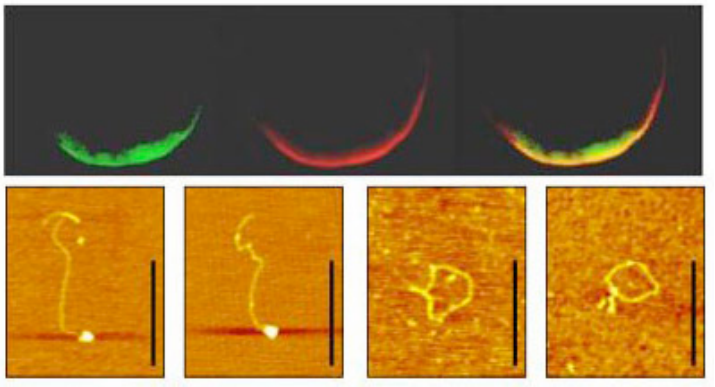
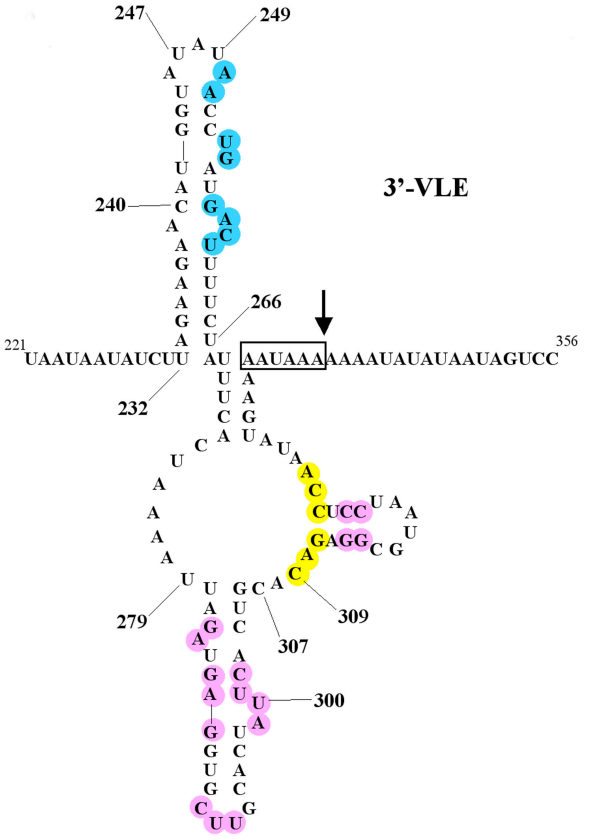
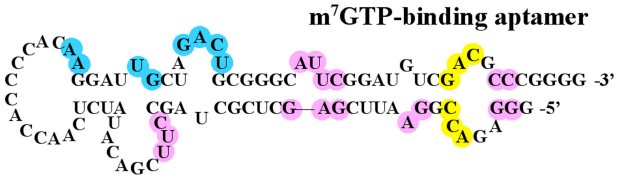
13th ANNUAL BIOCHEMISTRY RESEARCH FORUM

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







Potawatomi Inn
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Angola, IN
May 22-23, 2008

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Holly Goodson and Anthony Serianni

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Cover Illustration

Courtesy of the Huber Lab

Cover Legend. Vg1 mRNA (green) is localized to the vegetal cortex of *Xenopus* oocytes. The protein Prrp (red) binds to Vg1 and is colocalized with the RNA (yellow) (Zhao, Jiang, Kroll and Huber (**2001**) "A Proline-rich Protein Binds to the Localization Element of *Xenopus* Vg1 mRNA and to Ligands Involved in Actin Polymerization", *EMBO J.* **20**, 2315-2325). The binding site for Prrp in the 3'-UTR of Vg1 mRNA has structural elements found in an aptamer selected for binding to the m^7GTP cap structure located at the 5'-end of most mRNAs. AFM images of single Vg1 mRNA molecules (N.G. Kolev and P.W. Huber). Prrp (two rightmost images) binds and promotes circularization of the RNA. This observation suggests a mechanism for repressing translation during localization.

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 Keynote Lecture ♦

Christian R. H. Raetz

George Barth Geller Professor of Biochemistry
Department of Biochemistry
Duke University Medical Center

"Structure and inhibition of enzymes that assemble Gram-negative endotoxin: A potent lipid activator of innate immunity"

Keynote Lecture Abstract

The Raetz laboratory is studying the structures and mechanisms of enzymes required for the biosynthesis of outer membrane lipids (also known as endotoxins) in Gram-negative bacteria. In addition to their well known role in causing some of the complications of sepsis, these lipids are required for Gram-negative bacterial growth, and consequently, inhibitors of these enzymes have utility as novel antibiotics. The most advanced inhibitory compounds block an enzyme called LpxC, which catalyzes the second step of the endotoxin pathway, the deacetylation of UDP-(3-*O*-acyl)-GlcNAc. The antibiotic activity of the best LpxC inhibitor is comparable to that of ciprofloxacin. Furthermore these inhibitors are active against multi-drug resistant strains of *Pseudomonas*. For a recent review, see: Raetz *et al. Annual Review of Biochemistry* **2007**, *76*, 295-329.

Biography of Chris Raetz

Chris Raetz received his B.S. in Chemistry from Yale in 1967. He received his M.D. and Ph.D. from Harvard in 1973, where he worked with Eugene Kennedy on the enzymatic synthesis of phosphatidylserine. Following a two-year postdoctoral fellowship at NIH, Chris joined the Department of Biochemistry at the University of Wisconsin-Madison where he rose through the ranks to full professor. In 1987, Chris left academia to join Merck and Co. Inc., where he became vice-president for basic research in the areas of biochemistry and microbiology. In 1993, Chris returned to academia as George Barth Geller Professor and Chair of Biochemistry at Duke University. Starting with his independent work at NIH in 1975, Chris devised the first high-throughput screening methods for finding mutants in phospholipid biosynthesis. By combining genetics and chemistry, he elucidated the ten-step enzymatic pathway by which Gram-negative bacteria make the lipid A portion of lipopolysaccharide, a complex glycoconjugate of outer membranes that stimulates immune cells and is a target for new antibiotics. For more details, see: N. Zagorski *PNAS* **2007**, *104*, 17252-54.

