MOLECULAR MECHANISMS OF DISEASE

Predoctoral Training Program

Annual Symposium

April 21, 2021

8:00 AM - 5:30 PM Nebraska Innovation Campus

Program Contacts

Director
Donald Becker
Charles Bessey Professor of Biochemistry
N258 Beadle Center
(402) 472-9652
dbecker3@unl.edu

Co-director
Edward Harris
Professor Biochemistry
N133 Beadle Center
(402) 472-7468
eharris5@unl.edu

Training Program Summary

The goal of the program is to develop outstanding new scientists who work in collaborative multi-disciplinary teams to research disease mechanisms using quantitative approaches that ultimately yield tangible strategies for prevention and therapy. Participants are highly qualified, motivated students with a strong interest in the underlying causes of human disease. The program provides a framework to encourage all students to assemble a broad knowledge base, actively seek research collaborations, produce an outstanding record of original published research, and develop presentation, proposal-writing, and leadership skills that position them for future excellence as independent researchers focused on mechanisms of disease progression.

Administrative Advisors

Ron Yoder
Associate Vice Chancellor, Institute of Agricultural and Natural Resources

Bob Wilhelm
Vice Chancellor for Research and Economic Development

Daniel Duncan
Executive Director of Nebraska Innovation Campus

Tim Carr
Dean of Graduate Studies

David Hage
James Hewett University Professor of Chemistry

Oleh Khalimonchuk
Susan Rosowski Professor of Biochemistry

Angie Pannier
Maxcy Professor of Agriculture and Natural Resources, Biomedical Engineering

Jennifer Wood
Professor, Animal Science

External Advisors

Leslie Poole
Professor, Biochemistry, Wake Forest School of Medicine

Melanie Simpson
Professor and Head**,** Molecular and Structural Biochemistry, North Carolina State University

Molecular Mechanisms of Disease Mentoring Faculty

Jiri Adamec, Biochemistry
Mitochondrial dysfunction biomarkers link to environmental stressors

Matt Andrews, School of Natural Resources
Induction and maintenance of hibernation in mammals

Jennifer Auchtung, Food Science and Tech
Microbiome-targeted therapies

Donald Becker, Biochemistry
Proline metabolism in health and disease, enzyme mechanisms of substrate channeling

David Berkowitz, Chemistry
Chemical biology and synthetic organic chemistry

Nicole Buan, Biochemistry
Contribution of methanogenic bacteria to gut function

James Checco, Chemistry
Neuropeptides and peptide hormones as cell-to-cell signaling molecules

Andrea Cupp, Animal Science
Role of VEGF in testis morphogenesis

Eric Dodds, Chemistry
Chemical glycobiology

Liangcheng Du, Chemistry
Discovering new anti-infective agents from *Lysobacter*

Catherine Eichhorn, Chemistry
RNA structural dynamics and intermolecular recognition

Rodrigo Franco, Vet and Biomedical Science
Signaling mechanisms and neurotoxicity

Jiantao Guo, Chemistry
Protein tyrosine-O-sulfation, molecular interaction probes

David Hage, Chemistry
Chromatographic automation of immunoassays

Ed Harris, Biochemistry
Liver endothelial scavenger receptor function; systemic clearance of heparin

Tomas Helikar, Biochemistry
Dynamics of molecular and cellular mechanisms, Immune systems

Nicole Iverson, Biol Systems Engineering
Delivery, monitoring and analysis of *in vivo* nanoparticles that act as biological sensors

Oleh Khalimonchuk, Biochemistry
Mitochondrial oxidative function and protein homeostasis in health and sickness

Sri Kidambi, Chem & Biomol Engineering
Tissue engineering, stem cells

Forrest Kievit, Biol Systems Engineering
Nanoparticle-based delivery vehicles for brain cancer and brain injury treatments

Jaekwon Lee, Biochemistry
Mechanistic insights in homeostatic copper acquisition and cellular metal detoxification

Yuguo Lei, Chem & Biomol Engineering
Cell biomanufacturing for disease studies and therapeutic applications

Colin Meiklejohn, Biological Sciences
Genetic basis of speciation and regulatory evolution between nuclear and mitochondrial genomes

Angela Pannier, Biol Systems Engineering
Non-viral gene delivery, synthetic extracellular matrices, protein-cell adhesion

Kurt Piepenbrink, Food Science and Tech
Glycoproteins in bacterial pathogenesis

Robert Powers, Chemistry
NMR metabolomics

Amanda Ramer-Tait, Food Science and Tech
Host-microbial interactions in inflammatory bowel disease

Wayne Reikhof, Biological Sciences
Lipid distribution and storage mechanisms

Seung-Hyun Ro, Biochemistry
Sestrins and TORC signaling in metabolism

Jay Storz, Biological Sciences
Functional genetic variation in high-altitude mammals and birds, hypoxia adaptation

Xinghui Sun, Biochemistry
Long non-coding RNA regulation of gene expression in obesity and disease

Alex Vecchio, Biochemistry
Catalytic asymmetric hydroboration and “green chemistry” synthesis

