LIFE SCIENCES

RESEARCH SYMPOSIUM XIII

Program & Abstracts

January 14, 2022

9:00 am - 5:00 pm

Webinar:

https://albany.zoom.us/j/91522066896?pwd=bllicXpqSHVQL3M5OHl6YVFxQkZmQT09

University at Albany

State University of New York

College of Arts & Sciences

Program

9:00 - 9:10 **Opening Remarks:** **Dr. Jim Dias**, Vice President for Research, SUNY Albany

9:10 - 9:15 **Postdoc Association:** **Gabriele Baniulyte**

9:10 - 9:15 **Scientista**, **Lindsey Tolman**

9:20 - 9:40 **Speed Talks I : Moderator Cheryl Andam**

9:20-9:25 **Kristen Kaytes -*Biology*,** 'The role of conserved poly(A) sequences in the 3' UTR of Zika virus gene expression'

9:25-9:30 **Alyssa Hoy –*Chemistry*,** 'Click Chemistry backbone linkage provides a split-and-click strategy to modified CRISPR sgRNA'

9:30-9:35 **Alex Lemus -*Biology*,** 'Multispecies aggregates from human dental plaque nucleate highly diverse spatially structured oral biofilms on saliva coated surfaces'

9:35-9:40 **Joseph Ellis –*RNA Institute*,** 'Transcriptome-wide assessment of MBNL-dependent alternative splicing in mouse and humans

9:45 - 10:50 **Keynote Speaker I : Dr. Julie Biteen, Professor of Chemistry and Biophysics, University of Michigan**

'Understanding how subcellular components interact and organize in microbiology with single-molecule microscopy '

10:50 - 11:10 ***Coffee break***

11:10 - 12:10 **Selected Student Presentations I : Moderator Jeremy Feldblyum**

11:10-11:30 **Lindsey Tolman - *Biomedical Sciences*,** 'Eliciting respiratory immunity by single-dose immune complex vaccination'

11:30-11:50 **Allix Coon** - ***Chemistry*,** 'GC×GC-MS in the clinic? Towards diagnosis of Ménière's Disease'

11:50 - 12:10 Speed Talks II : Moderator Cheryl Andam

11:50-11:55 **Humphrey Omega**—***Biology,*** ' Defining the regulatory roles of the mnm cluster enzymes and RNA modifications during bacterial stress'

11:55-12:00 **Afrooz Golestanian** -***Biology,*** ' Analysis of gene expression and environmental exposure responses in myotonic dystrophy type 1 patient cells'

12:00-12:05 **Zhihua Chang** –***Chemistry,*** 'Development of a fluorescence assay for high-throughput screening of small ligands targeting CUG and CCUG RNA repeats'

12:05-12:10 **Eduardo Peru** –***Psychology,*** 'A comparison of afferent projections to amygdala from prefrontal cortex and ventral CA1 in the male and female rat'

12:10 - 1:30 *Lunch break and Student Flashtalks (2-min presentation + 1-min Q&A)*

12:25-12:28 **Samantha Hetherington -** 'Stress enhanced fear learning in juvenile and adult male and female rats'

12:28-12:31 **Jacob Schroader -** 'Disease-associated inosine misincorporation into RNA hinders translation'

12:31-12:34 **Nichole Traver -** 'Spatial structure of dental plaque biofilms in canines with periodontal disease'

12:34-12:37 **Emmanuel Adade -** 'FISH probe optimization for mapping the spatial structure of the gut microbiome in gnotobiotic zebrafish'

12:37-12:40 **Shu Jun Lin -** 'Noncanonical translation initiation of SARS-CoV-2 5' UTR '

12:40-12:43 **Shawn Gianola** - 'Zika virus gene expression differentially regulated by the 4-acetylcytosine reader NAT10'

12:43-12:46 **Nicholas Moskwa** - 'FGF2 stimulated mesenchyme controls salivary acinar differentiation through BMP signaling'

12:46-12:49 **Raghu Rum Katreddi** - 'Notch signaling determines cell-fate specification of the two main types of vomeronasal neurons of rodents'

12:49-12:52 **Rachel Lange** - 'Reemergence of Powassan Virus lineage 1 in New York State'

12:52-12:55 **Rachel Fay** - 'Unique interactions between viral genotype and mosquito population determine regional variability in the effect of increased temperature on vectorial capacity of Cx. pipiens for West Nile virus'

12:55-12:58 **Samuel Sears** - 'Raman Spectroscopy for the study of fingermark aging: Universal method development'

12:58-1:01 **Lamyaa Almehmadi** - 'SERS, a single-molecule and label-free technique for drug discovery'

1:01-1:04 **Luis Perez-Almodovar** - 'A novel detection method of Fish Exposed to Perfluoroalkyl Substances (PFAS: Raman Spectroscopy of blood plasma and chemometrics)'

1:04-1:07 **Tuan Tran** - 'Investigate protein protein interaction via machine learning framework'

1:07-1:10 **Olivia Buyea -** 'Demonstrating that insulin-like growth factor 2 enhances glucose uptake in primary hippocampal neurons via the IGF2 receptor'

1:10-1:13 **Paige Grane -** 'The effect of developmental exposure to synthetic progestin on depressive-like behavior in adolescent rodents'

1:13-1:16 **Emily Davey -** 'A 12-week strength exercise program improves transcriptome-level changes present in men with myotonic dystrophy type 1'

