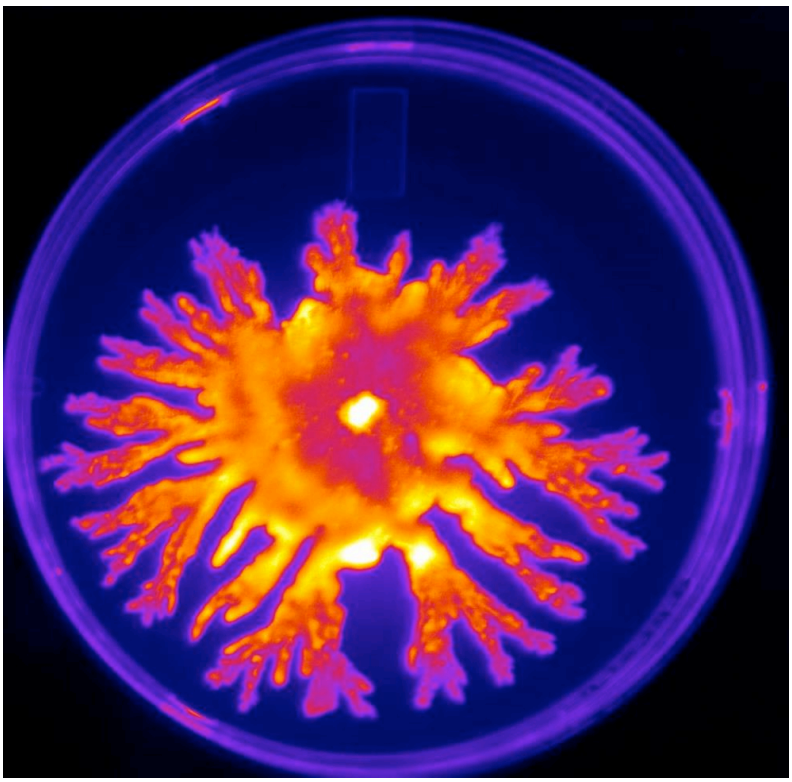
15th ANNUAL BIOCHEMISTRY RESEARCH FORUM

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



DeBartolo Performing Arts Center and Eck Visitors' Center
University of Notre Dame
April 30, 2010

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Cover Illustration

Courtesy of the DuBois and Shrout Laboratories

Cover Legend. *Pseudomonas aeruginosa* displays a type of multicellular coordinated motility called swarming, which has been associated with virulence. This motility has been found to be dependent on many factors, including iron availability and speciation. Work by Carolyn Dehner, laboratory of Professor Jennifer Dubois, and Professor Joshua Shrout (Department of Civil Engineering and Geological Sciences). Image taken by Matthew Leevy of the Notre Dame Integrated Imaging Facility.

Previous Keynote Speakers

1996: Nicholas Paoni (Genentech)
1998: Les Kozak (Jackson Laboratory)
1999: Andy Mesecar (IU-Chicago)
2000: Thomas Kelly (Case Western)
2001: Kwok Yeung (Bayer)
2002: John Beals (Lilly)
2003: Dudley Strickland (Red Cross)
2004: Elizabeth Komives (UCSD)
2005: Erik Zuiderweg (Michigan)
2006: Lila Gierasch (U. Mass)
2007: Shelagh Ferguson-Miller (Michigan State)
2008: Christian R. H. Raetz (Duke)
2009: John L. Wang (Michigan State)

♦ 2010 Keynote Lecture ♦

Thomas A. Gerken
Departments of Pediatrics, Biochemistry and Chemistry
Case Western Reserve University

Mucin Type O-Glycosylation: Cracking the Glycocode

Mucin-type *O*-glycosylation is one of the most common post-translational modifications of secreted and membrane associated proteins. A large family (~20 members) of polypeptide GalNAc transferases (ppGalNAc Ts) initiates the first step of mucin-type *O*-glycosylation by adding α-GalNAc to Ser or Thr residues of the peptide core. Subsequent transferases elongate the glycan chain by sequentially adding sugars to peptide-linked GalNAc. Glycoproteins containing heavily *O*-glycosylated mucin domains serve many important biological roles chiefly due to their unique biophysical and structural properties, which include an extended peptide conformation and robust resistance to proteases. It is also likely that *O*-glycosylation and heavily *O*-glycosylated domains may further present a molecular code for the specific recognition or modulation of additional binding partners, enzymes, receptors or even other glycosyltransferases. Hence, mucin-type *O*-glycosylation may be sufficiently regulated to serve as a modulator of complex biological processes and perhaps even signaling. Unfortunately, to date, very little is known quantitatively of the sites of *O*-glycosylation, their glycan structures, and of the specificity of the glycosyltransferases that initiate mucin type *O*-glycosylation.

The work of the Gerken laboratory has focused on filling in this lack of knowledge. Previously we have developed chemical and enzymatic methods for characterizing the site-specific glycosylation pattern of the simple mucins, which revealed highly reproducible sequence specific glycosylation patterns (*1*). Subsequent modeling of *in vitro* mucin glycosylation, by purified transferases, revealed a role for neighboring glycosylation and peptide sequence (*2*). Most recently we have focused on utilizing random peptide and glycopeptide substrates to quantify the peptide substrate preferences of the individual ppGalNAc T isoforms (*3,4*). These studies and those in progress further reveal that peptide sequence and overall charge serve to modulate each ppGalNAc T isoform's peptide substrate specificity. Coupled with each isoform's variable sensitivity to prior substrate glycosylation, a wide range of unique and specific substrate preferences is achieved across of the ppGalNAc T family of transferases. As a result of our studies, a basic understanding of the role of peptide sequence, neighboring glycosylation and local environment on the modulation of the initial steps of *O*-glycan elongation is being obtained. It is anticipated that tools for the rational prediction of site-specific *O*-glycosylation will result from these studies that will assist the elucidation of the protein substrates and biological role(s) of these transferases.

1) Gerken, T. A., Gilmore, M., and Zhang, J. (**2002**) Determination of the site-specific oligosaccharide distribution of the *O*-glycans attached to the porcine submaxillary mucin tandem repeat: Further evidence for the modulation of *O*-glycan side chain structures by peptide sequence, *J. Biol. Chem.* 277, 7736-7751.

2) Gerken, T. A., Tep, C., and Rarick, J. (**2004**) Role of peptide sequence and neighboring residue glycosylation on the substrate specificity of the uridine 5'-diphosphate-α-*N*-acetylgalactosamine: Polypeptide *N*-acetylgalactosaminyl transferases T1 and T2: Kinetic modeling of the porcine and canine submaxillary gland mucin tandem repeats, *Biochemistry* 43, 9888-9900.

3) Gerken, T. A., Raman, J., Fritz, T. A., and Jamison, O. (**2006**) Identification of common and unique peptide substrate preferences for the UDP-GalNAc: polypeptide α-*N*-acetylgalactosaminyltransferases T1 and T2 (ppGalNAc T1 and T2) derived from oriented random peptide substrates, *J. Biol. Chem.* 281, 32403-32416.

4) Perrine, C. L., Ganguli, A., Wu, P., Bertozzi, C. R., Fritz, T. A., Raman, J., Tabak, L. A., and Gerken, T. A. (**2009**) The glycopeptide-preferring polypeptide GalNAc transferase-10 (ppGalNAc T10), involved in mucin type *O*-glycosylation, has a unique GalNAc-*O*-Ser/Thr binding site in its catalytic domain not found in ppGalNAc T1 or T2, *J. Biol. Chem.* 284, 20387-20397.

Biography of Thomas A. Gerken

Thomas (Tom) Gerken received his undergraduate degree in 1972 from Grove City College, Grove City, PA, where he majored in Chemical Engineering. He did his graduate studies at Case Western Reserve University, Department of Chemistry, receiving his Ph.D. in 1977 studying copolymer structure by carbon-13 NMR. He began his postdoctoral studies in the Department of Pediatrics at Case Western Reserve as the Kathy Graub Memorial Research Fellow of the Cystic Fibrosis Foundation, characterizing mucin structure by carbon-13 NMR. In 1983 he joined the faculty of the Departments of Pediatrics and Biochemistry at Case Western Reserve as Assistant Professor, where he has remained, becoming Professor in 2005.

Tom has served on the Editorial Board of the *Journal of Biological Chemistry* (1998-2003 and 2004-2009) and has been a regular reviewer for the journals *Biochemistry* and *Glycobiology*. He is presently a member of the NIH Cell Biology Integrated Review Group, Intercellular Interactions (ICI) Study section (2010-2014).