Molecular Mechanisms of Disease Mentoring Faculty-Continued

Bill Velander, Chem & Biomol Engineering
cGMP recombinant factor IX for IV and oral hemophilia B therapy

Rebecca Wachs, Biol Systems Engineering
Tissue engineering and biomaterials-based therapeutics

Mark Wilson, Biochemistry
Redox regulation of DJ-1 function

Jennifer Wood, Animal Science
Steroid hormone sensing and signaling

Dustin Yates, Animal Science
Metabolic fetal programming related to maternal stress

Limei Zhang, Biochemistry
Structural biology, Mycobacterium tuberculosis infectivity

Schedule of Events

WEDNESDAY, April 21, 2021

8:00 AM TO 5:30 PM

University of Nebraska Innovation Campus

8:00-8:45 AM Breakfast and Poster Set-Up

8:45-9:00 AM Welcome and opening remarks

Dr. Donald Becker, Program Director

9:00-9:50 AM MORNING PLENARY PRESENTATION (ZOOM)

SLIDO # 171203

Toward personalized microRNA therapeutics

Frank Slack, Ph.D. Professor
Director, HMS Initiative for RNA Medicine
Department of Pathology BIDMC Cancer Center/Harvard Medical School

9:50-10:35 AM PREVIOUS TRAINEES: 2019-2020

SLIDO # 621615

David Lillyman
DEVELOPMENT AND CHARACTERIZATION OF A RODENT MODEL OF DISC DEGENERATION WITH LOW BACK PAIN

Gloricelly Roman-Arocho
ENGINEERING FLUORESCENT PROTEINS THROUGH NON-CANONICAL AMINO ACID INCORPORATION FOR BIOSENSING AND IMAGING OF REACTIVE SPECIES

Katie Bidne
MURINE DIET-INDUCED MATERNAL OBESITY ALTERS PLACENTAL LYSOPHOSPHATIDYLCHOLINE PROFILES, LIPID STORAGE, AND THE EXPRESSION OF GENES ASSOCIATED WITH LIPID METABOLISM

10:35-11:35 AM SESSION I POSTER VIEWING (IN PERSON)

11:35-12:15 PM STUDENT PRESENTATIONS I: SENIOR TRAINEES

SLIDO # 199408

Morgan Cade
OPTIMIZATION OF A MOUSE MODEL TO INVESTIGATE THE EFFECTS OF *BIFIDOBACTERIUM INFANTIS* ON THE SEVERITY OF PEANUT ALLERGY

Amanda Miller
IDENTIFICATION, BIOSYNTHETIC MECHANISM AND BIOLOGICAL FUNCTION OF LYSOCHELIN IN *LYSOBACTER*

12:15-1:00 LUNCH (BOX LUNCH PROVIDED)

1:00-1:50 PM AFTERNOON PLENARY PRESENTATION (ZOOM)

SLIDO # 629688

SeXX affects immunity and outcomes of respiratory virus infections

Sabra L. Klein, Ph.D. Professor
Department of Molecular Microbiology and Immunology
Co-Director, Johns Hopkins Center for Women's Health, Sex, and Gender Research
Johns Hopkins Bloomberg School of Public Health

1:50-2:20 PM AFTERNOON PREVIOUS TRAINEES: 2018-2020

SLIDO # 345898

Alicia Johnson
CHANGES IN THE LIPIDOMIC PROFILE OF A KV1.1 KO MODEL OF EPILEPSY

Kari Heck - Zoom
BACTERIAL-DERIVED OUTER MEMBRANE VESICLES AS A NOVEL ORAL GENE DELIVERY SYSTEM

2:20-3:05 PM SESSION II POSTER VIEWING (VIA ZOOM)

SLIDO # 389418

3:05-3:20 PM SHORT BREAK

3:20-4:20 PM STUDENT PRESENTATIONS II: JUNIOR TRAINEES

SLIDO # 848289

Alejandra Agosto-Maldonado
FUNCTIONAL ALTERATIONS OF CXCR4 BY CHEMOKINE HETERODIMERS ASSISTED BY LONG-CHAIN THIOL-CONTAINING UNNATURAL AMINO ACIDS

Timothy Hackett
STIFFNESS INDUCES AGING-LIKE PHENOTYPIC CHANGES IN MICROGLIA

Darcy Cochran
UTLIZING MSI TO DETERMINE LIPID DISTRIBUTION IN A TBI MOUSE MODEL

4:20-5:10 PM AFTERNOON PLENARY PRESENTATION (ZOOM)

SLIDO # 511152

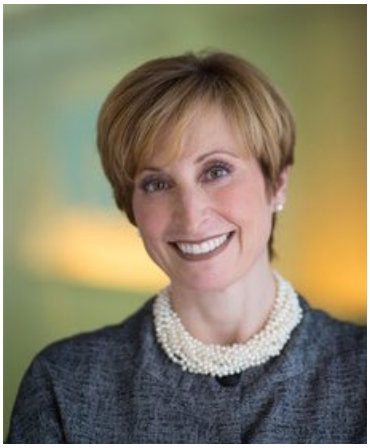
Open-Source Mass Spectrometry for Clinical Applications in Developing Countries

