17th Annual Undergraduate Research Symposium in the Chemical and Biological Sciences

The College of Natural and Mathematical Sciences;
The Department of Chemistry and Biochemistry &
The Department of Biological Sciences

UMBC
AN HONORS UNIVERSITY IN MARYLAND

Sponsored by:

National Institute of General Medical Sciences of the National Institutes of Health (NIGMS/NIH)

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Schedule of Events

Time	Event
8:00 am	**SYMPOSIUM CHECK-IN & ON-SITE REGISTRATION** *Lobby, University Center, 3rd Floor*
8:00 am – 9:00 am	**LIGHT CONTINENTAL BREAKFAST** *UC312, University Center, 3rd Floor*
9:00 am – 9:30 am	**OPENING REMARKS & WELCOME ADDRESS** Dr. Freeman Hrabowski, President, University of Maryland, Baltimore County (UMBC) Dr. William R. LaCourse, Dean, College of Natural & Mathematical Sciences, UMBC *ENG027, Engineering Bldg., (Lecture Hall 5)*
9:45 am – 11:45 pm	**MORNING POSTER SESSION** A – G: Biological Sciences H – L : Chemical Sciences M - R: Biochemistry and Molecular Biology *Ballroom, University Center, 3rd Floor*
10:30 am – 11:30 am	**WORKSHOPS**
	Professional Communication: Making the Right Impression Susan Hindle, Career Services, UMBC *UC310, University Center, 3rd Floor*
	A very, very short introduction to ethics for scientists Dr. Simon Stacey, Director, The Honors College *CASTLE, UC115, University Center, 1st Floor*
11:45 pm – 12:35 pm	**BUFFET LUNCH** *(gratis for registered guests with symposium name badge)* *The Commons – Main Street*
12:45 pm – 2:45 pm	**AFTERNOON POSTER SESSION** S – X: Biological Sciences Y – DD : Chemical Sciences EE – JJ: Biochemistry and Molecular Biology *Ballroom, University Center, 3rd Floor*
1:30 pm – 2:30 pm	**WORKSHOPS** *Repeat of workshop titles and locations above*
3:00 pm – 4:00 pm	**PLENARY TALK** **"Transition states, Hilde Mangold, and you"** Dr. David Asai, Howard Hughes Medical institute *ENG027, Engineering Bldg., (Lecture Hall 5)*
4:00 pm	**AWARDS PRESENTATION** *ENG027, Engineering Bldg., (Lecture Hall 5)*

Morning Poster Session
Group A - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

1. **OVEREXPRESSION OF FRUCTOSE BIS-PHOSPHATE ALDOLASE IN CHLAMYDOMONAS**
Abravi Rose Gbemafu[1], Rudolph Park[2], Amrita Madabushi[1], and Stephen M. Miller[2]
[1]Baltimore City Community College, Life Science Institute, 801 West Baltimore Street, Baltimore, MD 21201
[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

2. **ANTI-HIV THERAPY**
Deirdre Johnson and Ihid Leao
Department of Natural Sciences, UMES, Student Services Center Lane, Princess Anne, MD 21853

3. **C-PEPTIDE SECRETIONS AND DIFFUSION FROM RAT INSULINOMA CELLS USING A 3-D PRINTED FLUIDIC PLATE DEVICE**
Azel King[1], Chengpeng Chen[2], Yueli Liu[2], and Dana Spence[2]
[1]Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225
[2]Department of Chemistry, Michigan State University, 578 Shaw Lane, East Lansing, MI 48824

4. **OVEREXPRESSION OF CAH1 IN CHLAMYDOMONAS REINHARDTII**
Nicholas Often[1], Rudolph Park[2], Amrita Madabushi[1], and Stephen M. Miller[2]
[1]Baltimore City Community College, 2901 Liberty Heights Avenue, Baltimore, MD 21215
[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

5. **BLOOD SENSORS: DEVELOPMENT OF BIOSENSORS FOR THE MEASUREMENT OF FACTOR XA AND THROMBIN CONCENTRATIONS IN BLOOD**
Ashley N. Stewart and Peter B. Berget
Department of Biological Sciences, University of the Sciences, 600 S 43rd Street, Philadelphia, PA 19106

6. **DESIGNING AN ELECTROSPUN SCAFFOLD FOR TREATMENT OF DIABETICS' WOUNDS**
Juliet Strauss, Jennifer R. Weiser, and W. Mark Saltzman
Department of Biomedical Engineering, Yale University, 55 Prospect Street, New Haven, CT 06511

7. **TESTING AFFINITY OF COMPUTATIONALLY-DESIGNED HUMANIZED ANTIBODIES TO A TUMOR ANTIGEN**
Hannah C. Wastyk[1], Margie E. Ackerman, Ph.D. [2,3,4], Yoonjoo Choi[5], Chris Bailey-Kellogg[5], Ming-Ru Wu[3,4], Albert Gacerez[3,4], Charles Sentman[3,4], and Casey K. Hua[2]
[1]Department of Chemistry and Biochemistry, University of Delaware, 102 Brown Laboratory, Newark, DE 19716
[2]Department of Cellular and Molecular Biology, Dartmouth College, 7560 Remsen Building, Hanover, NH, 03755
[3]Thayer School of Engineering, Dartmouth College, 14 Engineering Drive, Hanover, NH 03755
[4]Department of Microbiology and Immunology, Geisel School of Medicine, 1 Rope Ferry Rd, Hanover, NH 03755
[5]Department of Computer Science, Dartmouth College, 6211 Sudikoff Lab, Hanover, NH 03755

OVEREXPRESSION OF FRUCTOSE BIS-PHOSPHATE ALDOLASE IN CHLAMYDOMONAS

Abravi Rose Gbemafu[1], Rudolph Park[2], Amrita Madabushi[1], and Stephen M. Miller[2]
[1]Baltimore City Community College, Life Science Institute,
801 West Baltimore Street, Baltimore, MD 21201
[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Algae hold great promises for production of liquid fuels but current yields are not high enough to compete with fossil fuels. Therefore there is a need to engineer algae to accumulate more biomass more quickly. *Chlamydomonas reinhardtii* is the *E. coli* of algae—many molecular genetic tools have been developed to manipulate it and its genome is sequenced and well characterized. In this study we are focusing on the Calvin cycle, a photosynthetic pathway localized to the stroma of the chloroplast that fixes carbon dioxide and produces sugar. The enzyme fructose bis-phosphate aldolase (FBA) is believed to carry out a key control step in the Calvin cycle. FBA combines glyceraldehyde-3- phosphate and dihydroxy acetone phosphate to form fructose-1,6-bisphosphate, a precursor to the 5-carbon sugar ribulose bisphosphate (RuBP) that must be regenerated in each round of the Calvin cycle. The main aim of our study is to determine whether we can increase the growth rate of *C. reinhardtii* by increasing the expression of FBA. To that end we have gene synthesized the coding sequence for *C. reinhardtii* FBA using *C. reinhardtii* chloroplast codon bias and are subcloning this sequence into our chloroplast transformation vector. Progress toward this goal will be reported. After transforming and selecting for spectinomycin resistant colonies we will use western analysis to determine protein expression and growth curve and dry weight analyses to determine whether FBA-expressing transformants are able to grow faster than wild type *C. reinhardtii*. Future work might involve overexpressing FBA together with other Calvin cycle enzymes.

These results were obtained as part of the Research Experience and Mentoring (REM) program the Department of Biological Sciences at UMBC. This program is funded by a grant (REM supplement to NSF-EFRI-1332344) from the National Science Foundation (NSF) Directorate for Engineering (ENG) Office of Emerging Frontiers in Research and Innovation (EFRI).

ANTI-HIV THERAPY

Deirdre Johnson and Ihid Leao
Department of Natural Sciences, UMES, Student Services Center Lane,
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Online access of this abstract is restricted at the request of the Principal Investigator.

C-PEPTIDE SECRETIONS AND DIFFUSION FROM RAT INSULINOMA CELLS USING A 3-D PRINTED FLUIDIC PLATE DEVICE

Azel King[1], Chengpeng Chen[2], Yueli Liu[2], and Dana Spence[2]
[1]Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225
[2]Department of Chemistry, Michigan State University, 578 Shaw Lane, East Lansing, MI 48824

C-peptide is a 31 amino acid peptide that is co-secreted with insulin by the beta cells in the pancreas. It was found that its main role was to facilitate folding of the pro-insulin molecule. Recently, research has shown that C-peptide has beneficial effects *in vivo* and may be a valuable source in diabetes therapy. Research has shown that C-peptide has an effect on erythrocytes (red blood cells, RBCs) by stimulating ATP release from the cells, which stimulates nitric oxide (NO) production in endothelial cells, which further relaxes smooth muscle cells on vessel walls. In this study, a cell line known as INS-1 was used to produce endogenous C-peptide that would be used on a 3D-printed circulation mimic fluidic device. We hypothesize that C-peptide should increase linearly upon stimulation over time before it plateaus. The secretion pattern of C-peptide as a function of time was first studied, by measuring C-peptide in the solution above cultured cells at different time points. Then the cultured INS-1 cells were integrated onto the circulation device to mimic the endocrine process of C-peptide existing pancreatic islet and entering circulation. Specifically, the amount of C-peptide that diffused from INS-1 cell culture inserts into the circulation was quantified against time with enzyme linked immunosorbent assays (ELISA). Results showed that 25 nM c-peptide was produced by the cells and approximately 2 nM diffused into the device channel. The 3-D printed fluidic device can be a good mimic for real time beta cell secretion and can be used for looking at the downstream bio-effect of RBCs and endothelial cells.

OVEREXPRESSION OF CAH1 IN CHLAMYDOMONAS REINHARDTII

Nicholas Often[1], Rudolph Park[2], Amrita Madabushi[1], and Stephen M. Miller[2]
[1]Baltimore City Community College, 2901 Liberty Heights Avenue, Baltimore, MD 21215
[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Algae hold great promise as a potential source for renewable liquid fuels, with most current research focused on increasing lipid content of natural or genetically engineered species. However, another important strategy for developing algae as a platform for biofuels production is to improve their growth rate. Our immediate goal is to determine if we can increase the growth rate of model organism *Chlamydomonas reinhardtii* through targeted genetic modifications, and our long-term goal is to apply successful modifications to other algae that naturally produce large amounts of lipids. Like many other phototrophs, *Chlamydomonas* has evolved a trait to survive under low carbon dioxide (CO_2) levels in the atmosphere. The CO_2 concentrating mechanism (CCM) works to accumulate inorganic carbon inside the chloroplast in the form of bicarbonate (HCO_3^-) at a much higher concentration than atmospheric CO_2 levels. We aim to repurpose the CCM to function with greater efficiency and under high CO_2 conditions to stimulate rapid growth in *Chlamydomonas*. Within the CCM, we are targeting a carbonic anhydrase gene (CAH1) for overexpression. The CAH1 enzyme is located in the periplasmic space and is the first enzyme of the CCM to handle the conversion of CO_2 to HCO_3^-. To prepare our overexpression construct we cut our gene synthesized CAH1 coding sequence out of a pUC57 vector and ligated it into our nuclear expression vector pUC-ARG. Using the ARG7 gene as our selection marker, we will perform a nuclear transformation of *Chlamydomonas* then analyze transformants by western blot to determine if any overexpress CAH1. Any such transformants will subsequently be analyzed for growth rate and biomass production. If this effort is successful, the next step will be to generate a vector that overexpresses additional CCM genes in hopes of improving growth rates even more.

These results were obtained as part of the Research Experience and Mentoring (REM) program in the Department of Biological Sciences at the University of Maryland Baltimore County. This program is funded by a grant (REM supplement to NSF-EFRI-1332344) from the National Science Foundation (NSF) Directorate for Engineering (ENG) Office of Emerging Frontiers in Research and Innovation (EFRI).

BLOOD SENSORS: DEVELOPMENT OF BIOSENSORS FOR THE MEASUREMENT OF FACTOR XA AND THROMBIN CONCENTRATIONS IN BLOOD

Ashley N. Stewart and Peter B. Berget
Department of Biological Sciences, University of the Sciences,
600 S 43rd Street, Philadelphia, PA 19106

Hemostasis is the process by which the body forms blood clots and terminates bleeding after blood vessel injury. It involves a cascade of reactions catalyzed by several serine proteases known as coagulation factors. Two of these, Factor IIa, more commonly identified as thrombin, and Factor Xa, have become the chemotherapeutic targets of direct inhibitor anticoagulant drugs. These drugs aim to lessen the body's ability to form blood clots by reducing the activity levels of these proteases in the blood. Currently, no method exists by which healthcare professionals can directly measure the activity of thrombin or Factor Xa circulating in the blood at a given time. This presents a potential danger to patients undergoing anticoagulant therapy, as the prescribing doctor has no direct way of measuring the efficiency of the prescribed dose. Our FAP-based biosensors serve as protease detectors by increasing the fluorescence of organic dyes in the presence of specific proteases. In this project, four biosensors were designed: two for the detection of thrombin and two for the detection of Factor Xa. One of each pair of sensors fluoresces in the blue wavelength of the visible light spectrum, while the other fluoresces red. After treating the red thrombin sensor with a sample of human blood, an increase in fluorescence was observed, indicating a potential clinical use of the sensor. Future directions will involve testing the specificity of the sensor, as well as its sensitivity to concentration of protease.

University of the Sciences, The Melvin Firman Undergraduate Research Scholarship and NIH Grant U54-RR022241.

DESIGNING AN ELECTROSPUN SCAFFOLD FOR TREATMENT OF DIABETICS' WOUNDS

Juliet Strauss, Jennifer R. Weiser, and W. Mark Saltzman
Department of Biomedical Engineering, Yale University,
55 Prospect Street, New Haven, CT 06511

Diabetes is a metabolic disease that afflicts 22.3 million people in the US, and is a heavy financial burden at 245 billion dollars annually. A major complication among diabetic patients is wounds that fail to heal, which can lead to distress, amputation, and additional medical costs. Our aim is to develop a therapeutic controlled release dressing to improve the healing of diabetic wounds, with the goal of increasing patient comfort, improving clinical outlook, and reducing the effective cost of treatment.

Currently, we are focusing on the development of a wound dressing formed from electrospun scaffolds. In particular, we are exploring scaffolds composed of two polymers, polycaprolactone (PCL) and chitosan (CS). PCL and CS were chosen for their mechanical properties, biocompatiblity, biodegradability, and their non-toxic nature. Furthermore, CS offers antimicrobial protection. Fibers were produced from various ratios of these two polymers: we, discovered that a 14 wt% PCL, 4% CS based on the PCL wt% resulted in the most uniform scaffold.

Additionally, we manipulated other parameters of the electrospinner in search for the best scaffold. Needle gauge size (16, 18, 20), electrospinner voltage (22.5-27.5 kV), distance between needle and mandrel, spin time, and solvent system ratios (acetic acid: formic acid) were assessed.

Initial characterizations of the manipulated scaffolds were performed via microscopy. Using scanning electron microscopy (SEM), we found scaffolds with minimized beading and webbing. A hydrolytic degradation study was performed to assess the scaffolds' mass and structural changes over time. In conjunction, mechanical strength tests from t=0 to t=10 weeks will be performed to characterize durability.

The goal of this project is to create a scaffold that not only protects wounds, but also offers a platform from which to deliver therapeutics in order to offer clinically relevant treatment options for diabetic wounds.

This research was supported in by a training grant fellowship from the NIH (T32 DK101019)

TESTING AFFINITY OF COMPUTATIONALLY-DESIGNED HUMANIZED ANTIBODIES TO A TUMOR ANTIGEN

Hannah C. Wastyk[1], Margie E. Ackerman, Ph.D. [2,3,4], Yoonjoo Choi[5], Chris Bailey-Kellogg[5], Ming-Ru Wu[3,4], Albert Gacerez[3,4], Charles Sentman[3,4], and Casey K. Hua[2]
[1]Department of Chemistry and Biochemistry, University of Delaware, 102 Brown Laboratory, Newark, DE 19716
[2]Department of Cellular and Molecular Biology, Dartmouth College, 7560 Remsen Building, Hanover, NH, 03755
[3]Thayer School of Engineering, Dartmouth College, 14 Engineering Drive, Hanover, NH 03755
[4]Department of Microbiology and Immunology, Geisel School of Medicine, 1 Rope Ferry Rd, Hanover, NH 03755
[5]Department of Computer Science, Dartmouth College, 6211 Sudikoff Lab, Hanover, NH 03755

Monoclonal antibody (mAB) therapeutics leverage high target specificity and innate immunity to combat complex diseases. While easy to produce, murine antibodies lead to a host of different problems in humans including: xenograft immune responses, short half-life, and weak effector function. By humanizing antibodies with the conventionally-used complementary-determining region (CDR) grafting, these pitfalls may be overcome, allowing new drug development efforts. With CDR grafting, however, the humanized antibody may still elicit high levels of anti-antibody response, and affinity to the targeted antigen can decrease by as much as 97%, in comparison to the murine antibodies. Another method of humanizing a murine antibody uses Human String Content (HSC), which optimizes every 9-mer sequence to parallel 9-mer identity in all available databases of human antibody sequences. A novel computational model, created by collaborators in the Bailey-Kellogg Lab at Dartmouth College, was used in this work to optimize both humanness (using HSC score) and structural stability, which improved upon previous HSC models by maximizing retention of antigen-binding during the humanization process.

In this work, forty different computationally designed, humanized antibodies were constructed and their binding to a cancer-cell ligand was assessed through two methods. Protein-to-cell interaction and binding was quantified through antibody staining of ligand-expressing-cells, followed by flow cytometry. Protein-to-protein interaction and binding was assessed through kinetic analysis, using the ForteBio Octet Bio-Layer Interferometry technology. At least five designs were shown to have significant binding to the cancer cell ligand compared to the non-transfected cells, suggesting significant retention of antigen-binding with the novel computational algorithm used to construct the designs. Given this successful test case, this computational algorithm may be used to more quickly obtain humanized mAbs with stable structure and antigen specificity than what is currently available through CDR-grafting/affinity maturation methods.

The authors thank the MD/PhD Undergraduate Summer Fellowship at Dartmouth for providing summer funding for the internship

Morning Poster Session
Group B - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

8. **DIFFERENTIAL EFFECTS OF INHIBITORS AND ANTIBODIES ON MOTILITY AND PROLIFERATION OF L1-POSITIVE VS. L1-NEGATIVE GLIOBLASTOMA CELLS**

Hannah Anderson and Deni Galileo
Department of Biological Sciences, University of Delaware, 248 Wolf Hall, Newark, DE 19716

9. **EFFECTS OF LSD1 IN HEMATOPOIESES DEPEND UPON ENZYMATIC AND NON-ENZYMATIC LSD1 FUNCTIONS**

Maria Julia Bianca Corpuz[1,2], David McClellan[2], Matthew Velinder[2],
Diana Bareyan[2], and Michael Engel, M.D., Ph.D[3]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Oncological Sciences, University of Utah,
2000 Circle of Hope Drive, Salt Lake City, UT 84112
[3]Department of Pediatrics, University of Utah, 2000 Circle of Hope Drive, Salt Lake City, UT 84112

10. **DIFFERENTIAL METHYLATION OF GNG7 IN NORMAL B6 MOUSE TISSUES**

Jeffrey Dailey, Ali Hussain, and William F. Schwindinger
Department of Biological and Allied Health Sciences, Bloomsburg University,
400 E. Second Street, Bloomsburg, PA 17815

11. **TARGETING TRIPLE NEGATIVE BREAST CANCER IN AFRICAN-AMERICAN WOMEN**

Michelle A Jaldin[1,2], Kenny Regis[2], Akanksha Mahajan[2],
Anju Duttargi[2], and Luciane R. Cavalli[2].
[1]Biology Department, Trinity Washington University, 125 Michigan Avenue, Washington, DC 20017
[2]Department of Cancer Genetics and Epidemiology,
Georgetown University- Lombardi Comprehension Cancer Center,
3970 Reservoir Road NW, Washington, DC 20007

12. **OVER-EXPRESSION OF APE1 IN RELATIONSHIP TO DISEASE**

Bre'Anna James[1], David M. Wilson III[1], and Jennifer Illuzzi[2]
[1]Department of Biology, Trinity Washington University,
125 Michigan Avenue NE, Washington, DC 20017
[2]Department of Molecular Gerontology, National Institutes on Aging,
251 Bayview Boulevard, Baltimore, MD 21224

13. **MICROTUBULES CONTRIBUTE TO THE ABNORMAL NUCLEAR MORPHOLOGY IN HUTCHINSON GILFORD PROGERIA SYNDROME**

Zeshan Tariq[1], Haoyue Zhang[1], Wolfgang Losert[2], and Kan Cao[1]
[1] Department of Cell Biology and Molecular Genetics, University of Maryland,
Bioscience Research Building, College Park, MD 20742
[2] Department of Physics, University of Maryland, Paint Branch Drive, Building 223,
University of Maryland, College Park, MD 20742

14. **DETERMINING THE ROLE OF A SILENT, INTRONIC SINGLE NUCLEOTIDE POLYMORPHISM (SNP) IN PANCREATIC CANCER**

Katlyn S. Tyrpak and John F. Harms
Department of Biological Sciences, Messiah College, 1 College Avenue, Mechanicsburg, PA 17055

DIFFERENTIAL EFFECTS OF INHIBITORS AND ANTIBODIES ON MOTILITY AND PROLIFERATION OF L1-POSITIVE VS. L1-NEGATIVE GLIOBLASTOMA CELLS

Hannah Anderson and Deni Galileo
Department of Biological Sciences, University of Delaware, 248 Wolf Hall, Newark, DE 19716

The L1CAM cell adhesion/recognition protein (L1, CD171) is abnormally expressed by high-grade gliomas and contributes to their aggressive motility and proliferation. The transmembrane form is proteolyzed to release a soluble ectodomain fragment that binds to both integrins and fibroblast growth factor receptors (FGFRs) and stimulates cells by an autocrine/paracrine mechanism. We hypothesized that blocking the interactions between the L1 ectodomain and these receptors or downstream effectors of their interactions would reduce the motility and proliferation of glioblastoma cells expressing L1. We compared T98G human glioblastoma cells infected with a lentiviral vector of short hairpin RNA with control cells to measure the differential effect of reagents on L1-negative and L1-positive cells. One attempted method of blocking L1-receptor interactions was the treatment of cells with two commercial anti-L1 antibodies, 5G3 and UJ127. Time-lapse cell motility studies demonstrated a reduction in velocity only for cells expressing L1. Flow cytometry cell cycle analyses showed, however, that the proliferation of both cell types was stimulated by the addition of anti-L1 antibodies. This result implies that anti-L1 antibodies have limited potential for treatment of glioblastoma. Two inhibitors were tested: PF431396, an inhibitor of focal adhesion kinase (FAK), a downstream effector of L1-integrin interactions, and PD173074, an inhibitor of FGFR autophosphorylation. Both cell cycle analyses and time-lapse motility studies showed that FAK inhibitor PF431396 had a significantly greater inhibitory effect on the cells expressing L1. A more pronounced difference was seen with the FGFR inhibitor PD173074, which had an inhibitory effect on velocity and proliferation of the L1-positive T98G cells but no significant effect on the L1-negative cells. From these results, we conclude that inhibitors of FAK and FGFR have chemotherapeutic potential for glioblastoma, especially those exhibiting L1-mediated stimulation.

Financial support for this project was provided by the 2014 Delaware Governor's Bioscience Fellowship and the University of Delaware Undergraduate Research Program.

EFFECTS OF LSD1 IN HEMATOPOIESES DEPEND UPON ENZYMATIC AND NON-ENZYMATIC LSD1 FUNCTIONS

Maria Julia Bianca Corpuz[1,2], David McClellan[2], Matthew Velinder[2],
Diana Bareyan[2], and Michael Engel, M.D., Ph.D[3]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Oncological Sciences, University of Utah,
2000 Circle of Hope Drive, Salt Lake City, UT 84112
[3]Department of Pediatrics, University of Utah,
2000 Circle of Hope Drive, Salt Lake City, UT 84112

Growth Factor Independence 1 (GFI1) is a zinc finger transcriptional repressor critical for the proliferation of T-cell leukemia and cell fate specification. Lysine Specific Demethylase-1 (LSD1), a histone 3 lysine 4 (H3K4) demethylase, down regulates expression of its target genes by removing methyl groups from H3K4. Our laboratory has explored the binding of Growth Factor Independence 1 (GFI1) to LSD1, which is an important epigenetic effector of the GFI1 repressor complex. The GFI1—LSD1 complex regulates normal hematopoietic development and is required for survival of T-ALL cells in vitro and in vivo. However, how LSD1 recruitment and activity at promoters regulated by GFI1 contributes to cell fate specification is not known.

In zebrafish, our laboratory has shown that catalytically inactive LSD1 (LSD1-K661A) partially restores wild type LSD1's role in neutrophil development. As such, we hypothesize that LSD1 has important functions in hematopoietic cell fate determination distinct from its enzymatic activity. We have also shown that an inhibitor of LSD1 (HCI-2509) effectively kills T-cell leukemia cell lines and decreases tumor burden in a mouse xenotransplant model. Whether this effect can be attributed specifically to inhibition of the enzyme or to some other aspect of LSD1 function following inhibitor treatment has not been explored. To begin to address these issues, we sought to determine the impact of LSD1 inhibition on the stability of LSD1 protein and to test the ability of enzymatically inactive LSD1 to bind GFI1. We show that LSD1 inhibitor treatment of T-ALL cells has no apparent effect on the stability of LSD1 up to 24hours. Notably, enzymatically inactive LSD1 binds GFI1 comparable to the wild type protein. When viewed in the context of partial complementation of neutrophil development by LDS1-K661A, these data suggest that LSD1 may have non-enzymatic functions that contribute to hematopoietic cell fate.

DIFFERENTIAL METHYLATION OF GNG7 IN NORMAL B6 MOUSE TISSUES

Jeffrey Dailey, Ali Hussain, and William F. Schwindinger
Department of Biological and Allied Health Sciences, Bloomsburg University,
400 E. Second Street, Bloomsburg, PA 17815

Cellular communication can involve externally transmitted chemical signals such as hormones and neurotransmitters, which are bound by receptors expressed on cell surfaces. The largest class of cell surface receptors is known as G-protein coupled receptors. When the ability to communicate is altered, cells may exhibit unregulated growth resulting in the formation of tumors and cancers. Epigenetic regulation of expression, such as methylation of cytosine residues to 5-methylcytosine, in guanine nucleotide binding protein gamma subunit 7 (GNG7) has been linked to cancers of the head, neck and pancreas in humans. Our research seeks to confirm that promoter methylation is linked to down regulation of expression and to determine whether methylation has a tissue specific role in expression in normal tissues.

The methylation state of Gng7 was studied at the two accepted promoters and an intron CpG island using complementary techniques: 1) restriction enzymes that were either blocked or cut methylated restriction sites and 2) treatment of DNA with sodium bisulfite followed by sequencing PCR products. DNA from the NIH 3T3 fibroblast cell line along with DNA from tissue samples including the caudate nucleus, prefrontal cortex, cerebellum, muscle, liver and pancreas were examined. In the intron, NIH 3T3 DNA showed complete methylation in contrast to tissues which showed little to no methylation. The downstream promoter region exhibited differential methylation with almost complete methylation in the prefrontal cortex, liver and NIH 3T3 DNA and a high degree of methylation (>50%) in the striatum and pancreas. The upstream promoter was highly methylated (50%) in NIH 3T3 while tissue samples ranged from 13 to 41%. Total levels of expression found in literature were not correlated to the degree of methylation. Further work will be done to examine expression due to the individual promoters of Gng7 in mice.

We would like to acknowledge Bloomsburg University for supporting our research efforts by providing the funding necessary to do so.

TARGETING TRIPLE NEGATIVE BREAST CANCER IN AFRICAN-AMERICAN WOMEN

Michelle A Jaldin[1,2], Kenny Regis[2], Akanksha Mahajan[2],
Anju Duttargi[2], and Luciane R. Cavalli[2]
[1]Biology Department, Trinity Washington University,
125 Michigan Avenue, Washington, DC 20017
[2]Department of Cancer Genetics and Epidemiology,
Georgetown University- Lombardi Comprehension Cancer Center,
3970 Reservoir Road NW, Washington, DC 20007

Triple negative breast cancer (TNBC) accounts for about 10-17% of all breast cancers and is defined by the lack of ER, PR and HER2 receptors expression. In the African-American (AA) population, an increased incidence and shorter survival rate of TNBC has been reported when compared to White women. The main objective of our study is to identify molecular markers in TNBC in AA patients that may be associated with their observed disparity in incidence and mortality rates. To accomplish our goal we initially studied two groups of tumors from AA women from the Washington DC area, TNBC and non-TNBC, to better understand their biology. We hypothesized that a higher level of genomic instability, as evaluated by DNA copy number changes, will be observed in the TNBC group when compared to the non-TNBC group. Pathology archived tumor sections were obtained from 17 TNBC and 17 non-TNBC cases and analyzed by array-CGH, to identify the presence of genomic regions with gains/amplifications and/or losses/deletions. Our analysis showed a higher level of genomic instability in the TNBC tumors when compared to the non-TNBC, corroborating with our hypothesis. Although some alterations were common to both groups, a distinct DNA copy number profile was observed between the groups studied. The alterations observed in the TNBC cases are being compared with a group of TNBC from White patients, to determine whether these alterations are specific to the AA women. In addition, we are evaluating its association with the clinical-histopathological parameters from the patients, to determine their potential prognostic role. We believe that our study, in addition to provide a better understanding of the biology of the TNBC, will lead to the identification of relevant molecular markers with a potential prognostic and therapeutic role in the AA population, ultimately leading for the reduction of their observed cancer disparity.

I appreciate the support and guidance provided by Dr. Luciane Cavalli and her lab research staff for letting me contribute in this research and guiding me through the research process. I would also like to thank Mr. Kenny Regis and Olusayo Lousie Oluwasanni for helping me generate statistical graphs for my data. Finally, I would like to thank Dr. Blancato for giving me the opportunity to intern at Georgetown University and the NIH. This study is supported by the Advanced Research Training Corps: A Novel Initiative for URM Students, NIH- NIGMS GM101997; Susan Komen Foundation: Post Baccalaureate Training in Disparities Research and FDA Center of Excellence in Regulatory Science and Innovation (CERSI); grant number: FDA U01 FD004319.

OVER-EXPRESSION OF APE1 IN RELATIONSHIP TO DISEASE

Bre'Anna James[1], David M. Wilson III[1], and Jennifer Illuzzi[2]
[1]Department of Biology, Trinity Washington University,
125 Michigan Avenue NE, Washington, DC 20017
[2]Department of Molecular Gerontology, National Institutes on Aging,
251 Bayview Boulevard, Baltimore, MD 21224

Base Excision Repair (BER) is responsible for removal of damaged DNA from the cell. Individuals with improperly functioning BER are vulnerable to cancer, neurodegeneration, and premature aging. The initiation of BER requires a damage-specific DNA glycosylase. The DNA glycosylase is responsible for recognizing the base damage and removing it, creating an apurinic/apyrimidinic (AP) site. The APE1 protein recognizes the AP site and then cleaves the phosphodiester backbone, creating a DNA strand break. DNA polymerase β cleans-up the strand break terminus and incorporates the correct nucleotide into the DNA. A protein complex of Ligase III and XRCC1 assure the correct nucleotide, and the DNA is subsequently sealed. The BER protein, APE1, is prominent to the survival of many species.

In a mouse model, the deletion of both APE1 alleles leads to embryonic lethality. We are pursuing the hypothesis that an over-expression of APE1 activity will contribute to heightened cell survival after exposure to DNA damage. To address this issue, we have designed a cell line that expresses APE1 in a doxicyclin-regulated manner and are now assessing cellular end-points related to the level of APE1 protein and disease etiology. In conclusion, quantification of AP-sites, DNA damage by comet assay, and a transformation assay assessing the contribution that APE1 over-expression has on cells transforming into a cancer-like state allows insight to APE1's relationship to disease.

MICROTUBULES CONTRIBUTE TO THE ABNORMAL NUCLEAR MORPHOLOGY IN HUTCHINSON GILFORD PROGERIA SYNDROME

Zeshan Tariq[1], Haoyue Zhang[1], Wolfgang Losert[2], and Kan Cao[1]
[1] Department of Cell Biology and Molecular Genetics, University of Maryland, Bioscience Research Building, College Park, MD 20742
[2] Department of Physics, University of Maryland, Paint Branch Drive, Building 223, University of Maryland, College Park, MD 20742

HGPS Syndrome is a genetic disorder characterized by rapid, premature aging and has been an area of interest due to its potential as a model system for normal aging. One of the hallmarks of HGPS is an abnormal nuclear shape which appears to hold clinical significance in itself, especially since a similar phenotype arises in cancer. Previous studies of this abnormality in HGPS have pointed to the accumulation of a mutant protein, progerin, as the major culprit. However, the potential interplay between the nucleus and the cytoskeleton has not been fully explored. Here we perturbed the microtubule network in both HGPS and control fibroblast cells through either microtubule drug treatments (nocodazole and taxol) or through an siRNA treatment against SUN1, an adaptor protein connecting the microtubule network and nuclear scaffolding. The nuclei of these cells then underwent two types of imaging: live cell deconvolution microscopy and fluorescent microscopy. The resulting movies/images were then analyzed by an in-house MATLAB program, thereby allowing for a sensitive quantification of the nucleus. Surprisingly, we find that the microtubule network plays a positive role in normal cells and a negative role in HGPS cells in regulating nuclear shape.

This work was supported by an NIH/NIA grant AG029761, and a grant by the University of Maryland, Howard Hughes Medical Institute Undergraduate Studies Education Program.

DETERMINING THE ROLE OF A SILENT, INTRONIC SINGLE NUCLEOTIDE POLYMORPHISM (SNP) IN PANCREATIC CANCER

Katlyn S. Tyrpak and John F. Harms
Department of Biological Sciences, Messiah College,
1 College Avenue, Mechanicsburg, PA 17055

Online access of this abstract is restricted at the request of the Principal Investigator.

Morning Poster Session
Group C - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

15. **DOCUMENTATION OF FEMALE SONG IN THE TROPICS: THE CASE OF THE PUERTO RICAN ORIOLE**
Susanna Campbell and Kevin Omland
Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

16. **CHARACTERIZATION OF STEROID SULFATASE IN *MUS MUSCULUS* TISSUES**
Joseph N. Hornak and Kyle W. Selcer
Department of Biological Sciences, Duquesne University,
600 Forbes Avenue, Pittsburgh, PA 15282

17. **ANALYSIS OF MODIFIED HISTONE DISTRIBUTION AT THE TISSUE-SPECIFIC IMPRINTED RASGRF1 LOCUS USING ALLELE-SPECIFIC QPCR FOLLOWING CHIP**
Nicole Hamagami and Tamara L. Davis
Department of Biology, Bryn Mawr College, 101 North Merion Avenue, Bryn Mawr, PA 19010

18. **INHIBITING THE EFFECTS OF TRAMATIC BRAIN INJURY THROUGH THE DESIGN OF GluR2 ANTAGONIST**
Abraham Isak, Keval Patel, Karan Arora, Earl Benjamin, and Ellis Benjamin
School of Natural Sciences and Mathematics, Richard Stockton College,
101 Vera King Farris, Galloway, NJ 08205

19. ***Borrelia burgdorferi:* VlsE1 AS A POTENTIAL GANGLIOSIDE BINDING PROTEIN**
Drew Phelan and Christopher W. Reid
Department of Science and Technology, Bryant University, 1150 Douglas Turnpike, Smithfield, RI 02917

20. **CollecTF: A DATABASE OF EXPERIMENTALLY – VALIDATED BACTERIAL TRANSCRIPTION FACTOR BINDING SITES.**
Dinara Sagitova[1], Sefa Kilic[2], and Ivan Erill[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

21. **CHARACTERIZATION OF STARCH DEGRADATION IN *CELLVIBRIO JAPONICUS***
Gisele Toumi and Jeffrey Gardner
Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

DOCUMENTATION OF FEMALE SONG IN THE TROPICS: THE CASE OF THE PUERTO RICAN ORIOLE

Susanna Campbell and Kevin Omland
Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Evolutionary biologists often assume that elaborate traits that cause sexual dimorphism evolve through male competition for females. However, recent work from our lab showed that the males and females of the common ancestor of all song birds both sang. Therefore, the dimorphism that is observed in many temperate breeding songbird species is due to loss of female song and not the gain of song by males (Odom et. al 2014, Nature Communications).

We investigated whether Puerto Rican Orioles (*Icterus portoricensis*), a tropical songbird, exhibits both male and female song. For this project we marked individuals with sex specific color bands and collected DNA samples. We successfully isolated CHD, a gene that is present in different lengths on either of the sex chromosomes in birds and determined the sex of our banded individuals. In March 2014, we repeatedly observed and recorded female song as well as male song of the Puerto Rican Oriole. Our findings show that both male and female Puerto Rican Orioles sing. This information helps show that the common ancestor to the oriole genus had both male and female song and that dimorphism has evolved due to loss of female song.

This work was funded, in part, by The Explorers Club Youth Activity Fund, Sigma Xi Grants-in-Aid of research, an Undergraduate Research Award from the UMBC Office of Undergraduate Education, an NSF Research Experience for Undergraduates and NIH/NIGMS MARC U*STAR T34 08663 National Service Award to UMBC.

CHARACTERIZATION OF STEROID SULFATASE IN *MUS MUSCULUS* TISSUES

Joseph N. Hornak and Kyle W. Selcer
Department of Biological Sciences, Duquesne University,
600 Forbes Avenue, Pittsburgh, PA 15282

Steroid hormones are often present in the plasma as inactive sulfated forms, such as estrone sulfate (E_1S) and dehydroepiandrosterone sulfate (DHEAS). These inactive sulfated steroid hormones can be activated in the peripheral tissues by removal of the sulfate group. Steroid sulfatase (STS) is the enzyme responsible for this conversion in the tissues. This study investigated the tissue distribution of STS in the laboratory mouse, *Mus musculus*. Homogenates prepared from various tissues exhibited widely different STS activities, as determined by an ^3H-E_1S conversion assay. Among the tissues, STS activity was highest in liver and testes, but also was present in heart, small intestine, skeletal muscle and ovarian tissues. STS activity in liver microsomes, prepared by differential centrifugation, increased with both time and temperature. Two known STS inhibitors (EMATE and Coumate 667) substantially decreased enzyme activity, indicating that the observed conversion represented authentic STS. STS kinetics of liver microsomes were analyzed using a Lineweaver-Burk plot, which indicated a higher K_m value for E_1S than that reported for human placental STS. The differential tissue distribution of STS suggests that there are different functions of this enzyme in various tissues. Because STS plays an important role in regulating active estrogen levels, characterization of this enzyme in the widely used mouse model can provide insight into the overall physiological function of STS, as well as into its role in hormone-dependent cancers and osteoporosis.

ANALYSIS OF MODIFIED HISTONE DISTRIBUTION AT THE TISSUE-SPECIFIC IMPRINTED RASGRF1 LOCUS USING ALLELE-SPECIFIC QPCR FOLLOWING CHIP

Nicole Hamagami and Tamara L. Davis
Department of Biology, Bryn Mawr College, 101 North Merion Avenue, Bryn Mawr, PA 19010

Genomic imprinting is an epigenetic mechanism that facilitates the expression of one parental allele over another by attracting or repelling various elements required for DNA transcription. Specifically, posttranslational histone modifications structurally alter the chromatin itself or regulate enzymatic chromatin binding factors that play a role in genetic expression and imprinting.

The imprinted mouse gene *Rasgrf1* is paternally expressed in a tissue-specific manner. Previous studies on the *Rasgrf1* gene have shown that DNA methylation is present on the paternal allele in tissues with both imprinted and non-imprinted expression, indicating that DNA methylation is not solely responsible for the tissue-specific expression patterns observed at this gene locus.

Histone modifications are additional epigenetic factors that may explain the tissue-specific imprinted expression seen at *Rasgrf1*. To determine whether histone modifications explain these tissue-specific expression patterns, we coupled chromatin immunoprecipitation (ChIP) with allele-specific amplification of DNA using quantitative PCR to locate modified histones that are preferentially distributed on either or both parental alleles. Since the chromatin structure at the promoter tends to reflect the expression patterns of the gene itself, we expect to see permissive and repressive histone modifications on the promoter region of the paternal and maternal chromosomes, respectively, in mono-allelic tissues, and permissive modifications on both alleles for bi-allelic tissues. However, we are unsure how these modified histones will be distributed at the DMR site. If the modified histone distribution appears to reflect that of DNA methylation at these regions, then we may conclude that histone modifications are not involved in the differential expression seen in mono-allelic and bi-allelic tissues. Differences in distribution in mono-allelic versus bi-allelic tissues, however, may suggest the involvement of these modified histones in tissue-specific gene expression. Therefore, the goal for our research is to determine whether histone modifications directly correlate with DNA methylation in regulating differential gene expression.

We thank Paige De la Rosa (BMC ’14), Aimee Heerd (BMC ’14), and Carolyne Face (BMC ‘15) for their contributions towards the development of this project. We also would like to thank Rachel Shields (BMC’15) and Ekaterina Vlasova (BMC ‘15) for their ongoing work towards the Davis Lab. Support for this research was provided by an award to TLD from NSF grant #1157819. In addition, student research was supported in part by the Bryn Mawr College Summer Science Research program.

INHIBITING THE EFFECTS OF TRAMATIC BRAIN INJURY THROUGH THE DESIGN OF GluR2 ANTAGONIST

Abraham Isak, Keval Patel, Karan Arora, Earl Benjamin, and Ellis Benjamin
School of Natural Sciences and Mathematics, Richard Stockton College,
101 Vera King Farris, Galloway, NJ 08205

Recent understanding of the detrimental effects of traumatic brain injury has been linked to a dramatic increase in glutamate receptor (GluR2) production and a decrease in the inhibitory GABAergic proteins of excitatory interneurons. The overproduction of GluR2 increases the likelihood of uncontrolled interneuronal excitation resulting in a seizure. This research sought to gain a better understanding of GluR2 structural interactions to design a more effective antagonist. A total of 1172 molecules separated into a series of 8 drug targeting groups that included alkaloids, imides, lactams, lactones, NSAIDS, sulfanilamide, flavonoids and FDA approved pharmaceuticals were selected. Using a series of computational docking methods including IGemDock and Pyrx - Autodock Vina yielded a small subset of molecules that showed improved docking interaction over standard GluR2 pharmaceuticals. These molecules were studied to determine structural interaction that can improve efficacy over current GluR2 drug design.

This Project was supported by The College of Natural Science and Mathematics at The Richard Stockton College of New Jersey.

Borrelia burgdorferi: VlsE1 AS A POTENTIAL GANGLIOSIDE BINDING PROTEIN

Drew Phelan and Christopher W. Reid
Department of Science and Technology, Bryant University,
1150 Douglas Turnpike, Smithfield, RI 02917

The Center of Disease Control (CDC) estimates that there are around 35,000 cases (including unreported cases) of Lyme disease each year in the United States. Caused by *Borellia burgdorferi sensu lato,* this disease, if left undiagnosed, can cause a number of neurological affects from myelitis to facial nerve paralysis. The goal of this research is to identify proteins involved in *B. burgdorferi* recognition of gangliosides. Through the process of bioinformatics and careful analysis of the results, the gene *vlsE1* was identified as a potential ganglioside binding protein. VlsE1 is known to be expressed during late stage Lyme disease and is an important virulence factor for establishing infection [1]. The *vlsE1* gene was synthesized and sub cloned into the pET28 expression vector to produce an N-terminally His_6-tagged construct. After sub-cloning VlsE1 we began establishing the expression and purification conditions that produce the best results. Ganglioside binding was tested using membranes enriched in recombinant VlsE1 in an ELISA assay.