Program

Friday Morning
DeBartolo Performing Arts Center – Decio Mainstage Theatre
Session Chair: Brian Baker

7:15-8:00 Continental breakfast
(outside Decio Mainstage Theatre)

8:00-8:10 Introduction and Orientation

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8:10-9:00 **Special Lecture**
Professor Subhash Basu
Induction of Apoptosis in Cancer Cells by Inhibitors of DNA and GSL Biosynthesis
Introduction by Professor Roger K. Bretthauer

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9:00-9:20 Jeffery A. Mayfield, Rosanne E. Frederick, Bennett R. Streit, Timothy A. Wincewicz and Jennifer L. DuBois
Spectroscopic, Steady-State, and Transient Kinetic Studies of a Representative Siderophore-Associated Monooxygenase

9:20-9:40 Ian Sander, Jenna L. Rose, Thomas F. Clarke IV, and Patricia L. Clark
Investigating the Role of Rare Codon Clusters in Protein Biogenesis

9:40-10:00 Oleg Y. Borbulevych and Brian M. Baker
Mystery Solved: X-ray Crystallography Explains the Cross-Reactivity Between Structurally Diverse Immunodominant MART-1 Epitopes

10:00-10:45 Mid-Morning Break

10:45-11:15 **Guest Speaker**
Professor Shaun Lee, Department of Biological Sciences, University of Notre Dame
Biosynthesis of Streptolysin S: Uncovering a New Class of "WMDs"

11:15-11:35 Zhi Xu, Francis J. Castellino and Victoria A. Ploplis
Plasminogen Activator Inhibitor-1 Stimulates Macrophage Activation Through Toll-like Receptor-4

11:35-11:55 Aranda Slabbekoorn and Holly Goodson
Interactions Between Tau and Microtubules

11:55-12:15 Jeanette Young, Shwan Rachid, Richard E. Taylor and Rolf Müller
Towards Characterization of the Polyketide Synthase Gene Cluster Responsible for the Production of Gephyronic Acid

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12:15-2:15 Lunch and Recreation
DeBartolo Performing Arts Center – Upper Level

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Friday Afternoon
DeBartolo Performing Arts Center – Decio Mainstage Theatre
Session Chair: Patricia Clark

2:15-2:45 **Guest Speaker**
Professor Amanda Hummon, Department of Chemistry and Biochemistry, University of Notre Dame
Transcriptomic and Proteomic Profiling of Cancer Cells

2:45-3:05 Krastyu Ugrinov and Patricia Clark
Co-Translational Folding Increases GFP Folding Yield

3:05-3:25 Leticia Llarrull and Shariar Mobashery
Unveiling Details of the Signal Transduction Pathway on the bla β-Lactam Resistance System in Staphylococcus aureus

3:25-3:45 James M. Kasuboski, Jason R. Bader and Kevin T. Vaughan
Aurora B is Required for Assembly of a Dynein-Binding Platform at Kinetochores

3:45-4:05 Carolyn Dehner, Jon Awaya, Lauren Barton, Patricia Maurice, and Jennifer Dubois
Iron Acquisition by an Environmental Aerobe: The Complex Role of Siderophores

4:05-4:45 Late Afternoon Break

Friday Evening

4:45-5:45 **Keynote Lecture**
Thomas A. Gerken, Departments of Pediatrics, Biochemistry and Chemistry, Case Western Reserve University
Mucin Type O-Glycosylation: Cracking the Glycocode

5:45-7:00 Poster Session and Reception
DeBartolo Performing Arts Center – Lower Level

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7:15-9:15 Dinner
Eck Visitors' Center – Main Lobby

9:15 Departure

ABSTRACTS: GUEST SPEAKERS

Transcriptomic and Proteomic Profiling of Cancer Cells

Amanda Hummon, Department of Chemistry and Biochemistry,
University of Notre Dame, Notre Dame, IN 46556 USA

The Hummon Research Group will develop methods to evaluate both the transcriptome and the proteome in mammary and colorectal cancer cells. We will develop and adapt current mass spectrometric and sampling protocols for global molecular profiling to understand cancer systems. Tandem measurements of mRNA and protein equilibria obtained following silencing of individual genes with RNA interference-mediated perturbations will elucidate the genetic regulation of cancer-associated signaling pathways. This translational research will assess the contributions of mRNA and protein levels to the global molecular environment in complex biological systems like colorectal cancer, improve understanding of the suppression mechanism of the endogenous RNAi pathways, as well as potentially having major contributions to human health in the identification of novel genetic targets for therapeutic intervention.

Biosynthesis of Streptolysin S: Uncovering a New Class of "WMDs"

Shaun Lee, Department of Biological Sciences, University of Notre Dame,
Notre Dame, IN 46556 USA

Bacteriocins are a large class of ribosomally synthesized toxins that serve as effective antibiotics for the producing organism against similar species (narrow spectrum) or across genera (broad spectrum). It is our primary research goal to gain a better understanding of the biosynthetic mechanisms underlying bacteriocin production. Our focus is on a particular class of bacteriocins that use a conserved mechanism of post-translational modification to produce the active toxin. One important member of this family is the highly active cytolysin Streptolysin S (SLS), an important virulence factor produced by the human pathogen Group A *Streptococcus pyogenes* (GAS). GAS is a leading human pathogen causing common infections such as pharyngitis and impetigo, as well as invasive syndromes such as necrotizing fasciitis and toxic shock syndrome. *In vitro* reconstitution of the SLS toxin has demonstrated that a precursor peptide and three conserved enzymes work in concert to modify the precursor into an active toxin. Gene clusters that resemble the SLS biosynthesis complex are present in several important human pathogens, such as *E. coli, Staphylococcus aureus, Listeria monocytogenes,* and *Clostridium botulinum*. Importantly, this class of peptide antibiotics is produced ribosomally, and thus will be amenable to genetic engineering strategies. Thus it is our goal to exploit this mechanism of bacteriocin biosynthesis to generate a library of artificial peptide antibiotics, many of which will undoubtedly have novel therapeutic value. It is likely that the discovery of similar peptidic antibiotics will rapidly expand as more genomes are sequenced.

ABSTRACTS: ORAL PRESENTATIONS

Spectroscopic, Steady-State, and Transient Kinetic Studies of a Representative Siderophore-Associated Monooxygenase

Jeffery A. Mayfield, Rosanne E. Frederick, Bennett R. Streit, Timothy A. Wincewicz and Jennifer L. DuBois
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Many siderophores used for the uptake and intracellular storage of essential iron contain hydroxamate chelating groups. Their biosyntheses are typically initiated by hydroxylation of the primary amine side chains of L-ornithine or L-lysine. This reaction is catalyzed by members of a family of flavin adenine dinucleotide (FAD) dependent monooxygenases. The kinetic mechanism for a representative family member has been studied by spectroscopic, steady-state, and transient kinetic methods for the first time, using the expressed N^5-L-ornithine monooxygenase (OMO) from the pathogenic fungus, *Aspergillus fumigatus*. Unlike previously characterized family members, this enzyme binds FAD with high affinity. The hydroxylation reaction occurs via the ordered addition of reductant (NADPH), then random binding by hydroxylatable substrate and O_2, followed by a chemically irreversible step resulting in production of the hydroxylating flavin-hydroperoxy species (FAD-OOH). Hydroxylation is followed by the ordered loss of the hydroxylamine and $NADP^+$. The siderophore-associated monooxygenases (SMOs) therefore share a sequential *ter-ter* kinetic mechanism with the hepatic microsomal flavin monooxygenases (FMOs) and the bacterial Baeyer-Villiger monooxygenases (BVMOs), with which they share moderate homology and from which they are distinguished by their acute substrate specificity. The reactive FAD-OOH is stabilized in the absence of hydroxylatable substrate by the presence of bound $NADP^+$ ($t_{1/2}$ = 0.94 min, 25°C, pH 7.4). $NADP^+$ therefore is a likely regulator of O_2 and substrate reactivity in the SMOs. The significantly shorter half-life of the reactive species and the remarkable specificity of the OMO-catalyzed reaction suggest added means of control beyond those of monooxygenases with similar kinetic mechanisms.

Investigating the Role of Rare Codon Clusters in Protein Biogenesis

Ian Sander, Jenna L. Rose, Thomas F. Clarke IV and Patricia L. Clark
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Protein sequences are encoded genetically using a redundant set of trinucleotide codons. However, not all synonymous codons are used with equal frequency. Rare codons have historically been regarded as detrimental to protein expression. Surprisingly, however, rare codons often cluster together, leading to transient pauses in protein synthesis (translation) [1]. While the reasons underlying rare codon clustering remain unclear, one possible role is to modulate co-translational protein folding. But such an effect on folding might be subtle, as the amino acid sequence should not be affected by use of rare versus common codons. We tested the effects of rare codon clusters *in vivo*, at the top-most level: cellular fitness. We constructed two otherwise isogenic strains of *E. coli* that differ only in the codon usage in one essential gene. Each strain also expresses a fluorescent reporter protein. Upon growth in competition, the strain bearing the wild type gene, which includes significant clusters of rare codons, dramatically out-competes the strain bearing the gene enriched in common codons. The wild type strain shows higher β-galactosidase activity than the common codon strain as well. We are currently determining the molecular mechanism underlying this competitive advantage.