1:30 - 2:00 Speed Talks III : Moderator Jeremy Feldblyum

1:30-1:35 **Ed Zandro Taroc –*Biology,*** 'Gli3 has a dose dependent and cell autonomous role in OEC development and GnRH-1 migration'

1:35-1:40 **Alexis Weber -*Chemistry,*** 'Raman spectroscopy to tackle the analysis of bloodstains in crime scene conditions'

1:40-1:45 **Sean Bialosuknia –*Ecology and Evolutionary Biology,*** 'Adaptive evolution of West Nile virus facilitated increased transmissibility and prevalence in New York State '

2:00 - 3:00 Selected Student Presentations II : Moderator Cheryl Andam

2:00-2:20 **Amber Altrieth - *Biology,*** 'Endothelial cell contributions to salivary gland injury'

2:20-2:40 **Ruogu Wang - *Mathematics and Statistics,*** 'Sparse Poisson regression approach to biological spectral imaging'

2:40-3:00 **Subodh Mishra - *RNA Institute,*** 'A CTG repeat-selective screen of a natural product library reveals dietary natural compounds as potential therapeutics for Myotonic Dystrophy'

3:00 - 3:20 *Tea time*

3:20 - 4:30 Keynote Speaker II : Dr. Victoria D'Souza , Professor of Molecular and Cellular Biology, Harvard University

'Alternate structure in RNA'

4:30 - 4:50 Award Ceremony : Dr. Jeanette Altarriba, Dean of the College of Arts & Sciences, SUNY Albany

Keynote Speakers

Dr. Julie Biteen

Professor of Chemistry and Biophysics, University of Michigan

'Understanding how subcellular components interact and organize in microbiology with single-molecule microscopy '

Dr. Victoria D'Souza

Professor of Molecular and Cellular Biology, Harvard University

'Alternate structure in RNA'

Abstracts

Ed Zandro Taroc ***Biology***, *etaroc@albany.edu*

'Gli3 has a dose dependent and cell autonomous role in OEC development and GnRH-1 migration'

Gonadotropin releasing hormone-1 (GnRH-1) is the master regulator hormone of sexual development and pubertal onset in most vertebrate species. This hormone is released by a subset of neurons known as the GnRH-1 neurons (GnRH-1ns), which controls the hormonal axis between the hypothalamus, pituitary gland, and the gonads. The GnRH-1ns reside within the hypothalamus but originate from the olfactory placode in the nose. During embryonic development the GnRH-1ns migrate with a neural crest derived glial cell type known as olfactory unsheathing cells (OECs) along the axons of the terminal nerve to get to the brain and eventually to their final positions within the hypothalamus. Perturbations in the development and/or migration of the GnRH-1ns or their ability to release GnRH-1 in humans leads to a disorder known as hypogonadotropic hypogonadism (HH), characterized with delayed or absent puberty. Previous studies have shown that OECs are crucial for GnRH-1ns migration. We recently demonstrated that loss of transcription factor Gli3 in mouse caused a loss of OECs that led to GnRH-1 migratory defects. Whether cell autonomous loss of Gli3 in the OECs is the cause of this defect is still unknown. To test what role Gli3 has in OEC development and GnRH-1 migration we utilized two mouse model 1) Gli3Pdn/Pdn which is a hypomorphic Gli3 model and will elucidate if Gli3 has a dose dependent effect and 2) Sox10Cre/Gli3Flx that will conditionally knockout Gli3 in all neural crest derived cells. Preliminary data suggest Gli3 having a dose dependent and cell autonomous effect on OECs development and GnRH-1 migration.

Afrooz Golestanian ***Biology***, *agolestanian@albany.edu*

'Analysis of gene expression and environmental exposure responses in myotonic dystrophy type 1 patient cells'

Myotonic dystrophy (DM) is the most common form of muscular dystrophy. DM is a neuromuscular disease caused by microsatellite repeat expansions, and it can represent as a multi-systemic autosomal dominant disease with DM1 and DM2 subtypes. DM1 is caused by a cytosine-thymine-guanine (CTG) triplet repeats in the 3' untranslated region (3'UTR) of myotonic dystrophy protein kinase (DMPK) gene, which leads to the production of a longer abnormal and toxic mRNA. The toxic DMPK mRNA sequester the splicing proteins Muscle blind-like (MBNL) and rbFOX, which alters gene expression. Repeat associated non-AUG (RAN) translation also occurs in DM1, and we postulated that translational regulation will be globally affected. Further mitochondrial dysregulation has been reported in DM and we predict that DM1 patient cells will be sensitive to environmental agents that promote increased ROS and poison the mitochondria. Transcripts up-regulated in DM1 cells are involved in multi cellular organism development, muscle tissue, organ, and structure development, system development, and development processes. Transcripts down-regulated in DM1 cells represent pathways such as regulation of multi cellular organismal process, response to stimulus, cell communication and signaling. We have used cell viability assays and the mitochondrial toxicant NaAsO2 to analyze if patient derived cells are sensitive to this stressor. While patient derived fibroblasts do not show clear sensitivity trends, we are expanding studies to myotubes to further our analysis. We are currently using computational approaches to analyze mRNA-seq data for gene expression trends that will inform on translation. Our work is significant because we are exploring new biology and exposure response specific to Myotonic Dystrophy type1.

