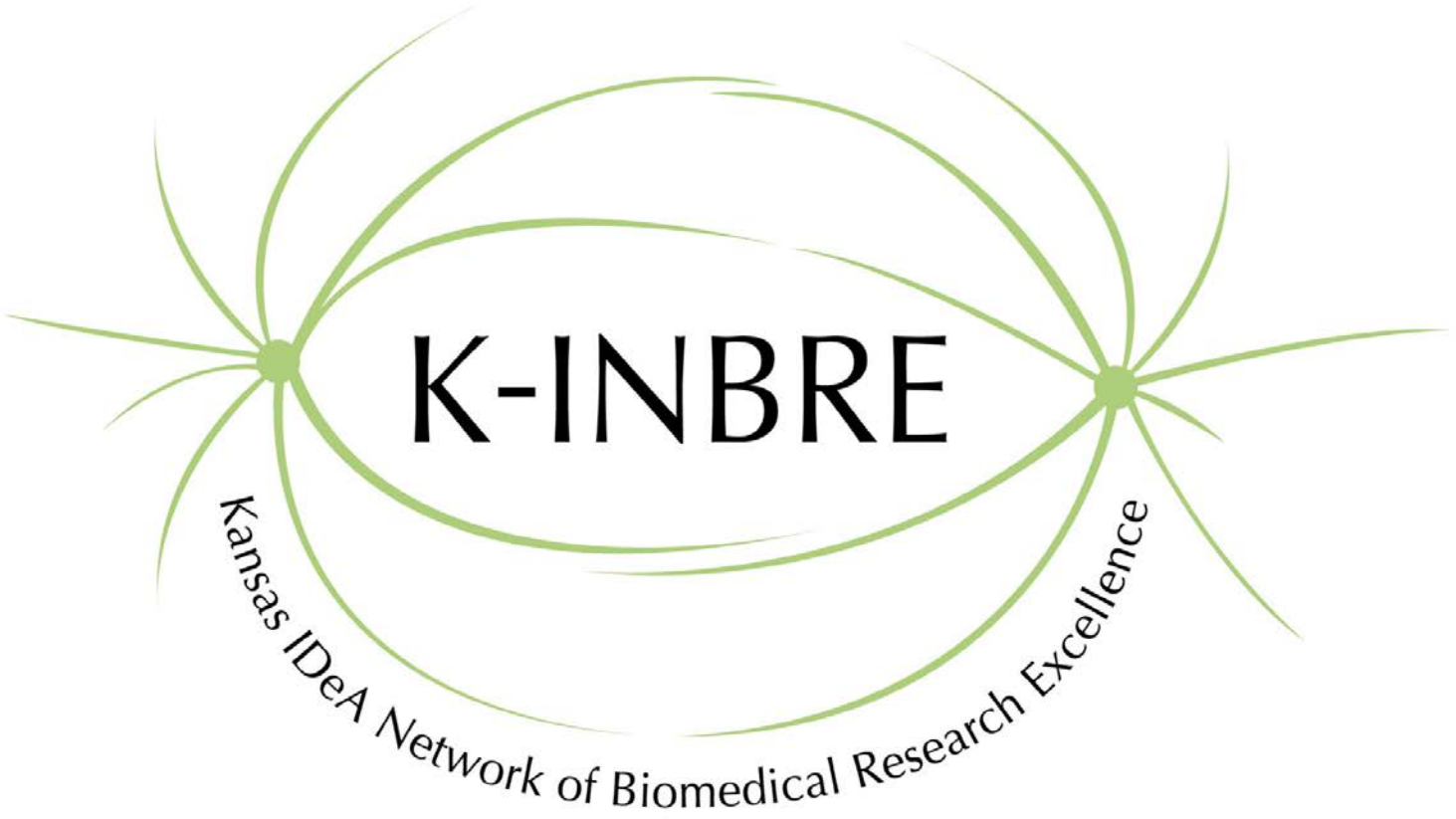
The 18th Annual Kansas-IDeA Network of Biomedical Research Excellence Symposium

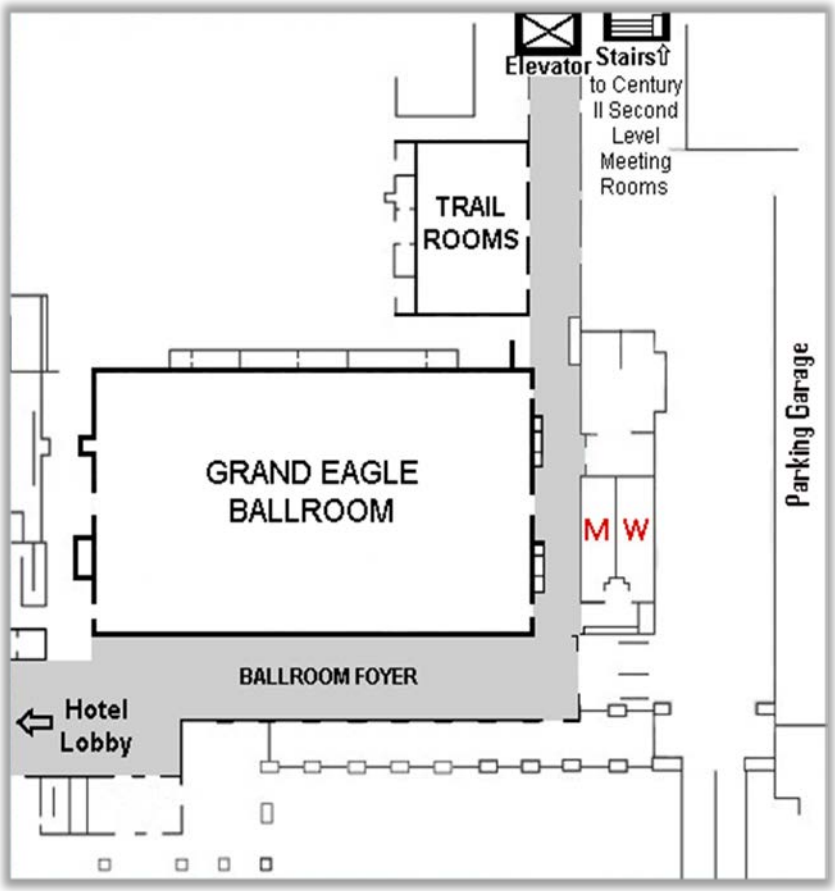


January 18-19, 2020

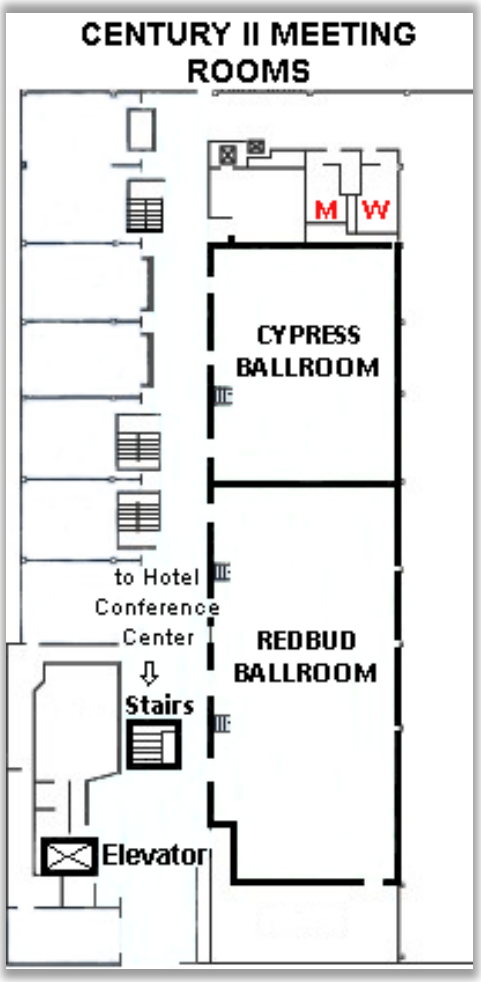
Hyatt Regency Wichita
Wichita, KS

This program was made possible by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH) under grant number P20 GM103418. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Hyatt Regency Wichita and Century II Performing Arts & Convention Center Floor Plan



Hyatt Regency Hotel Conference Center



Century II Second Level Promenade Meeting Rooms

LOCATION OF EVENTS:

- **Registration (Friday):** Grand Eagle Ballroom Foyer
- **Registration (Saturday/Sunday):** Redbud Ballroom Foyer
- **Friday Night Events:** Grand Eagle Ballroom
- **Breakfast:** Cypress Ballroom and Foyer
- **General Session:** Redbud Ballroom
- **Breaks:** Redbud Ballroom Foyer
- **Lunch:** Cypress Ballroom and Foyer
- **Poster Session/Reception:** Grand Eagle Ballroom
- **Dinner:** Redbud Ballroom & Foyer
- **Boxed Lunches:** Redbud Ballroom Foyer

K-INBRE 2020 Symposium
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Poster Presentations

Saturday, January 18, 2020

Poster Session I (1:00-2:00 PM) Abstracts Numbered 1-40

Poster Session II (2:00-3:00 PM) Abstracts Numbered 41-80

Poster Session III (4:50-5:50 PM) Abstracts Numbered 81-120

Poster Session IV (5:50-6:50 PM) Abstracts Numbered 121-160

See Alphabetical list for monitor assignment.
Abstract# = Monitor #

Please be near your monitor during your assigned session until the judges have visited.
Feel free to visit other boards during the alternate sessions.

IMPORTANT:

Please ensure that all publications resulting from INBRE funds are in compliance with the NIH Public Access Policy. Future awards from NIH will be delayed until evidence of compliance has been demonstrated. For more information on the Public Access policy, please visit this link: http://publicaccess.nih.gov/policy.htm

When K-INBRE funds have supported your research, please remember to acknowledge this support by including the grant number P20 GM103418, regardless of the time period between receipt of funding and the publication or presentation.

K-INBRE 2020 Symposium
Program Schedule

Hyatt Regency Wichita
Wichita, KS

Friday, January 17, 2020

3:00 PM	**Early Registration**	**Grand Eagle Ballroom Foyer**
	Open Poster viewing	**Grand Eagle Ballroom**
4:30 PM	**Early Registration Closes**	
6:00 PM	**Friday Night Dinner**	
8:00 PM	**Poster Viewing, Friday Night Dinner Ends**	

Saturday, January 18, 2020

7:30 AM	**Breakfast Buffet**	**Cypress Ballroom/Foyer**
	Registration	**Redbud Ballroom Foyer**
8:30 AM	**General Session**	**Redbud Ballroom**
	Doug Wright, Ph.D., K-INBRE Principal Investigator, University of Kansas Medical Center Opening Remarks	
8:45 AM	*Coleen Pugh, Ph.D., Dean Graduate School and Associate Vice President for Research & Technology Transfer, Wichita State University* Welcome from Wichita State University	
9:00 AM	*Doug Wright, Ph.D., K-INBRE Principal Investigator, University of Kansas Medical Center* Moderator: Keynote and Regional Scientist Presentations	
9:05 AM	*Sonia Hall, Ph.D., President and CEO, BioKansas* Title: Building a career: the value of mistakes, perspective, courage, and exploration	
9:35 AM	*Moriah R. Beck, Ph.D., Associate Professor, Wichita State University* Title: New actin branching mechanism facilitated by palladin	
10:05 AM	**Break**	**Redbud Ballroom Foyer**
	University Photos	
10:10 AM	Wichita State University Photo	
10:15 AM	Fort Hays State University Photo	
10:20 AM	Kansas State University Photo	
10:25 AM	Pittsburg State University Photo	
10:30 AM	University of Kansas Medical Center Photo	
10:35 AM	**General Session**	**Redbud Ballroom**
	Marija Kuna, Ph.D. Postdoctoral Fellow, University of Kansas Medical Center Moderator: Regional Scientist and Trainee Presentations	
10:40 AM	*Robert Unckless, Ph.D., Assistant Professor, University of Kansas, Lawrence* Title: Recurrent evolution of a virulent viral haplotype in a *Drosophila*/DNA virus system	
11:10 AM	**Trainee #1** *Morgan Bretches, Wichita State University, Wichita, KS* Title: Peripheral Nerve-derived Pluripotent Stem Cells as Potential Cell Source to Treat Segmental Bone Defect	

Saturday, January 18, 2020

11:30 AM	**Trainee #2** *Hunter Woosley, University of Kansas, Lawrence, KS* Title: The role of the hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) on HSV-1 infection	
11:50 AM	**Lunch**	**Cypress Ballroom/Foyer**
12:50 PM	**Poster Judge Meeting**	**Trail Rooms**
1:00 PM	**Poster Session I**	**Grand Eagle Ballroom**
2:00 PM	**Poster Session II**	
3:00 PM	**Poster Session II ends**	
3:05 PM	**General Session**	**Redbud Ballroom**
	Raja Veerapandian, Ph.D. Postdoctoral Fellow, Kansas State University Moderator: Regional Scientist and Trainee Presentations	
3:10 PM	**Trainee #3** *Stacii Cross, Langston University, Langston, OK* Title: Complement Activation in Rat Pre-Eclampsia	
3:30 PM	**Trainee #4** *Priyanka Radadiya, University of Kansas Medical Center, Kansas City, KS* Title: Ciclopirox-olamine alters ferritin trafficking and plays a protective role in polycystic kidney disease	
3:50 PM	**Trainee #5** *Vedant Jain, Pittsburg State University, Pittsburg, KS* Title: Nanozyme: A Developing Nanotechnology for the Detection of Food-Borne Pathogens	
4:10 PM	**General Session Concludes/Break**	**Redbud Ballroom Foyer**
	University Photos	
4:15 PM	Langston University Photo	
4:20 PM	Emporia State University Photo	
4:25 PM	University of Kansas, Lawrence Photo	
4:30 PM	Washburn University Photo	
4:35 PM	Haskell Indian Nations University Photo	
4:40 PM	**Poster Judge Meeting**	**Trail Rooms**
4:50 PM	**Reception/Poster Session III**	**Grand Eagle Ballroom**
5:50 PM	**Reception/Poster Session IV**	**Grand Eagle Ballroom**
6:50 PM	**Poster Session Ends**	
7:00 PM	**Dinner**	**Redbud Ballroom/Foyer**
7:30 PM	**Award Presentations**	**Redbud Ballroom**
	John Stanford, Ph.D., K-INBRE Associate Director, University of Kansas Medical Center	
8:00 PM	*Doug Wright, Ph.D., K-INBRE Principal Investigator, University of Kansas Medical Center* Closing Remarks	

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Hyatt Regency Wichita
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Sunday, January 19, 2020

7:30 AM	**Breakfast Buffet**	**Cypress Ballroom/Foyer**
8:30 AM	**General Session**	**Redbud Ballroom**
	John Stanford, Ph.D., Associate Director, University of Kansas Medical Center Opening Remarks	
8:35 AM	*Adam Scheid, Ph.D. Postdoctoral Fellow, University of Kansas Medical Center* Moderator: Regional Scientist and Trainee Presentation	
8:40 AM	**Trainee #6** *Alexis Winter, Fort Hays State University, Fort Hays, KS* Title: MRSA YjbIH coordinate expression of hemolysins through global networks	
9:00 AM	**Trainee #7** *Colby Spiess, University of Kansas, Lawrence, KS* Title: Overexpression of the RNA binding protein HuR promotes chemoresistance by stabilizing AKT in colorectal cancer cells	
9:20 AM	**Trainee #8** *Isabel Lewis, Kansas State University, Manhattan, KS* Title: CRISPR Cas12a endonuclease in a gene drive: cuts only as good as the guide.	
9:40 AM	*Eric T. Gillock, Ph.D., Professor of Biological Sciences, Fort Hays State University* **Title:** The potential spread of porcine endogenous retrovirus C among feral swine populations	
10:10 AM	**Break**	**Redbud Ballroom Foyer**
10:30 AM	**General Session**	**Redbud Ballroom**
	John Stanford, Ph.D., Associate Director, University of Kansas Medical Center Introduction of Speaker	
10:35 AM	*David Long, Ph.D., Assistant Professor, Wichita State University* Title: Generating morphological models of endothelial monolayers from limited data: A computational framework for mechanobiology to understand and predict cell behaviors	
11:05 AM	*James Balthazor, Ph.D., Assistant Professor, Fort Hays State University* Title: Probing the Unfolded Protein Response: mRNA knockdowns in *Acyrthosiphon pisum*	
11:35 AM	*John Stanford, Ph.D., Associate Director, University of Kansas Medical Center* Oral Presentation Awards	
11:45 AM	*Doug Wright, Ph.D., K-INBRE Principal Investigator, University of Kansas Medical Center* Closing Remarks	
12:00 PM	**Boxed lunches available for pickup**	**Redbud Ballroom Foyer**

Saturday, January 18, 2020

Peripheral Nerve-derived Pluripotent Stem Cells as Potential Cell Source to Treat Segmental Bone Defect

Morgan Bretches[1], Sunaina Shrestha[1,2], Bradley Dart[2], Michael Heggeness[2], Shang-You Yang[1,2]
[1]Wichita State University, Wichita, KS, [2]University of Kansas School of Medicine-Wichita, Wichita, KS

A population of cells within peripheral nerves has recently been identified to express stem cell markers Oct4, Sox2, Klf4, and c-Myc when the nerve is physically injured or exposed to BMP-2 in mice. These cells have been named Nerve Derived Adult Pluripotent Stem (NEDAPS) cells. The objective of this study is to explore the differentiation potential of NEDAPS cells to osteoblastic cells and their clinical application for bone healing. Briefly, NEDAPS cells were cultured in stem cell medium or in osteoblast medium for 7 days. These cells were also labeled with PKH26 fluorescent cell membrane linkers before infused to the fracture sites of a mouse fibular fracture model. The animals were sacrificed at 1, 2, and 4 weeks after cell therapy treatment. The results indicated that NEDAPS cells can be induced to functional osteoblasts representing the phenotypic and genotypic features of normal osteoblast. They expressed various genes that could be normally found at different stages of osteoblasts maturation by the process of RT-PCR, and functionally expressed proteins like type I collagen and alkaline phosphatase. Introduction of the NEDAPS cells and derived osteoblasts to the mouse fibular model resulted in ubiquitous survival of the bone matrix-forming cells up to 6 weeks post-surgery. However, the negative microCT results among groups warrant further investigation. It is possible that a proper scaffold to retain the therapeutic cells at the bone void is critical to achieve the successful clinical outcomes.
ACKNOWLEDGEMENTS: Supported by a WCGME Rising Star grant and by the Kansas INBRE, P20 GM103418

The role of the hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) on HSV-1 infection

Hunter Woosley[1,2], Christos Dogrammatzis[2] and Maria Kalamvoki[2]
[1] University of Kansas at Lawrence
[2]University of Kansas Medical Center, Department of Microbiology, Molecular Genetics and Immunology

Herpes simplex virus-1 (HSV-1) infection has been found to affect the properties of extracellular vesicles (EVs) released by infected cells, including biogenesis, cargo, and functions. These EVs appear to control virus dissemination. Following entry of the virus into the cells, its genome is released in the nucleus where viral gene transcription, replication, and capsid assembly occurs. The capsids of the virus acquire their envelope from the trans-Golgi network and endosomal compartments before released to the extracellular space. Data collected in our laboratory suggest that late gene expression and capsid formation during HSV-1 infection cause increased production of EVs. These data indicate that virion morphogenesis and EVs biogenesis are interwoven processes. Indeed, HSV-1 utilizes the Endosomal Sorting Complex Required for Transport (ESCRT) pathway for virion formation but the ESCRT pathway is important for EV biogenesis. The hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) is a key factor involved in protein sorting into EVs that functions early during the EV biogenesis process. Our goal through these studies was to investigate the role of Hrs on HSV-1 infection. We have made two major discoveries. First, that in Hrs-knockdown cells there is defect in virus release out of the cells, but virus replication and virion formation remain unaffected. Second, we observed that in Hrs-knockdown cells HSV-1 infection cannot anymore stimulate production of EVs. These data suggest that Hrs is important for HSV-1 release out of the infected cells, and for EV biogenesis during HSV-1 infection.

Complement Activation in Rat Pre-Eclampsia

Stacii Cross and Sherry Fleming, Department of Biology
Kansas State University, Manhattan, Kansas 66506

Pre-eclampsia is a multisystem disorder that occurs in pregnant women. This disorder causes the mother's blood pressure to become life-threateningly high with the only treatment being an early delivery for the baby; as a result pre-eclampsia is a major cause of premature births. Pre-eclampsia is characterized by the over-activation of the immune system, which includes complement killing cells located in the placenta. Complement is activated by IgM recognition of an antigen. We hypothesize that the antigen is found on a protein that is always found in the blood in high concentrations called Beta 2 Glycoprotein 1 (Beta 2). To test this hypothesis we subjected epithelial cells to hypoxia and tested for the presence of Beta 2, through the process of immunohistochemistry (IHC). We then tried to block the binding of Beta 2 to the cells with a peptide, developed by our lab. This research found that the peptide effectively blocks the binding of Beta 2 to the cells which prevents antibody-mediated activation of the innate complement system and cell death. We expect that this process can lead to a novel therapeutic for pre-eclampsia.

Ciclopirox-olamine alters ferritin trafficking and plays a protective role in polycystic kidney disease

Priyanka Radadiya,[1,5,6] Rajni Puri,[1,5] Brenda Magenheimer,[2,5] Dharmalingam Subramaniam,[4] Darren P Wallace,[1,5] Scott Weir,[3,5,6] James P Calvet,[2,5] and Madhulika Sharma,[1,5]
[1]Departments of Internal Medicine, [2]Biochemistry and Molecular Biology, [3]Cancer Center, and [4]Institute of Advancing Medical Innovation, University of Kansas Medical Center, [5]The Jared Grantham Kidney Institute, [6]The University of Kansas-Lawrence

Despite the recent launch of Tolvaptan, the search for safer polycystic kidney disease (PKD) drugs continues. We have recently shown that the Notch3 signaling pathway is activated in renal cells in PKD. Thus, we searched for a repurposed drug to serve as a Notch pathway inhibitor. Here we report the effects of Ciclopirox-olamine (CPX), an antifungal agent, shown to inhibit gamma secretase (activator of Notch signaling) by its property to chelate iron and inhibit activity of iron dependent enzymes. We found that as low as a 0.2uM dose of CPX inhibited cystogenesis in primary cells from ADPKD patients that were induced to form cysts in a 3D collagen gel system. Intraperitoneal injections of CPX in a PKD mouse model revealed a marked reduction in cystogenesis and the kidney to body weight ratio. This was associated with decreased cell proliferation, however not through Notch pathway inhibition. Given the role of CPX in iron chelation, we focused on ferritin. Ferritin levels were significantly elevated in the kidneys from PKD mice which were reverted through CPX treatments. Ferritin reduction by CPX was associated with increased ferritin-autophagy marker, NCAO4. A biphasic response of CPX dose on ferritin levels was observed. Our data suggest that CPX confers protection against ADPKD pathology with increased ferritinophagy as one of the mechanisms. These data also indicate that CPX, a drug used to treat skin infections and currently in clinical trials for cancer, has the potential to treat ADPKD.

Nanozyme: A Developing Nanotechnology for the Detection of Food-Borne Pathogens

Vedant Jain, Nilam Panchal, Tuhina Banerjee, and Santimukul Santra*
Department of Chemistry, Pittsburg State University, Pittsburg, KS 66762.
Email: ssantra@pittstate.edu

Nanozymes have recently emerged as promising alternatives to conventional enzymes for rapid detection of various analytes. Specifically, iron oxide nanoparticles and gold nanoparticles have long been known for their peroxidase- mimetic enzymatic property. However, their synergistic activity has never been employed in real-time for the detection purposes. We have combined these excellent catalytic activities as well as improved their limit of detection by integrating into a new nanozyme, named as Magneto-Plasmonic Nanosensor (MPnS). This newly formulated nanozyme will offer an alternative approach to the conventional ELISA for the detection of specific antigens. The detection performance of the optimized MPnS-based ELISA for O157:H7 strain of E. Coli will be demonstrated in this presentation.

Sunday, January 19, 2020

MRSA YjbIH coordinate expression of hemolysins through global networks

Alexis Winter, Crystal M. Austin, and Jeffrey L. Bose
Department of Molecular Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS

Staphylococcus aureus is a commensal bacterium that consistently colonizes 25-30% of the human population. For reasons that remain unclear, *S. aureus* can switch from a commensal to a pathogenic state and cause a multitude of diseases. Two key virulence factors that *S. aureus* secretes are α-hemolysin (Hla) and phenol soluble modulins (PSMs), two types of hemolysins. Our preliminary data suggests that mutants lacking YjbIH have altered hemolysis activity that is indicative of changes in Hla and PSM activity. Both Hla and PSMs are influenced by global regulators and we hypothesized that they contribute to the hemolysis phenotypes of the *yjbIH* mutant. We investigated this connection with the generation of mutants in combination with *yjbH*. We used qualitative Hla and PSM activity assays to identity regulators involved in Hla and PSM activity. We also used quantitative assays to determined that the *yjbIH* mutant has altered hemolysin activity. Importantly, we identified decreased Hla activity in an *yjbIH* mutant and determined that this was due in part to transcriptional differences using a β-galactosidase reporter of *hla* promoter activity. These results are the first to identify a role for YjbIH in *S. aureus* hemolysin production and shed new light on virulence factor regulation in this important human pathogen.
Supported by the Kansas INBRE, P20 GM103418.

Overexpression of the RNA binding protein HuR promotes chemoresistance by stabilizing AKT in colorectal cancer cells

Colby Spiess[1], Vikalp Vishwakarma[1], Ranjan Preet[1], Dan A. Dixon[1,2]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS and [2]The University of Kansas Cancer Center, Kansas City, KS.

Post-transcriptional regulation of proinflammatory gene expression is an important feature in colorectal cancer (CRC) development. HuR (*ELAVL1*) is an RNA-binding protein that stabilizes such genes by binding to AU-rich elements within the 3' untranslated region (3'UTR) of their mRNAs. HuR is often overexpressed and abnormally present in the cytoplasm during CRC development, where it interferes with rapid mRNA decay, allowing for enhanced oncogenic gene expression. Hyperactivity of the Pi3K/AKT/mTOR axis has been previously shown as a poor prognostic marker, and to confer resistance to chemotherapeutic treatment. We hypothesize that HuR overexpression modulates key CRC signaling pathways via mRNA stabilization, promoting cell survival and tumor progression. To explore this, we screened a library of established kinase inhibitors on HuR-overexpressing HCT-116 wild-type CRC cells and CRISPR/Cas9 HuR-knockout HCT-116 cells, then assessed for a differential response. HCT-116 WT cells demonstrated resistance to multiple kinase inhibitors, whereas deletion of HuR sensitized cells. While exploring specific signaling pathways, western blot analysis showed that Pan-AKT and Phospho-AKT(S473/T308) were significantly upregulated in HCT116 WT cells. Abundance of AKT mRNA was also shown with qPCR, and confirmed with single-cell RNA sequencing of these cells. Furthermore, compared to HuR-KO cells, single-cell RNA sequencing showed upregulation of the Pi3K/AKT/mTOR axis in WT cells. We performed an mRNA immunoprecipitation followed by cDNA synthesis and qPCR, which establishes AKT mRNA as a binding target of RNA stabilizing factor HuR, which likely contributes to aberrant AKT expression and chemoresistance. This may present novel combinatorial chemotherapy targets for CRC tumors overexpressing AKT and HuR.

CRISPR Cas12a endonuclease in a gene drive: cuts only as good as the guide

Isabel Lewis, Yao Yan, Gregory Finnigan
Kansas State University, Biochemistry and Molecular Biophysics Department

The CRISPR Cas9 system, a new and powerful gene editing technology, has been integrated into a gene drive" system using the type V Cas12a nuclease in S. cerevisiae. Cas12a creates a double strand break in DNA in living cells. It has a number of differences compared to the popular type II Cas9 enzyme: (i) Cas12a requires a PAM sequence (TTTV) on the 5' end of the target DNA while Cas9 requires a PAM sequence (NGG) on the 3' end, (ii) Cas12a can generate multiple guide RNAs included within the same expression cassette for multiplex editing and (iii) Cas12a creates a staggered (not blunt-ended) DNA break. In this study, guide RNA specificity was the main focus. We tested various lengths of guide RNA sequences that could be used for editing within a Cas12a-based gene drive system in diploid yeast cells. Mutagenesis of the guide RNA sequence at each position determined that the "seed region" was made up of the first 7-8 base pairs. The central portion of the guide (positions 8-17) displayed varying levels of tolerance and editing depending on the identity of the base substitution. Finally, the Cas12a nuclease could tolerate single guide RNA mismatches within the 3' end (positions 18-25) of the crRNA sequence. These findings provide insight into Cas12a specificity and will inform guidelines for optimized editing using this alternate type V nuclease.

Poster Presentations

1. Umama Ali[1], David S. Long[1]

[1]Biomedical Engineering Department, Wichita State University: **Quantification and Analysis of Morphological Changes in Microvascular Endothelial Cells After Exposure to Unidirectional or Pulsatile Flow**

2. Jaden Anderson[1] and Joanna S.G. Slusky[1,2]

[1]Center for Computational Biology, The University of Kansas, [2]Department of Molecular Biosciences, The University of Kansas: **Understanding Outer Membrane Protein Folding Using Sequence Coevolution**

3. [1]Bates, Janee, [2]Paige C. Geiger, [2]Fengyan Deng, [2]Josh Miller, [2]Danielle Rehor
[1]Haskell Indian Nations University, [2]University of Kansas Medical Center: **Heat Treatment and Neuronal Cell Health**

4. Jordan Block, Patil Tawidian, Kristin Michel
Department of Biology, Kansas State University: **Fungal friends and foes – larval source reduction using mosquito associated fungi**

5. Brodsky, Christine,[1] Morgan Smith,[1] Summer R. King,[2] and Kelly Mallatt[1]

[1]Department of Biology, Pittsburg State University, [2]Quapaw Nation Environmental Office: **Remediation of Tar Creek: Improving environmental quality and diversity over time**

6. Thai Butcher, Zachary Shaw, Arth Patel, Tuhina Banerjee, and Santimukul Santra*
Department of Chemistry, Pittsburg State University, Pittsburg, KS 66762: **Design and Synthesis of Functional Nanomedicine for the Targeted Treatment of Prostate Cancer**

7. Megan N. Campbell and A. Lorena Passarelli
Division of Biology, Kansas State University, Manhattan, Kansas: **Sulfhydryl oxidation and viral infectivity: Defining viral oxidoreductase substrates**

8. Maci Carlson, Patrick Lansdon, and Brian D. Ackley
Department of Molecular Biosciences, University of Kansas, Lawrence, KS: **Evaluating the Genetic Basis of Microbial Pathogenicity in Caenorhabditis Hosts**

9. Bradley Corbett, Matthew Mers, Qiyang Zhang
Department of Physical Sciences, Emporia State University: **ICP Analysis of Clay-Layer Sediments for the Identification of the K-Pg Boundary and the K-Pg Mass Extinction**

10. Andres Cordova, Patrick Lansdon, and Brian D. Ackley
Department of Molecular Biosciences, University of Kansas, Lawrence, KS: **Investigating Host-Pathogen Interactions using *Caenorhabditis elegans***

11. Rik Dhar[1], Meghan Franklin[2], Joanna Slusky[1,2]

[1]Department of Molecular Bioscience, [2]Center for Computational Biology, University of Kansas: **Making repeat protein topology with non repeating genetic material**

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Department of Biological Sciences, Fort Hays State University: **Enzymatic Tolerance to Sulfide, Lactic Acid, and Ethanol in Corn and Catfish Tissues**

13. Rachel Elting, Dr. James R. Walters
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14. Ernst, Nicholas[1*], Jeremy Goering[1*], Luke Wenger[1*], Yomna Badawi[1], Preethi Kunchala[1], Everett G. Hall[1], Nathan R. Wilson[1], Irfan Saadi[1], Hiroshi Nishimune[1].

[1]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS. *Equal contribution: **Molecular Changes underlying the Neuronal Deficits in *Specc1l*-deficient mice**

15. Freitas N, Zueckert W, Huan H, Wiepen J
Department of Microbiology, Molecular Genetics, and Immunology. Kansas University Medical Center**: Purification of *Borrelia burgdorferi* LptD homolog BB-0838**

16. Megan Goeckel, Erianna Basgall, Isabel Lewis, Madison Schrock, Kathrine Leonard, Yao Yan, and Gregory Finnigan
Kansas State University, Department of Biochemistry and Molecular Biophysics: **Examination of multiple septin-association domains within Bud3 in *S. cerevisiae***

17. Joshua Habiger,[1] Zhaoyang Ren,[1] Bende Zou,[2] Kelsey Erickson,[3] Zongbo Tong,[1] Huafang Fan,[1] Williams Cao,[2] Conrado Pascual,[2] Izumi Maezawa,[3] Lee-Way Jin,[3] Xinmin Simon Xie,[2] and Duy H. Hua[1]

[1]Department of Chemistry, Kansas State University, Manhattan, KS 66506
[2]AfaSci Research Laboratories, Redwood City, CA 94063
[3]M.I.N.D. Institute, 2805 50th Street, UC Davis Medical Center, Sacramento, CA 95817: **Pharmacokinetics and bioactivities of tricyclic pyrone molecules in Alzheimer's disease TgF344 rat model**

18. Jennifer L. Hackett[1,2,3,4], Erik A. Lundquist[1,2,4], Susan M. Lunte[1,5,6]

[1]Center for Molecular Analysis of Disease Pathways, [2]Genome Sequencing Core, [3]Higuchi Biosciences Center, [4]Department of Molecular Biosciences, [5]Department of Chemistry, [6]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence KS, USA: **Next Generation Sequencing at KU Genome Sequencing Core**

19. Madelyn Hilgers[1], Chingakham Ranjit Singh[1], Sarah Gillaspie[1], Mackenzie Thornton[1], Maureen Schick[1], Giovanni Di Pasquale[2], Sandra Afione[2], John A Chiorini[2], and Katsura Asano[1]

[1]Molecular Cellular and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66583
[2]Adeno-Associated Virus Biology Section, NIDCR, NIH, Bethesda, MD20892: **Translational control of Adeno-Associated Virus 2 by eIF5-mimic protein**

20. Courtney Hill[1], Berenice Jimenez-Marin[1], Tara Marriage[1], Bradley JSC Olson[1]
[1]Department of Biology, Kansas State University, USA (courtneyhill@ksu.edu): **Cooption of genes for cell-cell adhesion results in multicellularity for colonial alga *Gonium pectorale***

21. Joshua Lingo,[1] Pavithra Natarajan,[2] Dr. John Tomich,[2] Dr. Sherry D. Fleming[1]
[1]Kansas State University, Division of Biology, [2]Kansas State University, Department of Biochemistry: **Unraveling RD-p9 bioavailability and biodistribution: attenuating excessive innate immune response to tumor-associated hypoxia**

22. Aaron Morgan[1], Christopher Fischer[1], Allen Eastlund[2], Paul Jardine[2]
[1]Department of Physics and Astronomy, University of Kansas,
[2]Department of Diagnostic and Biological Sciences, University of Minnesota: **Kinetics of Nucleotide Binding to the gp16 ATPase**

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Dept. of Molecular & Integrative Physiology[1], University of Kansas Medical Center, Kansas City, Kansas, Dept. of Biostatistics[2], University of Kansas Medical Center, Kansas City, Kansas, Dept. of Pediatrics[3], Children's Mercy Hospital, Kansas City, MO, Sable Systems International[4], North Las Vegas, NV, Dept. of Anatomy & Cell Biology[5], University of Kansas Medical Center, Kansas City, Kansas, Kansas City VA Medical Center-Research Service[6], Kansas City, Missouri: **Sexual dimorphism and housing temperature modulate acute diet-induced weight gain through divergent energy expenditure.**

24. Megan Myers[1]; Noraida Martinez-Rivera, Ph.D.[1]; Aidyn Medina-Lopez[1]; Elias Michaelis, Ph.D. [1]; Yana Mikhaleva, Ph.D.[2]; Oleg Tolstenkov, Ph.D. [2]; Joel Glover, Ph.D. [2]; and Eduardo Rosa-Molinar, Ph.D.[1]
[1]University of Kansas, Lawrence, KS and [2]University of Bergen, Bergen, Norway: **Insight into the evolution of excitatory synapses using the larvacean tunicate, Oikopleura dioica**

25. Mariaelena Nabors, Robert Unckless
Department of Molecular Biosciences, University of Kansas: **The genetic basis of divergence in immune defense between *Drosophila* species**

26. Nansel-Lantz, Sara C. and Brian R. Maricle
Fort Hays State University Department of Biological Sciences: **Determining effective levels of antibacterial qualities of Kansas honeys**

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*Department of Chemistry, Pittsburg State University, Pittsburg, KS 66762: **Functional MRnS for the rapid detection of zika virus and assessment of cross-reactivity**

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Department of Biology, Pittsburg State University, Pittsburg, KS: **Assessing the Bioremediation Potential of Bacterial Strains Isolated from An Abandoned Coal Mine Following the Whole Genome Sequence Analysis Approach**

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[1]University of Kansas Department of Molecular Biosciences: **Temperature-sensitive screen for cell specification mutants**

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Department of Chemistry, Pittsburg State University, Pittsburg, KS 66762: **Magneto-Plasmonic Nanosensors (MPnS) for the Multiparametric Detection of E. Coli O157:H7**

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Department of Molecular Biosciences, University of Kansas, Lawrence, KS: **APC controls Wnt-induced β-catenin destruction complex recruitment in human colonocytes**

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[1]Higuchi Bioscience Center, University of Kansas, Lawrence, KS, USA; [2]KU Synthetic Chemical Biology Core Laboratory, University of Kansas, Lawrence, KS, USA: **The Synthetic Chemical Biology Core (SCB): A Resource for Research in Chemical Biology**

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[1]Departments of Molecular Biology, University of Kansas, Lawrence, KS 66045 and [2]Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160: **The RNA Binding Protein HuR Regulates Exosome Secretion in Colorectal Cancer via Rab 27B and can Serve as a Potential Biomarker.**

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[1]Department of Cancer Biology, University of Kansas Medical Center, Kansas City, KS
[2]Computational Medicine Center, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA: Investigating Polymorphic **Mitochondrial tRNA-Derived Fragments (mt-tRF) as Mitochondrial Signaling Molecules**

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[1 2] Division of Biology, University of Kansas State: **Generating Cell Lines Expressing Vaccinia Virus Decapping Enzymes**

36. Mingjing Sun
Department of Physical Sciences, Emporia State University: **Separation of inositol hexakisphosphate stereoisomers**

37. Spencer Tye, Jackie Dyke, Kistie Brunsell, Robert Ward
Department of Molecular Biosciences, University of Kansas, Lawrence, KS: **Larval *Drosophila* Trachea as Model for Post-embryonic Tissue-specific Allometric Growth**

38. Sarah Veesart and James McAfee
Department of Chemistry
Pittsburg State University: **Quantitative Analysis of hnRNPC Transcripts in Normal and Cancer Uterine Tissues**

39. Sarah E. Velasquez, MAB, MS, MLS
University of Kansas Medical Center: **K-INBRE Communications Core: Activities & Evaluation**

40. Katie Zimmerman[1,2], Kyle Boone Ph.D.[2,3], Candan Tamerler Ph.D.[2,3]
Department of Molecular Biosciences[1], Institute for Bioengineering Research[2],
Department of Mechanical Engineering[3], University of Kansas: **Design of the ATCUN Motif in Antimicrobial Metallopeptides**

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Department of Chemistry Pittsburg State University: **MALATI, A Triple -Stranded Cancerous RNA, a Triple Threat, Needs a Triple Solution**

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[1]Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS; [2]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ: **Spx and YjbIH mediate virulence factor production in *Staphylococcus aureus***

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Department of Biological Sciences, Department of Chemistry, Wichita State University: **Assessing the Timeframe for Formation of the Phi-clamp in Protective Antigen**

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[1]Department of Biomedical Engineering, Wichita State University, Wichita, KS: **Classifying Benign and Malignant Melanomas in Genetically Engineered Mouse Models using a Radio Frequency (RF) Resonator.**

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[1]Center for Computational Biology, The University of Kansas, [2]Department of Molecular Biosciences, The University of Kansas, [3]Department of Chemistry, University of Michigan: **Colicin E1 Fragments Potentiate Antibiotics by Plugging TolC**

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Department of Chemistry, Wichita State University: **Effect of Single Point Mutations in the Mobile Loop of Lactate Dehydrogenase**

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[1]Department of Pathology and Laboratory Medicine, [2]Department of Anatomy and Cell Biology, [3]Institute for Reproduction and Perinatal Research, University of Kansas Medical Center, Kansas City, Kansas, USA: **ESR2 regulation of primordial follicle preservation**

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Department of Chemistry, Emporia State University. Emporia, KS: **Identification of Ingredients in CBD Oil Sold in Emporia Through Analytical Techniques**

49. Joseph T Cornelius[1], Luciane M Silva[1], Wei Wang[1], Tana S Pottorf[1], Darren P Wallace[2], Pamela V Tran[1]
[1]Department of Anatomy and Cell Biology, Kidney Institute, University of Kansas Medical Center, Kansas City, KS
[2]Department of Integrative and Molecular Physiology, Kidney Institute, University of Kansas Medical Center, Kansas City, KS: **Investigating primary cilia structure in Autosomal Dominant Polycystic Kidney Disease**

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[1]Dept. of Molecular & Integrative Physiology, University of Kansas Medical Center: **Investigating the impact of exercise on hepatic mitophagy utilizing the autophagy inhibitor leupeptin.**

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[1]The Center for Molecular Analysis of Disease Pathways, [2]The Ralph N. Adams Institute for Bioanalytical Chemistry, [3]Department of Pharmaceutical Chemistry, [4]Department of Chemistry, University of Kansas: **Equipment and Services of the Ralph N. Adams COBRE Core Nanofabrication Facility**

