Abstract #1
Krista Armbruster, BMMB
Identification and Characterization of the Lipoprotein N-Acyl Transferase Gene in Low G+C Firmicutes
Krista M. Armbruster and T. C. Meredith

Approximately 1-5% of all bacterial proteins are lipoproteins, characterized by an N-terminal lipidated cysteine residue anchoring the globular domain to the surface of the cell membrane. Lipoproteins have a wide variety of cellular functions, including nutrient uptake, signal transduction, adhesion, and virulence. They also serve as potent ligands for Toll-like receptor 2 (TLR2), inducing a host innate immune response. Previously, lipoproteins were categorized as either diacyl or triacyl based on the absence or presence of an amide-linked fatty acid. In E. coli, addition of this N-acyl chain is catalyzed by the integral membrane protein lipoprotein N-acyl transferase (Lnt). Despite lacking an lnt sequence ortholog, recent structural analyses of lipoproteins from certain low-GC Gram-positive Firmicutes have demonstrated N-acylation, suggesting a unique lipoprotein biosynthetic pathway specific to these organisms. To determine the Enterococcus faecalis and Bacillus cereus gene(s) responsible for lipoprotein N-acylation, two genomic libraries were constructed and screened for Lnt-type activity. Our results revealed a single candidate protein demonstrating Lnt-type activity encoded by an unannotated sequence harboring a domain of unknown function. Genetic and functional characterization of this candidate protein will be presented.
Abstract #2
Alexis Baxter, Chemistry
Elucidating the Effects of Lipid Structure on Metal:Ion Complexes
Alexis J. Baxter, Tinglu S. Yang, Paul S. Cremer

The main goal of this project is to elucidate the effect of lipid head group structure on the formation of transition metal ion complexes, which has yet to been well explored. By utilization of supported lipid bilayers (SLBs), we have been able to probe the interactions of Cu2+ with lipids while varying head group structure. SLBs are two dimensional systems on a glass substrate containing lipids of interest as well as a fluorescently labeled lipid (Texas-Red DHPE) probe. This novel assay uses fluorescence microscopy to visualize the bilayer while the binding of copper (II) causes quenching of the fluorescence via resonance energy transfer. By better understanding metal:lipid interactions, one can provide insight as to how neurodegenerative diseases develop or worsen with hopes of developing more effective treatments in the future.
Abstract #3
Marcia Buanafina, Research Scientist
The impact of reduced cell wall feruloylation by expression of a fungal ferulic acid esterase on plant growth and the turn over
M. Fernanda Buanafina
Prashanti R Iyer
Erica A. Shearer
Marcia M de O. Buanafina

Plants rely on their cell walls to provide shape and strength to cells, to glue cells together, to give rigidity to the whole plant; all of which are of fundamental importance to plant growth and development1,2. Plant cell walls are complex structures composed of cellulose microfibrils, noncellulosic polysaccharides, proteins and phenolic substances3. An interesting feature of Arabinoxylans (AX) in grasses is the presence of ferulic acid4 which upon oxidative coupling by the action of peroxidases5 form deferuloyl bridges between formerly separated AX molecules. This creates intra- and intermolecular cross-links between the AX backbone. The specific role of feruloylation in various plant processes, however, has been established largely by indirect experiments.
Buanafina et al. (2010)6 have previously shown that intracellular targeted expression of Aspergillus niger ferulic acid esterase (FAE) to the apoplast or Golgi in tall fescue is an attractive strategy for decreasing the level of cell wall ferulates and diferulates, thereby targeting the inter-chain interactions as the cell wall polymers form. This situation has allowed us to test the direct role of ferulates on cell wall degradability 6-8.
In the present study we report on the role of cell wall feruloylation in two major processes in tall fescue: leaf growth and cell wall structure. This has been done by using transgenic plants where the level of cell wall ferulates have been specifically targeted by the expression of Aspergillus niger FAEA. By isolating cell walls from leaf blades at different developmental stages and quantifying the level of ferulates, arabinose and xylose, we have been able to associate the reduction in the levels of cell wall ferulates and sugar composition with changes in leaf morphology and in the growth rate of leaves, providing direct evidence for a significant impact of ferulates in leaf-elongation and cell wall sugar turnover, starting from very early stages of plant growth.
Abstract #4
Spencer Carran, Ecology
Unintended Consequences and the Paradox of Control: Managing Emerging Pathogens with Age-Specific Virulence
Spencer Carran
Matthew Ferrari
Tim Reluga

The intuitive response to an emerging outbreak is to halt, or at least reduce, transmission. However, in some circumstances, reducing overall transmission and incidence may be counterproductive from a public health perspective as public health interventions affect both the total level and the distribution of disease burden. Using the introduction of Zika Virus to Latin America as a case study, we consider the short- and long-term consequences of changes in transmission intensity for a pathogen with varying impacts among different age groups.
Abstract #5
Jasmine Caulfield, Neuroscience
Asthma during adolescence contributes to adult anxiety behavioral and neurobiological phenotype
Jasmine I. Caulfield, Mike J. Caruso, Rebecca A. Bourne, Sonia A. Cavigelli

Adolescence is a developmental period sensitive to perturbations that can affect adult neuronal and behavioral processes associated with internalizing disorders, like anxiety and depression. Asthma is a common chronic health challenge during adolescence, affecting 9% of U.S. adolescents, and often comorbid with anxiety and depression. However, little is known about the neurobehavioral impacts of this chronic adolescent challenge. Microglia, the resident immune cells of the brain, become activated in response to peripheral insult, and their over-activation has been implicated in neuropsychiatric disorder development. The mechanism underlying the comorbidity of asthma and internalizing disorders, and the involvement of microglia in this relationship, has not been established. The present study implemented a mouse model of chronic adolescent asthma to investigate the physiological properties that underlie the connection between asthma and anxiety, as well as the potential involvement of microglia. Three experimental groups, consisting of male and female BALB/c mice, were designed to examine the components of an asthma attack: (1) “Airway inflammation” via repeated house dust mite extract (HDM) exposure; (2) “Labored breathing” via methacholine (MCH) exposure; and (3) “Airway inflammation and Labored breathing” via both HDM and MCH exposure. As adults, MCH animals demonstrated an anxious phenotype, spending 30% less time on open arms of the elevated plus maze compared to non-MCH animals. These mice also had decreased serotonin transporter gene expression in the brainstem, which is consistent with findings supporting low serotonin transporter activity as a risk for developing anxiety. Additionally, MCH animals demonstrated elevated serotonin receptor 1a, mineralocorticoid, and Cd11b expression in the hippocampus compared to non-MCH mice (Cd11b expression indicates microglia activation). HDM-exposed mice exhibited 50% less basal circulating corticosterone levels compared to controls. Preliminary results for hippocampal gene expression of Cd11b revealed a sex difference in HDM-MCH animals, with females exhibiting higher levels. The results of the present experiment indicate that clinical symptoms of chronic asthma, particularly labored breathing, during adolescence lead to increased adult anxiety-related behavior and brain function.
Abstract #6
Xiaoheng Cheng, MCIBS
Fast and Robust detection of ancestral selective sweeps
Xiaoheng Cheng
Cheng Xu
Michael DeGiorgio
Abstract #7
Fengping Dong, Biology
Polycistronic tRNA and CRISPR guide-RNA enables highly efficient multiplexed genome engineering in human cells
Fengping Dong*, Kabin Xie*, Yueying Chen, Yinong Yang, Yingwei Mao

CRISPR/Cas9 has been widely used for genomic editing in many organisms. Many human diseases are caused by multiple mutations. The CRISPR/Cas9 system provides a potential tool to introduce multiple mutations in a genome. To mimic complicated genomic events in human diseases, such as multiple gene deletions or mutations, two or more small guide RNAs (sgRNA) must be introduced all together. This can be achieved by separate Pol III promoters in a construct. However, limited enzyme sites and the increased insertion size lower the efficiency to make a construct. Here, we report a strategy to quickly assembly multiple sgRNAs in one construct using a polycistronic-tRNA-gRNA (PTG) strategy. Taking advantage of the endogenous tRNA processing system in mammalian cells, we can efficiently transcript multiple sgRNAs driven by only one Pol III promoter. By a multiple sgRNAs within an all-in-one construct, we disrupt the deacetylase domain in multiple histone deacetylases (HDACs) in human cells simultaneously. We demonstrate that multiple HDAC deletions significantly affect the activation of the Wnt-signaling pathway. Thus, this method enables to efficiently target multiple genes and provide a useful tool to establish mutated cells mimicking human diseases.
Abstract #8
Hillary Figler, Immunology and Infectious Disease
Escherichia coliO157:H7 Shiga toxin production is enhanced by a phylogroup B2 E. coli isolate
Hillary Figler, Edward Dudley

*Escherichia coli* serotype O157:H7 is a food-borne pathogen. Symptoms vary greatly among individuals, even those infected with the same strain. It has been shown that the production of the main virulence factor, Shiga toxin (Stx), can increase due to other bacteria present in the environment. Shiga toxin is encoded on an inducible prophage, therefore, toxin expression is linked to phage induction. The hypothesis of this study was that commensal *E. coli* secrete Stx2 phage inducing molecules. This research aims to identify new secreted factors that can worsen O157:H7 infections.

To test this hypothesis, thirteen human *E. coli* isolates were screened for their ability to amplify Shiga toxin production. A co-culture protocol was used where an O157:H7 strain, PA2, was grown with the whole cells (16 hours) or cell-free supernatant (8 hours) of each isolate at 37°C. An *E. coli* strain designated 0.1229, produced 33.7 µg/mg of Stx2 when co-cultured with PA2 (compared to 6.1 µg/mg for PA2 alone) and 33.96 µg/mg of Stx2 when PA2 was grown in cell-free supernatant of 0.1229 (compared to 5.51 µg/mg for PA2 in LB media). Next, whole genome sequencing was employed to identify putative bacteriocin producing genes encoded in the *E. coli* 0.1229 genome, revealing one known bacteriocin, microcin B17. However, knockouts of the precursor gene *mcbA* and the entire operon MccB17, the mutant strains still increased Stx2 production by PA2 in the co-culture and cell-free supernatant assays however, suggesting molecule(s) other than microcin B17 are responsible for this phenotype. Treatment of the supernatant from 0.1229 with Proteinase K (1mg/ml) however decreased Stx2 production by PA2 approximately 20-fold in the cell-free supernatant assay (1.47 µg/mg of Stx2 was produced using Proteinase K treated supernatants).

From the data, we conclude that 0.1229 produces a Proteinase K sensitive molecule that is able to induce Shiga toxin production of O157:H7. *E. coli* O157:H7 infections are difficult to treat because antibiotics exacerbate the infection. This research could lead to alternative treatments, such as beneficial probiotics. Future experiments include identifying the sequence of the secreted molecule, potential receptors for this molecule on O157:H7, and determining the frequency by which commensal *E. coli* secrete similar molecules.
Abstract #9  
Waylon Hastings, Biobehavioral Health & Bioethics  
Differential CD8+ Response to Stress is Moderated by Exposure to Early-Life Adversity  
Waylon J. Hastings  
Susan Rutherford Siegel  
Idan Shalev  

Background: Exposure to early-life adversity (ELA) can result in long-term changes in physiological systems, which predispose individuals to develop physical and mental health problems in adulthood. Two systems that are highly susceptible to early life influences are the endocrine and immune systems, as evidenced by dysregulated stress reactivity and chronic inflammation characteristic of individuals with a history of ELA. While these changes are known to exist on the systemic level, little is known how the endocrine and immune system modulate specific subpopulations of cells. In this pilot study we investigated whether early-life adversity modulated immune cell dispersion in response to an acute laboratory stressor, compared to controls.

