STUPKA
UNDERGRADUATE RESEARCH SYMPOSIUM
April 7, 2016
Presented by the Undergraduate Club of the Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology
EVENTS SCHEDULE

THURSDAY

9:00 am  Breakfast  
*Keynote and Alumni speaker meet and greet*  
Atrium

11:00 am  Stupka Family Welcome  
Atrium

12:10 pm  Weibel Lunch  
*Undergraduates & Graduates Only*  
1102 MBB

1:10 pm  Kuriyan Lunch  
*Undergraduates & Graduates Only*  
1102 MBB

2:10 pm  Poster Session 1  
Atrium

3:10 pm  Poster Session 2  
Atrium

4:10 pm  Welcome and Introductions  
*Dr. Kristen Johansen*  
*Provost Jonathan Wickert*  
1414 MBB

4:20 pm  Natalie Whitis – STUDENT SPEAKER  
*Control of CRISPR Activity by a Cascade Switch*

4:35 pm  Dr. Douglas Weibel – KEYNOTE SPEAKER  
*Mechanical genomics*

5:15 pm  Adrienne Smith – STUDENT SPEAKER  
*Expression and Characterization of Xyloglucan Xylosyltransferases*

5:30 pm  Break and Refreshments

5:50 pm  Sam Schulte – STUDENT SPEAKER  
*Controlling Product Stereochemistry in Class II Diterpene Cyclases*

6:05 pm  Mariah Lawler – ALUMNA SPEAKER  
*Defining miRNA in motor neurons in ALS*

6:30 pm  Dr. John Kuriyan – KEYNOTE SPEAKER  
*Protein kinases as highly adaptable molecular switches*

7:10 pm  Poster Awards  
1414 MBB

7:15 pm  Dinner  
Atrium
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**save the date for the 12th annual Stupka Symposium**

**Thursday, April 6, 2017**

email: stupkaugrs@iastate.edu  
website: stupka.las.iastate.edu
Rob Stupka was an undergraduate student majoring in Biochemistry at Iowa State University. His passion for science and research led the effort to establish the BBMB research symposium. His unparalleled enthusiasm propelled the planning process forward. Rob was the chair and with fellow students Tony Cyr, Claire Kruesel, Adam Krupicka and Jordan Witmer, they planned the first symposium for spring 2006. Their vision for this symposium was for it to be a medium for scientific research interactions between undergraduates and professionals. Rob, also, wanted this to be a place where undergraduate researchers could be recognized for their achievements in science.

Rob’s life was tragically cut short, on November 30, 2005, after a pedestrian vehicle accident in front of the Molecular Biology Building. In tribute to Rob’s drive and passion to create this event, the BBMB Undergraduate Symposium bears his name. BBMB undergraduates, who are inspired by Rob’s story, continue to organize this event. We believe that through your attendance and participation, we continue to honor Rob’s memory.

The 2016 Stupka Undergraduate Research Symposium would like to thank you for being here. We hope you enjoy the wonderful research presented by our promising young scientists.

**LIPID TRANSFER PROTEIN**

Rob and his father visited several universities in early 2002. In June of that year, they visited Iowa State University. The beauty of the campus and the friendliness of the staff convinced them that this was Rob’s new home. Rob initially entered ISU in the Fall of 2002 as a chemical engineering student. During his first semester, he took BBMB 101: Introduction to Biochemistry as an elective. Rob was so captivated by the biochemical discoveries explained in class, that he became a biochemistry student at the end of his first semester. He worked on making cDNA libraries from floral nectaries. This molecule is the nectary Lipid Transfer Protein (LTP2). The cDNA that encodes this protein was made by Rob. It was subsequently cloned and characterized. It is currently being expressed in bacterial cells with the goal of determining its antimicrobial activity.
Middle: Kia Barry, Alissa Mathisen, Lauran Chambers, Alex Donelson, Natalie Whitis, Tyler Gilbreath
Back: Brittany Bolerjack, Drew Tonsager, David Rosenthal, Adrienne Smith, Ben Brown, Jeff Carley
Not Pictured: Julia Nguyen, Bailey Mooney, Maddison Wild, Sarah Brinkman

2016 Committee Leadership

Symposium Chairs: Amanda Gorniak, Flora Yen
Speaker Chair: Adrienne Smith, Tyler Gilbreath
Publicity Chair: Morgan Barrett
Funding Chair: Victoria Ridout
Operations Chair: Lauran Chambers
Food Chair: Natalie Whitis
Secretary: Jeff Carley
Treasurer: Ben Brown
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Poster Coordinator: Kia Barry
Volunteer Coordinator: Julia Nguyen
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TShirt Coordinator/ Risk Management: David Rosenthal
Advisers: Desi Gunning, Gustavo MacIntosh
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Natalie R. Whitis

Natalie Whitis is a senior at Iowa State and is majoring in Biophysics. She is the Breakfast Club Chair for the BBMB Club as well as Food Chair for the Stupka Symposium. After she graduates from Iowa State she plans to attend graduate school and work towards a PhD. Then, she plans to work in academic research. Since the fall of 2014, she has been an undergraduate research assistant with the Sashital Lab studying the mechanism of the CRISPR-Cas immune system in E. coli.