Humphrey Omeoga ***Biology***, *homeoga@albany.edu*

'Defining the regulatory roles of the mnm cluster enzymes and RNA modifications during bacterial stress'

The gram-negative bacteria, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa and Salmonella typhimurium contain unique wobble uridine (U34) modifications and writer enzymes, compared to other bacteria and humans. The mnm-cluster enzymes, mnmH, mnmA, mnmC, mnmE, and mnmG modify E. coli tRNA at the U34 of tRNALys, tRNAGlu, and tRNAGln. The chemistry of the tRNA wobble U34 nucleotide in E. coli can contain thiolation, geranylation or selenation products at position 2, as well as distinct groups (methyl and carbonyl) on position 5, producing 12 different modified uridines. We predict that the mnm enzymes play roles in stress responses. In support we have previously shown that mnmH E. coli cells are sensitive to the antibiotic CAM. Recently we have shown that E. coli mnmA cells are sensitive to heat, and that mnmH, mnmE, mnmA, mnmC, and mnmG cells are sensitive to the free radical-generating compounds, menadione and hydrogen peroxide. Using mRNA-seq we will determine if the transcriptome is reprogrammed in specific mutants to adapt to writer loss, and if there are any markers of stress. Further, we hypothesize that E. coli position 2 (s2U, ges2U and se2U) and position 5 [nm5s2U, nm5ges2U, nm5se2U, c(mnm5s2U, mnm5ges2U and mnm5se2U)] - based tRNA modifications are altered in response to heat, CAM, hydrogen peroxide and menadione to regulate the translation of stress response proteins. To test, we will expose cells to different stressors, RNA will be purified, and tandem LC-MS/MS will be used to characterize tRNA modifications in the wild type and mutant strains. We predict that RNA modifications generated by the mnm-cluster enzymes promote the synthesis of key stress-response proteins, and will use polysome RNAseq, to determine if the translation of specific transcripts is perturbed in modification-deficient cells. In addition, the WES ProteinSimple automated western blot systems will be employed for detection of specific proteins. Our work is significant because it will describe the tRNA modifications in bacteria under environmental stress and highlight the proteins that play roles in attenuating the effect of stress on bacteria. It will also serve to define salient mechanisms involved in translational regulation of the bacterial system, which can present as potential targets of some antibiotics.

Nicole Traver ***Biology***, *ntraver2@albany.edu*

'Spatial Structure of Dental Plaque Biofilms in Canines with Periodontal Disease'

Periodontal disease is an infection of the gums that can lead to bone resorption in the jaw, which can then lead to tooth loss. A contributor and indicator of periodontal disease is the buildup of plaque, which is a microbial biofilm that colonizes on the tooth enamel and leads to infection of the gums. About 44-63.6% of adult canines suffer from periodontal disease, with smaller and toy breed dogs being more at risk, as well as older dogs. It has been shown that humans and canines share similarities in oral taxa at the phylum level, and even more specifically at the genus level, such as Neisseria and Actinomyces. We hypothesize that conserved features of the oral microbiota in canines and humans, including the spatial structure of dental plaque biofilms, correlate with microbial community function in the periodontal disease process. To test our hypothesis, we began by performing a meta-analysis of the literature to determine the top 15 most abundant and most prevalent genera of bacteria in supragingival and subgingival plaque. The programs ARB and MathFISH were used to design genus-level probes, which will then be used in CLASI-FISH to label the plaque on teeth extracted from canine periodontitis patients. 80 probes were designed for 19 different genera and 4 families. Once the teeth are extracted from patient dogs, they will be immobilized and fixed in Histogel, preserving the dental plaque biofilms, to allow us to perform FISH. We found that cells could be labelled by CLASI-FISH through a layer of Histogel, further supporting that Histogel is an ideal embedding agent for the histological processing of canine teeth. In conclusion, a comprehensive understanding of the structure of periodontitis-associated dental plaque in canines may allow earlier diagnosis of the disease and development of more specific therapeutics for both dogs and humans.

Emmanuel Adade ***Biology***, *eadade@albany.edu*

'FISH probe optimization for mapping the spatial structure of the gut microbiome in gnotobiotic zebrafish'

The colonization pattern and spatial structure of the microbiota is important to host health and reflects bacteria-bacteria interactions. In communities with phylogenetically closely related organisms, the design of species-specific FISH probes for discriminating community members is challenging due the high level of homology in the 16S rRNA gene. We hypothesize that the spatial structure of the gut microbiome is highly non-random and reflects different patterns of growth and intercellular interaction that promote colonization resistance to infections. To test this hypothesis, we will map the spatial structure of 10-member consortium bacteria and a pathogen, Flavobacterium columnare in a gnotobiotic zebrafish model. Fluorescence in situ hybridization (FISH) with probes for the 16S and 23S rRNA of 11 isolated bacteria in our gnotobiotic zebrafish will be designed and validated for their specificity to target organisms. Probes are designed de novo and tested them against isolates of each species grown in pure culture. We found that while some of our probes bind specifically to the target (measured as florescence intensity), some demonstrate non-specific binding, presumably due to high 16S homology. We have validated 3 out of 20 probes that can uniquely bind to their targets. To increase specificity, we are optimizing a high throughput screening experiment using High Phylogenetic Resolution (HiPR) FISH probes which will allow us to screen many different probes in parallel. In addition, we are exploring a machine learning approach to classify labeled organisms in the presence of cross-reactive probes. Our validated probe repertoire will allow us to map the spatial structure of the gut microbiome in gnotobiotic zebrafish to achieve a comprehensive understanding of microbial community structure and the contribution of the normal microbiota to infection resistance in the host.