Mystery Solved: X-ray Crystallography Explains the Cross-Reactivity Between Structurally Diverse Immunodominant MART-1 Epitopes

Oleg Y. Borbulevych and Brian M. Baker
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Adaptive immunity mechanisms are based on antigen recognition by cytotoxic T cells. T-Cell receptors (TCR) interact with certain peptide epitopes presented by class I MHC molecules, leading to an intracellular signaling cascade and a subsequent immune response. The MART-1 antigen is overexpressed by the majority of melanoma cells, and hence represents an attractive target for cancer immunotherapy using cytotoxic T cells. Immunodominant epitopes of MART-1 presented by the class I MHC HLA-A2 proteins comprise the decameric MART-1_{26-35} epitope EAAGIGILTV and the nonameric MART-1_{27-35} epitope AAGIGILTV. Our previous crystallographic study indicated that the conformations of those peptides in the HLA-A2 peptide binding groove differ significantly. Paradoxically, most MART-1 specific cytotoxic T cells can still cross-reactively recognize both MART-1 epitopes.

In the present communication we report, for the first time, the crystallographic structures of the clinically relevant TCR DMF5 in the complex with MART-1_{27-35}/HLA-A2 and MART-1_{26-35}/HLA-A2 to 2.3 Å and 2.7 Å resolution, respectively. These structures allow us to unravel the mechanism of cross-reactivity. Notably, recognition of the MART-1_{27-35} nonamer peptide by DMF5 is accompanied by a significant rearrangement of the peptide backbone. In contrast, the conformation of the decameric peptide remains unchanged upon recognition, indicating that cross-reactivity occurs via an "induced molecular mimicry" mechanism.

Plasminogen Activator Inhibitor-1 Stimulates Macrophage Activation Through Toll-like Receptor-4

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

Aside from its traditional role in regulating the fibrinolytic pathway, PAI-1 has been implicated in inflammatory responses. While inflammation is often associated with elevated levels of PAI-1, the functional consequence of PAI-1 in inflammation has yet to be fully defined. In this study, PAI-1 was shown to activate monocytes and macrophages, as demonstrated by a PAI-1 induced increase in various pro-inflammatory cytokines at both the mRNA and protein levels in monocyte and macrophage cell lines. This finding was further confirmed using primary bone marrow-derived macrophages. PAI-1 induced a dose-dependent upregulation of PAI-1 expression, suggesting an autocrine signaling effect. Utilizing various PAI-1 mutants, the proteolytic inhibitory activity, as well as LRP and VN binding functions, were not found to be involved in PAI-1-mediated activation of macrophages. However, the effect of PAI-1 on macrophage activation was partially blocked by a TLR4 neutralizing antibody. Furthermore, PAI-1-induced MIP-2 and TNF-α expression, and secretion were significantly reduced in $TLR4^{-/-}$ macrophages compared to WT macrophages. This suggests that TLR4 is an important mediator of PAI-1-induced macrophage activation. Additionally, direct interaction between PAI-1 and TLR4 was observed. These results demonstrate, for the first time, that PAI-1 is critically involved in the regulation of host inflammatory response through TLR4-mediated macrophage activation, and suggests a functional crosstalk between two highly conserved systems: hemostasis and the Toll-family signaling pathway.

Interactions Between Tau and Microtubules

Aranda Slabbekoorn and Holly Goodson
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Tau is a lattice-binding microtubule (MT) associated protein that provides cytoskeletal stability in neurons by preventing MT depolymerization. Tau has been the focus of a great deal of attention because it is found aberrantly assembled into paired helical filaments (PHFs) detached from MTs in the brains of all Alzheimer patients. However, many aspects of its functions and behavior remain poorly understood. We are studying interactions between tau and MTs and are focusing on the sensitivity of tau to different tubulin conformations. We are also investigating competition between tau and +TIPs for MT binding, and the possibility that tau binds to microtubules cooperatively. This work will help elucidate the physiological function of tau as a MT-binding protein in neurons, and may help to provide insight into how tau goes awry in Alzheimer's disease.

Towards Characterization of the Polyketide Synthase Gene Cluster Responsible for the Production of Gephyronic Acid

Jeanette Young[+], Shwan Rachid[‡], Richard E. Taylor*[+] and Rolf Müller*[‡]
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[‡]Institut für Pharmazeutische Biotechnologie, Universität des Saarlandes,
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Gephyronic acid is an antibiotic natural product isolated at the HZI (Helmholtz-Zentrum für Infektionsforschung) by Sasse and Höfle from the myxobacterium *Archangium gephyra* in 1995. Gephyronic acid has been shown to inhibit the growth of yeast and mold in the micromolar range; it also exhibits a cytostatic effect on mammalian cell cultures (human cervix, human leukemia, hamster kidney, hamster ovary, mouse connective tissue, and monkey kidney cancer cells) in the nanomolar range. An *in vitro* translation assay demonstrated that gephyronic acid is a specific inhibitor of eukaryotic protein synthesis. Interdisciplinary efforts within our laboratory are directed to the evaluation of gephyronic acid as a potential anti-cancer agent and include the development of a synthetic route to prepare quantities of material for further biological evaluation. Moreover, we seek to identify and characterize the polyketide synthase (PKS) gene cluster responsible for gephyronic acid production in the producing organism. Our proposed biosynthetic pathway involves seven elongation modules and two post-PKS modifying enzymes. We seek to validate this postulate via genetic analysis through complementary use of whole-genome sequencing and the use of a cosmid library derived from the myxobacteria strain *Cystobacter* cbvi 76, another known producer of gephyronic acid. This method for gene cluster analysis also will allow us to confirm several other unique features that comprise the gephyronic acid structure. Identification of the gene cluster is the key step in a heterologous expression system, which will provide an alternative source of the natural product and a fermentation system for precursor-directed biosynthesis of gephyronic acid analogues.

Co-Translational Folding Increases GFP Folding Yield

Krastyu Ugrinov and Patricia Clark
University of Notre Dame, Department of Chemistry and Biochemistry,
Notre Dame, IN 46556 USA

A growing amount of evidence suggests that the folding of nascent polypeptides may be influenced by the vectorial appearance of the polypeptide at the surface of the ribosome. Our goal is to determine the effects of the ribosome on the conformations and folding properties of newly synthesized polypeptide chains. We are using green fluorescent protein (GFP) as a model for co-translational folding of β-sheet structures. GFP is an excellent model for these studies because the residues that make contacts in the native structure are often far apart in the primary sequence. This high 'contact order' renders GFP (and many other β-sheet proteins) prone to aggregation during refolding *in vitro*, and suggests co-translational nascent chain conformations may be particularly important for productive folding. In this study, we assess the folding abilities of ribosome-bound GFP with the *C*-terminus either buried in the ribosomal exit tunnel, or exposed on the ribosomal surface. Our results show that GFP can fold completely to its native structure on the ribosomal surface. However, complete folding is only possible if the entire GFP sequence is exposed outside of the ribosome exit tunnel via the extended stall sequence. Importantly, stalled ribosome-bound GFP nascent chains that have their *C*-terminus buried in the ribosomal tunnel remain competent for folding, and fold to a native conformation with very high efficiency after release from the ribosome. The role of the ribosome and the ribosome-related chaperones in the *de novo* folding of ribosome-released GFP nascent chains is discussed.

Unveiling Details of the Signal Transduction Pathway on the *bla* β-lactam Resistance System in *Staphylococcus aureus*

Leticia Llarrull and Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556 USA

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a globally important pathogen that is resistant to all classes of commercially available β-lactam antibiotics. The basis for resistance is acquisition of a pair of signal sensing/transducing systems that unleash two separate and complementary antibiotic-resistance mechanisms: the production of a β-lactamase, and the expression of a novel penicillin-binding protein, which is not inactivated by the β-lactams. The expression of these antibiotic-resistance determinants is induced by the presence of the antibiotic in the milieu. The BlaR1 receptor has been implicated primarily in induction of β-lactamase expression (*bla* operon). The antibiotic irreversibly acylates the extracellular sensor domain of BlaR1 ($BlaR^S$). $BlaR^S$ responds to this acylation by a protein conformational change. It is our goal to elucidate how the signal transmits from the extracellular sensor domain to the cytoplasmic domain, which is proposed to be a metallo-protease, activated in the presence of the antibiotic.