Special thanks for funding to Rhode Island SURF Program. The Reid Research Group and the Glycomics Lab at Bryant University. Special appreciation to Bryant University Academic Affairs for their support.

1. Bacon, R., Biggerstaff, B., Schriefer, M., Gilmore, R., & Philipp, M. (2002). Serodiagnosis of Lyme Disease by Kinetic Enzyme- Linked Immunosorbent Assay Using Recombinant VlsE1 or Peptide Antigens of Borrelia burgdorferi Compared with 2- Tiered Testing Using While-Cell Lysates.

CollecTF: A DATABASE OF EXPERIMENTALLY – VALIDATED BACTERIAL TRANSCRIPTION FACTOR BINDING SITES.

Dinara Sagitova[1], Sefa Kilic[2], and Ivan Erill[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

With the advent of high - throughput data and the need for complex models to describe transcriptional regulation in bacteria, new challenges for transcription factor binding site databases are posed. The computational analysis of the interaction of prokaryotic transcription factors with their binding sites is limited by the availability of experimental data on transcription factor binding sites. Although several databases for bacterial transcription factor binding sites exist, they tend to focus on model organisms and/or combine in silico approaches with experimental knowledge, hindering the ability to employ them in comparative genomics analyses. CollecTF is a database that compiles data on experimentally validated, naturally occurring prokaryotic transcription factor binding sites and aims to provide broader coverage than current databases by implementing a more open and flexible system. A strong emphasis is placed on the transparency of the curation process, the quality and availability of the stored data, and fully customizable access to its records.

CollecTF incorporates multiple sources of data automatically, allowing users to redefine binding motifs and their experimental support base. Validation and curation of published literature on CollecTF is done by a dedicated team of curators. In addition, direct submissions of data by experimentalists are encouraged. Validated CollecTF entries are periodically submitted to NCBI for integration into RefSeq complete genome records as link out features. This enriches the annotation of RefSeq files with regulatory information and maximizes the visibility of data. The current CollecTF release provides a comprehensive species-wide mapping of two transcription factor families (LexA and Fur), as well as in-depth coverage of clinically important bacterial families, the Vibrionaceae and the Pseudomonadaceae. Currently, CollecTF contains over 3,000 experimentally-validated binding sites for more than 200 transcription factors across 130 bacterial strains. CollecTF is accessible at: http://collectf.umbc.edu

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CHARACTERIZATION OF STARCH DEGRADATION IN *CELLVIBRIO JAPONICUS*

Gisele Toumi and Jeffrey Gardner
Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Online access of this abstract is restricted at the request of the Principal Investigator.

Morning Poster Session
Group D - Biological Sciences

Poster #	Title, Author(s) & Affiliation(s)
22.	**DIFFERENTIATING INDUCED PLURIPOTENT STEM CELLS INTO CEREBRAL ORGANOIDS** Yu-Rei Raymond Chang[1], Ivy Mead[2], Sam Pendergraft[2], Ashley Wagoner[2] and Colin Bishop, PhD[2] [1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21042 [2]Wake Forest Institute for Regenerative Medicine, 391 Technology Way, Winston-Salem, NC, 27157
23.	**INVESTIGATING THE ROLE OF HIF1-α IN ANOXIA TOLERANCE IN ZEBRAFISH EMBRYOS** Catrina Johnson and Rachel Brewster Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
24.	**EXAMINATION OF TRANSGLUTAMINASE ISOFORM EXPRESSION AND ACTIVITY IN NEURITE OUTGROWTH** Holly Langdon and Kristen L. Boeshore, PhD Department of Biology, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003
25.	**A GAIN OF FUNCTION SCREEN TO IDENTIFY TRANSCRIPTIONAL REGULATORS OF AXON GUIDANCE IN *DROSOPHILIA*** Akua Nimarko[1], Celine Santiago[2], and Greg J. Bashaw[2] [1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250 [2]Department of Neuroscience, Perelman School of Medicine at the University of Pennsylvania, 415 Curie Boulevard, Philadelphia, PA 19104
26.	**DYNAMIN AND ITS ROLE IN *DROSOPHILA MELANOGASTER* TUBE MORPHOGENESIS** Kamsi Odinammadu[1], Nathaniel Peters[2], and Celeste Berg[2] [1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250 [2]Department of Genome Sciences, University of Washington, 3720 15th Avenue NE, Seattle WA 98195
27.	**EXAMINING O-XYLOSYLTRANSFERASE SHEDDING IN *DROSOPHILA*** Brooke Palus and Erica M. Selva Department of Biology, University of Delaware, Newark, DE 19716
28.	**TUBB6 AND KIF1A MAY BE INVOLVED IN LENS FIBER ELONGATION** Troy Rubenstein, Dylan Audette, and Melinda K. Duncan Department of Biological Sciences, University of Delaware, Wolf Hall, The Green, Newark, DE 19716

DIFFERENTIATING INDUCED PLURIPOTENT STEM CELLS INTO CEREBRAL ORGANOIDS

Yu-Rei Raymond Chang[1], Ivy Mead[2], Sam Pendergraft[2],
Ashley Wagoner[2] and Colin Bishop, PhD[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21042
[2]Wake Forest Institute for Regenerative Medicine,
391 Technology Way, Winston-Salem, NC, 27157

By introducing somatic cells to specialized transcription factors, somatic cells can be reprogrammed into induced pluripotent stem cells (IPSCs). Given the pluripotential abilities of IPSCs, assorted tissues comprised of numerous cell types can be generated for cell replacement therapy, tissue morphogenesis, and disease modeling. Neural tissues are among the many tissue types that can be generated. My experiments this summer utilized an integration-free, episomal derived IPSC line that has been genetically engineered to fluoresce in the presence of calcium flux. Cerebral organoids were generated by culturing the cells into a spheroid, providing an ideal environment for differentiating the IPSCs into neural cells by using neural induction and neural differentiation mediums. The novel, fluorescent capacity of the IPSC-derived cells will allow for live imaging of neural calcium signaling. In order to characterize the organoid, we used reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry methods to analyze levels of various neuronal, forebrain, hindbrain, and progenitor markers and ensure differentiation of the IPSCs into specific neuronal subtypes. Successful brain characterization of a cerebral organoid derived from human IPSCs has tremendous implications in the field of medicine and would allow for not only cell replacement therapies, preliminary drug testing, and disease modeling, but also the bypass of many ethical issues surrounding embryonic stem cell testing and human trials.

INVESTIGATING THE ROLE OF HIF1-α IN ANOXIA TOLERANCE IN ZEBRAFISH EMBRYOS

Catrina Johnson and Rachel Brewster
Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Anoxia tolerance is a phenomenon exhibited by a several species, including turtles and carp as a survival mechanism in an environment lacking oxygen. Some species show behavioral adaptations to survive an anoxic environment, but zebrafish embryos undergo suspended animation. Suspended animation is a survival mechanism that temporarily halts development and movement, including heartbeat. To understand mechanisms underlying anoxia tolerance, we are exploring the role of Hypoxia-inducible Factor 1α (HIF1-α). This transcription factor is stabilized under anoxic/hypoxic conditions and is thought to regulate the transcription of genes that promote survival in low-oxygen conditions, including glycolysis enzymes that enable ATP synthesis in an oxygen-independent manner, and vascular endothelial growth factor (VEGF), which promotes angiogenesis. Despite its protective role in low oxygen environments, it remains controversial whether HIF1-α plays a significant role under anoxic conditions, as regulation of transcription is ATP-dependent and levels of this high energy metabolite are very limited in absence of oxygen. We therefore hypothesize that HIF1-α may not be required for anoxia tolerance in zebrafish embryos. To test this, we analyzed the expression of the HIF1-α downstream target *insulin-like growth factor binding protein 1α (IGFBP1-α)* in day old embryos that were subjected to anoxia and control embryos that were maintained under normoxic conditions. Consistent with our hypothesis, wholemount in situ hybridization of control and anoxia-treated embryos showed no significant increase in *IGFBP1-α* expression following 1 or 2 hours of anoxia. These results prompt further investigation of the regulatory mechanism associated with anoxia tolerance and the onset of suspended animation. Findings stemming from this research are relevant to stroke and ischemic injury, which have in common damage associated with anoxia and the sharp onset of reactive oxygen species (ROS) upon re-oxygenation.

This work was supported by the National Institute of General Medical Sciences grant 5R01-GM085290.

EXAMINATION OF TRANSGLUTAMINASE ISOFORM EXPRESSION AND ACTIVITY IN NEURITE OUTGROWTH

Holly Langdon and Kristen L. Boeshore, PhD
Department of Biology, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

Retinoic acid (RA) has been shown to promote axonal regeneration from injured neurons after an acute spinal cord injury. In the work presented here, the role of RA in promotion of neurite regeneration was examined in PC12 cells, a well-established cell line used for studying the mechanisms involved in differentiation of neurons. Specifically, the ability of RA to induce transglutaminase-2 (TG-2) expression and activation was investigated. Western blot analysis was used to determine if RA stimulation of injured cells increases expression or alters the relative expression of the long (TG-2L) and short (TG-2S) isoforms. Since transamidation activity of TG-2 has been linked to promotion of neurite outgrowth, it was expected that RA stimulation would increase expression of TG-2S, a form which exhibits greater transamidation activity. Additionally, *in situ* transamidation assays were carried out to examine if TG-2 activity is increased in RA-treated cells. Both isoforms of TG-2 were detected in injured cells following RA treatment. Replication of the experiment and quantification of the isoforms is ongoing. Activity levels of TG-2 were low across all conditions, and therefore optimization of the transamidation assay using higher numbers of cells is continuing.

This work was supported by the Arnold Experiential Grant Program at Lebanon Valley College.

A GAIN OF FUNCTION SCREEN TO IDENTIFY TRANSCRIPTIONAL REGULATORS OF AXON GUIDANCE IN *DROSOPHILIA*

Akua Nimarko[1], Celine Santiago[2], and Greg J. Bashaw[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Neuroscience, Perelman School of Medicine at the University of Pennsylvania, 415 Curie Boulevard, Philadelphia, PA 19104

The establishment of the neuronal circuitry of the central nervous system (CNS) requires the projection of axons to specific targets. Conserved families of axon guidance receptors regulate specific targeting of axons in the CNS by signaling attraction or repulsion in response to extracellular cues. In addition, studies suggest that transcription factors also play a role in establishing neuronal connectivity by regulating key axon guidance genes. Notably, it has been recently established that the Drosophila homeodomain transcription factor Hb9 regulates the expression of the Roundabout (Robo) 2 and Robo3 receptors in the CNS to control precise connectivity. However, few such regulatory relationships have been demonstrated and the mechanisms by which these transcription factors act during axon guidance are poorly understood. To identify additional axon guidance transcription factors that regulate connectivity, we used the Gal4-UAS system to overexpress candidate transcription factors in small subsets of neurons in the CNS and observed the effects on axon guidance. We found that the over expression of transcription factors *eyeless* and *glial cells missing* caused axons to display a lateral shift phenotype while the overexpression of transcription factors *teashirt*, *gooseberry-neuro*, *dichaete*, *hunchback*, and *twin of eyeless* produced abnormal midline crossing. Our results suggest that these transcription factors may play a key role in axon guidance.

This research was supported by NIH/NIGMS MARC U*STAR T34 08663 National Research Service Award to UMBC (AN) and NIH 2R01NS054739 (GJB).

DYNAMIN AND ITS ROLE IN *DROSOPHILA MELANOGASTER* TUBE MORPHOGENESIS

Kamsi Odinammadu[1], Nathaniel Peters[2], and Celeste Berg[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Genome Sciences, University of Washington,
3720 15th Avenue NE, Seattle WA 98195

Epithelial tubes are essential for normal function in almost all animals. Mutations in tube-forming genes can cause major developmental defects. To better understand tube formation, we utilize a model system that looks at the formation of dorsal appendages (DA) on the eggshell of fruitfly (*Drosophila melanogaster*) eggs. The DAs are formed by a subset of epithelial cells surrounding the oocyte. These epithelial cells pattern themselves into two groups that rearrange and form tubes as the oocyte develops. Later, the cells secrete protein into the tube lumens, and then the tube cells degrade, leaving the DAs. Many genes play a role in the development of these structures. Genetic analyses suggest that the gene *shibire,* which codes for dynamin, a protein that has a hand in endocytosis and other processes, affects the formation of the DAs. To investigate the importance of dynamin in DA morphogenesis, we asked: What cells require dynamin for proper DA formation? We hypothesized that dynamin is needed in different cells for different reasons. We tested our hypothesis by using a method that involves a GAL4-UAS system expressing a dominant-negative dynamin protein. We gathered GAL4 construct strains that drive expression of GAL4 in different parts of the *Drosophila* egg chamber and crossed them with UAS constructs to produce dominant-negative dynamin. In this way we knocked down dynamin function in cells specified by the GAL4 construct. We then collected the progeny's eggs and scored them. We found that the constructs that drove dominant-negative dynamin expression in some parts of the tube cells, as well as the ones that drove expression in the stretch cells, which act as a substrate for tube elongation, showed dramatic defects. We also found that disrupting dynamin in other cells produced DA defects of different, less significant types.

The authors acknowledge funding from the NIH (NIH R01-GM079433) and thank Greg Martin at the Keck Imaging Center for his useful contributions to this work.

EXAMINING O-XYLOSYLTRANSFERASE SHEDDING IN *DROSOPHILA*

Brooke Palus and Erica M. Selva
Department of Biology, University of Delaware, Newark, DE 19716

O-xylosyltransferase (Oxt) is a transmembrane glycosyltransferase that initiates the first step in heperan and chrondroitin sulfate (HS and CS) proteoglycan biosynthesis. Elevated levels of circulating Oxt have been detected in human osteoarthritis (OA) patients and in mouse injury models of OA, suggesting shed Oxt may have an extracellular function. However, little is known about Oxt behavior or function once it is released from the plasma membrane. Understanding the role of extracellular Oxt may provide further insight to how cell signaling is disrupted in OA patients. Cell culture experiments have shown that Oxt, normally located in the Golgi and the endoplasmic reticulum (ER), can be shed by proteolysis activity and move into the media. It is therefore likely that under the appropriate conditions *in vivo* Oxt will also be shed and become an extracellular protein. The overall aim of this project is to determine if Oxt released from expressing cells *in vivo* and what, if any, function extracellular Oxt has in organismal development. The data collected thus far shows localization of Oxt to the Golgi and the ER where it could have an active role in HSPG and CSPG biosynthesis. Movement of Oxt from expressing cells to non-expressing cells is observed in the imaginal wing disc of *Drosophila* larva. Extracellular staining shows that Oxt is found predominantly on the apical surface and can be detected in the peripodial space suggesting it is released from expressing cells. Further, preliminary Western analysis of *Drosophila* hemolymph (larval blood) indicated Oxt is released into hymolymph (blood), as observed in mice and humans. The results suggest that cleaved Oxt is found in the hymolymph of *Drosophila* and future studies will examine whether shed Oxt has a function.

TUBB6 AND KIF1A MAY BE INVOLVED IN LENS FIBER ELONGATION

Troy Rubenstein, Dylan Audette, and Melinda K. Duncan
Department of Biological Sciences, University of Delaware,
Wolf Hall, The Green, Newark, DE 19716

The lens is comprised of two types of cells; lens epithelial cells cap the anterior of the lens and differentiate at the equator into elongated lens fibers which fill the center of the lens. While the process that drives fiber cell elongation is not completely understood, prior studies demonstrated that microtubules are required for this event. Our prior work analyzed gene expression in a fiber cell elongation deficient mouse model as compared to a wild type, and identified several microtubule associated genes whose expression was down regulated. Among these were the atypical microtubule subunit, Tubb6, and the lens specific kinesin, Kif1a. We characterized the expression of Tubb6 and Kif1a in wild type lenses using a fluorescent stain as well as immunohistochemistry. This allowed us to observe that both genes are expressed in the elongating secondary lens fiber cells and showed a marked decrease in expression in fiber cell deficient lenses. These results show that the proteins are expressed within the lens extending fiber cells and it supports the hypothesis that they are involved in the elongation of lens fiber cells. We plan on furthering our investigation to demonstrate that these genes are essential for fiber cell elongation by knocking down these genes and observing if fiber cell elongation still occurs.

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Morning Poster Session
Group E - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

29. **LINGUISTIC HOMOGAMY EXPLAINS THE RECENT INCREASE IN PHENOTYPIC DEAFNESS, BUT DOES NOT PREDICT AN INCREASE IN ALLELE FREQUENCY**

Samir Jain[1], Eric Epstein[2], and Derek Braun[3]
[1] Department of Biomedical Engineering, Georgia Institute of Technology, North Avenue NW, Atlanta, GA 30332
[2]Department of Communication, Rochester Institute of Technology, 1 Lomb Memorial Drive, Rochester, NY 14623
[3]Molecular Genetics Laboratory, Gallaudet University, 800 Florida Avenue NE, Washington, DC 30097

30. **SILENCING OF ESSENSTIAL GENES IN NEMATODE SPECIES RESEMBLING PARASITES**

Anna Kania and Dr. Theresa Grana
Department of Biology, University of Mary Washington, 1301 College Avenue, Fredericksburg, VA 22401

31. **USING NUCLEAR INTRONS TO INFER THE EVOLUTIONARY HISTORY OF AN UNDERSTUDIED GENUS, *PETROICA***

John Malloy[1], Anna Kearns[1,2], Matthias Gobbert[3], Amy Driskell[4], and Kevin Omland[1]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Natural History Museum, University of Oslo, P.O. Box 1172, Blindern, Oslo, Norway
[3]Department of Mathematics and Statistics, UMBC, 1000 Hilltop Circle, Baltimore MD 21250
[4]Smithsonian Institution, Laboratories of Analytical Biology, National Museum of Natural History, P.O. Box 37012 MRC 183, Washington DC

32. **ANALYSIS OF THE STABILITY OF A TYPE III SECRETION SYSTEM CONTAINING PATHOGENICITY ISLAND IN THE HUMAN PATHOGEN *VIBRIO CHOLERAE*.**

Molly C. Peters, Megan R. Carpenter, and E. Fidelma Boyd
Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19716

33. **SPECIATION REVERSAL: THE CASE OF THE COMMON RAVEN**

Hayley Richardson[2], Jin Ah Kim[1], Anna Kearns[1], Matthias Gobbert[2], and Kevin Omland[1]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Mathematics and Statistics, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

34. **USING THE *AMBORELLA TRICHOPODA* EXPANSIN SUPERFAMILY TO ELUCIDATE THE HISTORY OF ANGIOSPERM EXPANSINS**

Victoria H. Seader[1], Jennifer M. Thornsberry[2], and Robert E. Carey[2]
[1]Program in Biochemistry and Molecular Biology, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003
[2]Department of Biology, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

35. **USING STRUCTURAL PARAMETERS IN TRANSCRIPTION FACTOR BINDING SITE PREDICTION**

Nicholas Stewart[1] and Ivan Erill[2]
[1]Department of Computer Science, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

LINGUISTIC HOMOGAMY EXPLAINS THE RECENT INCREASE IN PHENOTYPIC DEAFNESS, BUT DOES NOT PREDICT AN INCREASE IN ALLELE FREQUENCY

Samir Jain[1], Eric Epstein[2], and Derek Braun[3]
[1] Department of Biomedical Engineering, Georgia Institute of Technology,
North Avenue NW, Atlanta, GA 30332
[2]Department of Communication, Rochester Institute of Technology,
1 Lomb Memorial Drive, Rochester, NY 14623
[3]Molecular Genetics Laboratory, Gallaudet University,
800 Florida Avenue NE, Washington, DC 30097

Eugenicists like Alexander Graham Bell argued against deaf-by-deaf marriage: "A number of successive generations should result in the formation of a deaf variety of the human race." Bell's Volta Bureau funded Fay's study of 4,471 deaf marriages, which he hoped would illustrate his point. Despite Fay's conclusion, published in 1898, that deaf marrying deaf did not appreciably increase the chances of having deaf children, during the early-twentieth-century eugenics movement, Germany and 30 United States passed laws to involuntarily sterilize congenitally deaf individuals. Recently, Arnos et al. have reported that deaf couples are twice as likely to have deaf children now than they were 200 years ago. It has been proposed that by concentrating signing deaf individuals in a residential environment, deaf schools may have created an example of linguistic homogamy, a form of assortative mating where individuals preferentially marry those who share their own language, which may have increased the frequency of the commonest deafness-causing allele. The linguistic homogamy hypothesis is important in anthropological genetics because it purports to explain the rapid fixation of the human *FOXP2* gene some 100,000 years ago, which was key to the evolution of human speech and language. We tested the hypothesis that linguistic homogamy alone is sufficient to influence the gene pool by conducting forward-time mating simulations. We found that linguistic homogamy increased the number of deaf individuals, corroborating previous findings, but that the allelic frequency did not increase. We found that the allele frequency increased the most with doubled reproductive fitness. Therefore, our results modify the linguistic homogamy hypothesis.

We thank NASA, the D.C. Space Grant Consortium and the Sorenson Legacy Foundation for funding this project.

SILENCING OF ESSENSTIAL GENES IN NEMATODE SPECIES RESEMBLING PARASITES

Anna Kania and Dr. Theresa Grana
Department of Biology, University of Mary Washington,
1301 College Avenue, Fredericksburg, VA 22401

The technique of RNA interference (RNAi) has been widely used to identify gene functions in *Caenorhabditis elegans* as well as in a number of other species. However, this valuable reverse genetic tool has been either unsuccessful or unreliable in the study of human- and animal-parasitic nematodes. *Rhabditis* nematode species, whose reproductive strategies parallel those in many parasitic nematodes, have the potential to serve as excellent models for the study of parasitic species. Because susceptibility to experimental RNAi varies among nematodes, the primary task of this project is to evaluate the sensitivity to RNAi silencing of five *Rhabditis* species resembling parasites. This task will involve 1) construction of plasmid containing a fragment complementary to the gene of interest, 2) transformation of the plasmid construct into feeding bacteria and induction of double stranded RNA (dsRNA) synthesis, and 3) feeding the *Rhabditis* species as well as the *C. elegans* (a positive control for RNAi efficiency) with the engineered bacteria in an attempt to induce RNAi mechanism, which would result in altered phenotypes of the worms. Knock-down of genes associated with the life cycle in the RNAi susceptible *Rhabditis* species would provide other researchers with a way to identify genes that could serve as potential drug targets and thus contribute to the eradication of many diseases caused by vertebrate-parasitic nematodes. Furthermore, the optimized methods utilized throughout this project would allow future students in Dr. Grana's research lab to evaluate the RNAi susceptibility of the 150 free-living nematode strains in our unique collection; such information would add to the literature and help trace the evolution of the RNAi mechanism.

This project was funded by UMW Undergraduate Research Grant and Chi Beta Phi Research Award. A few strains were provided by the Caenorhabditis Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). Several other strains were obtained from Dr. Diana Shakes at the University of William and Mary.

USING NUCLEAR INTRONS TO INFER THE EVOLUTIONARY HISTORY OF AN UNDERSTUDIED GENUS, *PETROICA*

John Malloy[1], Anna Kearns[1,2], Matthias Gobbert[3], Amy Driskell[4], and Kevin Omland[1]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Natural History Museum, University of Oslo, P.O. Box 1172, Blindern, Oslo, Norway
[3]Department of Mathematics and Statistics, UMBC, 1000 Hilltop Circle, Baltimore MD 21250
[4]Smithsonian Institution, Laboratories of Analytical Biology,
National Museum of Natural History, P.O. Box 37012 MRC 183, Washington DC

The Australasian robin genus *Petroica* is commonly used as a textbook example of island speciation due to the radiation of the species outward from the Australian mainland (Mayr, Animal Species and Evolution, 1950). Although their common name sounds familiar, the Australian Robins are a distinct group not at all closely related to our American Robin. Recently, based on evolutionary analyses, a previous subspecies in the *Petroica* genus, the Australian mainland Scarlet Robin, was raised to a full species while the closely related Norfolk Island Robin remains a subspecies. The Norfolk Island Robin is considered vulnerable (Norfolk Island Robin Recovery Plan, 2004) and conservation efforts are therefore critical. However, the evolutionary analyses of this group that resulted in this new taxonomy were performed solely with mitochondrial DNA. Previous work done by the Omland Laboratory at UMBC has shown mitochondrial analyses to potentially be misleading. This project uses nuclear intron DNA (noncoding nuclear DNA) from a wide sampling of Australian Robins. These DNA samples will be analyzed using various evolutionary software programs, such as mrBayes and *BEAST, in order to infer a more conclusive and thorough evolutionary history of the genus as a whole, as well as to determine whether the Norfolk Island Robin should be considered a distinct species.

This project was funded, in part, by the UBM program at UMBC, an interdisciplinary biology and math training grant funded by the National Science Foundation (NSF) as well as by an NSF grant to Dr. Kevin Omland. Results were obtained with the use of UMBC High Performance Computing Facility, supported by NSF with additional substantial support by UMBC.

ANALYSIS OF THE STABILITY OF A TYPE III SECRETION SYSTEM CONTAINING PATHOGENICITY ISLAND IN THE HUMAN PATHOGEN *VIBRIO CHOLERAE*.

Molly C. Peters, Megan R. Carpenter, and E. Fidelma Boyd
Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19716

Vibrio cholerae is an enteric pathogen that is the causative agent of the secretory diarrhea, cholera, which affects millions each year. While the O1 serogroup *V. cholerae* strains are the most well studied due to their propensity to cause epidemic and pandemic cholera, a second pathovar of *V. cholerae* has been identified that causes inflammatory diarrhea. These strains encode a Type III Secretion system (T3SS). This system is present on a Pathogenicity Island (PAI), a mobile genetic element integrated into the chromosome of some non-O1 serogroup strains. The aim of this research is to study the island region of strain NRT36S containing the T3SS, named VPI-3, and understand the excision behavior of the region.

After the deletion of the cognate integrase of the island, *intV2*, the excision of the VPI-3 island of NRT36S was examined using a two stage nested PCR reaction. The results of this assay show that *intV2* is necessary for the excision of VPI-3. Additionally, another aim of this work is to examine the evolution of this island region in order to understand the role that HGT has on the divergence of PAIs amongst *Vibrio* and other species of bacteria.

A phylogenetic analysis of the conserved T3SS ATPase protein shows the presence of two different variants of T3SS within *V. cholerae*, T3SSα and T3SSβ. Additionally, the grouping pattern on the tree shows the close relationship between the T3SS ATPase of *V. cholerae* NRT36S, *V. parahaemolyticus,* and *V. mimicus* suggesting that this virulence system was passed horizontally between these different species of bacteria in the past.

SPECIATION REVERSAL: THE CASE OF THE COMMON RAVEN

Hayley Richardson[2], Jin Ah Kim[1], Anna Kearns[1], Matthias Gobbert[2], and Kevin Omland[1]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Mathematics and Statistics, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Speciation reversal results when two or more distinct species interbreed to form one species. This phenomenon is most widely known in human history; our evolutionary background consisting up to four separate lineages, now reduced to a single species. Speciation reversal is also noted in other organisms, such as fish and birds. However, unlike fish, the documented cases of speciation reversal in birds are not influenced by humans, and reflect a natural occurrence. We focus on the case of Common Ravens, which suggests a likely case of speciation reversal.

The Common Raven (*Corvus corax*) has a very wide range, spanning throughout North America, Europe, and Asia. Within this species, there exist two deep mitochondrial lineages, the California clade (found exclusively in south-western U.S.) and the Holarctic clade (found throughout the entire range). The estimated time of divergence is calculated to be 2 million years between these two lineages. These two genetically distinct clades now have overlapping ranges and Holarctic and California alleles are intermixing, which is likely causing the merging of these two lineages.

To test this hypothesis, we sequence ACO1, a nuclear intron, to evaluate specific clusters of alleles (haplotypes) that are present in our raven sample on a haplotype network. We then compare these data to the known mitochondrial haplotypes to determine if the nuclear genome also indicates a similar story of divergence. Using the program GENELAND, we map the geographic ranges of Holarctic and California clade ravens and visualize the genetic mixing between the groups, using both mitochondrial and nuclear data. These data are the first step in understanding the evolutionary history of the Common Raven and elucidating the process of speciation reversal.

J. Kim and H. Richardson were supported by the NSF funded training grant: "Interdisciplinary Training for Undergraduates in Biological and Mathematical Sciences - UBM" NSF DBI 1031420.

USING THE *AMBORELLA TRICHOPODA* EXPANSIN SUPERFAMILY TO ELUCIDATE THE HISTORY OF ANGIOSPERM EXPANSINS

Victoria H. Seader[1], Jennifer M. Thornsberry[2], and Robert E. Carey[2]
[1]Program in Biochemistry and Molecular Biology, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003
[2]Department of Biology, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

Expansins are a superfamily of proteins found in plants that assist in cell wall loosening. The superfamily is divided into four families: EXPA, EXPB, EXLA, and EXLB.[1] Previous work on *Arabidopsis thaliana*, rice, and *Populus trichocarpa* has clarified the evolutionary history of expansins in angiosperms.[2] *Amborella trichopoda* is a very early diverging flowering plant. Thus, it is a sister lineage to all other extant angiosperms.[3] Because of this relationship, comparing the *Amborella* expansin superfamily to those of other flowering plants can suggest which expansin genes were present in the last common ancestor of all angiosperms. The *Amborella* expansin superfamily was assembled from the *Amborella* genome by using BLAST searches with angiosperm expansin queries. The results of these BLAST searches were analyzed and annotated to isolate the complete *Amborella* expansin superfamily. This superfamily is smaller than other angiosperm expansin superfamilies. This is probably due to an absence of genome duplication events in *Amborella's* history.[3] Phylogenetic and synteny analyses of *Amborella* expansins have improved our understating of the evolutionary history of expansins in angiosperms. It was possible to place all of the *Amborella* expansins into an existing *Arabidopsis*-rice expansin clade, with the exception of *Amt*EXPA4. This expansin was not placed into any clade by either synteny or phylogenetic analysis, which suggests that it may belong to another clade of expansins not present in either *Arabidopsis* or rice. The results of phylogeny and synteny analyses allow us to estimate the number of expansins found in the last common ancestor of all angiosperms at 8-9 EXPA genes, 2 EXPB genes, 1 EXLA gene, and 2 EXLB genes.

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This project was funded by an Arnold Student-Faculty research grant from Lebanon Valley College.

USING STRUCTURAL PARAMETERS IN TRANSCRIPTION FACTOR BINDING SITE PREDICTION

Nicholas Stewart[1] and Ivan Erill[2]
[1]Department of Computer Science, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

This project addresses the problem of identifying transcription factor binding sites using physical parameters, as opposed to current methods that only use nucleotide sequences to predict possible binding sites. Proteins called "transcription factors" bind to sites on the DNA strand and control whether the transcription of genes will be promoted or inhibited. Identifying the sites where they bind gives researchers a clearer picture of how genes are being regulated. Typically, prediction is done by analyzing DNA sequences, using a sequence-based model of transcription factor binding. Research has shown that some transcription factors will bind to specific sites at one genomic location, but not to another location with the same nucleotide sequence. This shows that some transcription factors are dependent (for binding) on the structure of the DNA molecule and not just nucleotide sequence.

The goal of this project is to calculate structural parameters from a nucleotide sequence, and then use those parameters to enhance the prediction of binding sites. This project builds on previous research by creating portable libraries for the inference of structural parameters that can be deployed inside of existing prediction programs. Currently, these curvature profiles are being used as features that are fed to Machine Learning algorithms for binding site prediction. These algorithms "learn" from existing data, and use that knowledge to predict future classification. Specifically, an Artificial Neural Network, the Random Forest algorithm, and genetic algorithms are being used for prediction.

Morning Poster Session
Group F - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

36. **MICROCLIMATIC CONDITIONS AMONG TREES ON PARKING LOT "ISLANDS"**
Dennis C. Alvarez and Carl R. Pratt
Department of Biology, Immaculata University, Immaculata, PA 19345

37. **ORANGE PEEL DEPOSITION ON CATTLE PASTURES AS A METHOD FOR CATALYZING TROPICAL FOREST SUCCESSION AND RESTORATION**
Jonathan Choi[1], Tim Treuer[1], Daniel Perez Aviles[2], Leland Werden[2], Jennifer Powers[2], and David Wilcove[1,3]
[1] Department of Ecology and Evolutionary Biology, Princeton University, 106A Guyot Hall, Princeton, NJ 08541
[2] Department of Ecology, Evolution and Behavior, University of Minnesota, 100 Ecology Building, 1987 Upper Buford Circle, Saint Paul, MN 55108
[3] Woodrow Wilson School of Public and International Affairs, Princeton University, Robertson Hall, Princeton, NJ 08541

38. **SURVEY OF PHOTOSYNTHETIC MICROBES AT STEELE CREEK LAKE**
Caleb Corona and Dr. Han Chuan Ong
Department of Biology, King University, 1350 King College Road, Bristol, TN 37620-2699

39. **CONFOUNDS IN THE THIONIN-BASED ASSAY OF CHLORINATION ACTIVITY IN COMMON GREENSHIELD LICHEN**
Marisa Dunigan and Alessandra Leri
Department of Natural Sciences, Marymount Manhattan College
221 East 71st Street, New York, NY 10021

40. **OFFSPRING PHENOTYPE DEPENDS ON RESOURCE QUALITY IN THE DUNG BEETLE *ONTHOPHAGUS TAURUS***
Adrienne Muetterties and Patrice Ludwig
Department of Biology, James Madison University, 800 South Main Street, Harrisonburg, VA 22801

41. **NUTRIENT CYCLING IN THE BLACKISTON TAX DITCHES**
James Welsh[1] and Dr. Stephanie Stotts[2]
[1]Department of Biology, Wesley College, 120 North State Street, Dover, DE 19901
[2]Department of Environmental Studies, Wesley College, 120 North State Street, Dover, DE 19901

MICROCLIMATIC CONDITIONS AMONG TREES ON PARKING LOT "ISLANDS"

Dennis C. Alvarez and Carl R. Pratt
Department of Biology, Immaculata University, Immaculata, PA 19345

These results are part of an ongoing analysis to determine the microclimatic conditions experienced by trees placed in potentially stressful conditions such as automobile parking lots and sidewalk areas. Environmental parameters (soil surface temperature, air temperature, wind speed, relative humidity) and physiological parameters (leaf-xylem potential, leaf temperature) were compared among red maple trees (*Acer rubrum*) growing on two adjacent sites: one set of trees was in confined planting bed within a parking lot surrounded by asphalt and the other site contained similar trees (trunk diameter, stature, and age) planted along a lawn area. Wind speed, air temperature, and relative humidity were measured using a hand-held Kestrel 4500 Pocket Weather Tracker. Environmental surface and leaf temperatures were determined using a Fluke IR thermometer (model 561). Leaf potentials were measured at approximately 2:00 PM once a week during the summer of 2013 using a Pressure Chamber (PMS model 600). Trees on the parking lot site experienced significantly higher mean leaf potentials [7.50 $\pm$ 0.24 mBar] as compared to those trees in lawn area [5.94 $\pm$ 0.27 mBar] as determined by ANOVA and student t-test. Leaf potentials increased during the summer from May through August in both sets of trees, reaching a maximum in August. These measurements suggest trees in the parking lot islands were subject to potential drought stress as compared to trees in the lawn. Mean surface temperatures on the parking lot surface beneath trees [36.6 $\pm$ 1.9° C] were significantly higher than those beneath trees on the lawn site [23.9 $\pm$ 1.1° C]. However, mean leaf surface temperatures did not differ significantly among trees on the two sites [parking lot: 24.3 $\pm$ 0.4° C; lawn: 23.1 + 0.35 °C].

We would like to thank the Department of Biology at Immaculata University for providing the necessary resources to carry this project. We would also like to thank Deborash Tischler for coordinating the times to work on the project, and I, Dennis Alvarez, would like to thank Dr. Carl R. Pratt for his support and guidance during the length of time when this research was being conducted.

ORANGE PEEL DEPOSITION ON CATTLE PASTURES AS A METHOD FOR CATALYZING TROPICAL FOREST SUCCESSION AND RESTORATION

Jonathan Choi[1], Tim Treuer[1], Daniel Perez Aviles[2], Leland Werden[2], Jennifer Powers[2], and David Wilcove[1,3]
[1] Department of Ecology and Evolutionary Biology, Princeton University, 106A Guyot Hall, Princeton, NJ 08541
[2] Department of Ecology, Evolution and Behavior, University of Minnesota, 100 Ecology Building, 1987 Upper Buford Circle, Saint Paul, MN 55108
[3] Woodrow Wilson School of Public and International Affairs, Princeton University, Robertson Hall, Princeton, NJ 08541

Tropical forests have been a target for restoration due to their high biodiversity, carbon storage potential and the ecosystem services they provide. Many old cattle pastures and other degraded landscapes can be potentially restored, but nutrient limitations and the cost of fertilizer prevent many large scale restoration projects. Could agricultural waste restore lost nutrients and catalyze forest succession in a cost effective manner?

A partnership between the Área de Conservación Guanacaste (ACG) and the Del Oro orange juice company in northwest Costa Rica offers a rare case study. In 1998, the Del Oro orange juice company gave $480,000 USD of secondary forest to the ACG in exchange for permission to dump orange peels on a pasture within the park. In the summer of 2014, we conducted vegetation surveys to study the efficacy of this deposition in promoting regrowth of trees. Three 100m x 6m transects were established within the area of orange peel deposition with replicates in a cattle pasture 100m away from the site. All trees with a DBH greater than 5cm were measured and identified ($n_{fertilized} = 112$, $n_{unfertilized} = 111$). All saplings greater than 1.3m in height but smaller than 5cm DBH were also measured, but they were not identified ($n_{fertilized} = 820$, $n_{unfertilized} = 353$). Light reaching the forest floor was measured with hemispherical photography (n = 33 per treatment).

Surveys revealed an increase from 8 to 24 tree species, a two-fold increase in aboveground biomass by conservative estimates, and a three-fold decrease in visible sky on the fertilized treatment compared with the unfertilized treatment. These initial data suggest that agricultural waste could be a major catalyst for forest regeneration with many policy implications, especially considering deforestation rates and agricultural activity in the tropics.

Research was made possible by financial assistance from the Princeton Environmental Institute, the Office of the Dean of the College and from the Department of Ecology and Evolutionary Biology at Princeton University.

SURVEY OF PHOTOSYNTHETIC MICROBES AT STEELE CREEK LAKE

Caleb Corona and Dr. Han Chuan Ong
Department of Biology, King University, 1350 King College Road, Bristol, TN 37620-2699

The advent of molecular biological research, especially in the area of DNA sequencing, has opened a new horizon for every branch of the biological sciences. Scientists estimate that there are between 10 and 30 million species living on Earth today, with only 2 million being identified to date. Of these 2 million species, 99% are plant and animal species. A huge amount of undiscovered species lie within the families of algae and photosynthetic bacteria. This fact calls for more research into these organisms and the environments in which they thrive. My research question was what types of photosynthetic microbial lifeforms live in the freshwater lake ecosystem within Steele Creek Park.

Water samples were collected using a Vernier Water Depth Sampler. After these samples were obtained, they were transported to the lab at King University and were inoculated onto agar plates consisting of BG11 media, a type of media that only allows growth of photosynthetic life. After a growth period, the isolated colonies were removed from the plate and using chemical processes, their DNA were extracted. The DNA will then be purified, amplified, and sent to the University of Arkansas to be sequenced. These resulting sequences will be returned to us at King University and will be used to identify the types of organisms living in the lake.

This project is still underway, so far this project has provided me with a great opportunity to obtain hands on experience conducting experiments outside the normal classroom setting, and as the project continues, will further prepare for my future career in Wildlife Biology.

CONFOUNDS IN THE THIONIN-BASED ASSAY OF CHLORINATION ACTIVITY IN COMMON GREENSHIELD LICHEN

Marisa Dunigan and Alessandra Leri
Department of Natural Sciences, Marymount Manhattan College
221 East 71st Street, New York, NY 10021

Flavoparmelia caperata, or common greenshield lichen, is the product of a symbiotic relationship between an ascomycete fungus and an alga. Many forest fungi, including some ascomycetes, are known producers of organochlorine in terrestrial ecosystems. We hypothesized that *F. caperata* produces organochlorine as well. Using synchrotron-based X-ray absorption spectroscopy, we measured high concentrations of aromatic organochlorine in the thallus of *F. caperata*. This led to a further hypothesis that the lichen produces organochlorine through a chloroperoxidase (CPO)-like enzyme.

To assess chlorination by *F. caperata*, we used a colorimetric assay with the substrate thionin acetate to measure chlorination activity. Thionin has a deep violet color that is lost in the presence of chloride, hydrogen peroxide, and CPO (Manoj and Hager, 2006). This is measured quantitatively by monitoring the absorbance of thionin at 598 nm. We had apparent success when substituting extracts of *F. caperata* for pure CPO in this assay; however, replications of this experiment in the absence of hydrogen peroxide or chloride still showed color loss. This led us to theorize that antioxidants in the lichen were reducing thionin acetate, causing color loss and confounding the chlorination assay.

Continuing the search for chlorination activity in *F. caperata*, we ran another spectrophotometric CPO assay using monochlorodimedone (MCD). This assay showed no detectable chlorination, ruling out CPO-like activity in common greenshield lichen. We are currently developing a thionin-based assay to measure antioxidant activity in lichen and other organisms.

K. M. Manoj, L.P. Hager. A colorimetric method for detection and quantification of chlorinating activity of hemeperoxidases (**2006**). *Analytical Biochemistry* 348, 84-86.

OFFSPRING PHENOTYPE DEPENDS ON RESOURCE QUALITY IN THE DUNG BEETLE *ONTHOPHAGUS TAURUS*

Adrienne Muetterties and Patrice Ludwig
Department of Biology, James Madison University,
800 South Main Street, Harrisonburg, VA 22801

The phenotype of organisms is often determined by the genetics of the organism and the environment in which those traits are expressed. The phenotype of the dung beetle, *Onthophagus taurus*, is heavily based on the environment. Adults construct brood balls to provision the offspring. The size of the brood ball affects the body size of all beetles and the presence or absence of horns in the males. These horns determine the mating strategy for the males; horned males guard tunnels, hornless males sneak matings with females. The quality of the dung, therefore, can have a large impact on the phenotype of the offspring and therefore the mating strategies employed. We tested the hypothesis that resource quality affects offspring size and horn length in male dung beetles. Previous work showed that horse dung was of higher quality than cow dung, however, the researchers used homogenized dung. This form is not the shape of what is observed in nature. We used dung in its natural state and compared the quality of horse versus cow dung on the presences or absence of and size of the brood balls. Many pairs of beetles failed to construct brood balls. Of the pairs that do construct brood balls, there is no clear pattern that emerges.

This research was funded by a Betty Jo Loving Butler ‘58 Endowment for Undergraduate Research Scholarship and Farrell Summer Research Scholarship award.

NUTRIENT CYCLING IN THE BLACKISTON TAX DITCHES

James Welsh[1] and Dr. Stephanie Stotts[2]
[1]Department of Biology, Wesley College, 120 North State Street, Dover, DE 19901
[2]Department of Environmental Studies, Wesley College,
120 North State Street, Dover, DE 19901

Tax ditches were implemented in the 1950s for agricultural drainage; they now drain roads and residential areas as well. In some instances, these ditches are surrounded by vegetated buffers that can protect the water body from nonpoint source pollution. The purpose of this project is to compare the water quality, including phosphate, nitrate, dissolved oxygen, and temperature, of three tax ditches with varying vegetated buffer widths. Water samples were collected from three tax ditches within the Blackiston Wildlife Preserve: one with no buffer, one with minimal buffer, and one with a relatively wide buffer. The samples were collected between June 24th and August 1st with duplicate samples collected each trip to the field. Nitrate levels were tested using the Lamotte's test kit, while the Hanna test was used to analyze the phosphate concentrations. The DO percent, DO level, and temperature for each tax ditch was obtained using the YSI electronic reader. Student's T-Tests were used to compare water quality parameters between sites.