Shawn Gianola ***Biology***, *sgianola@albany.edu*

'Zika virus gene expression differentially regulated by the 4-acetylcytosine reader NAT10.'

Chemical moieties or marks may be deposited on RNA to affect structure, localization, and function of the RNA. Although more than 160 chemical moieties are known to decorate RNA, the biological role for the vast majority of these modifications is unknown. This is in part due to the limited detection reagents, and knowledge of the enzymes involved in installing (writer), removing (eraser) or interpreting (reader) the specific RNA modification. The long-term objective in our lab is to elucidate the role RNA modifications on positive-sense RNA virus gene expression.

We recently completed a survey of the RNA modification landscape during infection with poliovirus, hepatitis C virus, Zika virus (ZIKV) and Dengue virus. We identified particular dimethyl-cytosine modifications (m5Cm and m44C) that appeared only during viral infection. We also observed that the transcriptome-wide abundance of 4-acetylcytosine (ac4C) modification decreased during ZIKV infection. Despite this decrease in total ac4C levels, ZIKV RNA was heavily decorated with ac4C. To investigate the role of ac4C and the putative writer enzyme NAT10 in Zika gene expression we first performed an analysis to predict the number of ac4C sites within the ZIKV genome. The PACES program predicted five putative ac4C marks within the ZIKV RNA, compared to the twelve ac4C moieties estimated by our mass spectrometry analysis . Next, we infected wild-type (WT) and NAT10 knock-out (KO) cells with ZIKV and examined the abundance of viral protein and RNA, and production of infectious virions. By western blot we observed that the viral protein levels increased during ZIKV infection in NAT10 KO cells compared to WT cells. In contrast, RT-qPCR analysis showed that the levels of ZIKV RNA did not change between WT and NAT10 KO cells. Similarly, quantification of the new viral particles produced by plaque assay revealed little to no change in the viral titers. Together these data suggest that NAT10 and possibly also ac4C modifications affect ZIKV translation. Our study provides new insight into the role of NAT10 and ac4C RNA modifications on ZIKV infection, which may establish the groundwork for a novel antiviral target.

Nicholas Moskwa ***Biology***, *nmoskwa@albany.edu*

'FGF2 stimulated mesenchyme controls salivary acinar differentiation through BMP signaling.'

Multicellular organisms exist because cells can differentiate and compartmentalize into specialized structures. How cells control this ordering is a key developmental biology question. Neural crest-derived stromal mesenchyme (NCS) critically signals embryonic oral epithelial differentiation into secretory epithelium. These NCS signals are incompletely understood and these NCS possible exist as a diverse community of cells that participate in specific controls. We identified distinct causal NCS subpopulations using single-cell RNA sequencing on embryonic salivary glands and organoids. We have previously developed a complex epithelial-stromal salivary organoid model for elucidating cellular compartment interactions. We know that the NSC require fibroblast growth factor 2 (FGF2) autocrine signaling and hypothesized that the NSC FGF2 signaling drives paracrine factor expression directly controlling epithelial morphogenesis and differentiation. In our organoids, FGF2 signaling selected for stromal cell subsets expressing platelet derived growth factor alpha (PDGFRα). This PDGFRα stromal subpopulation alone simulates epithelial AQP5 similarly to total stroma. Further stromal signaling analysis using whole-transcriptome microarrays revealed multiple FGF2-induced signaling factors. We tested these FGF2-dependent factors as a stromal signaling replacement, assaying for the water channel protein, Aquaporin 5 (AQP5) as an epithelial differentiation indicator and organoid size as a morphogenesis indicator. We found that NCS-driven epithelial AQP5 expression, but not morphogenesis, was dependent on bone morphogenetic protein (BMP) signaling. FGF2 and BMP7 synergy was required for proacinar differentiation. These data suggest that BMP7 together with other PDGFRα stromal FGF2-dependent factors synergize, promoting epithelial differentiation. Here, we demonstrate epithelial differentiation is driven by PDGFRα stromal cell signaling, which may be manipulated for future regenerative therapies.

Amber Altrieth *Biology, aaltrieth@albany.edu*

'Endothelial Cell Contributions to Salivary Gland Injury'

Normal injury repair requires extracellular matrix remodeling, however if extracellular matrix deposition is prolonged, it can lead to fibrosis. Fibrosis can impede proper organ function and is responsible for approximately 45% of deaths world-wide. Endothelial cells have been identified as a driver of fibrosis in many organs, including the liver, lung, and kidney. In the liver specifically, endothelial cells provide an important balance between a regenerative or a fibrotic response following chemical injury. However, the contribution of endothelial cells to salivary gland fibrosis is currently unknown. To define the contributions of endothelial cells to salivary gland fibrosis, we performed ductal ligation surgery on 12-week-old wild type (C57BL6/J) mice and 12-week-old double transgenic mice for lineage tracing of endothelial cells ((PAC)Cdh5CreERT2;(CAG)ROSA26TdTomato) mice. In the ductal ligation surgery, a metal clip is placed on the main ducts that transports saliva from the submandibular and sublingual glands to the mouth of the mouse; the clip remains in place for 14 days to induce a fibrotic response. Samples were then harvested for single cell RNA sequencing (scRNAseq) or immunofluorescent staining. Integration of mock and ligated scRNAseq data and k-means clustering using Seurat identified 29 different cell clusters, which included endothelial cells, immune cells, epithelial cells, and stromal cells. The endothelial cell data was subclustered to identify heterogeneity within this cell population. We identified multiple subclusters of endothelial cells, some of which were expanded following ductal ligation surgery. Gene pathway analysis using Metascape revealed different predicted functions for endothelial subsets. Immunostaining of lineage traced salivary gland endothelial cells following ductal ligation or mock surgery is in progress to examine predictions of scRNAseq analysis. Identifying the mechanisms of fibrosis in the salivary glands can inform future therapeutics for restoration of gland function following injury.