ABSTRACT
Control of CRISPR priming and interference activities by a Cascade-activated switch

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR-associated proteins) is an adaptive immune system in bacteria that scans DNA within the cell and integrates pieces of viral DNA into the bacterial genome (acquisition) in order to recognize and destroy the virus the next time it infects the cell (interference). The immune system differentiates the viral genome from its own CRISPR loci by recognizing PAM (protospacer-adjacent motif) sequences that are present in viral targets but not in the host genomic locus. Mutations in the PAM trigger a more efficient method of integrating new spacers (priming), which requires both the acquisition and the interference machinery. Based on structural analysis of the target-binding interference complex Cascade, we identified several amino acid residues that may have a role in PAM recognition and tested the effects of alanine mutations at these positions on Cascade function. Surprisingly, we identified several mutants with measurable defects in interference but no effects on priming. Our in vitro DNA-binding assays have revealed that these Cascade mutants cause modest defects in target binding, but completely block target cleavage by the interference endonuclease Cas3, consistent with the observed loss of interference in vivo. Together, these results suggest that Cascade may bind DNA in two alternate conformations that switches activity from interference to priming and that the PAM-recognition region of Cascade controls this putative conformational change.
Douglas B. Weibel is an Associate Professor of Biochemistry, Chemistry, and Biomedical Engineering at the University of Wisconsin-Madison. He received his B.S. degree in chemistry in 1996 from the University of Utah (with Prof. C. Dale Poulter). From 1996-1997 he was a Fulbright Fellow at Tohoku University, Japan and studied organometallic chemistry (with Prof. Yoshinori Yamamoto). He received his Ph.D. in chemistry from Cornell University in 2002 (with Prof. Jerrold Meinwald). During his graduate studies he was an intern at Orchid Biosciences Inc. (now LabCorp) and a visiting scientist at the Max Planck Institute for Chemical Ecology, Jena, Germany (with Prof. Wilhelm Boland). From 2002-2006 he was a postdoctoral fellow with Prof. George M. Whitesides at Harvard University and in 2005 he was a student in the Physiology Course ('Modern Cell Biology using Microscopic, Biochemical, and Computational Approaches') at the Marine Biological Laboratory at Woods Hole (Course Directors, Prof. Ron Vale and Prof. Tim Mitchison). Since joining the faculty at UW-Madison 2006, he has received a number of research and teaching accolades, including: the Class of 1955 Distinguished Teaching Award (2014), the Early Career Life Science Award from the American Society for Cell Biology (2013), the NIH Director's New Innovator Award (2011), a DuPont Professorship (2010-2013), an Alfred P. Sloan Fellowship (2009), the DARPA Young Faculty Award (2009), and a Search Scholar Award (2008). He was a visiting professor of physics at the University of Washington, Seattle and a principal scientist at Amazon.com, Inc. from 2014-2015. He has consulted for a range of public and privately held companies (including Google[X], Cubist Pharmaceuticals [now Merck] and Amazon.com, Inc.) in the areas of biotechnology, bioengineering, chemistry, applied biology, materials science, and manufacturing and has participated in a range of government advisory positions in the areas of biodefense, infectious diseases, and biomedicine. He is a co-founder of two start-up companies: Agri Diagnostics Inc. develops products that improve agricultural yields; Avilas Health Inc. develops quantitative, at-home fertility tests. His research interests span the fields of chemistry, biochemistry, biophysics, materials science and engineering, and microbiology.

ABSTRACT

Mechanical genomics

Bacteria and other microorganisms have to solve an important physical problem for their survival: how do they mechanically resist the large pressure drop across their cell wall (~105 Pa) that arises due to mismatch in concentrations of dissolved solutes inside and outside of the cell?

We still know very little about bacterial mechanics, and yet one of the most clinically used family of drugs discovered to-date (the beta-lactam antibiotics), alter cell wall mechanics, create defects, and cause cell lysis. Understanding how bacteria control their mechanical properties will define new targets for developing clinical antibiotics and lay the foundation for studying mechanobiology in the most tractable model organism available.