The temperature at the ditch without a buffer was significantly higher than the ditch with a wide buffer, and the oxygen levels from the wide buffer site were significantly higher than the minimally buffered site. However, the site with no buffer had significantly higher oxygen levels than the narrowly buffered site. The nutrient data did not support our hypothesis as the nitrate level at the ditch with a wide buffer was actually higher than both the ditches with no buffer and a narrow buffer. Also, the ditch with no buffer had significantly lower phosphate levels than both the narrow and large buffers. Our surprising results may be due to differences in ditch size as the ditch with a wide buffer is much larger than the other two sites.

I would like to thank National Science Foundation (NSF) EPSCoR Grant No. IIA-1301765 (DE-EPSCoR RII-3) for funding this very important research.

Morning Poster Session
Group G – Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

42. **IDENTIFICATION AND CHARACTERIZATION OF AQUIFER BACTERIA**
Cooper Anderson, Mohammed Reza, Louay Zumot, Thai Tran and Karen York
Biology Program, The Richard Stockton College of New Jersey,
101 Vera King Farris Drive, Galloway, NJ 08205

43. **HISTONE MODIFICATION AS A POTENTIAL REGULATOR OF THE TISSUE-SPECIFIC IMPRINTING OF *RASGRF1***
Carolyne Face and Dr. Tamara Davis
Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010

44. CANCELLED

45. **PRACTICAL AND INEXPENSIVE DNA FINGERPRINTING FOR UNDERGRADUATE SCIENCE MAJORS AND HIGH SCHOOL STUDENTS**
Christine McBride[1], Jennifer Chin[2,3], and Derek C. Braun[3]
[1]University of Alabama at Birmingham, 1720 2nd Avenue, South Birmingham, AL 35294
[2]Hogeschool Leiden, Zernikedreef 11, 2333 CK Leiden, the Netherlands
[3]Molecular Genetics Laboratory, Gallaudet University,
800 Florida Avenue NE, Washington, DC 20002-3695

46. **APPROXIMATE BAYESIAN COMPUTATION FOR CAUSAL INFERENCE VIA MENDELIAN RANDOMIZATION AND APPLICATION TO HEART DISEASE**
David Nicholson[1] and Benjamin F. Voight[2]
[1] Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2] Department of Pharmacology and Department of Genetics,
University of Pennsylvania – Perelman School of Medicine, 415 Curie Boulevard, Philadelphia, PA 19104

47. **DOMINANCE OF OLD END GROWTH IS INHERITED IN FISSION YEAST**
Julie Rich, Bin Wei, and Maitreyi Das
Department of Biochemistry and Cellular and Molecular Biology,
University of Tennessee, Knoxville, TN 37996

48. **JUNCTIONAL ADHESION MOLECULE A (JAM-A): EXPRESSION IN THE MURINE EPIDIDYMAL TRACT AND ACCESSORY ORGANS AND ACQUISITION BY MATURING SPERM**
Kathie Wu and Patricia A. Martin-DeLeon
Department of Biological Sciences, University of Delaware, 401 Academy Street, Newark DE, 19716

IDENTIFICATION AND CHARACTERIZATION OF AQUIFER BACTERIA

Cooper Anderson, Mohammed Reza, Louay Zumot, Thai Tran and Karen York
Biology Program, The Richard Stockton College of New Jersey,
101 Vera King Farris Drive, Galloway, NJ 08205

In 1994, Richard Stockton College of New Jersey installed a large geothermal heat exchange system to heat and cool the main academic buildings. Thermal energy is exchanged underground in a four acre well field located under a parking lot. During the first ten years of operation, more thermal energy was deposited underground than was extracted, which resulted in an increased temperature from 14 ^{o}C to 24 ^{o}C in the Upper Cohansey aquifer that traverses the well field. Six bacterial isolates (CU 2, 7, 8, 10, 18, 24) were collected from the Upper Cohansey aquifer in 1997 when the temperature of the aquifer was 14^{o}C. One isolate (73) was collected in 2005 when the Upper Cohansey aquifer temperature was 24^{o}C. All seven isolates of aquifer bacteria were initially identified in previous work as being related to the genus "*Acidovorax*" by cloning and DNA sequencing of the 16S ribosomal RNA gene.

The purpose of this project was to further characterize these seven bacteria isolates. The approach has been to amplify the *lepA* gene by polymerase chain reaction. *LepA* also known as translation elongation factor Tu (EF-Tu), is a highly conserved single copy protein encoding gene in bacteria. DNA sequences for *lepA* from the aquifer bacteria were compared to entries in the NCBI database and a phylogenetic analysis was performed. The bacteria were also characterized by their nutritional requirements on BiOLOG plates. Based on phylogenetic analysis of *lepA* DNA sequences and nutritional data each of the isolates are distinct from each other and not identical to any other known bacteria.

HISTONE MODIFICATION AS A POTENTIAL REGULATOR OF THE TISSUE-SPECIFIC IMPRINTING OF *RASGRF1*

Carolyne Face and Dr. Tamara Davis
Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010

Genomic imprinting is a relatively rare phenomenon that occurs when one copy of a gene is expressed while the other copy remains silent in a parent-of-origin-specific manner. This process is often controlled by epigenetic factors, such as DNA methylation and histone modification, which alter the structure of DNA and influence gene expression. Understanding the regulation of imprinted genes is critical because without the proper control of genomic imprinting, severe developmental disorders can occur.

A small subset of imprinted genes display tissue-specific imprinting, which occurs when gene expression is monoallelic in some tissues yet biallelic in others. To understand this phenomenon, the Davis lab is investigating one particular tissue-specific imprinted gene, *Rasgrf1*, which is paternally methylated in all tissues, suggesting that DNA methylation cannot be the only factor responsible for regulating gene expression. I am investigating histone modification as a potential regulator of *Rasgrf1* by studying the allelic distribution of modified histones in tissues showing monoallelic expression (liver and brain). At the promoter, we expect permissive modifications to be present on the expressed paternal allele and repressive modifications on the silent maternal allele. However, while DNA methylation is often associated with repressive modifications, it remains unclear whether this is true at the imprinting control region of *Rasgrf1* in both monoallelic and biallelic tissues.

We have used chromatin immunoprecipitation (ChIP) to isolate chromatin containing specific modified histones. Using the immunoprecipitated chromatin fractions, we have conducted allele-specific qPCR to determine the relative levels of maternal and paternal DNA containing modifications at the promoter and the imprinting control region. From these data, we will be able to determine whether histone modifications at various regions of *Rasgrf1* correlate with expression levels in a tissue and allele specific manner, providing insight into the role of histone modification in the tissue-specific imprinting of *Rasgrf1*.

Support for this research was provided by an award to TLD from NSF Grant # 1157819. Student summer research was also supported by the Bryn Mawr College Summer Science Research program.

Poster 44 - Cancelled

PRACTICAL AND INEXPENSIVE DNA FINGERPRINTING FOR UNDERGRADUATE SCIENCE MAJORS AND HIGH SCHOOL STUDENTS

Christine McBride[1], Jennifer Chin[2,3], and Derek C. Braun[3]
[1]University of Alabama at Birmingham, 1720 2nd Avenue, South Birmingham, AL 35294
[2]Hogeschool Leiden, Zernikedreef 11, 2333 CK Leiden, the Netherlands
[3]Molecular Genetics Laboratory, Gallaudet University,
800 Florida Avenue NE, Washington, DC 20002-3695

We aimed to develop effective and inexpensive DNA fingerprinting as a hands-on learning method for undergraduate biology majors and high school students. Current DNA fingerprinting kits for educational use, such as the Forensic DNA Fingerprinting Kit (Bio-Rad, Hercules, CA) and Whose DNA Was Left Behind? (Edvotek, Washington, DC) do not represent the art and instead use synthetic DNA or dyes to simulate DNA fingerprinting. We believe in introducing these students to modern genetic profiling. The inexpensive protocol we propose here uses a commercially available DNA purification kit followed by PCR with CODIS primer sets, and nondenaturing agarose gel electrophoresis. The price of several commercially available kits were compared. Qiagen's Gentra Puregene Buccal Cell Kit was found to be much cheaper than other kits on the market such as the ones offered by EpiCenter and Biosystems. The cost and assembly of this exercise is cost-efficient and safe for all students. The method described is easy to perform and fast to complete. Our protocol allows for hands-on learning experience for science students with a modern application of genetic analysis. Finally, this approach appeals to the students' interests by allowing them to use their own DNA.

I would like to thank the NASA/D.C. Space Grant Consortium and Beverley Taylor Sorenson Student Fellowship for funding this project. I would like to thank my mentors and other members of the lab for their help and support.

APPROXIMATE BAYESIAN COMPUTATION FOR CAUSAL INFERENCE VIA MENDELIAN RANDOMIZATION AND APPLICATION TO HEART DISEASE

David Nicholson[1] and Benjamin F. Voight[2]
[1] Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2] Department of Pharmacology and Department of Genetics,
University of Pennsylvania – Perelman School of Medicine,
415 Curie Boulevard, Philadelphia, PA 19104

Coronary heart disease (CHD) is a leading cause of death worldwide; thus, identifying risk factors that cause CHD is critical to improve patient care and develop new therapies. Epidemiological studies have demonstrated correlation between numerous biomarkers and CHD. However, these studies do not establish a *causal* connection, owing the issues of reverse causation, where the disease impacts the observed biomarker, or uncontrolled confounding from additional factors implicated in this disease. The standard approach to test a hypothesis of causality is the randomized clinical trial, though such trials are expensive and lengthy to perform. Recently, a statistical approach that mimics the analytical design of a clinical trial, dubbed *Mendelian Randomization* (MR), provides a computational approach for causal inference. Because alleles that an individual receive at birth assort randomly at meiosis, and by selecting genetic factors as 'instruments' that strongly associate with a biomarker of interest, one can perform causal inference while avoiding issues of confounding and reverse causality. Using this analytical framework, we aim to assess the causal role of triglycerides (TG) with respect to CHD. Because genetic variants used in the analysis affect both TG and other biomarkers (i.e. cholesterol levels), established approaches to calculating the likelihood of CHD given TG are analytically impractical. Thus, we utilize Approximate Bayesian Computation (ABC) to circumvent this issue by estimating the likelihood through the process of statistical rejection sampling. Through synergizing both approaches, we will evaluate the hypothesis that TG levels are a causal risk factor for CHD.

This research was funded by the MARC U*STAR Program through NIH Grant: t3408663.

DOMINANCE OF OLD END GROWTH IS INHERITED IN FISSION YEAST

Julie Rich, Bin Wei, and Maitreyi Das
Department of Biochemistry and Cellular and Molecular Biology,
University of Tennessee, Knoxville, TN 37996

Schizosaccharomyces pombe, commonly known as fission yeast, is an excellent organism for the study of eukaryotic cell growth and division due to its unicellularity and the ease with which it can be imaged. A fission yeast cell grows in a bipolar fashion until mitosis, after which the cell divides into two daughter cells. The end of a cell derived from a division site is its new end, and the end that persists from the previous generation is its old end. Immediately after cell division, the old end, which is dominant, resumes growth. The new end grows only after the cell attains a certain size by a phenomenon called NETO (New End Take-Off). Cdc42, a small GTPase, is the primary regulator of cell growth and division in eukaryotes. Our recent studies show that the old end and the new end compete with each other for active Cdc42; the old end, which is the dominant end, wins initially. What endows the old end with dominance in this competition is unknown.

Cdc42 is activated by the protein Gef1. We observe that *gef1* deletion mutants exhibit unique growth patterns. Our data suggests that the old end is dominant only if it grew in the previous generation. This indicates that there exists a memory of growth that determines dominance and is inherited from one generation to the next. Moreover, the degree of dominance is also inherited through the generations. Current studies in our lab are focused on understanding the molecular details and regulation of growth dominance at the cell ends.

JUNCTIONAL ADHESION MOLECULE A (JAM-A): EXPRESSION IN THE MURINE EPIDIDYMAL TRACT AND ACCESSORY ORGANS AND ACQUISITION BY MATURING SPERM

Kathie Wu and Patricia A. Martin-DeLeon
Department of Biological Sciences, University of Delaware,
401 Academy Street, Newark DE, 19716

JAM-A, Junction Adhesional Molecule A, has been shown to be a membrane protein that is essential for sperm motility by the DeLeon laboratory. It has also been reported to be involved in the regulation of Ca2+ homeostasis in murine sperm since it shares a common interacting partner with the major Ca2+ efflux molecule in sperm, PMCA4 (Plasma Membrane Ca2+ ATPase 4). Recently, PMCA4 was shown to be expressed in both the bovine and the murine epididymides and to be secreted in the latter, where it can be acquired by sperm. Based on the relationship between JAM-A and PMCA4 we hypothesized that JAM-A may also be expressed in the murine epididymis. The objective of this study was to determine if JAM-A is expressed and secreted in the epididymal tissues and the luminal fluid, as well as the accessory organs. We also sought to determine if it can be taken up by sperm from the luminal fluid in vitro. Our results show differential expression of JAM-A in the three regions of the epididymis, being highest in the caput and lowest in the cauda. Consistent with this, caput sperm showed a 6-fold increase in the level of JAM-A expression, compared to caudal ones. JAM-A was also found to be present in the accessory organs: prostate and seminal vesicle, and was shown to be secreted in the epididymal luminal fluid (ELF). When ELF was co-incubated with sperm in *in vitro* uptake assays, caput/corpus sperm showed a 12-fold increase in uptake levels of JAM-A compared to the PBS control, while there was only a 7-fold increase for caudal sperm. Upon spinning down the ELF, we were able to obtain membrane vesicles known was epididymosomes, which we characterized using CD-9, and have found the presence of JAM-A on these exosomes. Our results suggest that JAM-A expression is involved in epididymal sperm maturation.

Morning Poster Session
Group H – Chemical Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

49. **STUDYING REACTIVITY, SUBSTITUENT, AND LEAVING GROUP EFFECTS IN ESTERS**
Ariel Bilbrough, Dionne Williams, and Dr. Malcolm J. D'Souza
Department of Biological Chemistry, Wesley College, 120 N. State Street, Dover, DE 19901

50. **MULTISTEP ORGANIC SYNTHESIS: BROMINATION OF AROMATIC ESTERS**
Sarah Bonson, Ann Marie Martin, and Patricia Kreke
Department of Science, Mount St. Mary's University,
16300 Old Emmitsburg Road, Emmitsburg, MD 21727

51. **SYNTHESIS AND CHARACTERIZATION OF A NOVEL TRIPODAL NITROXIDE TITANIUM(IV) FLUORIDE COMPLEX AND ITS SELECTIVE FLUORINATION CHEMISTRY**
Michael A. Boreen, Justin A. Bogart, Patrick J. Carroll, and Eric J. Schelter
Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, PA 19104

52. **COMPLEXATION STUDIES OF BIPYRIDINE AZA-CROWN MACROCYCLES**
Benjamin Carpenter and Marc Harris
Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

53. **AN EVALUATION OF THE CHEMISTRY OF $RuCl_2(PPh_3)_3$ WITH NITRILES**
Santina S. Cruz[1], John W. Gilje[1], Donna S. Amenta[1], and Glenn P.A. Yap[2]
[1]Department of Chemistry and Biochemistry, James Madison University,
800 S. Main Street, Harrisonburg, VA 22807
[2]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716

54. **MICROWAVE ASSISTED ORGANIC SYNTHESIS OF HEPTAMETHINE CYANINE DYES**
Jahnn Drigo, and Angela Winstead, PhD
Department of Chemistry, Morgan State University,
1700 E. Cold Spring Lane, Baltimore, MD 21251

55. **INVESTIGATING THE BINDING AFFINITY OF NOD2 AND SOLUBLE BACTERIAL CELL WALL DIMERS**
Lauren Genova, James Melnyk, Vishnu Mohanan, and Catherine Leimkuhler Grimes
Department of Chemistry and Biochemistry, University of Delaware,
162 Academy Street, Newark, DE 19717

STUDYING REACTIVITY, SUBSTITUENT, AND LEAVING GROUP EFFECTS IN ESTERS

Ariel Bilbrough, Dionne Williams, and Dr. Malcolm J. D'Souza
Department of Biological Chemistry, Wesley College, 120 N. State Street, Dover, DE 19901

The 4-methoxyphenoxy (or *p*-methoxyphenoxy) carbonyl group is incorporated in a number of EPA authorized herbicidal and pesticide formulations. This group has additionally gained greater importance with the recent synthesis of functionalized carbazole alkaloids that have important photo physical and biological properties. This project evaluates the solvent nucleophilic substitution chemistry observed with the 4-methoxyphenoxy group in synthetically useful organic and aqueous mixtures.

Equimolar amounts of analytical grade 4-methoxyphenyl chloroformate and silver *p*-toluenesulfonate were allowed to react at room temperature. The silver chloride quickly precipitated out and the resultant (filtrate) 4-methoxyphenyl *p*-toleunesulfonate solution was used as a substrate. In this project, the solvolysis of 4-methoxyphenyl *p*-toluenesulfonate was followed in numerous binary solvents at 25.0 ^{o}C using the acid-base titration method. These acid-base reactions were followed using lacmoid in acetone as the indicator solution and sodium methylate as the base.

The results indicate that the rates between 4-methoxyphenyl chlorformate and 4-methoxyphenyl *p*-toluenesulfonate are very comparable to one another. This trend occurs in all of the tested solvents. As well as similar rates, water concentration follows an important trend that is presented in the table. The higher the concentration of water present in the solvent, the faster the reaction will occur.

Based on the solvolysis and rates of reactions of 4-methoxyphenyl chloroformate and 4-methoxyphenyl *p*-toluenesulfonate in various binary solvents, the leaving group has no effect on the rate of reaction. Future work on the highly ionizing 2,2,2-Trifluoroethanol and 1,1,1,3,3,3-Hexafluoro-2-propanol will be conducted during the fall semester. If there is a leaving group effect, the effect would be prominent in these strongly hydrogen bonding solvents.

Financial support was from the National Science Foundation (NSF) EPSCoR Grant No. IIA-1301765 (DE-EPSCoR RII-3 program).

MULTISTEP ORGANIC SYNTHESIS: BROMINATION OF AROMATIC ESTERS

Sarah Bonson, Ann Marie Martin, and Patricia Kreke
Department of Science, Mount St. Mary's University,
16300 Old Emmitsburg Road, Emmitsburg, MD 21727

This experiment explored the directive effects in the bromination of proplbenzoate and other related compounds through a series of trials. The differences in the directive effects of the EAS substitution reaction of bromine with the benzene ring of propyl benzoate and other related aromatic esters, such as ethyl benzoate, were studied. The effects of the variables in the reactions were evaluated in terms of yield as well as purity and directive effects as determined through infrared spectroscopy. Using different esters resulted in discrepancies in directive effects as well as yields. Starting with propylbenzoate, a mix of meta and ortho/para substitutions was obtained, and the effects of using ethyl benzoate as the starting reagent are being investigated further. A new EAS reaction sequence may be necessary in order to successfully direct the bromination of propylbenzoate.

SYNTHESIS AND CHARACTERIZATION OF A NOVEL TRIPODAL NITROXIDE TITANIUM(IV) FLUORIDE COMPLEX AND ITS SELECTIVE FLUORINATION CHEMISTRY

Michael A. Boreen, Justin A. Bogart, Patrick J. Carroll, and Eric J. Schelter
Department of Chemistry, University of Pennsylvania,
231 South 34th Street, Philadelphia, PA 19104

Online access of this abstract is restricted at the request of the Principal Investigator.

COMPLEXATION STUDIES OF BIPYRIDINE AZA-CROWN MACROCYCLES

Benjamin Carpenter and Marc Harris
Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

Alkali metal ion complexation by classic crown ether and azacrown host molecules has been well documented in the literature. These systems display excellent selectivity for various ions based on steric requirements and hard-soft interactions. Recently, functionalized versions of these macrocycles have been reported that either display enhanced ion-binding, improved selectivity, or an external response to report successful binding. To this end, a series of bipyridine containing azacrown-ether macrocycles were synthesized by reacting a dibromo-substitued bipyridine unit with a pre-organized N-nosyl or N-tosyl protected polyamine. These azacrown-bipyridine analogues are expected to display similar ion selectivity and binding strength based on the size and number of donor atoms created by the bipyridine-amine pockets. In addition, by metallating the outwardly directed bipyridine units with photoactive transition metal centers these complexes may function as chemosensory devices that visibly display the encapsulation of small cationic molecules and ions. This paper reports the complete synthesis and characterization of several tosyl-and nosyl-protected bipyridine-polyamine macrocycles.

This project was supported by the Neidig Research Fund.

AN EVALUATION OF THE CHEMISTRY OF $RuCl_2(PPh_3)_3$ WITH NITRILES

Santina S. Cruz[1], John W. Gilje[1], Donna S. Amenta[1], and Glenn P.A. Yap[2]
[1]Department of Chemistry and Biochemistry, James Madison University,
800 S. Main Street, Harrisonburg, VA 22807
[2]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716

Due in part to our interest in the metal catalyzed hydrolysis of nitriles, we are investigating the coordination chemistry of nitriles with ruthenium. A number of years ago Wilkinson [1] characterized by IR *cis*- and *trans*-$RuCl_2(PPh_3)_2(NCR)_2$ from the reaction of $RuCl_2(PPh_3)_3$ with excess RCN. We have restudied this reaction with benzonitrile in several solvents and have characterized the complex *trans*- $RuCl_2(PPh_3)_2(NCPh)_2$ by IR and NMR spectroscopy, elemental analysis, and x-ray crystallography. However, we have been unable to detect an isomer containing *cis*- nitrile ligands. This contrasts to acetonitrile, where the *cis* rather than the *trans* complex is obtained [2]. With excess $RuCl_2(PPh_3)_3$ quite a different product forms with benzonitrile. In this case the diruthenium, triply chloro-bridged $(Ph_3P)_2(Cl)Ru(\mu\text{-}Cl)_3Ru(PPh_3)_2(NCPh)$ forms in good yield and has been spectroscopically and structurally characterized. In solution, the ^{31}P NMR spectrum consists of two sets of doublets. This is consistent with the solid state structure and indicated that the molecule is not fluxional and does not undergo isomerization or ligand exchange in solution. A similar reaction with actetonitrile appears to produce an analogous diruthenium product as indicated by ^{31}P NMR spectroscopy.

This material is based upon work supported by the National Science Foundation under CHE-1062629 and the Research Corporation Department Development Grant 7957.

1. Gilbert, J.D.; Wilkinson, G. *J. Chem. Soc.(A), 1969*, 1749-1753.
2. Al-Far, *Acta Cryst.* **E64**, *2008*, m184.

MICROWAVE ASSISTED ORGANIC SYNTHESIS OF HEPTAMETHINE CYANINE DYES

Jahnn Drigo, and Angela Winstead, PhD
Department of Chemistry, Morgan State University,
1700 E. Cold Spring Lane, Baltimore, MD 21251

The need for cyanine dyes that can be used for fluorescent labeling of biological molecules is quite significant in the detection of pathogens. However, the pentamethine cyanine (Cy5) dyes currently being used for this purpose are susceptible to interference from many other biological molecules fluorescing in the same region (650nm-700nm). Heptamethine cyanine (Cy7) dyes, conversely, emit fluorescence in the region of 780nm-1000nm, thus exhibiting less interference. The focus of this research is to employ Microwave-Assisted Organic Synthesis (MAOS) in the production of several Cy7 benzoindoleninedyes. MAOS is a faster, more efficient, and more environmentally innocuous synthetic method, giving higher yields and shorter reaction times than the conventional method using reflux heating. Benzo ethyl and propyl salts were synthesized and characterized with yields of 89% and 86%, respectively, by combining 2, 2, 3-trimethylbenzoindolenine with an alkyl halide. These salts were then used to synthesize the dyes: condensation reactions between bisimine and the ethyl- and propyl-benzoindolenine salt precursors were performed by combining the salt and bisimine with ethanol and sodium acetate in a Biotage Microwave Initiator 2.0. Time and temperature studies were carried out to maximize the yields of the dyes: the 77% yield of benzo-ethyl dye obtained is an improvement upon current literature reporting 44% yields and the benzo-propyl dye was synthesized with 85% yield in only ten (10) minutes, an enormous reduction when contrasted with 18 hours observed in reports utilizing the traditional method. In the future, salts with other R-groups will be used to synthesize additional benzo dye derivatives.

The presenter wishes to acknowledge Dr. Angela Winstead, Dr. Richard Williams, Grace Nyambura, Tijesunimi Odebode, and the Department of Defense W911NF-11-1-0157.

INVESTIGATING THE BINDING AFFINITY OF NOD2 AND SOLUBLE BACTERIAL CELL WALL DIMERS

Lauren Genova, James Melnyk, Vishnu Mohanan, and Catherine Leimkuhler Grimes
Department of Chemistry and Biochemistry, University of Delaware,
162 Academy Street, Newark, DE 19717

The innate immune system is the body's first line of defense against pathogens. The innate immune system is triggered by pathogen associated molecular patterns (PAMPs) that are recognized by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and Nod-like receptors (NLRs). This research project focuses on providing a better understanding of how the innate immune system senses and responds to the presence of bacteria. Specifically, our group is interested in the relationship between the nucleotide-binding oligomerization domain-containing protein 2 (Nod2), an NLR protein found in the cytosol of mammalian host cells, and muramyl dipeptide (MDP), the smallest bacterial cell wall fragment known to elicit an immunological response. When Nod2 is mutated, the signaling pathway becomes disrupted and uncontrollable inflammation arises, leading to chronic inflammatory bowel disorders such as Crohn's disease. To discover how to better treat these diseases, it is imperative to learn more about how Nod2 and MDP interact, a mechanism which is currently unknown. The Grimes Lab has previously shown that Nod2 binds to MDP *in vitro*; however, research suggests that a heightened immunological response may be elicited in a host if molecules containing multiple MDP's are used, suggesting multivalency is at play. To test this hypothesis, a variety of novel MDP dimers were synthesized to be assessed via cell-based assays and *in vitro* SPR binding assays.

Gratitude is extended to the Howard Hughes Medical Institute (HHMI) for funding, as well as to the University of Delaware Undergraduate Research Summer Scholars Program.

Morning Poster Session
Group I – Chemical Sciences

Poster #	Title, Author(s) & Affiliation(s)
56.	*CANCELLED*
57.	**CONFIRMING THE USE OF PHENYL CHLOROFORMATE AS AN APPROPRIATE ADDITION-ELIMINATION STANDARD IN LFER ANALYSES** Jasbir K. Deol, and Dr. Malcolm J. D'Souza Department of Chemistry, Wesley College, 120 N. State Street, Dover, DE 19901
58.	**SYNTHESIS OF ETHYL BENZOATE WITH A REDUCED REFLUX TIME** Paola Kleimann and Patricia Kreke Department of Science, Mount St. Mary's University, 16300 Old Emmitsburg Road, Emmitsburg, MD 21727
59.	**APPLICATION OF ANIONIC UREAS AS ACTIVATORS FOR SmI_2 IN RADICAL INTERCEPTION CYCLIZATION REACTIONS** Joseph Mauck, Christopher McAtee, Erin Hale, and Dr. Chriss McDonald Department of Chemistry, Lycoming College, 700 College Place, Williamsport, PA 17701
60.	**SYNTHESIS AND SUPRAMOLECULAR STRUCTURES OF MANGENESE COMPLEXES WITH *N*-PYRAXOLYLPROPANAMIDE-DERIVED LIGANDS** Michael A. McDougal[1], Jennifer Mitchell[1], David J. D'Amico[1], Donna S. Amenta[1], John W. Gilje[1], Cristian G. Hrib[2], and Frank T. Edelmann[2] [1]Department of Chemistry and Biochemistry, MSC 4501, James Madison University, Harrisonburg, VA 22807 [2]Chemisches Institut der Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany
61.	**SYNTHESIS OF PRELIMINARY HETEROCYCLIC SALTS FOR CYANINE DYES** Dominique Munson and Dr. Angela Winstead Department of Chemistry, Morgan State University, 1700 E. Cold Spring Lane, Baltimore, MD 20774
62.	**SYNTHESIS OF HALOGENATED THIENO[3,2-*d*] PYRIMIDINES AS POTENTIAL ANTICANCER AGENTS** Matthew Shin, Brian Cawrse, and Katherine Seley-Radtke Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Poster 56 – Cancelled

CONFIRMING THE USE OF PHENYL CHLOROFORMATE AS AN APPROPRIATE ADDITION-ELIMINATION STANDARD IN LFER ANALYSES

Jasbir K. Deol, and Dr. Malcolm J. D'Souza
Department of Chemistry, Wesley College, 120 N. State Street, Dover, DE 19901

Chloroformate esters are used in peptide and related synthesis of prodrugs. Two decades ago, phenyl chloroformate (PhOCOCl) was extensively studied in a variety of solvent mixtures and an addition-elimination (A-E) mechanism with a rate-determining addition-step was proposed. Since then, it has served as a reference compound in linear free energy relationship studies (LFERs) of numerous acyl halides. In this project, Phenyloxy carbonyl tosylate (PhOCOOTs) was synthesized and its specific rates were determined in the same set of solvents as PhOCOCl at 25.0 ^{o}C. The principal goal of the project is to determine the presence or absence of any correlation trends between the rates of reaction (energy barriers) observed and the differences in the chloride and tosyl anionic leaving group abilities. PhOCOOTs was synthesized from additions of silver tosylate to phenyl chloroformate. A rapid precipitation of silver chloride occurred and the resulting filtered solution was used directly as the source of the substrate. The specific rates of reactions were determined by acid-base titrations. These titrations employed lacmoid in acetone as the indicator solution, and sodium methoxide as the base. The specific rates of PhOCOOTs in a wide range of solvents were found to be very similar to those observed for PhOCOCl (in the same set of solvents at 25.0 ^{o}C). A tosyl leaving group is a sulfonic acid derivative which is a better leaving group than the chloride anion as it considerably stabilizes the negative charge by resonance stabilization. If the leaving group played an important role in the rate-determining step, the rates of reaction of PhOCOOTs should have been much faster than those for PhOCOCl. We therefore conclude that the mechanism of reaction of PhOCOOTs is exactly the same as PhOCOCl, and hence, it is very appropriate to continue to use PhOCOCl as a standard for A-E reactions.

This research is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 8 P20GM103446-13 (NIH-NIGMS-DE-INBRE Program).

SYNTHESIS OF ETHYL BENZOATE WITH A REDUCED REFLUX TIME

Paola Kleimann and Patricia Kreke
Department of Science, Mount St. Mary's University,
16300 Old Emmitsburg Road, Emmitsburg, MD 21727

The synthesis of ethyl benzoate is a procedure that all organic chemistry students at Mount Saint Mary's University complete. The reflux step takes 4 hours to complete so shortening this step would make the experiment less time consuming for the students and professors.

This project is an effort to shorten the pre-existing procedure in the synthesis of ethyl benzoate. Alterations were made to the reflux step in order to shorten the time required. A stirring bar was used to test if stirring made the process more efficient and then the time was halved to observe changes in the purity and yield of the ethyl benzoate. Stirring did not have a significant effect on the yield and purity of the ethyl benzoate. When only half of the time was used in the reflux, the product was still as pure as when the full time was used and the yield was also similar. These results indicate that using half of the reflux time would be acceptable as it yields similar results.

APPLICATION OF ANIONIC UREAS AS ACTIVATORS FOR SmI_2 IN RADICAL INTERCEPTION CYCLIZATION REACTIONS

Joseph Mauck, Christopher McAtee, Erin Hale, and Dr. Chriss McDonald
Department of Chemistry, Lycoming College, 700 College Place, Williamsport, PA 17701

Online access of this abstract is restricted at the request of the Principal Investigator.

SYNTHESIS AND SUPRAMOLECULAR STRUCTURES OF MANGENESE COMPLEXES WITH *N*-PYRAXOLYLPROPANAMIDE-DERIVED LIGANDS

Michael A. McDougal[1], Jennifer Mitchell[1], David J. D'Amico[1], Donna S. Amenta[1], John W. Gilje[1], Cristian G. Hrib[2], and Frank T. Edelmann[2]
[1]Department of Chemistry and Biochemistry, MSC 4501, James Madison University, Harrisonburg, VA 22807
[2]Chemisches Institut der Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany

The syntheses and single crystal X-ray structures of the multifunctional acrylamide-derived ligands *N*-pyrazolyl-2-methylpropanamide (**1**, = *N*-ppa^{Me}) and a mixture of 3- and 5-methylpyrazolylpropanamide (**2a** and **2b,** = *N*-^{3Me}ppa/*N*-^{5Me}ppa), and their Mn^{2+} complexes are reported. Compound **1** was prepared by the reaction of 2-methylacrylamide with pyrazole. A mixture of **2a** and **2b** was obtained from the reaction of acrylamide with 3-methylpyrazole. In the latter reaction, the first step involves the deprotonation of 3-methylpyrazole, followed by tautomerization that ultimately results in the formation of both the 3- and 5-methyl isomers of 2. The general synthesis of the Mn^{2+} complexes involves treatment of $MnCl_2{\cdot}4H_2O$ with the appropriate ligand in ethanolic solution in the presence of triethylorthoformate as dehydrating agent. This way Mn_2Cl_4(*N*-ppa^{Me})$_2$(EtOH)$_2$ (**3**), and [Mn(*N*-^{3Me}ppa)$_2$(*N*-^{5Me}ppa)][$MnCl_4$] (**4)**. In **3** the *N*-ppa^{Me} ligand acts as *N,O*-chelating ligand whose oxygens bridge the two Mn^{2+} ions. The ligands are also chelating in **4**. The molecular and crystal structures of **1, 2a**, **4**, and **5** have been determined by X-ray diffraction. The extensive hydrogen bonding that occurs in these compounds is discussed.

The material is based on work supported by the National Science Foundation under CHE-1062629 and the Research Corporation Departmental Science Development Award 7957. The crystallographic data was obtained at the Otto-von-Guericke-Universität Magdeburg which financially supported this aspect of the project.

SYNTHESIS OF PRELIMINARY HETEROCYCLIC SALTS FOR CYANINE DYES

Dominique Munson and Dr. Angela Winstead
Department of Chemistry, Morgan State University,
1700 E. Cold Spring Lane, Baltimore, MD 20774

Heptamethine (Cy7) cyanine dyes are of interest to scientists because of their significant ability to luminescence in the near-infrared region (650-900nm). In the past, scientists have used pentamethine (Cy5) dyes as fluorescent probes for pathogen detection. Even though the Cy5 dyes have the ability to luminesce up to 650nm, some biomolecules are able to fluoresce in this region, whereas Cy7 dyes are not blocked by background interference from tissue. This study focuses on sulfoindocyanine dyes which are highly water soluble compounds that are ideal for biological life forms.

The specific aims of this approach are the synthesis of the potassium sulfoindole precursor and four sulfoindole heterocyclic salts. The precursor was synthesized via conventional organic techniques, yielding 68%, while the ethyl salt was prepared via microwave heating with an overall yield of 80%. All structures were determined by ^{1}H NMR.

I will like to acknowledge my mentor, Dr. Angela Winstead, MBRS RISE, and Department of Defense.

SYNTHESIS OF HALOGENATED THIENO[3,2-*d*] PYRIMIDINES AS POTENTIAL ANTICANCER AGENTS

Matthew Shin, Brian Cawrse, and Katherine Seley-Radtke
Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

As rates for cancer cases have steadily increased for the last 10 years, there is an ever-increasing need for newer and more effective therapeutics. Existing studies have shown that by introducing small molecules into cancer cells, competition occurs between the natural bases and modified base analogues for DNA-synthesizing enzymes. Halogens are of particular interest due to their intrinsic levels of high electronegativity, thereby making them susceptible to alkylation by nucleophiles. As a result, halogenated thieno[3,2-*d*]pyrimidine analogues offer a new and exciting approach to drug design and may successfully address an important unmet medical need for cancer patients.

The goal of this project is to synthesize and test modified 2,4-dihalogenated thieno[3,2-*d*]pyrimidine analogues as a potential anticancer agents. We will test the hypothesis that these analogues will be more efficacious than existing analogues, which already show greater efficacy of 6-10 times the current standard treatment. Additionally, we will be exploring the differential activity of substituents at the C2 and C4 positions by creating two separate compound lines - one that is homohalogenated at C2 or C4 and the other which is hetero- or mono- halogenated. This will enable us to determine whether either of these two positions has a variable impact on activity.

We believe that these analogues will produce a compound that that exhibits stronger, more favorable bonds in active binding sites than previously reported analogues due to the halogen's greater electronegativity. This class of analogues addresses an important unmet medical need for patients, specifically those with leukemia or melanoma. The implications of the findings from the synthesis of these analogues prove to be a vital step in the process of developing more effective treatments. If patients can take more efficacious dosages of potentially cytotoxic drugs to achieve the same results, then the quality of life for cancer patients will be improved through a safer and more tolerable treatment regimen.

The author would like to extend thanks to Dr. Katherine Seley-Radtke, and to the entire Seley-Radtke group. This research is supported from a UMBC Undergraduate Research Award.

Morning Poster Session
Group J – Chemical Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

63. **SELECTIVE SYNTHESIS AND DERIVATION OF RIBOSE VIA THE FORMOSE REACTION**

P. Steven Donmoyer and Dr. Anderson L. Marsh
Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

64. **THEORETICAL STUDIES OF THE EFFECT OF ISOSTERIC SUBSTITUTION ON THE GARRATT-BRAVERMAN CYCLIZATION**

Jade Marino Creto, Mikey Kwon and Dr. Edyta Greer
Department of Natural Sciences, Baruch College, 17 Lexington Avenue, New York, NY 10010

65. **THE INFLUENCE OF ARONIA CULTIVATION MANAGEMENT ON THE ANTIOXIDANT CAPACITY OF ARONIA**

Tina Ndam[1], Motunrayo Fadipe[1], Blessing Aroh[1], Andrew Ristvey[2], and Victoria Volkis[1]
[1]Department of Natural Sciences, University of Maryland Eastern Shore,
Princess Anne, MD 21853
[2]University of Maryland Extension, Wye Research & Education Center,
P.O. Box 169, Queenstown, MD 21658-0169

66. **TOWARD WATER-SOLUBLE, CONJUGATABLE BODIPY DERIVATIVES**

Anthony Palilla, Adam Meares, and Marcin Ptaszek
Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

67. **COPPER CATALYZED ENANTIOSELECTIVE ALKYNYLATION OF ISOQUINOLINE DERIVATIVES**

Clarissa A. Shoffler[1], Srimoyee Dasgupta[2], and Mary P. Watson[2]
[1]Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003
[2]Department of Chemistry and Biochemistry, University of Delaware,
102 Brown Laboratory, Newark, DE 19716

68. **HYDROBORATION OF ALKENES WITH AZAFERROCENE-BORENIUM IONS**

Dave Szymanik, Sarah Krause, and Dr. Timothy Brunker
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

69. **SYNTHESIS AND STUDY OF NOVEL AMPHIPHILES AS POTENT ANTISEPTICS**

Brenna J. C. Walsh[1], Kirstie Thompson[1], John N. Marafino[1,2], Kristin McKenna[1], Louis Damiano[1],
Tara M. Gallagher[2], Kyle Seifert[2], and Kevin L. Caran[1]
[1]Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807
[2]Department of Biology, 820 Madison Drive, MSC 7801, Harrisonburg, VA 22807

SELECTIVE SYNTHESIS AND DERIVATION OF RIBOSE VIA THE FORMOSE REACTION

P. Steven Donmoyer and Dr. Anderson L. Marsh
Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

Since the beginning of time, humankind has sought the answer to the question of why we are here. There have been many postulates pertaining to the answer; one such school of thought is that the answer is scientifically based. The purpose of this experiment is to explore one of these scientific theories and improve upon it. The main goal of this experiment is to selectively synthesize ribose using the Butlerov (formose) reaction. This reaction will be accomplished by refluxing 1.66M formaldehyde, 5 mg complex sugar initiator, and a number of different catalytic bases: calcium hydroxide, thiazolium salts, and some that are yet to be determined but share similar qualities to the others. The product will be analyzed and characterized via standard analytical techniques, including, but not limited, to UV/Vis Absorption Spectrometry and LC-UV. Expected results will vary based on which base is added to the formaldehyde; with CaO, the results outlined in a Zweckmair paper (<1% ribose) will be expected. However, a salt that produces greater than 50% ribose is desired; 25-30% is the next highest desired fraction of ribose beneath 50%.

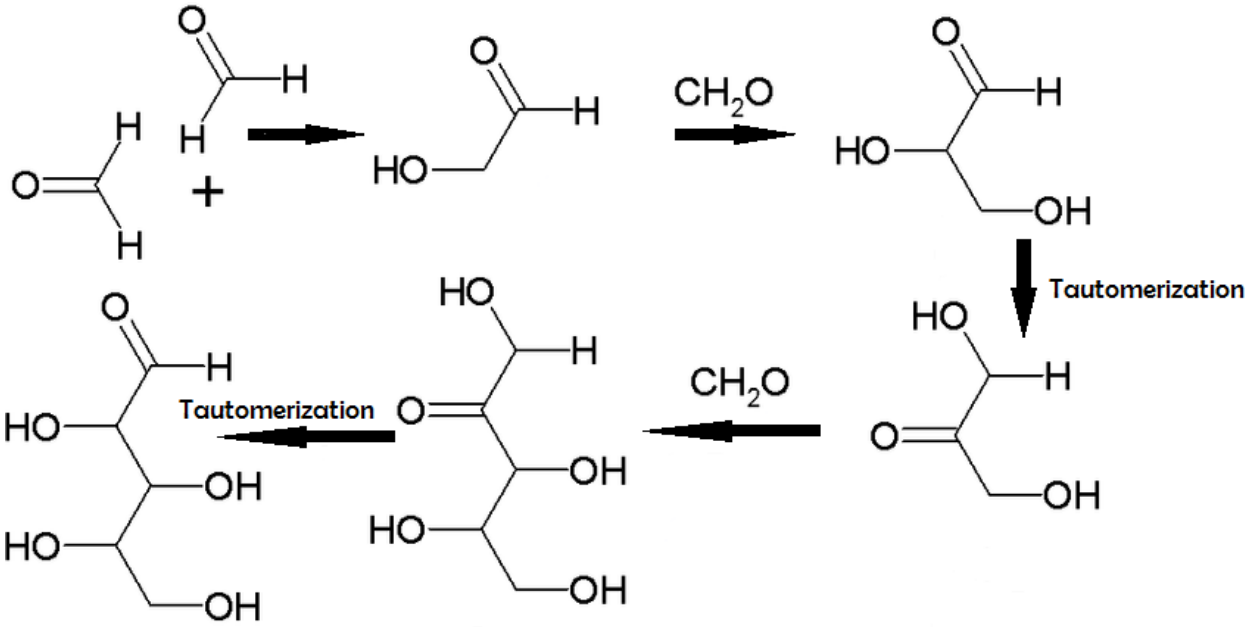


Figure 1. Example of a mechanism for the formose reaction.

THEORETICAL STUDIES OF THE EFFECT OF ISOSTERIC SUBSTITUTION ON THE GARRATT-BRAVERMAN CYCLIZATION

Jade Marino Creto, Mikey Kwon and Dr. Edyta Greer
Department of Natural Sciences, Baruch College, 17 Lexington Avenue, New York, NY 10010

The effect of isosteric substitution on the Garratt-Braverman Cyclization was investigated computationally. The initial investigation, which involves the parent diallene sulfone (CH_2=C=CH-SO_2-CH=C=CH_2) yielding diradical (Figure 1), shows that the energy of activation is $\Delta E^{\ddagger}$=30 kcal/mol and the reaction is exothermic by 6 kcal/mol. The groups that were chosen to replace the sulfone group include –O–, –N(CH_3)–, –S–, –(1,2-C_6H_4)–, –S(O)–, and –CH_2–. These groups have chemical and physical similarities to the sulfone. The thermochemical properties of species with isosteric substituents will be compared to the parent system.