Program

THURSDAY AFTERNOON
Session Chair: P. L. Clark

1:30 PM	WELCOME & INTRODUCTORY REMARKS (P. Huber)
1:40 PM	*Folding and secretion of the E. coli Pet autotransporter* J. P. Renn, M. Junker & P. L. Clark
2:10 PM	*Characterization of the effect of multiphosphorylation on Pin1 mediated isomerization in the proto-oncogene transcription factor c-Myc* B. Wilson & J. Peng
2:40 PM	*Role of Cu^{2+} on activation of PKCη – A resistance factor in lung cancer* C. G. Sudhahar and R. V. Stahelin
3:10 PM	BREAK
3:30 PM	*Hydroxyl group configuration/conformation and $^2J_{CCC}$ NMR spin-couplings in saccharides: DFT, NJC and NSA analyses of experimental data* T. Klepach, I. Carmichael & A. S. Serianni
4:00 PM	*An unprecedented MHC I α2 helix rearrangement as a novel mechanism for structural diversity between pMHC and TCR* O. Y. Borbulevych, K. H. Pipenbrink , A. Wojarnowicz & B. M. Baker
4:30 - 6:00 PM	OPEN TIME

THURSDAY EVENING

6:00 – 7:30 PM DINNER

7:30 – 7:45 PM OPEN TIME

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7:45 PM **KEYNOTE LECTURE**

Structure and inhibition of enzymes that assemble Gram-negative endotoxin: A potent lipid activator of innate immunity
Christian R. H. Raetz

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9:00 PM POSTER SESSION / SOCIAL

FRIDAY MORNING
Session Chair: B. M. Baker

7:30 - 9:00 AM BREAKFAST

9:00 AM **GUEST SPEAKER**
Engineering biomaterials for the treatment and diagnosis of skeletal disease and injury
Prof. Ryan K. Roeder, Department of Aerospace and Mechanical Engineering, Notre Dame

9:40 AM *Interactions between EB1 and MTs: Implications for EB1 Plus End tracking mechanism*
Z. Zhu, K. Gupta & H. V. Goodson

10:10 AM BREAK

10:30 AM	*Implications of single and combined murine deficiencies for coagulation factor XI and XII* F. A. Noria and F. J. Castellino
11:00 AM	**GUEST SPEAKER** *Unlocking how the immune system responds to intracellular pathogens: Do exosomes hold the key?* Prof. Jeff Schorey, Department of Biological Sciences, Notre Dame
11:40 AM	CLOSING REMARKS (H. Goodson)
11:50 AM – 12:30 PM	OPEN TIME
12:30 – 2:00 PM	LUNCH
2:00 PM	DEPARTURE

ABSTRACTS: GUEST SPEAKERS

Engineering biomaterials for the treatment and diagnosis of skeletal disease and injury

Ryan K. Roeder
Department of Aerospace and Mechanical Engineering
University of Notre Dame, Notre Dame, IN 46656

The ability to accurately diagnose fracture risk is severely limited. Accumulation of microdamage in bone tissue can lead to increased fracture susceptibility, including stress fractures in active individuals and fragility fractures in the elderly. Current methods for detecting microdamage are inherently invasive, destructive, tedious and two-dimensional. Therefore, we are investigating novel methods for detecting microdamage in bone using micro-computed tomography (micro-CT) and functionalized nanoparticle contrast agents with higher x-ray attenuation than bone.

Once a traumatic fracture or tissue degeneration has occurred, bone tissue may need to be replaced or augmented. The majority of all commercialized and FDA-approved orthopedic implants utilize relatively few biomaterials, with mechanical properties that typically deviate from bone tissue by an order of magnitude. Mechanical mismatch between a biomaterial and peri-implant tissue can lead in poor osteointegration. Therefore, we are also investigating hydroxyapatite (HA) whisker reinforced polymer biocomposites in order to engineer synthetic bone substitutes with tailored mechanical, biological and surgical function.

Unlocking how the immune system responds to intracellular pathogens: Do exosomes hold the key?

Jeffrey Schorey
Department of Biological Sciences
University of Notre Dame, Notre Dame, IN 46556

Exosomes are small vesicles released from hematopoietic and non-hematopoietic cells. Their release from cells promotes intercellular communication and they are involved in diverse functions including antigen presentation, protein secretion and RNA transport. Our studies suggest a novel mechanism of immune surveillance involving exosomes released from mycobacteria-infected macrophages and we have shown that these exosomes can activate innate and acquired immune responses *in vitro* and *in vivo*. Since these exosomes have both adjuvant and antigenic characteristics, we hypothesize that they may be excellent candidates for future vaccine studies.

ABSTRACTS: ORAL PRESENTATIONS

Folding and secretion of the *E. coli* Pet autotransporter

Jonathan P. Renn, Mirco Junker and Patricia L. Clark
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556

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 which 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, flanking the central passenger domain that forms the mature, secreted virulence factor. Intriguingly, there is no significant concentration of ATP in the periplasm, nor a proton gradient across the OM (outer membrane), so 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 α-helical structure, and the three crystal structures available for AT passenger domains each contain a long right-handed parallel α-helix. Previous studies have shown that the passenger domain of pertactin, an AT from *Bordetella pertussis*, exhibits three-state folding and has a C-terminal stable core structure. Here, we show that Pet, an unrelated AT from *Escherichia coli*, also exhibits three-state unfolding and also has a stable core structure. Mass spectrometry and N-terminal sequencing demonstrate that the Pet stable core is also located near the C-terminus of the passenger domain. Moreover, sequence analysis suggests that three-state folding and a C-terminal stable α-helix structure could be important general features of the biogenesis of AT proteins *in vivo*. We are currently investigating the role of other, non-α-helical parts of the passenger domain, and their influence, if any, on efficient OM secretion. Mutational analysis of the Pet N-terminal, globular protease domain suggests that the folding and/or stability of this domain may be important for efficient Pet OM secretion.

Characterization of the effect of multiphosphorylation on Pin1 mediated isomerization in the proto-oncogene transcription factor c-Myc

Brian Wilson and Jeff Peng
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556

The proto-oncogenic transcription factor c-Myc activates expression of several mitotic regulatory genes through binding E-box sequences and recruiting histone acetyltransferases. Degradation of c-Myc occurs by ubiquitination at the MB2 domain and its lifecycle is dependent on phosphorylation of Thr_{58} and Ser_{62} in MB1. *In vivo* and *in vitro* studies have shown that phosphorylation at Ser_{62} ($pSer_{62}$) stabilizes c-Myc while phosphorylation at Thr_{58} ($pThr_{58}$) is required for degradation by promoting the dephosphorylation of Ser_{62}. Upon phosphorylation, c-Myc is recognized by the peptidyl-prolyl isomerase Pin1, which accelerates the rate limiting *cis/trans* isomerization of phosphorylated Ser (Thr)-prolyl peptide bonds. Unlike the other families of isomerases (*e.g.*, cyclophilins, FKBP), Pin1's isomerase activity has been linked to several important cell cycle events, however, the overall mechanism of Pin1 is unknown. In the case of c-Myc, dephosphorylation at Ser_{62} occurs through the concerted activity of Pin1 and the trans phosphatase PP2A, leaving $pThr_{58}$ phosphorylated. The stability of $pThr_{58}$ and the relative spacing of phosphorylation sites raise questions about the mechanism by which c-Myc levels are regulated.

Using two-dimensional exchange spectroscopy (EXSY NMR) and other techniques sensitive to increased relaxation, catalytic rates can be characterized for the *substrate* over many time scales. We use 2D EXSY NMR to observe and compare the *cis/trans* isomerization of pSer(Thr)-prolyl peptide bonds in a minimalist c-Myc phospho-peptide (LLP$\mathbf{T_{58}}$PPL$\mathbf{S_{62}}$PS) in the presence and absence of Pin1. This provides insight into forward and reverse rates of isomerization by Pin1, and thus lays a foundation for understanding the role of phosphorylation in the c-Myc lifecycle and activity. The goal is to decipher the mechanism of Pin1-mediated phosphorylation-dependent isomerization in c-Myc using the single ($pThr_{58}$ or $pSer_{62}$) and double phosphorylated (pT_{58}-pS_{62}) states. These findings will be extended to analyze the effect of amino acid spacing between Pin1 recognition motifs, allowing hypothesis on the role of multiple phosphorylation in cellular signaling.