52. Evan Haas[1,3], Ember Krech[2], Elizabeth Friis[1,2]
University of Kansas, Department of Mechanical Engineering[1], Bioengineering Graduate Program[2], K-INBRE Research Program[3], Lawrence, Kansas: **Multi-Disc Voltage Analysis of Piezoelectric Composite Materials with Compliant Layers**

53. Arman A. Hadjian, Digamber Rane, and Blake R. Peterson
Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045: **Synthesis of molecular probes that accumulate in the endoplasmic reticulum of living eukaryotic cells**

54. Stacy D Holt Jr,[1] and James Beck,[1]
[1]Wichita State University, Department of Biological Sciences**: Understanding a local population of the invasive species Pyrus calleryana through genetic analysis**

55. Rajesh Kandel and Dr. Seid Adem, Chemistry Department, Washburn University: **Colorimetric Detection of Lead and Other Heavy Metals Ions Using Modified Gold Nanoparticles**

56. Marija Kuna[1, 2], Pramod Dhakal[1, 2], Lindsey N. Kent[1, 2], Regan L. Scott[1, 2], Khursheed Iqbal[1, 2] and Michael J. Soares[1, 2, 3, 4, 5]
[1]Institute for Reproduction and Perinatal Research, Departments of [2]Pathology and Laboratory Medicine, [3]Obstetrics and Gynecology, and [4]Pediatrics, University of Kansas Medical Center, Kansas City, Kansas; [5]Center for Perinatal Research, Children's Research Institute, Children's Mercy, Kansas City, MO: **CITED2 Regulation of Embryonic and Placental Development: Species Differences**

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[1]Department of Molecular Biosciences, University of Kansas: **The Role of Basement Membrane Proteins for Proper Q Neuroblast Migration in C. elegans**

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[1], Department of Anatomy & Physiology, Kansas State University College of Veterinary Medicine, Manhattan, Kansas 66506, United States of America
[2], euglena Co. Ltd., 5-29-11 Shiba, Minato-ku, Tokyo 108-0014, Japan: **Cell wall membrane fraction of Chlorella sorokiniana enhances host anti-tumor immunity and inhibits colon carcinoma growth in mice**

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Fort Hays State University, Department of Biological Sciences: **Relationship between food intake and expression of messenger RNA encoding aromatic amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), and catechol-O-methyltransferase (COMPT) in channel catfish.**

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[1]Department of Chemistry, Wichita State University: **Relationship of Oligomerization Status to the Catalytic Activity of Human Lactate Dehydrogenase (LDH)**

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[1]Department of Biology, Langston University, [2]Department of Plant Biology, University of California Davis, [3]Department of Biology, University of Oklahoma: **Investigating functions of BABY BOOM genes in embryogenesis**

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Kansas State University, Biology Department: **Defining the Mechanisms of eEF1A regulation by the Legionella pneumophila effector SidI**

63. Tabatha Polk[1], Sarah Schmitt[1], David Long, Ph.D.[1]
[1]Wichita State University, Department of Biomedical Engineering, Wichita, KS: **Effects of Fluid Shear Stress on Dermal Human Microvascular Endothelial Cell Morphology**

64. Collette L. Wright[1], Dr. Jeff McFarlane[1], Dr. Kathleen Meneely[1], Dr. Aron Fenton[2], Dr. Audrey L. Lamb[1]
[1]Molecular Biosciences, University of Kansas, Lawrence, KS; [2]Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS: **Is the Pyruvate Kinase of *Zymomonas mobilis* Allosterically Regulated?**

65. Derek Reese, Eric Trump
Emporia State University: **Synthesis of a Superstable Trioxatriangulenium Ion**

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[1]Dept. of Molecular Biosciences, Univ. of Kansas, [2]Inst. of Biomaterials and Biomedical Engineering, Univ. of Toronto, Toronto, ON, CA, [3]Dept. of Cell & Systems, Univ. of Toronto, Toronto, ON, CA, [4]Ted Rogers Centre for Heart Research, Univ. of Toronto, Toronto, ON, CA: **Septate junction proteins act at the leading edge to maintain tissue adhesion during *Drosophila* dorsal closure**

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University of Kansas Medical Center. Department of Radiation Oncology: **Macrophage directed therapy to ameliorate radiation induced rectal damage.**

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[1]Division of Biology, Kansas State University: **Mechanisms of LegC4-mediated attenuation of Legionella pneumophila replication within primary mouse macrophages**

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Wichita State University, Department of Biological sciences: **The differentiation and characterization of dental pulp stem cells: toward nucleus pulposus regeneration**

70. Michael Stricker, Nicholas Stewart, Bryant McAllister, Rebekah Rogers. Fort Hays State University, University of Iowa, University of North Carolina at Charlotte: **Identification of Genomic Regions that Influence Meiotic Drive Favoring Metacentric Chromosomes in *Drosophila Americana***

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Department of Biological Sciences, Wichita State University: **Investigating the effect of small interfering RNAs targeting cell wall biosynthesis on the growth of *Macrophomina phaseolina***

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[1]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS. *Equal contribution: **In-frame Genetic Disruption of SPECC1L Microtubule-Interaction Domain Caused Neural Tube, Palate, Ventral Body Wall and Optic Fissure Closure Defects**

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[1]Department of Molecular Biosciences, University of Kansas, Lawrence KS, [2]School of Medicine, University of Kansas Medical Center, Kansas City, KS and [3]University of Kansas Cancer Center, University of Kansas Medical Center, Kansas City, KS: **Role of RNA Binding Protein HuR as Facilitator in Non-alcoholic Fatty Liver Disease (NAFLD) Progression**

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Fort Hays State University: **Seeking Satiety: Dopamine Receptors 1,2 mRNA Expression in Channel Catfish**

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Fort Hays State University[1], University of North Carolina Charlotte[2]: **Karyotype Evolution and Meiotic Drive of Hybrid *Drosophila* Fruit Flies**

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Wichita State University Department of Biological Sciences: **Assessment of Bacterial Cell Survival and Vitrification in Salt Concentrations Relevant to Mars**

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Physical Sciences, Emporia State University: **Optimizations in Capillary Electrophoresis for Fast Determination of Amino Acids**

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Department of Internal Medicine, The Jared Grantham Kidney Institute, University of Kansas Medical Center, Kansas City, KS, United States: **The role of LKB1-AMPK signaling on cyst progression in PKD**

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University of Kansas, Department of Molecular Biosciences: **Evaluating the Role of Translational Efficiency in Synaptogenesis in *Caenorhabditis elegans***

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[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS
[2]Whitehead Institute for Biomedical Sciences, Cambridge, MA: **The RNA-binding protein Tristetraprolin: A key factor of intestinal cell differentiation and microbial homeostasis in colorectal cancer**

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[1]Department of Biological Sciences, Wichita State University, [2]Jet Propulsion Laboratory, [3]Space Science Institute: **First Demonstration of Bacterial Growth in Deliquescent Brines Relevant to Mars**

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Department of Biological Sciences, Wichita State University: **The migration of dental pulp stem cells in 3D culturing of chemotaxis chamber**

85. James Balthazor
Department of Chemistry, Fort Hays State University: **Probing the Unfolded Protein Response: mRNA knockdowns in Acyrthosiphon pisum**

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[1] Department of Biology, Washburn University, Topeka, KS: **Investigation of CHD7 homolog, kismet in Drosophila with respect to muscle growth and development**

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Kansas State University, Manhattan, KS, Department of Biochemistry and Molecular Biophysics: **Design of S. pyogenes Cas9 nuclease immune to anti-CRISPR inhibition**

88. Lia Boese, Jenna Placzek, Sarah Champagne
Fort Hays State University: Chemistry: **Development of a Green Double Allylation Methodology**

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Department of Psychological Sciences, Kansas State University: **Effect of Ethanol Exposure on CREB Levels in Enriched and Socially Isolated Rats**

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Physics Department, Washburn University, Topeka, Kansas: **An Introduction to Cellular Analysis Using Quantitative Phase Imaging**

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[1]Division of Biology, Kansas State University, [2]Department of Biology, Texas A&M University: **Role of Glycogen Metabolism in C. difficile Virulence**

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[1]Department of Biomedical Engineering, Wichita State University: **Development of an Automatic Computational Machine Learning Pipeline to Process Confocal Images for Virtual Cell Generation**

93. Carson Denney, Seid Adem, Washburn University Department of Chemistry: **Analysis of Dacthal in Various Food Samples from Local Stores Using GC-MS**

94. Dalton Doyle, Savannah Bender, Tim Burnett PhD.
Department of Biology Emporia State University: **Tracking mucosal T-cell subtype development in mice**

95. Nicole D'Souza[1], Sui Ching Phung[1], Mei He[1,2*]
[1] Department of Chemical and Petroleum Engineering,[2]Department of Chemistry, University of Kansas: **Optimization of Sucrose Cushions to Improve Efficiency of Exosome Isolation**

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Department of Chemistry, Wichita State University, Wichita KS: **Dependence of Proline Isomerization on the Kinetics of Folding of Anthrax Lethal Factor**

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1. Fort Hays State University Department of Chemistry
2. Fort Hays State University Department of Biological Sciences: **Effect of RNA Interference mediated knockdown of Protein Disulfide Isomerase on *Acyrthosiphon pisum* survival**

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Fort Hays State University Department of Biological Sciences: **Assessment of Environmental Bacteria for Resistance to Cetylpyridinium Chloride, Modes of Potential Resistance Transmission, and Clinical Applications**

99. Kaitlynn Hillery, Sierra Smith, Skyler Markham
Fort Hays State University; Chemistry: **Development of Novel Phosphonium Salts for use in Ionic Liquids and as Cationic Ligands**

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[1]Deparment of Chemistry, Emporia State University
[2]Department of Chemistry, Northwestern University: **Towards Peptide-polymer Nanoparticles of Different Charges for Studying Biological Interactions**

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Wichita State University, Department of Chemistry: **Monitoring Palladin's Effect on Actin Dynamics and Organization with TIRF Microscopy**

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[1]Wichita State University, Wichita, KS; [2]Institute of Genetic and Biomedical Research, CNR, Italy: **The Role of Myopalladin in Cardiac Muscle Function and Disease**

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[1]Department of Chemistry, Wichita State University: **Improving Personalized Medicine Through Systematic Protein Engineering of LDH**

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Department of Biological Science, Fort Hays State University: **Link between Nutritional Status and Gene Expression in Regulatory Associated Protein of Mammalian Target or Rapamycin mRNA (RPTOR) and Rapamycin-insensitive Companion of Mammalian Target of Rapamycin (RICTR) messenger RNA.**

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Emporia State Biology: **Soil microbiota use non-cellulolytic bacteria to synergistically enhance cellulose digestion**

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Department of Molecular Biosciences, University of Kansas: **Expression of human Tau mutants leads to synaptic loss in *Caenorhabditis Elegans***

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[1]Department of Molecular Biosciences, [2]HTS Laboratory, [3]Center for Bioinformatics, The University of Kansas, Lawrence, Kansas; [4]Program in Molecular Therapeutics, Fox Chase Cancer Center, Philadelphia, PA; [5]Department of Chemistry, [6]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA: **Identification and validation of an Aspergillus nidulans secondary metabolite derivative as an inhibitor of the Musashi1-RNA interaction**

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Department of Molecular Biosciences, University of Kansas, Lawrence, KS: **Transcriptomic Profiling Identifies Strain-specific Differences in the Response of *Caenorhabditis elegans* to Microbial Pathogens**

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Fort Hays State University; Chemistry: **The Formation of 2,2'-bipyridine by a Mild Phosphorus Extrusion Reaction**

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Department of Biology, Department of Chemistry, Fort Hays State University: **RNA Interference of Three Genes in the Unfolded Protein Response: Activating Transcription Factor 6 (ATF6), Prefoldin Subunit 2 (PFD2), and Tumor Necrosis Factor Receptor Associated Factor 2 (TRAF2) in Pea Aphids (*Acyrthosiphon pisum*)**

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[1] Center for Computational Biology, Kansas University, Lawrence, KS, 66045: **Synthetic Beta-barrel Protein Sequences for Bionanosensor Applications**

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Department of Molecular Biosciences, University of Kansas, Lawrence, KS: **Identifying regulators of directed neuroblast migration in *Caenorhabditis elegans***

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1) The University of Kansas Medical Center, Department of Molecular & Integrative Physiology: **Protein and Fat Analysis of Forelimb Muscles of Low and High Aerobic Capacity Rats Following Resistance Exercise**

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Department of Plant Pathology- Kansas State University. Department of Genetics- University of Melbourne: **The mechanism of nitrogen metabolite repression by the NmrA corepressor.**

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Department of Biology and Department of Chemical and Petroleum Engineering: **Single Cell Capturing for Molecular Profiling of Heterogeneous Extracellular Vesicles**

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[1]Department of Biochemistry & Molecular Biophysics , Kansas State University , Manhattan , Kansas 66506 , United States: **Inhibition of gC1qR to prevent role in blood coagulation systems**

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[1]Division of Biology, [2]Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS: **Identification of regulatory domains in HD-Zip IV transcription factor GLABRA2**

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Kansas State University: **A Method for Characterizing Membrane-Bound Proteins**

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Kansas State University, Department of Biology: **Dissecting functional domains of the Vaccinia Virus D10 Decapping Enzyme**

121. Adams, Rebecca[1] and Keri Maricle[1] [1]Department of General Education, North Central Kansas Technical College, Hays, KS: **Effectiveness of Common Household Cleaners in Preventing Microbial Growth**

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Department of Molecular Biosciences, University of Kansas, Lawrence, KS: **Characterization of novel role for Rab27B in autophagy regulation in colorectal cancer.**

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[1]Department of Chemistry, Wichita State University, Wichita, Kansas 67260, United States: **Synthesis of Phosphatidylglycerol Receptor: Precursor Preparation**

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Wichita State University, Wichita, KS: **Conflicting or Collaborating Roles for Palladin and VASP in the Regulation of Actin Filaments**

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[1]Department of Molecular and Integrated Physiology, University of Kansas Medical Center, Kansas City, Kansas: **Creating a Liver-Specific ERα Knockout Mouse (LERKO)**

126. Bioinformatics Core Personnel[1,2,3]
[1]Kansas State University, [2]University of Kansas and [3]University of Kansas Medical Center: **K-INBRE Bioinformatics Core**

127. Bijaya Basnet, Vincent Rossi, Dr. Long
Washburn University, Department of Physics, Wichita State University: **Observing Changes in Cellular Morphology via Digital Holographic Microscopy and Optical Scatter Imaging**

128. Nagendra Dhamala, Vincent Rossi
Washburn University: **Applying Machine Learning for Holographic Image Processing in order to Detect Morphological Changes in the Subcellular Environment**

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Fort Hays State University Department of Biological Sciences: **Results of soil nutrient additions in restoring Magnesium concentration to common crop species**

131. Eric Ebert, Bailey Lampton, Mulin He, Stephen Fields
Emporia State University: **The Role of Melatonin in Neuronal Development**

132. Engel, Ryan P. and Brian R. Maricle
Department of Biological Sciences, Fort Hays State University, Hays, Kansas: **The Influence of Land Use on the Pollen Diet of Honey Bee (Apis mellifera) Colonies in Ellis County, Kansas**

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Division of Biology, Kansas State University, Manhattan, KS 66506: **Translational control of Hri2 eIF2α kinase during amino acid starvation**

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Kansas State University Division of Biology: **Evolution of low and high virulence *Stenotrophomonas maltophilia* strains**

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Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045: **Selective Ubiquitination of Cellular Proteins by HSV-1 ICP0**

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[1]Department of Biology, Kansas State University, Manhattan KS, USA (jimenezb@ksu.edu)
[2]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson AZ, USA: **Reduction of functional content in volvocine genomes during the evolution of multicellularity**

138. Josephine Johnson and Qiyang Zhang
Emporia State University Department of Physical Science: **Determination of Heavy Metal Levels in Cosmetic Foundations & Mascaras Using ICP-AES Analysis**

139. Rachel Klausmeyer, Ravi Vattepu, Allan Ayella, Rahul Yadav, Joseph T. Dille, Moriah R. Beck
Chemistry Department, Wichita State University, Wichita, KS: **Discovery of Obscure Tyrosinate Fluorescence in Immunoglobulin Domain**

140. Price Kramer and Sam Leung
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141. Austin Landgraf, Dr. Nicholas Stewart, Dr. Yass Kobayashi
Fort Hays State University Biology Department
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142. Jacob Lutgen[1], Jared Ridder[1], James R. Balthazor[2]
[1] Fort Hays State University Department of Biological Sciences,
[2] Fort Hays State University Department of Chemistry: **RNA Interference of the Unfolded Protein Response in *Acyrthosiphon pisum***

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Pittsburg State University Biology Department: **CCR7 Expression in Pregnant Rat Uterine Tissue Prior to Embryonic Implantation**

144. Joshua Miller[1], Fengyan Deng[1], Brigid Flynn[2], and Paige Geiger[1]
[1] University of Kansas Medical Center - Department of Molecular and Integrative Physiology
[2] University of Kansas Medical Center - Department of Anesthesiology: **Investigation into the Efficacy of Heat Therapy to Improve Maximal Oxygen Consumption in Subjects ages 50 and older**

145. Seth T. Peery[1], and Kathrin Schrick[1,2].
[1]Department of Biochemistry and Molecular Biophysics, [2]Division of Biology, Kansas State University, Manhattan, KS: **HD-Zip Transcription Factors: Key Regulators of Development and Metabolism**

146. Isaiah Powell, Manish Bhatta and Achut Silwal
Department of Chemistry, Washburn University, Topeka, KS: **Raman Spectroscopy Analysis of Redox States and Mechanism of Flavin Cofactors**

147. Aaron J. Rudeen**[1]**, Minli Xing[2], Justin T. Douglas[2], Audrey L. Lamb[1] and Kristi L. Neufeld[1]
[1]Departmant of Molecular Biosciences, University of Kansas, Lawrence, KS
[2]Nuclear Magnetic Resonance Core Lab, University of Kansas, Lawrence, KS: **A domain of Adenomatous polyposis coli retained by colon tumors is intrinsically disordered, and can bind three β-catenin molecules**

148. Liana Savage, Gayani Wijegunawardena, Kandategе Wimalasena, PhD
Department of Chemistry, Wichita State University: **The Effect of Mitochondrial SOD-2 Knockout on the Dopaminergic Toxicity of MPP+ in C. elegans PD Model**

149. Sehgal, Chahat, Anna M. Brokesh, Cameron C. Hunter and Richard B. Todd.
Department of Plant Pathology, Kansas State University, Manhattan, Kansas: **Genetic interactions between three transcription factors involved in NmrA-mediated repression in *Aspergillus nidulans.***

150. Soto, Sergio J., Nayan Shrestha, and William J. Hendry
Department of Biological Sciences, Wichita State University: **Evaluation of differential *in vitro* culture dynamics and immunohistochemistry analysis results between human ovarian cancer and head and neck cancer cell lines**

151. Dipesh Thapa and Achut Silwal
Department of Chemistry, Washburn University, Topeka, KS: **Raman Spectroscopy Analysis of Biochemical States of Dopamine Neurotransmitters**

152. Mackenzie Thornton[1], Yuji Chikashige[2], Hiroaki Kato[3], Thomas D. Baird[4], Whitney Pepper[1], Madelyn Hilgers[1], Ariana Cecil[1], Izumi Asano[1], Haana Yamada[1], Cheyenne Brunkow[1], Carter Moravek[1], Ronald C. Wek[4], and Katsura Asano[1].
[1] Division of Biology, Kansas State University, Manhattan, KS 66506; [2] Advanced ICT Research Institute Kobe, National Institute of Information and Communications Technology, Kobe, Japan; [3] Department of Biochemistry, Shimane University School of Medicine, Izumo, Japan; [4] Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202: **Gcn2 eIF2α kinase mediates translational regulation through nucleotide motifs in the mRNA 5' untranslated regions.**

153. Lesly Torices[1]
[1]Kansas State University, Department of Biology, Kansas Lipodomics Research Center: **Analysis on the Separation/Identification of Arabidopsis Lipid Classes**

154. Vázquez Plaza, N., Bhakta, B., and Carvalho, C. M. (Faculty Mentor)
Department of Biological Sciences, Fort Hays State University, Hays, Kansas: **Examination of Prevalence and Associated Behaviors of MRSA Carriers Among College Populations**

155. Zoey K. Wallis, Nicholas B. Stewart, and Eric T. Gillock
Department of Biological Sciences, Fort Hays State University: **Genetic Analysis of the Porcine Shadow of Prion Protein Gene**

156. Jenika Wheeler, Antonio Zapata, and Qiyang Zhang
Department of Physical Sciences, Emporia State University, Emporia, Kansas 66801: **Injection Voltage and Time with Poly(dimethylsiloxane) and Flow Gating in Capillary Electrophoresis**

157. Emily White, Washburn University Biology Undergraduate
Takrima Sadikot, Department of Biology Associate Professor: **Genomic Annotation of 45,600 bp region of 3L chromosome in D. *takahashii***

158. Caleb Winscott, Abigail Morgan, Phillip Harries
Department of Biology, Pittsburg State University: **Optimization of a lead biosensor in E. coli**

159. Wei Wu,[1] Kaimin Jia,[1] Gaochao Huang,[1] Ruben Shrestha,[1] Bingbing Wu,[1] Yulan Xiong,[2] and Ping Li[1]
[1]Department of Chemistry, [2]Department of Anatomy and Physiology, Kansas State University, Manhattan, KS, 66506: ***In Vivo* N-Terminal Methylation of OLA1 Revealed by Target Profiling of NTMT1**

160. Marrissa Raynesford, Mulu Bannister, Taylor Weidenhaff,
Hays High School, USD 489: **The Warning Signs of Lung Disease**

1. Quantification and Analysis of Morphological Changes in Microvascular Endothelial Cells After Exposure to Unidirectional or Pulsatile Flow

Umama Ali[1], David S. Long[1]
[1]Biomedical Engineering Department, Wichita State University

The objective of this project is to quantify and analyze the changes in the cell membrane and nuclear morphology in human microvascular endothelial cells after exposure to unidirectional laminar flow or pulsatile laminar flow. The microvascular cells will be cultured into *ibidi* µ-Slide I Luer using protocols previously developed in Dr. Long's lab. The perfusion system will consist of the µ-Slide, and a pump, fluidic units, and perfusion tubing sets. The *ibidi* perfusion system is capable of both undisturbed flow and disturbed flow. The experiments will be divided into three groups: (1) no-flow (control); (2) unidirectional flow; and (3) pulsatile flow. The cell membrane nuclei will be visualized using, respectively, What Germ Aggulutinin (WGA) (Biotum) and Hoechst and live fluorescence microscopy at different time points after exposure to flow/shear. Tetraspeck fluorescent microspheres will be used as fiducal markers, adhered underneath the cells using Poly-L-Lysine, to identify a starting point in imaging the cells. Images of the cell membrane and nuclei will be acquired at each time point. Then nuclear and cell membrane morphology will be characterized and compared between control (no flow), unidirectional flow, and pulsatile flow conditions using *FIJI/imageJ.*

2. Understanding Outer Membrane Protein Folding Using Sequence Coevolution

Jaden Anderson[1] and Joanna S.G. Slusky[1,2]
[1]Center for Computational Biology, The University of Kansas, [2]Department of Molecular Biosciences, The University of Kansas

A unique characteristic of outer membrane proteins (OMPs) is that they almost exclusively exist as β-barrels. In *E. coli*, the β-Barrel Assembly Machine (BAM) complex catalyzes insertion of OMPs from the periplasm into the outer membrane. Experimental evidence has shown that the outer membrane embedded β-barrel portion of the BAM complex BamA is essential for insertion function (1,2). However, the precise mechanism of insertion is still unknown. To better understand the interactions between BamA and its client proteins, we explore a computational approach analyzing sequence co-evolution in Multiple Sequence Alignments (MSA) to determine inter-protein residue contacts between BamA and its client proteins. Our results using RaptorX describe a client protein c-terminal interaction with strand 5 of BamA. To validate these inter-protein contacts, we probe the *in vitro* and *in cells* folding of the client protein FadL by mutational analysis.

3. Heat Treatment and Neuronal Cell Health

[1]Bates, Janee, [2]Paige C. Geiger, [2]Fengyan Deng, [2]Josh Miller, [2]Danielle Rehor
[1]Haskell Indian Nations University, [2]University of Kansas Medical Center

Alzheimer's Disease (AD) is a progressive disease that is the accumulation of insoluble plaques consisting of Amyloid-beta proteins that destroys memory and other important mental functions. It is the most common dementia in the senior population affecting 5.2 million Americans over the age of 65. With the help of Heat Shock Proteins (HSP's) this number could possibly be minimized. HSP's are cellular chaperones that assist in protein-protein interactions that help with protein misfolding. For this experiment SH-SY5Y were used as a model because they have simple neurotransmitters. If SY5Y cells are exposed to heat then the expression of HSP's would increase and have a positive effect on cell mitochondrial activity. The goal of this experiment is to develop basic heat treatment data that can potentially be used in therapy for AD.

4. Fungal friends and foes – larval source reduction using mosquito associated fungi

Jordan Block, Patil Tawidian, Kristin Michel
Department of Biology, Kansas State University

The control of mosquito-borne diseases mainly relies on vector control through the application of insecticides. However, several mosquito species have developed insecticide resistance necessitating the use of alternative control measures, including the use of bacterial and fungal entomopathogens that serve as environmentally safe alternatives for insecticides. Entomopathogenic fungi such as Beauveria bassiana have been used successfully for the control of adult and larval stages of several mosquito species. The overall goal of this study is to determine the effects of mosquito associated fungi on mosquito immunity to potentially design new insecticides. Several fungal morphotypes were isolated from field collected Aedes albopictus larvae. The fungal species were identified through amplifying and sequencing the Internal Transcribed Spacer Region 2 (ITS2). A total of sixteen fungal morphotypes have been successfully isolated into pure cultures from the field collected Ae. albopictus larvae carcasses several of which are potential food sources for mosquito larval development such as Penicillium sp. Additionally, several potential mosquito entomopathogens and entomotoxigens were identified among the isolated fungal strains, including B. bassiana and Trichoderma atroviride, respectively. This study provides a glimpse into the diversity of fungi associated with field collected mosquito larvae and the effect on mosquito larval development. Ultimately, this knowledge can be applied to the use of fungal insecticides for mosquito control.

5. Remediation of Tar Creek: Improving environmental quality and diversity over time

Brodsky, Christine,[1] Morgan Smith,[1] Summer R. King,[2] and Kelly Mallatt[1]
[1]Department of Biology, Pittsburg State University, [2]Quapaw Nation Environmental Office

The Tar Creek Superfund Site in Picher, Oklahoma was once the world's largest lead and zinc mining areas. Since 1983, large-scale remediation efforts have occurred throughout the landscape, such as cleaning up mining waste and planting native grass mixtures. In this study, we asked how habitat remediation of a heavy-metal contaminated area impacted environmental quality, specifically bird community diversity over time. Since 2017, we surveyed 24 locations in various stages of remediation. We sampled each location's bird community in May – July with three, 5-minute point count surveys. We evaluated habitat resources by measuring ground vegetation cover and composition, canopy cover, and shrub and tree species composition. We observed 69 bird species across the mined area, with an average of 15.8 species per site. Remediation efforts attracted more bird species to the sites, particularly for sites with more grass and forb cover; however, some sites without remediation provided adequate habitat resources and hosted diverse bird communities. Construction sites with bare ground had the least amount of bird activity, highlighting the importance of leaving the ground bare for as little time as possible. Additionally, wildlife once found at the site are no longer present after remediation. Wildlife are meaningful to the Quapaw tribe and the loss of these species on their landscape may result in a cultural loss, in addition to changes in environmental quality. Our goal is to combine our data with conversations had with Quapaw tribe members to determine if remediation changes are beneficial to the public and to wildlife.

6. Design and Synthesis of Functional Nanomedicine for the Targeted Treatment of Prostate Cancer

Thai Butcher, Zachary Shaw, Arth Patel, Tuhina Banerjee, and Santimukul Santra*
Department of Chemistry, Pittsburg State University, Pittsburg, KS 66762.

In this study, a hyperbranched polyester co-polymer was designed using a proprietary monomer and diethylene glycol or triethylene glycol as monomers. The synthesis was carried out using standard melt polymerization technique and catalyzed by □-Tolulenesulfonic acid. The resulting polymer was chosen for further modification into folate-functionalized polymeric nanoparticles for targeted drug delivery to prostate cancer cells. We hypothesized that due to the 3D structure of the diacid A2B monomer, we expect a pseudo-branched polymer that is globular in shape which will be ideal for drug carrying and delivery. We used a solvent diffusion method, wherein the polymer can be simultaneously converted into water-dispersible nanoparticles and therapeutic agents (doxorubicin) can be encapsulated into the polymeric nanocavities. The efficacy of this delivery system was gauged by treating LNCaP prostate cancer cells with the drug-loaded nanoparticles and assessing the results of the treatment. The experimental results collectively show a nanoparticle that was biocompatible, target-specific, and successfully initiated apoptosis in an in vitro prostate cancer cell model.

7. Sulfhydryl oxidation and viral infectivity: Defining viral oxidoreductase substrates

Megan N. Campbell and A. Lorena Passarelli
Division of Biology, Kansas State University, Manhattan, Kansas

Baculoviruses are enveloped viruses with double-stranded DNA genomes that infect insects. They have two virus forms: budded (BV) and occlusion-derived viruses (ODVs). The BV specializes in cell-to-cell infection within the insect, while the ODV specializes in infection between insects. The baculovirus ac92 gene is a sulfhydryl oxidase present in all baculoviruses sequenced to date, suggesting that it has an essential function for baculovirus replication. During sulfhydryl oxidation, thiol groups from cysteines in specific proteins are oxidized to form disulfide bonds within or between proteins. Ac92, the product of ac92, is an envelope-associated protein of the BV and ODVs; its oxidation substrates may also be envelope-associated or virion proteins. Deletion of ac92 affects the phenotype of each virus form: lack of infectious BV production and singly-enveloped instead of multiply-enveloped nucleocapsids in the ODV, suggesting defects in assembly. The goal of this project is to identify ODV viral proteins oxidized by Ac92. Per os infectivity factors (PIFs) are ODV proteins with conserved cysteines required for infection of the insect at the primary site of infection and potential Ac92 substrates. Selected pif genes will be cloned in bacteria and proteins produced. Ac92 will be tested for its ability to form intramolecular disulfide bonds in PIFs, using an in vitro assay. Subsequently, PIF cysteines will be mutated and their requirement for Ac92 function will be tested in vitro and in vivo. This information may translate into methods important in expressing correctly folded proteins in heterologous systems and designing vaccines and therapeutic agents.

8. Evaluating the Genetic Basis of Microbial Pathogenicity in *Caenorhabditis* Hosts

Maci Carlson, Patrick Lansdon, and Brian D. Ackley
Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Microbial pathogens pose an evident threat to organismal survival. However, the discrepancies that exist within a specific host's responses to one pathogen over another has remained unclear. Due to its microbivorous behavior and observably consistent contact with many potentially pathogenic microbes, the anatomically simplistic and transparent nematode, *Caenorhabditis elegans*, serves as an excellent model organism to study human diseases and behaviors. Survival assays have provided a means for identifying a multitude of genetic factors and molecular pathways that influence the animal's physiology, particularly that of stress responses and immunity against pathogens. To evaluate interspecific differences in pathogen response across the *Caenorhabditis* genus we are employing a two-pronged approach. Firstly, we are assessing the survival of several *Caenorhabditis* strains infected with a given pathogenic bacteria to identify strain-specific differences in pathogen susceptibility. Secondly, we have begun to assay the survival rates of crossbred strains and compared them to that of native strains. This has allowed us to view trends in pathogenic susceptibility and make inferences about allelic patterns. Currently we are performing genomic mapping of F2 heterozygous offspring generated from these crossbred animals to identify genetic loci overrepresented in animals exposed to pathogens relative to non-pathogenic *E. coli*. Ultimately, our study seeks to understand the specific genetic mechanisms of host defenses against infection as well as explore any ambiguity regarding mammalian-specific defenses.

9. ICP Analysis of Clay-Layer Sediments for the Identification of the K-Pg Boundary and the K-Pg Mass Extinction

Bradley Corbett, Matthew Mers, Qiyang Zhang
Department of Physical Sciences, Emporia State University

The current leading theory for the mass Cretaceous-Paleogene (K-Pg) extinction is by an impact from a massive asteroid. Asteroids contain higher amounts of heavy metals than the earth's crust; a resulting collision from an asteroid would deposit Iridium (Ir) into the corresponding ash layers (Alverez et al.,1980). This increase in Ir has been discovered by other studies and along with other findings have supported the theory of the K-Pg extinction resulting from an asteroid (Alverez et al., 1980; Miller et al., 2010). Simple confirmation of increased iridium is vital to identifying the K-Pg boundary and determining if a fossil dates to pre-extinction or post-extinction event. The goal of this project was to determine the Ir levels in different clay layers from different locations in order to properly identify the K-Pg boundary. Ten total samples from 2 different sites from the Hell's Creek area in Montana were tested. The samples were powdered, heated, and soaked in acids and filtered. The sample solutions were diluted in volumetric flasks and were run on ICP. The results showed that iridium levels ranged from 0-17 ppm in the samples.

10. Investigating Host-Pathogen Interactions using *Caenorhabditis elegans*

Andres Cordova, Patrick Lansdon, and Brian D. Ackley
Department of Molecular Biosciences, University of Kansas, Lawrence, KS

The relationship between host organism and microbial pathogen is complex and not fully understood. On the one hand, the host possesses physical barriers (*e.g.* the epidermis) to prevent microbial colonization and a sophisticated immune system to recognize and respond to pathogens that have bypassed those physical barriers. On the other hand, microbial pathogens possess a suite of virulence factors allowing them to evade the host immune system and colonize the organism. With its anatomical simplicity, rapid maturation time, and short lifespan, *Caenorhabditis elegans* is a great model organism to study the relationship between host and pathogen. Using survival assays we sought to answer two questions: 1) Are there strain-specific differences in host response to pathogens and 2) Does removal of specific virulence factors affect the ability of the host to survive microbial infection. To evaluate these questions, we assessed the survival of the *Caenorhabditis elegans* strains N2 and CB4856 and the *Caenorhabditis briggsae* strain AF16 following infection with the pathogenic microbes *Erwinia amylovora, Enterococcus faecalis,* or *Pseudomonas syringae*. Further, we obtained mutated strains of these bacteria lacking specific virulence factors and tested the survival of the three *Caenorhabditis* strains on these, presumably, less pathogenic microbes. Currently we are expanding our study of *C. elegans* survival to other pathogenic microbes and virulence mechanisms. Ultimately, our research aims to identify host-specific responses to microbial pathogens and how specific virulence factors play a role in the interface between pathogen and host.

11. Making repeat protein topology with non repeating genetic material

Rik Dhar[1], Meghan Franklin[2], Joanna Slusky[1,2]
[1]Department of Molecular Bioscience, [2]Center for Computational Biology, University of Kansas

Outer membrane proteins present as an unusual type of repeat protein. Almost all outer membrane proteins share a topology of repeated sets of antiparallel beta strands curving around a central axis to make a barrel. Although all other known instances of repeat protein topology arise from genetic duplication events, previous work in our lab showed that in outer membrane proteins some sets of antiparallel strands can be formed through the mutation of long loops. This presents as an unusual circumstance of repeated topology but not repeated genetic material. In this study we aim to validate this alternate evolutionary mechanism through an experimental approach. Here we replace the loop of one outer membrane protein with the beta strand hairpin of another outer membrane protein, that were found to align with each other in the sequence alignment study. We expect to see a transition of the 16 stranded barrel PorB from *Neisseria gonorrhea* into an 18 stranded barrel after a loop to hairpin replacement. And also, the transition of 18 stranded barrel OpdH from *Pseudomonas aeruginosa* into a 20 stranded barrel through similar modification. From this we hope to better understand the plausibility and folding mechanisms of this unusual type of repeat protein topology.

12. Enzymatic Tolerance to Sulfide, Lactic Acid, and Ethanol in Corn and Catfish Tissues

Elliott, Jorja E., Naomi C. Quispe, Kalyn D. Compton, Yasuhiro Kobayashi, and Brian R. Maricle
Department of Biological Sciences, Fort Hays State University

The mechanism by which sulfide, ethanol, and lactic acid inhibits cellular respiration has not been characterized. The objective of this study was to examine sulfide, ethanol, and lactic acid toxicity on activities of enzymes of anaerobic (lactate dehydrogenase, LDH) and aerobic (citrate synthase, CS, and cytochrome *c* oxidase, CytOx) metabolism. The activity of respective enzymes was measured in tissue homogenates from corn roots, as well as catfish liver and muscle samples in the presence of increasing concentrations of sulfide (0 to 20 µM), lactic acid (0 to 100 mM), and ethanol (0 to 100 mM). Results indicated that sulfide and lactic acid, but not ethanol, inhibited CytOx, CS, and LDH activities to different degrees. Activity of CS and CytOx in catfish liver samples was 10-fold higher than in corn roots and was 50-fold more sensitive to sulfide. The inhibition constant (K_i) for sulfide on CytOx activity was 0.75 µM in catfish liver and 4.6 µM in corn roots. In contrast, the K_i for lactic acid on CytOx activity in catfish liver and corn roots was approximately 100,000 to 800,000 times higher than that of sulfide (approximately 80 mM and 50 mM, respectively). Compared to sulfide and lactic acid, ethanol did not inhibit the activity of these enzymes at the concentrations tested. Enzymatic activity in catfish is significantly more susceptible to sulfide and lactic acid toxicity compared to corn roots. This indicates environmental and physiological constraints on the metabolism of the organisms examined and offers insight into evolution of eukaryotic respiration.

13. Identifying W-linked sequence reads in the butterfly, *Heliconius melpomene*

Rachel Elting, Dr. James R. Walters
University of Kansas, Department of Ecology & Evolutionary Biology

Sex chromosomes are distinct from the other chromosomes within a genome. In female heterogametic species, males have two Z-chromosomes, while females have a Z- and a W-chromosome. This unequal distribution of sex chromosomes means that selective pressures may cause female-specific genes to evolve on the W-chromosome. Discovering W-linked genes is crucial if we wish to study the genetic components of sex-specific behavior and morphology. However, generating a reference genomic assembly for the W-chromosome with standard “short-read” sequencing technology has long proven difficult due to the repetitiveness of its DNA content. The recent advent of “long-read” sequencing provides great promise for overcoming these challenges. A useful first step in assembling the W-chromosome is to isolate the DNA sequences that originate from it. We aim to discover female-specific long-read sequences in the butterfly *Heliconius melpomene* that can be used to assemble its W-chromosome. This is accomplished by separately aligning many short-read DNA sequences from males and females to each female long-read DNA sequence. The ratio of successfully aligned male and female short-read sequences is called the *Chromosome Quotient* (CQ). This value predicts if the long sequence read originated from the W-chromosome (CQ<<1), the Z-chromosome (CQ=2), or elsewhere within the genome. Results show that the chromosome quotient method has great promise in identifying W-specific sequences in *H. melpomene*. Identification of these sequence reads is a significant step towards the future goal of assembling its W-chromosome.

14. Molecular Changes underlying the Neuronal Deficits in *Specc1l*-deficient mice

Ernst, Nicholas[1*], Jeremy Goering[1*], Luke Wenger[1*], Yomna Badawi[1], Preethi Kunchala[1], Everett G. Hall[1], Nathan R. Wilson[1], Irfan Saadi[1], Hiroshi Nishimune[1].
[1]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS. *Equal contribution.

Pathogenic variants of *SPECC1L* have been identified in patients that exhibit structural and behavioral anomalies, including autism spectrum deficits. SPECC1L is a cytoskeletal scaffolding protein that associates with both actin and microtubules. In this study we used a mouse model with a 200bp deletion in Exon 4 of *Specc1l,* which results in a 510 amino acid truncation of the protein's C-terminal tail (*Specc1l*$^{\Delta C510}$). *Specc1l*$^{\Delta C510/\Delta C510}$ homozygous mutants show perinatal lethality, but heterozygotes (*Specc1*$^{\Delta C510/+}$) survive and show several behavioral deficits. These deficits include severe hyperactivity, decreased marble-burying (object avoidance) as well as abnormal social behavior. To analyze the CNS defects in *Specc1l* mutant mice with behavioral deficits, we analyzed SPECC1L expression in the brain. Immunostaining of wildtype brain tissue showed that SPECC1L is strongly expressed in Purkinje cells of the cerebellum and colocalizes with neurofilament proteins. Hyperactive *Specc1*$^{\Delta C510/+}$ mice show a significantly reduced number of cerebellar Purkinje cells as well as mislocalization of neurofilament expression. Western blotting of embryonic *Specc1l*$^{\Delta C510/\ \Delta C510}$ brain tissues showed an overall reduction in neurofilament expression. Interestingly, co-immunoprecititation analysis showed that mutant truncated SPECC1L-ΔC510 can still physically interact with neurofilaments. This physical interaction with mutant SPECC1L suggests a potential dominant-negative mechanism for neurofilament reduction. We also observed reduction in expression of three proteins in the endocytic pathway, including Clathrin-Heavy Chain, Rab11, and LC3B. This is a novel finding indicating a potential function of SPECC1L in the endocytic pathway. Together, these molecular changes help identify a novel role for SPECC1L in the central nervous system.