Dr. Abraham K. Badu-Tawiah Associate Professor
The Ohio State University
Department of Chemistry and Biochemistry

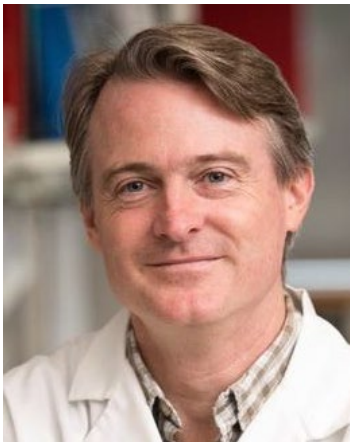
5:10-5:30 PM AWARD PRESENTATIONS

5:30-6:00 PM POSTER TAKE-DOWN

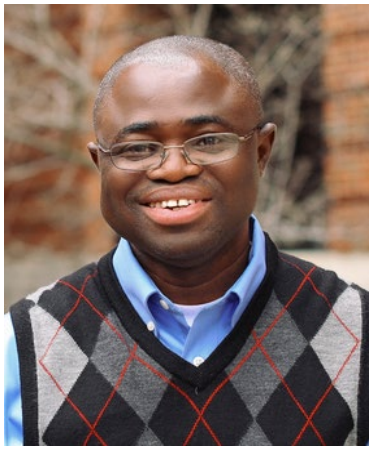
PLENARY SPEAKER PROFILES



Dr. Sabra Klein has a research program with an overarching goal to uncover the mechanisms that mediate how males and females differ in their immune responses to viral infection and vaccination. They hypothesize that sex chromosome complement and X-linked genes as well as sex steroids and signaling through sex steroid receptors are critical pathways modulating immune responses to viruses. They consider how immunological, hormonal, and genetic differences between males and females affect sex differences in susceptibility to viruses, including influenza, Zika, and SARS-CoV-2 viruses. They also study the mechanisms mediating altered immune responses and infectious disease pathogenesis during pregnancy. Her research indicates that non-pregnant females typically mount more robust immune responses than males, which can be beneficial for the efficacy of vaccines and clearance of viruses, but also can be detrimental by causing immunopathology.



Dr. Frank Slack's laboratory is at the forefront of the small RNA revolution. They co-discovered the first human microRNA, let-7 and showed that it is a tumor suppressor that controls key cancer genes, such as RAS, MYC and LIN28. They are developing let-7 and a second microRNA, miR-34 as novel cancer therapeutics with miR-34 already in Phase I clinical trials. They also proved that microRNAs act as key oncogenes and developed strategies to target these oncomiRs for cancer therapy. Their research also extends to discovery of additional novel small RNAs in development, cancer, aging and diabetes as well as identifying novel SNPs in the non-coding portions of the genome with an eye to identifying the next generation of actionable targets in cancer.



Dr. Abraham K. Badu-Tawiah's research group currently has three main research foci. First, diagnostic mass spectrometry (MS) - in this project they focus on the development of new MS tools for non-experts (e.g., surgeon, immunologist, neuroscientist, etc.), and to make MS data acquisition and interpretation easier for them. They are developing new chemical probes that enable mass spectrometric analysis of proteins, antibodies, DNA, and other disease biomarkers under ambient conditions. Second, the Badu-Tawiah group focuses on accelerated droplet reactions - here, they work to develop new tools to study organic/bio-molecular reactions using droplets as reaction vessels. They are interested in green catalysis, new reaction pathways, and the mechanism governing reactions in the micro-droplet environment. Finally, the third branch of their research looks at aerosol therapy - in which they utilize MS as a tool to characterize drug aerosols, and to improve on their efficacy.

Previous (2019-2020) MMoD Trainee Abstracts

Morning Session

DEVELOPMENT AND CHARACTERIZATION OF A RODENT MODEL OF DISC DEGENERATION WITH LOW BACK PAIN

David Lillyman[1], Tyler Miller[1], and Rebecca Wachs[1]
[1]Department of Biological Systems Engineering, University of Nebraska-Lincoln

One hundred million adults in the United States suffer from chronic pain with an estimated economic burden of $560-$635 billion. Of the one hundred million adults suffering from chronic pain, over one-third experience chronic low back pain (CLBP). CLBP is a complex problem with multiple etiologies, most involving chronic inflammation. The most common tissue perturbation associated with CLBP is degeneration of the intervertebral disc. Despite robust science supporting the link between disc degeneration and CLBP, little is known about development and systemic effects of painful degeneration. This lack of information is highlighted by the preponderance of animal models of disc degeneration which do not assess pain. Considering pain is the primary motivator for patients to seek medical attention, there is need for animal models with strong face and construct validity. To better understand disc degeneration, the emergence of pain, and systemic effects, we have developed an animal model of disc degeneration with low back pain. Our model induces disc degeneration via mechanical destabilization of the L5-L6 intervertebral disc in female, osseous mature, Sprague Dawley rats. To assess validity of our model, we measured pain hypersensitivity by assaying tactile allodynia and axial strength, spontaneous pain behavior using the open field test, and longitudinal signs of degeneration using μCT. We also probed the link between the microbiome and painful disc degeneration by employing 16s gene sequencing on fecal samples collected throughout the study. This model has enabled our group to observe the effects of disc degeneration on pain hypersensitivity, the microbiome, and overall animal well-being. Establishing this animal model has also served as an essential step towards testing therapeutics currently being engineered in the Wachs ONE lab.