Figure 1.

Acknowledgment is made to the Donors of the American Chemical Society Petroleum Research Fund for support of this research. We also acknowledge the City University of New York PSC-CUNY Research Award Program, and the Eugene Lang Foundation for financial support.

THE INFLUENCE OF ARONIA CULTIVATION MANAGEMENT ON THE ANTIOXIDANT CAPACITY OF ARONIA

Tina Ndam[1], Motunrayo Fadipe[1], Blessing Aroh[1], Andrew Ristvey[2], and Victoria Volkis[1]
[1]Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853
[2]University of Maryland Extension, Wye Research & Education Center, P.O. Box 169, Queenstown, MD 21658-0169

Aronia Mitschurinii commonly known as the Black Chokeberry is a fruit bearing woody perennial shrub in the rosacea family. Although the original *Aronia Melanocarpa* is native to Maryland and was used by Native Americans hundreds years ago, in the past decades it was more popular in Russia, Poland and the United Kingdom, after Russian Scientist breaded it with 25% of Mounting Ashe. Aronia is one of the richest plant sources in the world of phenolic and especially anthocyanins types of antioxidant. It's capability to capture free radical makes it an ideal plant for preventing and even treating diseases like cancer, heart disease, and genetic defects.

Recent studies has proven that this berry contain five times more antioxidants that assai berry even 40 times more than tomatoes, making it a future supper berry. The aim of this project is to develop and optimize the horticultural management program for growing aronia in Maryland small farms in order to produce the crop with as high as possible antioxidant capacity. This will help in improving sustainability of local farm business. Our preliminary study of antioxidant capacity of aronia as a function of bush age, sun-shadow ratio, fertilization and pest management influence significantly the antioxidant capacity.

Here we present data of the measurements of total polyphenols, anthocyanin content, total flavonoids, pH, juice/pulp ratio, some antioxidant profiles and the ORAC factor of Aronia based on the different levels of nitrogen and potassium treatments, conventional and organic, with or without Azomate ® additive. We are currently looking also at the effect of bush age on the antioxidant content of the berry and will provide three years comparison of data.

The project described was supported by the UMES MARC U* STAR Program, Award Number T34GM008411, from the National Institute of General Medical Sciences.

TOWARD WATER-SOLUBLE, CONJUGATABLE BODIPY DERIVATIVES

Anthony Palilla, Adam Meares, and Marcin Ptaszek
Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

The goal of this research was to synthesize water-soluble, bioconjugatable 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) derivatives to integrate them into energy transfer arrays, for multicolor bioimaging. BODIPY is a compound that absorbs and emits light in the 500-550 nm range. These fluorescent characteristics make BODIPY a suitable fluorophore for diverse applications. However, BODIPY is water-insoluble and for it to be applied to biomedical imaging it needs to be soluble and fluorescent in aqueous media. In order to achieve BODIPY's water solubility, an ethylene glycol (PEG) ester of isophthalic acid was attached to BODIPY's core through a Sonogashira coupling reaction. NMR proved that the PEG was successfully installed on the BODIPY core. The optical and photochemical properties of the resulting BODIPY derivative have been studied.

This was supported by the National Cancer Institute of the National Institutes of Health under Award Number U01CA181628.

COPPER CATALYZED ENANTIOSELECTIVE ALKYNYLATION OF ISOQUINOLINE DERIVATIVES

Clarissa A. Shoffler[1], Srimoyee Dasgupta[2], and Mary P. Watson[2]
[1]Department of Chemistry, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003
[2]Department of Chemistry and Biochemistry, University of Delaware,
102 Brown Laboratory, Newark, DE 19716

Many bioactive molecules, including pharmaceuticals, are chiral. Because the biomolecular targets of these molecules (proteins, DNA, carbohydrates, etc.) are often also chiral, only one enantiomer of the molecule will have the desired bioactivity. Therefore, pursuing synthetic pathways for the efficient preparation of enatiopure compounds has important biomedical applications. Currently, we are developing an enantioselective, copper-catalyzed route to form tetrasubstituted stereocenters via the addition of terminal alkynes to 1-aryl isoquinolines. This novel method allows for the attainment of previously inaccessible tetrahydroisoquinolines with tetrasubstituted α-stereocenters. Our goal is to enable efficient conversion of our readily available starting materials into these valuable, chiral products with high levels of enantioselectivity. We hypothesize that this reaction proceeds via an iminium ion intermediate, which is attacked by a chiral copper acetylide formed in situ from a copper salt, chiral PyBox ligand, alkyne, and base. Our synthesis of a wide variety of 1-aryl isoquinoline substrates will be presented, along with preliminary results in the enantioselective alkynylation.

I would like to acknowledge the University of Delaware's 2014 Chemistry REU Program, funded by the National Science Foundation.

HYDROBORATION OF ALKENES WITH AZAFERROCENE-BORENIUM IONS

Dave Szymanik, Sarah Krause, and Dr. Timothy Brunker
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

Online access of this abstract is restricted at the request of the Principal Investigator.

SYNTHESIS AND STUDY OF NOVEL AMPHIPHILES AS POTENT ANTISEPTICS

Brenna J. C. Walsh[1], Kirstie Thompson[1], John N. Marafino[1,2], Kristin McKenna[1], Louis Damiano[1], Tara M. Gallagher[2], Kyle Seifert[2], and Kevin L. Caran[1]
[1]Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807
[2]Department of Biology, 820 Madison Drive, MSC 7801, Harrisonburg, VA 22807

Online access of this abstract is restricted at the request of the Principal Investigator.

Morning Poster Session
Group K – Chemical Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

70. **ION CHROMATOGRAPHIC DETERMINATION OF NITRATE-NITROGEN AND AMMONIUM-NITROGEN IN LAKE SEDIMENT**

Kelsey L. Berrier, and Dr. Daniel M. Downey
Department of Chemistry & Biochemistry, James Madison University,
901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807

71. **CHARACTERIZING THE FUNCTION OF A PUTATIVE NAD(P)H NITROREDUCTASE**

Sarah Gregory and Dr. Alison Dewald
Department of Chemistry, Salisbury University, 1101 Camden Avenue, Salisbury, MD 21804

72. **BIOCOMPATIBLE CHITOSAN-BASED BLENDS FOR THE REVERSIBLE CAPTURING OF CARBON DIOXIDE**

Haneef Muhammad, Dawn Turnquist, and Victoria Volkis
Department of Natural Sciences, University of Maryland Eastern Shore,
Maryland Route 822, Princess Anne, MD 21853

73. **DETECTION OF SACCHARIDES IN FRESH WATER USING HPAEC-PAD**

Rebecca L. Neubauer, Joshua A. Wilhide, and William R. LaCourse
Molecular Characterization and Analysis Complex, UMBC
1000 Hilltop Circle, Baltimore MD 21250

74. **THE NATURE OF THERMOCHROMIC EFFECTS IN DYEINGS WITH INDIGO, 6-BROMOINDIGO, AND 6,6'-DIBROMOINDIGO, COMPONENTS OF TYRIAN PURPLE†**

John Scalise[1], Derrick Claye[1], Aygul Islamova[1], Irina Mironova[1], Olga Lavinda[2], Jacopo Samson[3], Federica Pozzi[4], Dimitrios Mantzouris[5], Ioannis Karapanagiotis[6], Christopher Cooksey[7], and Keith Ramig[1]
[1]Department of Natural Sciences, Box A0506, Baruch College of the City University of New York, 1 Bernard Baruch Way, New York, NY 10010
[2]Department of Chemistry, New York University, 100 Washington Square East, New York, NY 10003
[3]Department of Chemistry, Hunter College and the Graduate School, City University of New York, 695 Park Avenue, New York, NY 10065
[4]Department of Conservation, Solomon R. Guggenheim Museum, New York, NY 10128
[5]Ormylia Foundation, Art Diagnosis Center, Ormylia 63071, Greece
[6]University Ecclesiastical Academy of Thessaloniki, Thessaloniki 54250, Greece
[7]59 Swiss Avenue, Watford, Hertfordshire, WD18 7LL, England

75. **BROWN CARBON FORMATION IN SECONDARY ORGANIC AEROSOL FROM HETEROGENEOUS REACTIVE UPTAKE OF ISOPRENE EPOXYDIOLS**

Richard A. Siejack[2], Ying-Hsuan Lin[1], Sri Hapsari Budisulistiorini[1], Kevin K. Chu[1], Haofei Zhang[1], Zhenfa Zhang[1], Avram Gold[1], Jason D. Surratt[1], and Kathryn E. Kautzman[2]
[1]Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
[2]Department of Chemistry, Towson University, Towson, MD 21252

76. **COPPER (I) PHOTOREDOX CATALYSTS IN C-C BOND-FORMING REACTIONS: A ONE-POT ENOL BROMINATION/PHOTOREDOX APPROACH TO THE MODIFICATION OF ARYL KETONES**

Cody Lloyd, Andrew Stoltzfus and Dr. Timothy Peelen
Department of Chemistry, Lebanon Valley College, N. College Avenue, Annville, PA 17003

ION CHROMATOGRAPHIC DETERMINATION OF NITRATE-NITROGEN AND AMMONIUM-NITROGEN IN LAKE SEDIMENT

Kelsey L. Berrier, and Dr. Daniel M. Downey
Department of Chemistry & Biochemistry, James Madison University, 901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807

Measurements of ammonia-nitrogen and nitrate-nitrogen nutrients in lakes and reservoirs can provide information for management decisions concerning health and biological productivity. Traditional methods of nutrient determination in soil and sediment samples require a preliminary extraction step essential for freeing anionic and cationic species from the exchange sites on soil particles. Following extraction, nutrient determination generally requires time and labor consuming colorimetric quantification. Ion chromatography (IC) has become the method of choice for anion and cation analyses in aqueous samples due to its simplicity, automated system and convenience. However, the high salt concentration required for the extraction step has excluded IC as a technique for sediment nutrient determination due to column and detector saturation. Recently solid phase extraction cartridges for salt removal have been marketed, including silver, barium, hydronium and mixed bed cartridges. The purpose of this study is to develop methods to use these cartridges post-extraction in sediment analysis, followed by IC for nutrient determination. It has been found that at least 99.9% of chloride in the solution can be removed using silver cartridges. Nitrate and ammonium standards were assayed in a similar way with no loss of nitrate ($p<0.001$). However, in the process of extraction salt removal, ammonia (as ammonium) is lost. Currently an alternative extraction method with strontium chloride is being investigated to avoid loss of ammonia. Field collected and test sediment samples have been assayed by both colorimetric and IC methods for statistical evaluation.

CHARACTERIZING THE FUNCTION OF A PUTATIVE NAD(P)H NITROREDUCTASE

Sarah Gregory and Dr. Alison Dewald
Department of Chemistry, Salisbury University, 1101 Camden Avenue, Salisbury, MD 21804

This research focuses on characterizing the functions of protein 3GBH. For over a decade the NIH has funded the Protein Structure Initiative (PSI), whose goal is the 3D structure determination of a broad range of proteins. Of the approximately 4,000 PSI determined protein structures, over 1,500 are of unknown function. Linking protein function to structure and eventually gene sequence would have major impact on biology and medicine, though currently protein function must be characterized using classic biochemical techniques. The protein of interest for our research, protein data bank id 3GBH, is a putative enzyme found in the *Staphylococcus Epidermis* bacteria. Based on amino acid sequence homology, 3GBH is predicted to be a nitroreductase, which is an enzyme that move electrons from one molecule to another. While most nitroreductases function with either NADH or NADPH substrates, 3GBH exhibited no activity with NADH under a variety of conditions and with varied co-substrates. Structural alignment of 3GBH with known nitroreductases suggested that 3GBH was similar in structure to a nitroreductase with a unique fold rendering it specific for NADPH only. An assay was designed to test the nitroreductase activity of 3GBH with NADPH and Flavin mononucleotide (FMN) as substrates. Activity was tested at various pHs, with a Km (FMN) of 0.008 mM and a turnover number of 417 s^{-1} at optimal pH 7. These values are consistent with those reported for known FMN nitroreductases. Assays with additional substrates, temperatures, and salinities are currently underway.

BIOCOMPATIBLE CHITOSAN-BASED BLENDS FOR THE REVERSIBLE CAPTURING OF CARBON DIOXIDE

Haneef Muhammad, Dawn Turnquist, and Victoria Volkis
Department of Natural Sciences, University of Maryland Eastern Shore, Maryland Route 822, Princess Anne, MD 21853

The pollution of CO_2 into the atmosphere due to the utilizing of fossil fuels for the generation of electricity has been directly correlated with the steady increase in the global warming rate over the past 25 years. According to the National Oceanic and Atmospheric Administration (NOAA), the global warming rate has risen to about 2 °C per century. Such a steady increase in the global warming rate may be attributed to the residual effects of the burning of fossil fuels, as each power plant is capable of emitting several million tones of CO_2 annually. To control pollution of greenhouse gases and in particularly CO_2 and to further decrease quantities of CO_2 to be released, many approaches has in turn been developed. Carbon capture and storage (CCS) is one of such approaches.

The overall goal of this project is to prepare biocompatible polymeric sorbents made of blends of chitin, natural and modified chitosan with other polymers for the reversible carbon dioxide capturing, allowing to capture and store CO2 from one source and later release it back under mild conditions to be further used in industrial processes. In addition, the structure properties and the structure-property correlations for all of the prepared polymers and blends and model sorption-desorption processes will be further studied and analyzed.

For this project, I would like to acknowledge the University of Maryland Department Natural Sciences, the Louis Stokes Alliance for Minority Participation (LSAMP) program, and my mentor Victoria Volkis for the funding and overall support.

DETECTION OF SACCHARIDES IN FRESH WATER USING HPAEC-PAD

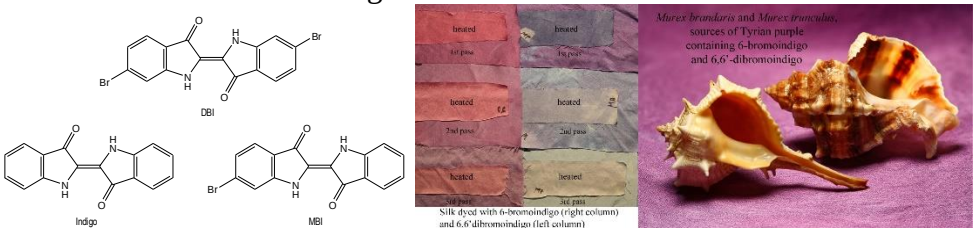
Rebecca L. Neubauer, Joshua A. Wilhide, and William R. LaCourse
Molecular Characterization and Analysis Complex, UMBC
1000 Hilltop Circle, Baltimore MD 21250

While freshwater ecosystems are rich with biodiversity, they are susceptible to chemical and environmental stresses which can impact their overall health. The concentration of carbohydrates in freshwater environments could potentially be a new area of biomonitoring, as saccharides are essential components of the metabolisms of all living organisms. The goal of this project is to apply a chromatographic assay to the quantification of free saccharides in freshwater aquatic environments. Separation of mono- and di-saccharides can be achieved using High Performance Anion Exchange Chromatography (HPAEC), which exploits the anionic properties of carbohydrate molecules under highly alkaline conditions. Pulsed Amperometic Detection (PAD) is then utilized to electrochemically detect and quantify the analytes of interest. A method was developed and optimized to separate three monosaccharides; glucose, fructose and galactose and the disaccharide sucrose. Calibration curves were then established determining the linear ranges of detection for the HPAE-PAD system for the target analytes. For proof of concept, this method was applied to samples procured from various regions of Pig Pen pond as well as a nearby stream. In order to confirm the identities of the saccharides and overcome the complex matrices of the water samples, spiking experiments with standard solutions were performed. An additional confirmation of the presence of glucose was established through a glucose-oxidase enzymatic assay. Future work will include continued sampling of the same and new regions of the pond in order to validate the method and to assess spatial and temporal differences in saccharide levels. The ability to quantify a variety of saccharides can be utilized to map differences in concentrations throughout a freshwater environment and determine how changes in concentration over time are correlated to other factors which monitor the health of the ecosystem.

THE NATURE OF THERMOCHROMIC EFFECTS IN DYEINGS WITH INDIGO, 6-BROMOINDIGO, AND 6,6'-DIBROMOINDIGO, COMPONENTS OF TYRIAN PURPLE†

John Scalise[1], Derrick Claye[1], Aygul Islamova[1], Irina Mironova[1], Olga Lavinda[2], Jacopo Samson[3], Federica Pozzi[4], Dimitrios Mantzouris[5], Ioannis Karapanagiotis[6], Christopher Cooksey[7], and Keith Ramig[1]
[1]Department of Natural Sciences, Box A0506,
Baruch College of the City University of New York,
1 Bernard Baruch Way, New York, NY 10010
[2]Department of Chemistry, New York University,
100 Washington Square East, New York, NY 10003
[3]Department of Chemistry, Hunter College and the Graduate School,
City University of New York, 695 Park Avenue, New York, NY 10065
[4]Department of Conservation, Solomon R. Guggenheim Museum, New York, NY 10128
[5]Ormylia Foundation, Art Diagnosis Center, Ormylia 63071, Greece
[6]University Ecclesiastical Academy of Thessaloniki, Thessaloniki 54250, Greece
[7]59 Swiss Avenue, Watford, Hertfordshire, WD18 7LL, England

Tyrian purple is a dye from antiquity with a fascinating history. It is obtained from *Murex* sea snails, and can contain primarily 6,6'-dibromoindigo (DBI), with lesser amounts of 6-bromoindigo (MBI) and indigo. It is known that wool dyed with MBI, initially a deep violet color, will turn blue upon heating in water. We reveal here that the thermochromic effect is not particular to the wool/MBI combination: A variety of other fabrics dyed with these three dyes show thermochromicity as well. Upon heating, many indigo- and MBI-dyed fabrics turn blue, while DBI-dyed fabrics turn red. The dyed fabrics are characterized by their reflectance spectra and colorimetric analysis. An HPLC study shows that the dyes do not decompose significantly after heating the fabrics, indicating that the color changes are physical rather than chemical phenomena. Electron microscopic analysis of MBI- and DBI-dyed carbon nanotubes, used as a fabric surrogate, indicates that changes in particle size of dye aggregates upon heating may be responsible for the observed color changes.



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†This poster is dedicated to Professor Theodore Cohen of University of Pittsburgh, on the occasion of his 85th birthday.

BROWN CARBON FORMATION IN SECONDARY ORGANIC AEROSOL FROM HETEROGENEOUS REACTIVE UPTAKE OF ISOPRENE EPOXYDIOLS

Richard A. Siejack[2], Ying-Hsuan Lin[1], Sri Hapsari Budisulistiorini[1], Kevin K. Chu[1], Haofei Zhang[1], Zhenfa Zhang[1], Avram Gold[1], Jason D. Surratt[1], and Kathryn E. Kautzman[2]
[1]Department of Environmental Sciences and Engineering,
Gillings School of Global Public Health,
The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599
[2]Department of Chemistry, Towson University, Towson, MD 21252

Light-absorbing organic carbon is an important climate warming agent that contributes to positive radiative forcing, but to date it is generally not represented in current climate models in part due to lack of understanding in its sources and formation processes. Secondary organic aerosol (SOA) produced from heterogeneous reactive uptake of isoprene epoxydiols (IEPOX) has been found to substantially contribute to the organic aerosol mass over the Southeast U.S. where SOA formation from isoprene is significantly enhanced by biogenic/anthropogenic interactions. Brown carbon (BrC) in rural areas in this region has been reported to be associated with secondary sources in summer when influences from biomass burning are low. We demonstrate the formation of light-absorbing (290 nm<λ<700 nm) SOA constituents resulting from reactive uptake of *trans*-β-IEPOX onto preexisting aerosols as a potential source of regional BrC. IEPOX-derived BrC formed in chamber experiments under dry conditions (RH<10%) in the presence of acidified seed aerosol have averaged mass absorption coefficients ~300 cm^2 g^{-1}. Chemical analyses of light-absorbing SOA constituents using diode array detection and mass spectrometric techniques indicate the formation of IEPOX-derived BrC is strongly associated with 3-methyltetrahydrofuran-3,4-diols, which are products of acid-catalyzed intramolecular rearrangement of IEPOX in the particle phase. Oligomeric species separated by mass units of 100 ($C_5H_8O_2$) and 82 (C_5H_6O) are measured in SOA with high levels of unsaturation, suggesting the presence of polymeric conjugated systems as chromophores. Field observations of these conjugated oligomers from the rural southeast U.S. support the atmospheric relevance of this secondary BrC source.

This work was supported by the U.S. Environmental Protection Agency, and in part by Electric Power Research Institute (EPRI). This work was also supported in part by Towson University startup funds. RAS gratefully acknowledges support from The Donald and Linda Raspet Undergraduate Research Fellowship. The authors would like to acknowledge Mr. George Kram for useful discussions and assistance with ATR-FTIR measurements.

COPPER (I) PHOTOREDOX CATALYSTS IN C-C BOND-FORMING REACTIONS: A ONE-POT ENOL BROMINATION/PHOTOREDOX APPROACH TO THE MODIFICATION OF ARYL KETONES

Cody Lloyd, Andrew Stoltzfus and Dr. Timothy Peelen
Department of Chemistry, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003

Transition metal complexes have been widely used to promote single electron redox processes photochemically through metal-to-ligand-charge-transfer (MLCT) processes. Typically, these processes have used iridium and ruthenium complexes; however these metals are not very abundant and consequently are costly. By comparison, copper, a far more abundant and cheaper metal, has recently been used in several photoredox reactions.

In this project, we aim to modify aryl ketones through enol bromination at the α-position followed by photoactivation of the C-Br bond to produce a reactive radical intermediate. Conceptually, we envision this process as a radical chemistry alternative to enolate chemistry for the modification of ketones.

Copper (I) photoredox catalysts were screened and optimized for activity in the allylation of bromoacetophenone using allyltributyl tin. A novel catalyst, $Cu(dap)(BINAP)^+$, with high activity was identified. Simultaneously, conditions for the efficient bromination of simple aryl ketones were identified. Coupling the two reactions together in a single pot, bromination followed by allylation, afforded allylated acetophenone in good yield. We have extended this two-step reaction sequence to electronically-diverse substituted acetophenones and investigated the mechanism of the photochemical reaction.

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Morning Poster Session
Group L – Chemical Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

77. **MANGANESE ACCUMULATION IN LEAVES AND RADISHES GROWN IN MANGANESE SUPPLEMENTED SOILS**
Hasani Douglas, Kortni Garcia, Shellyann Clarke-Lambert, Karl Ruddock and Dereck Skeete
Department of Environmental Sciences, Medgar Evers College,
1638 Bedford Avenue, Brooklyn, NY 11225

78. **DETERMINING EXTRACTION EFFICIENCIES FOR THE TRACE ANALYSIS OF ORGANICS IN AIRBORN PARTICULATE MATTER**
Morgan Dundon, Richard Siejack, Robert Ishakis, and Kathryn Kautzman
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

79. **FT-IR AND X-RAY SPECTROSCOPIC ANALYSIS OF CRUDE OIL COMPOSITION**
Katherine Ness and Alessandra Leri
Department of Natural Sciences, Marymount Manhattan College
221 East 71st, New York, NY 10021

80. **PROBING POLYMERIC BLENDS WITH NATURAL EXTRACTS FROM SPONGE, ALGAE, BERRIES, AND HERBS FOR ANTIFOULING PROTECTION**
So Jin Park, Diamond Nwaeze, Baruch S. Volkis, Paulinus Chigbu, and Victoria V. Volkis
Department of Natural Sciences, University of Maryland Eastern Shore,
Maryland Route 882, Princess Anne, MD 21853

81. **INFLUENCE OF IONIZATION CONDITIONS ON ION FORMATION IN ELECTROSPRAY MASS SPECTROMETRY**
Samantha Norris, Tim Hulyk, and Kevin H. Bennett
Department of Chemistry and Physics, Hood College, Frederick MD 21701

82. **THE INVESTIGATION OF CHARGE TRANSFER BETWEEN COBALT CHALCOGENIDE CLUSTERS AND THIOPHENE BASED POLYMERS**
Devon M. Shircliff, Brian J. Reeves and Brycelyn M. Boardman
Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807

83. **SYNTHESIS AND CHARACTERIZATON OF CONJUGATED POLYMETHINE DYES**
Gaige VandeZande and Dr. Anderson L. Marsh
Department of Chemistry, Lebanon Valley College, 101 N. College Avenue,Annville, PA 17003

MANGANESE ACCUMULATION IN LEAVES AND RADISHES GROWN IN MANGANESE SUPPLEMENTED SOILS

Hasani Douglas, Kortni Garcia, Shellyann Clarke-Lambert, Karl Ruddock and Dereck Skeete
Department of Environmental Sciences, Medgar Evers College,
1638 Bedford Avenue, Brooklyn, NY 11225

Manganese is a naturally occurring element, essential in trace amounts for living organisms, but is potentially toxic in high concentrations. Certain occupations including mining, welding and steel manufacturing can expose workers to chronically high levels of airborne manganese, leading to a clinical condition known as Manganism, which has Parkinson like symptoms. Recent studies report that excess dietary manganese can impair immune and reproductive functions in birds. Previously we showed that manganese is present in some commercially available fertilizers. We hypothesize that plants grown in soils high in manganese or supplemented with fertilizers containing manganese will accumulate manganese in their leaves and fruits. To test this we grew radishes in soils supplemented with fertilizers containing manganese, as well as in soil without added manganese. Samples (0.5 g) of each of the fertilizers as well as radish leaves were digested with nitric acid in a CEM Discovery Microwave Digester. Digested samples were analyzed for manganese levels using electrothermal vaporization with deuterium lamp background correction in a Perkin Elmer AA800 Atomic Absorption spectrophotometer with a THGA graphite furnace. We found that leaves accumulated manganese up to about 129.7 µg/gm tissue. The Control leaves contained 66.50 µg/gm of manganese, which was significantly less than all the experimental groups. Soil that were supplemented with fertilizers containing manganese had a significantly higher concentration of manganese than the plant tissue. The highest concentration of manganese recorded in the soil was 371.8 µg/gm, while the lowest concentration was 61.6 µg/gm. The study shows that plants will accumulate manganese from the soil and that use of fertilizers with high concentrations of manganese will increase the accumulations, possible creating a situation where animals and people ingesting the fruits and vegetables might be subjected to elevated manganese levels.

This work was supported by grant 0516041071 of NYSDOE.

DETERMINING EXTRACTION EFFICIENCIES FOR THE TRACE ANALYSIS OF ORGANICS IN AIRBORN PARTICULATE MATTER

Morgan Dundon, Richard Siejack, Robert Ishakis, and Kathryn Kautzman
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

Chemical speciation of atmospheric aerosols is often accomplished through high volume filter sampling whereby airborne particles are deposited on quartz or Teflon filters. The filters are extracted and subsequently analyzed by a variety of analytical techniques. The use of high-volume filtering sampling techniques thus provides a facile and common method for characterizing local atmospheric chemistries. There are two common strategies for extracting and analyzing high-volume filters, the first uses Soxhlet extraction and the second uses sonication. No work has been done to examine the relative efficiencies of the two extraction techniques. Our work to validate extraction methods examines extraction efficiencies for a range of organic and inorganic target compounds of both anthropogenic and biogenic origin. We have investigated extraction efficiencies over a range of solvents and with a variety of experimental parameters. Extraction efficiencies for aerosol tracer species extracted in methanol, dichloromethane, and 50-50 methanol-dichloromethane are determined by analysis of derivatized samples using Gas Chromatography-Mass Spectrometry (GC -MS). Extraction efficiencies for inorganic salts using Ion Chromatography (IC) have also been quantified.

We would like to acknowledge the Towson University Faculty Development and Research Committee, The Jess and Mildred Fisher College of Science and Mathematics Undergraduate Research Grant Program, and Towson University startup funds for funding this project.

FT-IR AND X-RAY SPECTROSCOPIC ANALYSIS OF CRUDE OIL COMPOSITION

Katherine Ness and Alessandra Leri
Department of Natural Sciences, Marymount Manhattan College
221 East 71st, New York, NY 10021

Crude oils are composed of aromatic and aliphatic hydrocarbons and may also contain nitrogen, sulfur, halogens, and other heteroatoms. The chemical composition of crude oils is important to the petroleum industry in areas from refining to combustion. For example, polynuclear aromatic solids called asphaltenes can clog oil wells [1] and halogens can cause corrosion during the refining process. The chemical composition of crude oils, particularly their heteroatom content, is also important in determining the by-products of combustion and predicting potentially harmful environmental consequences.

FT-IR spectroscopy was used to analyze six crude oil samples from Louisiana, Texas, Nigeria, Saudi Arabia, Colombia, and Venezuela. The samples vary in their content of paraffins, naphthenes, and aromatics, hydrocarbons used for classification of crude oils. Such classifications also indicate the density, sulfur content, and total acid number of a crude oil. By monitoring the methylene (CH_2) and methyl (CH_3) bands at 2923 cm^{-1} and 2953 cm^{-1}, respectively, we found differences in the aliphatic content among the oils. Relative aliphatic chain lengths were calculated using the CH_3:CH_2 band ratio [2]. In addition, aromatic content was monitored using bands at 1605 cm^{-1} and 695 cm^{-1}. We used these absorbances to calculate aliphatic and aromatic indices for the oils.

This characterization of the hydrocarbon content of the oils laid the foundation for study of their halogen content using X-ray absorption spectroscopy. There was no chlorine found in any of the crude oils, and only a trace amount of organically bound bromine in the most asphaltene-rich crude sample. Given recent findings of high organobromine levels in decaying algal material [3], our results suggest that this organobromine may be broken down during the chemical and geological processes that transform marine organic material into fossil fuels. We are currently investigating halogens in sedimentary material as an intermediate stage between algal detritus and fossil fuels.

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PROBING POLYMERIC BLENDS WITH NATURAL EXTRACTS FROM SPONGE, ALGAE, BERRIES, AND HERBS FOR ANTIFOULING PROTECTION

So Jin Park, Diamond Nwaeze, Baruch S. Volkis, Paulinus Chigbu, and Victoria V. Volkis
Department of Natural Sciences, University of Maryland Eastern Shore, Maryland Route 882, Princess Anne, MD 21853

Marine biofilm formation, known also as fouling, is a process of accumulation of chemicals, bacteria, micro- and macroorganisms on submerged surfaces. This process is responsible for billions of dollars in damage to all kinds of man-made materials and constructions in the marine environment. Available protective coating contain such a danger chemicals as tributyl tin (TBT) and are toxic to marine environments. With the aim to find an alternative to available toxic protecting coatings, extracts of marine algae such as *Fucus sp.*, *Ulva lactuca* and *Clathria Prolifera* (red beard sponge), as well as specialty crops such as *Aronia Mitchurinii*, and *Ocimum Sanctum* were obtained by utilizing various extraction methods paired with solvents of different polarity and solubility. The extracts were analyzed for nutrient content and then incorporated into polymers commonly utilized in the paint industry. The resulted polymeric blends were then used to coat microscope glass sample plates and latter was exposed to sea water obtained from Assateague Bay. To estimate the antifouling properties of the extract, the surface of the pure glass plate, glass plate coated by polymer film only and the glass plate coated by the polymeric blend with natural extract were compared using confocal microscope imaging technology. In addition, albumin-ALEXA® precipitation on sample plates were performed and analyzed using fluorescent microscope to model the first stage of biofilm formation involving adsorption of secondary metabolites of marine micro-organisms on protected and not protected surfaces.

INFLUENCE OF IONIZATION CONDITIONS ON ION FORMATION IN ELECTROSPRAY MASS SPECTROMETRY

Samantha Norris, Tim Hulyk, and Kevin H. Bennett
Department of Chemistry and Physics, Hood College, Frederick MD 21701

Mass spectrometry can provide a wide range of information on compounds that produce either positive or negative ions upon electrospray ionization (ESI). Finding ideal ionization conditions for analytes is important to maximize data quality. This project centers on the study of a range of target compounds and the impact of ESI conditions on the observed data obtain from those analytes. For negative ion mode this research focused on the analysis of the pain relieving drugs naproxen and ibuprofen. Optimal ionization conditions were determined over a wide range of solution and instrument conditions. In addition this work attempted to use cyclodextrins to gain information of drug enantiomers. For positive ion mode the research focus was on the analysis of Polyethylene glycol (PEG) and polypropyleneglycol (PPG). Solutions of for each polymer (PEG 1000, PEG 400, PPG 1000, and PPG 400) where studied with different complexing cations added (Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+ and Ag^+). Analytes were run at a range of cone voltages to study the impact of each complexing cation on the observed polymer distribution.

This work was supported by the National Science Foundation through grant DUE-0836771 and by the Hood College Summer Research Institute.

THE INVESTIGATION OF CHARGE TRANSFER BETWEEN COBALT CHALCOGENIDE CLUSTERS AND THIOPHENE BASED POLYMERS

Devon M. Shircliff, Brian J. Reeves, and Brycelyn M. Boardman
Department of Chemistry and Biochemistry, James Madison University,
Harrisonburg, VA 22807

The bulk hetero-junction (BHJ) photovoltaic architecture is applicable to a wide variety of materials and recently conjugated polymers and inorganic semiconductor nanoparticle systems have been investigated. These materials are advantageous for organic photovoltaic (OPV) applications because they combine the strong light harvesting capabilities of the inorganic component with the ability to solution process well-ordered structures for efficient charge separation and migration of the organic component. Colloidal semi-conducting nanocrystals or nanoparticles are often used as the inorganic component in these types of devices. Nanoparticles are of interest in energy conversion and light harvesting applications because of their size-tunable IR band gaps and solution processability. While there has been some success with these materials, they are plagued by a few fundamental problems. Additionally the majority of these nanoparticles are solution processable, because of coatings or surfactants on the surface of the nanoparticles. These coatings make it very difficult to understand the interaction between the donor and acceptor from a chemical perspective. It is therefore of interest to utilize well defined nanoparticles or clusters that can be structurally characterized, are solution processable, and also have the ability to harvest a large amount of light.

A series of thiophene functionalized cobalt chalcogenide clusters were heterogeneously mixed with poly-3-hexyl thiophene (P3HT), poly[(3-phenylethyl 2-(thiophene-3-yl)acetate)-2,2'-thiophene-3-hexyl thiophene] (PPTATHT), poly[(3-phenylethyl 2-(thiophene-3-yl)acetate)-2,2'-thiophene-2,6-diyl-*alt*-2,1,3-benzothiadiazole-4,7-diyl] (PPTATBT) to investigate the charge transport of the system. Emission spectra were recorded for the addition of, $Co_6Se_8(PEt_3)_6$ (**1**), $Co_6Se_8(P(Et)_2(Th\text{-}Br)_6$ (**2**) (Th-Br=2-bromothiophene) and $Co_6Se_8(P(Ph)_2(Th\text{-}Br)_6$ (**3**) to 0.004 wt% of the polymers in toluene. The quenching of the polymers emission follows first-order like decay for each cluster. P3HT > PPTATHT > PPTATBT with respect to quenching efficiency regardless of the R group substituent of the cluster's axial phosphines. Clusters **2** and **3** are approximately twice as efficient at quenching the emission than cluster **1**.

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SYNTHESIS AND CHARACTERIZATON OF CONJUGATED POLYMETHINE DYES

Gaige VandeZande and Dr. Anderson L. Marsh
Department of Chemistry, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003

Polymethine organic compounds are commonly used as fluorescent dyes to label DNA and RNA and are used in biomedical imaging. These compounds have conjugated pi systems with varying absorbance intensities and excitation wavelengths depending upon the level of conjugation within the dye. These conjugated pi systems absorb photons causing excitation of electrons which then fluoresce upon return to the ground state. The amount of light energy absorbed represents the spacing between the energy levels and can be measured through UV/Vis.

In this project, the cyanine family of polymethine dyes was studied with the goal of synthesizing the uncommonly used tricarbocyanine iodide in order to study the effects of extended conjugated pi systems on the observed UV-Vis absorbance levels. The three shorter cyanine dyes within this dye series are commercially available; however the tricarbocyanine iodide is unavailable and difficult to synthesize.

The synthesis of tricarbocyanine iodide was attempted through the base-mediated condensation of a quaternary amine salt and a bisimine. NMR and UV/Vis characterization of the isolated product showed that the condensation reaction was occurring with only one side of the conjugated bisimine chain. While this product was not the desired product, this partial reaction showed a promising direction in future synthesis and characterization of tricarbocyanine iodide.

Morning Poster Session
Group M – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

84. **OUTER MEMBRANE PROTEIN FOLDING IN LIPOSOMES**
Stephanos Gozali, Mark Culver and Dr. Alison Dewald
Department of Chemistry, Salisbury University, 1101 Camden Avenue, Salisbury, MD 21801

85. **MODEL MEMBRANES TO PROBE FOR RETROVIRAL GAG MEMBRANE TARGETING**
Tarik Hawkins[1,3], Diana Bobb[2,3], Nadine Bucca[3], Mansi Mehta[3], Christy Gaines[3], Peter Mercredi[3], and Michael F. Summers[3]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Seed School of Maryland, 200 Font Hill Avenue, Baltimore, MD 21223
[3]Howard Hughes Medical Institute, Department of Chemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

86. **TRACKING FREE SULFITE LEVELS IN THREE OPENED WINES OVER FOURTEEN DAYS**
Laura Maurer and Stephen Robertson
Department Of Chemistry, McDaniel College, 2 College Hill, Westminster, MD 21157

87. **APPLICATION AND DEVELOPMENT OF TLC METHODS FOR DETECTION OF PROTEOLIPOSOMES**
Dominic Santoleri, Matthew Fritz, and Sharon Rozovsky
Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716

88. **BACTERIAL INHIBITIONS OF WATERMOLDS**
Kori Sye, Jacob Nesemeier, and Dr. Nancy Peterson
Department of Chemistry, North Central College, 30 N. Brainard Street, Naperville, IL 60540

89. **SOLVENT REACTIONS OF 4-METHOXYPHENYL CHLOROFORMATE IN PURE AND BINARY SOLVENS**
Dionne Williams, Ariel Billbrough, and Dr. Malcolm J. D'Souza
Department of Biochemistry, Wesley College, 120 N. State Street, Dover, DE 19901

OUTER MEMBRANE PROTEIN FOLDING IN LIPOSOMES

Stephanos Gozali, Mark Culver and Dr. Alison Dewald
Department of Chemistry, Salisbury University, 1101 Camden Avenue, Salisbury, MD 21801

Despite the vast importance of outer membrane proteins (OMPs), OMPs are understudied because they must first be folded into a liposome or other membrane mimetic. There is no one folding condition that works for all OMPs, so empirical screens are used to determine the proper salinity, pH, liposome size, and lipid type for each unique protein under study. The goal of this project is to analyze what happens to various OMPs (fold, aggregate, or lipid-associate without folding) in a variety of these conditions, towards understanding the protein, lipid, and buffer characteristics that determine the protein's fate. Theoretical understanding of this determination would significantly decrease the time needed to find a folding system for any particular OMP, which could expedite work in this area. Four OMPs were chosen for study: OmpA, OmpW, Opa_{60} and OmpX. OmpA and OmpX fold easily under a variety of conditions while Opa_{60} and OmpW are poor folders. All four are comprised of eight stranded β- barrels. The OMPs were each overexpressed to inclusion bodies then purified using multiple centrifugation and wash steps, with final purity demonstrated by SDS-PAGE. OMP aggregates were separated from liposome-folded or unfolded lipid-associated protein via ultra-centrifugation. Folded and unfolded protein in each fraction was determined by SDS-PAGE (fully folded β-barrels migrate as a different apparent molecular weight in SDS if the sample is not heated) and gel densitometry. We will present results to the fates of the OMPs over a range of pHs and lipid chain lengths. Further study will complete and replicate these results and will examine other factors known to affect protein folding. Finally, bioinformatics analysis of the loop composition of these OMPs was performed. Ultimately, this analysis will be used to determine if there is a correlation between loop characteristics and folding behavior.

This research was made possible through the Salisbury University Bridges to SUCCESS (Salisbury University's Connections to Careers for Every Stem Student) program, funded by the National Science Foundation.

MODEL MEMBRANES TO PROBE FOR RETROVIRAL GAG MEMBRANE TARGETING

Tarik Hawkins[1,3], Diana Bobb[2,3], Nadine Bucca[3], Mansi Mehta[3], Christy Gaines[3], Peter Mercredi[3], and Michael F. Summers[3]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Seed School of Maryland, 200 Font Hill Avenue, Baltimore, MD 21223
[3]Howard Hughes Medical Institute, Department of Chemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

A key step in the retroviral replication cycle involves the localization of the Gag polyprotein to the plasma membrane of infected cells. Gag trafficking to the plasma membrane is regulated by the phospholipid phosphatidylinositol (4,5) bisphosphate (PIP_2), a phosphatidylinositol known to recruit cellular proteins to the plasma membrane. Previous structural studies with truncated-PIP_2 support a mechanism where PIP_2 directly interacts with the N-terminal matrix (MA) domain of Gag, resulting in Gag multimerization at cholesterol rich liquid-ordered regions of the PM. Conversely, this hypothesis has not been tested and it is currently unknown if Gag initially targets lipid-ordered of lipid-disordered regions of the membrane. Here, we prepared large unilamellar vesicles and conducted solution-state nuclear magnetic resonance (NMR) spectroscopy to in order to investigate if the initial assembly site of Gag molecules. Ultimately, our findings suggest that HIV-1 Gag initially identifies a PIP_2 containing lipid-disordered region and that Gag lateral diffusion/multimerization results in localization at liquid-ordered regions.

This Research is supported by NIH/NIAID Grant# 5R37AI30917; and the Howard Hughes Medical Institute at the University of Maryland, Baltimore County.

TRACKING FREE SULFITE LEVELS IN THREE OPENED WINES OVER FOURTEEN DAYS

Laura Maurer and Stephen Robertson
Department Of Chemistry, McDaniel College, 2 College Hill, Westminster, MD 21157

The added sulfite compounds in wine help to preserve the product as the free sulfites bind to oxygen and other compounds to maintain color, taste and smell. This study focuses on how the free sulfites react over a two week period after bottles are opened; thereby focusing on how the wine is preserved from a consumer's end. The levels of free sulfites were measured in three local Maryland wines over a fourteen day period. The effectiveness of increased sulfite levels was assessed for preserving opened wine. Preliminary results of this study indicate that free sulfites in a sweet wine decrease linearly, where dry wines have more exponential decreases.

L.M. would like to thank Stephen Robertson for all of his help and guidance, and for the opportunity to conduct this research. L.M. would also like to express gratitude to Emma Rixham for her help with the actual running of the tests. Additional gratitude is given to the McDaniel Chemistry Department and the McDaniel Student-Faculty Collaborative Research Fund for the funding of this experiment and the use of their labs. Thanks are also given to Linganore Winecellars for the donation of the wine researched in this experiment, and for their information about the wine.

APPLICATION AND DEVELOPMENT OF TLC METHODS FOR DETECTION OF PROTEOLIPOSOMES

Dominic Santoleri, Matthew Fritz, and Sharon Rozovsky
Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716

Membrane proteins are often studied in their native membrane environment by using proteoliposomes, liposomes with proteins incorporated into them. In order to be able to validate the successful incorporation of proteins into lipid vesicles, different lipid stains were applied and tested on samples using thin layer chromatography (TLC). The model liposomes, consisting of an 85:15 (mole fraction) DOPC:DOPS lipid mixture were separated from free proteins (Tobacco Etch Virus protease) in the solution using a density gradient. Two density gradients were tested in the assays, Accudenz and sucrose. Both gradients serve to separate the solutions by their densities through centrifugation. While centrifuging, the different components of the sample travel through the gradient until they reach the part where the density of the gradient matches the density of the sample. The more dense proteins go to the bottom layer while the liposomes float near the top. The Accudenz gradient proved to be more difficult to work with than the sucrose solution as it does not form a gradient as easily but is less reactive than the sucrose with regards to the stains used on the plates.