15. Purification of *Borrelia burgdorferi* LptD homolog BB-0838

Freitas N, Zueckert W, Huan H, Wiepen J
Department of Microbiology, Molecular Genetics, and Immunology. Kansas University Medical Center

The goal of this project was the purification of antigenically significant polypeptide domains in the *Borrelia burgdorferi* protein transporter subunit LptD, allowing further study of this protein in *Borrelia* cells to be more specific and efficient. *Borrelia burgdorferi,* the primary causative agent of Lyme Disease, is a serious vector borne pathogen. These spirochetal bacteria possess unique morphologies in comparison to Gram-negatives. An important feature of *Borrelia* pathogenicity is the abundance of lipoproteins, this diverse group of proteins attributes all of the necessary functions which allow *Borrelia* to be transmitted from their tick vectors causing infection in humans. An area of study has been the mechanisms of lipoprotein secretion and association with the outer membrane. A mechanism of lipoprotein transport in *Borrelia* is a system homologous to the Lipopolysaccharide Transport System (LPS) in Gram-negative bacteria. This secretion system consists of six individual polypeptides which function in exporting Lipopolysaccharide from the cytoplasm to be anchored to the outer leaflet of the outer membrane. The proteins conserved in the *Borrelia* system are hypothesized to transport lipoproteins in a similar manner. One key homolog protein in this system, LptD, was knocked out using CRSIPR/Cas with the goal of determining its function in *Borrelia* protein secretion. This homolog was determined to act as a lipoprotein flippase: moving secreted proteins from the periplasm to the outer membrane. The purification of this protein will allow further study of *Borrelia* lipoprotein secretion in native *Borrelia* cells, without the need for cloning into a competent *E. coli* strain.

16. Examination of multiple septin-association domains within Bud3 in *S. cerevisiae*

Megan Goeckel, Erianna Basgall, Isabel Lewis, Madison Schrock, Kathrine Leonard, Yao Yan, and Gregory Finnigan
Kansas State University, Department of Biochemistry and Molecular Biophysics

Septins are highly conserved proteins found across many organisms ranging from unicellular members of the fungal kingdom to multicellular metazoans. In budding yeast, septins are required for completion of the cell cycle, including cytokinesis. Septins are GTP-binding proteins which localize to the plasma membrane and form a collar at the "bud neck." There, septins recruit many non-septin proteins – such as Bud3 - which assist information exchange between pathways including the cell cycle and membrane organization. It is unclear how Bud3 is able to directly bind to the septin structure at the bud neck. A previous study identified an amphipathic helix in the central region of the Bud3 protein that is required for membrane association. My research project involved creation and testing of over 150 GFP-tagged Bud3 deletion, fusion, and mutational constructs. I imaged expression of Bud3 with mCherry-tagged septins at the bud neck in living cells to determine if co-localization occurred during the cell cycle. This study has revealed there may be multiple domains within Bud3 that assist in septin recruitment. I have found that a fusion between the central amphipathic helix and an 82 residue-fragment within the C-terminal domain produced septin collar localization. This region also contains a highly conserved motif that has not been previously analyzed. Future work will utilize a split-GFP assay to determine which septin subunit(s) the Bud3 fusion protein interacts with. Understanding how different proteins are able to all bind and coordinate association with the septin filaments during the cell cycle is a critical goal.

17. Pharmacokinetics and bioactivities of tricyclic pyrone molecules in Alzheimer's disease TgF344 rat model

Joshua Habiger,[1] Zhaoyang Ren,[1] Bende Zou,[2] Kelsey Erickson,[3] Zongbo Tong,[1] Huafang Fan,[1] Williams Cao,[2] Conrado Pascual,[2] Izumi Maezawa,[3] Lee-Way Jin,[3] Xinmin Simon Xie,[2] and Duy H. Hua[1]

[1]Department of Chemistry, Kansas State University, Manhattan, KS 66506
[2]AfaSci Research Laboratories, Redwood City, CA 94063
[3]M.I.N.D. Institute, 2805 50th Street, UC Davis Medical Center, Sacramento, CA 95817

Currently available Alzheimer's disease (AD) medications include donepezil, rivastigmine, galantamine and memantine. These drugs modulate neurotransmission and temporarily ameliorate AD symptom, however they do not treat the underlying disease. The pathophysiology of AD may involve an aggregation of amyloid-β peptide (Aβ), which initiates a cascade of molecular changes leading to neurodegeneration. Current strategies include targeting multiple sites in Aβ metabolism, anti-inflammation, and modulation of calcium homeostasis. However, none of them has shown clinical benefit yet. We discovered a class of tricyclic pyrone (TP) molecules and identified several lead molecules, including CP2 and TP70. TP's initially screened for neuroprotection against Aβ oligomer toxicity. TP molecules found to have excellent oral bioavailability and brain penetration in rodents. TP blocked Aβ aggregate formation and preserved memory and motor function without detectable side effects following chronic oral administration in AD-transgenic mice. TP significantly decreased hyperphosphorylated tau (p-tau) as well as soluble and insoluble Aβ in the brain tissue of treated APP/PS1 mice. For drug development, we use TgF344 AD rat model and wide-type rats (control) to investigate pharmacokinetics (PK)/pharmacodynamics (PD), modulation of NMDA-mediated excitatory postsynaptic potentials, glucose levels, water maze test, memory extinction test, and step-through passive avoidance test. TgF344 rats showed significant memory deficiency in water maze test and contexture fear memory after an episode of foot shock. TP treatment improved the long- and short-term memory tests and enhanced the retention of fear conditioning. Moreover, TP treatment in drinking water caused a decrease of 5% body weight and glucose levels in TgF344 rats.

18. Next Generation Sequencing at KU Genome Sequencing Core

Jennifer L. Hackett[1,2,3,4], Erik A. Lundquist[1,2,4], Susan M. Lunte[1,5,6]
[1]Center for Molecular Analysis of Disease Pathways, [2]Genome Sequencing Core, [3]Higuchi Biosciences Center, [4]Department of Molecular Biosciences, [5]Department of Chemistry, [6]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence KS, USA

The Genome Sequencing Core (GSC) is one of three research core labs in the NIH COBRE Center for Molecular Analysis of Disease Pathways (CMADP) at the University of Kansas (KU). The major mission of the GSC is to provide researchers with next-generation sequencing (NGS) technologies. NGS, carried out in a massively parallel fashion, has been revolutionizing bio-medical research and used in a growing list of applications. Projects supported by the GSC include de novo genome assembly, genome re-sequencing for identification of mutations and polymorphisms, transcriptome analysis (RNA-seq), and epigenomic and gene regulation studies such as ChIP-seq, Methyl-seq, and small RNA analysis. The GSC enhances the genomics infrastructure already at KU by providing a range of Illumina sequencing platforms, including the NextSeq 550 (mid-sized genome re-sequencing or transcriptome projects) and the MiSeq (metagenomic or targeted amplicon sequencing projects), to researchers at KU-Lawrence and across the region. To capture the full power of NGS, we provide a range of project support, from experiment consultation, sample quality check, library construction, cluster generation, data generation, to preliminary data analysis. For latest pricing, current job queue, or other info, visit the core's website: https://gsc.ku.edu/.

19. Translational control of Adeno-Associated Virus 2 by eIF5-mimic protein

Madelyn Hilgers[1], Chingakham Ranjit Singh[1], Sarah Gillaspie[1], Mackenzie Thornton[1], Maureen Schick[1], Giovanni Di Pasquale[2], Sandra Afione[2], John A Chiorini[2], and Katsura Asano[1]
[1]Molecular Cellular and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66583
[2]Adeno-Associated Virus Biology Section, NIDCR, NIH, Bethesda, MD20892

Adeno-associated virus 2 (AAV2) is a ssDNA virus and a member of the family Parvoviridae. AAV2 encodes capsid protein, DNA-binding SF3 helicase Rep, and Assembly-Activating Protein (AAP). Capsid proteins are produced as three (VP1, VP2 and VP3) isoforms and Rep six. VP2 and AAP translation is initiated by ACG and CUG codons located within the transcript encoding VP2 and VP3. We report that AAV2 Rep78 isoform interacts with translational regulatory protein, eIF5-mimic protein 1 (5MP1). 5MP1 binds Rep78 through the acidic surface of its C-terminal domain resembling the structure of double-stranded DNA by surface charge distribution.

5MP1 represses translation from non-AUG codons. Thus, we are currently testing the model that Rep78 binding to 5MP1 sequesters 5MP1 away from the ribosome, thereby decreasing initiation accuracy. The initiation frequencies from the firefly luciferase plasmids bearing VP2 ACG or AAP CUG start codon and the 24-nt region preceding each codon are ~10% compared to that from the AUG codon under a typical Kozak context (CCACCAUGG), and are indistinguishable from those from ACG or CUG codon under the same Kozak context. When measured using the luciferase plasmids with a full-length VP2-VP3 mRNA leader region prior to VP3 AUG codon and its luciferase gene in-frame to AAP, VP2 or VP2 and VP3, the initiation frequencies from AAP CUG and VP2 ACG codons are 6% and 19%, compared to combined frequencies from VP2 ACG and VP3 AUG start codons. Based on these and other results, we discuss translational control of AAV2 by 5MP.

20. Cooption of genes for cell-cell adhesion results in multicellularity for colonial alga *Gonium pectorale*

Courtney Hill[1], Berenice Jimenez-Marin[1], Tara Marriage[1], Bradley JSC Olson[1]
[1]Department of Biology, Kansas State University, USA (courtneyhill@ksu.edu)

The transition to multicellularity is one of the major transitions in evolutionary history. However, little is known about how this transition occurred. The Volvocales are useful in studying the transition to multicellularity because they are closely related, show striking similarities in their genomes despite morphological differences, and because they evolved much more recently in evolutionary history than other multicellular organisms. This project aims to better understand the evolution of multicellularity by studying the molecular basis of the initial transition to undifferentiated multicellularity in Gonium through a candidate-gene based approach. Putative cell-cell adhesion factor GpFsl1 differs from its Chlamydomonas orthologs in its expression pattern and shows evidence for positive selection. GpFsl1 is also homologous to a Volvox protein involved in the formation of intercellular bridges. Chlamydomonas gain-of-function (GOF) mutants expressing GpFsl1 have a multicellular phenotype and Gonium loss-of-function (LOF) mutants have a unicellular phenotype. These results suggest that GpFsl1 was coopted from a unicellular ancestor and may be important in the transition to multicellularity.

21. Unraveling RD-p9 bioavailability and biodistribution: attenuating excessive innate immune response to tumor-associated hypoxia

Joshua Lingo,[1] Pavithra Natarajan,[2] Dr. John Tomich,[2] Dr. Sherry D. Fleming[1]
[1]Kansas State University, Division of Biology, [2]Kansas State University, Department of Biochemistry

Cancers are diversifying and increasing in prevalence at an alarming rate. Among the many physiological abnormalities associated with cancer, hypoxia remains an enigma in the scientific community. By creating acidic conditions in the extracellular environment, tumor-associated hypoxia triggers a conformational change in the serum protein B2-Glycoprotein I (B2-GPI). The resulting conformational change allows protein binding to the plasma membrane of hypoxic cells, which induces naturally occurring antibody binding and triggers the complement cascade. The Fleming lab previously showed that peptides from the binding domain of B2-GPI can attenuate the excessive innate immune response to damaged tissues. Through past studies, a retro-inverso sequence made of D-amino acids, called RD-p9, reduced the damage caused by ischemic/reperfusion injuries. Tumor-associated hypoxia resembles ischemic/reperfusion injuries pathologically and immunologically, leading us to hypothesize that RD-p9 may attenuate tumor growth by creating an immunosuppressive innate immune response to hypoxic conditions. Using IEC-18 rat epithelial cells as an *in vitro* model and the B16-F10 syngeneic mouse tumor model, we desired to study the bioavailability and biodistribution of RD-p9. We demonstrated that RD-p9 continued to inhibit the B2-GPI-associated immune response even after incubation in sera for 48 hours at 37℃. Biodistribution will be examined in the future *in vivo* mouse tumor model. Together, these data will show the efficacy and plausibility of RD-p9's use as a therapeutic for tumors and other hypoxia-inducing events.

22. Kinetics of Nucleotide Binding to the gp16 ATPase

Aaron Morgan[1], Christopher Fischer[1], Allen Eastlund[2], Paul Jardine[2]
[1]Department of Physics and Astronomy, University of Kansas,
[2]Department of Diagnostic and Biological Sciences, University of Minnesota

The gp16 ATPase is the constituent subunit of the dsDNA (Double-stranded Deoxyribonucleic Acid) translocation motor of the B. subtilis Φ29 bacteriophage. Although single molecule studies have provided tantalizing clues about the activity of this motor, the mechanism by which the motor couples the energy it obtains from the binding and hydrolysis of ATP to the mechanical work of dsDNA translocation remains unknown. To address this need, we have characterized the binding of MANT-ATP and MANT-ADP to monomeric gp16 using a stopped-flow fluorescence assay. In addition to answering questions about the activity of monomeric gp16, these results are also a necessary step in constructing a model for inter-subunit communication within the pentameric gp16 motor.

23. Sexual dimorphism and housing temperature modulate acute diet-induced weight gain through divergent energy expenditure.

E. Matthew Morris[1*], Roberto D. Noland[1], Julie A. Allen[1], Colin S. McCoin[1], Qing Xia[2], Devin C. Koestler[2], Robin P. Shook[3], John R.B. Lighton[4], Julie A. Christianson[5] and John P. Thyfault[1,6]
Dept. of Molecular & Integrative Physiology[1], University of Kansas Medical Center, Kansas City, Kansas, Dept. of Biostatistics[2], University of Kansas Medical Center, Kansas City, Kansas, Dept. of Pediatrics[3], Children's Mercy Hospital, Kansas City, MO, Sable Systems International[4], North Las Vegas, NV, Dept. of Anatomy & Cell Biology[5], University of Kansas Medical Center, Kansas City, Kansas, Kansas City VA Medical Center-Research Service[6], Kansas City, Missouri.

Objective: Increased physical activity may mediate weight gain through increases in energy expenditure (EE) and reductions in energy balance (EB). Methods for modulating mouse EE (e.g. – exercise, chemical uncouplers, etc.) have confounding effects, however, it is known that mouse EE linearly increases with decreases in housing temperature below the thermoneutral zone (~30ºC). **Methods:** Herein we performed indirect calorimetry experiments in male and female mice at two housing temperatures (20ºC vs. 30ºC) to assess how divergent baseline differences in EE driven by housing divergent housing temperatures impacts 7-day changes in weight and body composition with low-fat (LFD) and high-fat, high-sucrose (HFHS) diets. **Results:** As expected, mice housed at 30ºC have ~40% lower total EE and energy intake compared to 20ºC mice regardless of diet or sex. EB was increased with HFHS in all groups, with ~30% greater increases observed in 20ºC versus 30ºC mice. HFHS increased weight gain regardless of temperature or sex. Interestingly, no HFHS-induced weight gain differences were observed between females at different temperatures; while 30ºC male mice on HFHS gained ~50% more weight than 20ºC males, and ~80% more weight compared to 30ºC females. HFHS increased fat mass (FM) across all groups but 2-fold higher gains occurred in 30ºC mice compared to 20ºC mice, and female had ~35% less FM gain than males at both temperatures. **Conclusions:** Together, these data reveal an interaction between divergent housing induced EE and sex to impact diet-induced short-term weight gain and body composition patterns.

24. Insight into the evolution of excitatory synapses using the larvacean tunicate, *Oikopleura dioica*

Megan Myers[1]; Noraida Martinez-Rivera, Ph.D.[1]; Aidyn Medina-Lopez[1]; Elias Michaelis, Ph.D. [1]; Yana Mikhaleva, Ph.D.[2]; Oleg Tolstenkov, Ph.D. [2]; Joel Glover, Ph.D. [2]; and Eduardo Rosa-Molinar, Ph.D.[1]
[1]University of Kansas, Lawrence, KS and [2]University of Bergen, Bergen, Norway
Abstract:
The goal of this project is to elucidate the nanoscale organization of excitatory synaptic clefts using the larvacean tunicate, *Oikopleura dioica,* as a model to understand the evolution of chordate synapses. The synaptic cleft is an integral compartment of the synapses, yet the nanoscale organization of the cleft of excitatory synapses in vertebrates remains a conundrum. We hypothesize that the expression of exon 5 (ex5) and exon 21(ex21), splice variants of NMDAR1, a subunit of N-methyl-D-aspartate receptor, is critical in establishing the nanoscale organization and dynamics of excitatory synaptic clefts of *O. dioica*. Antibodies against the ex5 and ex21 peptide sequence were developed and used to analyze the clefts of excitatory synapses within the caudal ganglion of *O. dioica*. Ex5 and ex21 antibodies were characterized using cell lines as positive and negative controls, the specificity was confirmed through Western Blot analysis, and optimal antibody dilutions were identified. In addition, a novel post-embedding immuno-labeling workflow that combines automated serial sectioning with automated high-throughput scanning transmission electron microscopy (ssSTEM) was developed and patented. Our findings provide insights into the spatial separation between ex5 and ex21 and advances our understanding of the nanoscale organization of post-synaptic organizing proteins of excitatory synapses cleft of metazoans and, particularly, in the chordate lineage. Research supported in part by NIH (GM-115042; GM-078441; MH-106245), NSF (HRD-1137725), and Kansas INBRE (P20 GM103418).

25. The genetic basis of divergence in immune defense between *Drosophila* species

Mariaelena Nabors, Robert Unckless
Department of Molecular Biosciences, University of Kansas

There is ample evidence for rapid evolution of genes involved in immune defense in many species. However, specific examples that connect genetic and phenotypic divergence between species in the ability to fight diseases are relatively rare. It is presumed that this rapid evolutionary divergence is due to a host-pathogen arms race, but there is little direct evidence that this is true. Our work aimed to understand the molecular basis of divergence in immune defense and determine the extent to which fast evolving genes lead to this phenotypic divergence. To determine phenotypic divergence in immune defense, we performed systemic infections on *Drosophila simulans* and *Drosophila mauritiana,* and measured survival five days post infection. Interestingly, we found the species with greater resistance depended on the pathogen used. To better understand the evolution of immune defense between species, we performed interspecific genetic mapping to determine genomic regions associated with the divergence in immune defense between species. We tested for variation in immune defense within and between *D. mauritiana* and *D. simulans*. We then used a backcross design (hybrid males are infertile) through females to map this divergence in response to both pathogens to the chromosome and performed RNA-seq to identify potential candidate genes. To confirm promising candidate genes' roles in immune defense, we used CRISPR/Cas9 to move alleles from one species to the other. This study on interspecific divergence in immune defense is among the first of its kind and will provide an exciting new approach to comparative immunology.

26. Determining effective levels of antibacterial qualities of Kansas honeys

Nansel-Lantz, Sara C. and Brian R. Maricle
Fort Hays State University Department of Biological Sciences

Multidrug resistance in bacteria is a top threat to global public health, as many antibiotics no longer treat common infections. The necessity for alternatives to modern medicine leads to a re-evaluation of the therapeutic use of ancient remedies like honey. Natural honey has numerous antibacterial properties such as high sugar content, low pH, and glucose oxidase that is responsible for hydrogen peroxide (H_2O_2) production. The enzyme is almost inactive in full strength honey, however, becomes active on dilution. Variations in honey have been widely characterized, potentially due to geographical distribution and different floral sources. The objective of this study is to quantify antibacterial properties of honeys in Kansas based on their different floral sources, dilution strength, and location of hive. Effectiveness of obtained samples are tested on many common human pathogens and assessed by determining the degree of dilution to which a honey retains antibacterial activity, representing sequential dilutions of honey from 5-25%. Currently, 20 different honey samples have been collected from all regions of Kansas. There is vast diversity of honey just from a qualitative standpoint (i.e., color, opaqueness, and fluidity). Preliminary tests show significant differences in the effectiveness of honey based on type and dilution. The darker honey sample showed inhibition at 10% dilution whereas the lighter honey showed an increase of some bacterial growth compared to the control. These results show honey could be a promising antimicrobial treatment. Combining honey with traditional antibiotic treatments could increase effectiveness, accelerate healing processes, and diminish harmful after-effects.

27. Functional MRnS for the rapid detection of zika virus and assessment of cross-reactivity

Raghunath Narayanam, Tyler Shelby, Tuhina Banerjee and Santimukul Santra*
*Department of Chemistry, Pittsburg State University, Pittsburg, KS 66762

Accurate analysis of zika infection remains challenging in clinical settings. Current diagnostic approaches, including zika-RNA detection, have limitations especially in asymptomatic pregnant individuals. Likewise, recommended serological test that detects titers of IgM and IgG antibodies is often compromised in terms of specificity and sensitivity. To overcome these existing hurdles, we explore the new approach of MR technology which sensitively detects zika antibodies and differential antibody responses in ZIKV and DENV infections. In this presentation, we propose a library of customizable MRnS (ZENV/ DIII-ZENV/ NS1-MRnS) for the specific serological diagnosis with simultaneous cross-reactivity determination. The design of new MR nanosensors targeting different zika epitopes (NS1, envelop proteins) makes our detection assay a stand-alone test that can be used for the definitive diagnosis of ZIKV infection. The developed MRnS-based assays will be further applied for the rapid screening of zika's entry receptors associated with its altered tropism.

28. Assessing the Bioremediation Potential of Bacterial Strains Isolated from An Abandoned Coal Mine Following the Whole Genome Sequence Analysis Approach

William Niedens, Brady Steinbock, and Anuradha Ghosh
Department of Biology, Pittsburg State University, Pittsburg, KS

Recent scientific progressions in the field of whole genome sequencing (WGS) have allowed genomic studies to be applied to a multitude of industries, one of the most notable being microbial bioremediation. The abundance or absence of genes in a bacterial species provides insight to determine if it would be a viable candidate for bioremediation purposes. Goals of this study were to retrieve WGS data from ten bacterial isolates from an abandoned coal mine, compare their genome sizes and distribution patterns of selected genes, and determine which bacterial strains are suitable for bioremediation.

Total genomic DNA was isolated using a DNA isolation kit, quantified, and was sequenced using Illumina platform MiSeq. The genome sequences were annotated and analyzed using an array of Linux-based command line programs including concatenation, Trimmomatics, SPAdes, PROKKA, and QUAST.

Identification based on 16S rDNA and IF-2 genes revealed that the strains belonged to the genera of *Arthrobacter, Jeotgalibacillus, Kocuria, Microbacterium, Pantoea, Rhodococcus, Vibrio, Brevibacterium,* and *Paenibacillus.* Genome size ranged from 3.9-7.2 x 10^6 bases and a GC% range was 38-72 among these isolates while six to 19 rRNA molecules and 59-123 tRNA molecules were found. The abundance of the following genes encoding proteins such as oxidoreductase, monooxygenase, dioxygenase, dehydrogenase, transferase, hydrolase, lyase, isomerase, and ligase were also determined. The resistance and metabolic profile of these isolates make them suitable candidates for bioremediation of heavy metals and recalcitrant chemicals and this potential would be further assessed in follow-up studies.

29. Temperature-sensitive screen for cell specification mutants

Bryn O'Meara[1], Meagan Kurland[1], Brian Ackley[1]
[1]University of Kansas Department of Molecular Biosciences

The nervous system requires a high level of organization. In part, this organization is achieved through the generation of multiple cell types during development. Cells acquire their identity through several rounds of cell specification. However, the mechanisms and degree of cell specification is not well understood. We study the VD13 neuron, a GABAergic motor neuron in *Caenorhabditis elegans*, to better understand neuronal cell differentiation.

The canonical Wnt signaling pathway is involved in multiple aspect of neuronal development. In this pathway, Wnt ligands bind to Frizzled receptors, which activate Disheveled proteins, ultimately allowing the transcription factor β-catenin to enter the nucleus. Loss of function in Wnt ligand *lin-44* or in Disheveled *mig-5* results in variable expression of the VD13 specific marker *lhIs97*, while the expression of GABAergic motor neuron marker *juIs97* is maintained.

To identify additional genes in VD13 differentiation, we conducted a genetic screen and recovered 8 mutations with a loss of *lhIs97* without loss of *juIs97*. Notably, the genetic screen was conducted at 25 deg C to potentially isolate temperature sensitive mutants. Temperature sensitive mutants are a powerful tool to determine the time of gene expression during development. Here, we report the expression of the 8 genetic screen mutants at varying temperatures.

30. **Magneto-Plasmonic Nanosensors (MPnS) for the Multiparametric Detection of E. Coli O157:H7**

Nilamben Panchal, Vedant Jain, Tuhina Banerjee, and Santimukul Santra*
Department of Chemistry, Pittsburg State University, Pittsburg, KS 66762.

E. Coli O157:H7 is one of the most deadly foodborne pathogens with a very low cell count (10-100 viable cells) for infection. As of recent estimation from CDC, total of 67 people have been infected with this pathogen across 19 states. Such frequent outbreaks cause economic and health related inconveniences which incur high costs upon the society. Herein, we propose the development of new hybrid nanosensor (MPnS) comprising magnetic resonance imaging (MR) and surface plasmon resonance (SPR) technologies for the rapid and specific detection of this foodborne pathogen to enhance food-safety and biosecurity. New MPnS with featured multi-read out detection strategies (T2 MR, SPR and colorimetry) will enable ultra-sensitive detection at extremely low CFU counts and with wide detection window. Our newly designed platform can overcome a number of existing hurdles including false positives/negatives, extensive sample processing and complexity associated with conventional detection techniques.

31. APC controls Wnt-induced β-catenin destruction complex recruitment in human colonocytes

Taybor W. Parker, Kristi L. Neufeld
Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Wnt signaling is essential for intestinal homeostasis and is aberrantly activated in most colorectal cancers (CRC). In over 80% of CRC, the tumor suppressor Adenomatous Polyposis Coli (APC) is mutated, resulting in expression of a truncated protein product. APC is a key component of the β-catenin destruction complex, which maintains low cellular levels of β-catenin but is inhibited following Wnt ligand presentation. The precise mechanism underlying β-catenin destruction complex inhibition is not clear, nor is the exact role of APC in the complex. APC is primarily considered a core destruction complex component but is known to have other roles involving β-catenin nuclear import/export and cytoskeletal functions. Here, we use Wnt3a beads to study the response of endogenous Wnt components to a local Wnt cue. Using three CRC cell lines, each with a different Wnt pathway status, we demonstrate that localized Wnt redistributes pathway components toward the Wnt source in the presence of full-length, but not truncated APC. Further, use of the Wnt3a-beads to perform protein pull-down demonstrates that APC and β-catenin both associate with the Wnt-beads. APC depletion in nontransformed human colon epithelial cells diminishes this Wnt-induced redistribution. Our results suggest revision of the current model as follows. In response to Wnt, the β-catenin destruction complex: 1) maintains composition and binding to β-catenin, 2) translocates to the plasma membrane, and 3) requires full-length APC for this membrane trafficking. Currently, work is being performed to uncover mechanistic insights into the role of APC in destruction complex reorientation to a Wnt3a signal.

32. The Synthetic Chemical Biology Core (SCB): A Resource for Research in Chemical Biology

Chamani T. Perera[1,2]
[1]Higuchi Bioscience Center, University of Kansas, Lawrence, KS, USA; [2]KU Synthetic Chemical Biology Core Laboratory, University of Kansas, Lawrence, KS, USA

The Synthetic Chemical Biology Core strives to provide comprehensive synthetic chemistry capabilities to investigators under one roof. The synthetic expertise of the core includes, but is not limited to, novel and commercially unavailable small molecules, fluorescent molecules and peptides. The core assists in identifying hits for medicinal chemistry optimization in infectious disease targets and provides synthesis capabilities for structure activity studies of said hits. The core staff will work with investigators to design and synthesis novel molecular probes to facilitate their research. SCB core encompasses the Purification and Analysis Laboratory (PAL) that provides purification, analysis and quality control of compounds via HPLC-MS. The core utilizes automated mass directed fractionation for purification in both reversed and normal phases (including chiral separations), and also provides relative purity analysis by UPLC coupled to a high-resolution mass spectrometer for structure confirmation.

33. The RNA Binding Protein HuR Regulates Exosome Secretion in Colorectal Cancer via Rab 27B and can Serve as a Potential Biomarker.

Ranjan Preet[1], Vikalp Vishwakarma[1], Wei-Ting Hung[2], Lane K. Christenson[2] and Dan A. Dixon[1]
[1]Departments of Molecular Biology, University of Kansas, Lawrence, KS 66045 and [2]Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160

Enhanced secretion of exosomes by cancer cells is recognized as a means of transferring specific RNA and protein cargo to recipient cells, and is promising blood-based cancer biomarkers. We have established that colorectal cancer (CRC) cells and tumors overexpress the RNA-binding protein HuR (ELAVL1) early in GI tumor development. When overexpressed, HuR can promote mRNA stabilization of tumor-promoting genes through binding of 3'UTR AU-rich elements (ARE). These same mRNAs are within tumor-derived exosomes, suggesting role for HuR in RNA trafficking. To test this, exosome levels from CRC cells that endogenously overexpress HuR, were compared to normal human intestinal epithelial and myofibroblast cells. CRC cells secrete ~3-fold greater exosome levels than normal cells Furthermore, HuR was detected in exosomes produced *only* from HuR-overexpressing cells. It has also been investigated that HuR regulates exosomes secretion via Rab27B in CRC cells. These findings were reflected *in vivo* where GI-tumor bearing $APC^{Min/+}$ mice produced ~3-fold more serum exosomes, with HuR as exosome cargo in $APC^{Min/+}$ mice compared to wild-type mice. Organoids derived from $APC^{Min/+}$ mice adenomas showed ~3-fold higher number of exosomes released as compared to normal intestinal tissues. ELISA showed significantly higher HuR in serum exosomes derived from $APC^{Min/+}$ mice that correlates with increasing tumor burden. Plasma derived exosomes from CRC patients (Stage I and II) showed significant increase in exosomal HuR content as compared to control. This work has identified a novel connection between HuR-mediated post-transcriptional regulation and tumor-derived exosome secretion through Rab27B, along with providing preclinical and clinical evidence of exosomal HuR as a blood-based CRC biomarker.

34. Investigating Polymorphic Mitochondrial tRNA-Derived Fragments (mt-tRF) as Mitochondrial Signaling Molecules

Scheid, Adam D.[1], Carolyn J. Vivian[1], Victoria Marshall[1], Griffin Welfer[1], Connor Thellman[1], Jonas Rowland[1], Thomas C. Beadnell[1], Yi Jing[2], Isidore Rigoutsos[2], Danny R. Welch[1]
[1]Department of Cancer Biology, University of Kansas Medical Center, Kansas City, KS
[2]Computational Medicine Center, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA

Associations between mitochondrial DNA (mtDNA) variants and cancer have been reported, but underlying mechanisms are poorly understood. Crosstalk between nuclear DNA (nDNA) and mtDNA and mtDNA genetic engineering challenges have stymied efforts to elucidate direct mtDNA contributions to cancer. To address this we generated mitochondrial nuclear exchange (MNX) mice, in which mtDNA from various mouse strains is paired with a common nDNA background. MNX mice exhibit alterations in traits including cancer metastasis as well as transcriptomic and epigenetic profiles. Signals mediating mtDNA modulation of biological traits in MNX mice have yet to be discovered. Selective alterations in transcriptomic and epigenetic profiles among MNX mice suggest they may be regulated by sequence-specific signals. Accordingly, the only non-coding mtDNA polymorphisms among MNX mice exist in mt-tRNA^{Arg}. Given tRNA-derived fragment (tRF) functional versatility, we hypothesize that polymorphic mitochondrial tRF (mt-tRF) underlie phenotypic variability among MNX mice. tRF are a class of small non-coding RNA that derive from nuclear and mitochondrial tRNA. Mitochondrial and polymorphic tRF are each understudied. Northern blotting and sequencing demonstrate that 2 polymorphic mt-tRF - mt-tRF-RL3 and mt-tRF-Ri - are generated from mt-tRNA^{Arg} among MNX mice. Northern blotting also suggests that mt-tRF-Ri is differentially expressed among MNX mice. Current efforts focus on quantifying mt-tRF-RL3/mt-tRF-Ri among MNX mice, characterizing mt-tRF-RL3/mt-tRF-Ri biochemical characteristics, and identifying mt-tRF-RL3/mt-tRF-Ri intermolecular interactions. Future studies will query role(s) of polymorphic mt-tRF in cancer metastasis and other traits. Overall, our findings demonstrate the existence of polymorphic mt-tRF and establish their candidacy as contributors to metastasis and other traits.

35. Generating Cell Lines Expressing Vaccinia Virus Decapping Enzymes

Joshua Spradlin[1], Fernando Cantu[2], Zhilong Yang[2]
[1 2] Division of Biology, University of Kansas State

Poxviruses have significant impacts on public health with many currently being endemic and causing considerable diseases and mortality in humans and economically important animals. Poxviruses are also being developed as a vaccine vector and for treating cancers. Vaccinia virus (VACV) is the prototype poxvirus that was used as the smallpox vaccine to eradicate smallpox. Today, VACV is being actively developed as an oncolytic agent that can potentially treat many types of cancers. Oncolytic virus therapy focuses on viruses that can infect and kill cancer cells, but not normal cells. VACV decapping enzymes (D9 and D10) that can remove the 5' cap structure of mRNA, which leads to faster mRNA degradation. These proteins play an important role in the replication of VACV. These decapping enzymes can be potential targets for VACV genetic enginnering to limit cancer cell growth. The objective of this project is to create two cell lines that inducibley express D9 and D10. This project will use Clontech's Tet-Off expression system for this purpose. In this system, gene expression is turned on when tetracycline or doxycycline is removed from the culture medium. The purpose of these two cell lines is that they will be valuable in further understanding of the functions of D9 and D10. The outcome of the project will facilitate understanding of the functions of decapping enzymes.

36. Separation of inositol hexakisphosphate stereoisomers

Mingjing Sun
Department of Physical Sciences, Emporia State University

Inositol phosphates (IPx) play a critical role in cellular metabolism, with functions in many diverse aspects of cell biology, such as regulating cytoskeletal structure, ion channels and pumps, membrane dynamics and nuclear signaling. As the most abundant inositol phosphate form in nature, myo-IPx contains 63 possible inositol phosphate stereo-and regio-isomers due to the position and numbers of phosphate groups on the inositol ring. There are other stereoisomers of inositol phosphate found in natural environment and animal tissue, including scyllo, D-chiro and neo- IPx. Different IPx isomers show different physiological functions, even for IPx enantiomers. The enantiomers are chemically equivalent, but they behave selective in a chiral biochemical reaction. For example, enzyme can preferentially react with one IPx enantiomers and can have profound biological effects. Understanding of the structural, stereochemical and transformation of inositol and its phosphorous derivatives in cells and biological samples is essential to investigate their biological aspects. The challenge is the separation and detection of IPx due a large number of sophisticated stereochemical, prochiral, chiral, and conformational issues associated. Another challenge is that those compounds are difficult to detect by conventional unltraviolet-visible spectroscopy because they contain no chromophoric groups. In this study, different stereoisomers of IP6 were separated by ion chromatography and derivatized with Fe3+ and detection by UV detector. Furthermore, different dephosphorylation isomer from each stereoisomers were also separated an identified with liquid chromatography and mass spectrometry.

37. Larval *Drosophila* Trachea as Model for Post-embryonic Tissue-specific Allometric Growth

Spencer Tye, Jackie Dyke, Kistie Brunsell, Robert Ward
Department of Molecular Biosciences, University of Kansas, Lawrence, KS

In humans and many animals, post-embryonic development is achieved through allometric growth. Allometric growth is characterized by organs and tissues that grow at different rates relative to each other. In other words, Allometric growth is tissue-specific, and while it is known that the growth of each organ or tissue is dependent on its function in development and homeostasis, the mechanisms that control this growth are poorly understood. In order to elucidate tissue-specific growth mechanisms, we are using the larval trachea in *Drosophila melanogaster* as a model tissue. The genes *uninflatable* (*uif*) and *Matrix metalloproteinase 1* (*Mmp1*) have been identified as tissue-specific growth regulators of the larval trachea. Larvae with homozygous mutations in these genes have trachea that are about half the relative size of those found in wild type animals. To identify additional genes involved in larval trachea growth, we screened through a collection of EMS-induced larval lethal mutations, and identified seven mutants that have an abnormal ratio of trachea to body length in third larval instars. Three of the mutants show reduced tracheal growth similar to mutations in *uif* and *Mmp1*, whereas the remaining four mutant lines show increased tracheal growth. We are currently characterizing the terminal phenotypes of third instar larvae such as tracheal length relative to body length with brightfield microscopy and cellular phenotypes such as cell size, shape, and endoreplication via antibody staining associated with these mutations in order to uncover molecular mechanisms associated with tracheal-specific growth to better understand allometric growth in all organisms.

38. Quantitative Analysis of hnRNPC Transcripts in Normal and Cancer Uterine Tissues

Sarah Veesart and James McAfee
Department of Chemistry
Pittsburg State University

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are highly abundant proteins that saturate pre-mRNA immediately upon transcription, and are linked to numerous functions associated with mRNA biogenesis. The hnRNPC family, which consist of hnRNPC1, C2, hRaly, and hRaly, are by far the most abundant in this group, with nuclear concentrations rivaling histone levels. The hnRNPC family has been implicated in a wide array of RNA metabolic activities as well as playing a role in cancer biology. Using RNA dot blots containing multiple paired mRNA samples from normal and cancer cells, we have previously demonstrated a more than 10-fold enhancement of hnRNPC and hRaly mRNA in uterine cancer cells compared to their normal counterpart. To more rigorously investigate this observation, we report here the analysis of hnRNPC, hRaly, and hRalyl expression in normal and cancer uterine tissues using real time PCR.

39. K-INBRE Communications Core: Activities & Evaluation

Sarah E. Velasquez, MAB, MS, MLS
University of Kansas Medical Center

Background: The Kansas IDeA Network of Biomedical Research Excellence (K-INBRE) Communications Core aims to expand and advance biosciences education and research among Kansas (and Oklahoma) science researchers, educators, and students.
Method: With partner institutions and participants dispersed across Kansas and Oklahoma, the Communications Core has the complex role of: 1) evaluating the effectiveness of programmatic and collaborative activities for continued improvement and scholarship of the program; 2) enhancing operational and programmatic communications; 3) highlighting the role and accomplishments of K-INBRE on bioscience education, research, faculty, and students engaged in the project; and 4) advancing and leveraging the K-INBRE infrastructure.
Results: Continued assessment and evaluation of the K-INBRE, coupled with changes in technology, has illuminated unique opportunities for communications and professional development activities for the program.
Discussion: This poster will provide details on ongoing Communications Core activities.

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40. Design of the ATCUN Motif in Antimicrobial Metallopeptides

Katie Zimmerman[1,2], Kyle Boone Ph.D.[2,3], Candan Tamerler Ph.D.[2,3]
Department of Molecular Biosciences[1], Institute for Bioengineering Research[2],
Department of Mechanical Engineering[3], University of Kansas

Pathogen drug-resistance is a growing health problem leading medicine back to the pre-antibiotic era. The design of new antimicrobial peptides can address this issue through combining peptide functionalities. Metallopeptides, peptides that incorporate metals in their activities, gained increased attention in literature because of their uses in the generation and degradation of oxidative species. Amino terminal copper and nickel (ATCUN) motifs are known divalent metal-binding sites, such as a copper, which lead to reactive oxygen species (ROS) generation. These species signal adaptive and innate immune system responses. In addition, ROS reduces viability of DNA in bacterial cells targeted by antimicrobial peptides. This leads to reduced drug resistance by limiting the transmission of genetic material following treatment. However, no study has designed an approach for tuning the interfaces between peptides and divalent metal ions to combine metal-based activities with other activities. Combining ATCUN motifs with antimicrobial peptides is an opportunity to address drug resistance by reducing the viability of parental genetic material following antibiotic treatment. We have developed a method for estimating the copper binding of peptides from methods that estimate copper binding in proteins. We have further identified sequence features specific to antimicrobial peptides in the desired copper-binding sites through a rough set theory method. Then, we rank antimicrobial peptides by their copper-binding score to find candidates for ROS-generation studies. A ROS-generation prediction model of antimicrobial peptides built from ROS-generation studies enables the discovery of novel antimicrobial peptides with targeted ROS-generation properties.

41. MALATI, A Triple -Stranded Cancerous RNA, a Triple Threat, Needs a Triple Solution

Asauskas Ryan and Zegar Irene
Department of Chemistry Pittsburg State University

Long non-coding RNA molecules (lncRNA) are transcripts of more than 200 nucleotides in length. Up to date, there has been about 35,000 lncRNA identified, which is believed to be an underestimate as this class of RNAs makes up 98% of the noncoding transcriptome. Although very few lncRNAs have been characterized in detail, it is clear that they are important regulators of gene expression. It is believed that lncRNAs may carry out both gene inhibition and gene activation through a range of diverse mechanisms. Moreover, lncRNAs have been found to play important roles in the development and pathophysiology of a number of diseases including cancer. In fact, key oncogenes and tumor suppressors are now known to be regulated by lncRNAs. In addition, lncRNAs are known to have secondary structures that play key roles in their functions. Therefore, lncRNAs now represent a major group of biomolecules for drug targeting research. MALAT1, a 9000- nucleotide, highly abundant, nuclear lncRNA found has been associated with the regulations of the expression of metastasis-associated genes. This RNA is thermally stable with a half-life of about 29 days. Its stability has been associated with its role in cancer. The RNA's stability is be due to its 3'-end region that has a unique triple stranded structure that consists. In this work, we will examine the effect of potential anti-cancer agents on the stability of the triple-stranded region of MALAT1 in an effort to hinder its role in maintaining the vitality of metastasized cancer cells.