Previous (2019-2020) MMoD Trainee Abstracts

Morning Session

ENGINEERING FLUORESCENT PROTEINS THROUGH NON-CANONICAL AMINO ACID INCORPORATION FOR BIOSENSING AND IMGAGING OF REACTIVE SPECIES

Gloricelly M. Roman-Arocho[1], Wei Niu[2], and Jiantao Guo[1]

[1]Department of Chemistry, University of Nebraska-Lincoln
[2]Department of Chemical and Biomolecular Engineering, University of Nebraska-Lincoln

Fluorescent proteins (FPs) have been extensively used in biological research for the study of gene expression, protein function and trafficking, and protein-protein interactions. They have also been engineered to act as biosensing agents to detect intracellular signaling molecules and other small molecule metabolites often associated with the progression of diseases. Although FPs have been engineered extensively to obtain novel functionalities, they are traditionally modified using the 20 canonical amino acids. Thus, limiting the number of functional groups available to design and construct proteins. The expansion of the genetic code through the incorporation of non-canonical amino acids (ncAAs) allows us to introduce amino acids containing novel functional groups to develop FPs with improved or new chemical and photophysical properties. We aim to develop reaction-based biosensors by modifying key residues in the photoconvertable FP, mEos2. Our approach entails the introduction of ncAAs that contain chemical functional groups that modify the ability of FPs to fluoresce and have high reactivity with the analyte of interest. Upon interaction with the analyte, the light-emitting capabilities of the FP is restored or altered. Herein we report the development of a FP-based sensors through the incorporation of p-boronophenylalanine in the chromophore-forming Tyr66 position for the detection of hydrogen peroxide (H2O2). Under the presence of micromolar concentrations of H2O2, our “turn-on” sensor undergoes a dark-to-green change, followed by red photoconversion once irradiated with 405 nm wavelength. This design represents the generation of the first reaction-based photocontrollable FP for hydrogen peroxide detection, and future super-resolution imaging.

Previous (2019-2020) MMoD Trainee Abstracts

Morning Session

MURINE DIET-INDUCED MATERNAL OBESITY ALTERS PLACENTAL LYSOPHOSPHATIDYLCHOLINE PROFILES, LIPID STORAGE, AND THE EXPRESSION OF GENES ASSOCIATED WITH LIPID METABOLISM

K.L. Bidne[1], A.L. Rister[2], A.R. McCain[1], B.D. Hitt[3], E.D. Dodds[2], J.R. Wood[1]

[1]Department of Animal Science, University of Nebraska – Lincoln
[2]Department of Chemistry, University of Nebraska – Lincoln
[3]Department of Statistics, University of Nebraska – Lincoln

Dyslipidemia is a characteristic of maternal obesity and previous studies have demonstrated abnormalities in fatty acid oxidation and storage in term placentas. However, there is little information about the effect of pre-pregnancy obesity on placental lipid metabolism during early pregnancy. The objective of this study was to determine the relationship between lipid profiles and markers of metabolism in placentas from obese and lean dams at mid-gestation. Mice were fed a western (WD) or normal (ND) diet and lysophosphatidylcholines (LPCs) and/or phosphatidylcholines (PCs) were measured in dam circulation and placenta sections using liquid chromatography-tandem mass spectrometry and mass spectrometry imaging, respectively. In WD dam, circulating LPCs containing 16:1, 18:1, 20:0, and 20:3 fatty acids were increased and 18:2 and 20:4 were decreased. In WD placenta from both sexes, LPC 18:1 and PC 36:1 and 38:3 were increased. Furthermore, there were moderate to strong correlations between LPC 18:1, PC 36:1, and PC 38:3. Treatment-, spatial-, and sex-dependent differences in LPC 20:1 and 20:3 were also detected. To identify genes that may regulate the diet-dependent differences in placenta lipid profiles, the expression of genes associated with lipid metabolism and nutrient transport was measured in whole placenta and isolated labyrinth using ddPCR and Nanostring nCounter assays. Several apolipoproteins were increased in WD placentas. However, no differences in nutrient transport or fatty acid metabolism were detected. Together, these data indicate that lipid storage is increased in mid-gestation WD placentas, which may lead to lipotoxicity, altered lipid metabolism and transport to the fetus later in gestation.