Role of Cu^{2+} on activation of PKCη – A resistance factor in lung cancer

Christopher G. Sudhahar[2, 3] and Robert V. Stahelin[1, 2, 3]
[1]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, South Bend, [2]Department of Chemistry and Biochemistry and [3]The Walther Center for Cancer Research, University of Notre Dame, Notre Dame, IN 46556

Protein Kinase C (PKC) is a family of serine/threonine kinases that play an essential role in many of the signaling cascades that preside over cellular actions. PKC inhibition or activation has lethal consequences in one cell type and a beneficial effect in another. Specificity of PKC isoform's activation/inhibition is prerequisite for the goal of future therapies. In fact, PKCs harbor conserved modular domains that behave differently among the isoforms due to slight differences in primary sequences and how the modular domains interplay to target PKCs to the cellular activation site. The current studies aim at unraveling the differential roles of C2 domains in PKC isoforms. Canonically, the role of the C2 domain in classical isoforms (a, b and g) and mechanism of membrane binding has been well studied by several groups. However, C2 domains in novel PKCs (e, h, d and q) have not been studied in detail. As a first step in understanding the role of C2 domains in PKC e and h, targets in heart disease and cancer, respectively, we performed sequence alignment of all C2 domains. While these C2 domains harbor some conserved residues involved in lipid binding, they lack the consensus Asp for binding Ca^{2+} like most C2 domains. Because metal ions play important roles in the biological function of many enzymes, we performed a metal binding bioinformatics prediction on the C2 domains of PKCe and h. SVMProt: Protein Functional Family Prediction found both C2 domains were predicted to Cu binding protein. Based on this result we proceeded with subsequent experiments using fluorescence spectroscopy and surface plasma resonance (SPR) which have demonstrated the role of Cu^{2+} in coordinating these two C2 domains and inducing unique lipid binding properties. Future experiments are aimed at dissecting the role of metal transport in PKCe and h activation in cellular systems. These studies will be the first step in understanding the role of C2 domains in novel PKCs and the importance of Cu^{2+} in the spatial and temporal activation of these PKC isoforms.

Hydroxyl group configuration/conformation and $^{2}J_{CCC}$ spin-couplings in saccharides: DFT, NJC and NSA analyses of experimental data

Thomas Klepach[1], Ian Carmichael[2] and Anthony S. Serianni[1]
[1]Department of Chemistry and Biochemistry, and [2]Radiation Laboratory,
University of Notre Dame, Notre Dame, IN 46556

Interpretation of experimental NMR spin-spin coupling constants (SSCC; *J*-couplings) using theoretically derived correlations between SSCCs and geometric parameters such as bond length, bond angle and dihedral angle has led to quantitative conformational analyses of saccharides in solution. Despite the success of these phenomenologic correlations, the underlying mechanisms of spin-density transfer and their relationship to molecular geometry remain largely unexplored in saccharide systems. In our continuing efforts to develop NMR spin-couplings as experimental probes of carbohydrate structure, we sought to cross-validate experiment with theory for the dependence of intra-ring $^{2}J_{CCC}$ magnitude and sign on saccharide configuration at the coupled carbons (*J. Org. Chem*. **2007**, *72*, 7511-7522). We also investigated the dependence of $^{2}J_{CCC}$ on hydroxyl group conformation (exocyclic C-O bond rotation). Since empirical correlations between experimental *J*-couplings and molecular geometry are to some extent incidental, with the former dependent on, and the latter dictating the underlying electronic structure of the molecule, an attempt was made to interpret theoretical findings within the context of geometry-dependent electron occupancy variations in a natural bonding orbital (NBO) analysis as correlated with specific spin-density transfer mechanisms described by a finite field perturbation natural *J*-coupling (NJC) analysis. Within the NBO paradigm, two distinct spin-density transfer mechanisms were identified for both the configurational and conformational dependencies, namely, destabilizing steric (through-space) and stabilizing hyperconjugative (through-bond) interactions. The merits and weaknesses of the NBO interpretation will be discussed.

An unprecedented MHC I α2 helix rearrangement as a novel mechanism for structural diversity between pMHC and TCR

O. Y. Borbulevych, K. H. Pipenbrink, A. Wojarnowicz and B. M. Baker
Department of Chemistry and Biochemistry, [3]Notre Dame Cancer Institute, University of Notre Dame, IN 46556

Antigen recognition by the T cell receptor (TCR) is initiated by molecular contact between the TCR and the peptide/MHC complex, which leads to an intracellular signaling cascade resulting in a functional response by the T cell. Although antigen specificity is a distinct feature of the adaptive immune response, a growing number of evidences indicates that TCRs could bind and respond to structurally diverse antigens. Notably, it has been found that the HTLV-1 Tax_{11-19} (LLFGYPVYV)-specific TCR A6 could cross-reactively recognize the Tel1p peptide (MLWGYLQYV), both presented by the class I MHC molecule HLA-A2.

To unravel the structural details underlying the recognition of the Tel1p peptide we carried out crystallographic studies of Tel1p/HLA-A2 and its complex with the A6 TCR at 1.9 and 2.6 Å resolution, respectively. Both Tax and Tel1p peptides were found to adopt similar conformations within the MHC binding groove. However, in contrast with the Tax structure, the binding of A6 to Tel1p/MHC is accompanied by the transition of Tyr5 in the center of the peptide to a different rotamer ($\Delta\chi1$ of 120°). However, the most unexpected finding with the Tel1p structure is an unprecedented rearrangement of the MHC I α2 helix fragment spanning residues 149-155, resulting in a 5.5 Å shift at Ala150. Mutational studies with differential effects on recognition of the Tax and Tel1p complexes independently confirm the existence of the structural shift.

The analysis of the known structures of peptide/MHC class I free (115 PDB entries) or complexed with T cell receptors (32 PDB entries) has revealed that a MHC helical shift similar to that found in the Tel1p/MHC/A6 structure has never been observed before. We believe that the rotation of the Y5 side chain upon the TCR engagement triggers a series of structural changes resulting in this MHC helix rearrangement. Overall, these results demonstrate a novel mechanism for achieving structural diversity in antigen complexes with immune receptors.