We compared the expression of BlaR1 in *S. aureus* NRS128 in the presence of β-lactam antibiotics with the expression of recombinant BlaR1 in *E. coli*, where BlaR1 was detected in the membrane fraction. We also evaluated BlaI proteolysis in the presence of BlaR1. The analysis of the proteolysis patterns observed for BlaR and BlaI in the presence and absence of antibiotics open new questions on the signal regulation that culminates in manifestation of antibiotic resistance in this unique system. We have also studied the interaction of the repressor BlaI with the operator, in order to determine whether the elimination of the dimerization domain in BlaI upon proteolysis eliminates the ability of the repressor to bind to DNA. Our results show that both BlaI monomer and dimer bind to the operator region and argue against this hypothesis. Instead, our results suggest that BlaI proteolysis gives rise to an unstable fragment which is further degraded both in *S. aureus* and *E. coli* cells. The previously proposed site specific cleavage of BlaI would result in further proteolysis and in a substantial decrease in the concentration of the repressor in the cells, which would give rise to the expression of the β-lactamase.

Aurora B is Required for Assembly of a Dynein-Binding Platform at Kinetochores

James M. Kasuboski, Jason R. Bader and Kevin T. Vaughan
Department of Biological Sciences, University of Notre Dame,
Notre Dame, IN 46556 USA

Kinetochores are required for several aspects of mitosis, including initial interactions between chromosomes and microtubules (MTs), chromosome movement and activities associated with the spindle assembly checkpoint (SAC). Cytoplasmic dynein plays a role in one or more of these kinetochore functions, however, the specific contributions of dynein remain under investigation. The Vaughan laboratory recently identified a novel phosphorylation site in the dynein intermediate chains (ICs) that is required for initial recruitment to kinetochores during prometaphase. This phosphorylation site undergoes dephosphorylation in response to MT attachment and kinetochore stretch at metaphase. Because PP1g is implicated in catalyzing this dephosphorylation, we investigated the role of Aurora B (AurB) as a potential kinase in the kinetochore dynein pathway. AurB inhibition was achieved using either the small molecule inhibitor ZM443749 or transfection of a dominant-negative kinase-dead AurB construct. The impact of AurB inhibition was assessed by immunofluorescence microscopy (IFM) analysis or live-cell imaging of mitotic progression. AurB inhibition reduced the recruitment of phospho-dynein to kinetochores substantially, raising the possibility that AurB phosphorylates dynein or a dynein-binding protein. Western blot analysis revealed that AurB inhibition did not affect dynein phosphorylation directly, but rather affected the ability of kinetochores to recruit phospho-dynein. Assessing the chain of interactions needed for successful dynein recruitment, both *ZW10* and dynactin were reduced after AurB inhibition, suggesting that proteins that interface with dynein at kinetochores could be targets for AurB. In contrast, neither HEC1 nor Zwint-1 was affected suggesting that basic kinetochore structure is intact after AurB inhibition. Because AurB inhibition affected the interface between zwint-1 and *ZW10* most directly, we measured the ability of AurB to phosphorylate each *in vitro*. AurB phosphorylated zwint-1 but not *ZW10* in these assays, suggesting that phosphorylation of zwint-1 is required for outer kinetochore assembly. These findings suggest that AurB activity is required for assembly of a kinetochore dynein platform, perhaps through phosphorylation of zwint-1.

Iron Acquisition by an Environmental Aerobe: The Complex Role of Siderophores

Carolyn Dehner[1], Jon Awaya[1], Lauren Barton[2], Patricia Maurice[2] and Jennifer Dubois[1]
[1]Department of Chemistry and Biochemistry and [2]Department of Civil Engineering and Geological Sciences, University of Notre Dame, Notre Dame, IN 46556 USA

Nearly all living things have a requirement for iron (Fe). In aerobic, circumneutral environments, however, Fe is bound primarily in scarcely soluble minerals and amorphous solids and therefore is poorly bioavailable. Microorganisms are the gatekeepers for entry of Fe into global biogeochemical cycles. A primary means by which aerobic microbes enhance Fe mobility is by secreting siderophores, which are structurally diverse, low molecular weight chelating agents with extremely high affinities for Fe(III). However, siderophores alone solubilize minerals less efficiently than expected, due to weak adsorption to the iron mineral surface compared to small organic acids such as oxalate. Synergistic interactions between siderophores and other biogenic molecules have been proposed from *in vitro* studies of mineral dissolution as the means by which thermodynamic *and* kinetic barriers to Fe acquisition are overcome. The obligate aerobe, *Pseudomonas mendocina*, and genetically modified variants, including a site-directed mutant strain incapable of siderophore production, and a bioreporter strain that signals Fe status, were used to examine the role of siderophores, a reductant (ascorbate) or small carboxylic acid (oxalate), in Fe acquisition from the mineral hematite. We show that siderophores are indeed required for growth on hematite in the absence of exogenous reductant. A clear synergistic effect was observed in the presence of siderophore and oxalate, but not with siderophore and ascorbate. Interestingly, *P. mendocina* seems to secrete ~50-fold more siderophore than is metabolically useful, raising questions about regulation of the energy-taxing production of siderophores in this environmental organism. We have also examined the effect of mineral particle size using well-characterized 8 nm and 80 nm nanohematite, and found that the dependence on siderophores was greatly diminished with the smaller particles. Although the 8 nm nanohematite was found to dissolve at a 10-fold faster rate in the presence of excess siderophores, no dissolution was detected in its absence, as measured by atomic absorption spectroscopy. Possible contributing factors to the acquisition of Fe from the nanohematite by the siderophore(-) strain include production and secretion of extracellular polymeric substances (EPS) required for biofilm formation such as alginate, and penetration of the outer membrane by the 8 nm nanohematite, as shown by transmission electron microscopy. Overall, our results reveal some unexpected complexities of aerobic microbial acquisition of Fe.

ABSTRACTS: POSTERS

1. Investigating Conserved Motions in Proteins: Defining the "Signaling Conduit" in Pin1

Kimberly A. Wilson, Andrew T. Namanja and Jeffrey W. Peng
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Human Pin1 is a peptidyl-prolyl isomerase composed of a catalytic isomerase (PPIase) domain that is flexibly linked to a WW domain. Pin1 recognizes phospho Ser/Thr-Pro segments in signaling proteins regulating the cell cycle, and thus serves as both a potential cancer and Alzheimer's disease target. Our work using nuclear magnetic resonance (NMR) spectroscopy has demonstrated that substrate-induced inter-domain interactions can stimulate changes in internal conformational dynamics, generating a "conduit" for catalytic activity. Specifically, 13 out of the 17 residues displaying a loss in side-chain flexibility upon substrate binding are conserved, strongly suggesting that their dynamics is pertinent to the Pin1 mechanism. To test this notion, we have begun dynamics-function studies of Pin1 mutants that include site-substitutions of the 13 conserved residues. Here we present our initial results on M130A mutant, which includes an analysis of its backbone dynamics by ^{15}N NMR spin relaxation. Similarities and differences with wild type, and the implications for flexibility-function correlations in Pin1 will be discussed.

2. Protonation State of Penicillin-binding Protein 6 of *Escherichia coli* and its Implication to the Catalytic Mechanism

Weilie Zhang, Qicun Shi, Jed Fisher and Shahriar Mobashery*
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Penicillin-binding proteins (PBPs) are murein biosynthetic enzymes involved in the assembly of the bacterial cell wall. Acylation at the active site serine residue of these enzymes is the first step in their catalytic events. Penicillin-binding protein 6 (PBP6) is one of the two main DD-carboxypeptidases in *Escherichia coli*, which are known to perform a DD-carboxypeptidase reaction on the bacterial peptidoglycan, the major constituent of the cell wall. We investigated the roles of the two active site lysines, Lys 69 and Lys 235, in the catalytic machinery of soluble PBP 6 (sPBP6). By a sequence of site-directed mutagenesis and chemical modifications, we introduced γ-thialysine at each of these positions individually to study the roles of Lys 69 and Lys 235 in the catalysis of sPBP6. The pH dependence of k_{cat}/K_m and k_{cat} for sPBP 6 and for the two γ-thialysine mutants at positions 69 and 235 were determined. The optimum pH of this enzyme was 10-11. The perturbed pK_a of the residue 69 variant affects both limbs of the bell-shaped pH profile. The pH profiles revealed an approximately 0.4 - 0.5 pK_a decrease for the lower-pH limb and 0.6 - 0.9 decrease for the higher-pH limb. The optimum pH of both k_{cat}/K_m and k_{cat} was also shifted to lower pH values (pH 9 to 9.5). Lys 235 also contributes to the higher-pH limb of catalysis in the bell-shaped region. The pK_a attenuation of γ-thialysine 235 was 0.9 - 1.2 in the higher-pH limb only. Results indicated that Lys 69 plays a key role in proton transfer events in the course of catalysis during both the acylation and deacylation events.