The three stains — molybdenum blue, ninhydrin, and p-anisaldehyde — were tested for sensitivity through serial dilutions as well as several control experiments involving the potential gradients, liposomes, and buffer. p-anisaldehyde proved to be the most sensitive stain but possessed the drawback of staining the sucrose.

BACTERIAL INHIBITIONS OF WATERMOLDS

Kori Sye, Jacob Nesemeier, and Dr. Nancy Peterson
Department of Chemistry, North Central College, 30 N. Brainard Street, Naperville, IL 60540

Over the past 40 years, amphibian populations have been declining partly due to infections by water molds. Bacteria have been found to inhibit the growth of the water mold; we hypothesized that the inhibition is dependent on more than the ability of bacteria to change the pH of the environment. We grew Saprolegnia sp.-2013 on LB plates buffered at pH 6, 7, 8, and 9 and compared this growth to Saprolegnia sp.-2013 on buffered plates with Bacillus anthracis Sterne present. We found that the water mold was more inhibited on the buffered plates when B. anthracis was present ($F=15.507$, $p<0.001$). We also hypothesized that different water molds will be inhibited differently by bacteria. We found that Phytophthora sp.-2014 was inhibited more by bacteria on protein-rich media ($p<0.01$). To investigate whether or not water molds compete in nature, we plated two different water molds on vary media. On nutrient-poor and glucose-rich media, the water molds coexisted, while on protein-rich media, Saprolegnia sp.-2013 out-competed Phytophthora sp.-2014.

This research was funded by a North Central College Summer Research Grant.

SOLVENT REACTIONS OF 4-METHOXYPHENYL CHLOROFORMATE IN PURE AND BINARY SOLVENS

Dionne Williams, Ariel Billbrough, and Dr. Malcolm J. D'Souza
Department of Biochemistry, Wesley College, 120 N. State Street, Dover, DE 19901

Chloroformate esters are used to study substitution reactions and serve as stable intermediates. This project primarily focuses on the reaction rates of 4 -methoxyphenyl chloroformate (4-MeOPhOCOCl) in ethanol (EtOH), methanol, aqueous EtOH, aqueous acetone, aqueous 2,2,2-trifluoroethanol (TFE), aqueous 1,1,1,3,3,3-hexafluro-2-propanol (HFIP), and TFE-EtOH mixtures. 4-MeOPhOCOCl is beneficial in environmental science because of its ability to be recycled to create new chemicals. The reaction rates obtained in this project will be compared to previously published kinetic rate data.

In this study, the reaction rates will be measured using an acid-base titration method. A solution of sodium methoxide (sodium methylate) is used as the volumetric base solution. The position of the equilibrium in the reaction can be visually followed as lacmoid is used as the indicator solution.

A majority of the initial results obtained are consistent with prior work, however, significant experimental rate deviations (>20%) were observed in the aqueous ethanol solutions.

The new rate data obtained complements prior work and similarities in mechanistic trends are seen when this work is compared to the published data.

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Morning Poster Session
Group N – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

90. **INVESTIGATING THE ENZYMATIC HYDROLYSIS OF CHEMICALLY SYNTHESIZED PYROPHOSPHOPEPTIDES**

Ariunzaya Amgalan[1,2], Lisa M. Yates[2], and Dorothea Fiedler[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Chemistry, Princeton University, Princeton, NJ 08544

91. **SYNTHESIS AND CHARACTERIZATION OF AN ENZYME-ACTIVATED FLUOROPHORE FOR DETECTION OF PHOSPHOLIPASE A2**

Noah Bloch[1], Michael Chiorazzo[2], Anatoliy V. Popov[3], and E. James Delikatny[3]
[1]Department of Biology, Haverford College, 370 Lancaster Avenue, Haverford, PA 19041
[2] Department of Pharmacology, University of Pennsylvania, 3620 Hamilton Walk, Philadelphia, PA 19104
[3] Department of Radiology, University of Pennsylvania, 3620 Hamilton Walk, Philadelphia, PA 19104

92. **MEASUREMENT OF LEVEL OF BRANCHED CHAIN AMINO ACIDS IN NUTRITIONAL SUPPLEMENTS**

Daniel Lamela, Kyle Hubble, and K.A. Keenan, Ph.D.
NAMS, Richard Stockton College of New Jersey, 101 Vera King Farris Drive, Galloway, NJ 08205

93. **DETERMINING THE ROLE OF MELANOPSIN C-TAIL IN DEACTIVATION AND TRAFFICKING**

Elelbin Ortiz, Preethi Somasundaram, Evan Cameron, PhD, and Phyllis Robinson, PhD
Department of Biology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

94. **PURIFICATION OF UNMODIFIED HUMAN ELONGATION FACTOR 2 FOR THE *IN VITRO* RECONSTITUTION OF DIPHTHAMIDE SYNTHESIS**

Nirja B. Patel[1], Joshua R. Ostovitz[1], and John E.Weldon[1,2]
[1]Molecular Biology, Biochemistry and Bioinformatics Program, Towson University, 8000 York Road, Towson, MD 21252
[2]Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252

95. **REDOX-SENSITIVE GREEN FLUORESCENCE PROTEIN AS DETERGENT-COMPATIBLE REDOX INDICATORS**

Jay Subramoney, Jun Liu, Zhengqi Zhang, and Sharon Rozovsky
Department of Chemistry and Biochemistry, University of Delaware, 102 Brown Laboratory, Newark, DE 19716

96. **THE EFFECT OF MOTOR PROTEIN BINDING ON MICRTUBULE DEPOLYMERIZATION**

David Witte[1], Ningxi Yu[2], and Ruxandra Dima[2]
[1]Department of Chemistry, Immaculata University, 1145 King Road, Immaculata, PA 19345
[2]Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221

INVESTIGATING THE ENZYMATIC HYDROLYSIS OF CHEMICALLY SYNTHESIZED PYROPHOSPHOPEPTIDES

Ariunzaya Amgalan[1,2], Lisa M. Yates[2], and Dorothea Fiedler[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Chemistry, Princeton University, Princeton, NJ 08544

Online access of this abstract is restricted at the request of the Principal Investigator.

SYNTHESIS AND CHARACTERIZATION OF AN ENZYME-ACTIVATED FLUOROPHORE FOR DETECTION OF PHOSPHOLIPASE A2

Noah Bloch[1], Michael Chiorazzo[2], Anatoliy V. Popov[3], and E. James Delikatny[3]
[1]Department of Biology, Haverford College, 370 Lancaster Avenue, Haverford, PA 19041
[2] Department of Pharmacology, University of Pennsylvania,
3620 Hamilton Walk, Philadelphia, PA 19104
[3] Department of Radiology, University of Pennsylvania,
3620 Hamilton Walk, Philadelphia, PA 19104

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MEASUREMENT OF LEVEL OF BRANCHED CHAIN AMINO ACIDS IN NUTRITIONAL SUPPLEMENTS

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NAMS, Richard Stockton College of New Jersey,
101 Vera King Farris Drive, Galloway, NJ 08205

The goal of this project was to develop a laboratory experience suitable for college students using nutritional supplements. An active ingredient in protein based nutritional supplements are the branched chain amino acids (BCAA) which have been linked to a quicker recovery for muscle. BCAA consist of three amino acids: leucine, isoleucine and dehydrogenase. These supplements are usually made from whey protein in milk. The objective in this study was to measure the level of BCAAs in a variety of supplements and milks using the leucine dehydrogenase enzyme assay. Supplements were prepared and were first hydrolyzed to release the amino acids. BCAAs were measured using the leucine dehydrogenase enzyme assay and arginine was measured with a colorimetric test. The BCAA levels as well as arginine are lower than the manufacturer's claims. When this process was repeated on proteins of known amino acid sequence, the levels of BCAAs as well as arginine were consistent with the expected values. In order to prove that the protein hydrolysis was complete, protein level was measured before and after hydrolysis using the Lowry method. In addition, proteins were run on protein electrophoresis gels (SDS-PAGE).

DETERMINING THE ROLE OF MELANOPSIN C-TAIL IN DEACTIVATION AND TRAFFICKING

Elelbin Ortiz, Preethi Somasundaram, Evan Cameron, PhD, and Phyllis Robinson, PhD
Department of Biology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Melanopsin is a unique non-image forming visual pigment expressed in intrinsically photosensitive retinal ganglion cells in the vertebrate retina. These cells are involved in many non–image forming functions such as the photoentrainment of circadian rhythm and the pupillary light reflex. Melanopsin is deactivated through the phosphorylation of the C-tail followed by the binding of a β-arrestin molecule. β-arrestin contains a signal on its C-terminus that allows for internalization of G-protein coupled receptors (GPCRs) after inactivation. However, it is currently unknown whether melanopsin is internalized. Angiotensin II type 1A receptor (ATII1AR) and B2 adrenergic receptor (B2AR) are two GPCRs known to bind β-arrestin and undergo endocytosis. To study the role of the C-tail in melanopsin deactivation and trafficking, the C-tail of melanopsin is replaced with either ATII1AR or B2AR c-tail using cloning techniques. We then introduce our plasmids into Human Embryonic Kidney (HEK) cells to assess the localization and signaling of the constructs. Sequencing has confirmed that several chimeric constructs have successfully been made. Calcium imaging has confirmed that the Mel/B2AR chimeric constructs signals in a similar manner as melanopsin in the presence of light. These results will help determine the role of the melanopsin C-tail in its deactivation and trafficking.

This study was supported, in part, by the Howard Hughes Medical Institute program, the NIH/NIGMS MARC U*STAR T34 08663 National Research Service Award to UMBC, and a National Eye Institute grant to P.R.R. (R01EY019053).

PURIFICATION OF UNMODIFIED HUMAN ELONGATION FACTOR 2 FOR THE *IN VITRO* RECONSTITUTION OF DIPHTHAMIDE SYNTHESIS

Nirja B. Patel[1], Joshua R. Ostovitz[1], and John E.Weldon[1,2]
[1]Molecular Biology, Biochemistry and Bioinformatics Program, Towson University, 8000 York Road, Towson, MD 21252
[2]Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252

Elongation factor 2 (EF2) is an accessory protein that plays an important role in protein synthesis by moving the ribosome along the mRNA during translation. In eukaryotes and archaea, EF2 contains an entirely unique residue called diphthamide that is a post-translational modification of histidine. At least seven different proteins are needed to produce diphthamide in yeast, DPH1-DPH7. Previous work and circumstantial evidence suggest that the diphthamide is important, but its precise function remains elusive and its synthesis pathway has yet to be fully reconstituted *in vitro*. In order to study the synthesis of diphthamide, we intend to develop a protocol to reconstitute the human pathway of diphthamide synthesis *in vitro.* As a first step, we have inserted the coding region of the human EF2 gene into a T7 expression vector and expressed it in *E. coli*. The cell extracts were analyzed by SDS-PAGE to confirm the presence of the protein. Under several expression conditions, we discovered that our protein deposits in insoluble aggregates called inclusion bodies. We subsequently isolated these inclusion bodies and dissolved the protein in the strong denaturant guanidine hydrochloride. We are currently evaluating refolding conditions for human EF2 using solubility and catalytic activity to determine if the protein is properly folded. At the end of this stage, we expect to have properly folded form of unmodified human EF2 that will be evaluated for structural and functional differences from EF2 containing diphthamide. Future directions include purification of the enzymes involved in diphthamide synthesis.

The authors would like to thank Towson University's Office of Undergraduate Research Committee, the Jess and Mildred Fischer College of Science and Mathematics Undergraduate Research Committee, and the Department of Biological Sciences for supporting this research.

REDOX-SENSITIVE GREEN FLUORESCENCE PROTEIN AS DETERGENT-COMPATIBLE REDOX INDICATORS

Jay Subramoney, Jun Liu, Zhengqi Zhang, and Sharon Rozovsky
Department of Chemistry and Biochemistry, University of Delaware,
102 Brown Laboratory, Newark, DE 19716

Activity assays that test for the ability of enzymes to reduce disulfide bonds in proteins often use insulin as an indicator. These assays rely on the aggregation of insulin which occurs once its disulfide bonds are reduced. Insulin assays, however, cannot be carried out in the presence of most detergents, and because detergents are used to model the hydrophobic environment of the cell membrane, assays which measure the activity of membrane-bound enzymes cannot be run effectively in-vitro.

To develop effective activity assays for membrane enzymes in the presence of detergents we have tested an alternative approach – the use of a redox-sensitive green fluorescence protein (roGFP). We expected that roGFP could function in the presence of detergents due to its structural stability. The ability of roGFP to measure reductase activity of well-known redox enzymes, such as thioredoxin and glutaredoxin, was tested in both membrane and aqueous conditions. The data shows that roGFP works well as a redox indicator in detergent solution, which is important for the examination of proteins which function differently within a membrane. However, due to numerous limitations it is not an ideal replacement for the traditional insulin-based activity assays.

This research was made possible by the David M. Heitzer Award and the University of Delaware. I credit my faculty mentor, Dr. Sharon Rozovsky for her assistance with my research, as well as the Department of Chemistry and Biochemistry for their support.

THE EFFECT OF MOTOR PROTEIN BINDING ON MICRTUBULE DEPOLYMERIZATION

David Witte[1], Ningxi Yu[2], and Ruxandra Dima[2]
[1]Department of Chemistry, Immaculata University, 1145 King Road, Immaculata, PA 19345
[2]Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221

Our study focused on the role of kinesin-1 motors in depolymerization of curved microtubule (MT) protofilaments (PFs). In particular, we determined the effect of kinesin-1 on the mechanical behavior of PFs. We compared and contrasted this behavior with the mechanical response of apo PFs and PFs decorated with kinesin-13, a known efficient MT depolymerizing motor.

This research was undertaken at the University of Cincinnati Chemistry REU program during the summer of 2014. Funding for this program was provided by the National Science Foundation.

Morning Poster Session
Group O – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

97. **PHOSPHORYLATION OF PTP1B REGULATES CATALYSIS BY MODULATING ACID-LOOP DYNAMICS**

Sean Haynie[1,2], Kelly Bird[1,2], Dr. Patrick Loria[2], and Dr. James Lipchock[1,2]
[1]Department of Chemistry, Washington College, 300 Washington Avenue, Chestertown, MD 21620
[2]Department of Chemistry, Yale University, 225 Prospect Street, New Haven, CT 0651

98. **ASSESSING THE POTENTIAL ROLE OF cAMP IN REGULATING CheA AND OTHER BACTERIAL PROTEIN HISTIDINE KINASES**

Kai-li Liang and Richard Stewart
Department of Cell Biology and Molecular Genetics, University of Maryland College Park,
1109 Microbiology Building, College Park, MD 20742

99. **HOFMEISTER ION AND COSOLVENT EFFECTS ON THE STRUCTURE, AGGREGATION, AND BACKBONE SOLVATION OF RECA**

Taylor P. Light, Karen M. Corbett, Michael A. Metrick, and Gina MacDonald
Department of Chemistry and Biochemistry, James Madison University,
800 S. Main Street, Harrisonburg, VA 22807

100. **IDENTIFICATION OF THE STRESS-DEPENDENT INTERACTOME OF O-GLCNACASE**

Austin O. Maduka[1], Jennifer A. Groves[2], and Natasha E. Zachara, Ph.D.[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biological Chemistry, The Johns Hopkins University,
School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

101. **CHARACTERIZATION OF A DICTYOSTELIUM DISCOIDEUM DCP2 GENETIC KNOCKOUT**

Marlee Nelson, Kirsten Bickford, and Susan Parrish
Department of Biology, McDaniel College, 2 College Hill, Westminster, MD 21157

102. **STRUCTURAL AND FUNCTIONAL ANALYSIS OF DISEASE CAUSING MUTATIONS IN THE M10 DOMAIN OF TITIN**

Michael W. Rudloff and Nathan T. Wright
Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807

103. **SEARCHING FOR HEMIMETHYLATION IN IMPRINTED GENES: DNA METHYLATION PATTERNS AT *Gtl2***

Ekaterina Vlasova and Tamara Davis
Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010

PHOSPHORYLATION OF PTP1B REGULATES CATALYSIS BY MODULATING ACID-LOOP DYNAMICS

Sean Haynie[1,2], Kelly Bird[1,2], Dr. Patrick Loria[2], and Dr. James Lipchock[1,2]
[1]Department of Chemistry, Washington College, 300 Washington Avenue, Chestertown, MD 21620
[2]Department of Chemistry, Yale University, 225 Prospect Street, New Haven, CT 0651

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ASSESSING THE POTENTIAL ROLE OF cAMP IN REGULATING CheA AND OTHER BACTERIAL PROTEIN HISTIDINE KINASES

Kai-li Liang and Richard Stewart
Department of Cell Biology and Molecular Genetics, University of Maryland College Park, 1109 Microbiology Building, College Park, MD 20742

Over 10,000 protein histidine kinases (PHKs) have been identified in the genomic sequences of Bacteria and Archaea. Most of these are involved in signal transduction, allowing cells to respond to changes in environmental conditions. One well characterized example is CheA, an important component of the chemotaxis signaling pathway, whose autokinase activity is regulated by a set of chemoreceptor proteins. This mechanism controls the activity of downstream effector proteins that regulate cell swimming patterns. Our recent studies suggest that there may be another layer of control not directly involving the chemotaxis receptors, but rather mediated by the binding of the second messenger molecule cyclic AMP (cAMP) to CheA - an interaction that inhibits the autokinase activity of CheA purified from *Escherichia coli*. This possibility has interesting parallels to the role of cAMP in eukaryotic cells, where cAMP regulates certain protein kinases and ion channels. To explore the interaction of cAMP with CheA from other bacterial species (other than *E. coli),* we purified the CheA ATP-binding domains of several CheA proteins from *Vibrio cholerae* and *Pseudomonas aeruginosa*, and we investigated whether they could bind cAMP using a fluorescence competition assay. Analysis of these results allowed us to determine the binding affinity of the proteins for cAMP. Using a similar approach, we measured the affinity of these proteins for ATP and AMP. The dissociation constants (K_d) values for cAMP binding were 2-fold lower than those for ATP binding and 5-15 fold lower than those for AMP binding. To determine whether cAMP-mediated inhibition is an activity shared by PHKs other than CheA, we are also exploring the cAMP-binding activities of several other well-characterized PHKs: EnvZ, QseC, PhoQ, CpxA, which also operate in *E. coli*. Our in vitro results will increase our understanding of how the activities of PHKs are controlled in living cells.

This research was supported by the Howard Hughes Medical Institute Undergraduate Research Fellowship.

HOFMEISTER ION AND COSOLVENT EFFECTS ON THE STRUCTURE, AGGREGATION, AND BACKBONE SOLVATION OF RECA

Taylor P. Light, Karen M. Corbett, Michael A. Metrick, and Gina MacDonald
Department of Chemistry and Biochemistry, James Madison University,
800 S. Main Street, Harrisonburg, VA 22807

RecA is an *Escherichia coli* protein that catalyzes the strand exchange reaction utilized in DNA repair. Previous studies have shown that the presence of salts influence RecA activity, aggregation, and stability. Here we utilized attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy and circular dichroism (CD) to further investigate how various Hofmeister salts and cosolvents alter RecA structure, aggregation, and solvation. Spectroscopic studies performed in water and deuterium oxide suggest that salts alter amide I (or I') and amide II (or II') vibrations arising from the protein backbone. Specific infrared vibrations that may arise from protein-solvent interactions were identified. Infrared vibrations that correlate with protein desolvation were observed in the presence of strongly hydrated SO_4^{2-} anions. The vibrations that correlate with protein solvation were observed in the presence of weakly hydrated Cl^- and ClO_4^- anions. Additional experiments were performed under solution conditions known to influence protein-solvent and protein-water interactions. An increase in the infrared frequency of amide I (or I') correlated with increasing concentrations of trifluoroethanol (TFE) and sucrose. This result suggests an increase in desolvation of the amide backbone with an increase in the concentration of cosolvents. Additionally, increasing concentrations of TFE resulted in an increase in RecA aggregation. These results show that salts and cosolvents alter the solvation water surrounding proteins and influences overall structure and aggregation.

This research was funded by NSF-RUI grant CHE-0814716 and NSF-REU grant CHE–1062629.

IDENTIFICATION OF THE STRESS-DEPENDENT INTERACTOME OF O-GLCNACASE

Austin O. Maduka[1], Jennifer A. Groves[2], and Natasha E. Zachara, Ph.D.[2]
[1]Department of Chemistry and Biochemistry, UMBC,
1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biological Chemistry, The Johns Hopkins University,
School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

Cardiovascular disease, including ischemia-reperfusion injury (from heart attacks), is a leading cause of death worldwide. Recent studies of the endogenous protective mechanisms of the heart provide insight into the pathophysiology of heart attacks and highlight possible new directions for the development of innovative cardioprotective therapeutics.

O-linked-β-N-acetylglucosamine (O-GlcNAc) is a post-translational modification of nuclear, cytoplasmic, and mitochondrial proteins that is considered protective as it regulates the cell's response to various types of stress including ischemia-reperfusion injury. The O-GlcNAc transferase (OGT) and the O-GlcNAcase (OGA) enzymes are responsible for adding and removing the O-GlcNAc sugar modification from proteins, respectively. However, the mechanism by which cells/tissues communicate with these enzymes during times of stress remains elusive. The ultimate goal is to understand how cells regulate OGT and OGA in response to oxidative stress to promote cell survival and cardioprotection.

Our research focuses on identifying proteins that interact with and regulate OGA in response to oxidative stress. A mass spectrometry screen was previously performed using hydrogen peroxide stressed cells (to mimic ischemia-reperfusion injury), in which the BioID method was used to biotinylate and isolate interacting proteins of an OGA-biotin ligase fusion protein *in vivo*. This technique was used in conjunction with western-blotting to validate protein-protein interactions. At two hours of oxidative stress treatment, mTOR, a global regulator of metabolism, and GRP75, a mitochondrial protein of the HSP70 family, were biotinylated. Additionally, OGT was biotinylated under normal and stressed conditions. Validating additional protein-protein interactions, as well as understanding the physiological role of these interactions will provide insight into the mechanisms by which OGA is regulated in response to cellular stress, allowing the possibility to improve treatment for patients experiencing ischemia-reperfusion injury.

This research was supported in part by a training grant from the National Heart, Lung, and Blood Institute (NHLBI) to the Zachara Lab at the Johns Hopkins University School of Medicine (P01 HL107153).

CHARACTERIZATION OF A DICTYOSTELIUM DISCOIDEUM DCP2 GENETIC KNOCKOUT

Marlee Nelson, Kirsten Bickford, and Susan Parrish
Department of Biology, McDaniel College, 2 College Hill, Westminster, MD 21157

Dictyostelium discoideum is a unicellular amoeba that, when starved of nutrients, aggregates to form a multicellular fruiting body. *D. discoideum* encodes the putative DDB_G0283315 protein which is homologous to yeast DCP2, an enzyme that contains a conserved Nudix hydrolase motif, characteristic of enzymes that cleave nucleoside diphosphates linked to another moiety. The yeast DCP2 enzyme has been shown to cleave the 5' m7GpppNm-cap from mature mRNAs, thereby causing degradation of the mRNA and inhibiting translation. Hence, DCP2 regulates gene expression by modulating mRNA translation. Since *D. discoideum* development is accomplished by rapid changes in gene expression, the *D. discoideum* DCP2 may have a role in modulating gene expression during the transition to multicellularity. To delete the *D. discoideum* DCP2 gene from the genome, a cassette was created that contained the blasticidin resistance gene surrounded by the flanking regions of the *D. discoideum* gene. Following transformation and selection with blasticidin, the *D. discoideum* DCP2 gene was deleted from the genome by homologous recombination, creating the DCP2 knockout (DCP2 KO). PCR amplification of the DCP2 gene in the DCP2 KO did not produce the expected 1863 bp DCP2 band, confirming gene removal. The DCP2 KO cells grew in axenic cultures at a similar rate as wildtype cells, suggesting vegetative growth was not impaired. To evaluate the role of DCP2 during multicellular development, the DCP2 KO cells were starved in parallel to wildtype cells. Although DCP2 KO cells could complete development, there appeared to be a slight delay in the development process as the cells transitioned from tipped mounds to fingers; therefore the DCP2 enzyme may perform an important regulatory function during *D. discoideum* development by decapping mature mRNA transcripts and thereby enhancing mRNA turnover. Future studies will examine levels of developmentally regulated mRNAs in the DCP2 KO in comparison to wildtype cells.

STRUCTURAL AND FUNCTIONAL ANALYSIS OF DISEASE CAUSING MUTATIONS IN THE M10 DOMAIN OF TITIN

Michael W. Rudloff and Nathan T. Wright
Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807

Titin (3-4MDa) is the largest monomeric protein found in humans and is crucial for the overall structural stability and function of muscle tissues. Dubbed the "molecular ruler", titin sets the length of the sarcomere while ensuring the structure is organized. While titin binds to many targets, the extreme C-terminal domain (M10) provides a critical function by binding to the extreme N-terminal domain (Ig1) of the giant muscle protein obscurin. Mutations in the M10 domain of titin have been linked to cases of limb girdle muscular dystrophy 2J (LGMD2J), most likely due to an ablation of M10-Ig1 binding. Here, we have recombinantly expressed and purified wild type M10 and all known human M10 mutations. Initial structural characterization using Circular Dichroism (CD) indicates that while the wild-type domain is predominantly beta sheet, most mutations are only partially folded, although each has a distinct structural phenotype. Isothermal Titration Calorimetry (ITC) studies demonstrate that misfolded mutants do not bind to Ig1, however one mutant (Belgian) binds to Ig1 with a similar affinity to wild-type. Protein NMR spectroscopy confirms these findings, as sequence specifically assigned HSQCs of M10 unbound and bound to Ig1 show no differences when compared to Belgian mutation counterparts. NMR dynamic studies have also revealed Belgian mutation has identical slow-timescale dynamic motion and highly similar fast-timescale dynamic motion, with the exception of isolated residues near the mutation site. Altogether these data suggest that the Belgian mutation is likely silent and therefore not responsible for disease propagation.

SEARCHING FOR HEMIMETHYLATION IN IMPRINTED GENES: DNA METHYLATION PATTERNS AT *Gtl2*

Ekaterina Vlasova and Tamara Davis
Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010

A small fraction of mammalian genes is regulated via genomic imprinting. During oogenesis and spermatogenesis, modifications to the chromatin in the developing gametes influence which genes are accessible for subsequent transcription. The mechanisms for this epigenetic regulation include DNA methylation and histone modification. For approximately 150 genes, genomic imprinting results in monoallelic gene expression. Abnormalities in gene imprinting cause defects in growth, a variety of inherited diseases, and cancer.

Previous analysis of the differentially methylated region (DMR) of imprinted gene *Dlk1* revealed an unexpected asymmetry of DNA methylation: approximately 35% of the complementary CpG dyads were hemimethylated, a significant deviation from the expected 5% of hemimethylation typically attributed to flaws in methyltransferase activity. We hypothesized that this trend could result from several factors: (1) there may be a lower level of methyltransferase fidelity associated with the maintenance of DNA methylation at secondary DMRs, and (2) the presence of a novel type of methylation in the form of 5-hydroxymethylcytosine may prevent the methyltransferase from functioning to its expected capacity, resulting in elevated levels of hemimethylation.

In order to test whether this high degree of hemimethylation is unique to *Dlk1* or if it is a hallmark of other imprinted genes, we analyzed hemimethylation patterns at the *Gtl2*-DMR. We analyzed the *Gtl2*-DMR at three stages of development: 14.5 dpc embryo, 5 dpp liver, and adult liver in B6xC9C12 mice. We found that the average hemimethylation levels at the *Dlk1*-DMR and *Gtl2*-DMR across several developmental stages is greater than the standard genomic rate of hemimethylation (5%). Furthermore, we found that on average *Dlk1* has a higher level of hemimethylation than *Gtl2*. We concluded that imprinted genes *Gtl2* and *Dlk1* show unexpectedly high rates of hemimethylation. These findings warrant further examination of DNA methylation patterns at CpG dyads in imprinted genes.

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Morning Poster Session
Group P – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

104. **POSTJUNCTIONAL MUSCARINIC M2 RECEPTORS REGULATE AIRWAY SMOOTH MUSCLE CONTRACTION**

Olubukola Abiona[1], Qi Liu[2], and Brendan Canning[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Pulmonary & Critical Care Medicine,
Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle,
Baltimore, MD 21224

105. **CHARACTERIZING A NOVEL PROTEIN INTERACTION INVOLVING ACETYL-COA CARBOXYLASE**

Jarreau Harrison[1], Jay J Thelen[2], and Matthew Salie[2]
[1]Department of Biology, Medgar Evers College, Brooklyn, NY 11225
[2]Department of Biochemistry, University of Missouri-Columbia, MO 65211

106. **KSHV vFLIP INTERACTS WITH THE A20 UBIQUITIN EDITING COMPLEX**

Garrett Heck, Kevin Herold, Daniela Rey Ardila, and Elana S. Ehrlich
[1]Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252

107. **TARGETING AND BINDING OF THE FELINE IMMUNODEFICIENCY VIRUS MATRIX PROTEIN TO PLASMA MEMBRANE ASSEMBLY SITES**

Morgan B. Moser, Janae L. Baptiste, and Michael F. Summers
Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, UMBC,
1000 Hilltop Circle, Baltimore, MD 21250

108. **THE EFFECT OF NEIGHBORING RESIDUES ON PEROXIDASE ACTIVITIES OF SELENOPROTEINS**

Jasmin Philip, Fei Li, and Sharon Rozovsky
Department of Biochemistry, University of Delaware, 163 The Green, Newark, DE 19716

109. **CHARACTERIZATION OF THE HIV-1 5'UTR DIMERIZATION MECHANISM**

Justin Leonel C. Santos[1], Alyssa T. Florwick[1], and G. Campbell Carter[1],
Sarah C. Keane[1], Xiao Heng[1,2], and Michael F. Summers[1]
[1]Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, UMBC,
1000 Hilltop Circle, Baltimore, MD 21052
[2]Biochemistry 117, Schweitzer Hall, University of Missouri Columbia, MO 65211

110. **SEMIQUNONE STABILIZATION VIA *DE NOVO* DESIGN PROTEIN SCAFFOLD**

Ivan Sokirniy[1], Christen Biernat[1], Gözde Ulas[2], William F DeGrado[2], and Amanda J Reig[1]
[1]Department of Biochemistry and Molecular Biology, Ursinus College,
601 E. Main Street, Collegeville, PA 19426
[2]Department of Pharmaceutical Chemistry, 555 Mission Bay Boulevard South, San Francisco, CA 94158

POSTJUNCTIONAL MUSCARINIC M2 RECEPTORS REGULATE AIRWAY SMOOTH MUSCLE CONTRACTION

Olubukola Abiona[1], Qi Liu[2], and Brendan Canning[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Pulmonary & Critical Care Medicine,
Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle,
Baltimore, MD 21224

Online access of this abstract is restricted at the request of the Principal Investigator.

CHARACTERIZING A NOVEL PROTEIN INTERACTION INVOLVING ACETYL-COA CARBOXYLASE

Jarreau Harrison[1], Jay J Thelen[2], and Matthew Salie[2]
[1]Department of Biology, Medgar Evers College, Brooklyn, NY 11225
[2]Department of Biochemistry, University of Missouri-Columbia, MO 65211

The demand for oils for agricultural and industrial use is increasing due to increasing population. Crop plants used to produce these oils, such as soybean, are not able to provide enough oil to meet the growing global demand. The *de novo* fatty acid synthesis (FAS) pathway in the chloroplast is responsible for producing the oil that is stored in the seed. The first and committed step of this pathway is catalyzed by acetyl-CoA carboxylase (ACCase), where acetyl-CoA is carboxylated to malonyl-CoA. ACCase is known to be highly regulated, but the specific regulatory mechanisms are still unknown. In dicots and non-graminaceous monocots, chloroplast ACCase is made up of four subunits: biotin carboxylase, biotin carboxyl carrier protein (BCCP1 and BCCP2), and α- and β- carboxyltransferase. The BCCP subunit is biotinylated, which allows for the carboxylation of acetyl-CoA. In previous work, we precipitated ACCase from isolated *Arabidopsis thaliana* chloroplasts using a BCCP2-specific antibody and observed co-precipitation of a chloroplast-localized protein termed 'Protein X'. We hypothesize that protein X has a regulatory role in FAS by acting as another BCCP subunit. Since BCCP is biotinylated and able to dimerize, Protein X must also have these qualities. To test this hypothesis, we analyzed recombinant Protein X using western blotting and mass spectrometry. To determine if Protein X can dimerize with the BCCP isoforms, we performed *in vitro* dimerization assays and then resolved the resulting dimers with Blue Native PAGE. Western blotting and mass spectrometry analysis showed that recombinant Protein X can dimerize but is not biotinylated in *E. coli*. Amino acid sequence alignment shows evidence of a biotinylation site at Lys253 in Arabidopsis. This study confirms the homo dimerization of Protein X and the absence of biotinylation of Protein X expressed in E. *coli*.

KSHV vFLIP INTERACTS WITH THE A20 UBIQUITIN EDITING COMPLEX

Garrett Heck, Kevin Herold, Daniela Rey Ardila, and Elana S. Ehrlich
[1]Department of Biological Sciences, Towson University, 8000 York Road,
Towson, MD 21252

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TARGETING AND BINDING OF THE FELINE IMMUNODEFICIENCY VIRUS MATRIX PROTEIN TO PLASMA MEMBRANE ASSEMBLY SITES

Morgan B. Moser, Janae L. Baptiste, and Michael F. Summers
Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, UMBC,
1000 Hilltop Circle, Baltimore, MD 21250

The feline immunodeficiency virus (FIV) is a lentivirus that impairs the immune response of an infected cat, much like the human immunodeficiency virus (HIV) in humans. An integral step of this retroviral replication cycle is the localization of the Gag polyprotein (Gag) to the plasma membrane of the host cell. Gag is composed of three major components, one of which is the matrix domain (MA), a protein that is responsible for anchoring Gag to the plasma membrane. A mechanism by which HIV-1 MA binds to the plasma membrane, known as the extended lipid model, has been proposed. This work aims to analyze the method FIV MA utilizes to target and bind to the plasma membrane by employing liposomes as mimetic membranes. High resolution nuclear magnetic resonance (NMR) is used to investigate the interaction between FIV MA and the liposomes. By studying and comparing FIV MA membrane targeting to that of HIV-1 MA, cats may serve as an animal model for HIV-1 treatment.

Dr. Michael Summers, Dr. Holly Summers, Janae Baptiste, Colin O'Hearn, Tarik Hawkins, Howard Hughes Medical Institute, Chemistry and Biochemistry Department, NIH/NIAID 5R37AI030917

THE EFFECT OF NEIGHBORING RESIDUES ON PEROXIDASE ACTIVITIES OF SELENOPROTEINS

Jasmin Philip, Fei Li, and Sharon Rozovsky
Department of Biochemistry, University of Delaware, 163 The Green, Newark, DE 19716

Although the many functions of selenoproteins are largely unknown, they play an important role in organism's cellular functions. Some selenoproteins, like SelS, manage oxidative stress and are essential for fighting against free radicals in the human body. Therefore, knowing the peroxidase activity of these proteins is of the utmost importance, as it gives insight on how well these proteins fight oxidative stress. The protein used for this project is Sec-Trx, which is a scaffold protein that has a selenium-containing redox motif and is used for mass spectroscopy and for obtaining redox potentials. In order to determine the antioxidant activity of each Sec-Trx mutant, we conducted peroxidase assays to ascertain their peroxidase activities. From running these assays, it was determined that there is a slight difference in peroxidase activities for each Sec-Trx mutant.

CHARACTERIZATION OF THE HIV-1 5'UTR DIMERIZATION MECHANISM

Justin Leonel C. Santos[1], Alyssa T. Florwick[1], and G. Campbell Carter[1],
Sarah C. Keane[1], Xiao Heng[1,2], and Michael F. Summers[1]
[1]Department of Chemistry and Biochemistry, Howard Hughes Medical Institute,
UMBC, 1000 Hilltop Circle, Baltimore, MD 21052
[2]Biochemistry 117, Schweitzer Hall, University of Missouri Columbia, MO 65211

Human Immunodeficiency Virus Type-1 (HIV-1) is responsible for a pandemic that affects roughly 35 million people worldwide. To date, there is no cure, and current antiretroviral therapies only target four main stages of the viral life cycle: entry, reverse transcription, integration, and maturation. Genome selection for packaging, however, is a relatively less studied part of the viral life cycle, thus no current therapies that target this process have been developed. HIV-1 genome packaging has been shown to be a highly selective process, in which only dimeric, unspliced RNAs are recognized by the *Gag* polyprotein for encapsidation. The nature of dimerization, which occurs at the 5' untranslated region (5'-UTR) of the genome, is hypothesized to be either a "kissing" interaction involving the six nucleotide palindromic (GCGCGC) dimerization initiation site (DIS) sequence, or an extended dimer conformation featuring a more extensive intermolecular interface . Previous studies in our lab demonstrate that the HIV-1 5'-UTR exists as an extended dimer at high concentrations and after long incubation times at 37 °C. Furthermore, the U5:AUG (Unique 5' Element: Gag start codon) region has been identified as one of the sites with an intermolecular interface. Thus, we hypothesize that dimer formation is initiated by "kissing" interactions and transitions to an extended conformation over time. To test this we utilize NMR spectroscopy using a mutagenesis strategy known as long-range Adenosine Interaction Detection (lr-AID). Our preliminary 1D NMR data collected on purified small U5:AUG control RNAs containing either UUA/UAA or UUG/UAA lr-AID mutations reveal chemical shifts at 6.5 ppm, and 6.7 ppm, respectively. Our next step is to incorporate either the UUA/UAA or the UUG/UAA sequence within the U5:AUG region of the 5'-UTR. RNA samples will then be prepared with specific deuterium labeling in order to probe the dimer conformation.

This research was funded by NIH/NIGMS grant *1P50GM103297* and was conducted at the Howard Hughes Medical Institute at UMBC, with support from the Summer Biomedical Training Program (SBTP). Supported in part by the Howard Hughes Medical Institute's Precollege and Undergraduate Science Education Program. I'd also like to thank our research team – Seung Ho Choi, Sayo McCowin, and Briaunna Minor, for their assistance.

SEMIQUNONE STABILIZATION VIA *DE NOVO* DESIGN PROTEIN SCAFFOLD

Ivan Sokirniy[1], Christen Biernat[1], Gözde Ulas[2], William F DeGrado[2], and Amanda J Reig[1]
[1]Department of Biochemistry and Molecular Biology, Ursinus College,
601 E. Main Street, Collegeville, PA 19426
[2]Department of Pharmaceutical Chemistry, 555 Mission Bay Boulevard South,
San Francisco, CA 94158

Catecholic compounds are ubiquitous in nature. Enzymatic oxidation of catechol species is thought to play a role in formation of reactive oxygen species (ROS) in the cell. Certain natural metalloproteins form the semiquinone (SQ) radical as a catalytic intermediate, such as in catecholic estrogen-mediated cytotoxicity and mutagenesis. However, while several synthetic inorganic complexes have been shown to stabilize a SQ species, few protein-based systems are able to stabilize this intermediate. In this work, the well-established *de* novo designed Due Ferri (DF) protein scaffold, a diiron four helix bundle motif, was used to model the coordination chemistry involved in SQ radical formation and stabilization. The SQ complex was formed using a variety of first row transition metals and has been detected by electron paramagnetic resonance and ultraviolet-visible spectroscopy. Varying the ligand composition at the metal coordination site of the single stranded DF protein permitted fine-tuning of SQ radical stabilization. This type of protein design can allow us to elucidate structure-function relationship of many natural metal active sites.

This work was supported by Ursinus College, Pearlstine Faculty Fellowship Fund, and National Institutes of Health (GM110657).

Morning Poster Session
Group Q – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

111. **MUTATION IN MITF IMPLICATED IN DYSFUNCTIONAL PIGMENTATION AND AUDITORY SENSORY IN SPANISH MUSTANG PEDIGREE**

Alecia M Achimovich and Dr. Koren Lipsett
Department of Chemistry, Gettysburg College, 300 N. Washington Street, Gettysburg, PA 17325

112. **INVESTIGATING THE ROLE OF MIRNAS IN SIV-INFECTED AND IFN-ß TREATED MACAQUE ASTROCYTES**

Diego A. Espinoza, Erin L. Buchanan and Kenneth W. Witwer
Department of Molecular and Comparative Pathobiology, Johns Hopkins University,
733 N. Broadway, Edward D. Miller Research Building, Room 829, Baltimore, MD 21205

113. **THE DYNAMICS INVOLVED IN ALLOSTERIC INHIBITION OF THE HEPATITIS C VIRUS POLYMERASE**

Marie Espiritu, Daniel Dagenhart, Ester Sesmero, and Dr. Ian Thorpe
Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

114. **CHARACTERIZING EPIGENETIC REGULATION OF GENE EXPRESSION IN THE DEVELOPING CHICKEN RETINA**

E. Grunwald [1], N. Dunham[1], S. Brown[1], G. Espina[2], L. Liotta[2], and R. Enke[1]
[1]Department of Biology, James Madison University, 951 Carrier Drive, Harrisonburg, VA 22807
[2]Center for Applied Proteomics and Molecular Medicine, George Mason University,
10900 University Boulevard, Manassas, VA 20110

115. **IDENTIFICATION AND CHARACTERIZATION OF THE SMALL PROTEIN ENCODED BY THE *tor*R_*tor*C_0 GENE IN *E. coli***

Shawn Hirsch[1] and Matthew Hemm[2]
[1]Molecular Biology, Biochemistry and Bioinformatics Program, Towson University,
8000 York Road, Towson, MD 21252
[2]Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252

116. **CLONING OF THE HIGHLY ALTERNATIVELY-SPLICED *NEUREXIN* cDNAs IN *DANIO RERIO***

Majesta Kitts and Cheng Huang
Department of Biology, McDaniel College, 2 College Hill, Westminster, MD 21157

117. **INVESTIGATION OF DIMERIZATION MECHANISMS IN THE 5'-UNTRANSLATED REGION IN SIMIAN IMMUNODEFICIENCY VIRUS IN CHIMPANZEES**

Ae Lim (Ally) Yang, Jessica Zaki, Michelle Seu, Thao Tran, and Dr. Michael F. Summers
Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, UMBC,
1000 Hilltop Circle, Baltimore, MD 21250

MUTATION IN MITF IMPLICATED IN DYSFUNCTIONAL PIGMENTATION AND AUDITORY SENSORY IN SPANISH MUSTANG PEDIGREE

Alecia M Achimovich and Dr. Koren Lipsett
Department of Chemistry, Gettysburg College, 300 N. Washington Street, Gettysburg, PA 17325

It has been observed in a pedigree of Spanish mustangs that there is a correlation between congenital deafness and lack of pigmentation. It is postulated that an integral gene to melanocyte function or migration is dysfunctional in the affected individuals. A genetic basis for this correlation was investigated by conducting a candidate gene study in which genes were chosen on the basis of correlation to similar disease states in humans such as Waardenburg Syndrome and role in the pigment production pathway. MITF is a transcription factor which acts as a regulator of the expression of genes coding for proteins necessary for the differentiation of melanocytes. The amplification of this position in the genome across the pedigree of horses revealed a G to A single nucleotide polymorphism (SNP), resulting in a substitution of the glycine residue for serine. All but one horse possessed the non-polar gylcine bearing genotype. The polar substitution was found to not correlate with the observed white-deaf trait. However, published horse genomes characterize the position in question with the serine residue rather than the glycine residue. If the wild-type phenotype confers a necessary component of coloration or auditory homeostasis, it is possible that the mutated form of the gene present in both white, deaf horses participates in an additive effect with other active or unknown mutated genes, producing the disease state phenotype.