Raghu Ram Katreddi *Biology, rkatreddi@albany.edu*

'Notch signaling determines cell-fate specification of the two main types of vomeronasal neurons of rodents.'

The ability of terrestrial vertebrates to find food and mating partners and to avoid predators heavily relies on the detection of chemosensory information from the environment. Semiochemicals responsible for social and sexual behaviors are detected by chemosensory neurons of the vomeronasal organ that transmit information to the accessory olfactory bulb (AOB). The vomeronasal sensory epithelium (VSE) of most mammalian species contains a uniform vomeronasal (VN) system; however, rodents and some marsupials have developed a more complex binary VN system, containing vomeronasal sensory neurons (VSNs) expressing either receptors of the V1R or V2R family. In mice, V1R and V2R VSNs form from a common pool of progenitors but have distinct differentiation programs. We used single cell RNA sequencing to identify differential expression of Notch1 receptor and Dll4 ligand among the neuronal precursors at the VSN dichotomy. We further demonstrated that Notch signaling is required for effective differentiation of V2R+ basal VSNs. Forced Notch signaling in immediate neuronal precursors can divert them to the basal fate, while earlier ectopic Notch expression redirects progenitors to a non-neuronal fate. Together, these results demonstrate that Dll4-Notch1 signaling plays a crucial role in triggering the binary dichotomy between the two main types of VSNs in mice and implicate this signaling pathway in the evolutionary diversification of sensory neuron identity.

Kristen Kaytes ***Biology,*** *kkaytes@albany.edu*

'The role of conserved poly(A) sequences in the 3' UTR of Zika virus gene expression'

Zika virus (ZIKV) and Dengue virus (DENV) are flaviviruses within the family of Flaviviridae. These viruses have a single-stranded positive-sense RNA genome that contains a 5'-cap but lacks a poly(A) tail in the 3' untranslated region (UTR). This suggests that these viruses likely use a mode of translation distinct from that of cellular mRNAs. Despite the lack of a poly(A) tail, DENV is known to interact with poly(A) binding protein (PABP). Bioinformatic analysis of the 3' UTR of ZIKV and DENV revealed six short stretches (3-6 nucleotides long) of adenosines with unknown roles in flavivirus gene expression.

The goal of this project is to dissect the role of these adenosine (A)-rich regions in ZIKV subversion of host translation machinery. We posit that tdbhese A-rich regions within flavivirus 3'UTRs mimic the function of the poly(A) tail of cellular mRNAs. To investigate this, we used site-directed mutagenesis within the context of a ZIKV minimal genome construct that contained a nano luciferase gene flanked by the ZIKV 5' and 3' UTRs. We mutated three conserved A-rich regions, namely 6As before the pseudo (Ψ)-dumbbell (DB) structures (pre-Ψ-DB), 4As within the Ψ-DB (endo-Ψ-DB), and 3As after the dumbbell structure (post-DB). These regions were mutated to U-rich regions, the A-rich region deleted, or expanded to 12 adenosines, the minimal binding region for PABP. These mutants were then in vitro transcribed and nano luciferase activity used as a proxy for translation in rabbit reticulocyte lysate and following transfection into the human hepatoma cell line, Huh7.5. Mutation of the A-rich to an U-rich region or expansion of the A-rich region within the Ψ-DB RNA structure significantly increased translation of the ZIKV minigenome in Huh7.5 cells. In contrast, the same mutants present before the Ψ-DB and or after DB structure decreased in translation of the ZIKV minigenome. Individual deletion of all three the A-rich regions within the 3' UTR of the ZIKV minigenome decreased translation, with the post-DB mutant showing the most significant defect in translation. These data together show that the A-rich regions differentially affect translation of the ZIKV minigenome, with the A-rich region within the Ψ-DB structure being particularly responsive to mutations.