3. Identification of Outer Membrane Secretion Determinants for Autotransporter Virulence Proteins

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Autotransporter (AT) proteins are virulence factors in Gram-negative bacteria. They are synthesized with an *N*-terminal signal sequence that is cleaved after secretion through the inner membrane, a central passenger domain (the mature protein), and a *C*-terminal porin domain. The porin domain is inserted in the outer membrane (OM) and the passenger domain is secreted across the membrane upon porin insertion. OM secretion does not require ATP or a proton gradient, and therefore the driving force for efficient OM secretion remains unknown. It is also not known what prevents premature folding of the passenger domain in the periplasm. There is an ongoing debate in the literature about the role, if any, of interactions between AT proteins and periplasmic chaperones and/or essential OM proteins like Omp85/BamA and their effect on AT secretion. Here, we investigate in detail the precise role of the AT porin domain and its contributions to passenger domain secretion. Two models for AT OM secretion have been proposed that make different, testable predictions for the role of the porin domain. In the concerted model, the porin domain is inserted in the OM, followed by passenger domain secretion facilitated by Omp85/BamA. In this model, the porin domain merely tethers the passenger domain to the OM and delivers it in close proximity to Omp85/BamA. To test this model, we will exchange the porin domain of the model AT pertactin with a similar but non-AT porin domain, and test this chimera for porin domain OM insertion and subsequent passenger domain secretion *in vivo*. Alternatively, the hairpin model suggests that the passenger domain is secreted through its own porin domain. In this model, the porin domain plays a more central, mechanistic role, and we expect that this would place more constraints on the chemical and/or steric properties of the porin interior, for successful passenger domain transport through the pore. We have identified a region of conserved glycine residues in the porin domain interior, and will test whether these residues have an impact on passenger domain OM secretion.

4. Passenger Domain Stability Correlates with Autotransporter Secretion Efficiency

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In Gram-negative bacteria, a wide range of virulence proteins is secreted via the autotransporter (AT) pathway. AT pre-proteins are synthesized with an *N*-terminal signal sequence that facilitates transport across the inner membrane, and a *C*-terminal β-barrel domain that acts as a pore for the mature virulence factor to cross the outer membrane (OM), flanking the central passenger domain that forms the mature, secreted virulence factor. Intriguingly, there is no significant concentration of ATP in the periplasm, or proton gradient across the OM, and hence the energetic origin of efficient OM secretion of AT proteins is unknown. Yet more than 97% of AT proteins are predicted to contain right-handed parallel β-helical structure, and the four crystal structures available for AT passenger domains each contain a long right-handed parallel β-helix. We tested what role, if any, passenger domain stability plays in AT OM secretion, using two model ATs: pertactin from *Bordetella pertussis* and plasmid-encoded toxin (Pet) from *E. coli*. Both pertactin and Pet contain β-helical structure, however, Pet also has a *N*-terminal globular protease domain. Previously, we showed that both pertactin and Pet have a *C*-terminal stable core structure in their β-helical domains. Mutations that destabilize the pertactin stable core result in lower levels of OM secretion. In contrast, a mutation that lowers the stability of only the *N*-terminus of the pertactin β-helix has no effect on OM secretion. To investigate the role of the *N*-terminal globular domain of the Pet passenger, we replaced the Pet protease domain with dihydrofolate reductase (DHFR), a protein whose folding and stability are well characterized. The chimeric DHFR-Pet β-helix passenger domain is secreted across the OM. Addition of methotrexate (MTX), an OM-permeable inhibitor of DFHR whose binding stabilizes native DFHR, to the culture medium reduces the level of secreted chimera. Moreover, we altered the stability of the DHFR domain by constructing a series of point mutations, and observed a linear correlation between DHFR stability and OM secretion efficiency. These results suggests that while the stability of the *C*-terminal stable core provides a positive contribution to OM secretion, stabilizing more *N*-terminal portions of the AT passenger domain can be detrimental to efficient OM secretion. These results demonstrate for the first time that passenger domain stability plays a direct role in AT OM secretion efficiency.

5. Cap Structures Reduce β-helix Aggregation Propensity

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Examination of β-sheets in protein crystal structures has revealed that the edge β-strands are protected from solvent. Presumably, such protection of unpaired hydrogen bond donors and acceptors helps prevent intermolecular associations and therefore reduces aggregation of these proteins. Here, we have investigated the role of β-sheet capping mechanisms in the folding and aggregation properties of pertactin, an autotransporter virulence protein from *Bordetella pertussis.* Pertactin is a right-handed β-helix protein that folds extremely slowly, yet is surprisingly resistant to aggregation. Examination of the pertactin crystal structure suggests two different strategies are employed to prevent multimerization. The *C*-terminus has irregular β-helical structure that folds in on itself, hence avoiding both solvent exposure of the hydrophobic core of the β-helix and intermolecular hydrogen bonding. In contrast, the *N*-terminus contains an inward-pointing charged residue. Removal of the *C*-terminal cap led to greater aggregation propensity and the formation of soluble oligomeric species. We are currently mutating the charged residue in the *N*-terminal cap in order to examine whether such mutations will also increase multimerization and/or aggregation. Altering both ends of pertactin will allow us to determine to what extent pertactin, and by extension other β-helical proteins, employ capping mechanisms to reduce/prevent aggregation.

6. Characterization of the VirG Autotransporter from *Yersinia pestis*

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VirG is a virulence-associated protein of *Yersinia pestis*, the causative agent of bubonic plague. Its homolog IcsA from *S. flexneri* is known to bind the mammalian protein N-WASP to facilitate actin polymerization. VirG/IcsA belongs to a family of autotransporter (AT) proteins, and like other ATs, it consists of a signal sequence that directs secretion across the inner membrane (IM), a central passenger domain (the mature virulence protein), and a *C*-terminal porin that inserts and forms a β-barrel in the outer membrane (OM) and is essential for OM secretion. Like most other ATs, the VirG passenger is predicted to adopt β-helical structure, which we have proposed is important for AT biogenesis. Previous studies in our lab with two other ATs, pertactin from *B. pertussis* and Pet from a pathogenic strain of *E. coli,* have shown that the *C*-terminal, β-helical portion of the passenger has a stable core that is resistant to chemical denaturation. This study aims to determine if this *C*-terminal stable core is a general feature of AT proteins, and also to identify the unique features of VirG that are important for biogenesis. We PCR-amplified the VirG gene from *Y. pestis* genomic DNA, and established an expression system in *E. coli*. OM purification showed that VirG co-purifies with the OM. Furthermore, we have also shown that the full length VirG protein is surface exposed, and by indirect immunofluorescence that it is active and can recruit N-WASP. The VirG passenger domain has been purified, and its secondary structure and thermal stability have been measured by far-UV CD spectroscopy. Our preliminary results suggest that, like pertactin, the purified VirG passenger unfolds and refolds reversibly and has a β-sheet-rich structure. However, unlike pertactin, VirG does not seem to populate a stable core, which might reflect unique features of its OM secretion mechanism.

7. Elucidation of the Structure of the Membrane Anchor of Penicillin-binding Protein 5 of *Escherichia coli*

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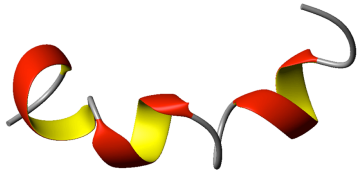
Penicillin-binding protein 5 (PBP 5) of *Escherichia coli* is a membrane-bound cell wall DD-carboxypeptidase that is localized in the outer leaflet of the cytosolic membrane of this Gram-negative bacterium. It is the most abundant PBP of *E. coli*, as well as a target for penicillins, and is the most studied of all of the PBP enzymes. PBP 5 is anchored to the cytoplasmic membrane by the 21 amino acids of its *C*-terminus. Although the importance of this terminus as a peripheral membrane anchor is well recognized, the structure of this anchor was previously unknown. Using natural isotope abundance NMR, the structure of the PBP 5 anchor peptide was determined within a DPC micelle. The structure conforms to a helix-bend-turn-helix motif and reveals that the anchor enters the membrane so as to form an amphiphilic structure within the interface of the hydrophilic/hydrophobic boundary regions of the lipid head groups. The bend and the turn within the motif allow the anchor to exit from the same side of the membrane that is penetrated. PBP anchor sequences represent extraordinary diversity, encompassing both *N*-terminal and *C*-terminal anchoring domains of these proteins. This study establishes a surface adherence mechanism for the PBP 5 *C*-terminus anchor peptide as the structural basis for further study toward understanding the roles of these domains in selecting membrane environments, and in the assembly of the multi-enzyme hyperstructures of bacterial cell wall biosynthesis.