Interactions between EB1 and MTs: Implications for EB1 Plus End Tracking Mechanism

Zhiqing Zhu, Kamlesh Gupta and Holly V. Goodson
Department of Chemistry and Biochemistry, University of Notre Dame, IN 46556

The microtubule (MT) cytoskeleton plays an essential role in many cellular processes, including intracellular membrane traffic, cytoplasmic organization, chromosome segregation and cell division. The cellular functions of MTs are highly dependent on their dynamic properties. Interestingly, MT dynamics are regulated by a number of proteins including the plus-end tracking proteins (+TIPs). These proteins are believed to act either by binding the plus end of MTs and inhibiting MT catastrophe, or by recruiting a MT destabilizer (such as stathmin/Op15) onto MTs. EB1 (End Binding protein-1) is a highly conserved +TIP. Studies reveal homologous proteins of EB1 can interact with MT plus ends in many organisms, from yeast to human. However, the mechanism of EB1 plus end tracking still remains unclear. Several studies have tried to elucidate this mechanism by investigating the interactions between EB1 and MTs; however, most of these studies have used EB1 proteins with affinity tags. We have found that tagged EB1 proteins with tags behave differently than tagless proteins. This discovery has raised questions about the interpretability of the existing work. We have studied tagless EB1 and find that EB1 binds weakly to both tubulin and MTs, but the EB1 which is missing the its tail region can interact strongly with tubulin/MT. This suggests that EB1 has a self-inhibited conformation that needs to be opened up ("turned on") to track the MT plus end.

Implications of single and combined murine deficiencies for coagulation factor XI and XII

Francisco A. Noria and Francis J. Castellino. Department of Chemistry and Biochemistry, University of Notre Dame and W.M Keck Center for Transgene Research, Notre Dame, IN 46556

The plasma contact system consist of a protein cofactor known as High Molecular Weight Kininogen (HK) and three zymogen serine proteases: coagulation Factors XII (FXII) and XI (FXI), and plasma Prekallikrein. Prekallikrein and FXI circulate in plasma bound to HK forming dual complexes that associate with cell surface receptors on EC, platelets and some leukocytes. The functions of the system have been identified first as a contributor to the secondary activation of prothrombin for augmentation of the initial thrombin generated after activation by the extrinsic pathway, Tissue Factor/FVIIa, and secondly in the generation of bradykinin from HK cleavage by kallikrein. These proteins exert their actions through binding to cell surfaces and contacting ECM components. These actions involve the cleavage of FXII after exposure to a negatively charged surface, kallikrein and FXI cleavage by FXIIa after binding to HK receptor on cell surfaces, and generation of bradykinin from the cleavage of HK by kallikrein. These actions make them likely to participate in additional cell adhesion, activation and other cell-cell and, cell-protein interactions. In humans, deficiencies of any of the contact system proteins do not present with severe symptoms with the exception of FXI, which can in some cases manifests a bleeding diathesis. Detailed phenotypic characterizations of mice lacking FXI revealed a series of vascular and hepatic abnormalities, as well as an augmented inflammatory response when a model after inducing arterial ischemia. Mice with a combined deficiency of FXII and FXI exhibit abnormal spleen morphology, an irregular CBC, and extended aPTT values compared to mice with either of the individual deficiencies. This data suggests the existence of extended roles for the contact system in inflammation and organ pathology.

ABSTRACTS: POSTERS

1. Mechanism, structure, and inhibition of siderophore-associated amine monooxygenases from pathogenic microbes

Rosie Frederick and Jennifer DuBois
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Notre Dame, IN 46556

Iron is an essential nutrient for all living things, and the ability to acquire it from the host is essential for the establishment and maintenance of infection by a pathogen. *Aspergillus fumigates*, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis* utilize secondary metabolites that competitively scavenge iron in dilute and tightly bound sources to sustain virulence in the host. Three homologous flavin-dependent enzymes from the biosynthetic pathways for siderophore production of these pathogens include L-ornithine monooxygenase (OMO), L-lysine monooxygenase (LMO), and mycobactin monooxygenase (MBMO), respectively. The kinetic mechanism for OMO is on the brink of elucidation and determination of effectors and inhibitors is in current progress. Also in progress are the kinetic mechanisms of LMO and MBMO, which will be determined concurrently as they hydroxylate different amine substrates, but are each L-lysine derived. Each of these enzymes pose as novel antimicrobial targets for common infectious agents as they spread globally and as resistance to available antibiotics grows.

2. Molecular basis for signal transduction in the bimodular protein Pin1

Andrew T. Namanja, Xiaodong J. Wang, Felicia A. Etzkorn, and Jeffrey W. Peng
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556

The three basic components making up any information system are input, processing and output. Connecting these components in the complex cellular networks of signal transduction proteins remains to be a challenge. A starting point includes the deciphering of the underlying molecular mechanism by which simple protein motifs build such complex information processing systems

Pin1 is a representative of many signaling proteins that adopt a modular design in which a docking module (WW domain) and a loosely connected catalytic module (peptidyl prolyl isomerase - PPIase domain) work in concert to counterbalance an offset in the equilibria of relative *cis* and *trans* populations of many target sequences. Specifically, Pin1 recognizes and catalyzes the *cis-trans* conversion of the peptidyl pSer/pThr-Pro bond. Some of Pin1 targets include: the amyloid precursor protein APP, the proto-oncogenic transcription factor c-Myc, the mitotic inducer Cdc25c phosphatase, the mitotic kinase Wee1, and the microtubule associating protein tau. Inhibition of Pin1 induces apoptosis of several cancer cell lines and contributes to the neuronal death in Alzheimer's disease. Pin1 has therefore gained popularity as an emerging drug target for the therapy of cancer and Alzheimer's disease.

Variations in the input substrate sequence induce differential processing by Pin1 and thereby modulating the resultant "output" activities for binding and catalysis. The absence of functional WW (lone PPIase or compromised WW) has deleterious consequences on the catalytic activity of Pin1. Hence, there must exist a mode of information transformation between Pin1 domains. Our previous studies of Pin1 interaction with Cdc25 peptide substrate using nuclear magnetic resonance (NMR) deuterium (^{2}D) sidechain relaxation suggested an alternative (novel) modular allostery mechanism in which domain interactions can stimulate changes in the internal conformational dynamics to allosterically modulate binding and catalytic activities. We hereby report NMR binding and spin relaxation (^{15}N, ^{2}D and ^{13}C) studies of full length Pin1 and PPIase upon interaction with *cis* (Z) and *trans* (E) locked amide isosteres of the Ac-FFpS-Y[(Z and E)CH=C]-PR-NH2 peptidomimetic. These isosteres are competitive PPIase inhibitors and induce different binding dynamics responses. They also represent the *cis* and *trans* ground state configurations of the peptide substrate FFpSPR and thus allow for the dissection of the binding step independent of the isomerase reaction. From this, new insights have emerged for the role of the WW domain as a heterotropic allosteric modulator for the PPIase. The molecular basis for the differential preference for binding *cis* or *trans* substrate ground configurations by the PPIase will be discussed.