I would like to thank the Gettysburg College Chemistry department, Howard Hughes Medical Institute through the Precollege and Undergraduate Science Education Program and Dr. Koren Lipsett for their support of the conducted research as presented.

INVESTIGATING THE ROLE OF MIRNAS IN SIV-INFECTED AND IFN-ß TREATED MACAQUE ASTROCYTES

Diego A. Espinoza, Erin L. Buchanan and Kenneth W. Witwer
Department of Molecular and Comparative Pathobiology, Johns Hopkins University,
733 N. Broadway, Edward D. Miller Research Building, Room 829, Baltimore, MD 21205

Background

The astrocyte, the most abundant cell of the central nervous system (CNS), is susceptible to infection by human and simian immunodeficiency viruses (HIV and SIV) but does not typically produce infectious virions. Since the astrocyte is a potentially vast latent reservoir of HIV, the mechanisms whereby it maintains HIV latency are of interest. microRNAs (miRNAs) are short effectors of RNA interference (RNAi) that respond to signals such as cytokines and may regulate the host response to infection as well as control the virus directly. In addition to host miRNAs, infected cells may contain small RNAs that are processed from viral transcripts.

Research question

How do miRNAs change during SIV infection and cytokine treatment of astrocytes, and do these changes have a possible impact on viral latency?

Approach and experiments

Primary rhesus astrocytes were infected or not with SIV and treated or not with the cytokine interferon beta (IFN-β). RNA was extracted from cells at time points from 2 to 21 days post infection. Microarrays, quantitative PCR, and RNA-Seq were used to profile small RNAs and longer RNAs.

Results and conclusions

Multiple profiling platforms confirm differential expression of miRNAs across the experimental conditions, while RNA sequencing suggests differentially expressed mRNA transcripts, such as MHC Class I genes. Interestingly, small RNA-Seq data indicate the presence of SIV-derived small RNAs that are distinct from small RNAs previously reported to be processed from HIV transcripts. The roles of these small RNAs in SIV infection and latency are the subject of planned experiments.

This project was supported by a Provost's Undergraduate Research Award (Johns Hopkins University), a Dean's Undergraduate Research Award (Johns Hopkins University), and the Center for AIDS Research Baltimore Scholars Program (Johns Hopkins University School of Medicine), all of which were awarded to Diego A. Espinoza.

THE DYNAMICS INVOLVED IN ALLOSTERIC INHIBITION OF THE HEPATITIS C VIRUS POLYMERASE

Marie Espiritu, Daniel Dagenhart, Ester Sesmero, and Dr. Ian Thorpe
Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

The Hepatitis C Virus (HCV) is a global epidemic, affecting 3% of the world's population. It is a blood-borne virus that ultimately leads to cirrhosis and liver cancer. HCV evades the body's natural defenses by mutating itself during the replication process. The HCV polymerase, NS5B, is critical to replication and is, therefore, a target to treat HCV. Non-nucleoside inhibitors (NNIs), like the recently approved drug Sofosbuvir, have been shown to be very good candidates for HCV treatment. These inhibitors bind allosterically, outside the active site of the NS5B enzyme. Two such NNIs are the ligands AG0 and AG6 that bind to the thumb and finger domain respectively. In our study, we utilize Molecular Dynamics (MD) simulations of AG0 and AG6 bound simultaneously to NS5B. With these simulations, our goal is to analyze the effects of AG0 and AG6 on the polymerase's structure, dynamics and function in order to determine how this differs from the effect of each ligand in isolation. Elucidating the mechanism of inhibition of these ligands may provide information that could be key in finding more effective treatments for HCV.

CHARACTERIZING EPIGENETIC REGULATION OF GENE EXPRESSION IN THE DEVELOPING CHICKEN RETINA

E. Grunwald [1], N. Dunham[1], S. Brown[1], G. Espina[2], L. Liotta[2], and R. Enke[1]
[1]Department of Biology, James Madison University, 951 Carrier Drive, Harrisonburg, VA 22807
[2]Center for Applied Proteomics and Molecular Medicine, George Mason University, 10900 University Boulevard, Manassas, VA 20110

The physical world is visualized through the retina, a layer of neuronal cells that lines the inside of the eye. These retinal neurons transmit signals to the brain creating images. Our lab focuses on how epigenetic modifications, namely DNA methylation, determine the fate of retinal neuron differentiation and the expression of retina-specific genes throughout the development of chicken embryos. In this study, whole retina samples were subject to DNA and RNA analysis. RNA expression and DNA methylation analysis were conducted for the *Rbp3* and *Pde6c* genes in early and late chicken embryo retinas. DNA methylation analysis was completed utilizing bisulfite pyrosequencing. RNA analysis was accomplished using quantitative reverse transcriptase PCR (qRT-PCR). Our results in whole retina demonstrate an inverse correlation between DNA methylation near the transcriptional start site and the expression of the retina-specific genes, *Rbp3* and *Pde6c*. We are currently analyzing DNA extracts from our cell-specific enriched laser capture microdissection (LCM) cell fractions to determine the identity of neurons with methylated versus demethylated genes. Future directions will focus on expansion of DNA and RNA analysis to other retina-specific genes and neurons from retinal tissue subpopulations, as well as characterizing DNA methyltransferase enzymes within the retina. Collectively, these data will help us better understand the differentiation process of neurons in the vertebrate retina. This information may be useful for developing novel early detection and therapeutic strategies for human diseases and disorders associated with retinal degeneration.

This project was funded by a 4-VA Minigrant and JMU startup funds. We also thank George's Foods for providing chicken eggs used in this project.

IDENTIFICATION AND CHARACTERIZATION OF THE SMALL PROTEIN ENCODED BY THE *tor*R_*tor*C_0 GENE IN *E. coli*

Shawn Hirsch[1] and Matthew Hemm[2]
[1]Molecular Biology, Biochemistry and Bioinformatics Program, Towson University, 8000 York Road, Towson, MD 21252
[2]Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252

Online access of this abstract is restricted at the request of the Principal Investigator.

CLONING OF THE HIGHLY ALTERNATIVELY-SPLICED *NEUREXIN* cDNAs IN *DANIO RERIO*

Majesta Kitts and Cheng Huang
Department of Biology, McDaniel College, 2 College Hill, Westminster, MD 21157

The genetic regulation of cell fate specification is a fundamental question in developmental biology. We use blood cell specification in the zebrafish *(Danio rerio)* as a specific model system to address this broad question, and have identified a novel genetic regulator of blood cell specification, the *neuer1* gene.

By performing protein domain analyses, we determined that the most prominent domain in the Neuer1 protein is shared by the Neurexophilin proteins, a family of cell-signaling ligands, raising the exciting possibility that Neuer1 may also function as a signaling ligand. Neurexophilins bind to the family of receptors known as Neurexins, begging the question of whether Neuer1 binds to one of the Neurexins as well. We reason that if we determine the expression pattern of each *neurexin* gene, the one that shares the same expression pattern as *neuer1* is likely to encode the receptor of the tentative Neuer1 ligand.

In order to determine the expression pattern of each *neurexin* gene using *in situ* hybridization, a riboprobe complimentary to each *neurexin* mRNA needs to be synthesized, mandating the cloning of each *neurexin* cDNA. Here we report our devised strategy to clone the *neurexin* cDNAs that represent an extremely high level of alternative splicing, and the successful cloning of some of these cDNA isoforms. This work lays important foundation for our efforts to identify the potential receptor for the novel Neuer1.

This undergraduate research project was funded by the Student-Faculty Collaborative Summer Research Fund, as well as the Department of Biology, of McDaniel College.

INVESTIGATION OF DIMERIZATION MECHANISMS IN THE 5'-UNTRANSLATED REGION IN SIMIAN IMMUNODEFICIENCY VIRUS IN CHIMPANZEES

Ae Lim (Ally) Yang, Jessica Zaki, Michelle Seu, Thao Tran, and Dr. Michael F. Summers
Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, UMBC,
1000 Hilltop Circle, Baltimore, MD 21250

Acquired Immunodeficiency Syndrome (AIDS) is caused by Human Immunodeficiency Viruses (HIV). HIV is the consequence of cross-species transmission of Simian Immunodeficiency Virus (SIV). SIVs have been present in monkeys for at least 32,000 years.

In order for a virus to thrive, it needs to go through a retroviral life cycle. Dimerization in the 5'- Untranslated Region (5'-UTR) is an essential process in the retroviral life cycle, but the mechanism is not clear. Therefore, we will be analyzing the viral dimerization using 5'-UTR RNA of SIVcpz_TAN1 strain. We hypothesize that dimerization of SIV constructs will behave similarly to the dimerization of its corresponding HIV.

Three SIVcpz RNA constructs: wild type 5'-UTR (wt), truncated AUG (trAUG), and deleted AUG (dAUG) were used to test the hypothesis. Using gel electrophoresis method, we investigated the difference in the dimerization of SIVcpz in TB and 0.2 TBM buffer. Thus far, the gels have shown dimeric confirmation in 0.2TBM but not in TB. However, in the same condition with HIV-1 constructs, the tests showed dimeric confirmation in both conditions. We suspect that SIV exhibits labile dimers. Further studies are in progress to explain the labile dimers.

Studying the 5'-UTR of SIVcpz will enhance our understanding of its structural biology and how it is related to HIV viral evolution. Ultimately, we seek to aid in the development of retroviral drug for AIDS and HIV patients.

This research was funded by NIH/NIGMS 5R01GM042561

Morning Poster Session
Group R – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

118. **STRUCTURE AND MOLECULAR DYNAMICS OF THE IG58/59 DOMAINS OF OBSCURIN**

Tracy A. Caldwell and Nathan T. Wright
Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807

119. **GLUTAMINE METABOLISM AS A DETERMINANT OF CELL PROLIFERATION, ABERRANT 2-HYDROXYGLUTARATE ACCUMULATION, AND PATIENT SURVIVAL IN BREAST CANCER.**

Ashlie Feldman[1,2], Tiffany Dorsey[2], Prachi Mishra[2], and Stefan Ambs[2]
[1]Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252
[2]Laboratory of Human Carcinogenesis, Center for Cancer Research (CCR),
National Cancer Institute (NCI), National Institutes of Health, 31 Center Drive, Bethesda, MD 20892

120. **EVALUATION OF HETEROAROTINOIDS FOR BIOLOGICAL RESPONSE IN HUMAN ERYTHROLEUKEMIA CELLS**

Jaclyn Highland and Paul J. Birckbichler
Department of Chemistry, Slippery Rock University, 1 Morrow Way, Slippery Rock, PA 16057

121. **REGULATION OF T-TYPE CALCIUM CHANNEL EXPRESSION BY DNA-ALTERING COMPOUNDS IN PROSTATE CANCER CELLS**

Ezechielle Kiessu[1], Erika Weaver[1], Jennifer Hearne[1], and Miguel Martin-Caraballo[2]
[1]Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne MD 21853
[2]Department of Pharmaceutical Sciences, University of Maryland Eastern Shore, Princess Anne MD 21853

122. **INVESTIGATING CELLULAR IMMUNITY USING THE SIMIAN VIRUS 40 LARGE TUMOR ANTIGEN**

Matthew Lauver[1], Caitlin Mason[2], Caitlin McGee[2], Caroline Passmore[1],
Stephanie Schell[1], and Lawrence Mylin[1]
[1]Department of Biological Sciences, Messiah College,
One College Avenue Suite 3030, Mechanicsburg, PA 17055
[2]Department of Chemistry and Biochemistry, Messiah College,
One College Avenue Suite 3030, Mechanicsburg, PA 17055

123. **CTL REACTIVITY AGAINST POTE-DERIVED PEPTIDES IN PROSTATE CANCER PATIENTS**

Yi Liu[1], Masaki Terabe[2], Lauren V. Wood[2], and Jay A. Berzofsky[2]
[1] Department of Biological Science, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Vaccine Branch, Center for Cancer Research, National Cancer Institute,
National Institutes of Health, Bethesda, MD 20892

124. **ADHESION ANALYSIS OF HUMAN ERYTHROLEUKEMIA CELLS TO HUMAN LUNG FIBROBLASTS**

Jessica Mols and Paul J. Birckbichler
Department of Chemistry, Slippery Rock University, 1 Morrow Way, Slippery Rock, PA 16057

STRUCTURE AND MOLECULAR DYNAMICS OF THE IG58/59 DOMAINS OF OBSCURIN

Tracy A. Caldwell and Nathan T. Wright
Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807

Obscurin (800-900 kDa) is a giant muscle protein vital to muscle cell maintenance and organization. It is the only known connection between the contractile apparatus and the sarcoplasmic reticulum and also binds to specific cytoskeletal, signaling, or membrane-associated proteins. Obscurin domains Ig58/59 binds to titin ZIg9/10, and this binding event is hypothesized to stabilize the sarcomeric cytoskeleton. Mutations in this obscurin region lead to malformed muscle architecture and, eventually, to hypertrophic cardiomyopathy (HCM). For obscurin/titin binding to occur, all four domains must be present. In order to fully characterize this physiologically important region of obscurin, and by extension determine the molecular factors that drive HCM, here we describe Ig59 structure determination via both X-ray crystallography and heteronuclear multidimensional NMR spectra. The dynamics and the energetics of unfolding of both obscurin domains are also analyzed through molecular dynamics and steered molecular dynamics.

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GLUTAMINE METABOLISM AS A DETERMINANT OF CELL PROLIFERATION, ABERRANT 2-HYDROXYGLUTARATE ACCUMULATION, AND PATIENT SURVIVAL IN BREAST CANCER.

Ashlie Feldman[1,2], Tiffany Dorsey[2], Prachi Mishra[2], and Stefan Ambs[2]
[1]Department of Biological Sciences, Towson University,
8000 York Road, Towson, MD 21252
[2]Laboratory of Human Carcinogenesis, Center for Cancer Research (CCR),
National Cancer Institute (NCI), National Institutes of Health,
31 Center Drive, Bethesda, MD 20892

Breast cancer cell metabolism is dependent on nutrients such as glutamine for continued proliferation. Thus, targeting glutamine metabolism may offer opportunities to slow tumor growth in patients. We recently observed that glutamine metabolism is linked to the formation of 2-hydroxyglutarate (2HG) in breast cancer. Increased glutamine consumption was found to be associated with poor breast cancer survival and aberrant accumulation of 2HG, an oncometabolite. Increased 2HG was present in a subset of human tumors and breast cancer cell lines and correlated with epigenetic reprogramming in the tumors. Here, we tested whether inhibition of glutaminase, a key enzyme in glutamine metabolism, would inhibit growth of human cell lines and also affect cellular levels of 2HG. We analyzed cell growth and observed that cells treated with the glutaminase inhibitor, compound 968, showed significantly decreased growth. This effect was dependent on the cell culture-medium. An apoptosis assay revealed that compound 968 did not induce apoptosis under the experimental conditions. Furthermore, we found that glutaminase inhibition causes a drop in intracellular 2HG. In summary, we observed that glutamine metabolism is linked to breast cancer outcome while glutaminase inhibition slows cell growth but does not cause apoptosis of breast cancer cells and decreases intracellular 2HG concentrations. Further experiments will test whether 2HG has oncogenic functions in breast cancer.

This work was supported by the Intramural Research Program of the Center for Cancer Research at the National Cancer Institute.

EVALUATION OF HETEROAROTINOIDS FOR BIOLOGICAL RESPONSE IN HUMAN ERYTHROLEUKEMIA CELLS

Jaclyn Highland and Paul J. Birckbichler
Department of Chemistry, Slippery Rock University, 1 Morrow Way, Slippery Rock, PA 16057

Retinoids are a class of molecules chemically related to Vitamin A which have demonstrated various medicinal applications. All-*trans* retinoic acid has shown anticancer activity; however, its usefulness in chemotherapeutic applications is limited by its toxicity. Heteroarotinoids—synthetic retinoids with one or more heteroatom—are structural analogues of retinoic acid. Heteroarotinoids exhibit lowered toxicity while maintaining significant biological activity. In this study, human erythroleukemia cells (K562) were evaluated for biological response following exposure to retinoic acid and heteroarotinoids. Cell cultures were evaluated for viability, growth, and induction of apoptosis (programmed cell death). Camptothecin was included as a positive control for the induction of apoptosis. Viability and growth were evaluated using a trypan blue exclusion assay. Relative induction of apoptosis was evaluated using flow cytometry. Treatment of cells with retinoic acid resulted in growth inhibition and induction of apoptosis, but also resulted in cell death by necrosis due to its toxicity. Treatment of cells with heteroarotinoids also resulted in growth inhibition and induction of apoptosis with lower levels of toxicity exhibited. Relative to the effects of retinoic acid, heteroarotinoids demonstrated increased growth inhibition and apoptosis induction.

This research was supported by funds provided by the Department of Chemistry and RFP Research Grants, Slippery Rock University, 1 Morrow Way, Slippery Rock, PA 16057.

REGULATION OF T-TYPE CALCIUM CHANNEL EXPRESSION BY DNA-ALTERING COMPOUNDS IN PROSTATE CANCER CELLS

Ezechielle Kiessu[1], Erika Weaver[1], Jennifer Hearne[1], and Miguel Martin-Caraballo[2]
[1]Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne MD 21853
[2]Department of Pharmaceutical Sciences, University of Maryland Eastern Shore, Princess Anne MD 21853

Gene-altering compounds are considered potential therapeutic tools in the treatment of various cancer tumors. Histone deacetylase inhibitors such as sodium butyrate (NaBu) and the DNA methyltransferase inhibitor 5-azacytidine (5-azaC) regulate cell survival and differentiation of cancer cells by yet to be fully understood mechanisms. Changes in intracellular calcium could potentially regulate regulate cell survival and differentiation by gene-altering compounds. It is becoming evident that T-type calcium channels constitute an important route for calcium influx in tumor cells that can trigger changes in cell proliferation and differentiation. This work was designed to test whether NaBu and 5-azaC promotes the expression of T-type Ca^{2+} channels in prostate cancer cells. Our study demonstrates that NaBu increases the expression of the Cav3.2 T-type Ca^{2+} channel subunit by a transcriptional mechanism. The effect of NaBu on Cav3.2 protein expression was concentration-dependent. NaBu stimulates the expression of the Cav3.2 T-type Ca^{2+} channels both in an androgen-dependent (LNCaP) and an androgen-independent (PC3) cell line. Inhibition of T-type Ca^{2+} channels had no effect on the number of apoptotic cell number. Further experiments will seek to determine the effect of 5- azaC on T-type Ca^{2+} channel expression. These results demonstrate that gene-altering compounds can regulate the expression of T-type Ca^{2+} channels.

This work was supported by funds provided by the UMES School of Pharmacy and grant P031B090209 from the US Department of Education

INVESTIGATING CELLULAR IMMUNITY USING THE SIMIAN VIRUS 40 LARGE TUMOR ANTIGEN

Matthew Lauver[1], Caitlin Mason[2], Caitlin McGee[2], Caroline Passmore[1], Stephanie Schell[1], and Lawrence Mylin[1]
[1]Department of Biological Sciences, Messiah College, One College Avenue Suite 3030, Mechanicsburg, PA 17055
[2]Department of Chemistry and Biochemistry, Messiah College, One College Avenue Suite 3030, Mechanicsburg, PA 17055

The Simian virus 40 large tumor antigen (SV40 T ag) is a viral oncoprotein that generates a strong cellular immune response in H-2^b (C57BL/6) mice directed against multiple immunodominant (I, II/III, IV) and immunorecessive (V) CD8+ T lymphocyte epitopes. Because of its ability to induce tumors and serve as the target for anti-tumor T cell responses, the SV40 T ag has been studied extensively as a model to better understand immune targeted control of solid tumors. Our laboratory has recently identified three CD4+ epitopes within the T ag and is conducting studies to characterize their role(s) in regulating cellular immunity to the T ag, including the generation of CD8+ T lymphocyte memory. Towards this goal, we have generated a cell line (C2a) that expresses a derivative of the T ag in which the three newly discovered CD4+ epitopes have been inactivated by substitution or deletion. The induction of SV40 T ag epitope I- and IV-specific CD8+ T cells has been compared by MHCI Tetramer staining and Intracellular Cytokine Staining (ICS; IFN-γ) following primary and secondary immunization using B6/WT-19 cells which express the intact SV40 T ag and/or the C2a cells which express the helper-deficient T ag derivative. The immunogenicity of the same cells has been compared following growth in traditional medium containing fetal bovine serum (FBS) vs. a serum-free HL-1 (Lonza) medium to remove potential adjuvant effects contributed by FBS proteins. Experiments are being conducted to investigate the role and importance of negative regulation by enumerating SV40 T ag-specific IL-10-secreting CD4+ under similar conditions and by searching for additional determinants in the T ag that stimulate IL-10 secreting T cells by ELISPOT using a 175 member peptide library corresponding to the SV40 T ag. Our studies continue to uncover additional intricacies in cellular immunity of the SV40 T ag.

This research was made possible by the Steinbrecher Undergraduate Summer Research Program at Messiah College.

CTL REACTIVITY AGAINST POTE-DERIVED PEPTIDES IN PROSTATE CANCER PATIENTS

Yi Liu[1], Masaki Terabe[2], Lauren V. Wood[2], and Jay A. Berzofsky[2]
[1] Department of Biological Science, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Vaccine Branch, Center for Cancer Research, National Cancer Institute,
National Institutes of Health, Bethesda, MD 20892

Online access of this abstract is restricted at the request of the Principal Investigator.

ADHESION ANALYSIS OF HUMAN ERYTHROLEUKEMIA CELLS TO HUMAN LUNG FIBROBLASTS

Jessica Mols and Paul J. Birckbichler
Department of Chemistry, Slippery Rock University, 1 Morrow Way, Slippery Rock, PA 16057

The study of attachment between human erythroleukemia (HEL) cells and human lung fibroblasts (WI-38) mimics a simulated *in vivo* environment for the study of metastasis. Exposing the adherent fibroblasts cells to the cancer cells growing in suspension provides an excellent metastatic model for adhesion of metastatic cells to normal tissues. The information obtained could possibly be a determining factor for an efficient treatment for cancer. Ninety percent of HEL cells adhered to the WI-38 cells, when the two cell types were mixed. In an attempt to determine the nature of the cell-cell adherence, HEL cells were grown in chamber slides coated with and without fibronectin, an extracellular matrix protein associated with WI-38 cells that plays a role in cell adhesion. Seventy percent of HEL cells added to the fibronectin-coated plate adhered to the plate suggesting that fibronectin might play a role in the adhesion of the two cell types. Dithiothreitol was ineffective in breaking the adhesion suggesting that disulfide linkages were not involved. These observations suggest the presence of a stronger linkage between the two cell types. Current studies are investigating the presence of such a bond. One such candidate is the isopeptide, €-(γ-glutamyl)lysine, catalyzed by tissue transglutaminase. WI-38 cells are rich in the enzyme and fibronectin is a substrate for the enzyme. Monoclonal antibodies to tissue transglutaminase, the isopeptide and the α5β1 integrin will be studied to investigate the involvement of these antigens in the binding of the two cell types. HEL attachment viability will be studied by an apoptosis assay to determine the deregulation of proliferation in HEL cells.

This research was supported by funds provided by the Department of Chemistry and RFP Research Grants, Slippery Rock University, 1 Morrow Way, Slippery Rock, PA 16057.

Afternoon Poster Session
Group S - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

125. **UNDERSTANDING HOW *STREPTOMYCES COELICOLOR* AVOIDS DESTRUCTION BY ENDOGENOUS-PRODUCED ANTIBIOTICS**
Kaeun Bae and Monica Chander
Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010

126. **EFFECTS OF PRE-WORKOUT SUPPLEMENTS ON METABOLISM IN THE EUKARYOTIC MODEL ORGANISMS, *SACCHAROMYCES CEREVISIAE***
Michael Bauer, Chet Kleynowski, and Dr. Bernadette Connors
Department of Biology, Dominican College of Blauvelt, Orangeburg, NY 10962

127. **ISOLATION OF NOVEL ARTHROBACTER SP. PHAGES WITH A ROBUST NEW METHOD**
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Department of Biological Sciences, Immaculata University, 1145 King Road, Immaculata, PA 19345

128. **COOPERATION AT THE MOLECULAR LEVEL AFFECTS YOUR PERFORMANCE AND HEALTH**
Andre Freligh and John R. Jungck
Department of Biological Sciences, University of Delaware, 5 West Main Street, Newark DE, 19716

129. **COMPETITIVE INHIBITION DRIVES UNIQUE CARBOHYDRATE METABOLISM AND MOTILITY OF *ESCHERICHIA COLI* WITHIN THE INTESTINE**
Ryan N. Montalvo, Cassandra Black, E.D. Richards, and Andrew J. Fabich
Department of Biology and Chemistry Liberty University,
1971 University Boulevard, Lynchburg, VA 24502

130. **ANTIBIOTIC EFFECTS OF CLOVE AND OREGANO OILS ON CARIOGENIC BACTERIA**
Marli F. Pimenta, Christine L. Cordi, and Anges T. Berki
Department of Natural and Physical Sciences, Caldwell University,
120 Bloomfield Avenue, Caldwell, NJ 07006

131. **SEROTONIN RECEPTOR DENSITY DIFFERENCES BETWEEN ACUTE CORONARY SYNDROME AND STABLE CORONARY ARTERY DISEASE PATIENTS**
Anna M. Roland[1] and Marlene S. Williams M.D.[2]
[1]Department of Biochemistry, Trinity Washington University,
125 Michigan Avenue NE,Washington, DC 20017
[2]Department of Pulmonary and Critical Care Medicine, Johns Hopkins Medical Institute,
5501 Hopkins Bayview Circle, Baltimore, MD 21224

UNDERSTANDING HOW *STREPTOMYCES COELICOLOR* AVOIDS DESTRUCTION BY ENDOGENOUS-PRODUCED ANTIBIOTICS

Kaeun Bae and Monica Chander
Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010

Online access of this abstract is restricted at the request of the Principal Investigator.

EFFECTS OF PRE-WORKOUT SUPPLEMENTS ON METABOLISM IN THE EUKARYOTIC MODEL ORGANISMS, *SACCHAROMYCES CEREVISIAE*

Michael Bauer, Chet Kleynowski, and Dr. Bernadette Connors
Department of Biology, Dominican College of Blauvelt, Orangeburg, NY 10962

The majority of pre-workout supplements on the market today contain several active ingredients such as β-Alanine, aspartame, and sucralose. The purpose of this study was to observe and interpret the effects of these common ingredients on anaerobic and aerobic metabolic processes. A fermentation assay in *Saccharomyces cerevisiae* was used to mirror an anaerobic intramuscular environment in humans. The results of the assay showed that β-Alanine treatment in yeast increased fermentation rates versus a glucose only control group by 73% in a 48 hour period. Fermentation using glucose plus aspartame (0.02M) showed a decrease of 60% in 24 hours versus the glucose only control. If the only carbon source available was aspartame, no fermentation occurred at concentration of 0.01M, 0.02M and 0.005M. The Sucralose fermentation utilized Splenda™ sweetener and glucose as carbon sources. The results indicated a decrease in fermentation rate as concentrations increased (1-5%), versus the glucose only control. Along with fermentation, a growth assay was conducted to determine if Aspartame inhibits growth of yeast cells. The results indicated a minimal difference between yeast grown in aspartame and without aspartame. Future studies are to include an more thorough analysis of the metabolic byproducts of Aspartame, namely, whether formaldehyde and formic acid are created as the artificial sweetener are used. In addition, DNA repair assays and a cell cycle delay analysis will be completed to determine if there is a change in the growth cycle of these cells in the presence of Aspartame

Funding was provided by the Department of Biology, Dominican College

ISOLATION OF NOVEL ARTHROBACTER SP. PHAGES WITH A ROBUST NEW METHOD

Dylan Chudoff and David Dunbar Ph.D.
Department of Biological Sciences, Immaculata University,
1145 King Road, Immaculata, PA 19345

Various species of *Arthrobacter* are ubiquitous in soil and display pleomorphic, Gram variable rods or cocci when grown in aerobic culture. This highly diverse genus of bacteria is known for its ability to survive in harsh conditions as well as degrade nitrogenous environmental toxins (Mongodin et al, 2006). Previous attempts at isolating *Arthrobacter* phages from soils have been met with little success and included lengthy incubations or studies resulted in little characterization of the phages found with these methods (Germida & Casida, 1981). Arthrobacter phage isolation done in the past has been focused mainly on developing methods for phage typing of Arthrobacter soil isolates or for control of microbial growth in industrial processes (Brown et al, 1978; Petrovski et al, 2011).

As Arthrobacter sp. bacteriophages are being discovered and investigated for their genomic diversity and host range specificities, increasing attention is placed on finding new phage isolates. Previous isolation procedures resulted in little success and required long incubations. Here, we present the discovery and initial characterization of multiple Arthrobacter phages found in southeastern Pennsylvania to be added to the current collection of 101 discovered and 18 sequenced Arthrobacter phages. Soils were gathered from several locations and phages were extracted from soil and filtered into enrichment culture with host strain *Arthrobacter* sp. KY3901, previously not attempted in Arthrobacter phage isolation. Initial attempts with this new method yielded several phage isolates showing varying plaque morphologies indicative of several putatively different phages. New phages were analyzed by restriction digest showing possible methylation of the genome. This enrichment procedure has shown to be effective in isolating various types of phages with Arthrobacter and other types of bacteria, also.

I would like to thank Dr. David Dunbar for his passion and knowledge behind the course, and South Eastern Pennsylvania Consortium of Higher Education (SEPCHE) institutions for funding the course.

COOPERATION AT THE MOLECULAR LEVEL AFFECTS YOUR PERFORMANCE AND HEALTH

Andre Freligh and John R. Jungck
Department of Biological Sciences, University of Delaware,
5 West Main Street, Newark DE, 19716

Many biological macromolecules are composed of subunits that function synergistically due to cooperativity. I have generated a spreadsheet model of the Hill Equation for the Biological Excel Simulations and Tools for Exploratory Experiential Mathematics (ESTEEM) modules page: http://bioquest.org/esteem/esteem_result.php in order for users to interactively develop a sense of nonlinear behavior of such macromolecules. This software is accessible to students and professors both nationally and internationally. Originally, the Hill equation represented the cooperative binding process of oxygen to hemoglobin molecules. Although Hill's model is not precise for modeling all forms of biological cooperativity, it is accurate enough that it is frequently utilized to describe the cooperativity of enzymes and conformational changes of their subunits. The parameters of the Hill equation, specifically the Hill coefficient and number of binding sites, are readily adjustable so that students can monitor the effects of adjusting such parameters. The Hill equation can be used as a logistic function and help users move from algebraic to differential equation models. Students will be able to view various plot types, decide which type of plot provides what information, and conclude which plot type is advantageous under different circumstances. Additional spreadsheets will incorporate real world data about the binding of oxygen to hemoglobin under various conditions, the binding of ligands to enzymes and genetic repressors whose data also fit the Hill equation. Medically, this model is relevant to the cooperativity of hemoglobin during intense anaerobic physical activity and is important to understanding the development of drugs for treating diabetes, sickle cell anemia, and thalassemia.

I particularly want to thank Dr. John Jungck and HHMI for making this opportunity possible, and for all of their help and support throughout the summer. I would also like to thank Dr. Anton Weisstein, Dr. Hal White, and Dr. Christopher Raymond for their assistance with excel, Hemoglobin, and Mathematics in that order. Also I would like to thank my colleagues Laura Cahill, Damir Creecy, Ross Sausa and all of the ISE 402G staff for being such a welcoming and helpful group.

COMPETITIVE INHIBITION DRIVES UNIQUE CARBOHYDRATE METABOLISM AND MOTILITY OF *ESCHERICHIA COLI* WITHIN THE INTESTINE

Ryan N. Montalvo, Cassandra Black, E.D. Richards, and Andrew J. Fabich
Department of Biology and Chemistry Liberty University,
1971 University Boulevard, Lynchburg, VA 24502

Mammalian intestines are host to a variety of microbes that comprise a dynamic community. Enteric microbes compete for carbohydrates. *Escherichia coli* MG1655 uses a quorum-sensing pathway similar to the pathogenic species *Escherichia coli* O157:H7, through the surface histidine kinase receptor QseC. Amongst other functions, QseC regulates carbohydrate metabolism and motility of MG1655 within the intestine through autophosphorylation of QseB and subsequent stimulation of *flhDC*. After seven days within the intestine MG1655, exhibits a predominantly non-motile phenotype (less than 5%). Motility is putatively used to access metabolites. However, we observe that it is not necessary for successful colonization. Whole-genome sqequencing of day 7 MG1655 non-motile strains reveals varying deletions of *flhDC*. To elucidate the consequences of non-motility, we competed non-motile (Δ*qseC*) and motile (WT) strains. In a feed-low competition assay of Δ*qseC* with the WT, the mutant outcompetes by ~2.5log/CFUg feces. Furthermore, the mutant similarly outcompetes the WT by ~2 log/CFUg feces in a feed-high (WT) vs. feed-low (Δ*qseC*) competition assay. Taking away MG1655's ability to communicate with the environment confers a persistent colonization advantage, often to a degree of over 100%. A diauxie assay revealed a possible mechanism that Δ*qseC* utilizes to benefit in colonization. WT was able to metabolize glucose more successfully than Δ*qseC* (110%). However, Δ*qseC* was able to metabolize the non-catabolite repressing sugar mannose more effectively (17%). Without *qseC* stimulation, MG1655 shunts its regulatory pathway to metabolize a different set of carbohydrates that will perpetuate colonization. Community analysis through qPCR displays a fluctuation in the relative concentrations of genera. The significant fold population changes observed is in *Bifidobacterium* and *Bacteroidales* with +7.23 and -200, respectively. We think *Bacteroidales* competes with MG1655 for specific carbohydrates and *Bifidobacterium* benefits. Ultimately, community antagonism causes MG1655 to alter genomic pathways away from motility to facilitate increased carbohydrate metabolism and, therefore, preserve competitive fitness.

This research has been funded by the Liberty University Provost Research Grant.

ANTIBIOTIC EFFECTS OF CLOVE AND OREGANO OILS ON CARIOGENIC BACTERIA

Marli F. Pimenta, Christine L. Cordi, and Anges T. Berki
Department of Natural and Physical Sciences, Caldwell University,
120 Bloomfield Avenue, Caldwell, NJ 07006

Bacterial resistance to existing antibiotics have led to a growing interest in natural remedies. Certain essential oils have demonstrated beneficial health effects. One such oil, Clove (*Syzygium aromaticum)* oil, has been shown to have antibacterial properties, and consequently, is used by dentists to treat toothaches. It is shown that formation of oral plaque, known as caries, and subsequent decay of the inner teeth layers by activities of the cariogenic bacteria are the common causes for toothaches. Oregano (*Origanum vulgare)* oil, another beneficial oil, has shown antimicrobial properties against certain bacteria as well as fungi. Although clove and oregano oils are well known, easily accessible, readily available, and indicated for anticariogenic treatment, they are not well studied. Thus, we decided to investigate their anticaries properties by observing their effects on the growth of bacteria found in the oral cavity. Microbiome of the mouth from three healthy individuals, as well as *Escherichia coli*, one of the most common bacteria, *Streptococcus sanguinis*, a bacterium of the healthy oral flora, and *Streptococcus salivarius*, a cariogenic bacterium were selected. These were subjected to the Kirby-Bauer antibiotic susceptibility test. In the assay, clove and oregano oils served as test samples, while ampicillin disks, and blank susceptibility disks were the positive and negative controls, respectively. Clove and oregano oils inhibited the growth of all strains tested in various degrees resulting in defined zones of inhibition measured in the Kirby-Bauer test. The results suggest that both clove oil and oregano oil could be used to inhibit bacterial strains of the oral cavity. Further research should be conducted for the deeper understanding of the mechanism of action, active components, and the enhancement of the antibiotic activity of these oils on the oral microbiome.

We would like to acknowledge the ICFNJ for providing support in the form of two Undergraduate Research Symposium Grants.

SEROTONIN RECEPTOR DENSITY DIFFERENCES BETWEEN ACUTE CORONARY SYNDROME AND STABLE CORONARY ARTERY DISEASE PATIENTS

Anna M. Roland[1] and Marlene S. Williams M.D.[2]
[1]Department of Biochemistry, Trinity Washington University,
125 Michigan Avenue NE, Washington, DC 20017
[2]Department of Pulmonary and Critical Care Medicine, Johns Hopkins Medical Institute,
5501 Hopkins Bayview Circle, Baltimore, MD 21224

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Afternoon Poster Session
Group T - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

132. **DIOXIN EXACERBATES PROSTATIC DISEASE BY INCREASING COLLAGEN DEPOSITION IN MICE PROSTATES**

Isaac Beaubrun[1], Jalissa Wynder[2], Tyler Bauman[2], Jonathan Ewald[2], Kimberly Keil[2], Chad M. Vezina[2] and William A. Ricke[2]
[1]Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225
[2]Department of Urology, University of Wisconsin-Madison, 1685 Highland Avenue, Madison, WI 53705

133. **URICASE KNOCK OUT MICE DISPLAY ENHANCED ENDURANCE**

N. Eady[1], R.G. Cutler[2], M.P. Mattson[2], and S. Camandola[2]
[1] Department of Biology, Trinity Washington University, 517 Montana Avenue NE, Washington, DC 20017
[2] Laboratory of Neurosciences, National Institute on Aging, 251 Bayview Boulevard, Suite 100, Baltimore, MD 21224

134. **INTERPLAY BETWEEN *E. COLI* HSP90 AND A TUBULIN HOMOLOG DURING CELL DIVISION**

Robyn Jasper[1,2], Monica Markovski[2], and Sue Wickner[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD
[2]Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Bethesda, MD

135. **EFFECTS OF PKA PHOSPHORYLATION ON THE SUBCELLULAR LOCALIZATION OF SMN IN MOTOR NEURON-LIKE CELLS**

Ryan Kirk[1,2], and Matthew E. R. Butchbach[2,3,4]
[1]Neuroscience Program, University of Delaware, 108 Wolf Hall, Newark, DE 19716
[2]Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19716
[3]Center for Applied Clinical Genomics, Nemours Biomedical Research, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803
[4]Center for Pediatric Research, Nemours Biomedical Research, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803

136. **ANALYSIS OF THE ROLE OF RPS9 IN RIBOSOME ASSEMBLY AND rRNA PROCESSING IN *SACCHAROMYCES CEREVISIAE***

Alana Lescure, Brian Gregory, and Lasse Lindahl
Department of Biology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

137. **EFFECT OF FERMENTATION PARAMETERS ON PROTEASE ACTIVITY IN BEER**

Reecha Pandya, Matthew Farber, and Peter B. Berget
Department of Biological Sciences, University of the Sciences, 600 South 43rd Street, Philadelphia, PA 19104

138. **CHARACTERIZATION OF MECHANOSENSITIVE CHANNEL KNOCKOUTS IN *V. CHOLERAE***

Stephanie Sansbury[1], Ian Rowe[1], Shah Manzur Rashed[2], Simona Patange[1], and Sergei Sukharev[1]
[1]Department of Biology, University of Maryland, College Park, MD 20742
[2]Maryland Pathogen Research Institute, University of Maryland, College Park, MD 20742

DIOXIN EXACERBATES PROSTATIC DISEASE BY INCREASING COLLAGEN DEPOSITION IN MICE PROSTATES

Isaac Beaubrun[1], Jalissa Wynder[2], Tyler Bauman[2], Jonathan Ewald[2], Kimberly Keil[2], Chad M. Vezina[2] and William A. Ricke[2]
[1]Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225
[2]Department of Urology, University of Wisconsin-Madison, 1685 Highland Avenue, Madison, WI 53705

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental toxin capable of causing increased incidents of prostatic diseases such as benign prostatic hyperplasia (BPH) and prostate cancer (PRCA). BPH is a disease causing prostate enlargement leading to obstruction of the prostatic urethra impeding urine flow. PRCA is a disease described as overexpression of carcinoma cells that can metastasize to neighboring organs. Physiological levels of hormones are also associated with prostate disease in older males. We hypothesized that dioxin and adult hormone exposure will increase collagen deposition in the prostate. Here we investigated the effects on in utero dioxin exposure on collagen content in the prostate of genetically modified mice (GEM). GEM were administered TCDD while mice received oil as a vehicle. When male pups reached sexual maturity were surgically implanted with subcutaneous pellets containing Testosterone (T) and Estradiol (E_2). We examined the histology of the bladders, prostates, seminal vesicles and urethras stained using picrosirius red (PSR) to assess collagen fiber content of treated and untreated mice. As a result an increase in red birefringence was seen in the ventral prostate lope, and no indication of increased birefringence distribution was seen in the anterior and dorsolateral prostate lobes. In conclusion, TCDD induces collagen deposition in the ventral prostate lopes indicating bladder outlet obstruction and lower urinary tract symptoms in mice

URICASE KNOCK OUT MICE DISPLAY ENHANCED ENDURANCE

N. Eady[1], R.G. Cutler[2], M. P. Mattson[2], and S. Camandola[2]
[1] Department of Biology, Trinity Washington University,
517 Montana Avenue NE, Washington, DC 20017
[2] Laboratory of Neurosciences, National Institute on Aging,
251 Bayview Boulevard, Suite 100, Baltimore, MD 21224

The gene encoding the uric acid-degrading enzyme uric acid oxidase (UOX) is functional in rodents, but not in humans, as the result of multiple mutations that occurred in the UOX gene during the divergence of the living genera of hominoids (gibbons, orangutans, chimpanzees, gorillas and humans) from the Old World monkeys. As a consequence, uric acid (UA) is the end product of purine metabolism in humans resulting in circulating levels of uric acid 5 – 10 times greater than rodents. In humans in response to physical exercise, levels of circulating and skeletal muscle uric acid increase. It has been suggested that such elevation of UA may sustain muscle function and reduce cellular damage during intense physical exertion, possibly contributing to the superior endurance phenotype of humans. We have recently shown that UOX-/- mice with higher levels of UA displayed increased exploratory behavior compared to UOX+/+. Further testing of all UOX genotypes in cages equipped with running wheels showed significant positive correlations between UA levels and daily voluntary activity in wheel running in UOX +/- and UOX -/- but not in UOX +/+ animals. Furthermore, when the animals were tested for endurance significant increases in levels of UA and running time, with lower levels of oxidative damage were found in UOX+/- mice compared to UOX +/+. Skeletal muscles consist of heterogeneous myofibers those biochemical, physiological, and metabolic characteristics determine the muscles function, size, metabolism and fatigue resistance. Given our findings of increased voluntary running and endurance in UOX deficient mice we explored the role of UOX in skeletal muscle physiology.

This work was supported by the Intramural Research Program of the National Institute on Aging.

INTERPLAY BETWEEN *E. COLI* HSP90 AND A TUBULIN HOMOLOG DURING CELL DIVISION

Robyn Jasper[1,2], Monica Markovski[2], and Sue Wickner[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD
[2]Laboratory of Molecular Biology, National Cancer Institute,
National Institutes of Health, 37 Convent Drive, Bethesda, MD

Molecular chaperones are proteins that assist in the refolding and reactivation of misfolded or inactivated proteins. The Hsp90 family of ATP-dependent molecular chaperones are abundantly expressed and highly conserved. In eukaryotes, Hsp90 is essential and activates more than 300 client proteins. In several pathogenic bacteria, $Hsp90_{Ec}$ is an important virulence factor. However, little is known about the *Escherichia coli* homolog, $Hsp90_{Ec}$. Our group recently found that overexpression of $Hsp90_{Ec}$ causes cells to filament. Cells can filament due to defects in cell division. During normal cell division, the divisome, an essential complex of proteins, localizes to the middle of the cell to initiate and promote cell division. Inhibition of any step in the process can lead to filamentation. In support of this possible cause of filamentation, our group has evidence to suggest that overexpression of $Hsp90_{Ec}$ inhibits cell division early.