8. Bacterial AmpD at the Crossroads of Peptidoglycan Recycling and Manifestation of Antibiotic Resistance

Dusan Hesek, Mijoon Lee, Weilie Zhang, Cesar Carrasco-López, Alzoray Rojas, Bruce C. Noll, Allen G. Oliver, Bill Boggess, Juan A. Hermoso* and Shahriar Mobashery*
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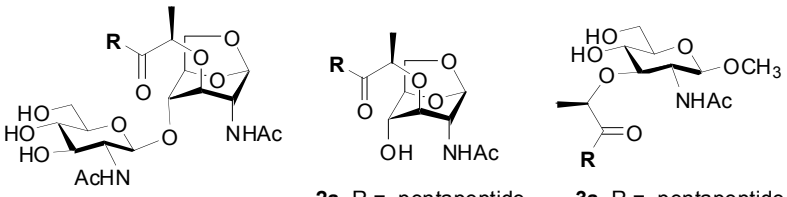
The bacterial cell wall is recycled extensively during the course of cell growth. The bacterial enzyme AmpD is an early catalyst in commitment of cell wall metabolites to the recycling events within the cytoplasm. The key internalized metabolite of cell wall recycling, β-D-*N*-acetylglucosamine-(1→4)-1,6-anhydro-β-*N*-acetylmuramyl-L-Ala-β-D-Glu-mesoDAP-D-Ala-D-Ala (compound **1**) is a poor substrate for AmpD. Two additional metabolites, 1,6-anhydro-*N*-acetylmuramyl-peptidyl derivatives **2a** and **2b**, served as substrates for AmpD with k_{cat}/K_m of $>10^4$ $M^{-1}s^{-1}$. The enzyme hydrolytically processes the lactyl amide bond of the 1,6-anhydro-*N*-acetylmuramyl moiety. This moiety is comprised of the sterically encumbered bicyclo system, with all its substituents in axial positions, which is in sharp contrast to the muramyl ring found in the peptidoglycan (and in **3a** and **3b**) with its all-equatorial substituents. Compounds **3a** and **3b** are not substrates for AmpD. The syntheses of compounds **1**, **2a/b** and **3a/b** are reported herein, which made the characterization of the enzymic reaction possible.



1a, R = pentapeptide

2a, R = pentapeptide
2b, R = tripeptide

3a, R = pentapeptide
3b, R = tripeptide

tripeptide = L-Ala-γ-D-Glu-*m*-DAP
pentapeptide = L-Ala-γ-D-Glu-*m*-DAP-D-Ala-D-Ala

9. Probing the Energetic Basis for T-cell Recognition

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T-Cell receptors (TCRs) are clonotypic, heterodimeric receptors on the surface of T-cells that interact with peptides presented on major histocompatiblity complexes (MHCs) on the surface of most nucleated cells through complementarity determining region (CDR) loops similar to immunoglobulins. The TCR binds to an MHC presenting a "non-self" peptide with a stronger affinity than MHCs presenting "self" peptides. The TCR must be able to distinguish between these two cases despite the fact that the majority of the potential interface is conserved between the two. The literature has traditionally drawn a distinction between contacts between the TCR and the peptide and contacts between the TCR and the MHC; even going as far as positing a two-state binding model where one state includes the TCR-MHC contacts but not the TCR-peptide contacts.

To assess to what extent the different portions of the TCR-pMHC interface contribute to binding, we quantify the contributions to binding of the side-chain side-chain contacts between the residues at the interface through double-mutant cycles. In a double mutant cycle between two residues, the free energy change upon binding is measured for i) the native partners, ii) the two partners with one residue mutated to alanine, iii) the two partners with the other residue mutated to alanine, and iv) the two partners with both residues mutated to alanine. The interaction energy between those two residues is defined as native free energy change minus the free energy changes of the two single mutants, plus the free energy change of the double-mutant: $\Delta\Delta G_{int} = \Delta G(X_{wt}\text{-}Y_{wt}) - \Delta G(X_{z\rightarrow A}\text{-}Y_{wt}) - \Delta G(X_{wt}\text{-}Y_{z\rightarrow A}) + \Delta G(X_{z\rightarrow A}\text{-}Y_{z\rightarrow A})$. By comparing the interaction energies of the TCR/peptide residue pairs and the TCR/MHC residue pairs, we can approximate the relative contributions of the peptide and the MHC to TCR/pMHC binding.

Double-mutant cycles allow us to compare the contributions to affinity from contacts made by the germline CDR 1and 2 loops and the variable CDR3 loops between the A6 TCR and the HLA-A2/tax9 pMHC. Both because the CDR3 loops are more variable and because of their position relative to the peptide in the available crystal structures. Although much work remains to be completed, our results so far show favorable interactions between CDR3β and the peptide, but also strong interaction energy between CDR1β and the periphery of the peptide. We have also found unfavorable interactions between CDR3α and the peptide and, surprisingly, a large favorable interaction energy between CDR3α and the MHC. These data suggest that despite the strong immunological pressures, the energetic profile of TCR recognition may be more malleable or variable than previously thought. In the case of A6 binding HLA-A2/tax9, CDR3/peptide contacts do not seem to be uniquely important and, in fact, a great deal of the "glue" holding A6 and HLA-A2/tax9 together appears to come from a CDR3 – MHC hydrogen bond between Arginine 65 on HLA-A2 and Aspartate 95 on CDR3α. In the future, we look forward to completing our study of A6 and HLA-A2/tax9, and to comparing the energetic distributions of other TCR-pMHC complexes.

10. Expression and Purification of the Cytoplasmic Domain of BlaR1 from Methicillin-resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a globally important pathogen that is resistant to all classes of commercially available β-lactam antibiotics. The basis for resistance is acquisition of a pair of signal sensing/transducing systems that unleash two separate and complementary antibiotic resistance mechanisms. One of these systems, the bla system, involves the expression of the PC1 β-lactamase, which hydrolyses the β-lactam ring, rendering the antibiotic ineffective. The expression of these antibiotic-resistance determinants is induced by the presence of the antibiotic in the milieu. The BlaR1 receptor has been implicated primarily in induction of β-lactamase expression (*bla* operon). The antibiotic acylates the extracellular sensor domain of BlaR1 ($BlaR^s$) irreversibly. $BlaR^s$ responds to this acylation by a protein conformational change that would be transmitted to the cytoplasmic domain to activate it. It has been proposed that the cytoplasmic domain is a metalloprotease, activated in the presence of the antibiotic. All previous attempts to express the cytoplasmic domain of BlaR1 have failed. Here we report the expression of the cytoplasmic domain of BlaR1 as different fusion proteins, and the purification of some of these fusion proteins in the presence of detergents mixtures.

11. NMR Backbone Dynamics of VEK-30 Bound to the Human Plasminogen Kringle 2 Domain

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Kringle domains are responsible for the binding of Pg and Pm to effector molecules, among which ligands are lysine and its analogues. The group A streptococcal surface protein PAM binds Pg with high affinity. A 30-residue internal polypeptide of PAM, VEK-30, presents the major binding determinant of the PAM/Pg interaction. Interestingly, while VEK-30 binds to Pg via its lysine binding sites, this peptide does not contain a *C*-terminal lysine residue, and, in addition, the binding of VEK-30 to Pg binding is highly specific to the kringle 2 domain of Pg ($K2_{Pg}$). To gain insights into the mechanisms for the tight and highly specific interaction of $K2_{Pg}$ with VEK-30, the dynamic properties of free and bound $K2_{Pg}$ and VEK-30 were investigated using backbone amide ^{15}N NMR relaxation measurements. Dynamic parameters, viz., the generalized order parameter, S^2, the local correlation time, τ_e, and the conformational exchange contribution, R_{ex}, were obtained for this complex by Lipari-Szabo model-free analysis. The results show that VEK-30 displays distinctly different dynamic behavior as a consequence of binding to $K2_{Pg}$, manifest by decreased backbone flexibility, particularly at the binding region of the peptide. In contrast, the backbone dynamics parameters of $K2_{Pg}$ displayed similar patterns in the free and bound forms, but, nonetheless, revealed interesting differences. Based on our previous structure-function studies of this interaction, we also made comparisons of the VEK-30/$K2_{Pg}$ dynamics results from different kringle modules complexed with small lysine analogs. The differences in dynamics observed for kringles with different ligands provide new insights into the interactions responsible for protein-ligand recognition and a better understanding of the differences in binding affinity and binding specificity of kringle domains with various ligands.