42. Spx and YjbIH mediate virulence factor production in *Staphylococcus aureus*

Crystal M. Austin[1], Siamak Garabaglu[2], Miranda J. Ridder[1], Mary A. Markiewicz[1], Jeffrey M. Boyd[2] and Jeffrey L. Bose[1]
[1]Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS; [2]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ

To cause disease, *Staphylococcus aureus* relies on its ability to precisely fine-tune virulence factor expression. During an unbiased transposon mutant screen, we observed that disruption of the two-gene operon, *yjbIH*, resulted in decreased pigmentation and aureolysin (Aur) activity. Further analysis revealed that YjbH, a predicted oxidoreductase, is mostly responsible for the observed *yjbIH* mutant phenotypes, though a minor role exists for the putative truncated hemoglobin YjbI. Using reporter plasmids, we found that the decreased pigment and Aur activity phenotypes were due to significantly decreased activity of the *crtOPQMN* and *aur* promoters. Previous studies found that YjbH targets the disulfide- and oxidative-stress responsive regulator Spx for degradation. The absence of *yjbIH* resulted in altered sensitivities to nitrosative and oxidative stress, iron deprivation, and aconitase activity. Decreased pigmentation and Aur activity in the *yjbH* mutant was found to be Spx-dependent and to involve the alternative sigma factor, σ^B. Lastly, we used a murine model to determine the effect of the *yjbIH* deletion on pathogenesis. These studies identify changes in pigmentation and protease activity in response to YjbIH and are the first to show a role for these proteins during infection.

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43. Assessing the Timeframe for Formation of the Phi-clamp in Protective Antigen

Meron T. Ayalew, James G. Bann
Department of Biological Sciences, Department of Chemistry, Wichita State University

The aim of this experiment is to provide a more detailed thermodynamic understanding of the timeframe needed to obtain the phi-clamp structure (ring of Phe427) that is viewed when the protein known as protective antigen (PA), a crucial unit involved in the toxin nature of anthrax, undergoes structural changes due to a drop of pH and eventual formation of a pore structure. Analysis of this phi-clamp structure may provide a deeper understanding of the full-time course of molecular changes that occur along the pathway to the formation of the pore state, which is an essential step in getting the toxic components of anthrax into a cell.
The hypothesis for the experiment is that the formation of the phi-clamp occurs at a later stage within pore maturation and that the structure's formation is preceded by the movement of the second domain within the pre-pore structure away from domain 4. To directly follow these two processes, we are taking a fluorescent based approach, which utilizes the known quenching of bimane (a suitable florescent probe) by tryptophan and measure it as a function of pH. The bimane will be utilized after making a F427C mutant of the regular PA protein and labeling the probe at the cysteine residue, forming F427C-Bimane. Our expectation is that as the pH is lowered and transition to the pore state occurs, the bimane fluorescence will be significantly quenched. Comparative kinetic experiments will also be done on the labeled protein as a function of pH from 8 to 7.

44. Classifying Benign and Malignant Melanomas in Genetically Engineered Mouse Models using a Radio Frequency (RF) Resonator.

Subash Bhandari[1], Bernardo Villafana[1], Kim, Cluff[1]
[1]Department of Biomedical Engineering, Wichita State University, Wichita, KS

Background: Melanoma cancer is an aggressive type of skin cancer. Current methods for diagnosis rely on visual inspections, which are highly subjective and depend on the physician's ability. The objective of this study was to investigate the difference in dielectric properties associated with malignant, benign and healthy skin tissues using Genetically Engineered Mouse Models (GEMMs) and develop a novel method for skin cancer diagnosis.
Methods: An electromagnetic resonant sensor was fabricated as a square planar spiral to be placed on the GEMM models to collect data using a vector network analyzer. Our model consists of one female *Braf*^{tm1Mmcm}*, Pten*^{tm1Hwu}*,* Tg(Tyr-cre/ERT2)13Bos and one *Braf*^{tm1Mmcm}*, Pten*^{tm1Hwu}-only Transgenic JAX® mouse.
Results: A mathematical simulation was done using FEKO electromagnetic simulation software to study the interaction of electromagnetic waves with benign and malignant skin cancer types. The simulation frequency was a resonance at 272.241 MHz.
Discussion: The simulation frequency was selected based on preliminary studies conducted on benign and malignant oil-in-gelatin phantoms. The mathematical simulation was used in order to quantify the sensor design to be placed on the mice models. The results obtained from preliminary phantom study and mathematical simulation will be used to design RF resonator properties to harness the interaction of electromagnetic waves with benign and malignant moles in the GEMM models. These changes in resonant frequency as a result of change in dielectric properties between normal cells and melanoma could be used to screen melanoma with a novel biosensor.
Acknowledgements: Institutional Development Award (IDeA): P20 GM103418

45. Colicin E1 Fragments Potentiate Antibiotics by Plugging TolC

S. Jimmy Budiardjo[1], Jacqueline J. Deay[2], Anna L. Calkins[3], Virangika K. Wimalasena[2], Daniel Montezano[2], Julie S. Biteen[3] and Joanna S.G. Slusky[1,2]
[1]Center for Computational Biology, The University of Kansas, [2]Department of Molecular Biosciences, The University of Kansas, [3]Department of Chemistry, University of Michigan

The double membrane architecture of Gram-negative bacteria forms a barrier that is effectively impermeable to extracellular threats. Accordingly, researchers have shown increasing interest in developing antibiotics that target the accessible, surface-exposed proteins embedded in the outer membrane. TolC forms the outer membrane channel of an antibiotic efflux pump in Escherichia coli. Drawing from prior observations that colicin E1, a toxin produced by and lethal to E. coli, can bind to the TolC channel, we investigate the capacity of colicin E1 fragments to 'plug' TolC and inhibit its efflux function. First, using single-molecule fluorescence, we show that colicin E1 fragments that do not include the cytotoxic domain localize at the cell surface. Next, using real-time efflux measurements and minimum inhibitory concentration assays, we show that exposure of wild-type E. coli to fragments of colicin E1 indeed disrupts TolC efflux and heightens bacterial susceptibility to four common classes of antibiotics. This work demonstrates that extracellular plugging of outer membrane transporters can serve as a novel method to increase antibiotic susceptibility. In addition to the utility of these protein fragments as starting points for the development of novel antibiotic potentiators, the variety of outer membrane protein colicin binding partners provides an array of options that would allow our method to be used to inhibit other outer membrane protein functions.

46. Effect of Single Point Mutations in the Mobile Loop of Lactate Dehydrogenase

Osiel Cecenas, Moriah R. Beck
Department of Chemistry, Wichita State University

Lactate Dehydrogenase (LDH) catalyzes the reversible conversion between pyruvate to lactate and NADH to NAD^+. This study aims to explore the role of single point mutations on the non-conserved regions of mobile loops in the enzyme near the active site. These loops contain amino acids that directly interact with the active site to stabilize the incoming substrate by altering the conformation. In a similar experiment with malate dehydrogenase, a conserved residue of the mobile loop was mutated yielding a significantly decreased affinity for oxaloacetate and increased thermal inactivation. We hypothesize that non-conserved residues in this mobile loop are able to modify the thermal stability of the protein without significantly altering its enzymatic activity. The LDH protein sequence was compared with a variety of other species to determine variances in the amino acid sequence of non-conserved regions on the mobile loop. The loop sequence of the extremophile Tardigrade contained an aspartate instead of serine at residue 111. In efforts to observe differences in thermal stability and enzymatic function, tardigrade LDH was mutated to serine (D111S). The enzymatic activity of Tardigrade LDH was measured by monitoring production of NADH spectrophometrically. Circular Dichroism spectroscopy monitored the protein's secondary structure resiliency using a variable-temperature study. These experiments revealed a slight decrease of thermal inactivation and enzymatic activity on the D111S LDH, when compared to the wild-type. Future experiments will explore further mutation sites on the mobile loop's non-conserved residues to investigate the role of single point mutations in protein function and thermal stability.

47. ESR2 regulation of primordial follicle preservation

V. Praveen Chakravarthi,[1] Subhra Ghosh,[1] Katherine F. Roby,[2,3] and M. A. Karim Rumi[1,3]
[1]Department of Pathology and Laboratory Medicine, [2]Department of Anatomy and Cell Biology, [3]Institute for Reproduction and Perinatal Research, University of Kansas Medical Center, Kansas City, Kansas, USA

Primordial follicles serve as the ovarian reserve. Molecular mechanisms regulating the maintenance of dormancy and the steady activation of primordial follicles remain largely unknown. We recently observed that estrogen receptor β (ESR2) plays a gatekeeping role during the recruitment of primordial follicles. An increased number of primordial follicles develop into primary follicles in *Esr2*-null (*Esr2-/-*) rats. However, follicle assembly was not affected, and the total follicle counts remained intact. Primordial follicle activation was also increased in *Esr2*-mutants lacking the DNA-binding domain suggesting a role for the transcriptional activation function of ESR2 in maintaining the dormancy. Disruption of ESR2-signaling with a selective ESR2-antagonist increased the number of activated follicles in wildtype rats, whereas a selective ESR2-agonist deceased the activation. In contrast, primordial follicle activation was not increased in the absence of ESR1, which indicates that the regulation of primordial follicle activation is ESR2-specific. An excessive recruitment of primordial follicles in *Esr2-/-* rats resulted in loss of their follicle reserve and premature ovarian senescence associated with reduced levels of serum AMH and estradiol. Finally, a candidate approach was employed to elucidate the underlying molecular mechanisms. We detected an abundant expression of ESR2 in primordial follicles suggesting a potential regulatory role within the follicles. Disruption of ESR2-signaling markedly augmented the activation of AKT and mTOR pathways. Our results demonstrate that the lack of ESR2 upregulated the expression of *Npm2, Aldh1a7,* and *Adcyap1r1* in *Esr2-/-* ovary, which might have facilitated the PI3K and AKT activation resulting in increased primordial follicle activation.

48. Identification of Ingredients in CBD Oil Sold in Emporia Through Analytical Techniques

Ashley Clifton and Kim Simons
Department of Chemistry, Emporia State University. Emporia, KS.

Cannabidiol (CBD) is one of the most prevalent compounds extracted from the Cannabis plants. The regulatory status of Cannabidiol products in the United States is still evolving. Loose guidelines are set for manufacturers when distributing CBD, for example, using non-psychoactive hemp and a limit of 0.3% Tetrahydrocannabinol (THC) within the product. THC is the psychoactive ingredient within Cannabis plants that produces a high and is federally illegal. Kansas's guidelines for the dispense of CBD products is for the products to contain less than 0.3% and to disclose the levels of THC and CBD on the bottle. However, there is no standardized procedure to analyze the ingredients or CBD/THC concentrations in CBD products. By performing instrumental analysis of CBD oil sold in Emporia, KS. through fluorescent spectroscopy, UV-VIS spectroscopy, IR spectroscopy, and Gas Chromatography with Flame Ion Detection (GC-FID), the ingredients can be determined. The goal of this research is to compare the ingredients displayed on CBD products to the analyzed ingredients shown through instrumental analysis. By analyzing which brands are reputable, consumers of Emporia will be able to make more educated decisions on their CBD purchasing.

49. Investigating primary cilia structure in Autosomal Dominant Polycystic Kidney Disease

Joseph T Cornelius[1], Luciane M Silva[1], Wei Wang[1], Tana S Pottorf[1], Darren P Wallace[2], Pamela V Tran[1]
[1]Department of Anatomy and Cell Biology, Kidney Institute, University of Kansas Medical Center, Kansas City, KS
[2]Department of Integrative and Molecular Physiology, Kidney Institute, University of Kansas Medical Center, Kansas City, KS

The primary cilium is a small, antenna-like sensory organelle that protrudes from most mammalian cells and mediates signaling pathways. Cilia are built by intraflagellar transport (IFT) multiprotein complexes B and A, which have separate roles. Dysfunction of IFT and cilia results in ciliopathies, disease syndromes which commonly cause renal cystic disease. Intriguingly, in mice, deletion of *Ift-B* genes, which causes loss of primary cilia, can markedly attenuate Autosomal Dominant Polycystic Kidney Disease (ADPKD), the most common renal cystic disease and a leading cause of renal failure. These data indicate that targeting primary cilia genes can have immense therapeutic potential in ADPKD through mechanisms that remain elusive. To help elucidate these mechanisms, we ablated an *Ift-A* gene, *Thm1*, in an ADPKD mouse model. Deletion of *Thm1* together with *Pkd1*, the most commonly mutated gene in ADPKD, dramatically attenuated the cystic disease, and cysts originating from the collecting duct and loop of Henle were reduced in size. While loss of *Thm1* results in shortened cilia, renal primary cilia were lengthened in *Pkd1* knockout mice, but normalized in *Pkd1;Thm1* double knock-out mice. Further, we observed longer cilia in kidney sections of human ADPKD than in those of normal human kidney, revealing that increased cilia length is a feature also of the human disease. We propose that THM1 regulation of cilia length, and in turn, of downstream ciliary-mediated signaling molecules, contributes to renal cystogenesis in ADPKD. Understanding the relationship between cilia length and regulation of signaling molecules may reveal potential therapeutic targets.

50. Investigating the impact of exercise on hepatic mitophagy utilizing the autophagy inhibitor leupeptin.

Edziu Franczak[1], Colin S. McCoin[1], Kelly N.Z. Fuller[1], Adrianna Maurer[1], Kevin Schwartze[1], Julie Allen[1], John P. Thyfault[1].
[1]Dept. of Molecular & Integrative Physiology, University of Kansas Medical Center

Autophagy is a highly conserved process that sequesters cellular material into autophagosomes for degradation/recycling. Mitochondrial autophagy, or mitophagy, specifically regulates mitochondrial turnover essential for removal of damaged or improperly functioning mitochondria. Impairment of mitophagy causes accumulation of dysfunctional mitochondria and increases susceptibility for hepatic steatosis. Mitophagy is putatively activated by exercise but this has yet to be directly examined in the liver. Leupeptin, a cysteine, serine and threonine protease inhibitor that blocks lysosomal activity, elicits the accumulation of mitophagy markers microtubule-associate protein 1A/B light chain (LC3-II) and sequestosome 1 (p62), thus allowing the measurement of mitophagy flux. LC3-II is crucial for autophagosome formation and p62 is an adapter protein that aggregates ubiquitinated proteins to autophagosomes. First, to understand the effective working time of leupeptin, we designed a time course examining 30-minute, 1, 2, and 4 hour time points post injection. Results indicate that leupeptin inhibits mitophagy in as early as 30 minutes and remains viable at least up to 4 hours post injection. A separate cohort of mice received leupeptin injections and then 30 minutes later performed 1 h treadmill exercise or remained sedentary. Immediately after exercise and 2 hours post, mitochondria were isolated from the liver and examined for LC3-II and p62. Our results suggest that acute exercise has no impact on LC3-II however p62 appears to be elevated 2 hours post exercise in the presence of leupeptin, suggesting greater hepatic mitophagy flux following exercise.

51. Equipment and Services of the Ralph N. Adams COBRE Core Nanofabrication Facility

Ryan Grigsby[1] and Susan M. Lunte[1,2,3,4]
[1]The Center for Molecular Analysis of Disease Pathways, [2]The Ralph N. Adams Institute for Bioanalytical Chemistry, [3]Department of Pharmaceutical Chemistry, [4]Department of Chemistry, University of Kansas

The Adams Nanofabrication Core Lab, is a core lab within the Kansas University Nanofabrication Cleanroom Facility, and is supported by the Center for Molecular Analysis of Disease Pathways COBRE. The Adams Nanofab primarily caters to researchers who are manufacturing micro- and nanofluidic devices for biomedical research, but has the equipment and resources to accommodate broad research applications with micro- and nanofabrication needs. The core lab consists of about 1,300 ft^2 of ISO class 5, 1,700 ft^2 of ISO class 6 and 1,250 ft^2 of ISO class 7 cleanroom space, housing tools and materials for techniques including photolithography, nano-imprint lithography, plasma (dry) etching (ICP-RIE), wet etching, metal and dielectric material thin film deposition, ellipsometry, profilometry, wafer dicing, laser ablation and engraving, 3D printing, hot embossing, and COMSOL software for device modeling. In addition, the facility has numerous microscopes for general inspection, ovens and furnaces, ultrapure water, dedicated process fume hoods and filtered lighting for photolithography.
This facility is under the direction of Dr. Susan Lunte. Services and usage of the facility are available to researchers from all Kansas universities. Training is provided to new investigators and graduate students in the use of micro- and nanofabrication procedures and equipment. In addition, researchers from both non-Kansas academic and private industry institutions may contract with the facility for consultation and services. Hourly and per-use rates apply for facility access, equipment usage, and staff labor. Consultation is free.

52. Multi-Disc Voltage Analysis of Piezoelectric Composite Materials with Compliant Layers

Evan Haas[1,3], Ember Krech[2], Elizabeth Friis[1,2]
University of Kansas, Department of Mechanical Engineering[1], Bioengineering Graduate Program[2], K-INBRE Research Program[3], Lawrence, Kansas;

Previous research has shown that tough piezoelectric composites used inside orthopedic implants enhance bone integration and healing. The goal of this research is to understand composite piezoelectric stack generators to inform implant design decisions and understand effects on overall voltage output. The addition of compliant layers between piezoelectric discs (i.e., Compliant Layer Adaptive Composite Stacks or CLACS) in piezoelectric generators stacked mechanically in series and wired electrically in parallel significantly increased power output for a given PZT volume. This study was designed to elucidate the effects of individual discs within a CLACS stack over load and frequency ranges used in biomedical applications to optimize power generation and design.

New techniques of measurement and analysis of the voltage generation of individual discs in the CLACS were developed. These new techniques control for the capacitance of each PTZ disc, top and bottom encapsulation thickness, disc alignment, and allow for individual electrical connections to the discs. Specimens were dynamically tested under load-control at frequencies of 1, 2, 3 and 5 Hz through a sweep of resistances. Voltage data was acquired and analyzed using MATLAB. Data gathered was analyzed to track individual disc outputs during testing to compare maximum output voltages for each disc within stacks. Percentages of total voltage output from the stack were calculated for each disc as well as variation of each disc from the mean output voltage for each resistance, load, and frequency. Preliminary results suggest that there is a relationship between position and output, further data will be presented.

53. **Synthesis of molecular probes that accumulate in the endoplasmic reticulum of living eukaryotic cells**

Arman A. Hadjian, Digamber Rane, and Blake R. Peterson
Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045

The endoplasmic reticulum (ER) is the second largest organelle of eukaryotic cells. This organelle serves as a key site for cellular protein and lipid synthesis. The tremendous surface area of membranes of this organelle, ~30 times greater than of the plasma membrane, makes it an attractive target for developing molecular probes and cellular sensors. The laboratory of Blake R. Peterson has synthesized numerous novel green fluorescent compounds derived from fluorinated rhodols that accumulate in this organelle. However, it is not clear as to why these fluorinated rhodols preferentially localize in the ER. We are investigating the potential involvement of polyunsaturated fatty acids in this process. These unsaturated acids are key lipid components of ER membranes, and we hypothesize that they may participate in pi-pi interactions with electron-deficient aromatic rings of fluorinated rhodols to promote the selective localization of these molecules in membranes of the ER. To test this concept, we propose to synthesize three fluorescent probes based on a non-fluorinated rhodol fluorophore. The proposed probes will have linker groups that can be easily modified to incorporate fluorinated aromatic rings. These compounds will be compared in cell-based assays with fluorinated rhodols developed by the Peterson lab as well as ER-tracker BW, which is a commercially available dye that accumulates in the ER. We hope that these studies will shed light on the electronic requirements for selective localization of small molecules in ER membranes to improve the design of fluorescent sensors and drugs that target the ER.

54. Understanding a local population of the invasive species Pyrus calleryana through genetic analysis

Stacy D Holt Jr.[1] and James Beck,[1]
[1]Wichita State University, Department of Biological Sciences

Basic research into invasive species could help predict future invasions, and this study aims to better understand Callery pear (Pyrus calleryana) a non-native tree rapidly spreading throughout the United States. This research project has two long-term goals: 1) evaluate the role of hybridization in establishing a local invasive population of P. calleryana, and 2) document the spread of genotypes throughout the history of the invasion across the U.S. The first goal concerns the large population of Callery pear trees that have invaded the woodlots in Wichita's Chisholm Creek Park. Are these trees hybrids between planted trees? Can the planted parental trees be found nearby? How many genotypes can be found in the invasive trees? These questions will be answered by sequencing whole chloroplast genomes and genotyping 14 nuclear loci. I have collected plant material and extracted DNA from 48 individual trees, representing both invasive trees and those planted in adjacent neighborhoods. These samples are now being genotyped at a pilot set of 9 nuclear loci. In the assessment of allele patterns for planted and invasive tree samples we would like to compare within and between each tree type. If the hybridization hypothesis is supported by the data, we expect to see the same multilocus genotype in many planted individuals, reflecting only a few or even a single cultivar. In the invasive tree samples the alleles are expected to have more variation and will more than likely be combinations of genotypes from the surrounding planted trees.

55. Colorimetric Detection of Lead and Other Heavy Metals Ions Using Modified Gold Nanoparticles

Rajesh Kandel and Dr. Seid Adem, Chemistry Department, Washburn University

In this work, we are trying to develop a colorimetric detection method for heavy metal ions using modified gold nanoparticles. Hexadecyl trimethyl ammonium bromide (CTAB) and dithizone reagents were used to modify the gold nanoparticles. The modified gold nanoparticles show distinct color changes with various metal ions: green(Cu^{2+}), pink (Zn^{2+}), grey (Ni^{2+}) and red(Cr^{3+}), which will allow a simultaneous detection of a mixture of these metal ions. As soon as a solution of Pb^{2+} was introduced into the modified gold nanoparticle dispersion, a color change form red to dark pink was observed. There is also a shift and broadening of the surface plasmon resonance peak. Using the shift in the peak position and regression analysis, our preliminary results showed that the limit of detection (LOD) of Pb^{2+} is 9.38 µM. Because of the simplicity, fast and relatively cheap cost, we are working on this method for practical application in detection of heavy metal ions in real samples.

56. CITED2 Regulation of Embryonic and Placental Development: Species Differences

Marija Kuna[1, 2], Pramod Dhakal[1, 2], Lindsey N. Kent[1, 2], Regan L. Scott[1, 2], Khursheed Iqbal[1, 2] and Michael J. Soares[1, 2, 3, 4, 5]
[1]Institute for Reproduction and Perinatal Research, Departments of [2]Pathology and Laboratory Medicine, [3]Obstetrics and Gynecology, and [4]Pediatrics, University of Kansas Medical Center, Kansas City, Kansas; [5]Center for Perinatal Research, Children's Research Institute, Children's Mercy, Kansas City, MO

CITED2 possesses critical roles in cellular development and differentiation. These CITED2 actions are achieved through regulating transcription through the recruitment of CBP/p300 to transcription factor complexes. The purpose of this investigation was to examine roles for CITED2 in the regulation of placentation. CITED2 is expressed in homologous structures of rat and human placentas termed the junctional zone and the extravillous trophoblast column, respectively. The biology of CITED2 was investigated in CITED2 deficient mouse and rat models and human trophoblast stem cells. CITED2 deficiencies in the mouse and rat similarly exhibited deficits in placentation, intrauterine fetal growth restriction, dysmorphic lung development, and congenital heart defects. Prominent species differences were also observed. CITED2 disruption in the mouse resulted in prenatal lethality, exencephaly, and adrenal gland agenesis. In contrast, in the rat, CITED2 null fetuses progressed through pregnancy and died immediately after birth with intact adrenal glands and without signs of exencephaly. Trophoblast cells of the junctional zone exhibit characteristic patterns of gene expression and genome accessibility, which are regulated by CITED2. Disruption of CITED2 in human trophoblast stem cells interfered with extravillous trophoblast cell differentiation. In summary, comparison of mouse and rat models of CITED2 deficiency demonstrated some conserved roles for CITED2 in embryonic and placental development, and also unique roles. Within the placenta CITED2 contributes to growth and differentiation of trophoblast cells directed toward restructuring the uterine compartment. (Supported by HD020676, HD079363, HD099638; Sosland Foundation, P20 GM103418)

57. The Role of Basement Membrane Proteins for Proper Q Neuroblast Migration in *C. elegans*

Angelica E. Lang[1], Erik A. Lundquist[1]
[1]Department of Molecular Biosciences, University of Kansas

Neuronal migration is crucial for proper nervous system development. In *C. elegans*, neuronal migration can be modeled in the two Q neuroblasts, QR and QL. They originate in approximately the same location in the worm but, during development, QR migrates anteriorly and QL migrates posteriorly. The transmembrane proteins UNC-40 and PTP-3 have been previously shown to work in two separate molecular pathways to control this initial migration. DPY-17, a collagen protein found in the cuticle and basement membrane of the worm, also appears to influence the direction of Q cell migration. Our data indicates it is the structural orientation of DPY-17 within the basement membrane that provides directional information to migrating cells and that it is working within the PTP-3 pathway. The involvement of DPY-17 suggests a broader role for basement membrane proteins in Q cell migration. We found that other structural proteins in the basement membrane, such as the laminin protein EPI-1, have a permissive role in migration but do not influence direction. This is an important finding as it shows that disrupting the structure of the basement membrane is not enough to alter the direction of migration, as with DPY-17. Other basement membrane proteins may also influence migration direction, such as the perlecan protein UNC-52. These findings set up a model for neuronal migration in which migration is in part directed by proteins in the basement membrane of the worm.

58. Cell wall membrane fraction of *Chlorella sorokiniana* enhances host anti-tumor immunity and inhibits colon carcinoma growth in mice

Mayme Loyd[1], Susumu Ishiguro[1], Nicole Robben[1], Riley Burghart[1], Paige Cote[1], Sarah Greenway[1], Ravindra Thakkar[1], Deepa Upreti[1], Ayaka Nakashima[2], Kengo Suzuki[2], Jeffrey Comer[1], and Masaaki Tamura[1]
[1], Department of Anatomy & Physiology, Kansas State University College of Veterinary Medicine, Manhattan, Kansas 66506, United States of America
[2], euglena Co. Ltd., 5-29-11 Shiba, Minato-ku, Tokyo 108-0014, Japan

Chlorella is a genus of single cell green algae and has been used as a dietary supplement for its rich nutrients. Although multiple studies suggest that Chlorella has antitumor properties, its origin, chemical nature of bioactive substances, and mechanisms are yet to be fully clarified. Accordingly, the antitumor and immunomodulatory effects of the colon cancer growth inhibitor,partially purified from the isolated cell wall membrane fraction of *Chlorella sorokiniana*, here referred to as Chlorella membrane factor (CMF), was evaluated in cell culture and in a colon carcinoma mouse model. The CMF treatment dose (1-100 µg/ml)- and time (24-72 h)-dependently inhibited the growth of both murine and human colon carcinoma cells in two-dimensional cultures. Treatment with CMF also significantly inhibited the growth of colon carcinoma spheroids in three-dimensional cell culture. This spheroid growth inhibition was further pronounced in a co-culture with T lymphocytes. In a mouse CT26 colon carcinoma peritoneal dissemination model, intraperitoneal injection of CMF (10 or 30 mg/kg, every other day) significantly attenuated the growth of tumor *via* induction of tumor cell apoptosis. Evaluation of immune cell populations in ascites showed that CMF treatment tended to increase T lymphocytes but lower granulocyte populations. The present study suggests that the cell wall membrane fraction of *Chlorella sorokiniana* contains a bioactive material that inhibits colon carcinoma growth *via* direct cell growth inhibition and stimulation of host anti-tumor immunity. Hence, it is suggested that the Chlorella cell wall membrane extract or a bioactive substance in the extract is an attractive complementary medicine for cancer therapy.

59. Relationship between food intake and expression of messenger RNA encoding aromatic amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), and catechol-O-methyltransferase (COMPT) in channel catfish.

Moyers, Macy, Danica Kostner, Blaine Wertz, Taylor White, Dr. Yass Kobayashi
Fort Hays State University, Department of Biological Sciences

Obesity is linked to various metabolic disorders and associated complications, including major depressive disorder and anhedonia. How dopamine metabolism affects food intake and the development of obesity in fish, however, is not clear. Our objectives were to characterize the tissue expression of AADC, TH, and COMPT mRNA and if their expression is influenced by food intake in channel catfish. Expression of these mRNAs were examined in the brain, hypothalamus, liver, muscle, kidney, spleen, large intestine, and small intestine, as well as within different parts of the brain to examine their tissue expression profile. To study the relationship between hypothalamic expression of these mRNAs and food intake, samples were harvested at -1 hour prior to (-1), at feeding (0), and 1 hour after feeding (+1, n=4 per time point). Expression of all transcripts encoding these enzymes was detectable in all tissues examined, with increased expression in the liver. These mRNAs were more highly expressed in the hypothalamus compared to other parts of the brain examined. The expression of AADC mRNA and TH mRNA was not altered by timing of food exposure. Given that these mRNA transcripts had increased expression in the hypothalamus, the dopaminergic neural system may influence the regulation of food intake. Lack of changes of expression of these enzymes within the hypothalamus in response to food exposure may indicate that the changes in the dopamine system in response to food intake may be at the enzyme activity level rather than changes in transcription.

60. Relationship of Oligomerization Status to the Catalytic Activity of Human Lactate Dehydrogenase (LDH)

Sam A. Munsell[1], Jared Smith[1], Moriah R. Beck[1]
[1]Department of Chemistry, Wichita State University

Lactate dehydrogenase (LDH) is a key enzyme in the anaerobic steps following glycolysis and catalyzes the interconversion of pyruvate to lactate. In previous studies it has been shown that the natural oligomeric status of LDH is directly linked to the enzymatic efficiency in bovine and rabbit LDH. Humans have five common types of LDH in the body that are made up of a combination of different subunits, human LDH5 is a homotetramer and is the most common form of human LDH. Our research focuses on how a mutation in the key region of binding between the tetrameric subunits will affect the enzymatic activity of the LDH. In our research we focused on lysine 256 (K265) located in the middle of the subunit binding region. To test the effect of oligomer status we mutated lysine to arginine to due to similarities in pK_a and overall charges and to directly monitor the effect that the steric hinderance may have on the binding and catalytic efficiency of the LDH subunits. Both mutant and wildtype LDH proteins were purified from bacterial cultures and were used in gel filtration to further purify and confirm the oligomeric state of the proteins. The catalytic activity of the enzymes was monitored spectroscopically by monitoring the absorbance of NADH. Circular dichroism was used to determine secondary structures and melting temperature. We found that the wildtype tetramer was more efficient in the lactate to pyruvate conversion while the dimer mutant was more efficient in the pyruvate to lactate conversion.

61. Investigating functions of BABY BOOM genes in embryogenesis

Kendall Odle[1], Kyra Murray[1], Ryan Robinson[1] Kyle Shankle[2], Lynn Hagelthorn[2], Scott Russell[3], Venkatesan Sundaresan[2]
[1]Department of Biology, Langston University, [2]Department of Plant Biology, University of California Davis, [3]Department of Biology, University of Oklahoma

BABY BOOM 1(BBM1) is a well characterized gene expressed in rice sperm cells, zygotes, and early embryos. BBM1 is initially male expressed in zygotes, later becoming biparental.BBM1 plays an essential role in rice (*Oryza* sativa) embryogenesis. When expressed ectopically in the egg cell, BBM1 can initiate parthenogenesis. There are two other homologs of BBM1—BBM2 and BBM3. We focused on BBM3. It's our hypothesis that BBM3's role in embryogenesis is similar to BBM1. A CRISPR Cas9 construct with gRNAs targeted to BBM3 was transformed into wild type (WT) Kitaake rice to try knocking out BBM3 so we could study its function. We expect the knockout would exhibit disrupted embryo development or zygotic arrest. To confirm the rice plants were truly knocked out for BBM3, we genotyped both WT and CRISPR mutant plants by cloning BBM3 and sequencing the alleles. Our results confirmed we had a bbm3 plant. We observed a frame shift mutation that provided a reliable knockout. By confirming this, we could begin observing the zygotes or embryos to see if there were developmental defects. This research improves our understanding of rice embryogenesis. If BBM3's role in embryogenesis is vital, it could potentially be used to improve synthetic apomixis technology by improving the rate of parthenogenesis. Synthetic apomixis was achieved by combining MiMe (mitosis instead of meiosis) mutations with parthenogenesis to create clonally propagating seeds. This technology's important because it maintains hybrid vigor in each generation. Hybrid crops generally result in higher yield compared to their inbred parent lines.

62. Defining the Mechanisms of eEF1A regulation by the Legionella pneumophila effector SidI

Adrienne Pohl, Stephanie Shames
Kansas State University, Biology Department

Legionella pneumophila is an intracellular pathogen that causes inflammatory pneumonia in humans called. Legionnaires' disease. For L. pneumophila intracellular replication effectors are required. SidI is one of over 300 effector proteins that are translocated into infected host cells by L. pneumophila. SidI interacts with eukaryotic elongation factor 1A (eEF1A). eEF1A performs countless cell biological functions. The protein SidI can dysregulate eEF1A, which can result in serious cellular pathologies that can damage the host cell. To understand how SidI regulates eEF1A a genetic approach will be taken to try to reveal molecular details of the interaction between SidI and eEF1A. Truncations of SidI are going to be used to help identify which regions of SidI and eEF1A interact. The truncations were made and transformed into pGex constructs. Using glutathione magnetic beads binding experiments will be run to determine which truncations of SidI are binding to eEF1A. HEK 293T cells will be used as the source of the eEF1A. After the binding experiments involving the eEF1A and SidI, the binding between the truncations of SidI and lpg 2505 will also be run using the same technique as the eEF1A biding.

63. Effects of Fluid Shear Stress on Dermal Human Microvascular Endothelial Cell Morphology

Tabatha Polk[1], Sarah Schmitt[1], David Long, Ph.D.[1]
[1]Wichita State University, Department of Biomedical Engineering, Wichita, KS

As blood flows through blood vessels, the fluid exerts a shear stress on the endothelial cells (ECs) that line the vasculature. The traditional view on endothelial cells is that they align in the direction of applied fluid shear stress. However, recent studies have found that human brain microvascular ECs (HBMECs) are resistant to morphological change when under fluid shear stress. Therefore, further research is necessary to determine if other types of microvascular ECs react similarly to that of HBMECs. The objective of this research is to determine if unidirectional laminar shear stress causes morphological changes in dermal human microvascular endothelial cells (HMEC-1).
A monolayer of HMEC-1 was exposed to a fluid shear stress of 16 dyne/cm^2 and imaged for 73 hours. Phase-contrast images of the live cell membrane and fluorescence images of the nuclei were captured hourly. After 73 hours, the cells were fixed and the f-actin was stained and imaged by confocal microscopy. These images were then processed and segmented using *FIJI*. To determine if nuclear and cellular morphology differed in comparison to no-flow conditions, the following parameters were calculated: inverse aspect ratio; orientation of cells with respect to flow direction; area; circularity. In addition, the distribution of f-actin was compared between flow and no-flow conditions.
The results of this study will demonstrate the morphological response of dermal microvascular endothelial cells to fluid shear stress. Because these cells play an important role in angiogenesis and wound healing, these results could contribute to potential novel mechanotherapies of wound healing.

64. Is the Pyruvate Kinase of *Zymomonas mobilis* Allosterically Regulated?

Collette L. Wright[1], Dr. Jeff McFarlane[1], Dr. Kathleen Meneely[1], Dr. Aron Fenton[2], Dr. Audrey L. Lamb[1]
[1]Molecular Biosciences, University of Kansas, Lawrence, KS; [2]Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS

Glycolysis is a universal pathway of energy production. Pyruvate kinase catalyzes the final step of glycolysis, generating a molecule of ATP by transferring a phosphate group from phosphenolpyruvate to ADP. This reaction is highly regulated, with a variety of allosteric regulators, including fructose-1,6-bisphosphate (F1,6BP). The enzyme structure is also highly evolutionarily conserved. It has been proposed that the pyruvate kinase from *Zymomonas mobilis* (ZmPYK) is not allosterically regulated[1] and is a functional dimer instead of a tetramer. A primary goal of this research is to understand mechanisms of allosterism in pyruvate kinase. Therefore, studying a non-allosteric homologue provides an outstanding negative control for the human liver homologue (hL-PYK). We aim to determine if ZmPYK is allosterically regulated and determine the functional oligomeric state of ZmPYK. We present here the first protein crystal structure of ZmPYK, which crystallized as a tetramer. Furthermore, phosphate is observed in the allosteric site, analogous to F1,6BP phosphate binding in the human form. We are currently removing phosphate from all buffer systems and stripping cellular phosphate from the protein during purification. This new sample will be used to assess if phosphate is the allosteric regulator in ZmPYK or if the phosphate is structural. We will determine a protein structure and perform kinetic analyses on the phosphate-free enzyme. We also present Dynamic Light Scattering data, monitoring the oligomeric state under different buffer conditions.
[1]Steiner, P. et. Al. (1998) Cloning and Expression of the Zymononas mobilis Pyruvate Kinase gene in Escherichia coli Jour. Genes and Genomes 220 31-38

65. Synthesis of a Superstable Trioxatriangulenium Ion

Derek Reese, Eric Trump
Emporia State University

The synthesis of a super stable planar triarylmethane is described. The cation is especially stable because the para nitrogens are locked into place, providing maximum pi overlap between the lone pairs of the nitrogens and the aromatic pi electrons. Using a published procedure for the preparation of julolidine, 3,5-dimethoxy aniline was heated with an excess of 1-bromo-3-chloropropane to make 8,10-dimethoxy-1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolone. This was then stirred with phenyllithium for three days and reacted with dimethylcarbonate to yield tris (8,10-dimethoxy-1,2,3,5,6,7-hexahydopyrido[3,2,1-ij]quinolin-9-yl]methanol. Pi-donor substituents of the compound are able to stabilize the carbenium ions through resonance. Trioxatriangulenium ions (TOTA+) bind duplex DNA through intercalation with a preference for G-C base pairs. Strand cleavage can occur primarily at the G-C steps following piperidine treatment, irradiation of intercalated TOTA+ ions and subsequent charge (radical cation) injection.

66. Septate junction proteins act at the leading edge to maintain tissue adhesion during *Drosophila* dorsal closure

Rice, Clinton[1], Rodrigo Fernandez-Gonzalez[2,3,4] Teresa Zulueta-Coarasa[3,4] Robert E. Ward[1]
[1]Dept. of Molecular Biosciences, Univ. of Kansas, [2]Inst. of Biomaterials and Biomedical Engineering, Univ. of Toronto, Toronto, ON, CA, [3]Dept. of Cell & Systems, Univ. of Toronto, Toronto, ON, CA, [4]Ted Rogers Centre for Heart Research, Univ. of Toronto, Toronto, ON, CA

Septate junctions (SJs) have long been known to establish occluding barriers in invertebrate epithelia. Recently, we showed that SJ proteins are also required for morphogenetic processes such as dorsal closure (DC), a process during late *Drosophila melanogaster* embryogenesis by which the embryo closes a dorsal hole that is temporarily plugged by the extra-embryonic amnioserosa. This process occurs before the formation of mature functional SJs and therefore represents a separate and as-of-yet uncharacterized role for SJ proteins. Embryos lacking the SJ components Coracle, Macroglobulin complement-related, or Neurexin IV typically fail to complete DC and experience tearing in which the amnioserosa and lateral epidermis separate along their shared interface, known as the leading edge. This tearing is accompanied by loss of the adhesive protein DE-cadherin from the region of the dorsal hole and does not appear to be caused by increased tensile forces, suggesting that adhesion is decreased. We also show that septate junction proteins are expressed in both the amnioserosa and the basolateral regions of the dorsal-most epidermal cells that make contact with the amnioserosa during late DC, when tearing occurs. Additionally, the region of physical contact between these cells appears to be reduced in SJ mutant embryos. Taken together, these results suggest that SJ proteins play a critical role in maintaining adhesion between tissues, either by stabilizing other adhesive protein complexes or through a direct adhesive role.

67. Title: Macrophage directed therapy to ameliorate radiation induced rectal damage.

Authors: Felipe Rodriguez-Tirado, Payel Bhanja, Ximena Diaz, Subhrajit Saha
University of Kansas Medical Center. Department of Radiation Oncology

Homeostasis of the intestinal epithelium depends upon signaling crosstalk between the crypt intestinal stem cells (**ISC**) and the surrounding niche including intestinal sub-epithelial myofibroblasts, endothelial cells, and macrophages (**MΦ**). MΦ play an important role in promoting epithelial regeneration. However, the regenerative role of MΦ against colonic and rectal injury has not been studied extensively. Rectal epithelial injury is a major limiting factor for effective radiotherapy against pelvic malignancies such as prostate, urinary bladder or ovarian cancer (20-30% of all malignancies). Chemotherapy in addition to radiotherapy can also increase the risk of late rectal morbidity. Currently there are no FDA-approved treatments to mitigate rectal injury resulting from toxic agents. We demonstrated that conditional porcupine-deleted transgenic mice (*Csf1r-iCre;Porcn$^{fl/fl}$), a* model that inhibits all MΦ-derived WNT secretion, are sensitive to radiation-induced rectal injury and subsequent proctitis. We have demonstrated that WNT, packaged in extracellular vesicles (**EV**) isolated from bone marrow derived *MΦ* conditioned medium (CM), could induce rectal stem cell regeneration. In ex-vivo irradiated rectal organoids we have demonstrated that treatment with EVs purified from MΦ conditioned medium (**CM**) could ameliorate radiation toxicity and improve organoid growth. However, EVs purified from porcupine null bone marrow derived *MΦ CM failed to rescue rectal organoids.* Further studies are going on to develop EV mediated therapy to ameliorate radiation induced proctitis.

68. Mechanisms of LegC4-mediated attenuation of Legionella pneumophila replication within primary mouse macrophages

Abigail Salberg[1], Tshegofatso Ngwaga[1], Stephanie R. Shames[1]
[1]Division of Biology, Kansas State University

Legionella pneumophila (Lpn) is a natural pathogen of amoeba but an accidental pathogen of humans that causes Legionnaires' Disease through uncontrolled replication within alveolar macrophages. Lpn replicates within macrophages through formation of a replicative membrane-bound compartment called the Legionella containing vacuole (LCV), which does not fuse with lysosomes. To form the LCV, Lpn is dependent on translocation of effector proteins directly into the host cell via the Dot/Icm type IV secretion system. Lpn that cannot translocate these effector proteins are quickly degraded by lysosomes, preventing their replication. One of these effector proteins, LegC4, is important for replication of Lpn within its natural host amoeba but is detrimental to replication within cytokine-activated macrophages. To determine how LegC4 attenuates bacterial intracellular replication, we asked whether LegC4 increased lysosomal localization to the LCV. Anti-Lpn and -LAMP1 (a lysosomal protein), were used to quantify lysosomal localization. Bone marrow-derived macrophages were infected for 1 or 9 hours with either legC4-overexpressing Lpn, a legC4 mutant, wild-type Lpn, or a dot/icm mutant strain, which is degraded in lysosomes. Overexpression of legC4 resulted in a significant increase in lysosomal fusion to LCVs compared to the wild-type and legC4 mutant Lpn. The dramatic increase of LCV and lysosome co-localization suggests that legC4 function results in inhibition of intracellular replication through increased lysosomal recruitment to the LCV.