Previous (2018-2020) MMoD Trainee Abstracts

Afternoon Session

CHANGES IN THE LIPIDOMIC PROFILE OF A KV.1.1 KO MOUSE MODEL OF EPILEPSY

Alicia Johnson[a], Ryan A. Grove[a], Deepak Madhavan[b], Cory H.T. Boone[a], Camila Braga[a], Hannah Kyllo[b], Kaeli Samson[e], Kristina Simeone[e], Timothy Simeone[e], Tomas Helikar[a], Corrine K. Hanson[f] and Jiri Adamec[a,1]

[a]Department of Biochemistry, University of Nebraska-Lincoln, Lincoln NE, 68588
[b]Department of Neurological Sciences, University of Nebraska Medical Center, Omaha, NE, 68198
[c]Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, CZ
[e]Department of Pharmacology, Creighton University School of Medicine, Omaha, NE 68178
[f]College of Allied Health Professions, University of Nebraska Medical Center, Omaha, NE, 68198

One percent of the world's population has been diagnosed with epilepsy, a neurological disorder characterized by seizures, or the hypersynchronous neuronal activity. One-third of patients are resistant to traditional AEDs, or have medically refractory epilepsy (MRE). The ketogenic diet, a high fat low carbohydrate diet currently serves as one of the few treatments for MRE. The KD is suggested to function through improved metabolism and mitochondrial function. Potassium channels have served as a viable drug target for AEDs as they may have roles in metabolic sensing and controlling excitation in neurons. The *Kcna1,* which encodes the Kv1.1 channel, knockout model of epilepsy was used to explore changes in the lipidomic profile in both hippocampal and cortical tissue via FT-ICR/MS. Distinct metabolic profiles were observed with significant ($p<0.05$) features in the hippocampus being increased, and those in the cortex being decreased. A partition ratio analysis, the balance of a lipid species between the two regions, showed that most metabolites tended to be increased upon Kv1.1 knockout. Metabolites in hippocampal tissue were commonly upregulated, which suggests seizure initiation in this region. Specifically, mitochondrial impairment is implicated by the increase in cardiolipin which is localized in the mitochondrial membrane. Altogether, our study finds that the observed changes in the lipidomes of the hippocampus and cortex indicate impairments in structural integrity and synaptic transmission.

Previous (2018-2020) MMoD Trainee Abstracts

Afternoon Session

BACTERIAL-DERIVED OUTER MEMBRANE VESICLES AS A NOVEL ORAL GENE DELIVERY SYSTEM

Kari Heck[1], Amanda E. Ramer-Tait[2], Angela K. Pannier[1,3]

[1]Department of Biological Systems Engineering – University of Nebraska-Lincoln
[2] Department of Food Science and Technology – University of Nebraska-Lincoln
[3]Department of Surgery and Mary and Dick Holland Regenerative Medicine Program and Center for Drug Delivery and Nanomedicine – University of Nebraska Medical Center

Non-viral gene delivery via the oral route is a desirable delivery method due to the high rate of patient compliance, ease of administration and large cellular surface area conditions within the gastrointestinal tract. Applications for non-viral oral gene delivery include nucleic acid-based vaccination and gene therapy. To overcome challenges associated with oral gene delivery, we are developing a novel delivery system by loading bacterial-derived outer membrane vesicles (OMVs) with plasmid DNA to create DNA-loaded OMV nanocarriers (DNA-OMV NCs). OMVs are produced by blebbing of the outer membrane of gram-negative bacteria and have been shown to cross the mucosal barrier within the intestine and facilitate immune modulation after oral administration. Optimization of the electroporation loading process showed electroporation voltage and DNA: OMV ratio influenced DNA loading efficiency into OMVs, and the highest DNA loading at 31.8% ± 10.9% was achieved using a 1:2 DNA: OMV ratio. DNA-OMV NCs were able to mediate transfection of HEK 293T cells and Caco2 intestinal epithelial cells in vitro. In addition, DNA-OMV NCs showed low cytotoxicity when applied to Caco2 cells and were able to effectively protect loaded DNA from degradation when incubated in simulated gastric fluid. Together, these data suggest that DNA-OMV NCs are a promising new delivery platform for oral gene therapy capable of transfecting cells and protecting DNA cargo through the gastrointestinal tract.

Current Senior MMoD Trainee Presentations
Morning Session

OPTIMIZATION OF A MOUSE MODEL TO INVESTIGATE THE EFFECTS OF *BIFIDOBACTERIUM INFANTIS* ON THE SEVERITY OF PEANUT ALLERGY

Morgan Cade[1,3], Tasneem Ali[1,3], Anthony Juritsch[2,3], Kristin Beede[2,3], Robert Schmaltz[2,3], Bethany Henrick[4], Amanda Ramer-Tait[2,3]

[1]School of Biological Sciences, University of Nebraska–Lincoln, USA
[2]Department of Food Science and Technology, University of Nebraska–Lincoln, USA
[3]Nebraska Food for Health Center, Lincoln, Nebraska, USA
[4]Evolve Biosystems, Davis, California, USA