Methods: 12 young men (\(\mu = 21.25, SD = 2.3\)) participated in this pilot study. Using a validated screening instrument, we invited 6 participants who endorsed at least 3 early-life adverse events (‘risk group’), and 6 who confirmed zero (‘controls’). In a randomized within-subjects design, we induced acute stress (Trier Social Stress Test, TSST), and included a no-stress control condition one week apart. During both sessions, we obtained repeated measurements of physiological reactivity and peripheral blood mononuclear cells (PBMC), over a 4-hour window posttest. Independent fractions of PBMCs were further segregated into subclasses of cells (e.g. CD8+ cytotoxic T-cells) and analyzed with respect to total cell count and viability (live to dead ratio).

Results: In repeated measures models, a significant within-subjects effect was observed for salivary cortisol in response to the TSST, compared with a no-stress condition (F=5.88, p<.001, \(\eta^2 = .370\)). The risk group tended towards a higher cortisol response to the TSST (F=3.16, p=.106). While PBMC viability did not differ by group or session, between-group analysis revealed that CD8+ viability tended to be lower in the risk group as compared to control in the TSST session (t=1.89, p=.092), but higher in the no-stress session (t=1.77, p=.110). Furthermore, within-group analysis showed that the risk group tended to have lower CD8+ viability in the TSST session compared to the no-stress session (t=1.085, p=.306), while the control group had significantly higher viability in the TSST session compared to the no-stress session (t=2.711, p=.024). In repeated measures models, these differences translated to a significant within-subjects Session x Time x Status effect (F=3.80, p=.045, \(\eta^2 = .322\)). Across groups, CD8+ viability was negatively associated with gross cortisol production, wherein greater cortisol release was associated with decreased CD8+ viability (B = -0.515, p=.106).

Conclusions: These preliminary findings suggest that the immune impairment associated with early-life adversity may be mediated by greater cortisol reactivity during stress. This may involve a change in glucocorticoid receptor expression levels, which previous research has shown to modulate CD8+ cell processes and survival. Further investigation of gene expression in
a cell-type specific manner may provide more answers regarding the specificity of early programming of biological systems.
Abstract #10
Matthew Jensen, Bioinformatics and Genomics
Towards a complex genetic interaction model to explain phenotypic variability associated with rare copy-number variants
Matthew Jensen; Lucilla Pizzo; Dhruba Mayanglambam Singh; Janani Iyer; Qingyu Wang; Santhosh Girirajan

Rare copy number variants (CNVs) have been associated with several complex neurodevelopmental disorders, including autism, schizophrenia, intellectual disability (ID), and epilepsy. One class of rare CNVs is characterized by incomplete penetrance, phenotypic heterogeneity and the lack of known causative genes. For instance, the 16p11.2 deletion contributes to 1% of sporadic autism cases, and has been linked to cases of ID, epilepsy, obesity, and congenital malformations. We hypothesize that interactions between genes within rare CNVs (in cis) and at second sites outside the CNV (in trans) determine the neurodevelopmental phenotypes associated with these CNVs. In order to identify important interactions and underlying molecular pathways related to these CNVs, we performed RNA sequencing of 13 Drosophila knockdown models of orthologous genes within three rare CNVs (16p11.2, 16p12.1 and 3q29), and analyzed the differentially-expressed genes for enrichment of gene ontology, functional relevance to neurodevelopment, and association with neurodevelopmental disease.
Abstract #11
Yugian Jiang, Bioengineering
Human stem cells derived Sox17+ cells generate Isl1 heart progenitors
1. Yuqian Jiang (Department of Bioengineering, the Huck Institutes of The Life Sciences, the Pennsylvania State University)
2. Lauren Randolph (Department of Bioengineering, the Huck Institutes of The Life Sciences, the Pennsylvania State University)
3. Pei Wang (Assistant Professor of Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio)
4. Seung Kim (Department of Developmental Biology and Medicine (Oncology Division), Beckman Center B300, Stanford University School of Medicine)
5. Xiaojun Lance Lian (Assistant Professor of Department of Biomedical Engineering and Department of Biology, the Huck Institutes of The Life Sciences, the Pennsylvania State University)

A myocardial infarction (MI) transforms healthy and contractile myocardium into an akinetic, fibrotic tissue, resulting in a heart that cannot pump blood at full capacity. As human heart is one of the least regenerative organs in the body, myocardial infarction often leads to the development of heart failure. Recently, cell transplantation is considered to be one of the promising therapies for heart repair. Human stem cells derived cardiac progenitor cells (CPCs) are a good candidate to be transplanted due to their ability to proliferate and further differentiate into mature cardiomyocytes. Therefore, it is critical to identify the appropriate cardiac progenitor markers for isolation and purification of these cells for cardiac regenerative medicine. ISL1 was previously reported to be a cardiac progenitor gene and human ISL1+ cells can give rise to cardiomyocytes, smooth muscle and endothelial cell lineages, which are the three major cell types in the heart.

Here we reported a transient expression pattern of Sox17 during differentiation of human stem cells to cardiomyocytes. Using a human Sox17 APC-conjugated antibody, we demonstrated that 17.3% of cells expressed Sox17 on day 4 of differentiation. At the mRNA level, RNA sequencing results also showed that the peak of Sox17 mRNA expression was on day 4, which is earlier than the expression of ISL1 gene during cardiac differentiation. Furthermore, we used a Sox17-GFP knock-in human embryonic stem cell line to perform cardiac differentiation. On day 4, we then did live cell FACS sorting to collect both the pure population of Sox17+ and Sox17- cells. The replated Sox17+ cells gave rise to Isl1+ cells three days later, while the Sox17- cells showed minimal differentiation to Isl1+ cells. Our results contributed to the understanding of the atlas of cardiac progenitors during human heart development and diseases.
Abstract #12
Ehsan Mahdinia, ABE
Optimization of Bacillus subtilis natto Growth Parameters in Glycerol-based Medium for MK-7 Production in Biofilm Reactors
Ehsan Mahdinia
Ali Demirci
Aydin Berenjian

MK-7 is the key form of vitamin K used as a dietary supplement and its production revolves around Bacillus subtilis natto. Current fermentation strategies, which suggest static fermentations without aeration and agitation, can be problematic for large scale MK-7 production due to the design and scale-up issues. Therefore, applying biofilm reactors in this regard was proposed in this study, which utilizes both agitation and aeration without significantly interrupting MK-7 secretion. Preliminary studies for plastic composite support (PCS) and strain selections were presented in the past study. In this study, biofilm reactors were constructed using the selected PCS and B. subtilis natto strain for MK-7 production. Using response surface methodology (RSM), optimum growth parameters including temperature, pH, and agitation were determined in a glycerol-based medium. Results were presented in a statistical model (R²=0.90), leading to optimum growth conditions of temperature (35°C), agitation (200 rpm) and pH (6.58). Model predicted MK-7 concentration was validated and MK-7 concentrations produced in biofilm reactors (12.09±1.72) were 58% higher compared to the suspended-cell reactors (7.67±2.15), which is a critical step towards improved industrial scale productions.
Lipoyl synthase (LipA in bacteria, LIAS in humans, Lip5 in yeast) is a radical SAM (RS) enzyme that catalyzes the second step of the de novo biosynthesis of lipoic acid, an essential cofactor known for its prominent roles in energy metabolism and the degradation of certain amino acids, among others. All RS enzymes contain one [4Fe–4S] cluster typically coordinated by cysteine residues lying in a highly conserved Cx3Cx2C motif. This iron-sulfur (Fe–S) cluster supplies the electron during the reductive cleavage of SAM to generate a 5′-deoxyadenosyl 5′-radical (5′-dA•) capable of hydrogen abstraction from an unactivated carbon center. Lipoyl synthases contain a second [4Fe–4S] ‘auxiliary’ cluster that, though controversial, has been hypothesized to be the source of the attached sulfur atom. Consistent with this hypothesis, LipA typically catalyzes no more than one turnover in in vitro assays due to the obligate destruction of the auxiliary cluster. However, it is likely that a system exists to either repair the partially degraded cluster or insert a newly assembled [4Fe–4S] cluster into its active site to render it catalytic. Although Fe–S cluster assembly can occur spontaneously in vitro, it has been established that the in vivo process involves a complex network of proteins that is highly regulated to minimize formation of free iron and sulfide, which can be toxic to the cell. Herein, we provide evidence that E. coli NfuA, an Fe–S cluster-containing protein suggested to serve as an intermediate in Fe–S cluster delivery, confers catalytic properties to E. coli LipA, showing for the first time, that RS enzymes that degrade Fe–S clusters as sources of sulfur are not simply substrates consumed in the reaction, but true catalysts.
Abstract #14
Benjamin Muthambi, Applied Statistics
Mortality after Loss-from-HIV-care (LFHC): Association with Older Age, Late Diagnosis & Unsuppressed Viral Load at LFHC
Benjamin Muthambi, DrPH, MPH^; Ekezie Francis, MD**; Nathaniel Geyer, MS*; Paul Colson, PhD--; John Zurlo, MD** and Tonya Crook, MD, MS, DTM&H**
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OBJECTIVES: The occurrence and predictors of mortality after loss-from-HIV-care (LFHC) are not well elucidated, and were therefore assessed in this study.
METHODS: The retrospective cohort study included persons >12 years of age at the time of LFHC from a Pennsylvania clinic during 2003-2012. Predictor characteristics were reviewed, and follow-up outcomes were ascertained through 2014 by way of record-linkage of clinic Careware™ data with disease registries, claims databases of publicly-funded state health insurance, and death registry. Multivariate logistic regression analyses were performed to determine the likelihood of death after LFHC, and how this varies by several predictors.
RESULTS: Approximately 25% of 389 persons studied were deceased, and all others were presumed alive. Multiple logistic regression analyses showed greater likelihood of death for those whose status at the time of LFHC was: a) 45-55 years old (adjusted odds ratio/aOR=3.41;95%Confidence-Interval,CI=1.41—8.25) compared to those 13-34, and even greater for those >=55 (aOR=5.51;95%CI=2.11-14.41); b) Late diagnosed(HIV stage-3/AIDS) as indicated by concurrent HIV-AIDS diagnosis within 6 months, with unsuppressed viral load/VL (aOR=4.91;95%CI=1.14-21.23) compared to persons diagnosed before HIV stage-3 with suppressed VL, who were comparable to others. The likelihood of death was lower for: a) Persons whose probable mode of HIV acquisition was ‘not documented’, other than heterosexual contact or males who have sex with men (aOR=0.28;95%CI=0.10-0.80), compared to those with a history of injection drug use, b) A mostly Latino/Hispanic group including a few others of unknown race/ethnicity (aOR=0.42;95%CI=0.19-0.91), compared to Caucasians/whites, who were comparable to African-Americans/blacks, and c) Those with no documented re-engagement/not trackable after LFHC (aOR=0.51;95%CI=0.28-0.92), compared to persons with documented re-engagement at other clinics. There were no trends by time interval of LFHC or differences by other characteristics studied.
CONCLUSIONS: The higher occurrence of death associated with older age and late diagnoses with unsuppressed Viral Load/VL at the time of Loss-From-HIV-Care/LFHC may indicate a frailty effect suggesting a need for: a) Timely screening, early diagnoses of HIV and timely linkage to care; and b) Closer HIV case management support to address retention-in-care and viral suppression needs. Fewer deaths among those not re-engaged require further investigation, including impact of out-migration.
Abstract #15
Debmalya Nandy, Statistics
Covariate Information for Feature Screening in Ultrahigh Dimension
Debmalya Nandy, Runze Li, and Francesca Chiaromonte

In many contemporary scientific fields, regression with ultrahigh-dimensional covariates (p >> n) involves sparse signals, i.e. only a small share of the original p covariates is truly associated with the response. As the first step of a two-step procedure to perform sufficient dimension reduction in ultrahigh dimension, we propose to substantially reduce the number of covariates through a model-free screening procedure called Covariate Information Screening (CIS) based on an (Fisher) information-based marginal utility that we call Covariate Information Number. This first step is designed to minimize false negatives eliminating only redundant covariates. Our preliminary simulation results demonstrate competitive performance of CIS compared to popular screening procedures such as Sure Independence Screening (Fan and Lv, 2008, JRSS-B) and Sure Independent Ranking and Screening (Zhu et al., 2011, JASA). Our proposed iterative version of CIS (ICIS) also seems to improve CIS performance. We are currently investigating the theoretical properties of CIS.