69. The differentiation and characterization of dental pulp stem cells: toward nucleus pulposus regeneration

Richard Sandefur, Li Yao
Wichita State University, Department of Biological sciences

Human dental pulp stem cell-derived nucleus pulposus cell-like cells (HDPSCs) are an attractive postnatal type of stem cells for nucleus pulposus (NP) generation. The transplantation of hDPSCs-NPLCs encapsulated in type II collagen-hyaluronic acid hydrogels mimicking the native NP tissue is potentially a novel approach to regenerating the degenerated NP. The motility of transplanted cells is critical in order that the cells could migrate out of the hydrogels and disperse through the NP tissue. The purpose of the research is to determine the phenotype and motility of hDPSCs-NPLCs grown in an injectable type II collagen-hyaluronic acid hydrogel. Although NP cells are chondrocyte-like and expressed Sox 9, aggrecan and type II collagen markers, recent studies explored the characteristic NP markers and their physiologic relevance. The differentiation of DPSCs toward NP cells should generate better tissue engineering-based therapeutic effect. In our study, dental pulp stem cell pellets were with BMP13 or BMP14 growth factors to induce differentiation. Cells were then mechanically separated and grown in culture with a type II collagen coating. Differentiated cells stained positive for aggrecan and type II collagen, two known chondrocyte markers for NP cells. A control for analysis of motility of these cells was done with NP cells and recorded via microscopy. Recordings of these cells showed that NP cells are able to migrate in the type II collagen-hyaluronic acid hydrogel. This study demonstrated that BMP13 and BMP14 growth factors can induce HDPSCs toward a nucleus pulposus phenotype.

70. Identification of Genomic Regions that Influence Meiotic Drive Favoring Metacentric Chromosomes in *Drosophila Americana*

Michael Stricker, Nicholas Stewart, Bryant McAllister, Rebekah Rogers. Fort Hays State University, University of Iowa, University of North Carolina at Charlotte.

Research has shown that Mendelian's law of segregation can be broken by a phenomenon called meiotic drive. Meiotic drive is when one allele can manipulate meiosis to ensure preferential inheritance to offspring. Many examples of meiotic drive have been identified, however the genetic mechanisms have not been fully elucidated. Here we use the D*rosophila virilis* subgroup to identify genetic mechanisms of meiotic drive. In *D. americana*, heterozygous females with respect to having metacentric and acrocentric chromosomes transmit metacentric chromosomes ~57% of the time. While *D. americana* evolved two metacentric chromosomes, their sister species, *D. novamexicana,* has maintained acrocentric chromosomes. To investigate the mechanisms of meiotic drive, we introgressed the metacentric centromere fusing the X and 4th chromosomes in *D. americana* into a *D. novamexicana* acrocentric background. Transmission rates from heterozygous females to offspring were measured in 6 replicate introgression lines. One line maintained the ~57% bias for the metacentric chromosomes while the other 5 reverted back to 50/50 transmission. This indicates that genetic background influences the meiotic drive phenotype. To identify genetic factors influencing meiotic drive, we sequenced the introgression lines and the parental strains. We identified SNPs that were *D. americana* descent in the one introgression line that maintained drive but absent in the other introgression lines. Two large genomic regions showing high frequency of SNP calls with such a pattern were identified. Currently we are analyzing these large regions for candidate genes that influences the observed meiotic drive favoring metacentric chromosomes in *D. americana*.

71. Investigating the effect of small interfering RNAs targeting cell wall biosynthesis on the growth of *Macrophomina phaseolina*

Tomlinson, Trey and Bin Shuai
Department of Biological Sciences, Wichita State University

The necrotrophic fungus, *Macrophomina phaseolina*, is a widely distributed phytopathogen that causes the disease charcoal rot. This fungus has a broad host range that includes over 500 species, such as the agriculturally significant crops soybeans and corn. Worldwide, it is attributed to annual crop losses that are devastating. Crop rotation, natural resistance, and tillage are some traditional control measures; however, they have been largely ineffective.

Our research objective is to develop an effective management approach to control the disease. In our previous study, it has been shown that the application of exogenous small interfering RNA (siRNA) effectively suppressed the growth of *M. phaseolina in vitro* through knockdown of *CHS* and *GLS* individually. The two genes targeted encode the enzymes chitin synthase (CHS) and β-1,3-glucan synthase (GLS), both of which are important in the biosynthesis of cell wall components. In this investigation, we hypothesize that knocking down both *CHS* and *GLS* at the same time will impact cell wall biosynthesis in a more severe way, and potentially result in synergistic suppression of *M. phaseolina* growth. Knowledge gained from this research will benefit in the development of transgenic crop lines that are resistant to the disease in order to better control the pathogen in ways traditional control measures have failed to.

72. In-frame Genetic Disruption of SPECC1L Microtubule-Interaction Domain Caused Neural Tube, Palate, Ventral Body Wall and Optic Fissure Closure Defects

Zaid Umar[1*], Luke Wenger[1*], Everett Hall[1], Jeremy Goering[1], Michael Moedritzer[1], Shahnawaz Paroya[1], Irfan Saadi[1].
[1]Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS. *Equal contribution.

Failure of neural tube, secondary palate and ventral-body-wall closure leads to exencephaly, cleft palate, and omphalocele, respectively. Autosomal-dominant *SPECC1L* mutations have been identified in patients with syndromic malformations, including hypertelorism, cleft palate and omphalocele. These mutations cluster in the second coiled-coil (CCD2) and calponin homology (CHD) domains, which interact with microtubule and actin, respectively. To study SPECC1L function in mice, we first generated genomic deletions that resulted in out-of-frame truncations. Homozygous mutants for these truncations, including those lacking CCD2 and CHD, died shortly after birth without cleft palate or omphalocele. We then generated a series of targeted in-frame deletions in CCD2, homozygotes for which also exhibited perinatal lethality but now with ~50% exencephaly, ~50% cleft palate, and ~50% omphalocele. Interestingly, exencephaly and cleft palate were never observed in the same embryo, suggesting antagonistic tissue mechanics between neural tube and palate closure. Consistently, we found that the oral cavity was narrower in mutant embryos with exencephaly, allowing for the palatal shelves to close eventually. Furthermore, histological analysis of *Specc1l*$^{\Delta CCD2}$ mutants identified novel ocular defects, including coloboma – a failure of the optic fissure to close. Thus, loss of SPECC1L interaction with microtubules – while maintaining actin association – was more detrimental to cell and tissue movement than loss of SPECC1L protein entirely. Taken together, our results showed that in-frame perturbations of CCD2 in SPECC1L protein specifically affect embryonic closure of several tissues, suggesting that human *SPECC1L*-CCD2 missense mutations are gain-of-function.

73. Role of RNA Binding Protein HuR as Facilitator in Non-alcoholic Fatty Liver Disease (NAFLD) Progression

Vikalp Vishwakarma [1], Ranjan Preet [1], Erika Peters [2], Colby Spiess [1], Dan A. Dixon [1,3].
[1]Department of Molecular Biosciences, University of Kansas, Lawrence KS, [2]School of Medicine, University of Kansas Medical Center, Kansas City, KS and [3]University of Kansas Cancer Center, University of Kansas Medical Center, Kansas City, KS.

Nonalcoholic fatty liver disease (NAFLD) is characterized by the accumulation of lipids (steatosis) and cellular inflammation in liver. It includes a spectrum of diseases from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) with liver dysfunction leading to cirrhosis and hepatocellular carcinoma (HCC). Hepatic inflammation is a common feature found in all stages of NAFLD progression. The Human antigen R (HuR, *ELAVL1*) is a key RNA binding protein involved in mRNA stabilization of various pro-inflammatory and oncogenic genes. So far, the role of HuR in NAFLD progression hasn't been explored. Using immunohistochemistry for HuR expression in human liver tissues, significant increase in HuR expression was observed during transition from NAFL to NASH. Further, fatty acid-induced steatosis in both Huh7 and HepG2 cells resulted elevated HuR expression. A 4-fold increase in HuR mRNA expression was observed during fatty acid-induced steatosis. Using luciferase reporter constructs, we noticed that steatosis enhanced both human and mouse HuR promoter activity (2.2- and 2.5-fold, respectively) in-vitro. Further, CRISPR-Cas9 HuR knocked-out HCC cells showed significant decrease in fatty acid-induced steatosis, suggesting involvement of HuR in disease progression. Interestingly, inhibition of HuR using the small molecule natural product 15,16-dihydrotanshinone-I (DHTS) derived from *Salvia miltiorrhiza*, prevented fatty acid-induced steatosis in HCC cells. Early prevention from steatosis by using HuR inhibitors can potentially prevent its progression to steatohepatitis, cirrhosis and hepatocellular carcinoma. Overall, present findings implicate HuR as a facilitator of liver steatosis and potential novel therapeutic target for NAFLD treatment.

74. Seeking Satiety: Dopamine Receptors 1,2 mRNA Expression in Channel Catfish

Blaine Wertz, Danica Kostner, Taylor White, Macy Moyers, Dr. Yass Kobayashi
Fort Hays State University

The dopaminergic nervous system regulates pathways of reward, pleasure, and satiety, and is therefore strongly related to food intake and subsequent eating behavior in mammals. Pathologies of this system have been well-studied in both humans and rodents; however, little is known about this system in fish species, including channel catfish. To increase our understanding in the involvement of the dopaminergic nervous system in regulation of food intake in fish species, the expression of dopamine receptors 1 and 2 (DR1-2) mRNA, and their modulation in response to changes in food intake was examined. Samples of the brain, hypothalamus, liver, muscle, spleen, kidney, and two segments of intestine were collected from three fish to examine the tissue distribution of DR1 and DR2 mRNA. In addition, the expression of DR1 and DR2 mRNA was examined in four segments of the channel catfish brain. DR1 and DR2 mRNA was detected across all tissues examined, with readily detectable expression in the brain and hypothalamus. Among brain segments, DR2 mRNA was most readily detectable in the hypothalamus and forebrain. Hypothalamic expression of DR1 and DR2 mRNA was unaffected by food exposure when samples were taken at one-hour prior to feeding, at the time of feeding, and one-hour post feeding. Currently, we are examining the expression of DR1 and DR2 mRNA in relation to feeding frequency, as well as prolonged fasting to further elucidate the relationship between food intake and dopaminergic neuron function.

75. Effects of food intake on relaxin 3 (Rxn3) mRNA expression in the muscle of Atlantic salmon

Taylor White, Macy Moyers, Blaine, Wertz, Danica Kostner, Brian Peterson, and Yass Kobayashi
Fort Hays State University

The Rxn3 is a protein that belongs to the insulin-like/relaxin superfamily and affect various physiological functions, including growth, food intake, and reproduction. Evidence in rodents suggests that Rxn3 is involved in the regulation of food intake and critical for proper development of muscle and bones. The roles that the insulin-like/relaxin superfamily plays in regards to growth and metabolism is largely unknown in fish, including Atlantic salmon. Recently, we identified the mRNA sequence for the Atlantic salmon Rxn3 by screening the database using channel catfish insulin-like 5 polypeptide mRNA sequence. Given the possible role of Rxn3 in muscle function and nutrient metabolism, changes in the expression of Rxn3 mRNA in response to the changes in food intake were examined. Juvenile Atlantic salmon (n=72, 6 fish per tank) were assigned to either receive food every day (control, n=4 tanks) or fasted for 14 days (fasted, n=4 tanks). Additionally, fish were fasted for 7 days and fed daily for the subsequent 7 days (refed, n=4 tanks). Muscle samples were collected from fish at the end of 14 days, and expression of Rxn3 mRNA was measured using real-time PCR. Expression of Rxn3 mRNA was similar among the three feeding treatments, suggesting that muscle expression of Rxn3 is not affected by changes in food intake. Given the preliminary nature of the study, further investigations are warranted to define the role of Rxn3 on the regulation of growth, food intake, and nutrient metabolism in Atlantic salmon.

76. RNA binding protein, HuR-containing CRC-derived exosomes promote lung metastasis by regulating CDK2-dependent inhibition of p21.

Hui Xiao, Vikalp Vishwakarma, Ranjan Preet, Dan Dixon.
Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas 66045, United States.

Lung metastasis is one of the major causes of cancer-related deaths in patients with colorectal cancer (CRC). We have observed that HuR regulates exosomes (50-150 nm) secretion in CRC and HuR was detected in exosomes secreted only from HuR overexpressing cells. However, the role of exosomal HuR in the lung metastasis from colon cancer is not yet defined. Here, we study the role of HuR-containing CRC-derived exosomes on impacting lung metastasis. First, HuR expression was assessed using immu¬nohistochemistry in tumor tissue samples from twenty CRC patients both with and without metastasis. Overexpression of HuR was significantly higher in the colon tissues with lung metastasis. Along with this, HuR was highly expressed in lung tissue metastasized from colonic origins as compared to benign lung disease (BLD). Furthermore, normal human bronchial epithelial (BEAS-2B) cells were treated with exosomes derived from HCT116 (CRC) cells and CRISPR/Cas9 mediated HCT116 HuR-Knockout cells (HCT116 HuR-KO cells). The effect of HuR-containing CRC exosomes on wound closure was observed with enhanced proliferation as compared to HuR-deficient exosomes. Enhanced migration (~2.5 fold) and invasion (~2.3 fold) of BEAS-2B cells was noted when treated with HuR-containing exosomes. BEAS-2B cells showed similar uptake of exosomes explaining that the presence of exosomal HuR enhanced cell proliferation, migration, and invasion. In addition, HuR-containing exosomes downregulated the expression of p21 in the BEAS-2B cells. This work suggests a relationship between HuR-containing CRC-derived exosomes and distant lung metastasis and explains its central role in altering tumor microenvironment during lung metastasis.

77. Karyotype Evolution and Meiotic Drive of Hybrid *Drosophila* Fruit Flies

Kearyn Zahnter1, Nicholas Stewart1, Theresa Miorin2
Fort Hays State University1, University of North Carolina Charlotte2

While major changes to chromosomal form is generally deleterious, karyotypes are not static. Major chromosomal rearrangements contribute to genetic isolation and divergence within and between species. As mechanisms that drive these divergences have not been fully elucidated, we hypothesize that meiotic drive, ie. bias transmission from parent to offspring, has contributed to the reshaping of karyotypes. *Drosophila americana* and *Drosophila novamexicana* are sister species that present an excellent model to study such evolutionary forces. Since diverging from *D. novamexicana, D. americana* has had two major chromosomal rearrangements: centromere fusions between the 2nd and 3rd chromosome and the X and 4th chromosome. Previous research has shown that the 2-3 fused centromere is transmitted at a 60% rate from *D. americana/D. novamexicana* F1 hybrid females to offspring. However, there has been no research measuring the transmission of the 2-3 fused chromosome in a genetic background that has maintained unfused acrocentric chromosomes. We introgressed the fused *D. americana* allele into the *D. novamexicana* genome and the transmission rate of the fused 2-3 chromosome from heterozygous females to offspring was recorded. We used a molecular marker near the centromere on the second chromosome to track the fused 2-3 chromosome to offspring. Our results show a strong transmission bias against the 2-3 fused chromosome indicating that there may be strong meiotic drive preventing the prevalence of fused chromosomes within *D. novamexicana.*

78. Assessment of Bacterial Cell Survival and Vitrification in Salt Concentrations Relevant to Mars

Hassan Zbeeb, Tyler Nolan, Mark Schneegurt PhD
Wichita State University Department of Biological Sciences

It is known that the soils of Mars are rich in salts beyond NaCl, such as epsomites and (per)chlorates. This hygroscopic environment is likely to produce aqueous solutions with low freezing points, and thus opens opportunity for bacterial habitability despite the extreme conditions. Our team has investigated the effects of certain salt concentrations on the survival of chosen bacterial isolates under cold temperatures. These isolates are especially halotolerant and thrive in naturally salty environments. They have been obtained from the Great Salt Plains of Oklahoma, Hot Lake in Washington and Basque Lake in British Columbia, which are rich in NaCl and $MgSO_4$, respectively. Various liquid broth media were inoculated with an existing organism and were incubated in the dark at -20^{0}C. The cultures were stored inside cryogenic tubes submerged in a sand chamber. Thus far, cultures have been grown exposed to ion combinations consisting of anions chloride, sulfate, and (per)chlorates coupled with cations Na and Mg. Survivability was, and continues to be, analyzed through the standard plate count method. Survival of cells was shown over a 24-hour and week-long period and will be monitored for months. Cells grown in high-salt media appear to survive after hours of incubation. Meanwhile, growth of cells under low-salt media have shown inhibition. This could suggest that these hypertonic solutions drive the generation of compatible solutes to lower the freezing points of cells. Salt effects on vitrifying cells will be investigated further through calorimetry. Detecting the freezing transitions of bathing solutions and their submerged cells is an additional avenue to explore regarding microbial responses in potential Mars habitats.
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79. Optimizations in Capillary Electrophoresis for Fast Determination of Amino Acids

Qiyang Zhang, Silei Zhang, Antonio Zapata, Jenika Wheeler
Physical Sciences, Emporia State University

In human bodies, there are 20 standard amino acids are required in human bodies, they play necessary roles in human bodies. When taken up into the human body from the diet, the 20 standard amino acids either are used to synthesize proteins and other biomolecules or are oxidized to urea and carbon dioxide as a source of energy. However, there are nine essential amino acids could not be produced in human bodies, some amino acids need to be obtained from food supplies, for example, lysine, histidine, threonine, methionine, valine and so on. For investigating amino acids in food and nutrition supplies, we chose capillary electrophoresis (CE), a powerful chemical separation technique to determine the concentration of amino acids. For this research, the buffer solution was sodium tetraborate buffer. To prepare the sample, amino acids were reacted with NDA (naphthalene-2,3-dicarboxaldehyde) and CN- to produce fluorescence product, which can be detected by a laser-induced fluorescent detection. Besides, we presented the optimized experimental conditions, for example, the voltage for separation, the length of the column, injection voltage and injection time. These results showed that the separation of multiple amino acids can be performed within 20 seconds with high resolution and efficient separation. More optimization of the instrument can be performed towards detector, column size and buffer.

80. The role of LKB1-AMPK signaling on cyst progression in PKD

Yan Zhang, Yuqiao Dai, Emily A. Daniel, Gail A. Reif, Brenda S. Magenheimer,
and Darren P. Wallace
Department of Internal Medicine, The Jared Grantham Kidney Institute, University of Kansas Medical Center, Kansas City, KS, United States

Background: In polycystic kidney disease (PKD), mTOR activation in cyst-lining cells contributes importantly to cell proliferation and cyst growth. Fluid accumulation within the cyst cavity is driven by Cl^--dependent fluid secretion via CFTR Cl^- channel. Liver Kinase B1 (LKB1) directly phosphorylates and activates AMP kinase (AMPK), an important negative regulator of both mTOR and CFTR. We hypothesized the LKB1-AMPK pathway modulates cyst growth and the progression of PKD.
Methods: Renal epithelial cells cultured from *Lkb1$^{flox/flox}$:ROSA26-Cre*^{ERT2} mouse kidneys were treated with tamoxifen to delete LKB1 expression. We specifically knocked out LKB1 in collecting ducts (CDs) of wildtype (WT) and *Pkd1$^{RC/RC}$* mice, an orthologous model of PKD. We treated *PKD1*$^{RC/RC}$*:PKD2*$^{+/-}$ mice with BIT-11, a novel small molecule LKB1 activator for 15 weeks by daily gavage. At the end of treatment, kidneys were collected for analysis.
Results: The loss of LKB1 significantly decreased AMPK activity but had no effect on mTOR signaling in renal epithelial cells. At 10 weeks, CD-specific LKB1 deletion was not sufficient to induce cyst formation in WT mice but caused a significant increase in kidney weight to body weight ratio (KW/BW), cystic index, and cyst number in *Pkd1$^{RC/RC}$* mice. Whereas, treatment with BIT-11 caused a significant decrease in KW/BW, improved renal function indicated by lower blood urea nitrogen, and decreased interstitial fibrosis in PKD mice.
Conclusion: Deletion of LKB1 accelerated cyst growth in PKD; and direct activation of LKB1 using BIT-11 decreased cyst growth and restored renal function, suggesting this may be a potential therapeutic target for PKD.

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81. Evaluating the Role of Translational Efficiency in Synaptogenesis in *Caenorhabditis elegans*

Kasra Alizadeh, Brian D. Ackley
University of Kansas, Department of Molecular Biosciences

Nidogen is a conserved basement membrane protein required for the proper formation and organization of synapses. In *Caenorhabditis elegans*, nidogen functions to localize the presynaptic scaffold protein SYD-2/α-liprin at synaptic active zones. The synaptic phenotype in nidogen mutants is suppressed by loss-of-function in genes that encode a calcium channel (*unc-2* or *unc-36*), the calmodulin kinase (*unc-43)*, or calmyrin (*calm-1*), demonstrating that calcium signaling is important in synaptic morphogenesis. Previous work identified proteins that associate with CALM-1 in a calcium-dependent fashion, including RACK-1 and multiple ribosomal proteins. Loss of function in *rack-1* results in a synaptic phenotype equivalent to the loss of nidogen, and this phenotype can be suppressed by the loss of *calm-1.* Interestingly, RACK-1 is known to be a translational inhibitor in many contexts, although whether this is how it is functioning in synaptic nidogen pathway is unclear. To test this, we are using RNAi to inactivate the ribosomal proteins isolated in our biochemical screen. The viability of RNAi in studying these genetic interactions has been validated by our previous screens. Furthermore, there is the question of the range of activity of RACK-1. While the role of RACK-1 in synaptic development might be through global translation inhibition, this protein could also be associated with only certain ribosomes, in which case, identifying the mRNAs associated with these ribosomes can point at new genes important in synaptic development. We are purifying and studying these mRNAs using immunoprecipitation and sequencing. Results from this project are important in understanding synapse formation and organization.

82. The RNA-binding protein Tristetraprolin: A key factor of intestinal cell differentiation and microbial homeostasis in colorectal cancer

Jennifer Amrein[1], Vikalp Vishwakarma[1], Sandhya Sanduja[2], and Dan A. Dixon[1]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS
[2]Whitehead Institute for Biomedical Sciences, Cambridge, MA

Post-transcriptional regulation is a critical component of gastrointestinal (GI) homeostasis known to be dysregulated in colorectal cancer (CRC). Rapid mRNA degradation controls the expression levels of various growth factors and inflammatory mediators. The main factor known to promote decay of these transcripts is the RNA-binding protein Tristetraprolin (TTP; *ZFP36*). In normal colonic epithelium, TTP is expressed at moderate levels where it binds mRNAs through their 3'UTR AU-Rich Elements, targeting them for degradation. However, during CRC tumor development, the loss of TTP expression results in uncontrolled expression of inflammatory and tumor promoting transcripts. This is evident *in-vivo*, where loss of TTP promotes a systemic pro-inflammatory phenotype in mice. Examining the effects of TTP loss on GI physiology, we observed a significant increase in the marker of inflammation known to be increased in CRC, neutrophil associated gelatinase-lipocalin (NGAL) in fecal samples from TTP-knockout mice. Furthermore, we also observed a significant reduction in intestinal goblet cell numbers, implicating a new role of TTP in controlling intestinal cell differentiation via Notch signaling. This combined increase in GI inflammatory markers coupled with a reduction in goblet cell numbers and associated intestinal mucin layer, led to a TTP-dependent change in the intestinal microbiome in TTP-knockout mice as confirmed through fecal 16S bacterial rRNA sequencing. TTP loss resulted in microbial dysbiosis associated with intestinal inflammation and cancer. Collectively, these findings demonstrate a novel role for TTP in controlling intestinal cell differentiation and microbial homeostasis. Further, they suggest development of therapeutic interventions to control dysbiosis observed in CRC.

83. First Demonstration of Bacterial Growth in Deliquescent Brines Relevant to Mars

Ansari, Irfan[1], Robin Cesur[1], Fei Chen[2], Benton Clark[3], Mark A. Schneegurt[1]
[1]Department of Biological Sciences, Wichita State University, [2]Jet Propulsion Laboratory, [3]Space Science Institute

Hygroscopic salts can absorb moisture from the atmosphere to form saturated brines through the process of deliquescence. The surface of Mars is abundant in sulfate salts which can form saturated brines through deliquescence. The brines formed from the deliquescence of the salts can contain the liquid water necessary for life. We have studied the growth of halotolerant microbes (*Halomonas* and *Marinococcus*) under deliquescing conditions. Bacterial cultures were grown in 2 M MgSO4 medium and have shown to grow from multiple drying and rewetting cycles. While there is cell death with each cycle (usually less than 50%), many of the cells were able to survive after storage. When water or salt solution is added to dried cultures, the cells were able to survive and grow, reaching high culture densities. Deliquescence experiments were also tested, in which dried cultures were not directly rewetted but immediately stored instead. After drying, the cultures were kept in a sealed jar above a layer of water or salt solution, allowing the MgSO4 to create a saturated brine within a day through moisture absorption from the headspace. Surviving cells were revived and were able to grow, reaching high culture densities. Our experiments were the first laboratory demonstration of microbial growth in deliquescent brines. Our findings of the survival of microbes, which contaminate spacecraft, after drying and growth in deliquescent brines is potentially relevant to planetary protection protocols for missions to Mars and guidelines for potentially habitable regions on Mars. Supported by NASA ROSES PPR and KINBRE.

84. The migration of dental pulp stem cells in 3D culturing of chemotaxis chamber

Jannet Balderrama, Li Yao
Department of Biological Sciences, Wichita State University

The nucleus pulposus (NP) plays an essential role in the flexibility and stability of the intervertebral discs of the spine. Degenerative changes to the interverbal disc results in biomechanical alterations of the spinal column, which leads to low back pain. Current treatment and rehabilitation are focused on surgical procedures, including discectomy, spinal fusion, and nucleotomy. However, the outcome of these methods results in restriction in flexibility of the spine. Stem cell therapy holds promise for potential solutions to this problem. As stem cell therapy advances as a potential treatment for degenerative disc, it is imperative that the most appropriate methodology be used to assess possible in vivo responses to the cells. Our study focuses on dental pulp stem cell (DPSC) and DPSC-derived nucleus pulposus (NP) cells motility as examined by 3D chemotaxis assay using the µ-Slide chemotaxis chamber. This method allows for the observation of migrating cells using time lapse microscopy. In this study we optimized the technique for insertion and assessment of the type II collagen containing cells in the chamber. The results presented provide valuable information regarding the potential behaviors of differentiated DPSCs, if introduced into a living organism. This increased level of understanding DPSCs in vivo contributes to the collective efforts of scientists and medical professionals who hope to provide improved treatment options for individuals with degenerative disc disease.

85. Probing the Unfolded Protein Response: mRNA knockdowns in Acyrthosiphon pisum

James Balthazor
Department of Chemistry, Fort Hays State University

Pea aphids, (Acyrthosiphon pisum), one of 2000 species of aphids, are a significant agricultural pest and are the model organism of aphid species. Current management of A. pisum includes use of insecticides and the introduction of natural predators, neither of which is ideal. The utilization of RNA Interference (RNAi) presents an alternative, pest specific targeting method. The introduction of double-stranded RNA (dsRNA) complementary to each of the target genes of the Unfolded Protein Response (UPR) in the pea aphid, coupled with a carrier, has shown significant changes in expression of gene products and subsequently has shown decreased survivorship and reduced fecundity in the organism. Here we will demonstrate that our preliminary research shows promise in insect mitigation as well as insights into other knockdown projects ongoing within the research group.

86. Investigation of CHD7 homolog, kismet in Drosophila with respect to muscle growth and development

Jonathan Barnell[1] and Takrima Sadikot[1]
[1] Department of Biology, Washburn University, Topeka, KS

Drosophila melanogaster serves as a useful tool for studying disease causing homologs in humans. The *Drosophila* gene *Kismet* is homologous to the *CHD7* gene in humans, a chromosome remodeling gene. Mutations in this gene cause CHARGE syndrome in humans, which is often identified by its hallmarks: coloboma of eye structures, atresia, fused/missing kidney, various heart defects, and impaired cognitive and physical development. Individuals diagnosed with CHARGE exhibit swallowing difficulties, esophageal reflux, delayed fine and gross motor skills, and delayed walking. While some of these issues are neurological, not much is known about the associated abnormalities in muscle structure, though low muscle tone in skeletal and smooth muscles is apparent. In this project, mutated *Kismet*'s potential impact on muscular development is investigated in the fly model using balancer chromosomes to maintain and identify inheritance of the mutated gene and upstream activator sequences to selectively express or repress the mutated genotype. Studying how muscle structure development occurs in *Drosophila* under the *Kismet* mutation might help us understand how mutations in CHD7 might affect early human development.

87. Design of S. pyogenes Cas9 nuclease immune to anti-CRISPR inhibition

Erianna Basgall, Megan Goeckel, Isabel Lewis, Yao Yan, and Gregory Finnigan
Kansas State University, Manhattan, KS, Department of Biochemistry and Molecular Biophysics

CRISPR/Cas is a gene editing system that offers an ever-increasing range of molecular methods. CRISPR evolved as an immune response in bacteria to defend against bacteriophages. The system requires a nuclease and guide RNA to target, bind, and cleave a DNA sequence. Anti-CRISPRs are proteins that evolved in bacteriophages to counter the CRISPR systems; many of them directly target and inhibit the Cas nuclease. These proteins can be used to regulate genomic editing, serve as switches, or inhibit CRISPR gene drives in whole populations. Each anti-CRISPR appears to be highly specific to one nuclease.
Our lab aims to design a CRISPR nuclease that can no longer be targeted and inhibited by specific anti-CRISPRs without compromising function. We used the S. pyogenes Cas9 nuclease and two anti-CRISPRs from L. monocytogenes, AcrIIA2 and AcrIIA4. Crystal structures of SpCas9 and AcrIIA4 in complex have shown these inhibitors act as DNA mimics. Specific amino acids that interact with the anti-CRISPR structures were identified within Cas9, which we used to generate ten separate substitutions using mutagenesis. Multiple assays in budding yeast were used to assess whether the anti-CRISPR inhibits the mutated Cas9 allele. These included (i) haploid editing of the genome, (ii) diploid editing, and (iii) a cellular assay using fluorescently tagged proteins to test association. Four of the ten Cas9 mutants retained significant editing activity, but the current mutational set did not escape AcrIIA4-based inhibition.
Ongoing/future experiments include co-localization of mutant dCas9 fusions with AcrIIA4 using fluorescent protein tags and generation of more mutants.

88. Development of a Green Double Allylation Methodology

Lia Boese, Jenna Placzek, Sarah Champagne
Fort Hays State University: Chemistry

Allylation reactions are commonly found in organic chemistry for the construction of novel molecules and natural products. However, these reactions are typically run under anhydrous conditions and utilizing hazardous solvents. Herein we report the development of a novel "green" double allylation methodology. This methodology has been initially employed for the synthesis of 1,3-diols and also for the closure of all carbon containing rings.

89. Effect of Ethanol Exposure on CREB Levels in Enriched and Socially Isolated Rats

Emma Brase, Thomas Wukitsch, Jared Rack, and Mary Cain
Department of Psychological Sciences, Kansas State University

cAMP response element binding (CREB) protein is a transcription factor that mediates the effects of brain-derived neurotrophic factor. Both proteins influence synaptic plasticity and regulation of addiction-related behaviors. Research on the rearing environment-alcohol interaction affecting CREB levels is limited. This project examined differentially reared rats' CREB levels as a result of adolescent intermittent ethanol (AIE) exposure. Rats arrived on postnatal day (PND) 21 and reared in either isolated (IC), enriched (EC), or standard condition (SC) environments for 30 days. Rats then underwent AIE treatment, consisting of an injection of either ethanol or saline. One injection occurred every other day for four weeks to model binging. Following the last AIE treatment, rats underwent a 30-minute locomotor test to measure novelty response and anxiety-related behavior. EC rats had less overall total, margin, and center distances traveled than IC and SC rats, with no main effect of treatment and no condition-treatment interaction. The next day, rats were euthanized, and brains were extracted and flash frozen. Hippocampal, medial prefrontal cortex, and dorsal striatum tissue samples were collected, and protein was extracted. Western blots will quantify each experimental condition's total CREB levels. I hypothesize that AIE treatment will reduce CREB levels across environmental conditions, that ethanol exposed IC rats will have the lowest hippocampal CREB levels, and that control EC rats will have the highest CREB levels. This research will clarify the effects of alcohol and early-life environments on the plasticity of the developing brain.

90. An Introduction to Cellular Analysis Using Quantitative Phase Imaging

Matthew E. Christman, and Vincent Rossi
Physics Department, Washburn University, Topeka, Kansas

Quantitative Phase Imaging (QPI) has come forth as a useful tool in collecting data for cellular biology. Changes in cell depth and the internal arrangement of material (which impacts the cell's localized index of refraction) can alter the phase of light entering and exiting a cell. QPI allows for the collection and analysis of this change in phase, providing the ability to image cells based on depth and index of refraction. Here we provide an introduction to how a quantitative phase microscope may be designed, as well as examples of analysis using phase imaging.

91. Role of Glycogen Metabolism in C. difficile Virulence

Yusuf Ciftci[1], Kamrul Hasan[2], Revathi Govind[1]
[1]Division of Biology, Kansas State University, [2]Department of Biology, Texas A&M University

Clostridiodes difficile, a Gram-positive, anaerobic bacterium, is the leading cause of antibiotic-associated nosocomial diarrhea in North America. C. difficile causes around half a million infections per year and costs about 4.8 billion dollars in healthcare bills. C. difficile's major virulence factors are the extracellular toxins A and B. The disease is prevalent in the nosocomial environment and challenging to keep in check because of the highly resistant spores produced by the bacteria. Like many other pathogenic microbes, C. difficile virulence factors are strictly regulated in response to the nutrient availability to the cell. Glycogen is a storage carbon that many organisms use as a form of stored energy to use during the starvation condition. C. difficile genome harbors a glycogen biosynthesis operon, and we explored the role of glycogen in C. difficile growth and virulence by creating a mutant strain with a disrupted glgC gene of the operon. The resulting mutant was incapable of glycogen accumulation and produced very few spores, signifying glycogen is required for efficient sporulation in C. difficile. In correlation with glgC mutant's higher toxin production and faster growth rate compared to its parent counterpart in in vitro condition, our animal infection model study showed that glycogen mutants are significantly more virulent in in vivo conditions. Further, disruption of the adjacent glgP gene responsible for glycogen degradation resulted in a similar phenotype. Thus, glycogen availability and metabolism are essential for C. difficile spore formation.

92. Development of an Automatic Computational Machine Learning Pipeline to Process Confocal Images for Virtual Cell Generation

Contreras, Miguel,[1] and David Long[1]
[1]Department of Biomedical Engineering, Wichita State University

Microscopy plays a central role in cell and developmental biology. In particular, fluorescence microscopy can be used to visualize specific cellular components and subsequently quantify their morphology. However, there are challenges with these imaging experiments which can make it difficult to quantify cell morphology: inconsistent results, time-consuming and potentially costly protocols, and, limitation on number of labels due to spectral overlap. To address these challenges, the objective of this project is to develop an automatic computational machine learning pipeline to predict nuclear morphology for virtual-cell generation based on fluorescence cell membrane confocal z-stacks. A pre-trained machine learning algorithm developed by Christiansen *et al.* was used to train and predict morphology of nuclei using only fluorescence membrane images. In this method, registered confocal z-stacks of nuclei and cell membrane were obtained from confocal microscopy and normalized through software pipeline. The machine learning algorithm was trained using this set of normalized *z*-stacks. The trained algorithm was used to predict morphology of nuclei using cell membrane fluorescence images as input. Preliminary results show qualitatively good predictions on training set after one week of training. Improvement of membrane image quality proportionally improved nuclei predictions, reducing errors relative to ground truth. These results show the potential of pre-trained algorithms to predict cell morphology using relatively small amounts of data and training time. Future steps include validation of results on unseen fluorescence membrane images as well as further training for prediction of different labels (e.g. focal-adhesion sites).
Acknowledgements: 'In silico labeling' algorithm (Christiansen *et al.*, 2018, *Cell* **173**:1-12)

93. Analysis of Dacthal in Various Food Samples from Local Stores Using GC-MS

Authors: Carson Denney, Seid Adem, Washburn University Department of Chemistry

Abstract: This research aimed to test and quantify the pesticide dacthal (DCPA) in food samples. Dacthal is a herbicide banned from use in food products in the European Union in 2009 and is recommended not to be used in food crops. However, dacthal is still used on kale in the US. Dacthal is reported to be carcinogenic. Currently, the Environmental Working Group (EWG) has named Kale as one of the dirt dozen foods. This group analyzed 2017 tests from the USDA and found dacthal in 60% of the samples, despite the fact that the samples were thoroughly washed. In this work, different kale samples purchased from a local store in Topeka, KS, were tested for the presence of dacthal using GC-MS. We used QuEChERS instead of the usual liquid-liquid extraction method in the sample preparation. In all the samples tested, no Dacthal was found. It is hard to believe that Kale found in Topeka stores will be free of dacthal against the widespread findings across the nation. This requires testing samples from various stores and the use of a better sample preparation work to ensure complete extraction of dacthal for the analysis.

94. Tracking mucosal T-cell subtype development in mice

Dalton Doyle, Savannah Bender, Tim Burnett PhD.
Department of Biology Emporia State University

T-regulatory (T-Reg) cells play a role in immunological suppression with anti-inflammatory characteristics, while T-helper 17 cells (T_H-17), with pro-inflammatory characteristics, ramp up immunological responses. The opposing affects of these immune cells likely influences the establishment of mucosal tolerance and the mutualistic symbiosis with gut microflora. In this study, we determined the relative abundance of T cell subsets in the small intestine throughout mouse postnatal development. We quantified expression of T cell master gene regulators (*Foxp3, Ror*γ at various time points from birth through 7 weeks of age using qRT-PCR. *Foxp3* expression was detectable as early as day 3 and increased dramatically after day 14. In comparison, *Ror*γ expression had low but detectable levels throughout. These results suggest both T-Reg and T_H-17 cells are present in small intestine mucosa shortly after birth and T-Reg, but not T_H-17 cells increase as mice mature.

95. Optimization of Sucrose Cushions to Improve Efficiency of Exosome Isolation

Nicole D'Souza[1], Sui Ching Phung[1], Mei He[1,2*]
[1] Department of Chemical and Petroleum Engineering,[2]Department of Chemistry, University of Kansas

Abstract

Exosomes are naturally derived nano-vehicles secreted by living cells, ranging in size from 30-150 nm.[1]. They have the ability to undergo horizontal gene transfer and their origin in living cells and non-immunogenicity make exosomes a promising research area[1]. Ultracentrifugation combined with a density gradient is commonly used to isolate exosomes from free proteins and other extracellular vesicles. A density gradient bed separates particles based on particle density and provides a cushioning effect against the high forces of ultracentrifugation[2,3]. Sucrose facilitates efficient exosome isolation because sucrose density (1.1-1.2 g/mL) is similar to the density of exosomes (1.15-1.19 g/mL), but not other vesicles or proteins[2,3]. This research sought to determine the optimal sucrose gradient bed concentration for the isolation of exosomes. HeLa cells were used as the model study. Isolation of exosomes from HeLa cell culture media was compared across four different sucrose gradient bed concentrations. The size distributions of the isolated particles were measured using Nanoparticle Tracking Analysis (NTA). A 30% weight per weight sucrose: PBS gradient bed successfully isolated extracellular vesicles ranging in size from 30 -150 nm and provided a relatively small number of non-exosome particles. These findings will facilitate future research, such as studying the use of exosomes for interfering RNA delivery.

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96. Dependence of Proline Isomerization on the Kinetics of Folding of Anthrax Lethal Factor

Wyel Halimeh & James G. Bann
Department of Chemistry, Wichita State University, Wichita KS

The death that can come within a few days after exposure to the anthrax toxin from Bacillus anthracis is due to the presence and effects of anthrax lethal factor (LF). LF is a zinc metalloproteinase whose function depends upon the translocation of LF from the endosome of a host cell into the cytosol, where it cleaves mitogen-activated protein kinase kinases and disrupting cell signaling pathways. The translocation requires LF to unfold as it transits through the narrow channel of the pore, formed by the anthrax protective antigen (PA). Unfolding of LF has been shown to be pH-dependent, but little is known regarding the kinetics of unfolding and refolding of LF. Specifically, refolding of LF must occur fairly rapidly within the cell cytosol to prevent degradation of the protein by cellular proteases. The N-terminal PA binding domain of LF, LF, has a single cis-proline residue (Pro166), and we hypothesized that this cis proline must isomerize to trans during the unfolding and translocation process and that refolding would be slow, and perhaps dependent on cellular prolyl isomerases for refolding. To this end, we have performed a detailed kinetic refolding/unfolding study of LFN from urea solutions. Our preliminary experiments indicate that LFN refolding occurs rapidly (within 1 second), suggesting that Pro166 does not isomerize to an appreciable extent in the unfolded state, or if it does isomerize, that isomerization back to cis is a fast process. The implications of these experiments on the mechanism of anthrax toxin lethality will be discussed.