In this talk I describe two approaches we have developed to identify the biochemical regulators of mechanical properties in bacteria: 1) CLAMP (cell length analysis of mechanical properties) is a medium-throughput technique to measure the effective Young’s modulus of growing bacterial cells; and 2) GRABS (general regulators affecting bacterial stiffness) is a high-throughput technique that assigns stiffness scores to every gene in a bacterial genome.

Using this suite of tools, I describe the surprising regulators of stiffness in bacteria and how these techniques are enabling us to lay the foundation for understanding mechanobiology in microbes (from components of the human microbiome, to bacterial pathogens, to infectious eukaryotic microbes).
Adrienne is a senior pursuing a degree in Biochemistry with a minor in Microbiology. Her research studies the amino acids involved in substrate binding and enzymatic catalysis of the Arabidopsis protein Xyloglucan Xylosyltransferase under Olga Zabotina, Ph.D. Adrienne also serves the Biochemistry, Biophysics, and Molecular Biology Club as the Liberal Arts and Sciences Representative and is the chair for the Speaker Committee for the Stupka Symposium. In addition, she tutors Organic Chemistry for the Hixson-Lied Academic Success Center. She plans to continue undergraduate research at Iowa State in the hopes of attending a graduate program in molecular biology.

ABSTRACT
Expression and Characterization of Xyloglucan Xylosyltransferases
Xyloglucan (XyG) is the main hemicellulosic component of the primary cell wall in eudicots and nongraminaceous monocots. Three xyloglucan xylosyltransferases, including XXT2, are known to encode XyG xylosyltransferases, key enzymes in the process of XyG biosynthesis. A computational model of XXT2 was used to identify amino acids, F204, K207, D228, S229, D230, H378, putatively localized in the active site of XXT2 and mutagenesis study was performed to confirm predictions. All created mutants were expressed in E. coli SoluBL21 in pET20b plasmids containing an N-terminal GB1 fusion tag. To increase yield and purity for future biophysical experiments, such as isothermal titration calorimetry and circular dichroism, the mutated genes were amplified from the pET20b plasmids, ligated into pGen2 plasmids and transfected into HEK293 cells. Results of the enzyme assay demonstrated that K207, D228, D230, and H378 are critical for enzyme activity due to their proposed coordination of the divalent cation. Reduced activity seen for the mutation made at F204 suggests that while this site is important for enzyme activity, it is not as crucial as the other sites tested. In addition, cysteine residues were selected for mutagenesis to investigate their effect on disulfide bond formation shown to be involved in hetero- and homo- dimer formations. Expression and analysis of these mutants is currently in process.

Truncations of XXT2 from both the N- and C-ends were used to examine the contributions of these termini to protein solubility and activity. In N-terminal truncations longer than 31 residues, both protein expression and activity of XXT2 were negatively affected, indicating a possible effect on protein folding. Increasing sizes of truncations from the C-terminus showed an increase in protein expression but a decrease in its catalytic activity. We conclude that the C-terminus is not involved in protein folding but rather substrate binding. This truncation identified a series of six positively charged residues between 24 and 44 amino acids from the C-terminus that may play a role in substrate binding. Mutagenesis of these residues revealed that four of the mutants have reduced catalytic activity.

We conclude that the N-terminal of XXT2 is involved in protein folding while the C-terminal is involved in substrate binding. Furthermore, active site residues K207, D228, D230 and H378 are proved to be critical for enzymatic activity of XXT2.
Sam Schulte

Sam is a senior at Iowa State and is pursuing a Biochemistry degree and a Computer Science minor. Sam is involved in research in the laboratory of Dr. Reuben J. Peters, where he studies the catalysis of class II diterpene cyclases. Previously, he has worked as a Borlaug-Ruan Intern at the International Maize and Wheat Improvement Center in Mexico, as a USDA Wallace-Carver Fellow, and as a Discovery Research Intern at Kemin Industries. When outside the Molecular Biology Building, Sam is the Principal Euphonium in the Iowa State University Wind Ensemble and has been in Student Government for three years. After graduating, Sam intends to earn a Ph.D. in Biochemistry.