Lindsey Tolman ***Biomedical Sciences,*** *ltolman@albany.edu*

'Eliciting Respiratory Immunity by Single-Dose Immune Complex Vaccination'

Inhalation of ricin toxin (RT) elicits profuse inflammation and localized cell death within the upper and lower airways, ultimately culminating in acute respiratory distress syndrome (ARDS). We previously reported that, in mice, the effects of RT exposure are nullified by intranasal administration of a bipartite monoclonal antibody (MAb) cocktail consisting of PB10 against ricin's enzymatic subunit (RTA) and SylH3 against ricin's binding subunit (RTB). We now report that delivery of these two mAbs as an RT-immune complex (RICs) to mice by the intranasal (i.n.) or intraperitoneal (i.p.) routes stimulates the rapid onset of RT-specific serum IgG that persists for months. RIC administration also induced high titer toxin-neutralizing antibodies, as measured on days 30 and 90. Moreover, RIC-treated mice were immune to a subsequent 5 x lethal dose 50 (LD50) RT challenge days 30 or 90. Intranasal RIC delivery was more effective than intraperitoneal delivery at rendering mice immune to intranasal RT exposure, which we attribute to the formation of inducible bronchus-associated lymphoid tissue (iBALT). The onset of RT-specific serum IgG following RIC delivery was unusual in that it was independent of FcγR engagement, as revealed through FcγR knockout mice and RICs generated with PB10/SylH3 LALA derivatives. We speculate that the observed immunity to RT elicited following RIC treatment initiates via an uptake pathway possibly involving C-type lection receptors (CLRs) that recognize RT's high mannose side chains.

Alexis Weber ***Chemistry,*** *aweber@albany.edu*

'Raman spectroscopy to tackle the analysis of bloodstains in crime scene conditions'

Blood traces are commonly found at crime scenes and can provide substantial information about the event that occurred and individuals involved. Determining the time of crime is an important goal for crime scene investigations, which can be achieved by estimating the time since deposition (TSD) of bloodstains. If crime scenes contain multiple sets of bloodstains, the calculated TSD should allow for the selection of bloodstains relevant to the crime; and therefore, reduce the number of samples which should be collected, documented, and processed.
Vibrational spectroscopy paired with chemometrics has shown provide reliable, rapid, and non-destructive methodologies to determine the TSD of bloodstains. However, research conducted with these techniques so far have analyzed the aging of bloodstains, specifically the degradation of hemoglobin, in ambient conditions. However, crime scenes are not always in such pristine environments and degradation rate of hemoglobin is commonly affected by the surrounding environment. Therefore, it is necessary to develop a model that is capable of estimating the TSD of bloodstains in different environments.
There are infinite varieties of potential environmental conditions. Our goal is to determine how potentially extreme conditions affect the aging mechanism of bloodstains, high temperature in particular. For this purpose, fresh blood samples were collected so that no anticoagulants were present, which potentially can affect the ex vivo aging mechanism of blood. The bloodstains were then aged in a controlled heated environment and tested at numerous time points post deposition. After the spectra were collected, they were loaded into statistical software for preprocessing and modeling. The reproducibility of heated blood analysis and TSD determination model will be discussed

Allix Coon ***Chemistry,*** *acoon@albany.edu*

'GC×GC-MS in the Clinic? Towards Diagnosis of Ménière's Disease'

Blood and tissue remain the primary biological materials used for disease diagnosis. However, the pain, discomfort, invasiveness and cost often associated with their collection have prompted consideration of alternative approaches that utilize non-traditional matrices. Earwax is an underutilized matrix that is receiving increased attention due to its ease of accessibility. Because it is a lipid-rich material, it has the potential to reflect lipid profile changes that are correlated to disease. However, its chemical complexity makes it challenging to analyze using traditional techniques. Furthermore, to be useful, the profile of "normal" earwax must be established. We utilized two-dimensional gas chromatography-mass spectrometry (GC×GC-MS) to determine the chemical profile of earwax. From GC×GC-MS analysis of ethyl acetate extracts of samples acquired from healthy donors, 44 compounds, which is the greatest number ever detected or observed in un-saponified earwax samples, were identified. Represented classes included alkanes, alkenes, fatty acids, esters, triglycerides and cholesterol esters. Earwax from patients diagnosed with Ménière's disease, a disorder of the inner ear, was also analyzed using this technique. Visual comparison of the GC×GC-MS contour plots revealed recognizable patterns representing either earwax from healthy donors or that from individuals with Ménière's disease. Major distinctions between the two included differences in triglyceride profiles, and levels of fatty acids. The results demonstrate that earwax can serve as a viable and readily accessible matrix that can be analyzed to show the presence of disease states. These observations pave the way for the use of this biological matrix in medical diagnostics.

Lamyaa Almehmadi ***Chemistry***, *Lalmehmadi @albany.edu*

'SERS, a single-molecule and label-free technique for drug discovery'

The field of drug discovery relies on sensitive, specific, and fast methods to identify hits. The ultra-high sensitivity and rich information obtained using Surface-enhanced Raman spectroscopy (SERS) render it an emerging technique for drug discovery.

Previously, we have developed a SERS platform with single-molecule sensitivity to detect a protein-linker adduct at a single-molecule level. This platform has also shown the possibility of differentiating the protein's spectral contribution from the linkers *via* visual inspection and statistical analysis. Therefore, we extended the application of this platform to detect the binding of a peptide ligand to targeted RNA repeats at a nanomolar concentration. The selected ligands are potential drug molecules that interact with disease-related RNA repeats. The binding trends found using SERS detection correlated with the binding affinity studies of different ligands. Furthermore, the binding ligand was also differentiated from the non-binding ligand and the RNA based on the analysis of the collected SER spectra. These differentiations were possible via visual inspection and statistical analysis.