97. Effect of RNA Interference mediated knockdown of Protein Disulfide Isomerase on *Acyrthosiphon pisum* survival

[2]Paul Hess and [1]James R. Balthazor
1. Fort Hays State University Department of Chemistry
2. Fort Hays State University Department of Biological Sciences

Aphids are notorious agricultural pests. Multiple studies have shown the ability of RNA
interference (RNAi) mediated gene silencing to cause targeted death of these organisms in the place of toxic insecticides. The endoplasmic reticulum (ER) resident protein disulfide isomerase (PDI) plays an important enzymatic role in the folding of nascent proteins via disulfide bond formation and cyclic oxidation/reduction. Family member PDIA6 acts as an attenuator of inositol-requiring enzyme 1 (IRE1), which is a major stress sensor and mediator of the unfolded protein response (UPR). In the setting of ER stress, UPR machinery upregulates chaperones, halts protein translation, and increases disposal of misfolded proteins. IRE1 attenuation is critical to cell survival as excessive IRE1 activity can lead to apoptosis. By selecting PDIA6 for RNAi mediated knockdown, deficient cells may hyperrespond to ER stress with sustained activation of IRE1 resulting in increased apoptosis leading to downstream death of the organism. During the study, RNA will be isolated from pea aphids from which cDNA will be synthesized using reverse transcription. The cDNA template will be used as a template for synthesizing PDIA6 dsRNA to be used in feeding studies. RNA interference of PDIA6 may result in a decrease of fecundity and survival in pea aphids and show potential as a pest management strategy.

98. Assessment of Environmental Bacteria for Resistance to Cetylpyridinium Chloride, Modes of Potential Resistance Transmission, and Clinical Applications

Nathaniel Higdon, Eric Gillock
Fort Hays State University Department of Biological Sciences

Projections indicate that by the year 2050 the leading cause of premature death will be due to drug-resistant microbes (de Kraker MEA 2016). Antimicrobial resistance and its associated danger to human health increases daily with each new discovery of resistant pathogens. Opinions vary on how, and why, resistance is growing, but a new theory has gained traction. It is hypothesized that quaternary ammonium compounds (QACs) in the environment at subclinical levels may be promoting selection and differentiation of microbes and thereby lead to resistance. QACs work on a wide variety of microorganisms. Our aim was to enhance the body of knowledge of resistance in microbes by using cetylpyridinium chloride (CPC, C21H38NCl) to assess if CPC resistant bacteria are identifiable in the environment. To this end, we collected soil samples from 15 sites. 1:100 dilutions were made and used to make lawns on 0.35% CPC incorporated agar, and resistant colonies were streaked for isolation 3 times on CPC agar. 60 isolated bacterial colonies were obtained, and 10 were sent for 16S rRNA sequence analysis identification. This yielded three distinct genera, Pseudomonas, Enterobacter, and Pluralibacter, which have been shown to be pathogenic in humans, fish, and plants. Cross-resistance was assessed with a Kirby-Bauer assay and MICs, sole carbon source, and isolation of transferable plasmid tests were performed. Our results show that clinically relevant species and clinically relevant genera of CPC resistant bacteria are identifiable in the environment, and further research on their ability to transfer resistance should be conducted.

99. Development of Novel Phosphonium Salts for use in Ionic Liquids and as Cationic Ligands

Kaitlynn Hillery, Sierra Smith, Skyler Markham
Fort Hays State University; Chemistry

Ionic liquids are of paramount utility in a wide array of high temperature and pressure applications. Cationic ligands are a novel area of ligands which can be utilized to increase the overall reactivity of the metal center to which they are attached. Herein, we report the development of a class of mono- and di-pyridiylphosphonium salts which we intend to explore as potential ionic liquids and cationic ligands. This report details our efforts towards the synthesis of these species along with our initial efforts at utilizing the salts as cationic ligands

100. Towards Peptide-polymer Nanoparticles of Different Charges for Studying Biological Interactions

Shelby Innes[1], Alireza J. Nasrazadani[1], Emily L. Torrey[1], Hernan A. Hernandez[1], Matthew P. Thompson[2], Nathan C. Gianneschi[2], Andrea J. Luthi[1]
[1]Deparment of Chemistry, Emporia State University
[2]Department of Chemistry, Northwestern University

Peptide-polymer amphiphiles (PPAs) are tailorable polymers that can be formulated into nanoparticles with different properties. These types of nanoparticles have various biomedical applications including as therapeutics and drug delivery vehicles. In addition to using peptide-polymer nanoparticles as new ways to treat diseases, it is important to study the interactions between these nanoparticles and biological systems. The goal of this research project is to synthesize PPAs with a negative, positive, or zwitterionic charge and formulate them into peptide-polymer nanoparticles with different surface charges. Then, the effect of surface charge on the interactions of the nanoparticles with biological systems will be studied. The first polymers were synthesized using a phenyl monomer, which will form the core of the nanoparticle, by ring-opening metathesis polymerization. A modified Grubbs second generation catalyst was used for the polymerization. Different sizes of polymers were made to check for control of polymerization. The sizes of the polymers were several times larger than expected so additional studies are being done to determine the cause. The polymers were analyzed by gel permeation chromatography. In addition to making polymers, a new gel permeation chromatography setup has been calibrated and the dn/dc value for the phenyl polymers determined. Once control of the polymerization has been demonstrated, PPAs will be synthesized and formulated into nanoparticles for biological interaction studies.

101. Monitoring Palladin's Effect on Actin Dynamics and Organization with TIRF Microscopy

Abby Jurgensmeier, Samuel Womack, Moriah R. Beck
Wichita State University, Department of Chemistry

The actin-associated human protein palladin plays a critical role in cytoskeletal organization in both normal and cancerous cells. In both breast and pancreatic cancer cell lines, palladin expression levels have been shown to correlate with metastatic potential. We have recently established that palladin contributes to actin dynamics in three distinct ways: nucleation of actin, crosslink formation, and filament stabilization. We hypothesize that palladin directly influences cell motility through simultaneous regulation of actin polymerization and organization. In this work we build upon our kinetic assays of actin polymerization to directly visualize actin assembly and protein dynamics with total internal reflection fluorescence microscopy (TIRFM). Bulk co-sedimentation assays can only monitor an increase in the total amount of actin polymerized, whereas the TIRF assay allows one to follow polymerization dynamics and filament topology simultaneously. To gain insight into the role of palladin in the assembly process and in the dynamics of supramolecular actin structures, we imaged the polymerization of monomeric actin and palladin in real time with TIRFM. Our data show that palladin mediates the formation of junctions between filaments. Analysis of filament morphology reveals that palladin promotes side branching in addition to bundles. Through these methods, we hope to understand how palladin contributes to actin-based cell motility in metastatic cells, which will be important for developing new therapies to specifically target this step in cancer progression.

102. The Role of Myopalladin in Cardiac Muscle Function and Disease

Vinay K. Kadarla[1], Erina Kutilek[1], Marie-Louise Bang[2], Moriah R. Beck[1]
[1]Wichita State University, Wichita, KS; [2]Institute of Genetic and Biomedical Research, CNR, Italy

Most cardiac malformations remain a mystery as scientists continue to examine how mutations and aging affect normal biological processes. Recently, mutations in a muscle protein myopalladin have been linked to the pathogenesis of cardiomyopathy. Myopalladin and palladin belong to a family of closely related immunoglobulin (Ig)-domain-containing proteins that have essential, but unclear roles. Recent work in the Beck lab has shown that the C-terminal domains of palladin bind directly to actin and increase both the rate and stability of actin filaments. The fact that various mutations in myopalladin are located within the analogous actin-binding region suggests that a disruption in actin-regulation may occur in cardiomyopathy. Thus, we hypothesized that myopalladin may also share a similar role in actin regulation. To study the capability of myopalladin to bind and crosslink actin filaments, co-sedimentation assays were performed. The results suggest that the Ig3 domain of myopalladin is the minimal domain required for both binding and bundling of F-actin and mutations on Ig3 domain trigger significant decrease in both binding and bundling of F-actin. Pyrene fluorescence was used to monitor the polymerization rate of G-actin in the presence of various Ig domains of myopalladin. Our data reveals that, unlike palladin, myopalladin actually decreases the rate of actin polymerization showing that myopalladin uses a different mechanism than that of palladin which is involved in cell motility. Thus, we suggest that myopalladin may act both as a scaffold, binding directly to actin and other actin binding proteins at the Z-disc, and limiting actin filament turnover.

103. Improving Personalized Medicine Through Systematic Protein Engineering of LDH

Shamir Khan,[1] Casey Palmer,[1] Shreeya Dalla,[1] Frank Kutilek,[1] and Moriah R. Beck[1]
[1]Department of Chemistry, Wichita State University

Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion between lactate and pyruvate and is found in the cells of almost all living organisms. Recent studies have indicated that changes in conserved regions of proteins cause a toggle switch effect – modulating the function in an "on/off" fashion. Whereas changes to the sequence of non-conserved regions result in changes similar to a rheostat, where function gradually changes. Previous studies exploring the effect of changes to non-conserved regions have been limited and have not yielded conclusions regarding either the type of substitutions that favor gradual change over severe functional changes. Here, we plan to investigate the relationship between changes in protein sequence and overall function and stability to assist in building a database that will enable better predictions in the future by examining non-conserved residues. To accomplish this, wildtype and mutant versions of LDH were purified before performing kinetic assays, chemical denaturation, and a circular dichroism spectroscopy to determine the thermal stability. We confirmed that non-conserved mutations showed gradual changes in the catalytic rate and the stability of the protein. An interesting mutation engineered at residue 68 resulted in an enzyme that was completely inactive, but also extremely stable. Protein stability tests can be related to proteopathic diseases, where the main cause of disease is related to protein structural and stability changes. Therefore, our research seeks to provide a deeper understanding of the relationship between protein sequence variation with disease state to improve personalized medicine in the future.

104. Link between Nutritional Status and Gene Expression in Regulatory Associated Protein of Mammalian Target or Rapamycin mRNA (RPTOR) and Rapamycin-insensitive Companion of Mammalian Target of Rapamycin (RICTR) messenger RNA.

Kostner, Danica, Blaine Wertz, Rebekah Spainhour, Oaklee Abernathy, Jenna Ball, Megan Dougherty, Abigail Schmidtberger, Dr. Yass Kobayashi
Department of Biological Science, Fort Hays State University

RPTOR and RICTR form a complex with Mammalian Target of Rapamycin (mTOR) called mTORC1 and 2, respectively and activates the transcription of genes associated with different metabolic pathways, including autophagy. Previous studies showed the expression of mTOR mRNA in muscle was increased in response to reduced food intake in channel catfish. However, whether RPTOR and RICTR mRNA expression is influenced by changes in food intake in catfish is unclear. The objective of the current study was to explore the relationship of RPTOR and RICTR mRNA expression and food intake in the brain, liver, and muscle. For the first study, channel catfish were fed daily (control) or fasted for 28 days (fasted). Fish in the third treatment were fasted for the first 14 days then fed daily for 14 days (refed). For the second study, catfish were fed once every 12 (overfed), 24 (control), or 48 hours (underfed, n=4 tanks per treatment) for 28 days. Brain, liver, and muscle expression of RPTOR and RICTR mRNA was measured from the samples collected on day 28. Muscle expression of RPTOR and RICTR mRNA was unaffected by changes in food intake. In contrast, fasting increased brain expression of RICTR mRNA, and increased feeding frequency decreased RPTOR mRNA in the liver. Our results showed that changes in the expression of RPTOR and RICTR mRNA did not coincide with changes in the expression of mTOR mRNA. The exact relationship between food intake and expression of these mRNA species needs further investigation.

105. Soil microbiota use non-cellulolytic bacteria to synergistically enhance cellulose digestion

Joseph LaForge[1], Guanpeng Wang[2] and Stephen D. Fields[1,3]
Emporia State Biology

Cellulose is a major carbon source for many microbial soil communities, but the community interactions between cellulolytic and non-cellulolytic species during cellulose degradation is poorly understood. In this investigation, metagenomic sequencing revealed a high level of variability in species diversity and composition of ten microbial assemblages randomly established on carboxymethylcellulose (CMC) plates from the same temperate soil sample. The number of species, or operational taxonomic units (OTUs), per established assemblage ranged from 33 to 236, and three "assemblage clades" with distinct community structures were identified. Only two OTUs were common to all ten assemblages, but these appear to play a minimal role in cellulose degradation. Achromobacter (OTU7), a non-cellulolytic Betaproteobacterium, was the predominant component of the GW-C clade, which demonstrated the most consistent and rapid growth on three different cellulose-based substrates (Fig 2). Whole metagenomic sequencing of two of the GW-C assemblages identified two Actinobacteria species, Cellulosimicrobium sp. and Kitasatospora sp., which are particularly enriched with cellulolytic genes. A third Actinobacteria species, Gordonia sp., from the GW-A clade is also highly enriched in cellulolytic genes. Other species, mostly gram negatives were also isolated from the assemblages. All three Actinobacterial species were isolated and tested for CMC digestion rates in monoculture and in various combinations. Interestingly, the highest cellulolytic rates were only observed in the presence of Achromobacter. Integrated waste management practices demand for increased yield of fermentable sugars from waste plant biomass.

106. Expression of human Tau mutants leads to synaptic loss in *Caenorhabditis Elegans*

Natasha L. LaGrega, Molly B. Massengale, Brian D. Ackley
Department of Molecular Biosciences, University of Kansas

In Alzheimer's disease, FTDP-17, and many other neurodegenerative diseases, tau aggregation and mutations of human tau are often seen and linked to a decline in neuronal function. However, the association of tau aggregation to decline in neuronal function is not well understood, due to the fact that it is difficult to examine tau in the human brain. To further explore this, we have put the longest isoform of human tau (htau40) as well as a mutation of htau40 that accelerates tau polymerization in vitro into multiple transgenic *C. elegans* lines to better understand how the formation of the tau aggregates may lead to toxicity and a decrease in neuronal function. Our results indicate that mutations in tau lead to a decrease in synapses in the dorsal nerve cord of *C. elegans* as they age. The decrease in synapses corresponds to a shorter average life span and a decrease in movement mimicking the phenotypic effects of neurodegenerative diseases. Our results indicate that our system is a valuable tool to further explore the form and function of htau40 and htau40 mutants.

107. Identification and validation of an Aspergillus nidulans secondary metabolite derivative as an inhibitor of the Musashi1-RNA interaction

Lan Lan[1], Jiajun Liu[1], Amber Smith[1], Carl Appelman[1], Xiaoqing Wu[1], Ke Li[1], Anuradha Roy[2], Ragul Gowthaman[1,3], John Karanicolas[4], Amber D. Somoza[5], Clay C. C. Wang[5,6], Berl Oakley[1], Roberto De Guzman[1], Kristi Neufeld[1], and Liang Xu[1]
[1]Department of Molecular Biosciences, [2]HTS Laboratory, [3]Center for Bioinformatics, The University of Kansas, Lawrence, Kansas; [4]Program in Molecular Therapeutics, Fox Chase Cancer Center, Philadelphia, PA; [5]Department of Chemistry, [6]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA

RNA-binding protein Musashi-1 (MSI1) is a key regulator of several stem cell populations. It is involved in tumor proliferation and maintenance, and it regulates target mRNAs at the translational level. The known mRNA targets of MSI1 include *Numb*, *APC* and *P21^{WAF-1}*, key regulators of Notch, Wnt signaling and cell cycle progression, respectively. In this study, we aim to identify small molecule inhibitors of MSI1-mRNA interaction thus blocking the growth of cancer cells with high levels of MSI1. Using a fluorescence polarization (FP) assay, we screened small molecules from several chemical libraries for those that disrupt the binding of MSI1 to its consensus RNA binding site. Two clusters of hit compounds have been identified, one of which is composed of derivatives of secondary metabolites from Aspergillus *nidulans*. The top hit, Aza-9, from this cluster was further validated by surface plasmon resonance and nuclear magnetic resonance which revealed that Aza-9 binds to MSI1 directly, in the RNA binding pocket. Next, we tested whether Aza-9 has anti-cancer potential in cells and whether such activities work through MSI1. Due to poor cellular uptake, we used PEGylated liposomes to facilitate Aza-9 entry into the cells. Aza-9-lipo inhibits colon cancer cell proliferation, induces apoptosis and autophagy, and down-regulates Notch/Wnt signaling in colon cancer cell lines. In conclusion, we identified a series of potential lead compounds for inhibiting MSI1 function while establishing a framework for identifying small molecule inhibitors of RNA binding proteins using FP-based screening methodology.

108. Transcriptomic Profiling Identifies Strain-specific Differences in the Response of *Caenorhabditis elegans* to Microbial Pathogens

Patrick Lansdon, Maci Carlson, and Brian D. Ackley
Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Infection from microbial pathogens is a major threat to organismal survival. In its natural environment the microbivorous nematode, *Caenorhabditis elegans*, frequently encounters pathogenic bacteria. Although *C. elegans* possess physical barriers and exhibit coordinated behavioral responses to decrease the likelihood of infection, they must also recognize and respond to pathogens that have bypassed these defenses. This response is modulated through the innate immune system, a defense mechanism comprised of evolutionarily ancient components that are highly conserved across phyla. Yet, *C. elegans* do not exhibit obvious conservation of microbial defense pathways observed in arthropods and mammals, (*e.g.* Toll or NF-κB). Rather, pathogen detection occurs via many different systems that converge upon a core set of physiological responses as well as a set of pathogen-specific responses, some of which are conserved in other organisms (*e.g.* generation of reactive oxygen species, production of antimicrobial peptides, *etc.*). We recently found differences in survival of several *Caenorhabditis* strains following infection with microbial pathogens. To investigate these strain-specific differences in immunity, we performed RNA sequencing of whole animals following pathogen exposure. This analysis yielded a large set of differentially expressed genes, some of which have been previously implicated in pathogen response. Currently, we are working to identify genes that are differentially expressed in both a pathogen-specific and strain-specific manner. Ultimately, our study seeks to shed light on the evolutionary origins of innate immunity as well as reveal uncharacterized aspects of mammalian defenses against infection.

109. The Formation of 2,2'-bipyridine by a Mild Phosphorus Extrusion Reaction

Skyler Markham, Kaitlynn Hillery, Sierra Smith
Fort Hays State University; Chemistry

The formation of carbon-carbon bonds has long been an important reaction in organic chemistry. This report details or further efforts towards the development of a mild and generalizable phosphorus extrusion reaction for the synthesis of 2,2'-bipridine species. We have been able to optimize several areas of the reaction to arrive at synthetically useful yields. We are currently working towards applying this methodology towards new substituted substrates.

110. RNA Interference of Three Genes in the Unfolded Protein Response: Activating Transcription Factor 6 (ATF6), Prefoldin Subunit 2 (PFD2), and Tumor Necrosis Factor Receptor Associated Factor 2 (TRAF2) in Pea Aphids (*Acyrthosiphon pisum*)

Spencer R. McCue, James R. Balthazor
Department of Biology, Department of Chemistry, Fort Hays State University

Pea aphids (*Acyrthosiphon pisum*) are common crop pests that cause billions of dollars worth of damage each year. Control of these pests using non-pesticidal methods would be an incredible step forward in maintaining the quality of our crops as well as the volume those crops produce. One possible method for this control is RNA interference (RNAi). RNAi is a gene 'knockdown' method, meaning it allows us to inactivate portions of an organism's genome using said organism's own cellular machinery. Within the cellular mechanisms of a pea aphid, the unfolded protein response (UPR) stands out as a promising target for gene knockdown. The UPR is a homeostatic mechanism centered in the endoplasmic reticulum (ER). When the ER becomes stressed, a series of complementary and adaptive mechanisms will activate, collectively known as the UPR. I will be examining the results of using RNA interference on three genes (PFD2, TRAF2, and ATF-6) housed within the UPR. I hypothesize that we will see a decrease in pea aphid survivability upon knockdown of these genes. Each gene will presumably create a decrease in survivability. However, I believe ATF-6 will create the most significant reduction as it is the beginning of a pathway within the UPR.* The implications of this study apply to the agricultural industry and medical professions. Understanding the UPR can lead to advances in neurodegenerative disease treatment and understanding, as well as help to create a new GMO plant that is resistant to aphid infestation.

111. Synthetic Beta-barrel Protein Sequences for Bionanosensor Applications

Montezano, Daniel[1], Joanna S. G. Slusky[1]
[1] Center for Computational Biology, Kansas University, Lawrence, KS, 66045

Outer membrane proteins are a class of proteins whose members almost exclusively assume the barrel fold. They have high sequence variability and performing a number of different and important functions for the bacterial cell, such as transport, signalling and catalysis. These highly stable proteins are also used as scaffold templates for the design of bionanopores in biotechnology applications. These pores have great potential as sensors in single-molecule sensing applications. They can be used to detect disease biomarkers, toxic compounds and perform protein fingerprinting. The successful application of these biosensors commercially is reflected in nanopore DNA sequencing, a method providing real-time, fast and low cost sequencing, poised to replace traditional sequencing techniques. The number of scaffolds currently used for bionanopore design is very small, with only a handful of membrane proteins, such as porin MspA porin and monomeric OmpG. New scaffods are needed to expand this set and development of devices for sensing complex molecules such as carbohydrates, which are difficult to create with the available pores. Our work uses state-of-the-art adversarial generative models for the generation of synthetic amino acid sequences that assume the beta-barrel tertiary structure. Our goal is to explore the membrane protein sequence space without evolutionary or structural constraints in the search for new scaffolds that can successfully fold and insert *in vivo* into the outer membrane of the bacteria *Escherichia coli*.

112. Identifying regulators of directed neuroblast migration in *Caenorhabditis elegans*

Vitoria Paolillo and Erik A. Lundquist
Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Guided neuronal migration is an essential process during nervous system development. The Q cell neuroblasts in *Caenorhabditis elegans* provide a simple and experimentally tractable model system for studies of directed neuronal migration. The Q neuroblasts are born in the same region of the animal and have identical differentiation patterns, yet undergo left-right asymmetric migration, with QR on the right migrating anteriorly and QL on the left migrating posteriorly. QL descendants encounter a posterior EGL-20/Wnt signal, which activates a canonical Wnt signaling pathway to induce expression of the Hox gene *mab-5* in QL and QL descendants, but not in QR and QR descendants. MAB-5 is both necessary and sufficient for posterior Q cell descendant migration, as QL descendants migrate anteriorly in *mab-5* loss-of-function (LOF) mutants and QR descendants migrate posteriorly in *mab-5* gain-of-function (GOF) mutants. However, it is unknown what genes are regulated by MAB-5 in the Q cells to drive posterior migration. We isolated Q cells from wild-type, *mab-5* LOF, and *mab-5* GOF animals via fluorescence-activated cell sorting and completed RNA-seq to generate Q cell transcriptomes. We identified 222 genes that were differentially expressed in the *mab-5* LOF Q cells versus wild-type Q cells and 559 genes that were differentially expressed in the *mab-5* GOF Q cells versus wild-type Q cells. Our preliminary functional studies have revealed that several of these candidate genes regulate Q cell migration. We anticipate that further functional studies of these putative *mab-5* targets will reveal new insights into directed Q neuroblast migration.

113. Protein and Fat Analysis of Forelimb Muscles of Low and High Aerobic Capacity Rats Following Resistance Exercise

Julia Stopperan[1], Fengyan Deng[1], Colin McCoin[1], Kimberly G. Stanford[1], Paige C. Geiger[1], John P. Thyfault[1], John A. Stanford[1]
1) The University of Kansas Medical Center, Department of Molecular & Integrative Physiology

We conducted protein and fat analyses on the forelimb (biceps) muscles of aged low and high aerobic capacity rats following isometric resistance training. Eighteen-month-old low capacity runner (LCR) and high capacity runner (HCR) rats were trained to use their right forelimb to press a force-sensing disc for water access. Rats underwent daily resistance exercise sessions until they were 22 months-old. Left (untrained) and right (trained) forelimb muscles of the HCR and LCR rats were extracted and pulverized. Muscles from sedentary, age-matched LCR and HCR rats were also analyzed. A TAG assay was performed on the forelimb muscles to determine the glyceride concentration. Western Blots were used to measure oxidative phosphorylation, heat shock protein (HSP)25, HSP72, HSP105, Uncoupling Protein (UCP)2, Light Coupling (LC)3, PGC1-alpha and AMPK. Overall, glyceride content was greater in the LCR rats than the HCR rats. This measure did not differ between the trained and untrained forelimbs. HSP72, PGC1-alpha, and oxidative phosphorylation were higher in the HCR rats than LCR rats. When LCR trained and untrained muscles were compared to HCR untrained muscles, there were no significant differences. Likewise, there were no significant differences in these measures between the muscles from LCR trained and LCR sedentary controls. These results demonstrate that intramuscular glyceride, HSP72, PGC1-alpha, and oxidative phosphorylation differ as a function of aerobic capacity. They also suggest that resistance exercise does not affect these differences, at least under the conditions of this study. Funding for this project came from the NIH and the K-INBRE Summer Scholars Program.

114. The mechanism of nitrogen metabolite repression by the NmrA corepressor.

Tran, Daniel H., Anna M. Brokesh, Cameron C. Hunter, Joel T. Steyer, Damien J. Downes, Meryl A. Davis and Richard B. Todd.
Department of Plant Pathology- Kansas State University. Department of Genetics- University of Melbourne

Nitrogen is a macronutrient essential for life as an important component of the building blocks of DNA, RNA and proteins. In the fungus Aspergillus nidulans, the transcription factor AreA activates the expression of genes for uptake and breakdown of nitrogen nutrients. When a preferred source of nitrogen is present, NmrA represses AreA activity. Overexpression of NmrA from a xylose inducible promoter prevents AreA-dependent gene expression and prevents growth on alternative nitrogen sources. Whole genome sequencing of a suppressor mutant that disrupts the NmrA overexpression phenotype revealed mutations in the AN4210 and bglA genes, suggesting that one or both of these genes are needed for NmrA function. AN4210 encodes a transcription Mediator complex protein and bglA encodes a beta-glucosidase enzyme. We deleted AN4210 and bglA separately and together to determine which deletion affects the function of NmrA. Partial suppression of the NmrA overexpression growth phenotype occurs in the AN4210 deletion mutant but not in the bglA deletion mutant. Because NmrA represses AreA, we can assay the activity of an AreA-dependent reporter gene to quantify the effect of the deletion of bglA and AN4210 on NmrA. Protein extracts from the deletion strains and the wild type control were used to measure reporter gene specific activity. We expect the deletion of at least AN4210 to suppress the effect of NmrA overexpression on gene expression.

115. Single Cell Capturing for Molecular Profiling of Heterogeneous Extracellular Vesicles

Bryan Vasquez, SuiChing Phung and Mei He
Department of Biology and Department of Chemical and Petroleum Engineering

Immunotherapies naturally boost the body's immune system and help restore the body's natural defenses. The immune system is a vital network in our body that aids in fighting off any diseases. Cells from the immune system secrete extracellular vesicles that aid in intracellular communication. However these secreted extracellular vesicles are heterogeneous, in terms of bio-molecular cargo leading to a difference in gene expression, indicating that cellular functions are varied. . It is likely that mutational and epigenetic factors significantly alter communication pathways in the immune system. To fully understand this heterogeneity of extracellular vesicles a complete analysis of an individual cell is essential. Molecular profiling of extracellular vesicles from single cells can reveal a more in depth analysis of extracellular heterogeneity. By utilizing molecular engineering techniques, like electroporation, we can introduce genetic sequences that can lead to the production of target molecules. We hypothesize that the heterogeneity of the extracellular vesicles at the single cell level can be studied using a microfluidic device. We designed a single channel microfluidic device with a single cell capturing region that will allow us to capture single cell for downstream analysis of the exosomes secreted by the individual cell. . We will utilize COMSOL simulation to optimize the capturing region design. Preliminary results from the COMSOL have shown that specific geometry affects the capturing efficiency of the single cell in the microfluidic channel. The simulation results will then be used to fabricate a single channel device for experimental study follow by the fabrication of optimized an high-throughput single-cell capturing device.

116. Metabolic regulation and cell envelop stress as the mechanisms for *S. gordonii* biofilm inhibition by gymnemic acids

Raja Veerapandian[1], Sumedha Gunewardena[2], and Govindsamy Vediyappan[1],*. [1]Division of Biology, Kansas State University, Manhattan KS 66506; [2]Department of Molecular and Integrative Physiology, Kansas University Medical Center, The University of Kansas, Kansas City, KS 66160.

S. gordonii is a pioneer colonizing commensal oral bacterium known to interact with pathogenic oral bacteria (*e.g. Porpyromonas gingivalis*) and fungi (*e.g. Candida albicans*) forming mixed-species oral biofilms. We and others have shown that *S. gordonii* interacts synergistically with *C. albicans in vitro* and *in vivo* forming enhanced oral biofilms and augment *C. albicans'* virulence. Intervention strategies that abrogate biofilm virulence are highly sought for therapeutic purposes. Recently, we demonstrated that gymnemic acids (GAs) could inhibit *S. gordonii-C. albicans* mono- and dual-species biofilms *in vitro*. In this report, we determined the biofilm inhibitory mechanisms of GAs against *S. gordonii* biofilm formed on sHA by RNA Seq method. Based on the RNA Seq and KEGG pathway analyses, GAs inhibit *S. gordonii's* phosphotransferase (PTS) sugar uptake system, glycolytic pathway and the early steps of the citric acid cycle. The reduced amounts of glycolytic intermediates in *S. gordonii* biofilms and the regulatory roles of the PTS could affect the bacterial cell envelop synthesis and thus activate adoptive or stress response pathways. Some of them include upregulation of CiaH, CiaR, SGO_0779/0780 two-component systems, *dlt* operon, and the com QS systems thereby affecting biofilm growth. Results from cellular and secreted metabolite profiles of the above control and GAs treated biofilms correlate with their corresponding gene expressions. Taken together, GAs treatment to *S. gordonii* reduces its central carbon metabolism and activates the cell envelope stress response pathways thereby affecting bacterial adhesion and biofilm growth or maintenance. Our results may have broader impacts against pathogenic oral streptococci.

117. Inhibition of gC1qR to prevent role in blood coagulation systems

Alexander Vontz[1], Ethan Kallenburger[1], Brian V. Geisbrecht[1].
[1]Department of Biochemistry & Molecular Biophysics , Kansas State University , Manhattan , Kansas 66506 , United States.

Many immune cascades, including the complement system and Kallikrein-Kinin System, are triggered by specific protein-protein interactions. One such interaction involves the protein gC1qR (p33) binding to C1q in the bloodstream. While gC1qR is normally present inside the cell, an immune response causes blood vessel endothelium to upregulate gC1qR and localize the protein on the cell surface. The presentation of gC1qR on the cell surface allows for extracellular C1q binding; this interaction initiates the complement cascade and causes blood vessel dilation, coagulation, and angiogenesis. Certain cancers exploit this system to promote angiogenesis, proliferation, and metastasis. We propose inhibition of C1q/gC1qR interaction could block the hemodynamic complement response and prevent cancer proliferation. To test this, we engineered 12 different single chain monoclonal antibody fragments (scFv) that have been shown to potentially bind to gC1qR and prevent C1q binding. To measure the C1q/gC1qR interaction and monitor inhibition imposed by scFvs, we will use an α-assay protocol. In short, HIS-tagged C1q and biotinylated gC1qR will be purified, bound to respective photosensitized beads, and allowed to interact. The beads, in complex with one another, produce a measurable fluorescent signal representative of C1q/gC1qR interaction. The successful inhibition of interaction by scFvs would suggest these inhibitors could prevent the coagulation response and potentially slow cancer growth.

118. Identification of regulatory domains in HD-Zip IV transcription factor GLABRA2

Adara Warner[1] and Kathrin Schrick,[1,2]
[1]Division of Biology, [2]Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS

In plants, class IV homeodomain leucine-zipper (HD-Zip IV) transcription factors play a key role in regulation of gene expression during development. These proteins contain a steroidogenic acute regulatory (StAR) protein-related lipid transfer (START) domain that is necessary for transcriptional activity. GLABRA2 (GL2) is a class IV HD-Zip member from *Arabidopsis* which aids in the specification of epidermal cells in the shoot, root, and seed. At present, there have been no reported activation or repression domains for HD-ZIP IV transcription factors; however, chromatin immunoprecipitation (ChIP) experiments together with mRNA expression studies suggest that GL2 can function either as a transcriptional activator or repressor. Recent experiments suggest that an 18-amino acid acidic patch located in an intrinsically disordered region at the N-terminus of GL2 encodes a putative regulatory domain. *Arabidopsis* mutants for $gl2^{\Delta Nterm18}$ have shown to display a dominant gain-of-function phenotype with defects in growth and fertility. Future work will include constructing additional mutants using site-directed mutagenesis that are predicted to affect the N-terminal regulatory domain of GL2, as well as using a yeast assay to characterize transcriptional activation versus repression domains from GL2. This project is expected to advance our understanding of how HD-Zip IV transcription factors regulate gene expression during plant development. Further applications of this research include understanding abnormal expression patterns resulting from gene rearrangements, which has relevance to diseases such as cancer, as well as for synthetic biology applications across organisms.

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119. A Method for Characterizing Membrane-Bound Proteins

Wilson, Cole and Durrett, Timothy
Kansas State University

Membrane-Bound O-Acyl Transferases (MBOATs) are a broad class of enzymes with functions varying from hunger regulation to synthesis of storage lipids. Their membrane-bound properties make them difficult to purify and crystal structures unavailable for this family of proteins. They all serve the same basic function of transferring an acyl group from Coenzyme A to a hydroxyl group on either a lipid or protein. Since more traditional methods fail in characterizing these enzymes, a different method will be used to characterize them: chimeragenesis. As a concept, chimeragenesis involves taking two or more similar genes with different activities, creating hybrids incorporating sequence fragments from both, and characterizing the activity of the resulting chimeras in order to draw connections between specific regions and their corresponding functions. For these experiments, two plant genes, Euonymus fortunei Diacylglycerol Acetyl Transferase (EfDAcT) and Sorbus acuperia Diacylglycerol Acyl Transferase (SaDGAT), are used. Both function as the final step in the biosynthesis of the storage lipid triacylglycerol (TAG), but with a key difference in activity. SaDGAT transfers a long-chain fatty acid from CoA to the molecule diacylglycerol (DAG) to form the more common long-chain TAG (lcTAG), while EfDAcT catalyzes the transfer of an acetyl group to DAG, forming the unusual lipid AcTAG. For these experiments we create chimeras with regions from both SaDGAT and EfDAcT in order to isolate the regions for substrate specificity.

120. Dissecting functional domains of the Vaccinia Virus D10 Decapping Enzyme

Lake Winter, Josh Molina, Candy Hernandez, Zhilong Yang
Kansas State University, Department of Biology

Vaccinia virus was essential in the eradication of smallpox as a vaccine. Vaccinia virus hijacks cellular machinery to reach a higher replication rate. The vaccinia virus-encoded D10 decapping enzyme is capable of removing the 5' cap with the enzyme's nudix motif being the region largely responsible for the decapping. Removing the 5' cap of cellular mRNA leaves the sequences unprotected and much more likely to de degraded. With less cellular mRNA present, vaccinia virus can have its own mRNA translated more efficiently using the cell's translational machinery. Decapping enzymes are also shown to repress mRNA translation in general. Unexpectedly, this lab previously found that D10 selectively enhances vaccinia virus mRNA translation. The focus of this research is to determine the roles of different domains of D10 in selective translation of vaccinia virus mRNA during infection. Site-directed mutagenesis will be used to generate desired D10 mutants. The mutants will be isolated, verified, amplified, and tested for their effect on viral mRNA translation. We expect to identify domains and amino acids of D10 that are critical for selective translation enhancement of viral mRNAs.

121. Effectiveness of Common Household Cleaners in Preventing Microbial Growth

Adams, Rebecca[1] and Keri Maricle[1] [1]Department of General Education, North Central Kansas Technical College, Hays, KS

Household cleaners have changed over the years to address the continually evolving pathogenic microbes that can inhabit a variety of surfaces. Many cleaners are classified as disinfectants, which are chemical liquids that detour or prevent microbes (specifically, bacteria) from colonizing surfaces. Multiple cleaners exist claiming to prevent microbial growth. Therefore, the objective of this study was to determine effectiveness of five common household cleaners in preventing microbial growth. Vending machine buttons were swabbed for microbes before and after applying each cleaner. Growth of microbes was compared between treatments for each cleaner by allowing microbes to culture on tryptic soy agar plates in an incubator set at 37 degrees Celsius for 48 hours. Microbes grew even after cleaning with every disinfectant used in this study, but the nature of growth and type of microbe differed across cleaners. Vinegar and 409 seemed to be the least effective in preventing microbial growth whereas Great Value Disinfecting Wipes, Lysol and Seventh Generation Disinfecting Wipes appeared most effective in preventing microbial growth. Specifically, results indicate there was a strong selection against bacteria but not fungi. However, even with the most effective cleaners, not all bacteria were eliminated and some fungi was allowed to grow. These results indicate household cleaners may not be as effective at removing all microbes from surfaces. Furthermore, the species of bacteria and fungi that grew after cleaning were not analyzed in this study. Therefore, the pathogenic nature of the microbes growing after using each cleaner is unknown.

122. Characterization of novel role for Rab27B in autophagy regulation in colorectal cancer.

Sahida Afroz, Ranjan Preet, Vikalp Vishwakarma, Dan A. Dixon
Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

Autophagy is a conserved catabolic pathway that has multiple roles in carcinogenesis and cancer therapy. While autophagy is maintained at basal levels in all cells, it is activated at higher level in many cancer cells as a mean to promote tumor growth, deal with nutritional and hypoxic constraints and facilitate resistance to anti-tumor immune response and chemotherapy. It is a multi-step cellular recycling process that includes formation of autophagosomes, fusion of autophagosomes with lysosomes and finally, degradation of cellular components. Several Rab proteins (RAS like small GTPases) are known to regulate different stages of vesicle trafficking and autophagy. Rab27B is a member of this family that is shown to be overexpressed in various cancers including colorectal cancer (CRC), and involved in cellular vesicle trafficking and regulating exosome secretion. Our previous work showed that RNA-binding protein HuR regulates exosome secretion via Rab27B in CRC cells. It was also noted that Rab27B was overexpressed in colon adenocarcinoma as the disease progress through stages 1-4. Surprisingly, CRISPR/-Cas9 Rab27B knockout CRC cells showed an abnormal accumulation of autophagosomes. To assess this, western blot analysis of autophagy marker LC3B was performed. LC3B was upregulated by 3-fold in the Rab27B knockout cells as compared to the parental cells. Immunofluorescence imaging for LC3B also showed an increase in LC3B puncta in Rab27B knockout cells. These results implicate a new role for Rab27B in controlling autophagy in CRC, and future studies will further define the mechanism and role of Rab27B in normal and cancer-associated autophagy.

123. Synthesis of Phosphatidylglycerol Receptor: Precursor Preparation

Mahreen Ahsan[1], Krishna M. Donavalli[1], and Dennis H. Burns[1]
[1]Department of Chemistry, Wichita State University, Wichita, Kansas 67260, United States

Antimicrobial peptides have garnered interest as potential therapeutics to combat multidrug resistant bacteria. Through targeting the bacterial plasma membrane, antimicrobial peptides bind to phosphatidylglycerol (PG), the major anionic phospholipid found in bacterial membranes via Columbic interaction, followed by insertion into the membrane and killing the bacterial cell. However, antimicrobial peptides can be toxic, difficult and expensive to make, and in general exhibit low bioavailability; thus, the focus has shifted toward the development of small molecules that specifically bind to PG to disrupt the membrane. These small molecules serve as PG receptors that comprise Liptins, a new class of antibiotics. Previously prepared Liptin families that bind to PG head groups have displayed high bacteriostatic properties at low concentrations (1-4 μM). The observed antimicrobial effect makes plasma membrane more permeable which depolarizes the membrane with the aim to stop replication. Currently, a new Liptin family with a proprietary structure is undergoing precursor preparation. This structure is expected to similarly bind to PG and exhibit the same antimicrobial effects due to its commonality in binding pocket as the other Liptin families, but with a different direction movement. The precursors developed so far consist of the synthesis of bis-bromoanisole from bis-anisole via bromination and the transformation of pentane-1,5-diol into a THP ether-pentyl toluene sulfonate.

124. Conflicting or Collaborating Roles for Palladin and VASP in the Regulation of Actin Filaments

Sharifah Albraiki and Moriah R. Beck
Wichita State University, Wichita, KS

Metastasis is the most clinically significant step in cancer progression. Migration and metastasis are not fully understood, but it is clear that the actin cytoskeleton plays an essential role. Palladin is specifically involved in metastasis of cancer cells, but also co-localizes with actin stress fibers in normal cells. The 90kDa palladin isoform contains three Ig domains and one proline-rich regions. This proline-rich region has been shown to bind directly to the actin-regulating proteins VASP. In a previous paper, our lab showed that the Ig3 domain of palladin is the minimal binding site for F-actin. In this work we wanted to compare functions of the 90kDa palladin to the isolated actin binding domain. Here, we used our standard co-sedimentation assay to test the binding and bundling of 90kDa palladin to F-actin. We found that 90kDa palladin has a higher affinity in binding and bundling actin compared to the Ig3 domain. To understand the mechanism of action for how palladin can influence actin assembly, we used fluorescence spectroscopy to monitor pyrene actin polymerization. By using site-directed mutagenesis via PCR we were able to mutate the putative VASP binding site within the proline-rich region of the 90kDa palladin. We then examined binding between VASP and WT or mutant palladin using a pulldown assay and far Western blot. Both Palladin and VASP proteins are involved in the regulation of actin filaments and understanding the fundamental mechanism of these proteins will help us eliminate the progression of cancer invasion and metastasis.