To gain insight into the role of $Hsp90_{Ec}$ in cell division, we tested the hypothesis that $Hsp90_{Ec}$ overexpression inhibits divisome assembly by preventing the localization of FtsZ, a tubulin homolog and the first essential divisome factor. To investigate this, we constructed an inducible fluorescent version of FtsZ to control its expression. Using fluorescence microscopy, we found that GFP-FtsZ was localized at midcell and this localization was not dependent on $Hsp90_{Ec.}$ Thus, the construct was functional. To test our hypothesis, we overexpressed $Hsp90_{Ec,}$ induced expression of *gfp-ftsZ*, and used fluorescence microscopy to monitor the localization of GFP-FtsZ in cells. Under these conditions, the majority of cells were no longer filamentous and GFP-FtsZ localized to midcell. These results show that increased amounts of GFP-FtsZ cause partial suppression of the filamentous phenotype observed when $Hsp90_{Ec}$ is overexpressed, suggesting there is interplay between $Hsp90_{Ec}$ and FtsZ. Additionally, they suggest that $Hsp90_{Ec}$ may function to regulate cell division through sequestration of FtsZ.

EFFECTS OF PKA PHOSPHORYLATION ON THE SUBCELLULAR LOCALIZATION OF SMN IN MOTOR NEURON-LIKE CELLS

Ryan Kirk[1,2], and Matthew E. R. Butchbach[2,3,4]
[1]Neuroscience Program, University of Delaware, 108 Wolf Hall, Newark, DE 19716
[2]Department of Biological Sciences, University of Delaware,
118 Wolf Hall, Newark, DE 19716
[3]Center for Applied Clinical Genomics, Nemours Biomedical Research, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803
[4]Center for Pediatric Research, Nemours Biomedical Research, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803

The SMN protein, which is mutated in the genetic disorder Spinal Muscular Atrophy (SMA), is phosphorylated by protein kinase A (PKA) at several different amino acid residues. The functional significance of this specific phosphorylation event is unknown, but it has been implicated in SMN protein stability and protein complex formation. It has not been determined whether phosphorylation by PKA regulates the intracellular transport of SMN, which is found in distinct protein complexes in the cytoplasm, along axons, within axon terminals, and as gems in motor neuron nuclei. This study aimed to investigate the effects of phosphorylation by PKA on the distribution of SMN throughout the motor neuron-like cell line, NSC34. SMN protein levels and subcellular distribution were analyzed using immunoblots and immunostains following transient treatment with inhibitors and activators of PKA. Additionally, a set of mutant SMN proteins with alanine substitutions at threonine and serine residues phosphorylated by PKA was used to study which residues are critical for PKA regulation of SMN. Because motor neurons are the primary cell type affected in SMA, NSC34 cells were differentiated into motor neurons using a reduced serum media and retinoic acid in order to better visualize the axonal localization of SMN.

This project was supported by the Nemours Foundation and the NIGMS COBRE program (8P20GM103464-8).

ANALYSIS OF THE ROLE OF RPS9 IN RIBOSOME ASSEMBLY AND rRNA PROCESSING IN *SACCHAROMYCES CEREVISIAE*

Alana Lescure, Brian Gregory, and Lasse Lindahl
Department of Biology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Rps9, which is encoded for by the gene *RPS9*, is one of 33 ribosomal proteins on the small subunit of the yeast ribosome. Previous studies have shown that ribosome biogenesis is severely inhibited when individual ribosomal proteins (r-proteins) on either the large or small subunit are depleted. However, the characterization of the different r-proteins' exact roles in rRNA processing and ribosome assembly remains incomplete. In this study, I show through northern blot analysis and methylene blue staining of total RNA transferred to membranes that the depletion of RpS9 in the yeast *Saccharomyces cerevisiae (S. cerevisiae)* leads to a buildup of rRNA precursors as well as a loss of mature 18S rRNA. Additionally, sucrose gradient profiling and growth curve characterization show that depleting cells of RpS9 leads to a loss of small subunit formation and a decrease in growth rate.

EFFECT OF FERMENTATION PARAMETERS ON PROTEASE ACTIVITY IN BEER

Reecha Pandya, Matthew Farber, and Peter B. Berget
Department of Biological Sciences, University of the Sciences,
600 South 43rd Street, Philadelphia, PA 19104

During stressful fermentation conditions, yeast cells can release a protease that perturbs beer foam stability. This outcome is highly undesirable in the brewing industry because foam enhances organoleptic quality and consumer interest. Several brewing parameters, such as fermentation temperature, strain selection, inoculation rate, and wort gravity, have been tested to quantify their effects on protease activity in beer. A yeast protease sensor using Fluorogen Activating Protein technology was used to measure the protease activity under various temperatures, strains, inoculation rates, and gravities. Under stressful fermentation conditions, different strains exhibited different stress responses in terms of protease release. Initial findings have shown direct proportionality between pitch rate and yeast protease activity. Increasing or decreasing wort gravity relative to the normal gravity value has also been shown to have an effect on protease activity. Thus, this can be used to optimize fermentation conditions that minimize potential protease release, ultimately benefiting quality control for home brewers and commercial breweries.

This work was funded by the American Homebrewers Association and by NIH Grant U54-RR022241.

CHARACTERIZATION OF MECHANOSENSITIVE CHANNEL KNOCKOUTS IN *V. CHOLERAE*

Stephanie Sansbury[1], Ian Rowe[1], Shah Manzur Rashed[2], Simona Patange[1], and Sergei Sukharev[1]
[1]Department of Biology, University of Maryland, College Park, MD 20742
[2]Maryland Pathogen Research Institute, University of Maryland, College Park, MD 20742

In *Vibrio cholerae*, the etiological agent associated with pandemic cholera, mechanosensitive channels open to relieve the otherwise lethal osmotic pressure along the cell membrane that results from the bacterium's release into its natural aqueous reservoir of fluctuating salinity. This class of channel is thus crucial to *V. cholerae'*s environmental stability and transmission.

To elaborate on the importance of mechanosensation and its relationship to the resistance to lysis provided by the peptidoglycan layer, we adapted a recombineering system modeled after the lambda red bacteriophage to create two single and one double chromosomal knockout of the two main mechanosensitive channels in *V. cholerae*, MscL and MscS. At the organismal level, the subsequent reduction in hypo-osmotic shock tolerance is quantified with a series of survivability experiments. The interplay and overlapping function of these two channels is further characterized by electrophysiological studies using the patch clamp.

This project is supported by funding from the National Institutes of Health.

Afternoon Poster Session
Group U - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

139. **HISTAMINE AND HISTAMINE RECEPTOR INVOLVEMENT IN SENSORY-MOTOR INTEGRATION OF GILL LATERAL CELL CILIA ACTIVITY IN THE BIVALVE *CRASSOSTREA VIGINICA***

Beatrix Boisette, Ave Harris, Patrick Akande, Edward J. Catapane, and Margaret A. Carroll
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

140. **DETERMINING THE GENOTYPE-PHENOTYPE RELATIONSHIP OF *ATG18* MUTANTS**

Matthew Fischer and Erica Selva
Department of Biological Sciences, University of Delaware,
210 South College Avenue, Newark, DE 19711

141. **INVESTIGATION OF THE INTERACTION BETWEEN HISTONE METHYLTRANSFERASES Set1 AND Set5 TO IDENTIFY REGULATORY MECHANISMS OF ncRNA SYNTHESIS AT TRANSPOSABLE ELEMENTS**

K. Andrew Graham-Yooll and Erin M. Green
Department of Biology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

142. **HISTAMINE RECEPTORS IN GILL OF THE BIVALVE *CRASSOSTREA VIGINICA* AND THE ACTIONS OF HISTAMINE AT THE GILL INTERFILAMENTAL JUNCTIONS**

Ave Harris, Jarreau Harrison, Fabienne Mondelus, Edward J. Catapane, and Margaret A. Carroll
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

143. **WESTERN BLOT IDENTIFICATION OF DOPAMINE AND GABA RECEPTORS IN GILL OF THE BIVALVE *CRASSOSTREA VIGINICA***

Fabienne Mondelus, Beatrix Boisette, Fiana Bess, Margaret A. Carroll, and Edward J. Catapane
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

144. **B CELL LIPID METABOLISM DYSREGULATION INDUCES THE DEVELOPMENT OF SPONTANEOUSLY ACTIVATED B CELLS**

Melanie Shapiro, Grace Wo, and Wenxia Song
Department of Cell Biology and Molecular Genetics,
University of Maryland, College Park, MD 20742

145. **GESTATIONAL AND LACTATIONAL IRON DEFICIENCY ALTERS BEHAVIOR IN RATS**

Emily N. Spurlin, Timothy R. Monko, and Erica L. Unger
Department of Biology, Lebanon Valley College, 101 North College Avenue, Annville, PA 17003

HISTAMINE AND HISTAMINE RECEPTOR INVOLVEMENT IN SENSORY-MOTOR INTEGRATION OF GILL LATERAL CELL CILIA ACTIVITY IN THE BIVALVE *CRASSOSTREA VIGINICA*

Beatrix Boisette, Ave Harris, Patrick Akande, Edward J. Catapane, and Margaret A. Carroll
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

Crassostrea virginica gill lateral cells are innervated by serotonin and dopamine nerves. While motor aspects have been well studied, the sensory side has not. Histamine is a neurotransmitter and ligand for sensory receptors in invertebrates, but studies in bivalves have rarely been reported. We used HPLC to quantify histamine in ganglia and tissues of *C. virginica.* We also found *C. virginica* can adjust gill lateral cell cilia beating in response to chemical cues, including histamine applied to mantle. Histamine does not alter cilia beating when applied to gill. Applying histamine to mantle decreased cilia beating. Our studies show the mechanism involves sensory tentacles of mantle rim and the visceral ganglia, indicating histamine is a putative sensory neurotransmitter in mantle receptor cells synapsing with afferents going to the visceral ganglia where signals are integrated. We hypothesize histamine receptors are present in mantle. To test this we used histamine H1, H2 and H3 receptor agonists and antagonist at the mantle rim. Dose responses were conducted and cilia beating observed in gill. H2 agonists and antagonists had the strongest effects. For Western Blot analysis, mantle body and mantle rim lysates were prepared by polytron disruption in NP-40 detergent buffer containing protease inhibitor, followed by centrifugation to obtain supernatant with solubilized mantle body and mantle rim membrane proteins. Up to 30 µg of protein was subjected to SDS-PAGE with 10% acrylamide gels and electroblotted onto nitrocellulose. H2 receptor immunoreactivity was revealed after incubation with primary antibodies followed by HRP-conjugated secondary antibodies, then resolved via colormetric development using CN/DAB substrate kit. Western Blot showed a band at 70 kD corresponding to H2 receptors in mantle body and mantle rim. The study shows mantle body and rim contain H2 receptors and further demonstrates a role of histamine in sensory-motor integration of gill lateral cell cilia activity.

This work was supported by grants 2R25GM0600309 of the Bridge Program of NIGMS and 0516041071 of NYSDOE.

DETERMINING THE GENOTYPE-PHENOTYPE RELATIONSHIP OF *ATG18* MUTANTS

Matthew Fischer and Erica Selva
Department of Biological Sciences, University of Delaware,
210 South College Avenue, Newark, DE 19711

Autophagy is a highly regulated and conserved process that is active during conditions of starvation and normal cellular maintenance. 8J16 and 9E6 are two independent allelic point mutations in *Drosophila melanogaster autophagy-specific gene 18a* (*atg18a)* located in introns three and four, respectively, of its 5 exon locus at 66B11 on chromosome 3. These *atg18a* alleles were initially characterized as being involved in Wnt or Hedgehog signaling due to their embryonic phenotype. The $atg18^{8J16}$ and $atg18^{9E6}$ intronic mutations are not in any sequences expected to disrupt transcript processing, yet these mutations cause pupal lethality and neurodegenerative phenotypes. The exact molecular effects of these mutations are unknown. The purpose of this research is to investigate and determine the genotype-phenotype relationship of how $atg18^{8J16}$ and $atg18^{9E6}$ alleles give rise to *atg18a* loss of function and characterize the effect of these mutations on neuronal maturation using the *Drosophila* eye as a model. In adults, $atg18^{8J16}$ and $atg18^{9E6}$ homozygous mutant eyes display the rough eye phenotype. To better understand the basis of this phenotype mutant larval imaginal eye discs were examined to determine ommatidial organization and photoreceptor identity. ELAV, a pan neuronal marker, showed that photoreceptors differentiate normally, but those that differentiate early are being lost. Examination of cleaved caspase 3 revealed that photoreceptors were succumbing to apoptosis, especially at the morphogenetic furrow. These phenotypes could contribute to the adult small rough eye phenotype. These results suggest autophagy may be required for photoreceptor maintenance and its absence leads to apoptotic death. Examination of Senseless and Prospero expression showed that photoreceptors R8 and R7, respectively, are more refractory to apoptosis as these photoreceptors predominate near the optic stalk where the first photoreceptors differentiate. This suggests autophagy is more important for the maintenance of photoreceptors R1, R2, R3 R4, R5 and R6 than R7 and R8.

Acknowledgements and appreciations are extended to the Howard Hughes Medical Institute, University of Delaware Undergraduate Research Program, and the University of Delaware Summer Scholars Program for financial and academic support.

INVESTIGATION OF THE INTERACTION BETWEEN HISTONE METHYLTRANSFERASES Set1 AND Set5 TO IDENTIFY REGULATORY MECHANISMS OF ncRNA SYNTHESIS AT TRANSPOSABLE ELEMENTS

K. Andrew Graham-Yooll and Erin M. Green
Department of Biology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Histone methylating enzymes Set1 and Set5 have been implicated in the regulation of transposable elements (*Ty*) of *Saccharomyces cerevisiae*. The sense strand is transcribed along with the anti-sense transcript to regulate the rate of transposition. This anti-sense strand is degraded rapidly by the 5'-3' endonuclease Xrn1 shortly after transcription. Deletion of *XRN1* helps to stabilize ncRNA that would otherwise be degraded and allows the determination of genetic interaction between *SET1*, *SET5*, and *XRN1*. Generating double and triple mutants of *XRN1*, *SET1* and *SET5*, we conducted a series of experiments to test the hypothesis of *SET5* cooperating with *SET1* in regulating ncRNAs at *Ty* elements. We have identified a synthetic genetic interaction between *XRN1*, *SET1*, and *SET5*. To investigate this genetic interaction, we are performing analysis of transcription and transposition rates from *Ty* elements. Our preliminary results suggest that Set1 and Set5 may alter the transposition rate in *xrn1*Δ cells, indicating that these histone methyltransferases may regulate transposition of transposable elements, possibly by a mechanism involving *Ty* element ncRNA.

HISTAMINE RECEPTORS IN GILL OF THE BIVALVE *CRASSOSTREA VIGINICA* AND THE ACTIONS OF HISTAMINE AT THE GILL INTERFILAMENTAL JUNCTIONS

Ave Harris, Jarreau Harrison, Fabienne Mondelus, Edward J. Catapane, and Margaret A. Carroll
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

Ganglia and innervated organs of the bivalve mollusc *Crassostrea virginica* contain serotonin and dopamine, which mediate physiologic functions in gills and other organs. Histamine is a neurotransmitter in nervous systems and ligand for sensory receptors in invertebrates, but information on it in bivalves has rarely been reported. We showed in *C. virginica* histamine is involved in sensory reception in the sensory-motor integration of gill lateral cell cilia ctivity. We also used HPLC to quantify histamine in ganglia and tissues, and immunohistofluorescence to detect histamine and histamine H2 receptors in various tissue locations including gill interfilamental junctions. We hypothesize histamine H2 receptors in gill could be confirmed by Western Blot and histamine has a physiological action on gill interfilamental junctions. For Western Blot analysis, gill cell lysate was prepared by polytron disruption in NP-40 detergent buffer containing protease inhibitor, followed by centrifugation to obtain supernatant with solubilized membrane proteins. Up to 30 Φg of solubilized protein was subjected to SDS-PAGE with 10% acrylamide gels and electroblotted onto nitrocellulose. H2 receptor immunoreactivity was revealed after incubation with primary antibodies followed by HRP-conjugated secondary antibodies. Receptor proteins were resolved via colormetric development using CN/DAB substrate kit. To determine if histamine has a physiological effect of at interfilamental junctions we observed gill sections with a microscope, and photographed responses of interfilamental junctions to histamine and the histamine antagonist famotidine. Western Blot showed a strong band at approximately 70 kD in gill corresponding to H2 receptors. The physiology study showed histamine (10^{-3} - 10^{-5} M) caused a dose-dependent contraction of interfilamental junctions which were blocked when famotidine (10-3 - 10^{-5} M) was applied. The study confirms previous immunohistofluorescence findings of the presence of H2 receptors in gill of *C. virginica* and further identifies a specific physiological role of histamine in the animal's gill.

This work was supported by grants 2R25GM0600309 of the Bridge Program of NIGMS and 0516041071 of NYSDOE.

WESTERN BLOT IDENTIFICATION OF DOPAMINE AND GABA RECEPTORS IN GILL OF THE BIVALVE *CRASSOSTREA VIGINICA*

Fabienne Mondelus, Beatrix Boisette, Fiana Bess, Margaret A. Carroll, and Edward J. Catapane
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

It is well established that ganglia and innervated organs of the bivalve mollusc *Crassostrea virginica* contain serotonin and dopamine, which mediate physiologic functions in their gill and other organs. Gill lateral cells of *C. virginica* are controlled by serotonergic-dopaminergic innervations from their ganglia and regulate cilia beating rates. Dopamine slows down cilia beating rates and serotonin speeds up cilia beating. GABA is a neurotransmitter in the nervous system of vertebrates and many invertebrates, but studies in bivalves have rarely been reported. Recently we used HPLC to show that GABA also is present in ganglia and tissues of *C. virginica* and that GABA acts as a ganglionic neurotransmitter modulating gill lateral cell cilia activity. We also used immunohistofluorescence to localize GABA receptors in ganglia and gill, and identify the dopamine receptors in gill lateral cells that slow down cilia beating rates as D2-like (D2DR). We hypothesize that Western Blot analysis would verify the presence of D2DR and GABA receptors in gill of *C. virginica*. For Western Blot analysis, gill cell lysate was prepared by polytron disruption in ice-cold NP-40 detergent buffer containing protease inhibitor, followed by centrifugation to obtain supernatant with solubilized membrane proteins. Up to 30 Φg of solubilized protein was subjected to SDS-PAGE with 10% acrylamide gels and electroblotted onto nitrocellulose. D2DR and GABA receptor immunoreactivity was revealed after incubation with primary antibodies followed by incubation with HRP-conjugated secondary antibodies. The Western Blot studies showed strong bands between 70 - 75 kD corresponding to dopamine D2DR and GABA RA1-6 receptors in gill. The present project allows us to confirm our previous and immunohistofluorescence studies showing the presence of dopamine and GABA and furthers the understanding of their physiological roles in *C. virginica*.

This work was supported by grants 2R25GM0600309 of the Bridge Program of NIGMS and 0516041071 of NYSDOE.

B CELL LIPID METABOLISM DYSREGULATION INDUCES THE DEVELOPMENT OF SPONTANEOUSLY ACTIVATED B CELLS

Melanie Shapiro, Grace Wo, and Wenxia Song
Department of Cell Biology and Molecular Genetics,
University of Maryland, College Park, MD 20742

Type 2 diabetes (T2D) is a metabolic disorder characterized by insulin resistance or inadequate insulin production from pancreatic beta cells. Recent studies suggest that obesity-induced autoimmunity, initiated by B cells, plays a critical role in development of the disease. We hypothesize that obesity-associated metabolic changes in B cells cause deregulation of B cell activation, leading to the B-cell-mediated activation of diabetogenic T cells and production of autoantibodies, with consequent development of insulin resistance.

To test this hypothesis, we have generated a novel mouse model where B cell lipid metabolism is perturbed by B cell-specific knockout of CGI-58 (BKO), a gene necessary for triglyceride hydrolysis. We fed mice with a high fat diet to induce obesity and analyzed the effects of BKO on B-cell maturation and activation. Compared to control mice, BKO mice exhibit a significant increase in the number of spontaneously proliferating germinal center B cells in the spleen, as analyzed via flow cytometry. Consistent with this result, our immunohistochemical analysis found that the number of spontaneously formed germinal centers in the spleens of unimmunized BKO is higher than that in control mice. Moreover, BKO mice also exhibit splenomegaly. Both spontaneous germinal center formation and splenomegaly are typically indicative of autoimmunity.

We plan to further characterize the immunological defects in the BKO mice, including autoantibody production using ELISA, presence of glomerulonephritis in kidney by immunohistochemistry, development of insulin resistance via insulin tolerance tests, and alterations in B-cell lipid metabolism by quantifying involved genes using real time PCR. Our results thus far indicate that B-cell lipid metabolism dysregulation causes spontaneous B-cell activation without antigenic stimuli, and those spontaneously activated B cells likely include self-reactive B cells. Together, these studies will aid in elucidation of the mechanism underlying B cell-mediated autoimmunity in T2D, inspiring new strategies to prevent and treat metabolic diseases.

This research was supported by funding from the Howard Hughes Medical Institute Undergraduate Research Fellowship.

GESTATIONAL AND LACTATIONAL IRON DEFICIENCY ALTERS BEHAVIOR IN RATS

Emily N. Spurlin, Timothy R. Monko, and Erica L. Unger
Department of Biology, Lebanon Valley College,
101 North College Avenue, Annville, PA 17003

Iron deficiency anemia in early childhood has profound effects on cognitive and affective development. More recent evidence suggests that these behavioral outcomes may be irreversible. This study in rats addressed the question of whether the effects of dietary iron deficiency in utero, during lactation, or throughout these periods could be repaired with dietary intervention at weaning (postnatal day (P) 21). Male and female rats were subjected to one of four iron dietary treatments during gestation and lactation: 1) iron sufficient throughout gestation and lactation; 2) iron deficient beginning on gestational day (G) 15; 3) iron deficient beginning on P4; and 4) iron deficient beginning on G15 followed by an iron sufficient diet on P4. Each treatment group was then fed an iron sufficient diet from weaning (P21) until euthanasia at P49. Tests for novel object recognition memory, anxiety-like behavior and locomotor activity were performed between P42 and P47. Overall, there was a trend toward poorer recognition memory in the early iron deficient groups compared to rats that were iron sufficient throughout gestation and lactation. Differences in locomotor activity were also observed. These results suggest that iron deficiency in utero and/or during lactation is associated with altered behavioral characteristics in early adulthood.

This research was funded by the Paul Wolfe Research Fund and Lebanon Valley College.

Afternoon Poster Session
Group V - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

146. **SOX-17 TRANSGENIC MICE ARE USED TO ANSWER QUESTIONS ABOUT CELL SIGNALING PATHWAYS INVOLVED IN OLIGODENDROCYTE DEVELOPMENT.**

Sonia Garcia[1], Cynthia DeBoy[1], Li-Jin Chew[2]; and Vittorio Gallo[2]
[1]Trinity Washington University, 125 Michigan Avenue, Washington, D.C. 20017
[2]Children's National Medical Center, 111 Michigan Avenue, Washington, D.C. 20017

147. **A MATHEMATICAL MODEL OF MELANOPSIN PHOTOTRANSDUCTION**

Abigail Jackson[1], Jessica Ortega[1,2], Phyllis Robinson[1], and Kathleen Hoffman[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Mathematics and Statistics, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

148. **P-AMINOSALICYLIC ACID (PAS) REVERSES THE NEUROTOXIC EFFECTS OF MANGANESE ON DOPAMINE POST-SYNAPTIC RECEPTORS**

Kurt Loney-Walsh[1], Loren Dubose[1], Edward J. Catapane[2], and Margaret A. Carroll[2]
[1]Department of Biology, Kingsborough Community College, Brooklyn, NY 11235
[2]Department of Biology, Medgar Evers College, Brooklyn, NY 11225

149. **IMMUNOHISTOFLUORESCENCE LOCALIZATION OF HISTAMINE AND HISTAMINE RECEPTORS IN GANGLIA AND TISSUES OF THE BIVALVE MOLLUSC, *CRASSOSTREA VIRGINICA***

Ayana McLeod[1], Danellie Semple[2], Edward J. Catapane[1], and Margaret A. Carroll[1]
[1]Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225
[2]Department of Biology, Kingsborough Community College,
2001 Oriental Boulevard, Brooklyn, NY 11235

150. **BEHAVIORAL AGGREGATION, SEX PREFERENCE AND LEARNING AND MEMORY IN FEMALE *GROMPHADORHINA PORTENTOSA***

Victoria E. Meadows and Kenneth G. Sossa Ph.D.
Department of Biology, Notre Dame of Maryland University,
4701 North Charles Street, Baltimore, MD 21210

151. **STUDYING THE NEUROMUSCULAR JUNCTION IN ALS**

Natalie Steenrod[1], Muhamed Hadzipasic[2], and Arthur Horwich, Ph.D.[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Genetics, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06520

SOX-17 TRANSGENIC MICE ARE USED TO ANSWER QUESTIONS ABOUT CELL SIGNALING PATHWAYS INVOLVED IN OLIGODENDROCYTE DEVELOPMENT.

Sonia Garcia[1], Cynthia DeBoy[1], Li-Jin Chew[2]; andVittorio Gallo[2]
[1]Trinity Washington University, 125 Michigan Avenue, Washington, D.C. 20017
[2]Children's National Medical Center, 111 Michigan Avenue, Washington, D.C. 20017

Multiple Sclerosis (MS) is a neurological disorder that causes paralysis and sensory loss. The corpus callosum (CC) contains white matter, made up of myelinated axons. Myelin is a fatty substance produced by mature oligodendrocytes (OL). Demyelination in MS individuals is due to the loss of mature OLs and an abnormal increase of immature oligodendrocytes progenitor cells (OPCs). MS patients were reported to have low Sonic Hedgehog (SHH) levels, a developmental signal essential for OL formation. We investigated signaling changes in the CC of transgenic mice that over-express Sox-17, a member of the SRY (Sex Determining Region Y)-Box containing genes, to understand its role in regulating SHH.

We hypothesized that SOX-17 transgenic mice have higher SHH/GLI2 levels that are associated with attenuated WNT and demyelination compared to wild type (WT) mice. PCR was applied to genotype litters of mice to distinguish transgenic mice from WT littermate controls. Mice were subjected to 4 weeks of cuprizone demyelination, and analyzed for differences in myelin and OL loss by immunohistochemistry, using epifluorescent and confocal microscopy. Microscopy demonstrated that Sox-17 overexpression promotes remyelination after injury. Compared with adult CNPSox-17 transgenic mice, the cuprizone-induced loss of myelin protein was greater in WT mice. The demyelination-induced loss of OLs was also greater in WT. The CNPSox17 transgenic mouse was observed to possess enhanced levels of GLI2, as well as Axin2, an inhibitor of beta-catenin.

These results indicate that Sox-17 overexpression offers protective advantage in demyelination, by inducing changes in developmental signaling pathways and elevating SHH levels, which increases GLI2. GLI2 can antagonize WNT/beta-catenin activity, thus promoting OPC maturation to OLs. Understanding the protein signaling that allows OLs to mature is important. This study reveals the clinical potential of designing drugs to target WNT/ beta-catenin or enhance Gli2, thus promoting OL maturation and remyelination in MS patients.

I would like to thank Dr. Cynthia DeBoy for allowing me to be part of her research. I would also like to thank Dr. Li-Jin Chew for allowing and helping me work in her lab. Research supported by NIH Grant R01NS056427, National Multiple Sclerosis Society Grants RG3712A1 and RG3954A1/2, Clare Boothe Luce Foundation.

A MATHEMATICAL MODEL OF MELANOPSIN PHOTOTRANSDUCTION

Abigail Jackson[1], Jessica Ortega[1,2], Phyllis Robinson[1], and Kathleen Hoffman[2]
[1]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Mathematics and Statistics, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Melanopsin is a recently discovered photopigment found in intrinsically photosensitive retinal ganglion cells (ipRGCs). It is involved in non-image forming vision, including circadian photoentrainment and the pupillary light reflex. It is also involved in light-related disorders, such as seasonal affective disorder. When light isomerizes the photopigment, a phototransduction cascade is activated, which produces an electrical signal that is sent to the brain. We developed a mathematical model of melanopsin's phototransduction pathway by using the law of mass action to convert chemical equations describing the pathway to a series of differential equations that was solved with MATLAB. Model parameters of the activation and deactivation were determined by fitting the model results to experimental calcium imaging data collected from transfected human embryonic kidney (HEK) cells expressing the melanopsin gene as well as electrophysiological data collected from ipRGCs. Mathematical simulations of a single flash response produce results consistent with those seen in the experimental data.

This work was funded by NSF for Undergraduate Biology and Mathematics Research Training, NSF IOSO721608 to P.R.R., NEI R01Y019053 to P.R.R.

P-AMINOSALICYLIC ACID (PAS) REVERSES THE NEUROTOXIC EFFECTS OF MANGANESE ON DOPAMINE POST-SYNAPTIC RECEPTORS

Kurt Loney-Walsh[1], Loren Dubose[1], Edward J. Catapane[2], and Margaret A. Carroll[2]
[1]Department of Biology, Kingsborough Community College, Brooklyn, NY 11235
[2]Department of Biology, Medgar Evers College, Brooklyn, NY 11225

Manganese (Mn), a neurotoxin causing Manganism a Parkinsons-like disease, disrupts dopamine (DA) neurotransmission, but the neurotoxic mechanism is not fully resolved. Reports postulate Mn toxicity is related to dysfunction of DA D2 receptors (D2DR). Lack of effective treatments is an obstacle in managing Manganism. Lateral gill cell cilia of *Crassostrea virginica* are innervated by DA neurons from their ganglia, causing cilio-inhibition. Our lab showed Mn treatment blocks DA's inhibitory effects and the post-synaptic DA receptors in the cells are D2DR type. We also showed treating animals with Mn in the presence of the drug p-Aminosalicylic acid (PAS) prevented the toxic effects. We hypothesize PAS would effectively reverse Mn neurotoxicity on D2DR when applied after Mn treatments. To test this we treated *C. virginia* with Mn (500 mM) followed by PAS (500 mM). Three sets of controls were similarly treated: Mn alone, PAS alone, or neither. Gills were excised, fixed, exposed to 1E antibodies against D2DR and FITC-linked 2Eantibodies, paraffin embedded and sectioned. We visualized D2DR in gill cells on a fluorescence microscope with FITC excitation and emission filters. Gill treated with 1E and 2Eantibodies showed bright FITC fluorescence in lateral cells and other cells, including cells lining the blood channels. Fluorescence intensity of lateral cells was quantified using ImageJ software from NSF. Results showed fluorescence intensity from animals treated with Mn had a progressive decrease in fluorescence of up to 40% less than non-Mn treated controls. Animals treated with PAS after Mn exposure did not show reduced fluorescence, indicating PAS was able to reverse the Mn induced lose of post-synaptic D2DR fluorescence. This immunohistological study shows a positive correlation between the loss of D2DR fluorescence in gill lateral cells in Mn treated animals vs controls and that PAS can effectively reverse the toxic effects of Mn on D2DR.

This work was supported by grants 2R25GM06003 of the Bridge Program of NIGMS and 0516041071 of NYSDOE.

IMMUNOHISTOFLUORESCENCE LOCALIZATION OF HISTAMINE AND HISTAMINE RECEPTORS IN GANGLIA AND TISSUES OF THE BIVALVE MOLLUSC, *CRASSOSTREA VIRGINICA*

Ayana McLeod[1], Danellie Semple[2], Edward J. Catapane[1], and Margaret A. Carroll[1]
[1]Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225
[2]Department of Biology, Kingsborough Community College,
2001 Oriental Boulevard, Brooklyn, NY 11235

Histamine is a neurotransmitter in nervous systems and sensory receptors in invertebrates. Histamine has rarely been reported in bivalves. Our previous studies showed it is involved in sensory reception in sensory-motor integration of gill lateral cell cilia beating in the bivalve Mollusc, *Crassostrea virginica.* We also used HPLC to show it present in ganglia and tissues of *C. virginica*. We hypothesize histamine containing neurons and histamine receptors are present in ganglia and innervated tissue of *C. virginica*. We tested this by immunohistofluorescence using primary antibodies to histamine and histamine receptor, and fluorescently labeled secondary antibodies. Antibodies were purchased from Abcam and Santa Cruz Biotechnology. Tissues were dissected, snap frozen, sectioned on a cryostat, fixed with EDAC (N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride) or paraformaldehyde, treated with blockers, and incubated with primary then secondary antibodies. Whole mounts of gill and mantle also were similarly treated with antibodies and visualized. Cryostat sections and whole mounts were viewed with a Zeiss epilume fluorescence microscope fitted with a ProgRes C3 Peltier cooled camera, as well as on a Leica epilume fluorescence microscope fitted with a Leica DFC400 camera. Both scopes had 100 watt mercury lamps and FITC and Texas Red excitation/emission filters. The results show histamine and histamine H2 receptors are present in visceral ganglia gill, mantle body and sensory tentacles of the mantle rim. Of particular significance is their presence in the sensory tentacles as that correlates well with our previous sensory physiology studies, and their presence in gill interfilamental junctions, the physiology of which has not been well described. This project confirms the identity of histamine and H2 receptors in the nervous system and innervated organs, and coupled with our other work shows histamine to be an important endogenous biogenic amine in the bivalve *C. virginica*.

This work was supported by grants 2R25GM0600309 of the Bridge Program of NIGMS and 0516041071 of NYSDOE.

BEHAVIORAL AGGREGATION, SEX PREFERENCE AND LEARNING AND MEMORY IN FEMALE *GROMPHADORHINA PORTENTOSA*

Victoria E. Meadows and Kenneth G. Sossa Ph.D.
Department of Biology, Notre Dame of Maryland University, 4701 North Charles Street, Baltimore, MD 21210

Previously, aggregation experiments showed that *Gromphadorhina portentosa*, the Giant Madagascar Hissing Cockroach, exhibited intra-species aggregation behavior. Sex preferences among pregnant females have not been studied. While females aggregated with dominant hissing males for mating, their preferences during other social interactions and how their gestation status influences those preferences are unknown. We hypothesized that pregnant females would aggregate with other females. *G. portentosa* were presented with a preference and learning and memory test in a Y-maze discrimination choice task. Results showed that *Pregnant* females preferred the male incentive instead of the female incentive, indicating that the initial hypothesis should be rejected. Subsequent studies demonstrated that conditioned females go in the direction of where the male was previously placed regardless of olfactory cues, suggestive of a learned behavior. To study the neural substrate for preference and learning and memory, we recorded action potentials from antennae neurons. Comparison of action potential frequency between control and experimental groups showed that Y-maze conditioned roaches had a higher frequency, suggesting an experience-induced change in neuronal activity. We also recorded from leg sensory neurons and observed cross-modal changes in frequency. These studies implicate neuronal activity changes mediate preference selection, learning and memory, and cross-modal effects in female roaches. Future experiments will be conducted to explore the underlying mechanisms of behavior and neuronal plasticity in *G. portentosa*.

This project was funded by the Notre Dame faculty research & development grant.

STUDYING THE NEUROMUSCULAR JUNCTION IN ALS

Natalie Steenrod[1], Muhamed Hadzipasic[2], and Arthur Horwich, Ph.D.[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Genetics, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06520

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that progressively destroys the motor system. Histological studies of brain, spinal cord and muscle suggest dysfunction begins at the neuromuscular junction (NMJ) causing motor neurons to "die back," eventually leading to paralysis. In this study, we used several techniques to study the NMJ in a mouse model of ALS (G85R SOD1YFP) with an overall goal of describing pathological changes in synaptic vesicle release.

First we used in vivo, nerve-evoked electromyography (EMG) to quantify NMJ denervation and changes in whole muscle fatigue. Results showed that at symptom onset (4 months old) G85R SOD1YFP mice displayed a greater than 4-fold decrease in M wave (extracellular muscle action potential waveform) amplitude compared to control animals (WT SOD1YFP). This indicates significant denervation by 4 months. Additionally, mutants showed a faster M wave rundown and slower M wave recovery in response to 30Hz stimulation, indicating changes in presynaptic vesicle number or release function.

In an effort to understand these whole muscle changes at a single cell level, we assembled an in vitro preparation allowing sharp electrode recording from single muscle cells. In future studies, this will allow us to estimate the size of presynaptic vesicle pools and kinetics of trafficking between them via measurements of evoked and miniature (spontaneous) post synaptic potentials (EPSPs, mEPSPs respectively).

This research was supported in part by the Raymond and Beverly Sackler Institute for the Biological, Physical, and Engineering Science/NSF REU at Yale University, as well as a grant to UMBC from the Howard Hughes Medical Institute through the Precollege and Undergraduate Science Education Program.

Afternoon Poster Session
Group W - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

152. **ARABIDOPSIS WDR20 REGULATES THE DEUBIQUITINASE UBP3**
Andrew Baskerville, Heather Yerecic, and Dr. F. Les Erickson
Department of Biological Sciences, Salisbury University, Salisbury, MD 21801

153. **DELAWARE WETLAND RESTORATION STRATEGIES: DOES PLANTING MAKE A DIFFERENCE AFTER 15 YEARS**
John H. Dougherty and Dr. Stephanie Stotts
Department of Environmental Science, Wesley College, 120 North State Street, Dover, DE 19901

154. **OPTIMIZATION OF AN IMMUNOHISTOCHEMICAL FLUORESCENT STAINING PROTOCOL FOR USE ON CHIMERIC TISSUE**
Nimasha Fernando[1, 2], Holly Hewitt[3], and Walter Low[3]
[1]Department of Interdisciplinary Studies, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Life Sciences Summer Undergraduate Research Program (LSSURP), University of Minnesota, 1475 Gortner Avenue, Saint Paul, MN 55108
[3]Department of Neurosurgery, University of Minnesota, 2001 6th Street SE, Minneapolis, MN 55455

155. **THE EFFECTS OF CALCIUM IONS ON CADMIUM TOXICITY AND SHOOT GROWTH IN A301 RICE *ORYZA SATIVA* CULTIVARS**
Michael Gordon[1], Roy Nunez[2], Koli Machirouf[2], Anu Bolarinwa[2], Kameka Deans[2], Kwasi Boateng[2], and Charles Maliti, PhD[2]
[1]Department of Biology, Dominican College, 470 Western Highway, Orangeburg, NY 10962
[2]Department of Biology, Bronx Community College, 2155 University Avenue, Bronx, NY 10453

156. **DETERMINING BACTERIAL AND VIRAL UPTAKE IN HYDROPONICALLY GROWN MICROGREENS**
June Teichmann, Qing Wang, and Kali Kniel
Department of Animal and Food Sciences, University of Delaware, Newark DE 19717

157. **IDENTIFICATION AND CHARACTERIZATION OF MUTANTS RELATED TO *LRS1* IN ARABIDOPSIS THALIANA**
Heather Yerecic, Patti Erickson, and F. Les Erickson
Department of Biological Sciences, Salisbury University,
1101 Camden Avenue, Salisbury, MD 21801

ARABIDOPSIS WDR20 REGULATES THE DEUBIQUITINASE UBP3

Andrew Baskerville, Heather Yerecic, and Dr. F. Les Erickson
Department of Biological Sciences, Salisbury University, Salisbury, MD 21801

The small protein ubiquitin regulates proteins by marking them for proteasomal degradation or by altering their function. Ubiquitin is covalently attached to target proteins by ubiquitinases. On the other hand, deubiquitinases (DUBs) remove ubiquitin and save tagged proteins from degradation or restore them to prior function. We have identified an Arabidopsis WD40-domain protein predicted to be involved in regulating DUBs. WDR20 is a conserved protein that, in animal cells, binds to and activates the DUBs, USP12 and USP46, which are involved in numerous signal transduction pathways. We used the yeast two-hybrid method to show Arabidopsis WDR20 interacts with UBP3 and UBP4, two plant homologs of USP12 and USP46. We found that WDR20 stimulates the DUB activity of UBP3 *in vitro* by over four fold. To identify additional WDR20 interactions, an Arabidopsis prey cDNA library was screened using WDR20 as bait. Interestingly, this screen uncovered two ubiquitinases, MIEL1 and PGPD14, as WDR20 interactors, which suggests plant WDR20 may also play a role in regulating ubiquitin addition reactions.

DELAWARE WETLAND RESTORATION STRATEGIES: DOES PLANTING MAKE A DIFFERENCE AFTER 15 YEARS

John H. Dougherty and Dr. Stephanie Stotts
Department of Environmental Science, Wesley College,
120 North State Street, Dover, DE 19901

Wetland restoration projects have become popular in Delaware after a 200 year legacy of wetland destruction. Land managers have restored over 40 wetlands in the Blackiston Wildlife Area since 1992. Despite the widespread increase in wetland restoration projects, a restoration protocol has not yet been adopted with some projects restored through planting and others left to revegetate naturally.

In this project, we compared the vegetative composition of two restored wetlands in the Blackiston Wildlife Area. Both wetlands were restored in 1997, but one was planted and the other one was not. We determined the tree, shrub, and herbaceous community composition using the Stephenson-Adams vegetation sampling method consisting of a series of nested subplots within a 50m X 20m rectangle. The diameter at breast height was recorded for all trees within 4 10m X 25m subplots and all shrubs within 4 5m X 5m subplots. We recorded the Daubenmire cover class for all herbaceous plants within 10 1m X 1m subplots. Half plots were substituted in the event that a full plot did not fit within the restored wetland area. After collecting field data, we calculated the Shannon-Weiner Diversity index, evenness, and density and compared the results.

This study indicates that the planted wetland has more species diversity in both the tree and shrub category and higher density in the shrub category than naturally vegetated wetlands. However, the naturally revegetated wetland had a higher density in the tree category. Planting a wetland may result in greater plant diversity than a naturally revegetated wetland, but we will need to sample more restored wetlands and complete a statistical analysis before drawing any definitive conclusions.

I would like to thank National Science Foundation (NSF) EPSCoR Grant No. IIA-1301765 (DE-EPSCoR RII-3) for funding this research

OPTIMIZATION OF AN IMMUNOHISTOCHEMICAL FLUORESCENT STAINING PROTOCOL FOR USE ON CHIMERIC TISSUE

Nimasha Fernando[1, 2], Holly Hewitt[3], and Walter Low[3]
[1]Department of Interdisciplinary Studies, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Life Sciences Summer Undergraduate Research Program (LSSURP), University of Minnesota, 1475 Gortner Avenue, Saint Paul, MN 55108
[3]Department of Neurosurgery, University of Minnesota, 2001 6th Street SE, Minneapolis, MN 55455

Biomedical research applications and medical treatments generate significant demands for human tissues. Potentially, this need may be satisfied by developing human organs in chimeric porcine containing human and pig genetics. To help obtain this goal, we endeavored to optimize an indirect immunohistochemical fluorescent staining protocol to differentiate human cells from those of other model species. Primary (1º) antibodies including human nuclear MAB (monoclonal antibody) 1281, human mitochondrial MAB 1273, and Ku80 were tested in coordination with Alexa Fluor 555 Goat-anti-Mouse secondary (2º) antibody. Various species, tissue types, washing agents, block types and concentrations, and other variables were tested to discern optimal experimental conditions for human cell identification. We concluded that tissues thoroughly fixed upon harvest which experienced minimal time between cryo-sectioning, staining, and fluorescent microscopy displayed reduced nonspecific binding. Furthermore, liver tissues demonstrated excessive nonspecific binding while pancreatic specimens produced exceedingly clear staining, especially when using 5% Goat Block with Phosphate Buffer Solution (PBS) washes. Overall, our results suggest that human and non-human cells can be accurately distinguished. When utilized for chimeric tissue, our protocol will allow the extent of human cell incorporation to be quantitatively analyzed to determine the progress achieved towards development of human organs in other species.

Thank you to the National Science Foundation and National Institutes of Health for sponsoring the University of Minnesota Life Sciences Summer Undergraduate Research Program's Molecular Genomics and Proteomics and Heart, Lung, and Blood divisions.