12. Synthesis and Kinetic Characterization of Sulfonates-Thiiranes as Gelatinase Inhibitors

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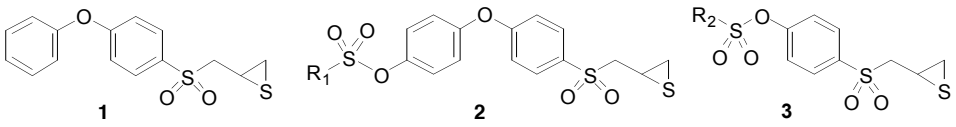
Matrix metalloproteinases (MMPs), a family of 26 closely related zinc-dependent endopeptidases, are involved in important pathological and physiological functions. Their unregulated and uncontrolled activities have been associated with a number of disease processes, including neurological disorders, arthritis, cardiovascular diseases, and cancer, just to mention a few. Virtually all known MMP inhibitors were developed to chelate the active-site zinc ions of these enzymes, and as such they are broad-spectrum inhibitors. This lack of selectivity has been problematic in clinical trials of MMP inhibitors, as the molecules show serious side effects. We have previously described the synthesis, MMP inhibitory activity, mechanism and metabolic studies of the thiirane-containing structure **1**, which is a potent and selective inhibitor of gelatinases (MMP-2 and MMP-9). We report herein the synthesis and evaluation of a series of sulfonyl-thiirane compounds of general structures **2** and **3**, which exhibit potent and selective inhibition of gelatinases. The detailed kinetics of inhibition for these compounds will be described. In the current study, kinetic analysis characterizing the rate of onset of inhibition (k_{on}), reversal of inhibition (k_{off}) and overall inhibition (K_i) was performed on each of these inhibitors. These inhibitors showed rapid onset of inhibition (k_{on} of 10^2 to 10^4 $M^{-1}s^{-1}$), slow reversal (k_{off} of 10^{-3} to 10^{-4} s^{-1}) and had K_i values in the range of 10 to 1000 nM. The kinetic parameters of the *p*-substituted derivatives **2** are comparable to those of compound **1**, but the single phenyl ring derivatives **3** have approximately 10-fold greater K_i values. No significant activity against MMP-1, MMP-3, and MMP-7 was observed. These results indicate that some of these compounds show promise as potent and selective inhibitors of gelatinases.



13. The Role of Molecular Flexibility in the Cross-Reactivity Between a T-Cell Receptor and its pMHC Ligands

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Selective recognition of an antigen by a T-cell is governed by the interaction of the T-cell receptor (TCR) with the targeted cell's major histocompatibility complex (MHC) and its bound peptide. The TCR binding surface is a composite of multiple, finger-like "loops" called complementarity determining regions (CDRs), which possess measurable degrees of flexibility. It is suspected that the conformational sampling by these CDR loops enable TCRs to bind to multiple antigens, thus accounting for the high level of cross-reactivity present in the T-cell repertoire. Examining the dynamics of CDRs in a TCR's unbound state ("free" TCR) should reveal pertinent details about how several peptide/MHC (pMHC) complexes can be recognized by a single TCR. Recent studies have demonstrated that cross-reactivity may also be dependent upon the dynamics of the antigen, making it necessary to assess the structural adaptability of both sides of the TCR/pMHC binding interface to more completely model T-cell recognition. This study utilizes time-resolved fluorescence anisotropy (TRFA) to characterize the mobility of CDRs and the peptide-presenting MHC platform on the nanosecond timescale.

Specifically, the A6 TCR is cross-reactive with two pMHC complexes, HLA-A2/Tax and HLA-A2/Tel1p, making these ideal systems for dynamic studies. TRFA results for both pMHC complexes indicate that the central region of the presentation platform of HLA-A2 is more dynamic when Tel1p is bound. The higher degree of flexibility measured for the HLA-A2/Tel1p binding surface aludes to the structural adaptation this pMHC complex undergoes in order to be recognized by A6 TCR. Crystal structures of free and bound HLA-A2 presenting both Tax and Tel1p corroborate this binding mechanism: HLA-A2/Tel1p has a different bound structure than its free state, whereas the Tax complex maintains its free conformation during recognition. Complementary measurements on the A6 TCR indicate that the CDR loops making contacts with the central region of the pMHC are more rigid than the more peripheral loops, which form fewer interactions with the pMHC complex. Minimizing the entropic penalties of conformational stabilization may explain why the inner loops are less flexible, as they must be structurally constrained in order to make interactions necessary for antigen recognition. Characterization of the flexibility of other TCRs will aid in possibly confirming a more general binding mechanism of rigid CDR loops at the point of peptide-specific contacts.

14. Synthesis, Kinetic Characterization and Metabolism of Diastereomeric 2-(1-(4-Phenoxyphenylsulfonyl)ethyl)thiiranes as Potent Gelatinase and MT1-MMP Inhibitors

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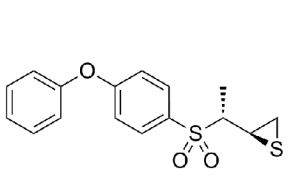
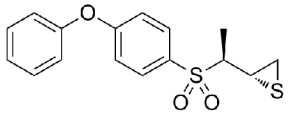
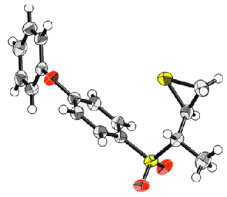
Gelatinases (MMP-2 and MMP-9) have been implicated in a number of pathological conditions, including cancer and cardiovascular disease. Hence, small molecule inhibitors of these enzymes are highly sought for use as potential therapeutic agents. 2-(4-Phenoxyphenylsulfonylmethyl)thiirane (SB-3CT) has previously been demonstrated to be a potent and selective inhibitor of gelatinases, however, it is rapidly metabolized due to oxidation at the *para*-position of the phenoxy ring and at the α-position to the sulfonyl group. α-Methyl variants of SB-3CT were conceived to improve metabolic stability and as mechanistic probes. We describe herein the synthesis and evaluation of these structural variants as potent inhibitors of gelatinases. Two (compounds **1c** and **1d**) among the four synthetic stereoisomers were found to exhibit slow-binding inhibition of gelatinases and MMP-14 (MT1-MMP), which is a hallmark of the mechanism of this class of inhibitors. The ability of these compounds to inhibit MMP-2, MMP-9 and MMP-14 could target cancer tissues more effectively. Moreover, metabolism of the newly synthesized inhibitors in *in vitro* systems showed that both oxidation at the α-position to the sulfonyl group and oxidation at the *para*-position of the terminal phenyl ring were prevented by the α-methylated analogs. Instead, oxidation on the thiirane sulfur is the only biotransformation pathway observed for these gelatinase inhibitors.

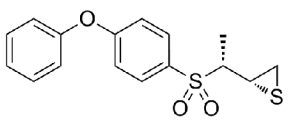


1a

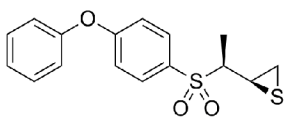


1b

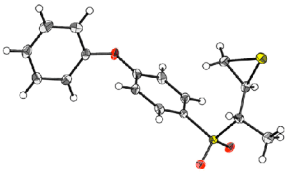




1c



1d



15. A Bioinformatics Approach Towards Characterizing ARP4-Ligand Interactions

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Actin is a fundamental component of the cytoskeleton that has been implicated in critical roles for an ever increasing number of processes, ranging from mitosis to muscle contraction. Although mostly studied in the context of the cytoplasm, it is now evident that actin is also present in the nucleus along with several actin-related proteins (ARPs). The ARPs represent a group of at least eight subfamilies that are evolutionarily related to conventional actin and thus have been named based on this similarity. Of particular interest is ARP4, the closest relative of actin that has been found in the nucleus. ARP4 is known to be involved in chromatin remodeling, although little is known about how it contributes to this process or what proteins it might bind to. In order to address these questions, we hypothesize that ARP4 is closely related enough to actin that the two proteins share some common ligands. This suggests that the surfaces that mediate protein-protein interactions are more likely to be conserved than surfaces which simply interact with solvent. To identify these conserved surfaces, a computational prediction of the ARP4 tertiary structure was built using a fungal subset of ARP4 sequences. Conservation scores among the fungal ARP4s were then mapped onto this homology model. A group of potential ARP4 ligands including actin, myosin, cofilin, and gelsolin were identified from the side-by-side comparison of the fungal ARP4 conserved surfaces to sites of known actin-ligand interactions. A workable model system is now being assembled to further investigate these ARP4 conserved surface amino acids using genetic and biochemical methods.

16. A Comparative Study of Different MART-1 Specific TCRs Interacting with Structurally Distinct Antigens

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T-Cells are the mediators of the immune response against pathogens and cancer cells. T-Cells use the T cell receptor (TCR) to bind antigens on the surface of diseased cells. TCRs are exquisitely specific for a particular antigen, yet many TCRs cross-react with multiple antigens. T-Cell cross-reactivity is a hallmark of the immune system and is necessary for maintaining the T-cell repertoire and initiating the cellular immune response. Though the importance of TCR cross-reactivity is well established, the molecular mechanisms underpinning TCR cross-reactivity remain unclear. The goal of my project is to determine how melanoma specific TCRs, which are highly cross-reactive, recognize structurally different tumor antigens. To accomplish this goal, I intend to quantify binding affinities between multiple TCRs and their cognate antigen complexes with surface plasma resonance (SPR). I also will use X-ray crystallography to solve structures of multiple cancer specific TCRs ligated to their cognate pMHCs. This will further our understanding of how these melanoma specific TCRs cross react with multiple tumor antigens. The results from this work will provide a molecular description of how TCRs achieve specificity and cross-reactivity in cellular immunity and inform the design of melanoma specific immunotherapies.