Keywords: Feature screening; Ultrahigh dimension; Fisher information; Model-free; False negatives; Iterative screening.
Abstract #16
Caitlin Nealon, Biomedical Sciences
Agonist-specific mechanisms of cannabinoid tolerance in desensitization resistant mice
Caitlin Nealon, Rebecca LaFleur, and Daniel J Morgan

The focus of this study was to better understand the mechanisms responsible for tolerance to cannabinoids such as delta-9-tetrahydrocannabinol (Δ9-THC). We used a newly developed knock-in mouse line which expresses a desensitization-resistant form of the cannabinoid receptor 1 (CB1) to investigate tolerance to WIN55,212-2, and CP55,940. These mutant mice (S426A/S430A) lack two serine residues that are phosphorylated by G protein-coupled receptor kinases (GRKs) and recruit β-arrestin2. Antinociceptive tolerance to repeated daily administration of WIN55,212-2 and CP55,940 was assessed in wild-type and S426A/S430A mice using the hot plate, tail flick, and formalin tests. S426A/S430A mutant mice exhibit a modest delay in tolerance for Δ9-THC (Morgan et al., 2014); interestingly, tolerance to the synthetic cannabinoid agonist WIN55,212-2 is profoundly delayed in S426A/S430A mutant males while tolerance to CP55,940, another synthetic full cannabinoid agonist, is only modestly affected. This finding suggests the likelihood of agonist-specific mechanisms of cannabinoid tolerance where tolerance to the antinociceptive effects of WIN55,212-2 are mediated entirely through a classic GRK/β-arrestin2 mechanism while tolerance to Δ9-THC and CP55,940 are mediated, in part, by other signaling pathways.
Abstract #17
Kelly Ness, Physiology
How Does Sleep Loss Impact Fat Metabolism?
Kelly M. Ness, Gregory C. Shearer, Orfeu M. Buxton

People who do not get enough sleep have a greater risk of developing obesity, type II diabetes, and cardiovascular disease. From laboratory studies, we know that people who have not gotten enough sleep do not clear glucose from their blood as quickly as healthy people. This is called insulin resistance. Insulin is a hormone that signals your body to absorb glucose and to shut down the release of fat from your adipose tissue. Our study will examine whether sleep impacts your body’s ability to shut down its release of fat in response to insulin.

We brought healthy, young subjects into our lab and only let them sleep for five hours per night for five nights in a row. We performed an intravenous glucose tolerance test (IVGTT) on each subject at baseline, after they had been sleep deprived, and after two nights of recovery sleep. We measured the amount of fat, called non-esterified fatty acids (NEFAs), being released from each subject’s adipose tissue during the IVGTT procedures. We then used compartmental modeling to assess the rate of production, rate of utilization and the degree of NEFA suppression (a measure of adipose insulin sensitivity) during baseline, sleep restriction, and recovery.

Sleep restriction increased the fractional rate of NEFA utilization by 30.7% compared to baseline. The inhibitory effect of remote glucose on adipose NEFA release was reduced by 45.8% during sleep restriction. These preliminary results indicate that lack of sleep impacts fat metabolism, a finding which may have implications for long-term health and clinical treatment.
Abstract #18
Nick Parekh, Biomedical Sciences
cGAS mediates a protective local response to Vaccinia independent of controlling virus replication
Nick J. Parekh, Irene E. Reider, Tracy E. Krouse, Chris C. Norbury

Immunization with Vaccinia virus (VACV) via damage to the epidermis is the most effective route to generate tissue-resident memory CD8+ T cells, but can lead to exaggerated tissue pathology at the immunization site in some individuals. It is unknown whether the complications following VACV immunization result from uncontrolled VACV replication or from a deficient local immune response.

VACV encodes numerous proteins that block pathogen recognition and subsequent activation of type-I interferon (IFN). Indeed, VACV-infected cells fail to produce type-I IFN in vitro. Expression of type-I IFN is rescued when VACV is either inactivated or immunomodulatory proteins are deleted, indicating that the virus actively attenuates production of type-I IFN. However, mice lacking the cytosolic DNA sensor cGAS are sensitive to intranasal VACV infection. Therefore, we sought to determine the mechanism of induction and action of type-I IFN during dermal VACV infection.

We found no induction of type-I IFN early after VACV infection, but IFN-β and -α4 were induced by 3 days post-infection. Type-I IFN was cGAS and STING dependent, but independent of TLR signaling. Mice lacking the type-I IFN receptor (IFNαR) exhibited a drastic increase in tissue pathology compared to wild-type mice. Surprisingly, increased tissue damage occurred independent of viral burden. Mice lacking either cGAS or IFNαR displayed impaired recruitment of inflammatory monocytes. Monocytes, which are the primary infected immune cell population, are crucial effectors in the local immune response to VACV. Tissue pathology in mice with an IFN-independent defect in monocyte recruitment closely resembled that of cGAS- or IFNαR-deficient mice. We examined downstream effectors uniquely induced by type-I IFN signaling following dermal VACV infection, and identified CCL4 as the requisite chemokine for monocyte recruitment. In summary, cGAS-mediated type-I IFN induces CCL4, facilitating recruitment of inflammatory monocytes, which reduce tissue pathology. Therefore, type-I IFN is required to orchestrate a protective immune response, independent of any role in control of virus infection or replication.
Iron deficiency, the most common nutrient deficiency worldwide, affects mostly children and women of reproductive age. Iron deficiency during pregnancy can lead to pre-term delivery and poor child cognitive development. The aim of this study was to assess the iron status of reproductive age Ghanaian women who plan to become pregnant in the next 6 months. A cross sectional study was carried out to recruit 100 prepregnant women between the ages of 18-35 years, who lived in Asesewa, in the Eastern Region of Ghana. Questionnaires were administered to collect sociodemographic data, health history, and food security data. Weight and height measurements were collected and body mass index calculated. Approximately eight milliliters of blood were taken from an antecubital vein. One drop was used for the assessment of hemoglobin (Hb) via a Hemocue. Subsequent analysis of iron biomarkers (serum iron, ferritin, total iron binding capacity (TIBC)) were performed in our laboratory using ELISA methods. Markers of inflammation such as C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) were assessed using radial immunodiffusion kits. Mean age was 26.5±5.1 years while mean body mass index was 25.4±5.3 kg/m2 with 39% overweight or obese. Food insecurity was reported among 24% of the households. Anemia was found among 34% of the women. After correcting for inflammation, 2% were iron deficient defined as two or more abnormal markers of iron status and 32% were iron deficient defined as just one abnormal iron status marker. Our findings reveal that, in prepregnant Ghanaian women, anemia is common but iron deficiency, as defined by two abnormal iron status markers, is lower than expected. Further research is needed to examine prevalence of iron deficiency in these women during pregnancy.
Abstract #20
Lauren Randolph, Bioengineering
Generation of MYL2eGFP/w reporter hPSCs via CRISPR-Cas9 technique for specific identification of ventricular myocytes
Lauren N. Randolph1,3, Xiaoping Bao4,5, Xiaojun Lian1,2,3,*

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The cardiomyocyte turnover rate is between 0.5% and 1% per year, even upon injury, meaning the loss of viable myocardial tissue during infarction is permanent.[1] Human pluripotent stem cell (hPSC) technology holds great promise and potential towards therapeutic development to treat degenerative or loss of function conditions, such as cardiovascular disease, due to hPSCs’ unlimited proliferative and differentiation capacity.[2,3] In 2012, Lian et al. published a chemically defined strategy for efficient cardiomyocyte differentiation from hPSCs, the GiWi protocol, expanding the potential of stem cell based therapy to the realm of cardiovascular disease.[4] However, subtype heterogeneity present in the stem cell derived cardiomyocytes challenges clinical applications as they have been showed to produce ectopic pacemaker activity and ventricular arrhythmias when transplanted into infarction sites in pig models.[5] Pure populations of appropriate cardiomyocyte subtypes are needed to further harness their therapeutic potential.

Identification and purification of ventricular myocytes using antibodies for ventricular markers, IRX4 and MYL2, requires cell destruction. In addition to identifying and isolating ventricular cardiomyocytes without loosing function, it would be beneficial to further increase the stem cell differentiation efficiency towards the ventricular subtype. Zhang et al. modulated retinoic acid signaling activation and inhibition to fluctuate population bias towards atrial or ventricular subtypes respectively.[6] Further experimentation and optimization has potential to provide higher subtype purity via differentiation and could allow for additional improvements to maturation time of cardiomyocytes. Here we present the development and characterization of MYL2eGFP/w reporter hPSCs via CRISPR-Cas9 technique. Additionally, we increased ventricular subtype efficiency of the GiWi protocol by stepwise optimization of retinoic acid signaling and characterized the maturation of cardiomyocyte populations via MLC2v expression.

REFERENCES
Abstract #21
Karly Regan, Entomology
Arthropod Response to Organic Cropping Systems
Karly Regan, Christina Mullen, Mary Barbercheck

Reducing synthetic chemical inputs and tillage can have numerous benefits in agroecosystems, such as building soil health, promoting biodiversity, and reducing non-target effects. In addition to these benefits, reduced-tillage cropping systems can enhance predator communities and biological control potential. While tillage can kill or disrupt invertebrates, planting a winter cover crop may help sustain invertebrate communities by providing habitat and nutritional resources. We investigated effects of tillage and cover crop management on herbivory and predation in corn. The experiment includes four management systems in an organic feed grain rotation that varied in preceding and in-season cover crop mixture and tillage. Hairy vetch (Vicia villosa) and triticale (Triticale hexaploide) was compared to red clover (Trifolium pratense) and timothy (Phleum pratense) prior to corn planting. One of the vetch and triticale systems was managed through rolling of the cover crop and no-till planted, while the other three systems were tilled before planting corn. In the tilled hairy vetch system, and one of the red clover systems, plots were interseeded with an orchardgrass (Dactylis glomerata), annual ryegrass (Lolium multiflorum), and forage radish (Raphanus sativus) mixture in early July. Sentinel predation rate was measure three times throughout the season. Additionally, damage to the crop from common pests was assessed. No significant differences were found for predation rate or herbivory for the four systems at any of the sampling dates. Further research in this project will investigate the role of environmental conditions and cumulative disturbance on arthropods and characterize the arthropod community contributing to predation in each system.
Abstract #22
Lila Rieber, Bioinformatics & Genomics
Computational approaches to gene regulation
Shaun Mahony, Naomi Yamada, Akshay Kakumanu, Divyanshi Srivastava, Lila Rieber

Our research aims to understand where transcription factors (TFs) bind in the genome, and what they do once they get there. There are many forces that can affect a TF’s choice of binding targets once it is introduced into the nucleus. The inherent DNA-binding preference of the protein will specify the sites that could potentially be bound, but the vast majority of high-affinity sequence sites will not in fact be occupied by the TF in any given cell type. Binding selectivity is thus determined by the regulatory environment of the cell: chromatin accessibility, interactions with co-factors, DNA methylation, and histone post-translational modifications all play roles in specifying the TF’s binding sites. These forces are context-specific, which allows the same TF to target different binding sites in different cell types. However, a TF’s choice of binding targets is only part of the equation; many bound sites do not seem to directly affect gene expression. We understand little about how enhancers can regulate genes that are thousands, sometimes millions, of bases away on the genome.