3. Development of a fluorescent anisotropy assay for peptide-MHC and TCR interactions

Brian E. Gloor and Brian M. Baker
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Notre Dame, IN 46556

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 (TCR) is a molecule found on the surface of T lymphocytes that is responsible for recognizing antigens presented by the major histocompatibility complex (MHC). The proteins encoded by the MHC are expressed on the surface of cells and display fragments of molecules from invading microbes or dysfunctional cells to the TCR and will induce apoptosis of the cell in question. Observations of TCR-pMHC kinetic parameters have been obtained through the utilization of surface plasmon resonance (SPR). Unlike pure solution methods, SPR does not allow for detailed resolution of the kinetic mechanism for the TCR-pMHC interaction. Through the use of fluorescence anisotropy, we hope to be able to obtain steady-state kinetic parameters and then broaden our understanding of TCR-pMHC interactions through fluorescence anisotropy. The need for such an assay is key to understanding the kinetic recognition mechanism of the TCR to the pMHC as well as enable further investigation of cross reactivity and specification within various TCRs. Fluorescence studies on the interaction will be completed using fluorescein (FAM)) to label a free cysteine *via* maleimide chemistry. Current data does show that as the concentration of A6 increases, a higher fraction of the analyte is bound in a larger protein-protein complex resulting in an increased anisotropy. This same trend is seen in various A6 constructs (A6 wt, A6 c134, A6 C/S wt, and A6 c134 C/S) that have been studied. The desire for such a development assay is unique to the studies completed. Once we have determined that an anisotropy reading is observed using our fluorescein derivative (FAM) we can utilize various peptides and their corresponding TCRs using our modified HLA-A2-S195C construct to determine their unique kinetic parameters and recognition mechanism.

4. The generation of a streptokinase sensitive mouse plasminogen

Diana Cruz Topete, Takayuki Iwaki and Francis J. Castellino
Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556

Plasminogen (Pg) is the precursor of plasmin (Pm), which is the main active enzyme of the fibrinolytic pathway. Pg is activated to Pm by the cleavage of the peptide bond Arg^{561}-Val^{562}. Group A streptococcus (GAS) is the etiologic agent responsible for a number of human diseases that range from common pharyngitis to severe infections. Streptokinase (SK) is a 414 amino acid protein secreted by several streptococcal species, and an efficient activator of plasminogen. Interestingly, SK is not an enzyme; it activates Pg indirectly by the formation of 1:1 complexes with Pg, which have amidolytic activity. Furthermore, Pg activation by SK is highly species specific with strong activity towards human Pg (hPg), and exhibiting little or no activity against other mammalian species including mouse plasminogen (mPg). In an attempt to define which amino acid regions within Pg may account for the species specificity of SK, several mutants, and chimeric mouse-human plasminogen constructs were generated utilizing standard molecular biology techniques. The hPg light chain was identified as the region responsible for SK sensitivity, specifically the amino acid sequences encoded by exons 16 and 18. In addition, surface plasmon resonance experiments (SPR) demonstrated high affinity binding between all plasminogen variants and SK, including mPg, indicating that human derived SK has the ability to form "catalytic complexes" in a non-species specific manner, however no active site is formed within the moiety of the activator complex. In summary, we identified the loci within human plasminogen that productively interacts with SK. The data generated herein presents novel insights for the understanding of the activation mechanism of hPg by SK.

5. Pyridoxamine protects proteins from functional damage by 3-deoxyglucosone: Mechanism of action of pyridoxamine

W. Zhang[1], S. V. Chetyrkin[2], B. G. Hudson[2], P. Voziyan[2] and A. S. Serianni[1]
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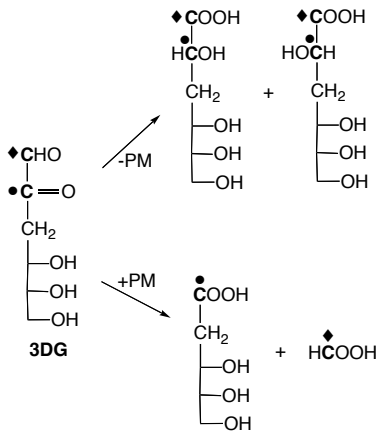
Pyridoxamine (PM) is a promising drug candidate for the treatment of diabetic nephropathy. The therapeutic effect of PM has been demonstrated in multiple animal models of diabetes and more recently in phase II clinical trials. However, the mechanism of PM therapeutic action is unknown. In the present study, we showed that the important physiologically reactive carbonyl species, 3-deoxyglucosone (3-DG), can damage protein functionality, including the ability of collagen IV to interact with glomerular mesangial cells. We also demonstrated that PM protects from 3-DG induced protein damage. The protection mechanism was investigated by monitoring *in vitro* reactions of PM with ^{13}C-labeled 3-DG. A novel reaction mechanism has been proposed which includes transient adduction of 3-DG with PM followed by irreversible PM-mediated oxidative cleavage of 3-DG to give non-reactive products. PM appears to modify the route of 3-DG degradation to produce C2-epimeric metasaccharinic acids to one favoring oxidative C-C bond cleavage to give the deoxyaldonate and formic acid. The mechanism of metasaccharinic acid formation from 3-DG was also investigated with [1-^{2}H; 2-^{13}C]3-DG.



S. V. Chetyrkin, W. Zhang, B. G. Hudson, A. S. Serianni and P. A. Voziyan, Pyridoxamine Protects Proteins from Functional Damage by 3-Deoxyglucosone: Mechanism of Action of Pyridoxamine, *Biochemistry* **2008**, *47*, 997-1006.

6. Observations of lysine NH_3 groups in human Pin1 upon substrate binding

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Human Pin1 is a peptidyl-prolyl isomerase composed of a catalytic isomerase (PPIase) and a WW domain. Pin1 recognizes phospho Ser/Thr-Pro segments in signalling proteins and thus serves as a potential cancer target. Here, we present a series of heteronuclear NMR experiments for the direct observations of lysine NH_3 groups in Pin1 with various phosphopeptide substrates. Historically, NMR observations of NH_3 groups have been hindered by rapid water exchange and associated scalar relaxation of the second kind. Due to this relaxation, the ^{15}N line shape from the lysine NH_3 are often broadened into oblivion. However, a recently developed pulse scheme by Iwahara *et al.*, instead observes in-phase ^{15}N transverse coherence (HISQC, Heteronuclear In-phase Single Quantum Coherence Spectroscopy). Such coherence is independent of scalar relaxation in the ^{15}N time domain, giving sharper ^{15}N line shapes and higher intensity NH_3 cross-peaks than the HSQC. We demonstrate these novel methods on Pin1 in the presence of various phosphopeptide substrates. These studies are novel for Pin1, and enable observation of functionally important NH_3 groups within the Pin1 active site. As such, these studies should enhance our understanding of Pin1 mechanism.