17. The Mechanism of Cytoskeletal "Cross-talk": Defining the Interactions among EB1, Actin and Microtubules

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It is known that two major cytoskeletal elements, actin and microtubules (MTs), are crucial for the survival of all eukaryotic cells: actin filaments provide mechanical structure and motility, while MTs drive essential cell activities ranging from separating chromosomes to transporting large particles. However, although actin filaments and MT polymers co-exist and cooperate in all animal cells, the mechanism of "cross-talk" between actin and tubulin still remains poorly understood. Interestingly, we have recently discovered that EB1, an essential MT binding protein (MTBP), can also promote actin polymerization. This observation suggests that EB1 can interact directly with both actin and MTs. Given that EB1 appears to play a fundamental role in controlling the stability of MT plus ends, we hypothesize that EB1 could be a major mediator (perhaps *the* major mediator) of actin-MT cytoskeletal "cross-talk." Thus, our goal is to define the interactions between EB1, actin, and MTs: Can EB1 bind actin directly? How strong is the interaction? Can actin or MTs "cross-talk" to each other through EB1? Overall, our studies will both elucidate EB1 function and help to clarify the mechanism of actin and MT "cross-talk." This in turn will contribute to the development of a coherent picture for the mechanisms of cell organization and differentiation in various cell types from ameobas to muscle cells.

18. A Yeast-Based Biosensor for Continuous Monitoring of Water Quality

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Water quality is an increasing concern in the modern world. As the human population proliferates, securing clean drinking water for the world's population is a daunting challenge. Industrial contamination, combined with shifting water table levels caused by unusual weather patterns, is increasingly releasing contaminants into the drinking water supply. In addition, there is the threat of terrorist attacks against the drinking water supply. Using current monitoring technologies, the first sign of a problem with drinking water is often mass illness. Therefore, there is an urgent need for new technologies that can alert us to contamination in the drinking water supply before they have a chance to impact human health.

We propose a new technology for continuous water quality monitoring based on a yeast-based biosensor. Our sensor will use genetically engineered yeast to respond to water contaminants by producing an easily detectable fluorescent protein signal. The yeast will be housed in a small chamber and water and nutrients will be constantly cycled through, providing continuous monitoring. The initial versions of this system will detect copper or estrogen, but future versions will be engineered to detect a variety of chemical water contaminants.

19. Molecular Architecture of Viral Assembly and Bud Site Formation

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Viruses cause an array of diseases, some fatal and others a minor nuisance, some transmitted through the air and others through bodily fluids. Strides have been made in eradicating some viruses from the planet through vaccination, but others such as HIV, Ebola or the Human T-cell Leukemia virus (HTLV) lack significant treatment options or preventive vaccination. The pathogenesis of these viruses depends on infection, replication and generation of new virions. This process requires concerted action of newly synthesized viral proteins and hijacking of mammalian machinery. The generation of new virions requires budding from the host cell membrane into a new particle with the ability to infect more cells. Over the last several years the matrix proteins (MA) and capsid proteins (CA) have been implicated in association with lipid membranes and budding from the host cell to create new virions, but mechanistic insight is lacking. To address basic questions underlying virion formation, we have used biochemical, biophysical, structural, cellular, and single molecule analysis to investigate how MAs bind lipid membranes and change their shape. We then map these interactions in human cells and in the formation of new virions.

20. Synthesis and NMR Studies of ^{13}C-Labeled Methyl Glucopyranuronides

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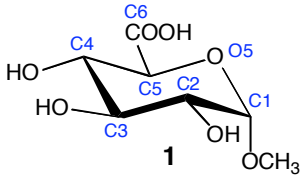
Methyl α-D-glucopyranuronic acid (**1**) and methyl β-D-glucopyranuronic acid (**2**) were prepared chemically containing single sites of ^{13}C-enrichment at C1, C2, C3, C4, C5 and C6. Aqueous solutions of these ^{13}C isotopomers were studied by ^{1}H and ^{13}C NMR spectroscopy at pD 2 and pD 7 to measure ^{1}H and ^{13}C chemical shifts, and J_{HH}, J_{CH} and J_{CC} scalar couplings, as a function of the ionization state of these molecules. Experimental studies were complemented by density functional theory (DFT) calculations in model structures of the same set of *J*-couplings in protonated and ionized **1** and **2** to determine how well theoretical predictions match the experimental findings in saccharide systems bearing ionizable functionality. Results show that all intra-ring J_{HH} values in **1** and **3** appear largely unaffected by solution pD. The solution pD exerts a relatively large effect on J_{CH} and J_{CC} values involving C5 and C6. Comparisons with methyl α- and β-D-glucopyranosides suggest that bonding within the C1-C4 fragment of the aldohexopyranosyl ring is minimally affected by conversion from CH_2OH to COOH at C6. This conversion, however, does affect the C4-H4, C5-H5, C4-C5 and C5-C6 bonds; the ionization state of **1** and **2** also affects these bonds significantly. The pK_a for **1** (3.2) and **2** (3.1) were estimated by fitting experimental ^{13}C/^{1}H chemical shift and *J*-coupling measurements to a modified Henderson-Hasselbach equation. This fundamental work establishes *J*-coupling behaviors in biologically important hexuronic acids, thus assisting in the structural interpretation of these NMR parameters in more complex biologically relevant oligosaccharides containing uronic acid constituents (*e.g.,* glycosaminoglycans). The present work focuses on pyranuronic acids that are relatively conformationally rigid (*gluco* configurations). Ongoing work aims to extend these findings to idopyranuronic acids, which are conformationally flexible; baseline studies of **1** and **2** are a prerequisite to experimental and theoretical investigations of the more complex *ido* configurations.



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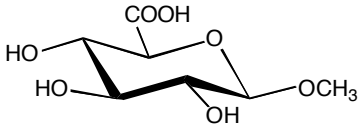

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21. Copper Sequestration and Delivery: The Yin and Yang of Breast Cancer and Cardiotoxicity

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Breast cancer is the leading killer disease in women. Breast cancer survivors may face increased risk of heart disease. It is essential to understand and improve the drug target in order to reduce side effect due to the current drugs used in treating breast cancer patients. The long-term goal of this study is to understand the molecular insight into selective regulation of protein kinase C in breast cancer etiology. Protein kinase C is a family of enzymes involved in several important cellular processes in both normal and cancer cell regulation. For instance, various studies have demonstrated the opposing role of protein kinase Cs in normal and various diseases states. A prime therapeutic target is protein kinase C-delta, a prosurvival factor in breast cancer cells, and a contributor to coronary heart disease, while protein kinase C-epsilon acts as a prosurvival factor in coronary heart disease and serves as a biomarker of aggressive breast cancer. These protein kinase C isoforms with different modes of activation and regulation demonstrate they can serve as unique therapeutic targets but must be addressed carefully and systematically due to their different roles in cell and disease states. Recently, we have found that metal ions play an important role in protein kinase -delta and -epsilon regulation. It is widely accepted that first, second and third hand smoke as well as industrial pollution are contributors to breast cancer incidence and heavy metal toxicity. Our results indicate that protein kinase -epsilon is positively regulated while protein kinase -delta is negatively regulated by various metal ions found in these pollutants. This exciting finding has opened an avenue to understand and further breast cancer treatment.

22. Optical Imaging of Tumors Using a Near-Infrared Probe for Cell Death

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Imaging probes that can determine, non-invasively, the amount of cell death in a specific tumor may have utility in clinical prognosis, as there is evidence that some highly malignant tumors have high rates of cell death, and in measuring anti-cancer treatment efficacy.[i,ii] Annexin V, a protein that binds exposed phosphatidylserine (PS) during cell death, is commonly used to determine drug efficacy and cell viability *in vitro*, and has been investigated for use *in vivo*. It is often challenging to optimize the formulation and pharmaceutical properties of proteins; therefore, we have developed a fluorescent small molecule mimic of Annexin V. Our near-infrared probe contains a carbocyanine dye conjugated to a zinc (II)-2,2-dipicolylamine (Zn(II)-DPA) coordination complex, which binds to anionic phospholipids on apoptotic and necrotic cells. Using tumor-bearing mouse and rat models, the probe (PSS794) was easily visualized at the site of the tumor. *Ex vivo* analysis revealed heterogeneity of probe localization in the tumor, with most of the probe concentrated in the necrotic core. Small molecules that can selectively target tumor anionic membrane surfaces and distinguish them from the near-neutral membrane surfaces of normal human cells have promising potential as imaging probes,[iii] drug delivery agents, [iv] and targeted molecular therapeutics.[v]

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