Fortunately, high-throughput sequencing assays are giving us unprecedented insight into the regulatory environment of the cell. ChIP-seq and ChIP-exo allow us to profile TF and histone modification occupancy at high resolution over the entire genome. RNA-seq lets us profile the global transcriptional activity. DNase-seq profiles the genome-wide accessibility landscape, while new assays such as ChIA-PET and Hi-C are opening a window on the three-dimensional architecture of the nucleus. The challenge will be integrating these voluminous data types into a cohesive understanding of cellular activity. We believe that integrative machine-learning approaches that model the biological and experimental processes that generate such data will help us to understand the context-specific activity of transcription factors.
Abstract #23
Prakash Timilsena, Biology
Phylogenomic analysis of monocots using multiple single copy genes elucidates the evolution of mycoheterotrophy
Abstract #24
Alex Weiner, MCIBS
Wnt Pathway Players Target Microtubule Regulators to Dendrite Branch Points
Alexis Weiner, Dylan Seebold, Nick Michael, Michelle Guignet, Chengye Feng, Brandon Follick, Christin Folker, Brandon Yusko, Chris Kozlowski, Dylan Barbera, Mit Patel, Pedro Torres, Melissa Rolls

In Drosophila neurons, dendrite branch points act as hubs for microtubule organization. For example, growing microtubules are actively directed towards the cell body at branch points by kinesin-2 attached to their plus ends. Apc acts as the linker between kinesin-2 and EB1 at the plus end, and is concentrated to branch points by Apc2. To understand how Apc2 is concentrated at its site of action, we performed candidate screens using the very clear localization pattern of Apc2-GFP as a readout. We identified several groups of proteins from the initial screen including proteins that regulate actin polymerization through the Arp2/3 complex, the scaffold ankyrin2 and its membrane protein partner neuroglian, frizzleds, heterotrimeric G proteins and axin. Importantly, several of the proteins identified in the screen themselves localized to dendrite branch points; these included axin and ankyrin2. In addition to microtubule steering regulators, dendrite branch points house microtubule nucleation sites. To determine whether the same suite of proteins used to localize Apc2 concentrates nucleation proteins, we depleted candidates in the presence of gamma-tubulin GFP. We found frizzleds, heterotrimeric G proteins and axin were also critical for localization of gamma-tubulin. Furthermore, these proteins were necessary for maintaining microtubule polarity in dendrites. We also tested a functional requirement for frizzleds and heterotrimeric G proteins in nucleation by assaying the nucleation-dependent increase in microtubule dynamics after axon injury, and found that, these proteins were required for the increase in nucleation in dendrites. Our data suggests that several different pathways cooperate to position Apc2 at dendrite branch points, and that one of these also localizes nucleation sites. We propose that frizzleds, heterotrimeric G-proteins and axin act as master regulators to manage microtubule nucleation and steering.
Abstract #25
Wen-Bin Yu, Visiting Scholar
Plastome reduction in parasitic plants: independent evolutionary events?
Wen-Bin Yu, Christopher P. Randle, Hong Wang, Jun-Bo Yang, Yu Song, Chao-Nan Fu, Claude W. dePamphilis, and De-Zhu Li

Many parasitic plants are characterized specialized organs (i.e. haustoria) by which they obtain water and/or nutrients from host plants. There are around 4000 parasitic flowering species placed in 12 orders and 19 families. Of them, about 90% of parasitic species (in four orders) retain photosynthetic capability (hemiparasites), while 10% of parasitic species (in 10 orders) have lost photosynthetic capability (holoparasites). In free-living plants, the plastome is very conserved in size (120 – 170 kb), gene contents (110 – 130 genes) and a quadripartite structure (LSC, IRA, SSC and IRB). Loss of photosynthetic capability in holoparasites is often correlated with degradation or loss of photosynthesis-related and other non-housekeeping genes. To date, plastomes of 11 parasitic orders have been published or examined (except Ehretiaceae, Boraginales). In hemiparasitic taxa such as Cassytha (Lauraceae, Laurales), Krameriaceae (Zygophyllales), Orobanchaceae (Lamiales) and Santalaceae s.l. (Santalales), plastome size and/or gene contents showed high similarity to its non-parasitic relatives. Some NADH-polymerase (ndh) genes were found to be degraded or lost in some species, Cassytha ssp. had no inverted repeat region, and gene rearrangements were found in hemiparastic Orobanchaceae. Genome reductions were found in all holoparasitic plants. Genome size in holoparasitic plants varied from 11,348bp (Pilostyles aethiopica, Apodanthaceae, Cucurbitales) to 150,504bp (Lathraea squamaria, Orobanchaceae). Photosynthesis-related genes were (partially) retained in Cuscuta spp. (Convolvulaceae, Solanales) and some holoparasitic Orbanachaceae (e.g. Harveya spp., Lathraea spp., Orobanche californica). Nevertheless, the photosynthesis-related genes, as well as some ribosomal protein genes, were lost in other holoparasitic lineages, i.e., Cynomorium coccineum (Cynomoriaceae, Saxifragales), Cytinus hypocistis (Cytinaceae, Malvales), Hydnora visseri (Hydnoraceae, Piperales), Mitratemon yamamotoi (Mitratemonaceae, Ericales) and Pilostyles spp., as well as in some Orobanchaceae, e.g. Christisonia hookeri, Cistanche phelypaea, Conopholis americana, Epifagus virginiana, and Orobanche spp. Moreover, a plastome was not detected in Rafflesiaceae (Malpighiales) and Balanophoraceae (Santalales). Phylogenetic analyses have demonstrated that parasitism has evolved independently at least 12 times. When considering the evolutionary ages of parasitic lineages, plastome degradation follows a pattern: degradation of ndh genes in hemiparasites, followed by photosynthesis-related genes in newly derived holoparasites, loss of protein synthesis and other functional genes, and finally retention of ribosomal RNAs in ancient holoparasites, or complete elimination or with possible incorporation of plastome remnants into mitochondria and nuclear genomes. Seven ancient holoparasitic lineages, i.e. Ericales, Cucurbitales, Malvales, Malpighiales, Piperales, Santalales, Saxifragales, have extremely reduced (or no) plastomes. By contrary, young holoparasitic lineages in Cuscuta and Orobanchaceae showed a gradual reduction pattern of plastomes.
Consensus sequences in the Pol II CTD are sufficient to mediate Drosophila development
Feiyue Lu, Bede Portz and David S. Gilmour

The CTD is a repetitive, intrinsically disordered region at the C-terminus of the largest subunit of RNA polymerase II (Pol II). It serves as a docking site for a myriad of factors involved in various co-transcriptional events. The CTD of yeast is comprised primarily of repeating heptads Y1S2P3T4S5P6S7 whereas the CTDs of higher eukaryotes have significantly more repeats, with many repeats divergent from the consensus ones at one or multiple positions. This increase in length and sequence complexity is thought to be essential for the evolution of complex patterns of gene expression in higher eukaryotes. However, the functional significance of the non-consensus heptads towards development remains largely untested. Here we show that regions that are comprised solely of non-consensus heptads are less important than a region that contains consensus heptads. In addition, the human CTD, which is composed of a different assemblage of non-consensus heptads than Drosophila, is able to support the development of Drosophila to adulthood. Remarkably, a viable homozygous fly line can be made by replacing the entire naturally occurring Drosophila CTD with 29 consensus heptads. Our results argue against the hypothesis that the non-consensus heptads in the CTD are required to mediate complex patterns of gene expression. We propose that the non-consensus heptads control the overall biophysical properties of the CTD and do not provide unique binding sites for factors involved in regulating gene expression.
Abstract #27
Eric Kohn, Rowan University
Effects of ionic liquids on myoglobin denaturation by a zwitterionic detergent
Eric M. Kohn, Anthony J. Calabro, Timothy D. Vaden, Gregory A. Caputo

Recent interest in the applications of ionic liquids have prompted investigations into their effects on biomolecules. Previous research has shown that ionic liquids can affect protein stability; however, the mechanism of this effect is unknown. The zwitterionic detergent N,N-Dimethyl-N-dodecylglycine betaine (Empigen BB) was used to denature myoglobin in the presence of aqueous ionic liquids, including 1-butyl-3-methylimidazolium tetrafluoroborate and 1-ethyl-3-methylimidazolium acetate, and the resulting denaturation profiles were examined. Detergents traditionally disrupt the hydrophobic contacts at the interior of a folded protein, causing the structure to denature. The hypothesis was that if the ionic liquids, or some component of the ionic liquids, were reacting through a similar mechanism, the detergent-mediated unfolding would be enhanced. Unfolding was monitored through a combination of absorbance, fluorescence, and circular dichroism spectroscopy. Absorbance and fluorescence quenching were used to measure changes in the distance between intrinsic heme and tryptophan residues before and after denaturation. The fluorescence of DPH was used to measure the critical micelle concentration of Empigen BB in the presence of various aqueous ionic liquid solutions compared to salt controls. CD spectroscopy was used to monitor the loss of the heme signal as well as the loss of secondary structure in control experiments. All three types of experiments showed similar denaturation profiles under the same ionic liquid conditions. The ionic liquids did not appear to have a significant impact on unfolding by Empigen BB, indicating their effects on protein stability may be through a different mechanism than that by which Empigen BB unfolds proteins. The presence of ionic liquids did not appear to influence the formation of detergent micelles. In all cases examined, the protein denaturation profile remained a traditional, cooperative process.
Abstract #1
Elizabeth Adams, Nutritional Sciences
Within-Person Patterns of Gestational Weight Gain for Multiparous Women Relate to Pregnancy Spacing
Elizabeth L. Adams, Michele E. Marini, Danielle S. Downs, Krista S. Leonard, Ian M. Paul, Jennifer L. Kraschnewski, Kristen H. Kjerulff, and Jennifer S. Savage

Purpose: Gestational weight gain (GWG) in the first and subsequent pregnancies is often examined using cross-sectional study designs and likely confounded by between-person variability. The purpose of this study was to examine within-person differences in patterns of GWG for multiparous women followed prospectively across their 1st and 2nd pregnancies.

Methods: Data were used from a subset of the First Baby Study, a 3-year prospective cohort of mothers delivering first live births at enrollment (n=691). Self-reported GWG was collected after each birth and characterized using Institute of Medicine (2009) GWG guidelines. Pre-pregnancy BMI and weight retention at 6 months postpartum (>0 kg above pre-pregnancy weight) were calculated. Chi square tests examined associations among pregnancy characteristics. Results: Only 25.9% of women met guidelines for both pregnancies, while 31.7% exceeded guidelines for both pregnancies (40.7% met in just the 1st pregnancy, 46.1% met in just the 2nd pregnancy). Of women who met guidelines in the 1st pregnancy, more met (63.6%) vs. exceeded (23.3%) guidelines in their 2nd pregnancy. Of women who exceeded guidelines in the 1st pregnancy, more exceeded (65.4%) vs. met (31.17%) guidelines in their 2nd pregnancy (p<0.01). About half (55.1%) of women retained weight at 6 months after their 1st pregnancy; these women had 1.9 times the odds of exceeding guidelines in their 2nd pregnancy, compared to women who did not retain weight (p<0.01). Pregnancy spacing (mean 16.3±6.0 months) did not affect GWG for overweight/obese women; however, for normal weight women, greater pregnancy spacing resulted in lower GWG during their 2nd pregnancy (p=0.05). Conclusion: Exceeding GWG guidelines in a 1st pregnancy and retaining weight at 6 months postpartum were associated with greater GWG in 2nd pregnancies. Pregnancy spacing was a significant determinant of GWG in women’s 2nd pregnancy among normal weight, but not overweight/obese women.
Abstract #2
Meridith Bartley, Statistics
A Bayesian penalized hidden Markov model for colony-level behavioral switching in ants
Sixtus Aguree and Alison D. Gernand