Zhihua Chang ***Chemistry,*** *zchang@albany.edu*

'Development of a Fluorescence Assay for High-throughput Screening of Small Ligands Targeting CUG and CCUG RNA Repeats'

Pathogenic CUG and CCUG RNA repeats have been associated with myotonic dystrophy type 1 and 2 (DM1 and DM2) respectively. Identifying small ligands that can bind these RNA repeats is of great significance to develop potential therapeutics to treat these neurodegenerative diseases. In this work, we developed an efficient fluorescence-based assay to improve the detecting sensitivity and reduce false positive readings. The system utilizes the changes of fluorescent signal that is initially minimized by covalently incorporating the fluorescein into the RNA repeat sequences and later increased upon ligands binding. Using a short peptide ligand that could bind to CUG repeat as the positive control, we demonstrate this new biding assay is consistent with other conventional binding characterization techniques such as ITC and gel shifting assay, indicating its feasibility for wide applications to measure the binding constants and kinetics in a simple and high-throughput fashion. In addition, we extend its application by successfully identifying a cyclic peptidomimetic ligand that also targets CUG repeats.

Alyssa Hoy ***Chemistry,*** *ahoy@albany.edu*

'Click Chemistry backbone linkage provides a split-and-click strategy to modified CRISPR sgRNA'

First discovered as a defense mechanism in Streptococcus thermophiles; clustered regularly interspaced short palindromic repeats (CRISPR) are being repurposed to transform genome editing at the single-nucleotide level. This mechanism can be used to virtually manipulate a short stretch of guide RNA in any genomic sequence, fashioning CRISPR to be optimal for large-scale genomic manipulation. In nature a dual RNA hybrid, consisting of two small noncoding RNAs the trans-activating crRNA (tracrRNA) and the CRISPR (crRNA), guides the Cas9 enzyme to cleave the target DNA sequence. Regardless of its great promise and advantages, there are still some challenges between CRISPR-Cas9 and its complete therapeutic potential. One of them is avoiding and decreasing unwanted off-target effects, which can potentially be addressed through chemical modifications of sgRNA. These modified sgRNAs, that are over 100-nt long, are difficult to synthesize and low yielding. This presentation will describe a convergent approach that splits the sgRNA into two constructs. The shorter segment (~30-nt, crRNA), containing RNA modifications can be easily synthesized using solid phase techniques. The longer segment (~70-nt, tracrRNA) does not contain any modifications and can also be chemically synthesized. The two segments will contain tetrazine (Tz) and trans-cyclooctene (TCO) at the 3' and 5' ends respectively. This allows them to be attached together using click chemistry between TCO and Tz forming a long sgRNA. Optimization of the split and click process, as well as CRISPR experiments involving modified RNA will be described.

Alex Lemus ***Biology,*** *alemus@albany.edu*

'Multispecies aggregates from human dental plaque nucleate highly diverse spatially structured oral biofilms on saliva coated surfaces'

According to the CDC, Periodontitis affects 47.2% of individuals aged 30 and over. By the age of 65, the number of people impacted by periodontitis jumps to 70.1%. Dysbiosis, shifts in biofilm composition leading to imbalances in microbial composition are precursors of widespread human diseases such as tooth decay (dental caries) and periodontitis. How shifts in biofilm spatial structure impact the progression from health to disease is unknown. We have developed an in vitro dental plaque culture system in which plaque samples obtained from healthy donors via flossing are used to seed complex communities. Quantitative analysis by Fluorescence in situ Hybridization (FISH), confocal microscopy, and spectral imaging demonstrated that model biofilms are highly diverse, spatially structured, and unexpectedly heterogeneous in spatial structure. We observed that the dental plaque inocula from healthy donors were composed of single cells and large multispecies coaggregates. We hypothesize that early stochastic events mediated by these multispecies coaggregates lead to highly spatially structured and heterogeneous biofilms depending on where these interacting communities land on nascent surfaces. To test our hypothesis, we developed a protocol for disrupting plaque coaggregates. We observed that biofilms derived from dental plaque inocula in which aggregates were disrupted before seeding in vitro cultures resulted in biofilms with decreased diversity and increased spatial homogeneity. Biofilms derived de-aggregated plaque inocula lacked many gram-negative, obligate anaerobe community members. Our results demonstrate a previously unknown role multispecies aggregates in structuring oral biofilms and may have clinical importance in the mouth.

Sean Bialosuknia ***Ecology and Evolution Biology,*** *seanbialosuknia@albany.edu*

'Adaptive evolution of West Nile virus facilitated increased transmissibility and prevalence in New York State'

West Nile virus (WNV; Flavivirus, Flavivridae) was introduced to New York State (NYS) in 1999 and rapidly expanded its range through the continental United States (US). NY10, an emergent genotype characterized by shared amino acid substitutions R1331K and I2513M, first appears in the genetic record of NYS WNV strains in 2010 and coincides with increased WNV cases in humans and an increased prevalence of the virus in mosquitoes. Excepting the documented selective sweep of the WN02 genotype displacing the NY99 genotype, there has been little evidence of adaptive evolution of WNV in the US. Previous data demonstrated increased prevalence of NY10 strains and evidence of positive selection. We expand our investigation of the genetic and surveillance record of WNV and investigate if NY10 genotype strains are associated with phenotypic change consistent with an adaptive advantage. WNV has shown a significant increase in prevalence in mosquito pools tested by NYS arbovirus surveillance, and updated sequencing of more recent mosquito pools shows a continued dominance of NY10. We tested NY10 strains in vivo in Culex pipiens mosquitoes to assess infectivity and transmissibility and found that the NY10 genotype strains infect a greater number of mosquitoes at a lower dose and are more transmissible than WN02. Experimental infection of American robins (Turdus migratorius) demonstrates an extended peak viremia for NY10 relative to WN02. Modeling the increased infectivity and transmissibility of the NY10 strains demonstrates a mechanistic basis for selection that has likely contributed to the increased prevalence of WNV in NYS.