125. Creating a Liver-Specific ERα Knockout Mouse (LERKO)

Geneva X. Allen[1], Kelly N.Z. Fuller[1], John P. Thyfault[1].
[1.] Department of Molecular and Integrated Physiology, University of Kansas Medical Center, Kansas City, Kansas

Previous studies have shown that female mice are protected against hepatic steatosis (fatty liver, triglyceride accumulation), a disease that affects 30% of U.S. adults. Furthermore, estrogen withdrawal via removal of the ovaries causes a two-fold increase in liver triglycerides, a finding that is reversed when estrogen is given back. Therefore, we hypothesize that female protection against steatosis results from the action of estrogen through ERα, the most widely expressed estrogen receptor in the liver. Thus, the goal of this project was to create a liver-specific ERα knockout mouse (LERKO) for future investigations into the role of estrogen action and how it relates to hepatic steatosis risk. We obtained whole-body ERα floxed mice on a C57BL6 background, originally created by Dr. Stavros Manolagos. Once in our hands, we genotyped the mice from tail snips using an electrophoresis agarose gel to determine if the animals were floxed or wildtype for the ERα gene. Next, we used an associated adenovirus (AAV) delivery of Cre recombinase (IP injection) to knockout the floxed ERα gene specifically in the liver. Two weeks after injection, mice were sacrificed, and liver and skeletal muscle tissues were harvested. We confirmed through PCR analysis of liver transcript that only ERα and not ERβ was knocked out. Additionally, we confirmed via western blotting that ERα was only absent from the liver by measuring protein expression in both liver and skeletal muscle. Our results indicated that the Cre injection of floxed mice was successful in creating a LERKO mouse model.

126. K-INBRE Bioinformatics Core

Bioinformatics Core Personnel[1,2,3]
[1]Kansas State University, [2]University of Kansas and [3]University of Kansas Medical Center

The K-INBRE Bioinformatics Core supports bioinformatics infrastructure, research and training for all member institutions. We purchase software licenses and computational equipment, write scripts and create data analysis pipelines to support research programs, and develop bioinformatics content for courses, workshops, and individualized training. One of our major projects in 2019 was designing and teaching a bioinformatics module on bacterial genome sequence assembly and analysis for undergraduate and graduate students. The content for the module was based on our Fall 2018 faculty Bioinformatics workshop that we developed in collaboration with Dr. Kelly Thomas, Bioinformatics Core Director for New Hampshire INBRE, and Dr. Tom Platt, microbiology faculty member at Kansas State University. Working with faculty members from five K-INBRE institutions, we presented the content three times, in three different formats, to a total of 22 students. We will present lessons learned from these pilot course, based on our experiences as well as exit surveys of students and participating faculty members.

127. Observing Changes in Cellular Morphology via Digital Holographic Microscopy and Optical Scatter Imaging

Bijaya Basnet, Vincent Rossi, Dr. Long
Washburn University, Department of Physics, Wichita State University

Digital Holographic Microscopy (DHM) in combination with Optical Scatter Imaging (OSI) can be used to measure the angle of scattering buy subcellular components, thereby affording the ability to measure morphological changes in subcellular structures such as nuclei and mitochondria. We designed a pair of DHMs—a Digital Fourier Holographic Microscope (DFHM) and a Phase Shifting Digital Holographic Microscope (PSDHM)—in order to monitor morphological changes in mitochondria and nuclei via their scattered fields. In addition to the OSI application, both the DFHM and PSDHM can be used for 3D imaging. Both the DFHM and PSDHM are calibrated and tested using polystyrene microspheres of known diameters before cellular applications. We are specifically interested in witnessing changes in mitochondrial morphologies of breast cancer cells at the onset of apoptosis as a response to Photodynamic Therapy. In addition, we are interested in changes in nuclear morphologies as a result of extended exposure to laminar flow and how those changes can be associated with transdifferentiation.

128. Applying Machine Learning for Holographic Image Processing in order to Detect Morphological Changes in the Subcellular Environment

Nagendra Dhamala, Vincent Rossi
Washburn University

Applying the MATLAB Machine Learning Toolbox to holographic images, we train the system to predict particle sizes based upon their corresponding optical scattering patterns. This is often called a supervised machine learning methodology. Specifically, we use Support Vector Machine techniques to address significant parts of the problem. Ultimately, this technique will be used in combination with Digital Holographic Microscopy and Optical Scatter Imaging in order to automate the detection of morphological changes in mitochondria and nuclei as a response to external stimuli. We begin here however with an introduction to Machine Learning via a simulated experiment.

129. Synthesizing Picket Porphyrin Molecules that Bind to the Anionic Phosphatidylglycerol (PG) Head Group in the Bacterial Plasma Membrane

An Do, Bhusi Seelam, Richard Nguyen, Dennis Burns
Wichita State University, Chemistry Department

A multidrug-resistant microorganism, colloquially known as “superbug,” is a nationally and globally recognized threat, causing more than 2 million cases of infections and a staggering 23,000 deaths annually. The significant impact of superbug has yet to reach its climax, and its growing number of infections—such as pneumonia, tuberculosis, gonorrhea, and salmonellosis—has resisted the efficacy of antimicrobial drugs. Synthesizing a series of picket fence porphyrins, which are highly stable and versatile molecules capable of transforming their characteristics by replacing their substituents, may result in the arrival of a suitable synthetic antibiotic. In Dr. Burns’s laboratory, we are currently investigating the specific binding between the phosphatidylglycerol (PG), a notable feature of the bacterial anionic membrane, and the picket fence porphyrins. The final artificial anion receptors are then linked to membrane disruptors, resulting in synthetic antibiotics that can degrade the CDC Category A-C bacterial cell wall. Consequently, the successful integration of this process tentatively suggests that porphyrin receptors can exert potent control on bacteria through PG interaction, bringing us one step closer to procuring a cost-effective clinical candidate for future antimicrobial therapeutics.

130. Results of soil nutrient additions in restoring Magnesium concentration to common crop species

Riley G Drees, Mitchell J. Greer
Fort Hays State University Department of Biological Sciences

Lack of nutrition is a problem that faces numerous people across the world. One of the most important macro minerals that much of the world’s population is lacking is Magnesium. Research has shown the importance that Magnesium has on several key functions within the body and more importantly, the issues caused by the absence of sufficient amounts. Past and current research shows that Magnesium is critical in performing hundreds of activities within the body. Humans receive only a percentage of the minimum amount of Magnesium needed on a daily basis from their diets. It may seem unusual that a mineral so important is so scarce in our food sources when in actuality, it is one of the most abundant elements on Earth. This decline is due to the overuse of agricultural soil throughout the last two centuries depleting many of the nutrients within the soil and as a result, in our food. We aim to determine what results traditional and Magnesium fertilizers have on a few common food crops. This experiment will focus on nutrient content of edible grain in plants exposed to magnesium fertilizer, traditional fertilizer (nitrogen, phosphorus, and potassium), and both fertilizers in combination. Preliminary results showed an average Magnesium concentration of 0.2355% in control treatments, 0.2332% in NP treatments, 0.2347% in Mg treatments, and 0.2569% in NPMg treatments in wheat. By increasing the Magnesium content in common crop species, we hope to help counteract the magnesium deficiency in the diet of much of the world’s population.

131. The Role of Melatonin in Neuronal Development

Eric Ebert, Bailey Lampton, Mulin He, Stephen Fields
Emporia State University

Melatonin is a hormone secreted by the pineal gland of vertebrates. Melatonin initiates a signaling cascade that leads to regulation of the circadian rhythm. Additionally, melatonin participates in a wide variety of other pathways including blood pressure regulation, fetal development, and neuronal development. The role of melatonin in neuronal development has not been well-characterized. We are seeking to elucidate the effects of melatonin on neuronal development in the model organism *Caenorhabditis elegans*. Previously, a bioinformatics study was completed that identified several putative *C. elegans* melatonin receptors. The F59D12.1 gene, coding for a G protein coupled receptor, was chosen for the current study as there is a commercially available mutant. The mutant strain has been outcrossed five times to remove background mutations, with the goal of outcrossing the strain with wildtype *C. elegans* 7 times. We also crossed in a fluorescent GFP construct that is expressed in all cholinergic neurons. This fluorescent mutant strain will be used to study the development of cultured neurons in the presence of melatonin. Our preliminary data indicate that the lifespan of *C. elegans* is altered in the presence of melatonin, but the locomotion behavior appears unaffected by the mutation. We will confirm that the F59D12.1 gene encodes a melatonin receptor by examining the mutant’s behavior in the presence and absence of melatonin. The lack of melatonin-dependent behaviors will indicate a role in melatonin signaling.

132. The Influence of Land Use on the Pollen Diet of Honey Bee (Apis mellifera) Colonies in Ellis County, Kansas

Engel, Ryan P. and Brian R. Maricle
Department of Biological Sciences, Fort Hays State University, Hays, Kansas

Pollinators are integral to plant ecology as well as to worldwide food availability and security. Understanding how human-driven land use change impacts the nutrition of managed honey bees would be important in pollinator conservation efforts and contribute towards combating recent pollinator declines. The objective of this study was to determine critical sources of forage for honey bees across different land use types in Ellis County, Kansas, through pollen analysis and taxonomic identification. Replicate study colonies were placed in one of three separate land use types: Urban, Cropland, or Native/Semi-native. Pollen was sampled every 7-14 days throughout the growing season (April 1 to September 30). Pollen abundance (mass) and diversity (number of taxa) was measured to assess the diet of honey bees throughout an entire growing season to identify key resources and their availability throughout time and space. Pollen abundance varied greatly throughout time and across treatments, although Urban colonies consistently displayed higher pollen abundance and Cropland colonies consistently displayed reduced pollen abundance. Understanding the availability of floral resources in prairies could help to inform conservation decisions and improve understanding of community ecology in prairies. Overall colony health and overwintering success could also be improved with a better understanding of how land composition influences honey bee nutrition. Seeing deficiencies in pollen abundance or diversity collected from hives in a specific environment could indicate similar deficiencies for native pollinators in prairies.

133. Translational control of Hri2 eIF2α kinase during amino acid starvation

Whitney Pepper, Mackenzie Thornton, Ariana Cecil, Madelyn Hilgers, Izumi Asano, Haana Yamada, Cheyenne Brunkow, Carter Moravek, and Katsura Asano.
Division of Biology, Kansas State University, Manhattan, KS 66506

Cells' immediate response to stress insults is to shut down protein synthesis or "translation" by phosphorylation of eukaryotic translation initiation factor (eIF) 2α. The first eIF2α kinase discovered is heme-regulated inhibitor (HRI), which shuts down translation in response to heme-depletion during erythrocyte development. Its universal role in eukaryotes is to sense oxidative stress through heme. Thus, in the fission yeast *Schizosaccharomyces pombe*, HRI is activated in response to oxidative stress and represses translation. We previously found that it is also activated during amino acid starvation in the absence of the Gcn2 protein kinase. Gcn2 is the master regulator of translation during nutrient limitation including amino acid starvation and plays an important role in cancer metastasis in humans. Activated Gcn2 also phosphorylates eIF2α, thereby repressing general translation. Therefore, the previous finding suggests the cooperation of two kinds of eIF2α kinases with distinct ligands during more general stress arrangement. Here we report that Gcb2 regulates Hri2 translation depending on upstream open reading frames (uORFs) in its leader regions during amino acid starvation. Our luciferase reporter assays, in combination with site-directed mutagenesis altering uORFs, show that Hri2 translation is activated during amino acid starvation, and identified the uORF responsible for translational induction during the stress. These results indicate that Hri2 regulation is an integral part of the stress response program allowing a positive feedback of eIF2α phosphorylation. In addition, we report the results of further mutagenesis studies designed to reveal the mechanism by which uORFs regulates *hri2* translation during amino acid starvation.

134. Evolution of low and high virulence *Stenotrophomonas maltophilia* strains

Hanson, Tyler, Aakash Pandey, Thomas G. Platt
Kansas State University Division of Biology

Virulence is the degree to which pathogens harm hosts. Theory predicts, and experiments demonstrate, a trend for pathogens to evolve toward a state of intermediate virulence in order to balance the benefits of high virulence with the costs of killing the host. However, these studies focus primarily on obligate pathogens and omit key life history features of facultative pathogens. We used interactions between *Caenorhabditis elegans* hosts and one strain of *Stenotrophomonas maltophilia* to investigate how the ability of these pathogens to live independent of hosts influences the evolution of their virulence. To do this we used an experiment in which treatments varied in the degree of selective dynamics occurring in environmental reservoir environments as oppose to in host environments. In this experiment, only the pathogen was able to evolve as all hosts were naïve. We expected virulence to trend toward similar intermediate levels across all treatments; however, virulence decreased in all treatments. This data suggests that environmental selection results in loss of virulence for this strain irrespective of the presence and infection of hosts. To test this hypothesis, we are currently evolving various strains of *S. maltophilia* that vary in their virulence on *C. elegans* in unstructured *in vitro* populations. We predict that virulence will trend toward similar low virulence levels across all strains in these unstructured environments that lack hosts.

135. Selective Ubiquitination of Cellular Proteins by HSV-1 ICP0

Bailey Huser, Cindy Ly, Vamsi Mangena, Heba Mostafa, and David Davido
Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045

Herpes simplex virus 1 (HSV-1) impacts 80% of the global population and causes recurring cold sores, life-threatening encephalitis, and ocular blindness. HSV-1 has evolved to exploit host cellular processes to ensure replication and spread. One example is infected cell protein 0 (ICP0), an important viral protein that is a potent transactivator to all HSV-1 genes and functions as an E3 ubiquitin ligase. ICP0 allows the virus to hijack parts of the host ubiquitination pathway by targeting and marking specific cellular proteins with the post-translational modification, ubiquitin. It is hypothesized the ICP0-directed ubiquitination of these cellular targets alters their stability and/or physiological function to enhance lytic infection. The first goal of our project is to identify the cellular protein(s) and pathways targeted by ICP0 for ubiquitination. Using a proteomics based approach, we have identified several cellular proteins that appear to be specifically ubiquitinated by ICP0. These analyses identified the nuclear pore protein, RANBP2, and the actin-binding protein, FLNA, as likely targets of ICP0-mediated ubiquitination. The second goal of our project is to determine the roles RANBP2 and FLNA play in the HSV-1 life cycle. We propose that this modification of RANPB2 and FLNA by ICP0 alters the nuclear environment of host cells to facilitate HSV-1 replication. Currently, we are examining the ubiquitination and kinetics of RANBP2 and FLNA stability during infection. We expect these studies will elucidate at least one novel mechanism of how HSV-1 establishes a productive infection via ICP0's interaction with RANBP2 and FLNA.

Keywords: HSV-1, virus-host interactions, ubiquitination

136. Charcoal Rot Resistant Transgenic Soybeans

Kyla Jantz, Zurek Daniel Pittsburg State University Department of Biology

Charcoal rot fungus is the leading cause of crop failure in soybeans in Kansas. Despite causing millions of dollars in loss to Kansas farmers each year, there are currently no cost-effective methods to prevent this disease. The purpose of this work is to create a transgenic soybean plant that overexpresses the normal soybean gene, BOZO, that should provide resistance against charcoal rot. BOZO is a B-1,4 glucanase whose function is to cleave load bearing bonds in plant cell walls during growth. Prior experiments revealed that the protein encoded by this gene inhibits fungal growth, presumably due to cleavage of charcoal rot fungal cell walls as well. Currently, experiments are being done to clone the BOZO gene under the control of a strong inducible promotor into a transformation vector for plant transformation.

137. Reduction of functional content in volvocine genomes during the evolution of multicellularity

Berenice Jiménez-Marín[1], Antariksh Tyagi[1], Jessica B. Rakijas[1], Aakash Pandey[1], Erik R. Hanschen[2], Jaden Anderson[1], Tom G. Platt[1] , Bradley JSC Olson[1]
[1]Department of Biology, Kansas State University, Manhattan KS, USA (jimenezb@ksu.edu)
[2]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson AZ, USA

The evolution of multicellularity is a major transition in biology that has occurred independently in multiple lineages. The volvocine algae comprise an excellent system for addressing multicellular evolution because member species exhibit an apparent increase in developmental complexity ranging from unicellular to differentiated multicellular organisms. Furthermore, the genomes of key volvocine species show notable similarity, which allow for comparative approaches to search for candidate genes for multicellular evolution. Our detailed analysis of the evolutionary trajectory of all genes in key volvocine species reveals that with increases in organismal complexity, there is a statistically significant reduction of functional content in the genomes, including regulatory genes such as transcription factors and protein kinases. Developmental transcriptomic analyses of volvocine algae indicate that, while the genomes have a high degree of similarity and reduction in functional content, nearly half of the genes in the genomes have different developmental expression patterns. When protein-protein interactions were examined by 2D-PAGE, we found that altered developmental expression patterns lead to differential protein complexes as multicellular complexity increases. We suggest that major evolutionary changes may not always be driven by increases in genome content, but rather may be impacted by the formation of novel protein complexes that provide new function in more complex species, while less useful or orphan functions are quickly lost. We term the mechanism “combinatorial cooption”.

138. Determination of Heavy Metal Levels in Cosmetic Foundations & Mascaras Using ICP-AES Analysis

By: Josephine Johnson and Qiyang Zhang
Emporia State University Department of Physical Science

Heavy metals are a part of the composition of various commercially available cosmetic products, which are oftentimes used on a daily basis. Over time, heavy metals in these products can be harmful to skin cells and can accumulate in tissues and organs over time giving rise to various health problems. Thus, it is important to monitor the level of heavy metals in makeup products so that health and quality control standards can be ensured. Here a study was presented of the heavy metal contents of five different cosmetic foundations, (Loreal True Match, Sassy & Chic, LA Colors, Younique Mineral Touch, Makeup 4 Ever) and eight different mascaras (Maybelline Colossal Volum' Express, It Cosmetics Superhero, Maybelline Total Temptation, Maybelline Lash Sensational, Maybelline Great Lash, Voluminous Lash Paradise, Endlessly Beautiful, Tetyana Naturals 4D Silk Fiber). Many of these metals could be added as ingredients while others are inadvertent contaminants from the production process. Through the use of USGS sample preparation methods, acid digestion, and ICP-AES analysis, the thirteen samples were examined, and the presence of thirteen metal elements was detected. Three of the five samples contained varying levels of arsenic and aluminum. All eight of the mascara samples contained varying levels of iron, potassium, magnesium, and vanadium. Arsenic was detected in three of the eight samples with It Cosmetics Superhero exceeding the FDA limit and lead was detected in Maybelline Great Lash exceeding the FDA limit.

139. Discovery of Obscure Tyrosinate Fluorescence in Immunoglobulin Domain

Rachel Klausmeyer, Ravi Vattepu, Allan Ayella, Rahul Yadav, Joseph T. Dille, Moriah R. Beck
Chemistry Department, Wichita State University, Wichita, KS

An anomalous protein fluorescence phenomenon was revealed while investigating a mutation to an absolutely conserved tryptophan in an immunoglobulin (Ig) domain. Ig domains are the most prevalent protein domain structure and share a highly conserved folding pattern; however, this structural protein family also displays vast diversity in biological roles and tissue expression. While Ig domains vary significantly in amino acid sequence, they share a set of highly conserved residues in the hydrophobic core. In fact, one core tryptophan is absolutely conserved across all Ig domains. A mutation of this core tryptophan was identified in palladin from a pancreatic cancer cell line. Palladin is an actin binding and bundling protein with five Ig domains and plays an important role in cell adhesion and motility. We investigated the role of this tryptophan in palladin Ig domain structure, stability, and function. Our results from circular dichroism and NMR spectroscopy show that this tryptophan mutation disrupts folding. Denaturation experiments provide evidence that this mutation decreases stability. Removal of this conserved tryptophan also decreases palladin’s actin binding and bundling function. Similar to other tryptophan-free proteins, this mutant palladin domain displays a tryptophan-like fluorescence credited to an anomalous tyrosine emission at 345nm. Our results indicate that this emission originates from a tyrosinate that may be formed in the excited state by proton transfer to a nearby glutamyl residue. Furthermore, this study emphasizes the importance of tryptophan in protein structure stability and how tyrosinate emission contributions may be overlooked during the interpretation of fluorescence properties of proteins.

140. Advancement in the Synthesis of Dipyrromethanes with a β-Diazo Linkage to a Methoxycarbonylphenyl Group

Price Kramer and Sam Leung
Department of Chemistry, Washburn University

Photodynamic therapy (PDT) is an alternative cancer treatment technique that relies on the use of a photosensitizer and a radiation source to incite localized apoptosis in cancer cells. Photosensitizers used in PDT are usually porphyrin compounds, which excite triplet state oxygen into its singlet state when exposed to light within their absorption bands. Current photosensitizers on the market weakly absorb when exposed to light of ~620 nanometers. This study involves the synthesis a porphyrin compound that contains a methoxycarbonyphenyl group at the beta position of the porphyrin ring via a diazo linkage. It is expected to have stronger absorption bands located at longer wavelengths. The diazo-linked methoxycarbonylphenyl group increases the overall conjugation of the porphyrin ring, and the compound should theoretically produce an absorption spectrum at wavelengths over 620 nm. The synthesis for the porphyrin could be accomplished by a 2+2 addition of dipyrromethanes. At this point, only small amounts of the required precursor dipyrromethane have been synthesized. Due to difficulties in purification of the reaction mixture, there have been two different synthesis routes attempted.

141. De Novo Walleye Transcriptomic Database Pairing and Genetic Market Identification

Austin Landgraf, Dr. Nicholas Stewart, Dr. Yass Kobayashi
Fort Hays State University Biology Department
Experimental Biology 2020
Abstract

Fish species such as yellow perch (*Perca flavescens*), Atlantic salmon (*Salmo salar*) are major sport fish and commercially important in the United States. This has led to extensive research on their genome and transcriptome. Between sport and commercial fishing, the walleye (*Sander vitreus*) has been established as one of the most popular fish in North America. Despite their value, no annotated transcriptome database is currently available to the public. In order to expand our understanding of walleye transcriptome, we generated a transcriptome using the Next Generation Sequencing technology. The *de novo* transcriptome assembly from walleye brain tissue was constructed using RNA samples harvested from sexually mature male and female walleye (n=5 per sex). Sequence from the walleye transcriptome was aligned to the yellow perch transcriptome. Over 8400 fully assembled transcripts from the walleye transcriptome were identified. We were able to recover over 90% of the BUSCO genes associated with bony fish, indicating a quality assembly. Currently, we are expanding upon our original list of 8400 annotated transcripts by using possible open reading frames as well as further identifying partially assembled transcripts in our assembly. Our annotated transcriptome should provide tools for further research in physiology, evolutionary history, and ecology.

142. RNA Interference of the Unfolded Protein Response in *Acyrthosiphon pisum*

Jacob Lutgen[1], Jared Ridder[1], James R. Balthazor[2]
[1] Fort Hays State University Department of Biological Sciences,
[2] Fort Hays State University Department of Chemistry

Pea aphids, *Acyrthosiphon pisum*, are a significant pest to legumes, *Febaceae*, throughout the world, primarily due to the species serving as a vector to many *Febaceae* viruses. *A. pisum* is a model organism for biological investigation because its genome is sequenced and annotated. Current management of *A. pisum* includes use of insecticides and the introduction of natural predators. The utilization of RNA Interference (RNAi) presents an alternative, pest-specific targeting of *A. pisum* rather than introducing natural predators or insecticides that can affect a wide variety of species. X-Box Binding Protein 1 (XBP1) is involved in the regulation of the unfolded protein response (UPR) to promote proper folding in the endoplasmic reticulum (ER). RNAi targeting the XBP1 gene could result in the accumulation of misfolded proteins in the ER lumen, which downstream results in death of the cell via apoptosis. XBP1 has been shown to be a promising target in *A. pisum* and combination knockdown studies with other UPR targets are underway. Combined targeting of UPR mRNAs could be an affective method to kill pea aphids and potentially provide tangible economic benefit to farmers across the world.

143. CCR7 Expression in Pregnant Rat Uterine Tissue Prior to Embryonic Implantation

Erick McCloskey, Virginia Rider
Pittsburg State University Biology Department

CCR7 is a T-cell homing receptor and regulates aspects of adaptive immunity. It is hypothesized T-cell recruitment to the uterus pre-implantation suppresses the local immune response and therefore allows implantation of the embryo. This is because the embryo is semi-allogeneic, meaning it only shares some of the DNA with its mother, and therefore can be recognized as not “self” and targeted by the immune system for destruction. Rat uterine tissue was removed from pregnant Sprague Dawley rats at days 3-6 of pregnancy and fixed (4% paraformaldehyde) and sectioned (6 µm), then affixed to slides and assessed using immunohistochemistry. The expression of CCR7, showing up as brown reaction product against a methyl green counterstain, was localized in the glands and luminal epithelium in day 3, with some expression at the mesometrial end. On day 4, the expression was entirely localized to the luminal epithelium and glands. Day 5 uteri showed expression in the luminal epithelium as well as diffuse reactivity among the stromal cells of the uterus, and less expression among the glands. Implantation occurs on the evening of day 5. This suggests that, prior to implantation, expression of CCR7 in the rat uterus tissue changes. Theoretically, by tracking CCR7 expression in uterine tissue, the recruitment of T-cells can be tracked pre-implantation, visualizing the local distribution of CCR7-positive cells. Further research is needed to outline the roles of the CCR7-expressing cells in the implantation process, as well as identifying the specific types of cells expressing CCR7.

144. Investigation into the Efficacy of Heat Therapy to Improve Maximal Oxygen Consumption in Subjects ages 50 and older

Joshua Miller[1], Fengyan Deng[1], Brigid Flynn[2], and Paige Geiger[1]
[1] University of Kansas Medical Center - Department of Molecular and Integrative Physiology
[2] University of Kansas Medical Center - Department of Anesthesiology

Cardiovascular disease is the leading cause of death for men and women in the United States, affecting approximately 84 million people. Endothelial dysfunction, which presents as arterial stiffness, inflammation, and lack of vascular relaxation, is implicated in the development of cardiovascular disease. Improvements in vasoreactivity and vascular stiffness have previously been shown after 8 weeks (4-5 times/week) of heat treatment (HT) via hot water immersion in young subjects. The purpose of this study was to first establish safety and efficacy of HT in older subjects, and to determine if prior reports of improved endothelial function with heat treatment translate into improved cardiovascular function as measured by a maximal oxygen consumption (VO_{2max}) test. Healthy male and female subjects 50-76 years of age underwent 8-10 bouts of HT (45 min, 1°C increase in core body temperature) over a 14-day period. Systolic blood pressure (SYS) , diastolic blood pressure (DIA), mean arterial pressure (MAP), systemic vascular resistance (SVR), heart rate (HR), and cardiac index (CI) were measured using the Clearsight© (Edwards Lifesciences, Irvine, CA) monitor during hot tub sessions. In addition, cardiac ultrasounds were taken pre- and post-intervention to detect structural and/or functional changes to the heart. Importantly, HT was well-tolerated by all subjects and resulted in acute increases of HR and CI with acute reductions in SVR, SYS, DIA, and MAP. In only a small subject pool, we observed no changes in resting BP, MAP, SVR, HR, or CI. It is possible that a longer duration of HT may be needed to observe improvements in cardiovascular function on healthy, older subjects.

145. HD-Zip Transcription Factors: Key Regulators of Development and Metabolism

Seth T. Peery[1], and Kathrin Schrick[1,2].
[1]Department of Biochemistry and Molecular Biophysics, [2]Division of Biology, Kansas State University, Manhattan, KS

About 450 million years ago, land plants evolved from freshwater charophycean green algae. This was potentially facilitated by transcription factors of the homeodomain leucine-zipper (HD-Zip) family that are highly conserved across all land plants. These developmentally important proteins contain a steroidogenic acute regulatory (StAR) protein-related lipid transfer (START) domain that is required for transcriptional activity. HD-Zip proteins regulate epidermal differentiation and drought tolerance in several crops, including rice, cotton, and maize. Arabidopsis glabra2 (gl2) mutants that exhibit defective trichomes due to deficiency of a class IV HD-Zip transcription factor were transformed with two other HD-Zip family members in an attempt to rescue the mutant phenotype. Transformants with SpHDZ4, from the charophycean green algae Spirogyra pratensis, failed to rescue the defects despite high levels of nuclear expression. However, multiple lines transformed with Arabidopsis PROTODERMAL FACTOR2 (PDF2) displayed an unexpected gain-of-function phenotype: stunted growth and malformed leaf rosettes. START domain mutation pdf2Q267K was identified, but was determined by comparison with a PDF2 control to not be causative. A new mutation, pdf2K107E, was generated, changing a key amino acid in the third helix of the homeodomain necessary for DNA binding. Plants transformed with this construct showed nuclear expression and normal growth. This result indicates that the transcription factor, and the novel phenotype associated with its ectopic expression, is dependent on the homeodomain's DNA binding ability. Future work will focus on the function of ancestral transcription factors in the charophycean green algae Penium margaritaceum, representing the closest evolutionary lineage to land plants.
Contact: peery@ksu.edu, kschrick@ksu.edu
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146. Raman Spectroscopy Analysis of Redox States and Mechanism of Flavin Cofactors

Isaiah Powell, Manish Bhatta and Achut Silwal
Department of Chemistry, Washburn University, Topeka, KS

Abstract
Flavin cofactor such as FMN and FAD are involved in many biological pathways, specifically metabolic pathways for the ATP synthesis which play a vital role as electron carrier through their redox process. The redox states of flavin cofactors has connections with several health problems which makes the study of redox states of flavin cofactor the important field of the research. The several experimental and computational approaches have been used for the probing of redox states of flavin cofactors. To understand the involvement and contribution of flavin cofactors in different biological processes, probing and characterizing of the associated redox states of cofactors using powerful experimental approaches are fundamental and crucial, and the use of Raman spectroscopy has grown as a powerful tool in recent years. In this study, we have generated a number of typical redox states of FMN in Britton–Robinson buffer at different pH environments. The pH-dependent events of protonation, deprotonation, and electron transfer process of FMN are probed and characterized by surface-enhanced Raman spectroscopy (SERS) using silica coated silver nanoparticles (AgNP@SiO2) as SERS substrate. In addition to experimental SERS analysis, we, also use the density functional theory to identify the spectral signatures of the FMN redox-state sensitive Raman modes.

147. A domain of Adenomatous polyposis coli retained by colon tumors is intrinsically disordered, and can bind three β-catenin molecules

Aaron J. Rudeen[1], Minli Xing[2], Justin T. Douglas[2], Audrey L. Lamb[1] and Kristi L. Neufeld[1]
[1]Departmant of Molecular Biosciences, University of Kansas, Lawrence, KS
[2]Nuclear Magnetic Resonance Core Lab, University of Kansas, Lawrence, KS

The tumor suppressor Adenomatous polyposis coli (APC) is a large, multi-domain protein with many identified functions within the cell. The best characterized role of APC is to scaffold a protein complex that negatively regulates Wnt signaling via β-catenin destruction. This destruction is mediated by β-catenin binding to centrally located 15- and 20-amino acid (aa) repeat regions of APC. Cancers of the colon and rectum present with APC mutations in greater than 80% of cases. Most carcinomas with mutant APC express a truncated APC protein which retains the ~200-aa long 15-aa repeat region. We show that the 15-aa repeat region of APC lacks well defined secondary and tertiary structure. We used NMR to assign the backbone chemical shifts for the 15-aa repeat region. Using these assignments, we identified residues and regions that may be involved in structural elements required for molecular recognition and thus, targets for future therapeutic intervention. Additionally, our data demonstrate that the 15-aa repeat region of APC interacts with a maximum of three β-catenin molecules at one time when β-catenin is at a molar excess. This study expands our understanding of how the 15-aa repeat region of APC contributes to the regulation of β-catenin.

148. The Effect of Mitochondrial SOD-2 Knockout on the Dopaminergic Toxicity of MPP+ in C. elegans PD Model

Liana Savage, Gayani Wijegunawardena, Kandategе Wimalasena, PhD
Department of Chemistry, Wichita State University

Parkinson's Disease (PD) is a leading neurodegenerative disease, associated with the degradation of dopaminergic neurons in the substantia nigra of the mid brain. The disease is linked to both environmental and genetic factors. The Parkinsonian toxin 1-methyl-4-phenylpyridinium (MPP^+) serves as an important tool in modeling the environmental causes of PD. The proposed mechanism of action for MPP^+ is the inhibition of mitochondrial complex 1, leading to increases in the level of reactive oxygen species (ROS) and the subsequent activation of an apoptotic pathway. The production of ROS and the initiation of the apoptotic pathway is more prevalent in dopamine neurons than other cells. This difference in their susceptibility could be explained by the amount of antioxidant enzymes expressed in various cell lineages. In this study the deficiency of the antioxidant enzyme superoxide dismutase (SOD) in a novel strain of *Caenorhabtis elegans* and its effects on the toxicity of MPP^+ is investigated. The new strain is generated by cross breeding of BY250 (dopaminergic neurons are GFP labelled) and GA184 (mitochondrial SOD-2 is knocked out) animals. The proposed outcome would be that there is a positive correlation between the increased intracellular ROS production and MPP^+ susceptibility in SOD knockdown strain. In future studies the role of ROS in dopaminergic neuro-degradation in the SOD-2/GFP labelled strain (generated in the lab) will be investigated.

149. Genetic interactions between three transcription factors involved in NmrA-mediated repression in *Aspergillus nidulans.*

Sehgal, Chahat, Anna M. Brokesh, Cameron C. Hunter and Richard B. Todd.
Department of Plant Pathology, Kansas State University, Manhattan, Kansas.

Nitrogen is important for the survival of all living organisms. It plays a crucial role in growth and is used for the production of various compounds. The fungal regulatory system nitrogen metabolite repression regulates the expression of nitrogen utilization genes by repressing them when a preferred nitrogen source is available. In *Aspergillus nidulans*, nitrogen metabolism genes are regulated by the transcription activator AreA. During nitrogen sufficiency the transcriptional activity of AreA is repressed by interaction with the co-repressor NmrA. The *nmrA*Δ mutant shows partial derepression of AreA-regulated genes. A mutation in *areA* that deletes the 3' untranslated region (3'UTR) also leads to partial derepression of AreA-regulated genes. An *areA-3'UTR*Δ *nmrA*Δ double mutant shows full derepression indicating that these mutations affect independent controls of AreA activity. It has been proposed that the AN4210 Mediator Complex protein is needed for NmrA-mediated repression. To assess the genetic interactions between AN4210, *areA* and *nmrA* we crossed an AN4210Δ mutant to an *areA-3'UTR*Δ *nmrA*Δ double mutant and isolated progeny with different combinations of these mutations. To observe the phenotypic effects of combining these mutations we are performing growth tests that compare derepression. We expect the AN4210Δ mutant and the AN4210Δ *nmrA*Δ double mutant to show the same partial derepression and the AN4210Δ *areA-3'UTR*Δ double mutant to show full derepression. This work will help to show that AN4210 plays a role in NmrA-mediated repression.

150. Evaluation of differential *in vitro* culture dynamics and immunohistochemistry analysis results between human ovarian cancer and head and neck cancer cell lines

Soto, Sergio J., Nayan Shrestha, and William J. Hendry
Department of Biological Sciences, Wichita State University

To develop an improved human cancer patient "avatar" system using the hamster cheek pouch as the xenotransplantation site, we first focused on ovarian cancer cell lines and recently expanded our focus to head and neck cancer cell lines. Some anomalous observations noted between those two developmental foci prompted us to conduct some direct comparisons between the two types of cultured cell lines: 1) high grade serous ovarian cancer - Kuramochi (KUR) and TOV-112D (TOV); and 2) head and neck squamous cell cancer – CAL27 (CAL) and FaDu (FAD). When cell cultures were established and maintained on standard polystyrene plastic culture dishes, all four cell lines existed as monolayers of spread cells. For immunohistochemistry (IHC) analyses, we used trypsin harvest cells from the plastic dishes and transfer/seed them into 8-well chamber slides (20,000 cells per chamber) where the cells rest on glass surface. Under those conditions both ovarian cancer cell lines still existed as spread monolayers while both head and neck cancer cell lines existed as distinct colonies of cell clusters. Those differential histomorphological states likely influences the differential patterns of IHC signals we observe between the two types of cell lines. An example of that phenomenon is what we observed for the tumor suppressor gene product, p53. For that protein, signals were cytoplasmic in KUR cells and often nuclear in TOV cells whereas they were quite variable and not clearly localized in both the CAL and FAD cell clusters. The basis of such plastic vs. glass substrate-dependent differences needs further investigation.

151. Raman Spectroscopy Analysis of Biochemical States of Dopamine Neurotransmitters

Dipesh Thapa and Achut Silwal
Department of Chemistry, Washburn University, Topeka, KS

Abstract
The catecholamine neurotransmitters such as dopamine (DA), epinephrine (EP) and norepinephrine (NEP) play a vital role in the central nervous system (CNS) of mammals including human beings. The proper interactions of dopamine (DA) with dopamine transporter (DAT) or dopamine receptors (DARs) are crucial to maintain the homeostasis of DA in synaptic cleft and produce the proper magnitude of dopaminergic signals. The impairments of these interactions and the dysfunctionality of DA system are associated with various neurological disorders such as Parkinson's disease, ADHD, Alzheimer's disease and Schizophrenia. Beside the improper DA-DAT or DA-DARs interactions, the oxidized state of DA in dopaminergic cells is also connected to neurodegenerative diseases. Our research program uses the combined approach of SERS, electrochemistry, fluorescence microscopy and DFT calculation to probe and analysis of DA-DAT and DA-DARs interaction in living cells, and different redox states of DA in a buffer solution. The outcomes of our research could be useful to understand the mechanism of neurodegenerative diseases and gain a vision for the development of new therapeutic treatments.

Keywords: Dopamine (DA), Dopamine Transporter (DAT), Dopamine Receptors (DARs), Parkinson Disease (PD), Attention Deficit Hyperactivity Disorder (ADHD), Surface enhanced Raman Scattering (SERS).

152. Gcn2 eIF2α kinase mediates translational regulation through nucleotide motifs in the mRNA 5' untranslated regions.

Mackenzie Thornton[1], Yuji Chikashige[2], Hiroaki Kato[3], Thomas D. Baird[4], Whitney Pepper[1], Madelyn Hilgers[1], Ariana Cecil[1], Izumi Asano[1], Haana Yamada[1], Cheyenne Brunkow[1], Carter Moravek[1], Ronald C. Wek[4], and Katsura Asano[1].
[1] Division of Biology, Kansas State University, Manhattan, KS 66506; [2] Advanced ICT Research Institute Kobe, National Institute of Information and Communications Technology, Kobe, Japan; [3] Department of Biochemistry, Shimane University School of Medicine, Izumo, Japan; [4] Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202

Cellular signaling controls translation by modulating mRNA recruitment though *cis* regulatory elements or utilizing delayed re-initiation mechanisms involving upstream ORFs (uORFs). Gcn2 is the master regulator of translation during nutrient limitation. Activated Gcn2 phosphorylates eIF2α, thereby repressing general translation while activating translation of specific mRNAs with uORFs in its leader regions.
Here we performed genome-wide measurement of mRNA translation during histidine starvation in fission yeast *Schizosaccharomyces pombe*. Polysomal microarray hybridization experiments revealed 1779 candidate genes whose translation is up-regulated in Gcn2-dependent manner. The GO enrichment analysis and ribosome distribution analysis show that translation is reprogrammed to enhance chromatin component translation and repress ribosomal protein synthesis. The 1779 genes included *gcn5* and *hri2* shown to be required for growth under histidine starvation.
Interestingly, MEME enrichment analysis identified 5'-UGACGG-3' as a motif promoting translation during starvation. The luciferase reporter analysis using *hrd1* 5'UTR with such a motif demonstrated requirement for this motif in Gcn2-dependent translational control. While CU-rich elements in the 5'-proximal region are responsible for translational activation through mTOR protein kinase, to our knowledge, this is the first nucleotide motif found to be responsible for translational control by Gcn2.

153. Analysis on the Separation/Identification of Arabidopsis Lipid Classes

Lesly Torices[1]
[1]Kansas State University, Department of Biology, Kansas Lipodomics Research Center

This research is aimed to determine suppressing genes in Arabidopsis through the separation of lipids using a solid core reversed phase HPLC column due to its high efficiency. Through the range of lipids derived from extract, lipid classes can be separated in order to produce high chromatographic performance. This method exceeds the typical LC-MS method due to the optimized results, without unwanted secondary effects taking place.

154. Examination of Prevalence and Associated Behaviors of MRSA Carriers Among College Populations

Vázquez Plaza, N., Bhakta, B., and Carvalho, C. M. (Faculty Mentor)
Department of Biological Sciences, Fort Hays State University, Hays, Kansas

According to the CDC, Methicillin-Resistant Staphylococcus aureus (MRSA) commonly colonizes the human nasal passages. In the U.S., about 5-10% of the population are carriers of MRSA (Pettengill, 2019). This bacterium causes infections in different parts of the body, especially the skin, and often, the transmission can occur by direct or indirect contact with others. MRSA is problematic to treat in comparison to other strains of *Staphylococcus aureus* because it is resistant to most commonly used antibiotics, such as oxacillin. The purpose of this study is to find MRSA carriers among the FHSU undergraduate population and to assess the associated behaviors of those carriers. We hypothesized that the volunteers that are carriers of MRSA strain acquired it from their household settings and physical activities, such as sports, resulting in community-acquired MRSA. One hundred nasal samples were collected from undergraduate volunteer students in the biology department. The samples were analyzed following a series of microbiological testings to identify MRSA strains. Twenty-eight samples were found to be carriers of MRSA, and seventy-two samples were labeled as negative. The common factors of students carrying MRSA were people living with roommates, playing sports, and working in a clinical setting based on the volunteer surveys. In conclusion, there was a higher percentage of MRSA carriers among the college population due to, perhaps, their environmental surroundings and daily close contact. Future studies include increasing the sample size of undergraduate student volunteers to assess the correlation between behaviors and carriers using SPSS analytic tool.