ABSTRACT

Controlling Product Stereochemistry in Class II Diterpene Cyclases

Class II diterpene cyclases (DTCs) catalyze the committed step in production of the large class of labdane-related diterpenoid natural products, many of which function as important primary and secondary metabolites in plants. Mechanistic studies of DTCs, which bicyclize geranylgeranyl diphosphate (20-C), have revealed the importance of a variable set of two amino acids comprising a catalytic base dyad. Here, the catalytic base dyads of the closely related DTCs DsCPS (Danshen copalyl diphosphate synthase) and MvPPS (Marrubium vulgare peregrinol diphosphate synthase) were probed to further elucidate the evolution and mechanisms of these enzymes. Such studies were completed using site-directed mutagenesis, with the mutated DTCs expressed in an Escherichia coli metabolic engineering system. DsCPS ordinarily produces copalyl diphosphate (CPP), which adopts the chair-chair conformation. However, upon mutating the catalytic base dyad residues (along with one other neighboring residue) to the amino acids of the catalytic base dyad in MvPPS, the DsCPS mutant produces terpentedienyl diphosphate (TPP). Intriguingly, TPP adopts the chair-boat conformation, corresponding to the stereochemistry of the MvPPS product. Thus, simply introducing the catalytic base dyad of one DTC into another is sufficient to change product stereochemistry in this instance, illustrating the critical role of these two amino acids. This research provides insight into how specific mutations in closely related DTCs have created the capacity to produce such a wide array of labdane-related diterpenoids.
Mariah Lawler

Mariah’s adventure at ISU began as a biology major. But – when everyone else in her introductory biology class was excited for the section on “mammals” – Mariah had the lurking feeling she was in the wrong place. After a BBMB student suggested meeting with Desi Gunning, Mariah knew she had finally found home. During her time at ISU, Mariah worked in the laboratory of Dr. Ravi Singh at the Vet Med School. Under the guidance of Dr. Natalia Singh, Mariah characterized a long-distance intronic interaction that contributed to pre-mRNA alternative splicing of Survival Motor Neuron 2, which is associated with Spinal Muscular Atrophy. Mariah became fascinated by the regulatory roles of RNA, particularly as they pertain to human disease.

Mariah is currently a fourth-year graduate student in the laboratory of Timothy Miller at Washington University in St. Louis. Her doctoral work focuses on the contributions of small regulatory RNAs, microRNAs, to the selective vulnerability of motor neurons to Amyotrophic Lateral Sclerosis (aka the “Ice Bucket Challenge” disease). She is the recipient of both a pre-doctoral fellowship from the NIH and from Sigma-Aldrich. Mariah has thoroughly enjoyed her time in St. Louis, which seems like big city living in comparison with her beloved Ames. Finally, to address the question on everyone’s minds (or at least her mother’s), Mariah does not yet know when she will graduate. TBD!

ABSTRACT

Cell type miRNA profiling reveals candidate biomarker of motor neuron disease

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder marked by the loss of motor neurons (MNs) in the brain and spinal cord, leading to fatally debilitating muscle atrophy. For diseases in which a single cell type is predominantly affected by disease pathology, characterizing the distinct expression profile of that cell type may serve to elucidate underlying disease mechanisms and identify novel targets of therapeutic or diagnostic utility. MicroRNAs (miRNAs) are short, non-coding RNAs that can shape the expression profile of a cell and, consequently, often exhibit cell type enriched expression. Because they are actively released from cells and detectable in clinically accessible fluids, such as cerebrospinal fluid (CSF), miRNAs have been increasingly used for diagnostic purposes. Utilizing Cre-dependent miRNA tagging and affinity purification in mice, we defined the in vivo miRNA expression of all neurons as well as MNs, astrocytes and microglia, leading to the specific identification of MN-enriched miRNAs. Characterizing the levels of the MN-enriched miRNAs in CSF harvested from models of MN disease led to the development of a novel biomarker (miR-218) that tracked with disease progression and was responsive to an ALS therapy in rodent models. Thus, we have employed cellular expression profiling tools to assess the distinct miRNA expression of our cell type of interest, which enabled a hypothesis-driven approach for development of a novel, drug-responsive biomarker of MN disease.
Dr. Kuriyan's research concerns the atomic-level structure and mechanism of the enzymes and molecular switches that carry out cellular signal transduction. His laboratory uses x-ray crystallography to determine the three-dimensional structures of proteins involved in signaling, as well as biochemical, biophysical, and cell biological analyses to elucidate mechanisms. Breakthroughs from the lab have included determining the auto-inhibited structures of several tyrosine kinases, including Src family kinases and elucidating the mechanism of allosteric activation of the kinase domains of the EGF receptor. His laboratory has provided a fundamental understanding of the structure and regulation of several other signaling proteins, including STATs, the Ras activator SOS, and calcium/calmodulin-dependent protein kinase-II. Their structural insights have helped understand how the misregulation of these enzymes is often coupled to cancer and immune diseases and has implications for the development of kinase-targeted drugs to treat these diseases. His lab has also made fundamental contributions to understanding the structural basis for high-speed DNA replication.