The incidence and prevalence of pediatric peanut allergy are increasing rapidly, with over 1.2 million children in the US having peanut allergy. Results from recent studies suggest a link between food allergies and changes in the gut microbiota and have subsequently led researchers to specifically hypothesize that interactions between gut microbes and early immune programming influence the development of food allergies in young children. *Bifidobacterium infantis* is a highly abundant member of the infant gut microbiota and is capable of metabolizing all human milk oligosaccharides, which are otherwise indigestible by the neonate. Preliminary data suggests this bacterium is also able to induce expansion of CD4+CD25+Foxp3+ regulatory T cells (Tregs) through a yet undefined mechanism. Tregs function to maintain oral tolerance and prevent immune responses against ingested food antigens by inhibiting antigen presentation and T helper 2 cell expansion. We hypothesize that *B. infantis* can limit the severity of anaphylaxis in a mouse model of peanut allergy through induction of Treg-mediated oral tolerance. To investigate the effects *B. infantis* on oral tolerance and food allergy, we established and optimized a mouse model of peanut allergy where the mice harbor a human infant microbiome lacking *Bifidobacteria*. Our results demonstrated that sensitization with lower doses of peanut protein, in combination with cholera toxin as a mucosal adjuvant, produced the most robust anaphylactic response in the mice. Strong anaphylactic responses were correlated with elevated levels of plasma IgE and decreased body temperatures following a challenge with a high dose of peanut protein. Future studies will use this new model to test if the development and/or severity of peanut allergy is attenuated following administration of *B. infantis* with or without human milk oligosaccharides and linked to changes in the abundance and/or function of Treg cells.

Current Senior MMoD Trainee Presentations
Morning Session

IDENTIFICATION, BIOSYNTHETIC MECHANISM AND BIOLOGICAL FUNCTION OF LYSOCHELIN IN *LYSOBACTER*

Amanda Miller, Yongbiao Zheng, Shanren Li, Richard Bell, Eric Dodds, Liangcheng Du

Department of Chemistry - University of Nebraska-Lincoln

Antibiotic resistance has become a constant threat to human health. The discovery of new antibiotics with novel modes of action is an urgent and continual need. *Lysobacter* is a genus of Gram-negative gliding bacteria that have been utilized as biocontrol agents because of the bacteria's ability to produce potent natural products and lytic enzymes. We recently identified a natural product, named lysochelin, from *Lysobacter enzymogenes* OH11. Structurally, lysochelin contains two units of 2,3-dihydroxybenzoic acid (2,3-DHB) linked together by spermidine. DHB is a common building block of iron-chelating siderophore, and spermidine is a known signal molecule in *Lysobacter*. Lysochelin provides a unique opportunity to understand how the bacteria may simultaneously sense (through spermidine) and control (through 2,3-DHB) the iron homeostasis in the cells. Indeed, our result showed that lysochelin has iron-chelating activity. We also identified the gene cluster responsible for the biosynthesis of lysochelin and proposed a biosynthetic pathway for lysochelin. Interestingly, the deletion of genes encode arginine decarboxylase and SAM decarboxylase eliminated lysochelin production in *L. enzymogenes* OH11. These two genes are known to play a key role in spermidine biosynthesis. Additionally, we were able to develop a Mass Spectrometry Imaging method to show the spatial distribution of lysochelin in colonies of *L. enzymogenes* OH11. Together, the research suggests that lysochelin may be a novel bioactive natural product involved in both iron homeostasis and cellular signaling.

Current Junior MMoD Trainee Presentations
Afternoon Session

FUNCTIONAL ALTERATIONS OF CXCR4 BY CHEMOKINE HETERODIMERS ASSISTED BY LONG-CHAIN THIOL-CONTAINING UNNATURAL AMINO ACIDS

Alejandra Agosto Maldondo[1], Wei Niu[2], and Jiantao Guo[1]

[1]Department of Chemistry, University of Nebraska-Lincoln
[2]Department of Chemical and Biomolecular Engineering, University of Nebraska-Lincoln

Chemokines and its receptors are crucial for the functionality of the immune system. However, their dysregulation can cause numerous diseases including cancer. The CXCR4/CXCL12 axis is overexpressed in cancerous cells, leading to cell growth, trafficking and migration, downregulation of tumor cell markers, and survival. CXCL12 can undergo both homo- and hetero-dimerization with other chemokine ligands, which leads to signaling alterations with the CXCR4 receptor. While the CXCL12 homodimer has been extensively studied, the functionality of the CXCL12-CXCL7 heterodimer and its interaction with CXCR4 has not been demonstrated in literature. Characterizing the structure and functionality of CXCL12 heterodimers is challenging due to continuous interference with monomers and homodimers. To overcome this limitation, “trapped” disulfide-linkage with cysteines can be used to maintain a stable chemokine heterodimer. Nonetheless, the short length of such disulfide linkage (~ 5.5 Å) makes the formation of the heterodimer difficult. Here, we report the synthesis and genetic encoding of a long-chain thiol-containing unnatural amino acid, 4-mercaptobutyriclysine (MBK), for the engineering of disulfide “trapped” CXCL12-CXCL7 heterodimer. This crosslinking strategy enables the formation of longer and flexible disulfide bonds (~ 11.0 Å) that can facilitate the formation of chemokine heterodimers. Hence, our method prevents possible conformational changes in their native fold and simplifies the selection of possible mutation sites. Furthermore, this approach can be used for the study of protein-protein or ligand-receptor interactions in living systems.