7. *J*-Coupling parameterization of exocyclic *N*-acetyl sidechains of saccharides

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Over the past 10 years, we have been interested in refining NMR *J*-coupling based analyses of specific conformational domains in saccharides in solution, including *O*-glycosidic linkages (*J. Phys. Chem. B* **2008**, *112*, 4447-4453; *J. Org. Chem*. **2008**, *73*, 3255-3257), exocyclic hydroxymethyl groups (*J. Org. Chem*. **2002**, *67*, 949-958; *J. Am. Chem. Soc*. **2004**, *126*, 15668-15685), and more recently hydroxyl groups (*J. Org. Chem*. **2007**, *72*, 7071-7082). A remaining key exocyclic appendage is the *N*-acetyl group, which is a common constituent of many protein- and lipid-bound oligosaccharides and which has been implicated in structure-function relationships in biopolymers such as hyaluronic acid. We are interested in investigating two core questions: (a) the effect of acyl structure on the *cis*-*trans* configurational equilibrium of these sidechains, and (b) parameterization of new carbon- and nitrogen-based *J*-couplings to help determine conformation about the C2-N2 bond (*syn*/*anti* conformation) in 2-acetamido-2-deoxy-aldohexopyranosyl residues and in other saccharides containing the *N*-acetyl group (*e.g*., *N*-acetyl-neuraminic acid; *J. Org. Chem*. **2008**, web-release May 20). To address these aims, we have applied experimental and theoretical approaches, the former involving the synthesis of ^{13}C- and ^{15}N-labeled aminosugars using recently reported general synthetic methodologies (*J. Org. Chem*. **2006**, *71*, 466-479), and the latter using density functional theory (DFT) to predict the structural dependencies of new couplings. In this as yet unpublished work, we have found that aldopyranosyl ring configuration and acyl sidechain structure affect *cis*/*trans* equilibria, and that at least six vicinal *J*-couplings involving *N*-acetyl sidechain atoms exhibit a strong dependence on the C2-N2 bond torsion; other non-vicinal couplings (^1J and ^2J) may do so as well. The latter dependencies have been parameterized to yield a new set of equations for use in quantitative treatments of the *N*-acetyl conformational domain.

8. Towards understanding carbapenem hydrolysis by GES β-lactamase

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The widespread use of antibiotics in the treatment of infections has resulted in the selection and spread of microorganisms resistant to these antibiotics. The major mechanism for resistance to β-lactam antibiotics by gram-negative bacteria is the production of β-lactamases, enzymes catalyzing the hydrolysis of the β-lactam ring thereby rendering the antibiotic inactive. Members of the recently discovered GES (Guiana extended-spectrum) β-lactamase family confer resistance to a wide-range of β-lactams, including penicillins, and first-, second-, and third-generation cepalosporins. In addition, a two of the nine members have evolved the ability to hydrolyze carbapenems (imipenem or meropenem), some of the most potent agents currently available for the treatment of infections. In the present study, we explored the basis for the differences in the ability of GES variants to hydrolyze β-lactams. GES-1, the first variant identified, weakly hydrolyzes carbapenems ($k_{cat} < 0.01$ s^{-1}). GES-5, containing a single amino acid substitution (G165N) relative to GES-1, hydrolyzes carbapenems much more rapidly ($k_{cat} = 0.1 – 0.4$ s^{-1}), however both enzymes have comparable values for K_m. Determination of the dissociation constant for the carbapenem substrate revealed that GES-1 has a higher affinity for carbapenems than GES-5, indicating it is not higher affinity which results in increased turnover. Product inhibition studies showed neither GES-1 or GES-5 to have any affinity for the hydrolyzed product up to 4 m*M*, indicating it is not product inhibition which influences the difference in turnover. Single-turnover experiments with GES-5 showed the rate constant for acylation to approach the value for k_{cat}, implying this step might be rate limiting. This is in sharp contrast to other β-lactamases, in which deacylation is the rate-limiting step.

9. Binding specificity and optical imaging of bacterial infections in murine models using fluorophores containing a bis-dipicolylamine-Zinc(II) affinity group

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Optical imaging of bacterial infection in living animals is usually conducted with genetic reporters such as light emitting enzymes or fluorescent proteins. However, there are many circumstances where genetic reporters are not applicable, and there is a need for exogenous synthetic probes that can selectively target bacteria. The foci of this study utilize fluorescent imaging probes that are composed of a bacterial affinity group conjugated to a number of fluorescent dyes. The affinity group is a synthetic zinc(II) coordination complex (Zn-DPA) that is shown to target the anionic surfaces of bacterial cells through model membrane studies using liposomes containing bacterial components. In addition, bacterial infection models were created in athymic nude mice using *Staphylococcus aureus*. The squaraine rotaxane fluorophore conjugated to the Zn-DPA affinity ligand is compared to previously tested cyanine dyes both of which emit light in the near-infrared range, a region essential to the *in vivo* success of near-infrared dyes. Using region-of-interest analysis, squaraine rotaxane fluorophores are shown to have higher target-to-nontarget ratios than that of cyanine dyes. The limits of detection in respect to bacterial concentration of an infection were also shown to be ~1 x 10^6 colony forming units. This work shows both the utility of the Zn-DPA affinity ligand as well as the superiority of the squaraine rotaxane fluorophore over the commercially available cyanine dye.

10. Sulindac treatment alters extracellular matrix remodeling in a colorectal cancer mouse model

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Sulindac, a non-steroidal anti-inflammatory drug (NSAID) has shown potential as a chemopreventive agent against cancer formation, especially colorectal cancers. However, the mechanisms by which this drug and NSAIDS in general act are not fully understood. In this study, $Apc^{Min/+}$ mice treated with sulindac demonstrated a tumor reduction of over 80%, consistent with previous reports. Gene microarray analyses of laser capture microdissected (LCM) tumor epithelial cells indicated differential expression of some extracellular matrix related genes. *Col1a2, Col5a2, Col6a2,* and *Col6a3* were upregulated, while matrilysin (*Mmp7*) was decreased in sulindac-treated mice. Real time RT-PCR of *Col6a2* and *Mmp7* validated the gene expression results. Confocal microscopy and immunofluorescence were used to further validate and localize collagens I, V, and VI, as well as MMP7 in epithelial and adenoma cells of the $Apc^{Min/+}$ intestines. All results were consistent with the microarray analyses. $Apc^{Min/+}/Mmp7^{-/-}$ mice, which have been shown to develop less tumors than $Apc^{Min/+}$ mice, were used to demonstrate the effect that lack of MMP7 has on collagen degradation *in vivo*. Collagens I and VI were significantly higher in $Apc^{Min/+}/Mmp7^{-/-}$ mice compared to $Apc^{Min/+}$ mice. Collagen V was also higher but without reaching statistical significance, implicating collagens I and VI as MMP7 substrates *in vivo*. These results demonstrate that sulindac is effective in increasing the expression of different collagens, and decreasing the expression of MMP7, which may contribute to altered tumor burden in cancer patients undergoing NSAIDS treatment.

11. Apoptosis of breast cancer cells by L-PPMP and D-PDMP: Translational and transcriptional regulation of glycosyltransferase genes

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Apoptotic effects of glycolipid biosynthesis inhibitor L-PPMP and D-PDMP in human breast cancer cells (MCF-7, MDA-468, and SKBR-3) were identified with techniques including phase contrast microscopy, DNA laddering, and the flopping of PS with PSS-380 fluorescent dye studies. Using a new fluorescent Dye (AKS-0) in the presence of these two apoptotic reagents, we observed a time-dependent scrambling of membranes of mitochondria, ER, and Golgi bodies. SA-Le^X (neolacto-series glycolipids) is distributed mostly on the outer lamella of plasma membranes of human breast and colon metastatic cells. Although exact functions of these glycoconjugates are not yet known, direct analyses of these glyco-molecules suggest they alter their structures during the onset of oncogenic processes. A time-dependent down-regulation of glycosyltransferase (GLTs) activities involved in the biosynthesis of SA-Le^X [GalT-4, GalT-5, and FucT-3] and gangliosides [SAT-4 and SAT-2] was observed. By using novel DNA-microarrays specifically designed for screening 359 Glyco-related genes, transcriptional up-regulation of several GLTs involved in the biosyntheses of Sialo-LeX and Sialo-Lea (cancer cell surface antigens) was observed. Down-regulation of GLT activities and up-regulation of some GLT mRNA suggest a tight regulation of these enzymes by signal transduction pathways.