Ruogu Wang ***Mathematics and Statistics,*** *rgwang@albany.edu*
'Sparse Poisson Regression Approach to Biological Spectral Imaging'

Genetic labeling with fluorescent probes for the 16S rRNA gene attempts to discriminate the different species of bacteria in a biological image. The concentrations of the fluorophore labels at each pixel in a digitally recorded microscope image can be determined through spectral unmixing methods. Most existing methods assume Gaussian noise in the recorded spectra and obtain fluorophore abundances through least-squares approaches. However, detected photon counts in fluorescence microscopy are known to follow a Poisson distribution. Moreover, most pixels only contain a few fluorophores in our specific application to classify microbes in images of complex communities. These motivate us to utilize a Poisson regression approach to tackle the unmixing problem and incorporate sparseness constraints to produce sparse solutions. The commonly used Lasso regularization, as a sparseness constraint, tends to select only one fluorophore from a group of spectrally overlapping fluorescent labels. As an alternative to the Lasso regularization, the elastic net regularization possesses comparable sparse representation ability while encouraging a grouping effect, meaning that the group of overlapping fluorescent labels can be selected simultaneously. In our study, we first extract the fluorophore endmember information from reference images through a multi-output Poisson regression. Based on the extracted endmembers, we then propose to learn the abundance information via an elastic net regularized Poisson regression. We demonstrate that our proposed method improved the accuracy of fluorophore discrimination as compared to existing methods. This talk is based on an ongoing project with Yunlong Feng and Alex Valm.

Subodh Mishra ***RNA Institute**, smishra@albany.edu*

'A CTG repeat-selective screen of a natural product library reveals dietary natural compounds as potential therapeutics for Myotonic Dystrophy'

Myotonic dystrophy (DM) is a multisystemic neuromuscular disease. The prevalence of DM can be as high as 1:2100 depending on the region. Expansion of a CTG and CCTG nucleotide tract in non-coding region of DMPK and CNBP gene, respectively is the responsible mutation for DM. Transcripts containing the expanded repeat form non-canonical structures that sequester the muscleblind-like (MBNL) family of splicing regulators into ribonuclear foci. This sequestration results in global aberrant splicing of pre-mRNA. Targeting expanded repeat transcription is a promising therapeutic approach for mitigating toxic RNA associated pathogenesis. Recently, we developed a DM1 HeLa cell model that permits repeat-selective screening by measuring the ratio-metric expression of r(CUG) 480 levels relative to an r(CUG)0 control following drug treatments. In the current study, we utilized this DM1 HeLa cell model to screen a natural product small molecule library obtained from National Cancer Institute (NCI). This study revealed NP3-NSA1 as a selective modulator of toxic CUG RNA abundance, as it reduces ~45% of the r(CUG)480. Further, NP3-NSA1 showed almost significantly rescue of DM associated mis-splicing events in DM patient-derived muscle and fibroblast cell lines. NP3-NSA1 significantly recused the mis-splicing in DM2 patient derived fibroblast as well. These results were further validated in DM1 mouse model, which showed that NP3-NSA1 significantly improved both molecular and phenotypic feature of DM1. Several widely available foods such as red onion, grapes and broccoli are rich in NP3-NSA1 and are consumed as part of the daily human diet. NP3-NSA1 was also recently labeled as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration). This excellent safety profile with little to no adverse effects, positions NP3-NSA1 as a potentially safe lead compound for therapeutic consideration in DM.

Joseph Ellis ***RNA Institute**, jaellis@albany.edu*

'Transcriptome-wide assessment of MBNL-dependent alternative splicing in mouse and humans'

The Muscleblind-like (MBNL) family of RNA-binding proteins is important in the regulation of alternative splicing (AS) events. In Myotonic Dystrophy (DM), a microsatellite repeat expansion disorder, sequestration of MBNL protein causing aberrant AS patterns which leads to a multi-systemic phenotype with myotonia being a hallmark symptom. A deeper understanding of how MBNL proteins regulate AS is important in development of both biomarkers as well as therapeutic strategies as there is currently no available treatment for DM. To further explore fundamentally how MBNL proteins regulate AS, we utilized two MBNL1-inducible cell lines (human and mouse) to generate RNAseq libraries at multiple concentrations of induced MBNL1. Using rMATS and comparing each MBNL1-induced library to no-induction control library, skipped exon events were identified and their respective exon percent spliced in (PSI) values obtained. Using these PSI values for overlapping events we fit dose-response curves to the RNAseq-derived data to characterize transcriptome-wide splicing outcomes of both human and mouse cell lines. From this, we can more broadly determine average EC50 values, slopes, upper and lower bounds for MBNL1-dependent alternative splicing regulation across the transcriptome. Additionally, we validated several events using RT-PCR and find that some AS exons are highly conserved between cell systems. Overall, we effectively characterize MBNL1-dependent splicing regulation across cell systems which is important in furthering our understanding of how sequestration of MBNL proteins and subsequent mis-splicing causes DM.