Current Junior MMoD Trainee Presentations
Afternoon Session

STIFFNESS INDUCES AGING-LIKE PHENOTYPIC CHANGES IN MICROGLIA

Timothy A. Hackett[1] and Srivatsan Kidambi[2]

[1]Department of Biochemistry, University of Nebraska-Lincoln
[2]Department of Chemical & Biomolecular Engineering, University of Nebraska-Lincoln

Aging is associated with tissue regeneration and significant loss of function in brain cells, including microglia. Microglia play a critical role in the primary immune response of the central nervous system which are highly active and motile cells interacting chemically and mechanically with their environment. Studies have shown that brain stiffness is significantly higher in aging brains compared to younger brains. The role of matrix stiffness as related to subtle but pivotal changes in microglia physiology and dysfunction is underexplored. The overall goal of our study is the development and implementation of a platform that enables the convergence of engineered cell microenvironments with the phenotypic and functional analysis of microglia. Using our innovative biomimetic model that allows modulation of substrate stiffness (2 kPa, 8 kPa, 15 kPa, and 25 kPa mimicking young, mid-age, middle-age, and aged brain tissue, respectively), we investigated the role of matrix stiffness in modulating microglial phenotype and function. We demonstrated that stiffness increased microglial proliferation and migration, elevated expression of inflammatory markers, and a heightened inflammatory M1 profile. We also demonstrated that microglia cultured on aging-like stiffness showed 1) increased ROS production, 2) impaired mitochondrial respiration, and 3) increased lipid droplet accumulation and total intracellular cholesterol. We observed a significant increase in oxidized glutathione (GSSG) in microglia cultured on aging-like stiffness compared to young brain stiffness. A similar effect has been observed in microglia isolated from aging murine models indicating a correlation to physiological conditions. Pretreatment with n-acetyl-cysteine (NAC) ameliorates stiffness-induced microglial growth and ROS generation. These data suggest a plausible mechanism that increased stiffness modulates microglial dysfunction. Understanding the impact of stiffness on microglial biology will provide significantly more nuanced data to intervene in an aging-related loss in brain function.

Current Junior MMoD Trainee Presentations
Afternoon Session

UTLIZING MSI TO DETERMINE LIPID DISTRIBUTION IN A TBI MOUSE MODEL

Darcy Cochran[1], Aria Tarudji[2], Forrest Kievit[2], and Eric Dodds[1]

[1]Department of Chemistry, University of Nebraska-Lincoln
[2]Department of Biological Systems Engineering, University of Nebraska-Lincoln

Traumatic Brain Injury (TBI) is a common head injury often caused through accidental means such as sports or car accidents. The primary or secondary symptoms of these range from mild to severe or fatal, and are often associated with causing lifelong dysregulation to brain function. There is no cure and past research has focused on finding ways to limit the extent of damage until patients can reach facilities equipped with more extensive treatment options. Through prior research, lipid levels have been shown to rise post-TBI and mice provided lipid treatments experienced better outcomes post-TBI compared to control groups. Thus, the neuroprotective properties of lipids contain a plethora of information yet to be elucidated regarding their role in TBI. The research discussed in this talk will cover mass-spectrometry imaging TBI in a mouse model. The biomolecules of interest are PC/LPC and PE/LPE as preliminary results show localization of the one-tailed species at the site of injury. Investigating the spatial profile of these lipids can provide valuable information about lipid degradation or accumulation post TBI that might be missed via standard quantification methodology. Challenges arise with method development for the MALDI-MSI imaging and future work would be focused in expanding the range of biomolecules to include lipids best ionized as negative ions.

Poster Presentation

In Person: Posters 1-15

1. *The Vaccinia B12 Pseudokinase Targets the Cellular VRK1 Kinase for Dysregulation During Poxviral Infection*

Linville AC[1,3], Rico AB[2,3], Wiebe MS[2,3]

[1]School of Biological Sciences – University of Nebraska Lincoln
[2]School of Veterinary Medicine and Biomedical Sciences – University of Nebraska Lincoln
[3]Nebraska Center for Virology – University of Nebraska Lincoln

2. *Analysis of N-Linked Glycoproteins via Two-Dimensional Fourier Transform on Cyclotron Resonance Mass Spectrometry*

Richard J. Bell V[1], Eric D. Dodds[1]

[1]Department of Chemistry – University of Nebraska Lincoln

3. *Ferrochelatase Performance is Supported by Mitochondrial Membrane Organization Complex*

Jonathan V. Dietz[1], Iryna Bohovych [1], Mathilda M. Willoughby [2,3], Robert B. Piel, III [4], James A. Wohlschlegel [5], Amit R. Reddi [2,3], Amy E. Medlock [4,6], Oleh Khalimonchuk [1,7,8]

[1]Department of Biochemistry, University of Nebraska, Lincoln, NE 68588
[2]School of Chemistry and Biochemistry and School of Biological Sciences, Georgia Institute of Technology
[3]Parker Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology
[4]Department of Biochemistry and Molecular Biology, University of Georgia
[5]Department of Biological Chemistry, University of California
[6]Augusta University/University of Georgia Medical Partnership
[7]Nebraska Redox Biology Center, University of Nebraska-Lincoln
[8]Fred & Pamela Buffett Cancer Center, Omaha, NE