Our objective was to pilot a simple, quick method for plasma volume measurement using ICG dye in healthy women of reproductive age and to assess the relationship between plasma volume and markers of body composition and nutritional status. Participants were a convenience sample of women with a regular menstrual cycle who responded to announcements at Penn State University. Participants visited the Penn State Clinical Research Center after overnight fasting, then their demographic and health data were self-reported and weight, height, blood pressure, and body fat composition were measured (n=8). Subjects rested for 15 minutes in a supine position, and blood samples were taken for nutritional biomarkers. Serial blood samples were collected for ICG concentration measurement following a bolus injection of ICG (0.25 mg/kg). Then a disappearance curve was fitted to extrapolate back to the concentration at injection. Mean (SD) age of participants was 24.3 (4.1) years, BMI of 23.8 (2.9) kg/m2, and body fat percentage of 28.5 (5.3)%. Women were mostly white, single, and nulliparous. Hemoglobin and hematocrit were 12.3 (0.7) g/dL and 37.1 (2.3) %, respectively. The mean plasma volume was 2029 (364) mL or 31.8 (3.4) mL/kg. Plasma volume was highly correlated with body weight (r=0.81, p=0.016), fat mass (r=0.78, p=0.024), body fat percentage (r=0.73, p=0.041), lean body mass (r=0.78, p=0.023), and body surface area (r=0.82, p=0.013). Plasma volume is positively correlated with body weight, body fat and body surface area. Plasma volume was not associated with micronutrient concentrations, however, serum zinc concentration showed a trend towards a positive significance association with plasma volume (r=0.70, p=0.08) among participants with normal body mass index (18.5-24.9kg/m2). Our future research will build an equation for predicting plasma volume using non-invasive body composition measures.
Abstract #3
Sixtus Aguree, Nutritional Sciences
Plasma Volume in Healthy Reproductive Age Women using Indocyanine Green Dye
Meridith L. Bartley
Ephraim Hanks
David Hughes

Interactions between social animals provide insights into the exchange and flow of nutrients, disease, and social contacts. We consider an analysis of trophallaxis interactions between carpenter ants (Camponotus pennsylvanicus) over 4 hours of second-by-second observations. The data show clear switches between fast and slow modes of trophallaxis; however, fitting a standard hidden Markov model (HMM) results in an estimated hidden state process that is overfit to this high resolution data, as the state process fluctuates an order of magnitude more quickly than is biologically reasonable. We propose a novel approach for penalized estimation of HMMs through a Bayesian ridge prior on the state transition rates. This penalty induces smoothing, limiting the rate of state switching to ensure more biologically feasible results. We develop a Markov chain Monte Carlo algorithm to perform Bayesian inference based on discretized observations of the contact network.
Abstract #4
Shi Chai, Physiology
Vitamin A Deficiency Alters Small Intestine Transcriptional Profile, but to Different Extents Depending on the Citrobacter rodent
Zhi Chai, Qiuyan Chen, Cheng-Hsin Wei, Lindsay Snyder, Veronika Weaver, Margherita T. Cantorna, A. Catharine Ross.

Vitamin A (VA) deficiency remains a public health issue in resource-limited areas. It is estimated that over 20% of preschool aged children have clinical VA deficiency (VAD). VAD is positively associated with severity of infectious diseases. Diarrheal related diseases are a major cause of mortality in VAD children. Citrobacter rodentium (C. rodentium) is a natural mouse enteropathogen mimicking enteropathogenic Escherichia coli (EPEC) infection in human. During C. rodentium infection, vitamin A sufficient (VAS) mice displayed enhanced survival rate and pathogen clearance compared to their VAD counterparts. As C. rodentium attachment subverts several signaling pathways in the intestinal epithelial cells and VA is a potent regulator of cell differentiation and mucosal immunity, we hypothesized that without C. rodentium infection, transcriptional profile in lower small intestine (LSI) will differ according to nutritional status (VAS vs VAD without infection = comparison 1); with the presence of C. rodentium infection, VAD will still affect transcriptional profile in LSI on post-infection day 5 (VAS vs VAD under infection = comparison 2), but this nutritional effect will be modified by the infection. We further hypothesized that the modifying effect may involve genes associated with actin polymerization, tight junction formation, Pattern Recognition Receptor (PRR) and cytokine receptor expression, as well as anti-microbial protein (AMP) and cytokine production. We observed that genes associated with actin polymerization and tight junction were exclusively shown in comparison 2, but not comparison 1. Additionally, PRR-, AMP- and cytokine-related genes were affected by VA status regardless of C. rodentium infection, but VAD effect was much modified by the infection status. In summary, VAD alters LSI transcriptome, but to different extents depending on the C. rodentium infection status.
Abstract #5
Mihaela Ciulei, Nutritional Sciences
The Prevalence of Postpartum Depressive Symptoms in Iron Deficient and Iron Sufficient Mothers from Rural Bangladesh
Mihaela A. Ciulei1, L.K. English1, S. Chung3, J. Hamadani4, S. El Arifeen4, A. Baqui2, R. Black 2, and Laura E. Murray-Kolb1
1The Pennsylvania State University, 2International Health, John Hopkins Bloomberg School of Public Health, 3The University of Oklahoma, 4International Centre for Diarrhoeal Disease Research

Background. More than half of pregnant women in developing countries suffer from iron deficiency (ID) and many are also affected by postpartum depression (PPD). The prevalence of PPD in Bangladeshi women ranges from 22 – 52%, depending on the study. PPD is detrimental to mother-infant interactions and subsequent child development and several studies report a relation between ID and depressive symptoms.

Objective. This analysis evaluates the relation between iron status and PPD in women of rural Bangladesh. We hypothesized that women with ID would have higher PPD scores (measured 3 times in the postpartum) than iron sufficient (IS) women.

Design. This study utilized data from a longitudinal, double-blind, intervention (in the infants only) study in rural Bangladesh. Mother-infant dyads were followed and PPD assessed via the Center for Epidemiologic Studies Depression Scale (CES-D) at baseline (6-18 months postpartum; n=113), 3 months (n=318), and 6 months (n=328) later. ID was assessed via ferritin and transferrin receptor at baseline (n=127) and +6 months (n=311).

Results. A high prevalence (70.3%) of PPD (CES-D > 16) but a low prevalence (17%) of ID was found in mothers. PPD was higher in ID than IS women at every time point (ANOVA, p<0.04). Multivariate regression showed that body iron was associated with the total CES-D scores at all time points (p<0.001) even after controlling for covariates (child age, sex, weight-for-age Z score, mean upper arm circumference, and assets).

Conclusions. Women who were ID reported higher PPD symptoms. Our findings support the need to prevent and control ID to positively influence mother and infant health from developing countries.
Abstract #6
Charles Crowe, Chemistry
Aqueous multiphase emulsion droplets as cellular mimics: Production and utilization
Charles Crowe, Paola Torre, Raghav Poudyal, Philip C. Bevilacqua, Sheref S. Mansy, Christine D. Keating

Non-membrane bound compartments within the cell have been shown to be vital to cellular function. Such intercellular organization is governed by physical influences of the crowded and phase-separated environment within cells. In order to study the effects of compartmentalization, cellular mimics demonstrating these aspects of cells are desired. Aqueous multiphase system water-in-oil emulsion droplets provide such a platform. These droplets are typically composed of multiple distinct phase-separated regions, each rich in a specific neutral polymer (such as poly(ethylene glycol), dextran, or Ficoll). These regions provide unique partitioning effects as well as crowded environments within which cellular reactions may be studied. Microfluidic techniques have been developed to exert control over droplet composition and morphology. The effect of these physical characteristics upon the extent of reaction and reaction rates is currently being explored. Previous work utilizing such a system has demonstrated successful observation of transcription and translation of fluorescent proteins within droplets. Expanding on these results, modifying the droplets’ attributes (such as phase composition, relative phase volume, and droplet size) changes the availability of reaction components due to phenomena such as diffusion effects and effective concentration. This work provides a foundation of a physical understanding of emulsion-based cellular mimics upon which many biological reactions may be studied.
Abstract #7
Adwitia Dey, Physiology
The protective role of ron receptor tyrosine kinase in cns inflammation
Adwitia Dey, Joselyn N. Allen, James W. Fraser, Lindsay M. Snyder, Limin Zhang, Yuan Tian, Andrew Patterson, Margherita T. Cantorna, Robert F.Paulson, Pamela Hankey-Giblin

Neurodegeneration is characterized by severe uncontolled central nervous system (CNS) inflammation. Macrophages are immune cells that closely regulate inflammation by taking on different activation states. The Ron receptor tyrosine kinase is expressed on tissue resident macrophages including microglia (predominant CNS macrophages). An in vivo deletion of Ron (Ron KO) promotes an inflammatory (M1) state and limits a reparative (M2) macrophage activation state, whether this response influences CNS inflammation is not characterized. Herein, the objective of this study was to elucidate whether Ron expression plays a critical role in CNS inflammation. Ron KO mice develop an inflammatory CNS niche with increased tissue expression of M1-associated markers TNFα, Cox-2 and iNOS when compared to WT-mice (P<0.05). The brain is a crucial metabolic organ, our preliminary NMR data highlights differentially expressed metabolites the CNS of the two genotypes (P<0.05). In a diet induced obesity(DIO) model of chronic inflammation, Ron KO mice exhibit exacerbated CNS inflammation with decreased expression of M2(Arg-1) and a robust increase in M1(markers compared to WT-mice following 27 weeks of DIO(p<0.05). Lastly, we evaluated the role of Ron in disease model of Multiple Sclerosis, experimental autoimmune encephalitis (EAE). Ron KO mice exhibited higher disease severity from peak disease state and onward when compared to WT-EAE mice (P<0.05). Ron KO mice had increased CNS expression of M1 markers relative to increased peripheral inflammation with increased IFNγ secretion from peripheral lymphoid organs(P<0.05). Collectively these results illustrate how maintenance of Ron expression in the CNS could then be a potential therapeutic approach to treating various grades of CNS inflammation.
Abstract #8
Juliana Fritts, Food Science
Herbs and spices increase school lunch vegetable acceptance at a rural public high school
Juliana Fritts, Clara Fort, Anne Quinn Corr, Qihan Liang, Terri Cravener, John E. Hayes,
Barbara J. Rolls, Kathleen L. Keller

Intake of vegetables in children and adolescents is below dietary recommendations. Following
the Healthy, Hunger-Free Kids Act of 2010, serving sizes and variety of school lunch vegetables
have increased. Nevertheless, more efforts are needed to increase vegetable consumption.
Previous studies used herb and spice flavored dips to increase vegetable intake in young
children, but no studies have used this approach to improve school lunch food choices in
adolescents. To address this gap, we developed and tested recipes for vegetables prepared with
herbs and spices for use in a future cafeteria-based intervention at a rural Pennsylvania high
school. Distinct spice blends (including dill, cardamom, cumin, etc.) were formulated for each
vegetable. We assessed liking (100 mm line scale) and preference (forced choice) from
adolescents (age 14-18 y) for the 7 control and 7 seasoned vegetables. The number of students
who participated in each taste-test ranged from 96 to 110. Liking ratings between control and
seasoned vegetables were compared with paired T-tests. Preference ratings were compared by
Chi Square. Results showed higher liking ratings for seasoned broccoli (P=0.02), vegetable dip
(P<0.0001), black beans and corn (P<0.001), and cauliflower (P= 0.01) compared to control
vegetables. Liking ratings between seasoned and plain varieties of corn and peas (P=0.1), green
beans (P=0.5), and sweet potatoes (P=0.8) did not differ. Students preferred the seasoned corn
and peas (66.3%, P=0.002), broccoli (62.4%, P=0.02), dip (83.7%, P<0.0001), black beans and
corn (69.5%, P<0.001), cauliflower (70.7%, P<0.0001), and green beans (61.7%, P=0.02) to
control, while no difference was found between seasoned and plain sweet potatoes. Results
support the use of herbs and spices to improve acceptability of some vegetables served to
adolescents in school lunch programs. Future research will test the impact of offering these
vegetables in the school cafeteria on student food choice and vegetable intake.
Abstract #9
Kahina Ghanem, Physiology
Evidence that Time of Recruitment of an Avian Ovarian Follicle to the Preovulatory Hierarchy May not Be Linked to Time of Ovulation
Kahina Ghanem
Alan Johnson