12. Insight into siderophore and reductant dependence in iron acquisition from hematite by *Pseudomonas mendocina ymp*

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Pseudomonas mendocina ymp was shown by Hersman *et al.* to produce a siderophore, possibly in conjunction with a reductant, to aid in iron acquisition from mineral. To further probe this interaction of bacteria, siderophore, reductant, and mineral, we constructed a *P. mendocina* mutant incapable of siderophore production, and restricted direct access of the bacteria to the mineral with dialysis tubing. We show here that the siderophore is required for iron acquisition; its loss abolished the bacteria's ability to grow on hematite. Interestingly, the amount of siderophore needed for complementation was far less than that secreted by the microbe. Surprisingly, the likely siderophore receptor is regulated directly by iron availability, rather than amount of siderophore in the medium. While the addition of an external reductant could partially restore growth, only addition of external siderophore resulted in wild-type growth. A reductase produced by *P. mendocina* was found in the supernatant prior to peak siderophore production, suggesting a possible iron mobilization role. Two Fur-regulated, flavin-dependent reductases were identified in the *P.mendocina* genome; one is up-regulated in the absence of iron, has been isolated by chromatography, and is under investigation.

13. Chlorite decomposition and alternate reactivity of the heme dependent enzyme Chlorite Dismutase

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The heme b enzyme chlorite dismutase (Cld) catalyzes the decomposition of chlorite (ClO_2^-) into Cl^- and O_2, a reaction uncatalyzed by any other heme enzyme. The dismutation reaction as well as the alternate reactivity of the enzyme has been studied using steady state kinetics and spectroscopy. Cld catalyzes the dismutation reaction with a viscosity independent k_{cat}/K_m of 3.52 x 10^7 $M^{-1}s^{-1}$, indicating that while the dismutation rate is fast, substrate binding is not rate limiting. The enzyme also undergos inactivation coincident with loss of heme absorbance suggestive of a mechanism resulting in scission of the heme. Using alternate oxidants (peracetic acid, peroxide, hypochlorite and mCPBA) Cld has been shown capable of forming a Compound II like (Fe(IV)= O) species as well as being capable of catalyzing the 1 e^- oxidation of typical peroxidase substrates. Peroxidative substrates also prevent enzyme inactivation under ClO_2^- turnover condition indicating that the inactivating species must be on the same reaction pathway as that which the peroxidative substrates react. The oxidation of thioanisole and halogens has also been studied to determine if Cld has peroxygenase and haloperoxidase activities using both chlorite and alternate oxidants. The enzyme appears incapable of haloperoxidase activity with any oxidant tested and only capable of utilization of chlorite as an oxidant of thioanisole < 0.01% of the time, with 99.9% of the time chlorite being dismutated. The lack of peroxygenase and haloperoxidase as well as the very minimal of peroxidase activity when chlorite is used as an oxidant suggests that Cld is finely tuned to nearly exclusive dismutate chlorite.

14. Human plasminogen and Group A streptococcal M-like protein. Interaction, conformational transition, and activation

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In vertebrate organisms, clot lysis or fibrinolysis is primarily carried out by the serine protease plasmin (Pm). Generation of this enzyme occurs by hydrolysis of the Arg^{561}-Val^{562} peptide bond and cleavage of the N-terminal 77-residues of the zymogen plasminogen (Pg). One of the most distinctive features of Pg/Pm is the presence of the 5 independent homologous kringle (K1-K5) domains. These elements possess conserved binding pockets, termed Lys binding sites (LBS), essential for interactions with substrates, activators, inhibitors and receptors. In human Pg (hPg), while K2 displays very weak ligand binding properties, this domain has been implicated in a peculiar interaction with the N-terminal end of a surface protein from bacterial origin known as PAM (Pg-binding group A streptococcal M-like protein). A direct correlation has been established between invasiveness of the group A streptococci and their ability to bind Pg. It has been demonstrated that a 30-residues peptide (VEK30) that includes the Pg-binding region of PAM can recapitulate most of the interaction of the full-length protein with Pg/Pm. Comparisons of the crystal structures of angiostatin (K1-K3) with and without VEK30 bound to K2 (Cnudde *et al*., 2006) show a significant kringle reorientation. Using analytical ultracentrifugation, we attempted to determine if conformational shifts occur in Pg upon VEK30 binding. Additionally, we studied the effects of these interactions on the activatability of Pg. Our data reveal a decrease in the sedimentation velocity of Pg when associated with VEK30, as well as a direct relationship between the concentration of VEK30 or PAM and the activation rate of Pg. From these results, a novel mechanism of Pg activation *in vivo* has emerged has advanced our understanding of streptococci virulence factors.

15. Understanding the specificity of MART1/Melan A pMHC recognition by a clonally distinct TCR

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The interaction of a T cell receptor (TCR) with a ligand bound to class I or II major histocompatibility complex molecules (pMHC) initiates T cell activation. We are interested in TCR binding specificity and cross-reactivity, focusing on epitopes from the MART-1/Melan-A tumor protein presented by the class I MHC HLA A2. The MART-1/Melan-A protein has two overlapping antigenic epitopes of particular interest: the 26-35 decamer and the 27-35 nonamer. These peptides have been shown in our previous work to adopt very different conformations when presented by HLA-A2; the decamer adopts a more bulged conformation compared to the nonamer, which adopts an extended structure. Nonetheless, many T cell clones cross-react with these two epitopes. Here, we present surface plasmon resonance (SPR) data for the cross-reactive TCR Lau444 with the MART1/Melan A peptide and its variants. The results indicate that minor amino acid substitutions on the peptide two MART1/ Melan A conformations have differential effects on TCR recognition. Our data is beginning to illuminate how TCRs are able to cross-react with structurally dissimilar ligands, yet differentiate between almost identical peptides.

16. Towards characterization of the polyketide synthase gene cluster responsible for the production of gephyronic acid

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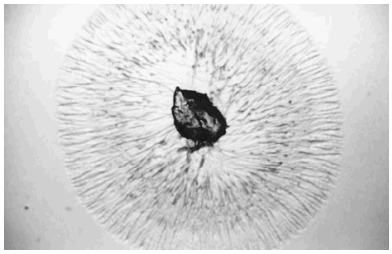
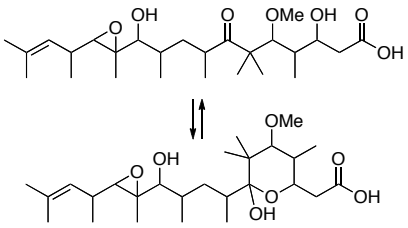
Gephyronic acid was isolated at the HZI (Helmotz-Zentrum fur Infektionsforschung) in Braunschweig by Sasse and Höfle[1] from the myxobacterium *Archangium gephyra* in 1995. Initial biological analysis suggests that gephyronic acid is a eukaryote-selective protein synthesis inhibitor. The focus of our studies is the identification and characterization of the polyketide gene cluster responsible for gephyronic acid production. Genetic analysis of a cosmid library derived from the myxobacteria strain *Cystobacter* cbv 76, another known producer of gephyronic acid, should validate the proposed biosynthesis involving seven elongation modules and two possible post-PKS modifying enzymes, as well as confirm several other unique features composing the gephyronic acid structure. Identification of the gene cluster is the first step of a broad-based program to generate gephyronic acid and analogues through heterologous expression systems and precursor-directed biosynthesis.