4. *Novel PYCR Enzyme Function – Oxidation of L-thioproline Substrate*

Sagar M. Patel[1], John J. Tanner[2], Kyle M. Stiers[2], Donald F. Becker[1]

[1]Department of Biochemistry and Redox Biology Center University of Nebraska – Lincoln
[2]Department of Biochemistry and Chemistry, University of Missouri

5. ***Identification, Biosynthetic Mechanism and Biological Function of Lysochelin in Lysobacter***

Amanda Miller[1], Yongbiao Zheng[1], Shanren Li[1], Richard Bell[1], Eric Dodds[1], Liangcheng Du[1]

[1]Department of Chemistry– University of Nebraska Lincoln

6. ***Engineering Fluorescent Proteins through Non-Canonical Amino Acid Incorporation for Biosensing and Imaging of Reactive Species***

Gloricelly M. Roman-Arocho[1], Wei Niu[2], Jiantao Guo[1]

[1]Department of Chemistry– University of Nebraska - Lincoln
[2]Department of Chemical and Biomolecular Engineering – University of Nebraska - Lincoln

7. ***Characterization of Receptor Activity-Modifying Proteins present in GCPRs***

Noelle Waltenburg[1], James Checco[1]

[1]Department of Chemistry– University of Nebraska - Lincoln

8. ***Development of Single Walled Carbon Nanotube Platforms to Quantify Extracellular Nitric Oxide Concentration Changes with Cancer***

Ivon Acosta[1], Becca Francis[1], Carley Conover[1], Nicole Iverson[1]

[1]Biological Systems Engineering, University of Nebraska Lincoln

9. ***Labeling Membrane Bound Proteins Using Azo-Coupling Based Proximity-Induced Labeling Strategy***

Sheryl Sharma[1], Makayla Gill[1], James Checco[1]

[1]Department of Chemistry – University of Nebraska Lincoln

10. ***Stiffness Induces Aging-Like Phenotypic Changes in Microglia***

Timothy A. Hackett[1], Srivatsan Kidambi[2]

[1]Department of Biochemistry, University of Nebraska - Lincoln
[2]Department of Chemical and Biomolecular Engineering, University of Nebraska – Lincoln

11. ***Kokumi-Active Dietary γ-glutamyl Peptide in Modulating Vascular Inflammation***

Snigdha Guha[1], Kaustav Majumder[1]

[1]Department of Food Science and Technology – University of Nebraska Lincoln

12. *Changes in the Lipidomic Profile of a Kv1.1 KO Mouse Model of Epilepsy*

Alicia Johnson[1], Ryan A. Grove[1], Deepak Madhavan[2], Cory H.T. Boone[1], Camila Braga[1], Hannah Kyllo[2], Kaeli Samson[3], Kristina Simeone[3], Timothy Simeone[3], Tomas Helikar[1], Corrine K. Hanson[4] and Jiri Adamec[1]

[1]Department of Biochemistry, University of Nebraska-Lincoln
[2]Department of Neurological Sciences, University of Nebraska Medical Center, Omaha, NE
[3]Department of Pharmacology, Creighton University School of Medicine, Omaha, NE
[4]College of Allied Health Professions, University of Nebraska Medical Center, Omaha, NE

13. *Proteomics & Metabolomics Facility: Nebraska Center for Biotechnology*

Sophie Alvarez, Mike Naldrett, Anne Fischer, Felicia Phares, Lori Laucks

14. *Kinetic Characterization of Proline Utilization A (PutA) Using Proline Analogues*

Yizi Mao[1], Donald Becker[1]

[1]Department of Biochemistry – University of Nebraska – Lincoln

15. Analysis of ALS-associated mutation in the conserved mitochondrial metallopeptidase Oma1 in yeast genetic model

Iryna Bohovych, Andrew Harrahill, Jonathan Dietz, Oleh Khalimonchuk

Zoom Poster Session

Zoom: Posters 16-18

16. Analysis of Hydroxychloroquine and Chloroquine Binding to Glycoforms of Alpha1-Acid Glycoprotein by High-Performance Affinity Chromatography

Kyungah Suh[1], Sadia Sharmeen[1], David S. Hage[1]

[1]Department of Chemistry – University of Nebraska Lincoln

17. Rapid Fluorometric Sandwich Assay for Detection of COVID-19 Antibodies

Ashley G. Woolfork[1], Saumen Poddar [1], Kyungah Suh [1], Sazia Iftekhar [1], Susan Obvude [1], Sadia Sharmeen [1], Jacob Jones [1], Isaac Kyei [1], David S. Hage [1], Katelyn Dial [2]

[1]Department of Chemistry – University of Nebraska Lincoln
[2]Ni2o, Inc, Providence, RI

18. High-Throughput Screening of Small Molecule Inhibitors of the Proline Dehydrogenase

Lu Zhang[1], David Lee Kelly [2], John Tanner [3], Donald Becker [1]

[1]Department of Biochemistry – University of Nebraska - Lincoln
[2]Molecular Biology Core Facility, University of Nebraska – Lincoln
[3]Department of Chemistry, University of Missouri

Program funded by NIGMS Award 5T32GM136593