To accommodate the demand for daily oviposition, the hen ovarian preovulatory follicles are organized into a hierarchy by size where each day the largest is ovulated. For hens laying long clutches the number of preovulatory follicles found in the ovary at one time is typically less than the total number of follicles ovulated by the end of a clutch. Despite daily ovulations the organization and number of preovulatory follicles is relatively constant. This is regulated by the process of recruitment of a single small undifferentiated pre-recruitment follicle to the hierarchy. Despite its crucial role in establishing and maintaining the hierarchy little is known about the most proximal event that leads to recruitment. Conversely, timing of ovulation has been well characterized and can reliably be predicted. The objective of this study was to determine if recruitment and ovulation are temporally linked in laying hens. It was first hypothesized that on days when ovulation does not occur recruitment also fails to occur. Laying hens 80 weeks of age were divided into 3 groups of 5 hens each, no ovulation, late ovulation, and first ovulation. The time and occurrence of oviposition and ovulation were recorded using cameras and palpation one hour after oviposition. Ovaries were collected during the last hour of photoperiod, and the reproductive tracts were examined to verify that no ovulation occurred. Ovarian follicles were collected and weighed. A follicle weighing between 0.3 g and 0.9 g, with a yolky appearance, and a diameter of 9-12 mm was considered to be recruited that day. To compare the rate of yolk uptake in the 9-12 mm follicles among the three groups, 60 week old hens (3 hens per group) were fed gelatin capsules containing 150 mg Sudan IV red dye 6 hours prior to ovary collection. The most recently recruited follicles were collected, weighed, boiled and sectioned. Pictures of the sections were taken under the dissecting microscope and analyzed using the image analysis software ImageJ. Here we report that a 9-12 mm follicle was found in all the ovaries collected in hens that did not ovulate with a weight comparable to the control groups (0.67 +/- 0.08 g, 0.65 +/- 0.12 g, and 0.56 +/- 0.13g) for no ovulation, late ovulation, and first ovulation, respectively. Furthermore the dye uptake measurements showed that the 9-12 mm follicles were actively incorporating yolk and there were no significant differences in the percent of dyed yolk uptake among the three groups (19.02 +/- 2.03%, 19.83 +/- 3.20%, and 18.02 +/-3.66%) no ovulation, late ovulation, and first ovulation, respectively. We therefore conclude that recruitment and ovulation are not temporally linked in the laying hen.
Abstract #10
Virginia Greenberger, Chemistry
Sensing Ions, Small Molecules, and Peptides at the Lipid Membrane Interface
Alexis J. Baxter, Virginia R. Greenberger, Anne M. Sendecki, Simou Sun, Tinglu S. Yang, Paul S. Cremer

Supported lipid bilayers offer a biomimetic platform that can be used to study interactions at the membrane surface. Using these models, we are able to elucidate the mechanisms of ion, small molecule and peptide binding to various phospholipid compositions. Fluorescent probes responsive to local stimuli conjugated onto a lipid headgroup provide a way to monitor surface interactions via fluorescence microscopy. By studying these binding events, we can isolate individual interactions that occur within a complex biological system.
Abstract #11
Kevin Hart, Immunology and Infectious Disease
CCR4-1 acts as a translational repressor and helps to regulate malarial transmission from host to vector
Kevin J. Hart, Michael P. Walker, Elyse E. Munoz, Mark Kennedy, and Scott E. Lindner

The transmission of the malaria parasite between mosquitoes and mammals requires translational repression to ensure that only the proper proteins are expressed at the right time, while still allowing the parasite to prepare the mRNAs it will need for the next developmental stage. With relatively few known specific transcription factors (ApiAP2 family) that may specifically initiate gene transcription, Plasmodium also regulates the stability and turnover of transcripts to provide more comprehensive gene regulation. We and others have demonstrated that the parasite uses both translational repression and transcript degradation mechanisms to achieve this control. Transcript degradation in eukaryotes typically begins with the removal of the Poly-A tail by deadenylases, especially CCR4 and Caf1 that are found in the CAF1/CCR4/NOT complex. We have bioinformatically identified four CCR4-domain containing proteins in the Plasmodium yoelii genome that are conserved across Plasmodium species that may provide specialized functions. Genetic deletion of PyCCR4-1, which we found associates with the CAF1/CCR4/NOT complex in cytosolic granules, results in significantly fewer exflagellating male gametes and a decreased productive transmission to the mosquito. Comparative RNA sequencing in gametocytes showed a threefold decrease in CDPK4, a known regulator of exflagellation in microgametocytes, along with a decrease in transcripts (e.g. NEK4, CelTOS, PSOP Family) that play important roles in early mosquito stage development and that match the observed phenotypes. It is clear from these data and the work of others that Plasmodium encodes specific proteins for the targeted preservation and degradation of specific mRNAs to promote and control facets of its development and transmission.
Abstract #12
Shue Huang, Nutritional Sciences
Longitudinal Study of alcohol consumption and HDL
Abstract #13
Hillary Koch, Statistics
Maximum likelihood estimation of species tree from gene trees in the presence of population structure
Hillary Koch, Michael DeGiorgio

Though large multilocus genomic datasets have led to overall improvements in phylogenetic inference, they have also made this task more difficult due to conflicting signals across the genome. In particular, ancestral population structure, which has been uncovered in a number of diverse species, can skew gene tree frequencies, thereby hindering the performance for estimating species trees. We developed a novel maximum likelihood method that can infer phylogenies under such scenarios, and find that it has increasing accuracy with increasing numbers of gene trees, contrasting with the poor performance of methods not tailored for ancestral structure.
Abstract #14
Kathleen Leamy, Chemistry
Cooperative tRNA Folding in Cellular Conditions Arises from Stabilization of Tertiary and Destabilization of Secondary Structure
Kathleen A. Leamy*, Neela H. Yennawar, Philip C. Bevilacqua

RNA folding is thought to happen in a hierarchical manner, where secondary structure forms before tertiary structure. The RNA folding process and the structures that RNA forms in vivo are not well understood. Several studies have shown that certain classes of RNAs adopt very different structures in vivo than predicted in vitro or in silico. The conditions typically used to study RNA folding in vitro are very dilute with non-physiological salt concentrations, often 1 M Na+. However, the conditions of the cell are very different. The predominant monovalent ion is 140 mM K+, there is only 0.5-1.0 mM Mg2+ (eukaryotes) and 1.5-2.0 mM Mg2+ (prokaryotes), and there is an estimated 20%-40% molecular crowding. In the work herein, the molecular mechanism behind tRNAphe folding in conditions that mimic the cellular environment is investigated using biophysical, structural, and RNA mapping methods on full length RNA and model oligonucleotides that represent secondary structure elements. Our results show that under dilute in vitro conditions tRNA folds in a hierarchical, non-cooperative manner, but in cellular ionic and crowding conditions it folds in a two-state, cooperative manner. Strong secondary structure has been suggested to drive RNA folding, but surprisingly we observe that cellular conditions destabilize RNA secondary structures, contributing to cooperative RNA folding. We also observe that co-transcription folding intermediates are destabilized in crowded conditions, resulting in folding only once full-length tRNAphe is transcribed. Overall, two-state RNA folding in cellular conditions is achieved by a combination of stabilization of tertiary structure and destabilization of secondary structure.
Abstract #15
Yaqi Li, Nutritional Sciences
Hepatic Uptake of Vitamin A is Independent of Retinoic Acid Pretreatment in Neonatal Rats
Yaqi Li, Michael H Green, Libo Tan, A. Catharine Ross

Previous studies have shown that total retinoid concentration in the liver of neonatal rats would increase in response to the VARA supplementation, which is a combination of vitamin A (VA) and retinoic acid (RA). Later studies have further demonstrated that a single dose of VA may stimulate the hepatic uptake, which in turn, significantly elevated the liver total retinol concentration. In current study, we gave the neonatal rats the RA treatment prior to the VA supplementation, and examined the distinct effect of RA on VA metabolism and kinetics in the liver tissue of these young animals. Sprague-Dawley male and female rats were mated, and the dams were fed a VA-marginal diet (0.35 mg retinol equivalents/kg diet) and were allowed to consume food and water ad libitum. On postnatal day (P) 2 and 3, pups from experimental group received an oral dose of RA (0.625µg /g body weight), while other pups received canola oil as the control treatment. Then, an oral dose of VA (6 µg retinyl palmitate/g body weight), containing 1.8 µCi of [3H]-retinol as a tracer, was administered to all pups on P4. Pups (n = 4-6/treatment/time point) were euthanized from 25 min to 30 h, a total of 12 time points, after dose administration and the liver were collected. Tissue total retinoid mass was determined by ultra-performance liquid chromatography (UPLC), and the tissue tracer level was measured by liquid scintillation counting. Results showed that in both oil-pretreated and RA-pretreated groups, the fraction of retinyl ester and retinol entering the liver increased continuously and peaked at 10 h. After that, the fraction of retinol slightly declined and remained at the same level until the end of the study. However, the fraction of retinyl ester decreased gradually until the 20hr time point and showed a trend of increase afterward. At the same time, liver vitamin A content of both groups increased gradually and peaked firstly at 10 h, after a slight decrease it reached a second peak at 20 h and declined in the following period of time. In conclusion, our findings indicate that liver uptake of dietary vitamin A may be tightly controlled and would not be affected by RA supplementation.
Abstract #16
Gregory Mountain, Chemistry
Coexisting Coacervate Systems to Model Non-Membranous Biological Compartments
Gregory Mountain, Dr. Christine Keating

The importance of liquid-liquid phase separation in biological systems has become a major topic of current research, however the underlying forces that govern the behavior of non-membranous liquid organelles (such as the nucleolus, stress granules, PML bodies, etc.) are not completely understood. Complex coacervate systems are an appealing model system for liquid organelles, as they can be composed of similar classes of molecules (e.g. peptides and nucleic acids) and behave similarly to the liquid organelles observed in biology. Through the use of synthetic polymers, model RNAs and peptides we are able to investigate the physiochemical properties of coacervate systems and develop an understanding of the potential mechanisms of liquid phase separation in biological systems. Complex coacervate systems composed of both synthetic and biologically inspired polyelectrolytes yield systems capable of coexisting coacervate phases with unique properties. I will discuss how kinetic trapping can be used to maintain multiple coacervate systems in the same solution simultaneously, outline and identify necessary conditions to assemble and manipulate multiple unique coacervate systems in the same environment.
Abstract #17  
Ester Oh, Nutritional Science  
The effect of spice consumption delivered in a high fat meal on pro-inflammatory cytokine secretion: A pilot study  
Ester S. Oh, Hannah L. VanEvery, Kristina S. Petersen, Emily Johnston Sheila G. West, Penny M. Kris-Etherton, Connie J. Rogers

Postprandial lipemia is a risk factor for cardiovascular disease. The postprandial inflammation that occurs concurrently with the lipidemia following ingestion of a high fat meal may be a causal factor in this association. Numerous individual spices have anti-inflammatory properties in vitro and in vivo in animal models and humans. However, the effect of a spice mixture containing popular herbs and spices consumed in the U.S. on inflammatory mediators has not been examined in humans in a randomized clinical trial. A pilot study was conducted to investigate the effect of acute spice consumption delivered in a high fat meal on inflammatory cytokine responses in the plasma and from isolated peripheral blood mononuclear cells (PBMCs). The spice blend consisted of black pepper, basil, bay leaf, cinnamon, cilantro, coriander, cumin, ginger, oregano, parsley, rosemary, red pepper (capsaicin), thyme and turmeric (curcumin). Five overweight/obese, nonsmoking, men (40-65 years old) with increased waist circumference (≥ 94 cm) and at least one other risk factor for metabolic syndrome were recruited for a 3-period cross-over study. In random order, participants consumed the following dietary interventions: 1) a 1000 kcal high fat meal containing 45 g fat (HFM), 2) a HFM containing 2 g of spice blend (HFM+2 g spice), or a HFM containing 6 g of spice blend (HFM+6 g spice), with a ≥ 3-day washout period between each test meal. Participants fasted overnight and blood was collected for baseline inflammatory cytokine assessment in the plasma and from cultured PBMCs. Participants then consumed the high fat test meal, and blood was drawn hourly for four hours to assess inflammatory cytokine responses. Baseline characteristics of the study population were as follows: age 51.2 +/- 4.7 years, body mass index 27.9 +/- 0.7 kg/m2, waist circumference 97.5 +/- 1.4 cm, serum triglyceride levels 111.8 +/- 23.7 mg/dL, high density lipoprotein levels 50.6 +/- 4.0 mg/dL, and low density lipoprotein levels 128.8 +/- 11.6 mg/dL. PBMCs were stimulated with 0.625 µg/mL lipopolysaccharide (LPS), cultured for 4 hours, and supernatants assayed by ELISA. Repeated measure ANOVA demonstrated differences in TNF-α secretion from subjects who consumed 6 grams of spice vs. no spice within the meal at 240 minutes after meal consumption (n=5; p=0.086). Inhibition of the pro-inflammatory cytokine, TNF-α suggests that consumption of 6 grams of spice may have the potential to prevent HFM-induced postprandial inflammation.
Abstract #18
Damie Pak, Entomology
Climate and the spring phenology of tortricid moths from 1981 to 2016: Local temperatures and the North Atlantic Oscillation
Damie Pak, Ottar N. Bjornstad, and David J. Biddinger

The timing of insect life-cycle events such as the annual spring flight is highly influenced by temperature. For predicting how future climate change will affect the phenological responses across insect species, it is crucial to investigate both the local temperatures and large-scale climatic indices such as the North Atlantic Oscillation (NAO) that drive the timing of biological events. In the northern hemisphere, the NAO exerts significant influence on the climatic fluctuations across Europe and eastern North America. As the NAO index shifts from its positive (linked with warmer, milder winters) to its negative phase (linked with colder, harsher winters), this could then affect the local temperatures which can consequently lead to the advancement or delayment in spring phenology.