Dr. Kuriyan’s achievements in science have been recognized by numerous honors, including:


**ABSTRACT**

**Protein kinases as highly adaptable molecular switches**

Protein kinases are signaling switches that phosphorylate proteins and thereby control decisions in the cell. The targets of protein kinases regulate most cell functions, and they are especially important in sending messages across the cell membrane. Ultimately, kinases can prompt cells to divide, move, and even die. Our studies on the structural mechanisms of kinases encoded by oncogenes have shown us how the structure of the catalytic core is flipped between off and on states by very different kinds of accessory elements. Understanding how to toggle these switches has helped us understand how drugs that block the catalytic activity of kinases work, and how they might be improved. I will discuss our current studies, which include analysis of the mechanism by which a cell-surface receptor, known as the epidermal growth factor (EGF) receptor, communicates across the cell membrane, how these two receptors form an active signaling complex, and how a calcium/calmodulin-dependent protein kinase II responds to activating signals.
Morgan Barrett

Morgan is a senior majoring in Biochemistry and Genetics. Throughout her time at Iowa State, she has been actively involved in the Stupka Symposium Planning Committee, where she is currently Publicity Chair, and the BBMB Undergraduate Club, where she is currently Vice President. She has been an undergraduate research assistant in Dr. Eric Underbakke’s lab since spring 2014. After graduating from Iowa State, Morgan plans on pursuing further education in forensic science with the ultimate goal of working as a DNA analyst/criminalist.

Adrienne Smith

Bio on page 10

Natalie Whitis

Bio on page 8

### PAST STUPKA SCHOLARS

**Claire Kruesel  2006**
Lecturer for the English Department at Iowa State University

**Mara Alexeev  2007**
Public health Masters student at University of California, Berkeley

**Luke Helgeson  2008**
Postdoctoral researcher in the laboratory of Dr. Trisha Davis at University of Washington studying the regulation of kinetochore microtubule attachments

**Mina Farahbaksh  2009**
6th year M.D./Ph.D. candidate at University of Kansas Medical Center graduating in 2019

**Dayna Peterson  2010**
5th year Biochemistry Ph.D. candidate at Arizona State University

**Jackie Rivas  2011**
Ph.D. candidate in immunology at UT Southwestern Medical Center

**Craig Brown  2011**
M.D. candidate at Pritzker School of Medicine at University of Chicago, graduating in 2016, will begin general surgery residency after graduation

**Johanna Jass Bailey  2011**
Laboratory and Site Manager at Elemental Enzymes in Columbia, MO, Master of Public Health, University of Missouri, 2015.

**Mollie Schubert  2011**
Research Scientist II in the Molecular Genetics research group at Integrated DNA Technologies in Coralville, IA

**Samson Condon  2012**
3rd year Ph.D. student in the University of Wisconsin Madison Department of Biochemistry

**Alana Jackson  2012**
Pursuing her M.D. at the University of Minnesota Medical School Duluth

**Kristen McKibben  2013**
2nd year Ph.D. student at University of Pennsylvania studying physical and organic chemistry

**Kinsey Cornick  2013**
2nd year osteopathic medical student at Des Moines University

**Jennifer Kaczynski  2013**
Working as a dermatology scribe at Unity and Mercy Hospital, MN

**Denis Tamiev  2014**
Senior at Iowa State University, graduating in May 2016 with a degree in Biochemistry and a minor Economics

**Zack Young  2014**
Research assistant at Oklahoma Medical Research Foundation

**Flora Yen  2015**
Senior in Biochemistry at Iowa State University, graduating in 2016 to pursue further education at University of Iowa studying Dentistry

**Adrienne Smith  2015, 2016**
Senior in Biochemistry at Iowa State University
Thank you!

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John and Mary Stupka • Robert and Diane Stupka
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Tom Bobik • Bayou Chen • Alan DiSpirito • Jack Girton  
Mark Hargrove • Richard Honzatko • Ted Huiatt • Robert Jernigan  
Jorgen Johansen • Kristen Johansen • Gustavo MacIntosh  
W. Alan Miller • Alan Myers • Scott and Marna Nelson  
Basil Nikolau • Marit Nilsen-Hamilton • Reuben Peters • Guru Rao  
Dipali Sashital • Yeon-Kyun Shin • Michael Shogren-Knaak  
Robert Thornburg • Eric Underbakke • Edward Yu • Olga Zabotina

**POSTER JUDGES**

Scott Nelson (head judge) • Linda Ambros • Adam Barb  
Bayou Chen • Mark Hargrove • Richard Honzatko • Dipa Sashital  
Robert Thornburg • Eric Underbakke • Olga Zabotina