155. Genetic Analysis of the Porcine Shadow of Prion Protein Gene

Zoey K. Wallis, Nicholas B. Stewart, and Eric T. Gillock
Department of Biological Sciences, Fort Hays State University

Prion diseases are a group of fatal, neurodegenerative diseases lacking a cure and have the potential to infect numerous species including the vast majority of livestock. A key event in development of prion diseases is the well-studied process of a conformational change of the cellular prion protein into a β-sheet rich form. However, there is a lack in understanding of the Shadow of Prion Protein gene and how this gene product contributes to prion disease pathogenesis. Previous research has showed the importance of the Shadoo protein (encoded by the Shadow of prion protein gene) in degrading the activity of the protease resistant remodeled Cellular Prion Protein. I hypothesize that since pigs are prion resistant—that is with no natural case of prions—Shadow of Prion Protein could play a major role in contributing to this lack of prion disease in pigs. By utilizing molecular and bioinformatic techniques, genomic sequences of the understudied Shadow of Prion Protein gene will be analyzed across various prion resistant and prion suspectable livestock species. Since it is an understudied gene, it is important to learn more about the composition of the Shadow of prion protein gene in species that are considered prion resistant to determine if it is a cause of prion resistivity.

156. Injection Voltage and Time with Poly(dimethylsiloxane) and Flow Gating in Capillary Electrophoresis

Jenika Wheeler, Antonio Zapata, and Qiyang Zhang
Department of Physical Sciences, Emporia State University, Emporia, Kansas 66801

Capillary electrophoresis (CE) is an innovative separation technique that utilizes an electrical field to separate the components of a mixture. In recent years, the flow-gated CE system has grown in popularity for biological and chemical analysis. A prototype CE system constructed with monolithic poly (dimethylsiloxane) (PDMS) in a cross-configuration injection system was combined with a flow-gated injection and tube-to-tube assembly. In this study, a sample of green food dye was used for visualization of injection. This procedure allowed injection voltage and time to be investigated and analyzed for optimization. Results revealed that an injection time of 0.3 to 0.5 seconds with an injection voltage of 4 to 10kV provided visible injection of the green food dye. Less than 2kV resulted in zero injection despite the longer injection time. The conclusion of this study found that both injection voltage and time are crucial to determine the quantity of injection. These two parameters complement each other. Longer injection time cannot compensate a lower injection voltage, which indicated a threshold voltage for injection into the separation capillary. Further study will include measuring the sample injection lengths in the separation capillary and will involve determining the relationship between the injection voltage threshold and the length of the capillary. This prototype offers a more efficient utilization of time during separation, reduces costs, decreases reagent consumption, and is more suitable for construction.

157. Genomic Annotation of 45,600 bp region of 3L chromosome in D. *takahashii*

Emily White, Washburn University Biology Undergraduate
Takrima Sadikot, Department of Biology Associate Professor

Drosophila melanogaster, the common fruit fly, is one of the most versatile model organisms in biology, used for a wide range of research centered around cellular mechanisms of complex eukaryotes. In this project we use the D. *melanogaster* genome as a reference to identify and annotate coding regions in Drosophila *takahashii*. This project is part of a collaborative effort of the Genomics Education Partnership (GEP). Here we use conservation-based analysis and various online tools such as the Drosophila melanogaster genome browser, Flybase, Gene Model Checker and Gene Record Finder to analyze a 45,600 base pair segment of chromosome 3L in *D. takahashii* labeled contig 42. The BLAST of D. *melanogaster* indicates the presence of genes ADP-ribosylation factor GTPase activating protein 3 (ArfGAP3), Maelstrom (mael), lethal(3)04053 (l(3)04053), CG32454, CG14451, CG11367, CG32452, CG14450, CG8745, Aminotransferase class-III (Oat) and CG11241 genes in the contig. The analysis of the *D. takahashii* sequence supports the BLAST results for the aforementioned genes except for CG8745 and Oat which are found in a different region in D. *melonogaste*r. Discrepancy in exon number and placement was resolved using the small exon finder and protein BLAST analysis to select the most conserved model. The results and the data collected from this research will be submitted for review and inclusion to the GEP data repository.

158. Optimization of a lead biosensor in E. coli

Caleb Winscott, Abigail Morgan, Phillip Harries
Department of Biology, Pittsburg State University

Lead contamination of water and soil poses a serious health risks to humans. Gas chromatography and atomic absorption spectrometry are typically used to detect lead in environmental (water and soil) samples. These detection methods require costly equipment and expertise. Here, we discuss preliminary attempts to optimize a lead biosensor generated as part of the international genetically engineered machine (iGEM) program in order to facilitate development of a cheaper and easier lead detection method. We will be working with a biosensor within the bacteria Escherichia coli (E.coli) which will allow for detection of lead by adding a soil or water sample into a growing culture of bacterium, making it a cost-effective method that takes minimal training. The biosensor consists of a plasmid containing a constitutively expressed repressor protein which binds to the promoter/operator unit from the chromosomal lead operon of Cupriavidus metallidurans. If lead is present, it binds to and inactivates the repressor, allowing for transcription of green fluorescence protein (GFP). Initial tests indicate that the amount of fluorescence produced by the biosensor is similar for all lead concentrations.

159. *In Vivo* N-Terminal Methylation of OLA1 Revealed by Target Profiling of NTMT1

Wei Wu,[1] Kaimin Jia,[1] Gaochao Huang,[1] Ruben Shrestha,[1] Bingbing Wu,[1] Yulan Xiong,[2] and Ping Li[1]
[1]Department of Chemistry, [2]Department of Anatomy and Physiology, Kansas State University, Manhattan, KS, 66506

N-Terminal methyltransferase 1 (NTMT1) catalyzes the N-terminal methylation of proteins with a specific N-terminal motif after methionine removal. Aberrant N-terminal methylation has been implicated in several cancers and developmental disease. NTMT1 is downregulated in patients with breast cancer and its loss promotes the growth and metastasis of breast cancer cells, suggesting that NTMT1 is a tumor suppressor. Conversely, NTMT1 is upregulated in colon cancer and has been proposed to function as a tumor promoter and oncogene. The NTMT1-knockout mice exhibited phenotype of premature aging. Thus far, more than 700 human proteins have been identified as potential substrates of NTMT1. Here we developed an activity-based protein profiling (ABPP) for NTMT1 using (*E*)-hex-2-en-5-ynyl-*S*-adenosyl-*L*-methionine (Hey-SAM). The compound synthesis achieved ≥ 98% yield based on SAH conversion, successfully eliminating HPLC purification normally associated with the preparation of SAM analogs. Together with motif sequence and signal peptide analyses, mass spectroscopy of ABPP revealed 72 potential NTMT1 targets, which include several previously confirmed ones and many unknowns. Target validation using normal and NTMT1-knockout HEK293FT cells generated by CRISPR-Cas9 demonstrated that Obg-like ATPase 1 (OLA1), an important regulatory protein involved in many protein-protein interactions, is methylated *in vivo* by NTMT1. Identification of reader proteins for OLA1 N-terminal methylation as well as the physiological function of this post-translational modification is still ongoing in our laboratory.

160. The Warning Signs of Lung Disease

Authors: Marrissa Raynesford[1], Mulu Bannister[1], Taylor Weidenhaft[1]
[1]Hays High School, USD 489

Our lungs are responsible for providing our cells with the oxygen that they need. Harmful pollutants in the environment can have an adverse effect on the health of the respiratory system. Research into this topic focused on causes, types of diseases, and prevention. The most common contributors to lung disease are discussed, including smoking, radon or asbestos exposure, and air pollution. The three main subdivisions of lung disease: airway, pulmonary/vascular, and lung tissue mention common diseases such as asthma and COPD. Prevention covers the key to avoid contracting most lung diseases along with the harsh reality that comes with diagnosis. Lastly, the spirometry testing of students and staff at Hays High School will be discussed.

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
1	Abraham	Kj	Langston	Associate Professor	kjabraham@langston.edu
2	Abrahamson	Dale	K.U. Medical Center	Chair, K-INBRE Committee, COBRE PI	dabrahamson@kumc.edu
3	Ackley	Brian	K.U. Lawrence	Faculty	bdackley@ku.edu
4	Adams	Rebecca	North Central Kansas Technical College	Undergraduate Student	adams21617@ncktc.edu
5	Adams	Rikki	Langston	Undergraduate Student	rikki.y.adams@langston.edu
6	Adem	Seid	Washburn	Associate Professor	seid.adem@washburn.edu
7	Afroz	Sahida	K.U. Lawrence	Graduate Student	sahida.afroz@ku.edu
8	Ahsan	Mahreen	Wichita State	Undergraduate Student	mxahsan@shockers.wichita.edu
9	Albraiki	Sharifah	Wichita State	Graduate Student	sealbraiki@shockers.wichita.edu
10	Ali	Umama	Wichita State	Undergraduate Student	uxali@shockers.wichita.edu
11	Alizadeh	Kasra	K.U. Lawrence	Undergraduate Student	k496a130@ku.edu
12	Allen	Geneva	K.U. Lawrence	Undergraduate Student	gxacollege@gmail.com
13	Ambardar	Medhavi	Fort Hays State	Assistant Professor	m_ambardar@fhsu.edu
14	Amrein	Jennifer	K.U. Lawrence	Graduate Student	jamrein@ku.edu
15	An	Chao	Kansas State	Research Assistant	anchao1995@gmail.com
16	Anderson	Briana	Langston	Undergraduate Student	briana.m.anderson@langston.edu
17	Anderson	Jaden	K.U. Lawrence	Graduate Student	jadena@ku.edu
18	Ansari	Irfan	Wichita State	Undergraduate Student	imansari@wichita.edu
19	Arastehnejad	Niloofar	Pittsburg State	Graduate Student	niloo.arasteh@gmail.com
20	Archer	Joshua	Langston	Undergraduate Student	joshua.arch27@gmail.com
21	Arukula	Ravi	Pittsburg State	Postdoc	ravigoud.iict@gmail.com
22	Asano	Katsura	Kansas State	Professor	kasano@ksu.edu
23	Asauskas	Ryan	Pittsburg State	Undergraduate Student	rasauskas@gus.pittstate.edu
24	Atkinson	Simon	K.U. Lawrence	Vice Chancellor for Research	satkinson@ku.edu
25	Austin	Crystal	K.U. Medical Center	Postdoc	caustin5@kumc.edu
26	Ayalew	Meron	Wichita State	Undergraduate Student	mtayalew@shockers.wichita.edu
27	Ayella	Allan	Washburn	Assistant Professor	allan.ayella@washburn.edu
28	Badawi	Yomna	K.U. Medical Center	Postdoc	ybadawi@kumc.edu
29	Balderrama	Jannet	Wichita State	Undergraduate Student	jxbalderrama1@shockers.wichita.edu
30	Balthazor	James	Fort Hays State	K-INBRE Campus Coordinator	jrbalthazor@fhsu.edu
31	Banerjee	Tuhina	Pittsburg State	Adjunct Instructor	tbanerjee@pittstate.edu
32	Bannister	Mulu	Hays High School	Night at the lab winner	20mbannister@usd489.com
33	Barnell	Jonathan	Washburn	Undergraduate Student	jonathanbarnell@gmail.com
34	Basgall	Erianna	Kansas State	Undergraduate Student	erianna@ksu.edu
35	Basnet	Bijaya	Washburn	Undergraduate Student	bijaya.basnet@washburn.edu
36	Bates	Janee	Haskell Indian Nations	Undergraduate Student	janee.bates@haskell.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
37	Beck	James	Wichita State	Associate Professor	james.beck@wichita.edu
38	Beck	Moriah	Wichita State	Associate Professor	moriah.beck@wichita.edu
39	Bhakta	Bhavisha	Fort Hays State	Undergraduate Student	b_bhakta2@mail.fhsu.edu
40	Bhandari	Subash	Wichita State	Undergraduate Student	sxbhandari3@shockers.wichita.edu
41	Bhatta	Manish	Washburn	Undergraduate Student	manish.bhatta@washburn.edu
42	Blanco	Gustavo	K.U. Medical Center	Professor, Kathleen M. Osborn Chair and Mentoring Core Director	gblanco@kumc.edu
43	Block	Jordan	Kansas State	Undergraduate Student	blocjord@ksu.edu
44	Boese	Lia	Fort Hays State	Undergraduate Student	liboese@mail.fhsu.edu
45	Bose	Jeffrey	K.U. Medical Center	Assistant Professor	jbose@kumc.edu
46	Bower	Elizabeth	K.U. Medical Center	K-INBRE Coordinator	ebower@kumc.edu
47	Brase	Emma	Kansas State	Undergraduate Student	ebrase@ksu.edu
48	Bretches	Morgan	Wichita State	Undergraduate Student	morgan.bretches@gmail.com
49	Brodsky	Christine	Pittsburg State	Assistant Professor	cbrodsky@pittstate.edu
50	Brown	Sue	Kansas State	University Distinguished Professor of Biology	sjbrown@ksu.edu
51	Budiardjo	Sandi	K.U. Lawrence	Postdoc	jbudi101@gmail.com
52	Burnett	Tim	Emporia State	Chair, Biological Sciences	tburnett@emporia.edu
53	Butcher	Thai	Pittsburg State	Undergraduate Student	tbutcher@gus.pittstate.edu
54	Cain	Mary	Kansas State	Professor	mecain@ksu.edu
55	Campbell	Megan	Kansas State	Undergraduate Student	megcampbell@ksu.edu
56	Carlson	Maci	K.U. Lawrence	Undergraduate Student	macicarlson@ku.edu
57	Carvalho	Claudia	Fort Hays State	Faculty	cmdasilvacarvalho@fhsu.edu
58	Cecenas	Osiel	Wichita State	Undergraduate Student	cecenas.osiel@gmail.com
59	Chakravarthi	V. Praveen	K.U. Medical Center	Postdoc	praghavulu@kumc.edu
60	Chapes	Stephen	Kansas State	Professor and Director, Johnson Cancer Research Center	skcbiol@ksu.edu
61	Chapin	Bridgett	Haskell Indian Nations	Professor of Environmental Science and Biology	bchapin@haskell.edu
62	Chapman	Heiata	K.U. Medical Center	K-INBRE Assistant Director	hchapman@kumc.edu
63	Christman	Matthew	Washburn	Undergraduate Student	matthew.christman@washburn.edu
64	Chung	Peter	Pittsburg State	Professor	pchung@pittstate.edu
65	Ciftci	Yusuf	Kansas State	Undergraduate Student	yciftci@ksu.edu
66	Clark	Matthew	Langston	Undergraduate Student	mattcl@langston.edu
67	Clifton	Ashley	Emporia State	Undergraduate Student	aclifto1@g.emporia.edu
68	Contreras	Miguel	Wichita State	Undergraduate Student	mxcontreras1@shockers.wichita.edu
69	Cook	Matthew	Washburn	Assistant Professor	matthew.cook@washburn.edu
70	Corbett	Bradley	Emporia State	Undergraduate Student	bcorbett@g.emporia.edu
71	Cordova	Andres	K.U. Lawrence	Undergraduate Student	andresc_ortiz@ku.edu
72	Cornelius	Joseph (J.T.)	Rockhurst University	Undergraduate Student	CorneliusJ@hawks.rockhurst.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
73	Cote	Paige	Kansas State	Undergraduate Student	Pecote@ksu.edu
74	Cross	Stacii	Langston	Undergraduate Student	staciicross@gmail.com
75	Crowell	Gaby	Emporia State	Undergraduate Student	gcrowell@g.emporia.edu
76	Dandreo	Lauren	K.U. Lawrence	Undergraduate Student	laurendandreo@ku.edu
77	Davido	David	K.U. Lawrence	Professor	ddavido@ku.edu
78	Denney	Carson	Washburn	Undergraduate Student	carson.denney@washburn.edu
79	Dhamala	Nagendra	Washburn	Undergraduate Student	nagendra.dhamala@washburn.edu
80	Dhar	Rik	K.U. Lawrence	Graduate Student	rdhar@ku.edu
81	Dible	Melissa	Fort Hays State	Graduate Student	Mrdible@mail.fhsu.edu
82	Dissanayake	Sunanda	Kansas State	Associate Dean of Graduate School	sunanda@ksu.edu
83	Dixon	Dan	K.U. Lawrence	Associate Professor	dan_dixon@ku.edu
84	Dixon	Grady	Fort Hays State	Professor and Interim Dean	pgdixon@fhsu.edu
85	Do	An	Wichita State	Undergraduate Student	axdo3@shockers.wichita.edu
86	Dogrammatzis	Christos	K.U. Medical Center	Graduate Student	cdogrammatzis@kumc.edu
87	Doyle	Dalton	Emporia State	Undergraduate Student	daltond003@gmail.com
88	Drees	Riley	Fort Hays State	Undergraduate Student	rgdrees@mail.fhsu.edu
89	D'Souza	Nicole	K.U. Lawrence	Undergraduate Student	nicole.dsouza@ku.edu
90	Ebert	Eric	Emporia State	Undergraduate Student	eebert1@g.emporia.edu
91	Ehling	Abby	K.U. Lawrence	University Administration	aehling@ku.edu
92	Elliott	Jorja	Fort Hays State	Undergraduate Student	jeelliott2@mail.fhsu.edu
93	Elliott	Rebekah	K.U. Lawrence	Research Assistant	relliott1@ku.edu
94	Elting	Rachel	K.U. Lawrence	Undergraduate Student	rachelelting@gmail.com
95	Engel	Ryan	Fort Hays State	Graduate Student	rpengel@mail.fhsu.edu
96	Ernst	Nicholas	K.U. Medical Center	Undergraduate Student	ernstn@hawks.rockhurst.edu
97	Fernando	Chamil	Emporia State	Undergraduate Student	cfernand@g.emporia.edu
98	Fields	Stephen	Emporia State	Assistant Professor	sfields1@emporia.edu
99	Finnigan	Gregory	Kansas State	Assistant Professor	gfinnigan@ksu.edu
100	Fleming	Sherry	Kansas State	Professor	sdflemin@ksu.edu
101	Franczak	Edziu	K.U. Medical Center	Undergraduate Student	franczake@hawks.Rockhurst.edu
102	Freitas	Nick	K.U. Medical Center	Undergraduate Student	nafreita@ksu.edu
103	Gamero	Pamela	K.U. Lawrence	Graduate Student	pgamerok@ku.edu
104	Gardner	Jamie	Kansas State	Research Assistant	jamieg09@vet.k-state.edu
105	Gardner	Stewart	Emporia State	Assistant Professor	sgardne4@emporia.edu
106	Ghosh	Anuradha	Pittsburg State	Assistant Professor	aghosh@pittstate.edu
107	Gillock	Eric	Fort Hays State	Professor	egillock@fhsu.edu
108	Gipper	Jordan	Kansas State	Graduate Student	jgipper@ksu.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
109	Goeckel	Megan	Kansas State	Undergraduate Student	mgoeckel97@ksu.edu
110	Goering	Jeremy	K.U. Medical Center	Research Assistant	jgoering2@kumc.edu
111	Gomez	Christian	K.U. Lawrence	Graduate Student	cgomez-2@ku.edu
112	Gong	Ying	Wichita State	Graduate Student	f938w972@wichita.edu
113	Goodrow	Emily	Emporia State	Undergraduate Student	egoodrow@g.emporia.edu
114	Gress	Joanna	Emporia State	Assistant Professor	jgress@emporia.edu
115	Grigsby	Ryan	K.U. Lawrence	Core Lab Director	ryan.grigsby@ku.edu
116	Gupta	Ram	Pittsburg State	Associate Professor	rgupta@pittstate.edu
117	Haas	Evan	K.U. Lawrence	Undergraduate Student	haas.evang@gmail.com
118	Habiger	Joshua	Kansas State	Undergraduate Student	jhabiger@ksu.edu
119	Hackett	Jennifer	K.U. Lawrence	Core Lab Director	jhackett@ku.edu
120	Hadjian	Arman	K.U. Lawrence	Undergraduate Student	arman.hadjian@gmail.com
121	Halimeh	Wyel	Wichita State	Undergraduate Student	halimehwyel@yahoo.com
122	Hall	Sonia	BioKansas	Non-profit professional	sonia@biokansas.org
123	Hanson	Tyler	Kansas State	Undergraduate Student	tlhanson@ksu.edu
124	Hao	Xinbao	K.U. Lawrence	Associate Researcher	haoxb@ku.edu
125	Hardwidge	Philip	Kansas State	Professor	hardwidg@vet.k-state.edu
126	Harries	Phillip	Pittsburg State	Associate Professor	pharries@pittstate.edu
127	He	Nan	K.U. Lawrence	Research Scientist	henan70@hotmail.com
128	Hendry	William	Wichita State	K-INBRE Campus Coordinator	william.hendry@wichita.edu
129	Hess	Paul	Fort Hays State	Graduate Student	pmhess2@mail.fhsu.edu
130	Higdon	Nathaniel	Fort Hays State	Graduate Student	nathaniel.higdon17@gmail.com
131	Hilgers	Madelyn	Kansas State	Undergraduate Student	madelynh@ksu.edu
132	Hill	Courtney	Kansas State	Undergraduate Student	courtneyhill@ksu.edu
133	Hillery	Kaitlynn	Fort Hays State	Undergraduate Student	kmhillery@mail.fhsu.edu
134	Hinds	Annie	Fort Hays State	Undergraduate Student	a_hinds@mail.fhsu.edu
135	Hober	Stephanie	Kansas State	University Support Staff	shober@vet.k-state.edu
136	Holt Jr	Stacy	Wichita State	Undergraduate Student	d987w662@wichita.edu
137	Hua	Duy	Kansas State	University Distinguished Professor	duy@ksu.edu
138	Huser	Bailey	K.U. Lawrence	Undergraduate Student	baileykh@ku.edu
139	Innes	Shelby	Emporia State	Undergraduate Student	sinnes@g.emporia.edu
140	Jabara	Selena	K.U. Medical Center	Photographer	sjabara@kumc.edu
141	Jain	Vedant	Pittsburg State	Undergraduate Student	vjain@gus.pittstate.edu
142	Jantz	Kyla	Pittsburg State	Undergraduate Student	kjantz@gus.pittstate.edu
143	Ji	Yan	Kansas State	Research Assistant	yanj@ksu.edu
144	Jimenez Marin	Leticia Berenice	Kansas State	Graduate Student	jimenezb@ksu.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
145	Johnson	Josephine	Emporia State	Undergraduate Student	jjohns75@g.emporia.edu
146	Jurgensmeier	Abby	Wichita State	Undergraduate Student	aljurgensmeier@shockers.wichita.edu
147	Kadarla	Vinay Kumar	Wichita State	Graduate Student	vxkadarla@shockers.wichita.edu
148	Kalamvoki	Maria	K.U. Medical Center	Assistant Professor	mkalamvoki@kumc.edu
149	Kanber	Duaa	Emporia State	Graduate Student	Dkanber@g.emporia.edu
150	Kandel	Rajesh	Washburn	Undergraduate Student	rajesh.kandel@washburn.edu
151	Khadka	Samiksha	Kansas State	Graduate Student	samiksha@ksu.edu
152	Khan	Shamir	Wichita State	Undergraduate Student	shamir.khan99@yahoo.com
153	Klausmeyer	Rachel	Wichita State	Graduate Student	raklausmeyer@shockers.wichita.edu
154	Klein	Robert	K.U. Medical Center	Vice Chancellor for Academic and Student Affairs	rklein@kumc.edu
155	Knoblauch	Samantha	Pittsburg State	Undergraduate Student	sknoblauch@gus.pittstate.edu
156	Kobayashi	Yass	Fort Hays State	Associate Professor	y_kobayashi@fhsu.edu
157	Kostner	Danica	Fort Hays State	Undergraduate Student	dlkostner@mail.fhsu.edu
158	Kramer	Price	Washburn	Undergraduate Student	price.kramer@washburn.edu
159	Kuna	Marija	K.U. Medical Center	Postdoc	mkuna@kumc.edu
160	LaForge	Joseph	Emporia State	Undergraduate Student	jlaforge@g.emporia.edu
161	LaGrega	Natasha	K.U. Lawrence	Undergraduate Student	natasha.lagrega@ku.edu
162	Lan	Lan	K.U. Lawrence	Postdoc	lan@ku.edu
163	Landgraf	Austin	Fort Hays State	Graduate Student	aelandgraf@mail.fhsu.edu
164	Lang	Angelica	K.U. Lawrence	Undergraduate Student	aelang@ku.edu
165	Lansdon	Patrick	K.U. Lawrence	Postdoc	patrick_lansdon@ku.edu
166	Leung	Sam	Washburn	Professor	sam.leung@washburn.edu
167	Lewis	Isabel	Kansas State	Undergraduate Student	icl@ksu.edu
168	Lewis	Sharon	Langston	Associate Professor	lewissa@langston.edu
169	Li	Ping	Kansas State	Associate Professor	pli@ksu.edu
170	Lingo	Joshua	Kansas State	Undergraduate Student	joshua.j.lingo@gmail.com
171	Long	David	Wichita State	Assistant Professor	david.long@wichita.edu
172	Loyd	Mayme	Kansas State	Undergraduate Student	loydmayme@gmail.com
173	Lundquist	Erik	K.U. Lawrence	Professor, Associate Vice Chancellor for Research	erikl@ku.edu
174	Lunte	Susan	K.U. Lawrence	Distinguished Professor	slunte@ku.edu
175	Lutgen	Jacob	Fort Hays State	Undergraduate Student	jdlutgen@mail.fhsu.edu
176	Luthi	Andrea	Emporia State	Assistant Professor	aluthi@emporia.edu
177	Macdonald	Stuart	K.U. Lawrence	Professor	sjmac@ku.edu
178	Maricle	Brian	Fort Hays State	Associate Professor	brmaricle@fhsu.edu
179	Maricle	Keri	North Central Kansas Technical College	Instructor	kmaricle@ncktc.edu
180	Markham	Skyler	Fort Hays State	Undergraduate Student	samarkham@mail.fhsu.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
181	Matoushaya	Calandra	Emporia State	Undergraduate Student	cmatoush@g.emporia.edu
182	Maxwell	Julianne	Haskell Indian Nations	Visiting Native Scholar Program Assistant	jmaxwell@haskell.edu
183	McAfee	James	Pittsburg State	Professor of Chemistry	jmcafee@pittstate.edu
184	McCloskey	Erick	Pittsburg State	Undergraduate Student	nmccloskey1@gmail.com
185	McCue	Spencer	Fort Hays State	Graduate Student	srmccue@mail.fhsu.edu
186	Meyer	Zachary	Fort Hays State	Undergraduate Student	zjmeyer3@mail.fhsu.edu
187	Miller	Joshua	K.U. Medical Center	Undergraduate Student	jmiller39@kumc.edu
188	Mims	MacKenzie	Langston	Undergraduate Student	mackenzie.mims@langston.edu
189	Monroe	Elissa	K.U. Medical Center	Photographer	emonroe@kumc.edu
190	Montezano	Daniel	K.U. Lawrence	Postdoc	montezano@ku.edu
191	Morey	Kinsey	Pittsburg State	Undergraduate Student	kmorey@gus.pittstate.edu
192	Morgan	Aaron	K.U. Lawrence	Graduate Student	a469m571@ku.edu
193	Morgan	Abigail	Pittsburg State	Graduate Student	abigail.morgan@gus.pittstate.edu
194	Morris	E. Matthew	K.U. Medical Center	Assistant Professor	emorris2@kumc.edu
195	Moyers	Macy	Fort Hays State	Undergraduate Student	mgmoyers@mail.fhsu.edu
196	Munsell	Sam	Wichita State	Undergraduate Student	munsesam@gmail.com
197	Musgrove	Chloe	Fort Hays State	Undergraduate Student	cmross@mail.fhsu.edu
198	Myers	Megan	K.U. Lawrence	Undergraduate Student	mjmyers@ku.edu
199	Nabors	Mariaelena	K.U. Lawrence	Undergraduate Student	mariaelena.nabors@ku.edu
200	Naidoo	Gnanambal	Langston	Associate Professor	g.naidoo@langston.edu
201	Nansel-Lantz	Sara	Fort Hays State	Graduate Student	scnansel@mail.fhsu.edu
202	Narayanam	Raghunath	Pittsburg State	Graduate Student	rnarayanam@gus.pittstate.edu
203	Neufeld	Kristi	K.U. Lawrence	Professor	klneuf@ku.edu
204	Niedens	William	Pittsburg State	Undergraduate Student	wniedens@gus.pittstate.edu
205	Nolan Jr.	Tyler	Wichita State	Undergraduate Student	txnolan@shockers.wichita.edu
206	Odle	Kendall	Langston	Undergraduate Student	kmodle97@gmail.com
207	O'Meara	Bryn	K.U. Lawrence	Undergraduate Student	bryn.omeara@ku.edu
208	Panchal	Nilamben	Pittsburg State	Graduate Student	ndpanchal@gus.pittstate.edu
209	Panovich	Peyton	K.U. Lawrence	Undergraduate Student	peypano@ku.edu
210	Paolillo	Vitoria	K.U. Lawrence	Postdoc	v136p507@ku.edu
211	Parker	Taybor	K.U. Lawrence	Graduate Student	parkertw@ku.edu
212	Passarelli	A. Lorena	Kansas State	Professor	lpassar@ksu.edu
213	Peak	Mandy	Pittsburg State	Associate Professor	mpeak@pittstate.edu
214	Peery	Seth	Kansas State	Undergraduate Student	sethpeery@gmail.com
215	Pepper	Whitney	Kansas State	Undergraduate Student	wmpepper@ksu.edu
216	Perera	Chamani	K.U. Lawrence	Core Lab Director	chamani@ku.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
217	Peterson	Alonzo	Langston	Dean School of Arts and Sciences	alonzfp@langston.edu
218	Phung	Sui Ching	K.U. Lawrence	Postdoc	suiching.phung@ku.edu
219	Platt	Thomas	Kansas State	Assistant Professor	tgplatt@ksu.edu
220	Pohl	Adrienne	Kansas State	Undergraduate Student	pohltwin2@gmail.com
221	Polk	Tabatha	Wichita State	Undergraduate Student	tlpolk@shockers.wichita.edu
222	Pomatto	Mary Carol	Pittsburg State	University Administration	mpomatto@pittstate.edu
223	Powell	Alexis	Emporia State	Assistant Professor	aapowell@emporia.edu
224	Powell	Isaiah	Washburn	Undergraduate Student	isaiah.powell@washburn.edu
225	Preet	Ranjan	K.U. Lawrence	Postdoc	rpreet@ku.edu
226	Pritchard	Michele	K.U. Medical Center	IGPBS Associate Director	mpritchard@kumc.edu
227	Pugh	Coleen	Wichita State	Dean of Graduate School & AVP for Research	coleen.pugh@wichita.edu
228	Radadiya	Priyanka	K.U. Medical Center	Undergraduate Student	p551r170@ku.edu
229	Raynesford	Marrissa	Hays High School	Night at the lab winner	21mraynesford@usd489.com
230	Reese	Derek	Emporia State	Undergraduate Student	dreese2@g.emporia.edu
231	Rice	Clinton	K.U. Lawrence	Postdoc	clinton-rice@ku.edu
232	Richt	Juergen	Kansas State	Director, CEEZAD	jricht@vet.k-state.edu
233	Rider	Virginia	Pittsburg State	K-INBRE Campus Coordinator	vrider@pittstate.edu
234	Rodriguez-Tirado	Felipe	K.U. Medical Center	Postdoc	frodrigueztirado@kumc.edu
235	Roettger	Paul	Langston	Undergraduate Student	Paul.roettger@langston.edu
236	Rossi	Vince	Washburn	Assistant Professor	vincent.rossi@washburn.edu
237	Ruan	Shaobo	K.U. Lawrence	Postdoc	ruanshaobo@ku.edu
238	Rudeen	Aaron	K.U. Lawrence	Graduate Student	aaron.rudeen@ku.edu
239	Rumi	Mohammad	K.U. Medical Center	Associate Professor	mrumi@kumc.edu
240	Saadi	Irfan	K.U. Medical Center	Associate Professor	isaadi@kumc.edu
241	Sadikot	Takrima	Washburn	Associate Professor	takrima.sadikot@washburn.edu
242	Saha	Subhrajit	K.U. Medical Center	Associate Professor	ssaha@kumc.edu
243	Salberg	Abigail	Kansas State	Undergraduate Student	asalberg@ksu.edu
244	Sandefur	Richard	Wichita State	Undergraduate Student	sandefurr@icloud.com
245	Santra	Santimukul	Pittsburg State	Associate Professor	ssantra@pittstate.edu
246	Saulsberry	Madyson	Langston	Undergraduate Student	madyson.as12@gmail.com
247	Savage	Liana	Wichita State	Undergraduate Student	ljsavage@shockers.wichita.edu
248	Scheid	Adam	K.U. Medical Center	Postdoc	ascheid@kumc.edu
249	Schmidt	Shaun	Washburn	Professor and Chair	shaun.schmidt@washburn.edu
250	Schmitt	Sarah	Wichita State	Undergraduate Student	snschmitt@shockers.wichita.edu
251	Schneegurt	Mark	Wichita State	Professor	mark.schneegurt@wichita.edu
252	Segaran	Devapriya	Pittsburg State	Graduate Student	dsegaran@gus.pittstate.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
253	Sehgal	Chahat	Kansas State	Undergraduate Student	chahat1@ksu.edu
254	Sharma	Madhulika	K.U. Medical Center	Assistant Professor	msharma3@kumc.edu
255	Shippy	Teresa	Kansas State	Bioinformatics Specialist	tshippy@ksu.edu
256	Shrestha	Nayan	Wichita State	K-INBRE Undergraduate Support Core Administrator	nayan.shrestha@wichita.edu
257	Silwal	Achut	Washburn	Faculty	achut.silwal@bgsu.edu
258	Simons	Kim	Emporia State	Associate Professor	ksimons@emporia.edu
259	Skinner	Nathaneil	Emporia State	Undergraduate Student	nskinne2@g.emporia.edu
260	Smith	Sierra	Fort Hays State	Undergraduate Student	sasmith15@mail.fhsu.edu
261	Soares	Michael	K.U. Medical Center	University Distinguished Professor	msoares@kumc.edu
262	Soto	Sergio	Wichita State	Undergraduate Student	ssoto3012@yahoo.com
263	Spiess	Colby	K.U. Lawrence	Undergraduate Student	colbyspiess08@ku.edu
264	Spradlin	Josh	Kansas State	Undergraduate Student	jsprad23@ksu.edu
265	Stanford	John	K.U. Medical Center	K-INBRE Program Director	jstanford@kumc.edu
266	Stephanie	Hober	Kansas State	University Support Staff	shober@vet.k-state.edu
267	Stewart	Nicholas	Fort Hays State	Assistant Professor	nbstewart2@fhsu.edu
268	Stopperan	Julia	K.U. Lawrence	Undergraduate Student	julia.stopperan@yahoo.com
269	Stricker	Michael	Fort Hays State	Undergraduate Student	mbstricker@mail.fhsu.edu
270	Sukthankar	Pinakin	K.U. Lawrence	Postdoc	pinakin@ksu.edu
271	Sun	Mingjing	Emporia State	Assistant professor	msun2@emporia.edu
272	Taber	Chuck	Kansas State	Provost and Executive Vice President	ctaber@ksu.edu
273	Tamura	Masaaki	Kansas State	Professor	mtamura@vet.ksu.edu
274	Thapa	Dipesh	Washburn	Undergraduate Student	dipesh.thapa@washburn.edu
275	Thomas	Brent	Emporia State	Dean, College of Liberal Arts & Sciences	rthomas2@emporia.edu
276	Thornton	Mackenzie	Kansas State	Undergraduate Student	mmthornt@ksu.edu
277	Todd	Richard	Kansas State	Associate Professor	rbtodd@ksu.edu
278	Tomlinson	Trey	Wichita State	Undergraduate Student	tdtomlinson1@shockers.wichita.edu
279	Torices	Lesly	Kansas State	Undergraduate Student	torices@ksu.edu
280	Tran	Dang	Kansas State	Undergraduate Student	dtran@ksu.edu
281	Tran	Pamela	K.U. Medical Center	Associate Professor	ptran@kumc.edu
282	Trump	Eric	Emporia State	Associate Professor	etrump@emporia.edu
283	Tye	Spencer	K.U. Lawrence	PREP Postbaccalaureate Scholar	tye.spencer.a@gmail.com
284	Umar	Zaid	K.U. Medical Center	Undergraduate Student	zoomar39@gmail.com
285	Unckless	Rob	K.U. Lawrence	Assistant Professor	unckless@ku.edu
286	Vasquez	Bryan	K.U. Lawrence	Undergraduate Student	b059v710@ku.edu
287	Vázquez Plaza	Natalia	Fort Hays State	Undergraduate Student	n_vazquezplaza@mail.fhsu.edu
288	Vediyappan	Govindsamy	Kansas State	Assistant Professor	gvediyap@ksu.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
289	Veerapandian	Raja	Kansas State	Postdoc	rveer001@ksu.edu
290	Veesart	Sarah	Pittsburg State	Undergraduate Student	sveesart@yahoo.com
291	Velasquez	Sarah	K.U. Medical Center	K-INBRE Communications Manager	svelasquez@kumc.edu
292	Vishwakarma	Vikalp	K.U. Lawrence	Postdoc	vikalp@ku.edu
293	Vontz	Alex	Kansas State	Undergraduate Student	avontz@ksu.edu
294	Waisner	Hope	K.U. Medical Center	Graduate Student	h721w416@kumc.edu
295	Wallace	Darren	K.U. Medical Center	Professor	dwallace@kumc.edu
296	Wallis	Zoey	Fort Hays State	Undergraduate Student	zoeywallis@hotmail.com
297	Ward	Joy	K.U. Lawrence	Associate Dean of Research and Professor	joyward@ku.edu
298	Ward	Robert	K.U. Lawrence	K-INBRE Campus Coordinator	robward@ku.edu
299	Warner	Adara	Kansas State	Undergraduate Student	adaraw@ksu.edu
300	Wei	Lanjing	K.U. Lawrence	Graduate Student	lanjingwei@ku.edu
301	Weidenhaft	Taylor	Hays High School	Night at the lab winner	20tweidenhaft@usd489.com
302	Welch	Danny	K.U. Medical Center	Professor	dwelch@kumc.edu
303	Welti	Ruth	Kansas State	Professor	welti@ksu.edu
304	Wenger	Luke	K.U. Medical Center	Research Assistant	lwenger3@kumc.edu
305	Werle	Michael	K.U. Medical Center	Dean of Graduate Studies	mwerle@kumc.edu
306	Wertz	Blaine	Fort Hays State	Graduate Student	btwertz2@mail.fhsu.edu
307	Wheeler	Jenika	Emporia State	Graduate Student	jwheele1@g.emporia.edu
308	White	Emily	Washburn	Undergraduate Student	white.e855@gmail.com
309	White	Taylor	Fort Hays State	Undergraduate Student	tawhite6@mail.fhsu.edu
310	Wiest	Tania	Emporia State	Undergraduate Student	tamw97@gmail.com
311	Wilson	Cole	Kansas State	Undergraduate Student	cole35@ksu.edu
312	Winscott	Caleb	Pittsburg State	Undergraduate Student	cwinscott@gus.pittstate.edu
313	Winter	Alexis	Fort Hays State	Undergraduate Student	alexwinter66@gmail.com
314	Winter	Lake	Kansas State	Undergraduate Student	lrwinter@ksu.edu
315	Winters	Bobby	Pittsburg State	University Administration	bwinters@pittstate.edu
316	Woosley	Hunter	K.U. Lawrence	Undergraduate Student	Hwoosley@ku.edu
317	Wright	Collette	K.U. Lawrence	Undergraduate Student	clwright99@ku.edu
318	Wright	Doug	K.U. Medical Center	Professor	dwright@kumc.edu
319	Wu	Wei	Kansas State	Graduate Student	gisswu@ksu.edu
320	Xiao	Hui	K.U. Lawrence	Postdoc	h981x263@ku.edu
321	Xiong	Yulan	Kansas State	Assistant Professor	Yulanxiong@ksu.edu
322	Xu	Liang	K.U. Lawrence	Professor	xul@ku.edu
323	Yanez	Darlene	K.U. Lawrence	Undergraduate Student	Darleneyanez1@gmail.com
324	Yang	Shang-You	Wichita State	Associate Professor	shang-you.yang@wichita.edu

Symposium Participants

#	Last Name	First Name	University	Identifier/Title	Email
325	Yao	Li	Wichita State	Associate Professor	li.yao@wichita.edu
326	Yeremuh	Rodaind	Langston	Undergraduate Student	yeremuhj@gmail.com
327	Yu	Jianzhong	Kansas State	Assistant Professor	Jianzhongyu@ksu.edu
328	Zahnter	Kearyn	Fort Hays State	Undergraduate Student	Ktzahnter@mail.fhsu.edu
329	Zapata	Antonio	Emporia State	Undergraduate Student	azapata@g.emporia.edu
330	Zbeeb	Hassan	Wichita State	Undergraduate Student	hzbeeb2@gmail.com
331	Zegar	Irene	Pittsburg State	Professor	izegar@pittstate.edu
332	Zhang	Qi	K.U. Lawrence	Graduate Student	q643z135@ku.edu
333	Zhang	Qiyang	Emporia State	Assistant Professor	qzhang2@emporia.edu
334	Zhang	Yan	K.U. Medical Center	Postdoc	yzhang8@kumc.edu
335	Zhang	Ying	Kansas State	Graduate Student	yingz@ksu.edu
336	Zhou	Qilong	Kansas State	Postdoc	qlzhou@ksu.edu
337	Zhu	Hongrui	K.U. Lawrence	Graduate Student	hongrui@ku.edu
338	Zimmerman	Katie	K.U. Lawrence	Undergraduate Student	kzimmerman199@ku.edu
339	Zimmerman	Lynn	Hays High School	Night at the lab sponsor	lzimmerman@usd489.com
340	Zueckert	Wolfram	K.U. Medical Center	Professor	wzueckert@kumc.edu
341	Zurek	Daniel	Pittsburg State	Professor	dzurek@pittstate.edu

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