In this study, we examined the relationship between the annual spring flights of five tortricid moth species and the climatic variables with trap data collected at the Fruit Research and Extension Center (Biglerville, Pennsylvania) from 1981 to 2016. We used a moving-window correlation to investigate the monthly temperatures which best predicted the timing of the annual spring flight across all species. Similarly, with a moving average of the monthly NAO index, we investigated which periods, if any, affect the species’ spring phenology.

For all tortricid species, we found the timing of spring flight to be affected by shared monthly temperature-windows and the North Atlantic Oscillation. Locally, March temperatures were the most important predictors with colder temperatures leading to later spring flights in all species. The multivoltine species appear to use supplementary cues such as January and February temperatures possibly due to their earlier spring flights suggesting that variation in life-history traits can influence phenological responses. Finally, November temperatures also significantly influenced the spring phenology of the tortricid moths with lower temperatures correlating to a delay in the timing of spring flights.

At the regional scale, the NAO has significant effects on four of the tortricid species with the timing of their spring flights affected by either the autumn (September to November) or winter period (January to February). The mechanism for how these climatic variables drive spring phenology is mostly likely due to the temperatures affecting the insect development rates, overwintering survivorship, and the induction and breaking of diapause.

In conclusion, our results show that the spring phenology of agricultural pests is best predicted by climatic variables at both the local and regional scale.
Abstract #19
Sarthok Rasique Rahman, Biology
The genetic mechanism underlying adaptive mimetic coloration in bumblebees
Li Tian, Sarthok Rahman, Briana Ezray, Heather Hines

There are numerous rich examples of adaptive variation in nature. Yet little is known how selection sculptures the most basic level of life, the genome, to promote phenotypic variation and diversification. To address this question, we are studying the genetic mechanisms underlying colour pattern polymorphism in bumblebees, an adaptive phenotypic diversification which is driven by Müllerian mimicry. Using a range of bioinformatic and genomic tools, we have identified a narrow locus driving a red/black abdominal hair colour polymorphism in the mimetic bumble bee species Bombus melanopygus. This is located in a regulatory region of the abdominal Hox genes, two major developmental genes that are highly evolutionarily conserved across arthropods. Using developmental genetic tools, we are investigating how changes in gene expression of these genes create distinct colour phenotypes. Examination of this locus in co-mimicking species revealed that they acquired the same colour patterns as B. melanopygus convergently, suggesting rampant mutations as opposed to the sorting of ancestral polymorphisms may be driving this variation. This research provides new insights into how the genome is modified under adaptive diversification, while also revealing processes involved in pigment and Hox gene regulation.
Abstract #20
Pamuru Ramachandra Reddy, Visiting Scholar
Perturbation of male reproduction in mice exposed to baicalein, a natural phytoestrogen in utero.
Pamuru RR, Vaadala S, Naveen P and Sowjanya MGS

Baicalein is a phytoestrogen belongs to flavonoid family and mimics like estrogen in animals. To elucidate the role of baicalein perinatally on male fertility in mice, exposed female pregnant mice to 30mg, 60mg and 90mg/Kg body weight of baicalein intraperitoneally on gestation day 11, 13, 15 and 17. Mice were allowed to deliver pups and the baicalein exposure was continued lactational till weaning. Male pups were separated and fed on the normal pellet diet, until the age of 50 days. Males were sacrificed and sperm parameters were measured after mating them with 45 days old control females. Observed significant decrease (p<0.0001) in the sperm count, sperm motility, sperm viability and HOS (hypo-osmotic swelling) in the baicalein exposed males when compared to controls. Exposed mice were shown a significant decrease (p<0.0001) in the activity of steroidogenic marker enzymes 17β-hydroxysteroid dehydrogenase and 3β-hydroxysteroid dehydrogenase than controls. Besides this, conception time and the number of implantations were decreased with increased pre and post implantation loss in a dose-dependent manner. Observed significant decrease (p<0.05) in the weight of reproductive organs (testis, epididymis, seminal vesicle and prostate gland) and the activity of testicular antioxidant enzymes superoxide dismutase (SOD) and catalase along with increased (p<0.05) levels of testicular lipid peroxidation in perinatal baicalein exposed males in a dose-dependent manner compared to controls. The results of the present study suggest that the failure in male reproduction in mice exposed to baicalein may be due to increase in oxidative stress causing failure in oxidative defense mechanism thereby suppressed steroidogenic pathway and male fertility.
Abstract #21
Laura Ritchey, Chemistry

Structure-seq2: Sensitive and accurate genome-wide profiling of RNA structure in vivo in plants and bacteria
Laura E. Ritchey; Zhao Su; Yin Tang; David Tack; Paul Babitzke; Sarah M. Assman; Philip C. Bevilacqua

RNA structure contributes to a variety of RNA functions.(1) To elucidate these structurally-correlated RNA functional trends, it is beneficial to determine the structure of the entire transcriptome. Additionally, as the physio-chemical environment can dramatically change RNA structure,(2) structural determination using in vivo analysis provides a more rigorous structure-prediction. Structure-seq2, a method developed by our labs,(3) uses DMS chemical probing followed by reverse transcription with a random hexamer to calculate reactivities that can be used to help predict RNA structure genome-wide and in vivo. Compared to the original Structure-seq,(4) two variations of Structure-seq2 have higher quality with four-fold less starting material than Structure-seq. This was accomplished by reducing the presence of a deleterious by-product, lowering ligation bias, reducing mismatches, and improving read coverage. Applying Structure-seq2 to rice provides evidence of hidden breaks present in chloroplast 23S rRNA and a previously unreported N1-methyladenosine in 25S rRNA. Structure-seq2 is also being used to determine the RNA structurome of Bacillus subtilis to uncover novel regulatory functions attributed to RNA structure in bacteria.

Abstract #22  
Maliheh Safari, PPEM  
Evolution of a Plant Persistent Virus and its Modification of Aphid Behavior  
Maliheh Safari, Marilyn J Roossinck

Most well studied plant viruses are acute viruses that cause disease in their host. However, plants are very frequently infected with cytoplasmic RNA viruses that persist for many generations through nearly 100% vertical transmission without producing any obvious symptoms. Movement between plant cells and transmission through grafting has not been observed in these persistent viruses; instead they are distributed to all host cells through host cell division. Peppers are perennial plants, native to the Americas, and as domesticated plants human selection accelerated their evolution, so codivergent timelines should be easier to follow. Jalapeño peppers (Capsicum annuum) are all infected with Pepper cryptic virus 1 (PCV1), which belongs to the Partitiviridae family; its genome consists of two dsRNAs that encode the RNA-dependent RNA polymerase (RdRp) and the coat protein. To investigate the evolution of this virus, dsRNA was extracted from over one hundred different pepper cultivars/landraces/wild plant materials including C. annuum, C. chacoense, C. chinense, C. frutescens, C. pubescens and C. baccutum. The presence of PCV1 was tested by RT-PCR using specific primers. The nucleotide sequence of the RT-PCR products was determined and their phylogenies have been analyzed. Here we present evidence for a remarkably slow evolution rate in PCV1.

Vectors play an important role in the transmission of acute plant viruses because of the immobility of plants, and aphids are the most common vectors of agriculturally important plant viruses. Several studies showed that viruses manipulate plant’s volatile compounds and plant quality to attract vectors or to deter vector feeding in order to enhance their transmission to healthy plants. The roles of plant persistent viruses in the ecology of their hosts have not been studied thoroughly, but their very long-term relationships with their hosts, and their high level of vertical transmission imply beneficial interactions. Studying the aphid-virus interaction revealed the beneficial role of PCV1 for its Jalapeño host.
Abstract #23
Rachel Walker, Nutritional Sciences
The Effect of Inflammation and Soluble Epoxide Hydrolase Inhibition on Fatty Acid Epoxide Incorporation into VLDL
Rachel E Walker, Olga V Savinova, Theresa L Pedersen, John W Newman, Gregory C Shearer

OBJECTIVE: We have previously observed fatty acid epoxides, a class of potent anti-inflammatory oxylipins, in circulating VLDL. The source of these epoxides is unknown. Cytochrome P450 (CYP450) produces them via oxygenation of polyunsaturated fatty acids (PUFAs), and soluble epoxide hydrolase (sEH) converts them to diols. Our objectives were 1) to investigate if incorporation of epoxides into VLDL occurs via hepatic VLDL synthesis and 2) to determine if incorporation is modulated by inflammation or by inhibition of hepatic sEH.

APPROACH AND RESULTS: A 2×2 factorial design was used for treatment assignment. Livers were isolated from rats treated with pro-inflammatory lipopolysaccharide (LPS, 10 mg/kg ip) or saline. AUDA, an inhibitor of sEH (10 μM), was included or excluded in the perfusate (Control, N=3; LPS, N=4; AUDA, N=4; LPS+AUDA, N=4). Livers were perfused for 180 minutes. VLDL was isolated by ultracentrifugation, then analyzed by LC-MS/MS for oxylipin content. Analyzed epoxides and diols were derived from alpha-linolenic acid (ALA), linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Two-way ANOVA’s were used with triglyceride concentration as a covariate. Concentrations (nM) are reported as mean [95% CI]. DHA-derived epoxides increased with AUDA treatment (3.91 [3.01, 5.07]) compared to livers without AUDA (2.06 [1.58, 2.67]) (p=0.004), but other epoxides were unchanged by AUDA. EPA and ALA-derived epoxides decreased with LPS treatment (0.32 [0.22, 0.47]; 2.44 [2.07, 2.87]) compared to animals without LPS (0.73 [0.46, 1.16]; 3.28 [2.71, 3.96]) (p=0.01; 0.02). AA and DHA-derived diols decreased with LPS treatment (1.01 [0.82, 1.25]; 0.21 [0.17, 0.26]) compared to animals without LPS (1.46 [1.15, 1.86]; 0.31 [0.24, 0.39]) (p=0.03; 0.03).