17. The Minimal Plus-End tracking unit of the cytoplasmic linker protein CLIP-170

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Because the molecular events at the microtubule (MT) plus-end govern whether it grows or shrinks, the proteins that localize specifically to this region are expected to play an important role in the regulation of the MT cytoskeleton. Understanding how proteins track MT plus ends should provide insight into the why they track plus-ends and the mechanisms by which they regulate MT dynamics. CLIP-170 is a MT plus-end tracking protein involved in regulating both MT dynamics and interactions between MTs and other cellular components. Recently, we provided evidence that CLIP-170 uses a preassociation and copolymerization mechanism to track MT plus-ends (Folker *MBOC* 16:5373, 2005). However, the yeast orthologs of CLIP-170 track MT plus-ends by a kinesin dependent mechanism, suggesting that CLIP-170 might track by multiple mechanisms in mammalian cells (Busch, Dev Cell 6:831, 2004). To further investigate the mechanisms by which CLIP-170 tracks MT plus-ends and alters MT polymerization, truncations of CLIP-170 head domain were made to separate the different subdomains. Each CLIP-170 head domain is characterized by two conserved CAP-Gly domains, surrounded by basic serine-rich regions. The ability of each construct to track MT plus-ends, induce tubulin polymerization, and bind microtubules was then tested. The conclusions drawn from these experiments are as follows. First, the plus-end tracking and efficient binding of CLIP-170 to tubulin/MTs is not only mediated by the CAP-Gly domains alone, but requires the presence of the basic serine rich regions in addition to the CAP-Gly domains. It appears that the second CAP-Gly is essential to plus-end tracking behavior; none of the constructs that contain only the first CAP-Gly were found to track MT plus-ends. Second, we provide evidence that a construct with single CAP-Gly domain in the presence of serine-rich region alone can induce tubulin nucleation/elongation *in vitro* and, MT binding and in some cases plus-end tracking in living cells. These observations provide new insights into the mechanisms by which CLIP-170 binds to tubulin/MTs and regulates microtubule dynamics.

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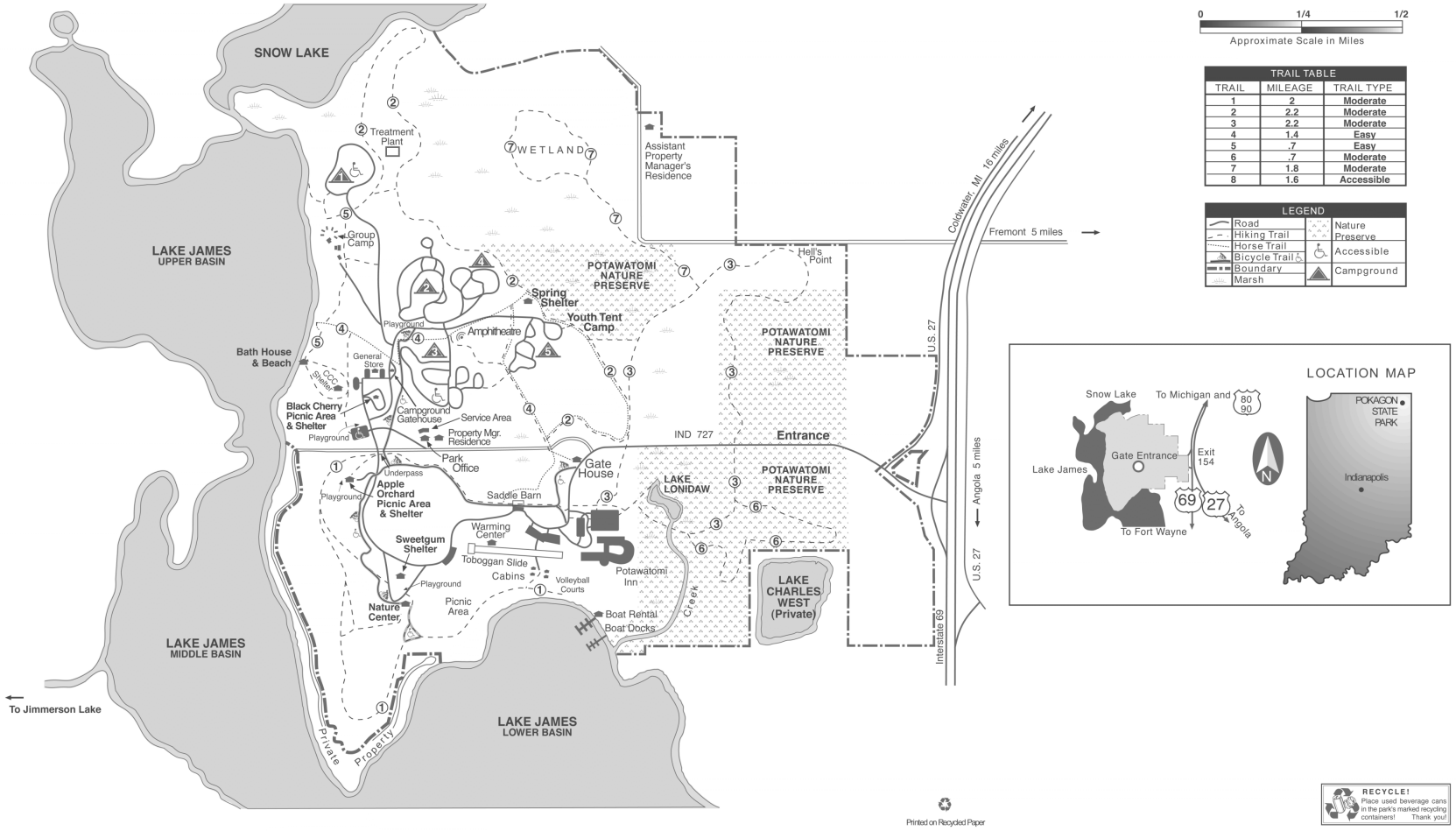

0 1/4 1/2
Approximate Scale in Miles

TRAIL TABLE		
TRAIL	MILEAGE	TRAIL TYPE
1	2	Moderate
2	2.2	Moderate
3	2.2	Moderate
4	1.4	Easy
5	.7	Easy
6	.7	Moderate
7	1.8	Moderate
8	1.6	Accessible

LEGEND			
	Road		Nature Preserve
	Hiking Trail		Accessible
	Horse Trail		
	Bicycle Trail		Campground
	Boundary		
	Marsh		

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