CONCLUSIONS: Treatment with LPS and AUDA have significant effects on incorporation of epoxides and diols into VLDL, supporting hepatic incorporation controlled by inflammation. Inflammation decreased select EPA- and ALA-derived epoxides. In contrast, sEH inhibition increased only DHA-derived epoxides. Surprisingly, in VLDL only epoxides derived from omega-3 fatty acids were affected by either inflammation or inhibition of sEH.
Abstract #24
Michael Walker, MCIBS
Defining Gene Regulatory Systems in Malaria Parasites with Single-Plasmid, Ribozyme-Guide-Ribozyme CRISPR Interference
Michael P. Walker, Scott E. Lindner

Malaria remains one of the world’s most daunting public health concerns, causing nearly half a million deaths and over 200 million new infections every year. Efforts to characterize essential genes in Plasmodium largely rely on genetic intervention to regulate expression of genes-of-interest. However, these approaches are limited by the time needed to generate transgenic parasite lines and the ability to utilize exogenous components without disturbing native regulatory elements. Furthermore, Plasmodium yoelii, a rodent-infectious species favored for rapid transfection and selection procedures, is significantly hampered by only having a single drug-selectable marker. Here, we show that CRISPR/Cas9 adapted for a single-plasmid intervention system can effectively disrupt expression of genes-of-interest. We have utilized a Ribozyme-Guide-Ribozyme (RGR) strategy in order to efficiently package the essential CRISPR/Cas9 elements with a drug-selectable marker onto a single plasmid. Furthermore, we have minimized the HDR template, as well as included a GFP tag in the HDR to optimize intervention for both “knock-outs” and “knock-ins”. Based on the editing efficiencies of these strategies (90-100%), we hypothesize that CRISPR interference (CRISPRi), using a dCas9 and sgRNAs that target the 5’UTRs of genes-of-interest, would allow us to define gene regulatory systems without genetic integration. We will use our GFP “knock-in” parasites (ALBA4::GFP) to assess the efficacy of this method. By tiling sgRNAs across the 5’UTR of ALBA4, we hope to observe a knockdown of ALBA4::GFP expression. This strategy will provide a “plug-and-play” gene regulation system where a synthetic DNA fragment can be inserted into a single plasmid to generate a panel of RGR-produced sgRNAs. This robust and flexible system would allow for in-depth investigation of essential genes in human-infectious Plasmodium species.
Abstract #25
Cheng-Hsin Wei, Nutritional Sciences
The Impact of Early Postnatal Vitamin A Supplementation on Childhood Obesity Risk — A Study in Young Rat Model
Cheng-Hsin Wei, MS
Alexis L. Weaver,
Connie J. Rogers, PhD
A. Catharine Ross, PhD

Childhood obesity is a serious medical concern because it is associated with many immediate and long-term health consequences, including diabetes, hypertension, and cardiovascular disease. However, studies are limited about the effects of early nutritional supplementation on later health outcomes. The objective of the study was to investigate whether early postnatal vitamin A (VA) supplementation alters metabolic functions related to childhood obesity risk later in life, using a young rat model. We hypothesized that early-life VA supplementation in rats is associated with altered expression of genes related to insulin resistance and thermogenesis at neonatal period, with carry-over effects at prepubescent age. In addition, previously in our laboratory, we showed that the early postnatal treatment with VARA, a retinoid combination of VA and 10% retinoic acid (RA), significantly decreases the expression of retinol-binding protein 4 (Rbp4) in the liver of neonates as well as adult rats and reduced liver Rbp4 expression is associated with improved glucose tolerance. A defined high-fat VA-marginal (HFVAM) diet, which is modified to contain 45 kcal% fat and a marginal level of VA (0.35 µg of retinol as retinyl palmitate per gram diet), was fed to Sprague-Dawley dams from pregnancy until the pups were euthanized at postnatal day (P) 12 or 5 weeks of age. Three doses of VA, VARA, or canola oil as control, were adjusted based on body weight (6 mg VA/kg of body weight) and administered orally to the pups on P0/1, P4, and P10. Plasma and tissues, including liver and adipose tissues, were collected on P12 and 5 weeks of age. Body composition of adolescent rats (5 weeks old) was measured by dual-energy X-ray absorptiometry (DEXA) to determine the effects of early VA supplementation on adiposity in prepubertal rats. Total retinoid concentrations in the plasma and tissues were determined by ultra-performance liquid chromatography (UPLC). Data were compared to age- and sex-matched normal chow-fed non-obese rats as references. There was no significant difference in body weight change between treatment groups, indicating similar growth patterns and successful randomization among pups. The adolescent rats fed a HFVAM diet had significantly higher body fat (%), as compared to rats fed a normal chow diet, but there was no difference in body fat between sexes in each treatment group. The VA-supplemented HFVAM diet-fed adolescent rats had significant higher serum total retinol concentration, as compared to normal chow-fed rats. Both neonatal and adolescent VA-supplemented rats had increased hepatic retinoid storage, as compared to the oil-dosed group, suggesting the possibility of “carryover” effects of early postnatal VA-supplementation on metabolic changes later in life. However, no difference was observed in the brown adipose tissue retinoid concentrations between treatment groups in adolescent rats. The relationship between early-life VA supplementation and metabolic functions related to obesity risk remains to be determined by gene expression analysis.
Abstract #26
Andi Wilson, Visiting Scholar
Unpacking the genetic mechanism behind unisexual reproduction in Huntiella moniliformis
Andrea Wilson 1
Magriet van der Nest 1
Markus Wilken 1
Michael Wingfield 2
Brenda Wingfield 1

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Unisexual reproduction is a reproductive strategy where an individual fungus is able to produce sexual offspring despite possessing genes that represent a single MAT idiomorph. This form of reproduction has been described in a few species of fungi, but was most recently observed in MAT2 individuals of the filamentous ascomycete; Huntiella moniliformis (Wilson et al. 2015. Fun. Genet. Biol. 80:1-9). This fungus is a member of the family Ceratocystidaceae, a group including well-known pathogenic fungi that infect a wide variety of economically important plants. The underlying molecular mechanisms responsible for unisexuality in filamentous fungi are poorly understood and this study aimed to elucidate these mechanisms using a comparative transcriptomics approach. By sequencing the mRNA from vegetative and sexually-competent cultures of H. omanensis, a strictly heterothallic relative of H. moniliformis, we were able to identify some of the genes that are important for sexual reproduction in these fungi. By comparing these data to similar data obtained from cultures of H. moniliformis, we were able to detect significant differences in gene expression between the heterosexual and unisexual pathways. Most notable was the mating-type-independent expression of both the α- and a-factor pheromone genes in the unisexual H. moniliformis. This was in contract to H. omanensis, were MAT1 individuals produce the α-factor pheromone and MAT2 individuals the a-factor pheromone in a manner similar to other heterothallic species such as Neurospora crassa. H. moniliformis cultures also expressed both pheromone receptors at a constitutive level during vegetative growth and sexual reproduction compared to the potentially mating-type-specific expression observed in H. omanensis. The results suggest that mating-type-independent expression of these pheromones plays an important role in the unisexual capabilities of H. moniliformis. This can be compared to one of the unisexual pathways in C. albicans where endogenous pheromone production allows for self-activation.
Intestinal colonization by the foodborne pathogen E. coli O157:H7 causes serious disease symptoms, including bloody diarrhea and severe abdominal cramps. The disease can further develop into hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC). Synthesis of one or more Shiga toxins (Stx) is essential for HUS and HC development. The genes encoding Stx, including Stx2a, are encoded by a lambdoid prophage integrated into the E. coli O157:H7 chromosome. Enhanced Stx2a expression was reported when specific non-pathogenic E. coli strains were co-cultured with O157:H7, and it was hypothesized that this phenotype required the former to be sensitive to infection by the Shiga toxin-converting phage. We tested this hypothesis by using a previously published method [1] to replace bamA (an essential gene and Stx phage receptor) in non-pathogenic E. coli strains with the ortholog from Salmonella enterica. Such heterologous gene replacement abolished the ability of E. coli strain C600 to enhance toxin production when co-cultured with E. coli O157:H7 strain PA2, which belongs to the hypervirulent clade 8 cluster. Two extracellular loops of BamA (loops 4 and 6) were further shown to be important for infection by Stx2a phage. However, gene replacement in other non-pathogenic E. coli strain revealed a bamA-independent mechanism for toxin amplification. Collectively, these data suggest that multiple mechanisms exist for commensal E. coli to increase Stx production when they co-exist with E. coli O157:H7.
Abstract #28
Xiaoyu Zhu, BMMB
Arabidopsis PASTICCINO2 (PAS2) is a multifunctional protein involved in cellulose biosynthesis
Xiaoyu Zhu, Shundai Li, Frédérique Tellier, Renhong Wu, Jean-Denis Faure, Kian Hematy, Ying Gu*

In higher plants, cellulose is synthesized by membrane spanning large protein complexes named cellulose synthase complexes (CSCs). Through a membrane-based split ubiquitin yeast two-hybrid screen, we identified the Arabidopsis PASTICCINO2 (PAS2) protein as an interacting partner of primary cell wall CSCs. Mutations in pas2 cause defective cell elongation that is associated with the reduction of cellulose content. PAS2 was previously characterized as the plant 3-hydroxy-acyl-CoA dehydratase, an ER membrane-localized dehydratase that is essential for very-long-chain-fatty acid (VLCFA) elongation. However, transgenic lines that specifically disrupted the activity of VLCFA dehydratase did not cause deficiency in cellulose synthesis, suggesting the function of PAS2 in cellulose is independent of its VLCFA elongase activity. Consistent with this hypothesis, pharmacologically inhibition of VLCFA elongase by Flufenacet did not interfere with cellulose synthesis. We also discovered that PAS2 localized to Golgi apparatus, which has not been shown previously. The novel localization of PAS2 in Golgi supports a unique function of PAS2 in the cellulose synthesis.
Abstract #29
Catherine Herzog, Biology
Distribution of peste des petits ruminants virus in a multi-species system in Northern Tanzania
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Modeling disease ecology in multi-host systems faces many challenges including identifying the population size required to support transmission, determining the maintenance community of hosts involved, and measuring cross species transmission in the field. For livestock disease, linking transmission models at multiple scales to data and applying these models in resource poor settings continue to be major challenges. The recent geographical expansion and endemic establishment of peste des petits ruminants virus (PPRV) in African livestock and wildlife is an example of a understudied multi-host disease system in a resource poor setting. Currently, PPRV has spread to more than 70 countries in Asia, the Middle East, and Africa. PPRV threatens 80% of the global small ruminant population of nearly 2 billion animals. Over 330 million farmers’ livelihoods rely directly on small ruminants and the demand for meat and milk is expected to rise 137-177% by 2030.

PPRV, a Morbillivirus in the same genus as rinderpest virus (RPV) and measles virus, is spread by direct contact with infected hosts, aerosols, or fomites. PPRV causes high morbidity and mortality in domesticated sheep and goats and can infect cattle subclinically. After the recent global eradication of RPV and end of RPV vaccination programs, there is increasing evidence that PPRV dynamics have changed in that PPRV outbreaks have increased in small livestock and serological evidence of PPRV infection has been found in cattle and wildlife. Tanzania first reported PPRV in 2008, with PPRV seroprevalence of 45.8% in small ruminants in northern districts. PPRV spread south, following trade of infected animals. While a few outbreak studies of PPRV in Tanzania have been reported, there are no large cross-sectional or longitudinal serosurveys or modeling studies of PPRV transmission in Tanzanian livestock. This study seeks to elucidate the epidemiology of PPRV in a large serosurvey of northern Tanzanian sheep, goats, and cattle. Furthermore, this research will explore the effects of herd demographic dynamics, geography, and herd management practices on PPRV seroprevalence, transmission, and persistence in this endemic region.

Serological samples, outbreak history, and vaccination surveillance data were collected from a total of 20 pastoral and agropastoral villages in northern Tanzania. Of the 7,570 sheep, goat, and cattle serum samples with extensive household survey data, a total of 7,542 samples with recorded species, age, and gender information were tested in duplicate for PPRV antibodies using a BDSL Pirbright competitive ELISA kit. Overall, PPRV seroprevalence ranged from 1-47%, with a range of 0-63% in sheep, 0-68% in goats, and 0-34% in cattle. Seroprevalence also varied by village management system and geography, with the pastoral communities having higher PPRV seroprevalence than the agropastoral villages. Together, these data suggest that there is wide variation in PPRV seroprevalence by both geography and management system and that the particular characteristics of the villages and practices of each management system should be examined further to determine their role in
PPRV transmission and aid in the development and parameterization of herd demographic composition models and compartmental disease transmission models.