THE EFFECTS OF CALCIUM IONS ON CADMIUM TOXICITY AND SHOOT GROWTH IN A301 RICE *ORYZA SATIVA* CULTIVARS

Michael Gordon[1], Roy Nunez[2], Koli Machirouf[2], Anu Bolarinwa[2], Kameka Deans[2], Kwasi Boateng[2], and Charles Maliti, PhD[2]
[1]Department of Biology, Dominican College, 470 Western Highway, Orangeburg, NY 10962
[2]Department of Biology, Bronx Community College,
2155 University Avenue, Bronx, NY 10453

Online access of this abstract is restricted at the request of the Principal Investigator.

DETERMINING BACTERIAL AND VIRAL UPTAKE IN HYDROPONICALLY GROWN MICROGREENS

June Teichmann, Qing Wang, and Kali Kniel
Department of Animal and Food Sciences, University of Delaware, Newark DE 19717

Microgreens are a rapidly growing industry with few specific regulations leaving growers caught between guidelines for sprouts and baby greens. For all leafy greens and sprouts, the two most common pathogenic bacteria found on them are *Salmonella* and *E.coli*. As the microgreen market grows, it becomes increasingly important to understand bacterial behavior specifically relevant for microgreens, because of some major differences from sprouts. An increasingly common way to grow microgreens is in hydroponics systems, which offer vast benefits for sustaining resources like water and space. Regarding food safety, in a controlled environment it should be easier to limit contamination, although if contamination should occur, the risk for subsequent illness associated with the product could be greater. In this study, separate hydroponics systems were set up for the analysis of *Salmonella* and *E. coli*. An additional trial tested *Norovirus*, which is the leading cause of acute gastroenteritis worldwide. Kale and mustard microgreens were studied (n=3 systems per treatment). Following inoculation on day 8, samples were collected from the water, roots, and leaves of each tray (days 9-12, n=9 for each pathogen). To determine bacterial or viral uptake and distribution, all samples were serially diluted, and bacteria and virus titers determined by enumeration or PCR. Results indicate the ability for pathogens to persist in a circulating water system and address the risk of uptake within microgreen tissues by bacteria and virus ($p<0.05$ compared to controls). Further knowledge of this risk is essential regarding the growth of hydroponically-grown leafy greens and microgreens.

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IDENTIFICATION AND CHARACTERIZATION OF MUTANTS RELATED TO *LRS1* IN ARABIDOPSIS THALIANA

Heather Yerecic, Patti Erickson, and F. Les Erickson
Department of Biological Sciences, Salisbury University,
1101 Camden Avenue, Salisbury, MD 21801

Lateral root stimulator 1 (LRS1) is a WD40-repeat protein that contains a Damaged DNA Binding (DWD) motif, suggesting an interaction with E3 ubiquitin ligases of the Damaged DNA Binding 1–Cullin 4 (DDB1-CUL4) type. Previous findings indicate LRS1 binds a deubiquitinase, suggesting a role in ubiquitin regulation. Plant lines containing Agrobacterium T-DNA insertions in *LRS1* have been identified for phenotypic analyses. Initial characterization of one *lrs1* line (FS16) showed reduced roots, as previously described for the *lrs1-1* allele. Altered leaf, flower, and seed morphologies, in addition to altered hormone responses, have also been observed. Comparison to wild-type sibling segregates (FS15), however, suggested the presence of additional mutations in the T-DNA line under study. To segregate the T-DNA insertion from unrelated mutations, FS16 plants are being back-crossed three times to wild-type Columbia (Col-0) and genotyped using PCR. In addition to these mutants, T-DNA lines mutated in genes encoding potential LRS1-interacting proteins, such as a RING (Really Interesting New Gene) E3 ubiquitin ligase and a histone methyltransferase, are also being analyzed. Characterization of mutant phenotypes will help elucidate the function of LRS1*in vivo* and allow the exploration of its role in ubiquitination.

I would like to thank The Henson School of Science for funding my research with the Undergraduate Research Grant.

Afternoon Poster Session
Group X - Biological Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

158. **PRESENCE OF OCTOPAMINE RECEPTORS IN HEART OF THE BIVALVE *CRASSOSTREA VIGINICA***

Fiana Bess, Ave Harris, Christopher Welsh, Margaret A. Carroll and Edward J. Catapane
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

159. **IDENTIFYING POTENTIAL TRANSCRIPTION FACTOR BINDING SITES IN SYNTHESIZED DNA**

Grace Chandler[2], Jacob O'Bott[1], Ivan Erill[2], and DoHwan Park[1]
[1]Department of Mathematics and Statistics, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Bioinformatics, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

160. **UPLAND FOREST BUFFER LOSES DUE TO INUNDATION CAUSED BY SEA LEVEL RISE IN THE UPPER AND LOWER SUB-WATERSHEDS OF THE BROADKILL RIVER**

Kyle Frame[1], Susan Love[2], and Stephanie Stotts[1]
[1]Department of Environmental Studies, Wesley College, 120 N. State Street, Dover, DE 19901
[2]Department of Natural Resources and Environmental Control, Dover, DE 19901

161. **P-AMINOSAYLISYLIC ACID (PAS) REVERSES NEUROTOXIC EFFECTS OF MANGANESE ON THE PHYSIOLOGICAL RESPONSE OF A DOPAMINERGIC SYSTEM**

Cassandra Mezalon, Toshanna McBean, Margaret A. Carroll, and Edward J. Catapane
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

162. **THE EFFECTS OF FOX P2 KNOCKDOWN ON BRAINSTEM MOTOR NEURON PROJECTIONS TO LARYNGEAL MUSCLES IN ADULT MICE**

Taylor Radden[1,3], Jonathan Chabout[1,2] , and Erich D. Jarvis[1,2]
[1]Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815
[2] Department of Neurobiology, 412 Research Drive, Duke University, Durham, NC 27710
[3] UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

163. **ANALYSIS OF DNA METHYLATION AT THE UDMR LOCUS OF THE IMPRINTED *RASGRF1* IN MOUSE EMBRYONIC DEVELOPMENT**

Rachel Shields and Dr. Tamara Davis
Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010

164. **ANTIFUNGAL COMPOUNDS PRODUCED BY *BACILLUS* SPP. ISOLATED FROM SOIL**

Sarah Yannarell, Jessica Keffer, Mengyin Yao, and Julia Maresca
Department of Civil and Environmental Engineering, University of Delaware,
301 DuPont Hall, Newark, DE 19716

PRESENCE OF OCTOPAMINE RECEPTORS IN HEART OF THE BIVALVE *CRASSOSTREA VIGINICA*

Fiana Bess, Ave Harris, Christopher Welsh, Margaret A. Carroll, and Edward J. Catapane
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

Octopamine, a biogenic amine first identified in octopus, is well studied in arthropods and gastropods where it functions as a neurotransmitter and hormone. The presence or function of octopamine has rarely been reported in bivalves. Previously, using HPLC we found octopamine in cerebral ganglia, visceral ganglia, gill, heart, palps and hemolymph of the oyster *Crassostrea virginica* and using immunohistofluorescence we visualized octopamine in cerebral ganglia, visceral ganglia, gill and heart. Our physiological studies also found that octopamine was cardio-active when applied to *C. virginica* and *Mytilus edulis* hearts. We hypothesize that octopamine receptors are present in the heart of *C. virginica.* To test this we did Western Blot analysis using and pan TAAR (trace amine-associated receptor) primary antibodies, which are reactive with octopamine, beta-phenylethylamine (b-PEA), p-tyramine (p-TYR) and tryptamine receptors, but unresponsive to classical biogenic amines and histamine receptors. For Western Blot analysis, heart tissue lysate was prepared by polytron disruption in ice-cold NP-40 detergent buffer containing protease inhibitor, followed by centrifugation to obtain supernatant with solubilized membrane proteins. Up to 30 Φg of solubilized protein was subjected to SDS-PAGE with 10% acrylamide gels and electroblotted onto nitrocellulose. Pan TAAR receptor immunoreactivity was revealed after incubation with primary antibodies followed by incubation with HRP-conjugated secondary antibodies. The Western Blot studies showed a strong band at 85 kD corresponding to octopamine receptors in heart. The present project, coupled with our immunohistofluorescence and cardio-physiology studies, confirms the presence of octopamine receptors and furthers the understanding of a physiological role for octopamine in *C. virginica*.

This work was supported by grant 0516041071 of NYSDOE.

IDENTIFYING POTENTIAL TRANSCRIPTION FACTOR BINDING SITES IN SYNTHESIZED DNA

Grace Chandler[2], Jacob O'Bott[1], Ivan Erill[2], and DoHwan Park[1]
[1]Department of Mathematics and Statistics, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Bioinformatics, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Metagenomics is the study of genetic material taken directly from an environment, analyzing collected data from a multi-species level. Transcription factors (TFs) are proteins that affect the transcription of related genes within a functionally transcribed group. The goal of this study is to properly identify putative transcription factor binding sites within a metagenome. By identifying putative binding sites, the functional relationship between environmental conditions and transcriptional behavior can be better characterized, eventually determining if environment, as opposed to phylogeny, has a greater effect on the composition of transcriptional systems.

Site identification is done statistically by determining scores of each potential TF binding site. Various statistical methods, including different order Markov models are being tested. In order to find these putative binding sites, a computer program was developed which reads DNA in a sliding window and sliding the scores based off of a position-specific-scoring matrix, PSSM, with higher scores signaling the location of a potential binding site. The PSSM uses a background probability model a Markov-1 model, as well as a motif probability, derived from known binding sites gathered from the CollecTF transcription factor database.

This program was tested using artificially created DNA sequence sets enriched with synthetic binding sites. These binding sites were synthesized using nucleotide frequencies trained from aligned binding sites. Moving forward, the PSSM method will be applied to metagenomic samples using a calculated threshold to determine whether a score is high enough to signal a binding site.

This work was funded in part through an Undergraduate Biology Mathematics (UBM) Research Award from the National Science Foundation under Grant No. DBI 1031420, PIs Drs. Leips and Neerchal. We would also like to thank our research mentors, Dr. Erill and Dr. Park, ErillLab Researcher Patrick O'Neil and Master Curator of CollecTF, Dinara Sagitova.

UPLAND FOREST BUFFER LOSES DUE TO INUNDATION CAUSED BY SEA LEVEL RISE IN THE UPPER AND LOWER SUB-WATERSHEDS OF THE BROADKILL RIVER

Kyle Frame[1], Susan Love[2], and Stephanie Stotts[1]
[1]Department of Environmental Studies, Wesley College, 120 N. State Street, Dover, DE 19901
[2]Department of Natural Resources and Environmental Control, Dover, DE 19901

Riparian buffer zones improve water quality by filtering and reducing nutrients that run off of adjacent properties. These important barriers are threatened in the Broadkill River Watershed by inundation from sea level rise and infringement by development, especially where buffer regulations are weak. A digitization of upland forested buffers around the high tide mark was done using 2012 aerial imagery and was overlaid with the Bathtub Model Sea Level Rise (SLR) scenarios of 0.5 meters, 1.0 meter, and 1.5 meters to calculate the geometric acreage of riparian forest inundation extent for both the Lower Broadkill River and the Upper Broadkill River sub-watersheds. Areas with potential development were overlaid with the SLR scenarios to estimate the maximum loss of riparian buffers. With a build out condition and the existing 50 foot buffer, up to 64.13% of the non-protected forested riparian area in the 2 watersheds could be inundated at 0.5 meters sea level rise; 82.53% at 1 meter and 91.51% at 1.5 meters. Similar analyses were run to determine extent of loss with wider buffer requirements. These results will help local governments to understand potential impacts of sea level rise on water quality and to plan for future regulatory or restoration archives.

This research was funded by the National Science Foundation (NSF) EPSCoR Grant No. IIA-1301765 (DE-EPSCoR RII-3)

P-AMINOSAYLISYLIC ACID (PAS) REVERSES NEUROTOXIC EFFECTS OF MANGANESE ON THE PHYSIOLOGICAL RESPONSE OF A DOPAMINERGIC SYSTEM

Cassandra Mezalon, Toshanna McBean, Margaret A. Carroll, and Edward J. Catapane
Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225

Manganese is a neurotoxin causing Manganism, a Parkinsons-like disease in humans. Manganese neurotoxicity involves disruption of dopaminergic neurotransmission. The mechanism how manganese is not fully resolved. Lack of effective treatment for manganese toxicity is a major obstacle in clinical management of Manganism. Recently, p-Aminosalicylic acid (PAS) was reported effective treatment of Manganism; however its mechanism of action is unclear. Gill lateral cell cilia of *Crassostrea virginica* are controlled by serotonergic-dopaminergic innervations from their ganglia. Dopamine is the neurotransmitter causing cilio-inhibition, serotonin cilio-excitation. Previous work of our lab showed manganese blocks cilio-inhibitory effects of dopamine and this is prevented by co-treatments with PAS. We hypothesize that PAS would effectively reverse the neurotoxic actions of manganese when applied after manganese. We treated *C. virginia* for up to 3 days with manganese (500 mM) followed by up to 5 more days with PAS (500 mM). Three sets of control animals were similarly treated with only manganese, or only PAS, or neither. Ciliary activity of gill lateral cells was measured by stroboscopic microscopy and expressed as beats/min $\forall$ sem. We found, in congruence with our earlier studies, that manganese treatments disrupted the dopamine (10^{-6} - 10^{-4} M) induced cilio-inhibitory dose response of the gill lateral cells. In addition our results show that PAS treatments, after manganese exposure, effectively reversed this neurotoxicity and the cilia of lateral gill cells responded normally to the dose response to dopamine with the appropriate decrease in cilia beating rates. The study shows that PAS can effectively reverse the neurotoxic effects of manganese on the physiological response of a dopaminergic system innervating gill lateral ciliated cells. These finding are helpful to furthering the understanding of the mechanism underlying manganese neurotoxicity and in the search for effective treatments for Manganism, and in particular concerning PAS as a therapeutic agent.

This work was supported by 2R25GM06003 of the Bridge Program of NIGMS and 0516041071 of NYSDOE.

THE EFFECTS OF FOX P2 KNOCKDOWN ON BRAINSTEM MOTOR NEURON PROJECTIONS TO LARYNGEAL MUSCLES IN ADULT MICE

Taylor Radden[1,3], Jonathan Chabout[1,2] , and Erich D. Jarvis[1,2]
[1]Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815
[2] Department of Neurobiology, 412 Research Drive, Duke University, Durham, NC 27710
[3] UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Vocal learning, a behavioral substrate of spoken language, is a rare trait found only in eight animal groups including humans. Across these groups, the neural framework supporting such ability is highly similar. The disruption, particularly through genetic manipulation, of comparable segments of the vocal system in non-human animals has led to further understanding of disorders of speech development in humans. One such example is the mutation of the transcription factor Fox-P2. As found originally in the KE family, in humans a R553H mutation leads to a reduced control of the orofacial muscles used in speech production as well as issues with language comprehension. In songbirds, FoxP2 knockdown has been shown to disrupt song learning and production. Here we studied mice carrying the heterozygous R553H FOXP2 mutation of the KE family, to understand the role of FOXP2 in the establishment of a direct projection from the nucleus ambiguus to the laryngeal muscles controlling vocalizations in mice. We used a GFP- expressing viral tracer PRV- Bartha that we injected into the vocal- controlling laryngeal muscles. Our preliminary results suggest that the nucleus ambiguus motor neurons are more scattered in the brainstem compared to wild type controls. We propose that this could be the cause of differences in vocal behavior of the FoxP2 mutant mice. Moving forward, we hope to reach a clearer understanding of the effects of genetic manipulation on the neural circuitry important to the mouse vocal system. With this understanding, the ease of genetic manipulation offered through mouse models may hopefully be applied to studies focused on uncovering the genetic aspects of speech development.

Funding for this work was provided by the Howard Hughes Medical Institute and the Duke Summer Research Opportunity Program.

ANALYSIS OF DNA METHYLATION AT THE UDMR LOCUS OF THE IMPRINTED *RASGRF1* IN MOUSE EMBRYONIC DEVELOPMENT

Rachel Shields and Dr. Tamara Davis
Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010

Genomic imprinting refers to the differential expression of the parental alleles, which is achieved through epigenetic modifications to the chromatin structure to distinguish the maternally vs. paternally derived alleles. DNA methylation and histone modification are two mechanisms through which the parent of origin-specific allele can be differentiated. *Rasgrf1* is a paternally-methylated, paternally-expressed imprinted gene located on mouse chromosome 9. This imprinted gene's differentially methylated region (DMR) is believed to be more expansive than originally proposed. The methylation patterns of this extended, paternally-methylated upstream DMR (uDMR) of *Rasgrf1* are still being investigated.

Paternal allele-specific DNA methylation at *Rasgrf1* is inherited through the sperm. Both the maternal and paternal alleles of the *Rasgrf1* uDMR in sperm cells are methylated, while oocytes exhibit low levels of DNA methylation on both parental alleles. The epigenetic modifications in gametic DNA have been compared to the methylation patterns of the uDMR in 12.5 dpc embryos, which depict different levels of allele-specific DNA methylation. It has been observed that DNA methylation at imprinted genes contracts and re-expands during early embryonic development, however it is not known at what developmental stage this occurs.

This study aims to examine how the DNA methylation pattern at the *Rasgrf1* uDMR is maintained during mouse embryonic development by utilizing bisulfite mutagenesis, which converts unmethylated cytosines to uracils. Subsequent sequence analysis is employed to identify the non-mutagenized, methylated cytosines. It is hypothesized that this reorganization of the DNA methylation occurs during the first 12.5 days of embryonic development. Therefore, this analysis will be done utilizing DNA extracted from blastocysts as well as 6.5 dpc, 9.5 dpc, and 14.5 dpc embryonic tissues in order to determine specifically when the DNA methylation contraction and re-expansion occur at the *Rasgrf1* uDMR.

ANTIFUNGAL COMPOUNDS PRODUCED BY *BACILLUS* SPP. ISOLATED FROM SOIL

Sarah Yannarell, Jessica Keffer, Mengyin Yao, and Julia Maresca
Department of Civil and Environmental Engineering, University of Delaware,
301 DuPont Hall, Newark, DE 19716

Developing new methods to control pathogens is vital in crop and health protection as some organisms are becoming resistant to current means of protection. Biologically active compounds produced in nature could be harnessed as control agents for plant pathogens. Fungus is the most prevalent type of plant pathogen due to its spore forming capability. *Magnaporthe oryzae* is a fungal pathogen that causes rice blast, a disease that damages rice plants through leaf lesions and seed blanking. The goals of this project were to screen strains of *Bacillus* spp. isolated from soil for antifungal activity against *M. oryzae* and determine the cause of fungal growth inhibition by investigating secondary metabolites with antifungal activity.

Fifteen strains of soil bacteria were screened against two unidentified fungi isolated from soil and *M. oryzae* using a whole colony assay. Four *Bacillus* spp. secreted soluble antifungal molecules shown by reduced fungal growth on solid plates.

When bacterial cultures were grown alone, the antifungal activity could not be located to either cell lysate or culture supernatant. However, activity was detected in *Bacillus* cultures grown with fungus, which indicated that antifungal molecules might only be secreted in the presence of fungus. Additionally, a foamy layer formed between the organic and combined culture supernatant layer, suggesting that a surfactant might be present in the supernatant. Other strains of *Bacillus* are known to produce biosurfactant lipopeptides that exhibit antifungal activity. To begin isolating the hypothesized lipopeptides, the supernatant was extracted using chloroform/methanol and each fraction was tested for antifungal activity.

Active fractions were analyzed by reverse-phase high performance liquid chromatography (HPLC), and common peaks were identified as the possible active lipopeptide. Future work includes determining when the antifungal molecules are produced and purifying and identifying the predicted lipopeptide using HPLC and mass spectrometry.

This work was made possible by the National Science Foundation EPSCoR Grant no. EPS-0814251, University of Delaware Summer Scholars Program, the DENIN Environmental Frontiers Grant Program, and the Department of Civil and Environmental Engineering, University of Delaware.

Afternoon Poster Session
Group Y – Chemical Sciences

Poster #	Title, Author(s) & Affiliation(s)
165.	**SURFACE TENSION OF SALT AND AMINO ACID SOLUTIONS** Diana Al Husseini and Yanjie Zhang Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807
166.	**THE CHEMISTRY AND SPECTROSCOPY OF OH RADICALS IN O_2, N_2, AND CO SOLID MATRICES** Derek Dwyer and Dr. Paul Cooper Department of Chemistry and Biochemistry, George Mason University, Fairfax, VA 22030
167.	**CHLORIDE (Cl^-) VS, TOSYLATE (OTs^-): LEAVING GROUP BATTLES AT THE ALKOXYCARBONYL CARBON** Catherine E. Gross and Dr. Malcolm J. D'Souza Department of Chemistry, Wesley College, 120 N. State Street, Dover, DE 19901
168.	**MODIFYING THE SURFACE CHEMISTRY OF TWO TYPES OF ELECTRODES: BORON-DOPED DIAMOND (BDD) AND GLASSY CARBON (GC)** Azeez Ibrahim[1] and Greg M. Swain[2] [1]Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225 [2]Department of Chemistry, Michigan State University, 578 Shaw Lane, East Lansing, MI 48824
169.	**TRANSITION METAL CATALYSTS FOR THE ELECTROCHEMICAL CONVERSION OF CO_2 TO CO** Thomas Keane, Jonnathan Medina Ramos, John DiMeglio, and Joel Rosenthal Department of Chemistry and Biochemistry, University of Delaware, 102 Brown Laboratory, Newark, DE 19716
170.	**TIME-RESOLVED INFRARED SPECTROSCOPY OF [FeFe]-HYDROGENASE MODEL COMPOUNDS** Rachel Meyer[1], Anet Zhandosova[1], Edwin J. Heilweil[2], and Christopher J. Stromberg[1] [1]Department of Chemistry and Physics, Hood College, 401 Rosemont Avenue, Frederick, MD 21701 [2]Radiation Physics Division, Physical Measurements Laboratory, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899
171.	**TRANSITION FROM B-DNA TO Z-DNA IN SODIUM ION-STABILIZED POLYNUCLEOTIDE DUPLEXES** Daniela Rey-Ardila, Aloise Diedrich, Kelsey Polak, and Dr. Richard Preisler Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

SURFACE TENSION OF SALT AND AMINO ACID SOLUTIONS

Diana Al Husseini and Yanjie Zhang
Department of Chemistry and Biochemistry, James Madison University,
Harrisonburg, VA 22807

Hofmeister series is a set of ions in order of their abilities to affect physical properties in aqueous solutions. Franz Hofmeister was the first to discover the specific ion effects on protein solubility in aqueous solution. Although the Hofmeister series' phenomena are general, their mechanisms are still not fully understood. Herein, we use optical tensiometry by pendant drop method to study the Hofmeister anion effects on the surface tension of water. It has been observed that the surface tension of water increases linearly as the salt concentrations increase. The effects of salts follow a specific trend with respect to the surface tension increments: SO_4^{2-} > CO_3^{2-} > $H_2PO_4^-$ > F^- > Cl^- > Br^- > I^- > NO_3^-> ClO_4^- > SCN^-. Using the same method, the effect of solution pH on the surface tension of different amino acid solutions is also being investigated. So far, it has been observed that the surface tension increments increase as pH of the solution increases. These studies will provide us with some valuable data that would help further the understanding of the behaviors of salts and amino acids at the air/water interface.

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THE CHEMISTRY AND SPECTROSCOPY OF OH RADICALS IN O_2, N_2, AND CO SOLID MATRICES

Derek Dwyer and Dr. Paul Cooper
Department of Chemistry and Biochemistry, George Mason University, Fairfax, VA 22030

The chemical formation of water in the Interstellar Medium (ISM) is of major importance in Astrochemistry. Many important chemical reactions in the ISM occur on grains that may be covered in ices such as O_2, N_2, and CO, as well as H_2O. The identification of OH radical, an intermediate in the formation of water, in these different ices can give a better understanding of the formation of water in the ISM. Matrix isolation experiments in O_2, N_2 and CO at low temperature and pressure were performed to simulate the icy conditions that would be found in the ISM. Samples were prepared in the gas phase in two separate gas lines: one for the gas to be used as the matrix; and the other containing an H_2O/He mixture that was passed through an RF discharge to generate OH. The samples were co-deposited onto a KBr window at low temperature and the resulting FTIR spectrum acquired.

The band position of matrix-isolated OH radical in O_2, N_2, and CO ices will be presented. These results will support further investigation of the reactions of OH in these ices by the unambiguous determination of the OH band position in the three different ices. However, our results also show that that OH radical may react with these ices themselves, thus producing new intermediates that may lead to new chemical pathways to produce stable molecules in the ISM.

I would like to thank the OSCAR (Students as Scholars) program at George Mason University for funding my undergraduate research project.

CHLORIDE (Cl^-) VS, TOSYLATE (OTs^-): LEAVING GROUP BATTLES AT THE ALKOXYCARBONYL CARBON

Catherine E. Gross and Dr. Malcolm J. D'Souza
Department of Chemistry, Wesley College, 120 N. State Street, Dover, DE 19901

The octyloxy carbonyl group is used in the synthesis of novel 2-oxy-bezoxazinone derivatives whose capability in the inhibition of enzyme to catalyse the hydrolysis of an ester functionality is used the treatment of obesity. In chemistry, tosylate is a better leaving group than the chloride anion since the displaced group is the resonance stabilized anion. In this project, we compare the rates of reaction of octyloxy carbonyl tosylate to octyl chloroformate at 25.0 oC.

Octyloxy carbonyl tosylate is prepared by mixing a equimolar solution of oxyl chloroformate and silver tosylate. The silver chloride precipitate forms immediately and is suction filtered out of the solution. In one project, our research group will compare the specific rates of solvolysis of octyl chloroformate in a variety of solvents with varying ionizing abilities. My project will study octyloxy carbonyl tosylate in detail and compare the rates against octyl chloroformate. These two compounds are studied in a wide variety of aqueous alcohols, aqueous acetone, and several mixed fluoro-alcohols using the acid-base titration method, with acetone diluted lacmoid as an indicator and sodium methoxide as the base.

Octyloxy carbonyl tosylate had similar reaction rates in some solvents, but reacted faster in fluoro-alcohols. Solvolysis data of such organic compounds are useful to effectively prepare a pharmaceutically acceptable carrier or diluent. In n-alkoxycarbonyl esters, the leaving group has an effect on the rate determining step in SN1 reaction in some solvents.

This research was funded by the National Institute of General Medical Sciences - NIGMS (8 P20 GM103446-13) from the National Institutes of Health (DE-INBRE program).

MODIFYING THE SURFACE CHEMISTRY OF TWO TYPES OF ELECTRODES: BORON-DOPED DIAMOND (BDD) AND GLASSY CARBON (GC)

Azeez Ibrahim[1] and Greg M. Swain[2]
[1]Department of Biology, Medgar Evers College, 1638 Bedford Avenue, Brooklyn, NY 11225
[2]Department of Chemistry, Michigan State University, 578 Shaw Lane, East Lansing, MI 48824

This research project investigated whether the surface of carbon electrodes can be chemically modified and, if so, what effect does the changes in surface chemistry have on basic electrochemical properties of a soluble redox system. Three surface terminations were studied: hydrogen-terminated, oxygen-terminated and amine-terminated. Hydrogen-terminated surfaces were introduced via microwave plasma in the presence of hydrogen, while the oxygen and amine-terminated surfaces were produced in radio frequency plasma using oxygen and ammonia, respectively. Once the surface chemistry was changed, the question of the effect the change some basic electrical chemical properties of the electrode were studied. Cyclic voltammetry (CV) was used to investigate the background current and Faradaic current response for two soluble redox systems: $Fe(CN)_6^{-3/-4}$ and $Ru(NH_3)_6^{+3/+2}$. I hypothesize that for both electrodes, the hydrogen-terminated surfaces should exhibit high activity for both redox systems. For the oxygen-terminated surfaces, we expected high activity for $Ru(NH_3)_6^{+3/+2}$ and more inhibitory effects on $Fe(CN)_6^{-3/-4}$. The activity of the amine-terminated surfaces is dependent on the solution pH. With the amine groups protonated (positive surface charge), high activity was expected for $Fe(CN)_6^{-3/-4}$ while more inhibited electron transfer was expected for $Ru(NH_3)_6^{+3/+2}$. To prepare the surface of the electrodes, they were purged with argon gas to remove contaminants. Surface of the carbon electrodes were then purged with their respective organic compounds (hydrogen, oxygen and nitrogen) and then analyzed through the use of an electrochemical cell. Our results showed distinctions between the two-carbon electrodes, proving to us that certain surface terminations cause changes to the surface chemistry of the respective carbon electrode, as well as affects certain electrochemical properties of the carbon electrodes. The significance of this research is that it will provide a greater understanding of modified electrodes. Such modification could serve as a foundation for electrochemical detection schemes of enzymes, proteins, and DNA.

TRANSITION METAL CATALYSTS FOR THE ELECTROCHEMICAL CONVERSION OF CO_2 TO CO

Thomas Keane, Jonnathan Medina Ramos, John DiMeglio, and Joel Rosenthal
Department of Chemistry and Biochemistry, University of Delaware,
102 Brown Laboratory, Newark, DE 19716

Electrocatalyzed CO_2 reduction to form CO is a process which can be applied to two current environmental problems: Rising atmospheric CO_2 concentrations, and the need for an efficient storage method for power produced by intermittent "clean" power sources (i.e. Solar and wind power). The CO produced in this process can be fed into well-established reactions in order to produce both liquid fuels and commodity chemicals. Electrodeposition is a process which allows for easy fabrication of catalysts with novel surface morphologies. Several transition metals were electrodeposited onto catalytically inactive substrates from organic solution. The abilities of these films to catalytically activate CO_2 were examined under various conditions.

This research was funded through the HHMI summer scholars program at the University of Delaware

TIME-RESOLVED INFRARED SPECTROSCOPY OF [FeFe]-HYDROGENASE MODEL COMPOUNDS

Rachel Meyer[1], Anet Zhandosova[1], Edwin J. Heilweil[2], and Christopher J. Stromberg[1]
[1]Department of Chemistry and Physics, Hood College,
401 Rosemont Avenue, Frederick, MD 21701
[2]Radiation Physics Division, Physical Measurements Laboratory,
National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899

Hydrogenases are naturally-occurring enzymes that catalyze the reversible reaction from H^+ to hydrogen gas. Synthetic models of [FeFe]-hydrogenase are being studied in hopes of developing a hydrogen source that is not reliant on fossil fuels. These model compounds have a wing-like structure with a Fe-Fe bond, three pendant ligands on each Fe, two sulfur atoms bridging the irons, and a short organic chain connecting the sulfurs. The turnover rates for these compounds are substantially lower than that of the biological catalyst, although varying the active site ligands can alter the catalytic properties.

One active area of research is to pair hydrogenase models with visible/UV absorbing chromophores, in a mechanism inspired by photosynthesis. To successfully create photo-driven hydrogenase catalysts, their behavior after light exposure must be understood. Our research involved studying $Fe_2(\mu\text{-}S_2C_2H_4)(CO)_4(PMe_3)_2$ using time-resolved ultraviolet/visible pump, infrared probe spectroscopy. We also investigated the isomerization properties of $Fe_2(\mu\text{-}S_2R)(CO)_4(M)_2$ and $Fe_2(\mu\text{-}S_2R)(CO)_5(T)$ models (R=ethyl or propyl; M=CO, $P(Me)_3$, or CN; T=CN) using infrared spectroscopy.

$Fe_2(\mu\text{-}S_2C_2H_4)(CO)_4(PMe_3)_2$, was dissolved in acetonitrile or n-heptane and excited with 532, 355, and 266 nm light. In both solutions, a decay to half the original concentration is present at ~1944 cm^{-1} in all observed wavelengths. A similar decay at 1850 cm^{-1} is only seen at 355 nm and 532 nm in both solvents. In heptane, there is a similar decay seen at 1900 cm^{-1} that is not seen in acetonitrile. These decay behaviors are similar to those observed in the propyl-bridged phosphine model compound.

The carbonyl and phosphine models were dissolved in acetonitrile and hexanes, and the cyanide models were dissolved only in acetonitrile. The phosphine models' isomer ratios are affected by the solvent polarity, while the carbonyl models, which only have one isomer, are not. Studies on the effects of solvent polarity on the cyanide models are in progress.

This work was funded in part by the National Institute of Standards and Technology through Cooperative Agreement number 60NANB130143. Additional funding was provided by Hood College's Summer Research Institute program.

TRANSITION FROM B-DNA TO Z-DNA IN SODIUM ION-STABILIZED POLYNUCLEOTIDE DUPLEXES

Daniela Rey-Ardila, Aloise Diedrich, Kelsey Polak, and Dr. Richard Preisler
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

The transition from B-DNA to Z-DNA is reversible and may act as a switch in regulating genetic expression. The Z-DNA conformation is found to form behind actively transcribing RNA polymerase. An important factor in this transition is hydration. Since osmolytes, like alcohols and diols, remove water from DNA, and Z-DNA is less hydrated than B-DNA, osmotic stress tends to favor Z-DNA. These osmolytes shift the equilibrium further toward Z-DNA in the presence of transition metal complexes, such as cobalt complexes with amine ligands. During this project, the +3 cobalthexammine complex was used because its interaction with DNA showed to be more effective than other metal complexes.

DNA poly[d(G-C)] was used because it forms the Z-conformation more readily. Circular dichroism spectroscopy (Jasco-815 spectropolarimeter) was used to determine the conformation of the DNA as B or Z.

The results obtained showed that at lower concentrations, osmolytes shift the equilibrium towards Z-DNA, as expected, but at higher concentrations, unusual spectra was obtained that may have shown either B-DNA or denaturation of the double helix. To solve this problem 25mM NaCl were added to our samples because cations like Na^+ are known to stabilize the double helix. In the presence of 25mM of NaCl the Z-DNA was stabilized by the removal of water.

Recently, another complex, trisethylenediaminecobalt has been examined. This complex has less conformational entropy than cobalthexammine due to the bonding of the ligands to the metal ion. It is less effective in driving the B to Z-DNA transition, but is more sensitive to osmotic stress. Sodium ions have been shown to compete with cobalt complexes for binding to DNA, so a decrease the NaCl concentration to 10 mM was necessary to observe a B-to-Z transition with trisethylenediaminecobalt. The effects of osmolytes in the presence of the trisethylenediaminecobalt are being studied.

Afternoon Poster Session
Group Z – Chemical Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

172. **A NOVEL APPROACH TO INCORPORATE CARBON NANOTUBES IN CARBON FIBER**
Bradley Baker[1], Jimmy Mays[2], and Benjamin Ripy[2]
[1]Department of Chemistry, King University, King College Road, Bristol, TN 37620
[2]Department of Chemistry, University of Tennessee, Knoxville, TN 37996-0230

173. **ISOLATION OF NICKEL(II) COMPLEXES OF COORDINATED YET UNCONDENSED SCHIFF BASE LIGAND PRECURSORS**
Eric Bowman and Peter Craig
Department of Chemistry, McDaniel College, 2 College Hill, Westminster, MD 21157

174. **EXAMINATION OF Mo COMPLEXES AND THEIR PROPERTIES: ANALYSIS OF MONODENTATE AND BIDENTATE PHOSPHINES ON TRI- AND TETRA-CARBONYLS**
Bryan Eck and Charles Mahler
[1]Department of Chemistry, Lycoming College, 700 College Place, Williamsport, PA 17701

175. **PREPARATION AND ANALYSIS OF MOLYBDENUM DISULFIDE NANOMATERIALS**
Amelework Habtemichael, Christopher Mbochwa, and Paul Sabila
Chemistry and Physics, Department of Science, Technology, and Mathematics (DSTM) Gallaudet University, 800 Florida Avenue N.E., Washington DC, 20002

176. *CANCELLED*

177. **INFLUENCE OF TEMPERATURE ON THE ANTIOXIDANT CAPACITY OF ARONIA MITSCHURINII**
Courtney Rhoades[1], Blessing Aroh[1], Kelsey Chandler[2], and Victoria Volkis[1*]
[1]Department of Natural Sciences, University of Maryland Eastern Shore, 1 Backbone Road, Princess Anne, MD 21853
[2]ACS Project SEED students, summer 2014

178. **A G4·K+ HYDROGEL STABILIZED BY AN ANION**
Luke P. Skala, Gretchen Marie Peters, and Jeffery T. Davis
Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742

A NOVEL APPROACH TO INCORPORATE CARBON NANOTUBES IN CARBON FIBER

Bradley Baker[1], Jimmy Mays[2], and Benjamin Ripy[2]
[1]Department of Chemistry, King University, King College Road, Bristol, TN 37620
[2]Department of Chemistry, University of Tennessee, Knoxville, TN 37996-0230

Carbon nanotubes (CNTs) are known to display exceptional mechanical properties including high modulus and tensile strength. However, these properties have yet to be exploited in a practical manner. Furthermore, although theoretical studies suggest that a fiber composed of CNTs should exhibit a tensile strength of at least 10x greater than tradition carbon fibers, current CNT-based fibers are only capable of providing ½ the tensile strength of traditional carbon fibers [1]. These CNT fibers fail due to the ease of which the parallel CNTs slide past one another [2].

To overcome this slippage, a method was employed involving the surface modification of the CNTs via the grafting of polyacrylonitrile (PAN) from the CNTs' surface. An initiator precursor, 4-hydroxyethyl benzocyclobutene (4-EO-BCB), was first obtained via Grignard synthesis by combining tetrahydrofuran, dibromoethane, Mg, and 4-bromobenzocyclobutene to produce a Grignard reagent; subsequently, the Grignard reagent was reacted with ethylene oxide to obtain the desired 4-EO-BCB. The 4-EO-BCB was then randomly attached to the surface of the multi-walled CNTs via Diels-Alder cycloaddition. The attached initiator precursor was then converted into the desired initiator via reaction with 2-bromoisobutyryl bromide in the presence of triethylamine. This allowed for polymerization of PAN from the initiator via ARGET polymerization. The resulting PAN-grafted CNTs were then dispersed into a PAN solution which could be spun, drawn, and carbonized into a testable fiber.

Thermal gravimetric analysis and transmission electron microscopy were utilized to determine polymer grafting levels and to characterize the grafted CNTs respectively. The production of the grafted CNTs has been successfully scaled up; dispersion and loading tests are being evaluated. Ultimately, this NASA-sponsored project aims to produce CNT fibers with a tensile strength of 7-10 GPa, about 5-7x greater than traditional carbon fibers. Achieving this goal would enable the production of a 30% lighter carbon reinforced composite [3].

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2. J. Mays, G. Baht, *Improved Carbon Nanotube Fibers through Crosslinking and Densification*, Proposal, 2013.
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Funding for this research was provided by NASA and TN-SCORE.

ISOLATION OF NICKEL(II) COMPLEXES OF COORDINATED YET UNCONDENSED SCHIFF BASE LIGAND PRECURSORS

Eric Bowman and Peter Craig
Department of Chemistry, McDaniel College, 2 College Hill, Westminster, MD 21157

Cobalt(III) complexes containing one tetradentate Schiff base ligand and two additional monodentate axial labile ligands have shown promise in clinical trials for the treatment of ocular herpes [1]. We were interested in preparing related complexes incorporating one pentadentate Schiff base ligand and one additional monodentate ligand, and comparing the spectroscopic properties and kinetics of monodentate ligand release for the two types of Schiff base complexes.

In the literature a one-pot synthesis of Nickel(II) complexes containing one pentadentate Schiff base ligand (that was the 1:1 condensation product of tris(2-aminoethyl)amine and acetylacetone or salicylaldehyde) and one potentially labile aqua ligand was reported [2]. Such compounds appeared to fit the profile for the type of compound we were looking to evaluate in our comparative study. However, we found that the complexes isolated in the way reported did not exhibit spectroscopic evidence for a coordinated aqua ligand even though the elemental analyses suggested that incorporation of one molecule of water into the molecular formulae was likely. The mystery was solved for one of the complexes prepared using X-ray crystallography – the aliphatic amine and carbonyl compound (together comprising the Schiff base ligand precursors) had coordinated to Nickel separately without undergoing a condensation reaction.

Details of the syntheses, elemental & spectroscopic analysis, and X-ray crystallography will be presented in an effort to validate this apparent contradiction of previous literature findings.

1. Cobalt derivatives as promising therapeutic agents, Marie C Heffern; Natsuho Yamamoto; Robert J Holbrook; Amanda L Eckermann; Thomas J Meade; Current Opinion in Chemical Biology. 2013;17(2):189-196.
2. Two novel nickel(II) complexes with Schiff base derived from tripod amine and acetylacetone or salicylaldehyde, Frantisek Brezina; Martine Biler; Richard Pastorek; Acta Universitatis Palackianae Olomucensis Facultas Rerum Naturalium.1998;Chemica 37:7-10.

We acknowledge the financial support of awards from the McDaniel College Student-Faculty Collaborative Summer Research Fund and the Jean and Donald Richards Student Research Fund.

EXAMINATION OF Mo COMPLEXES AND THEIR PROPERTIES: ANALYSIS OF MONODENTATE AND BIDENTATE PHOSPHINES ON TRI- AND TETRA-CARBONYLS

Bryan Eck and Charles Mahler
[1]Department of Chemistry, Lycoming College, 700 College Place, Williamsport, PA 17701

Many different properties of molybdenum carbonyls were examined and compared to one another. The change in enthalpy, cone angle of the phosphine, carbonyl stretch via IR and ^{31}P NMR are the most prominent of these characteristics. The two former are found in the literature and the latter two experimentally determined. These were measured and plotted against each other to look for possible correlations. If there is a trend, it is likely that there is a link between the two where one may be the cause and the other the effect.

The specific phosphines and phosphites used were PPh_3, $P(PhF)_3$, $P(PhCl)_3$, $P(PhCH_3)_3$, $P(PhCF_3)_3$, $P(PhOMe)_3$, PPh_2Me, $PPhMe_2$, PMe_3, PEt_3, PBu_3, PCl_3, $P(OPh)_3$, and $P(OMe)_3$. The chlelating phosphines tested were dppm, dppe, dppp, dppb, dppbz, and triphos. The overall reactions are $Mo(CO)_4(NBD) + 2PR_3 \rightarrow Mo(CO)_4(PR_3)_2 + NBD$ and $Mo(CO)_3(Tol) + 3PR_3 \rightarrow Mo(CO)_3(PR_3)_3 + Toluene$.

The two types of molybdenum complexes that were worked with are tetra- and tri-carbonyls with various phosphines attached to fill in the remaining cis- and fac- sites. When the phosphine attaches to the metal, backbonding occurs, weakening the overall carbon-oxygen bond. Comparing the carbonyl stretch to the phosphorus NMR chemical shift should determine if the strength of the phosphine-metal bond correlates with the strength of the carbonyl.

The characteristics that have the largest effect on the molybdenum-phosphine bond are electronics and sterics. These are determined by the groups attached to the phosphorus. The bigger the attached groups, the larger the cone angle; the stronger the electron withdrawing group, the less the phosphorus donates to the metal. This occurs with chelating phosphines as well. Ring strain and sterics when looking at bite angles may have a correlation with some of the other properties.

PREPARATION AND ANALYSIS OF MOLYBDENUM DISULFIDE NANOMATERIALS

Amelework Habtemichael, Christopher Mbochwa, and Paul Sabila
Chemistry and Physics, Department of Science, Technology, and Mathematics (DSTM)
Gallaudet University, 800 Florida Avenue N.E., Washington DC, 20002

Nanotechnology is a science that deals with the manipulation of matter at very small dimensions (a scale about the size of one billionth of a meter). It is currently being studied for many applications including medicine, semiconductor device fabrication and electronics. Nanomaterials are considered two dimensional (2D) materials and have interesting properties compared to their bulk properties. Molybdenum disulfide (MoS_2) is the most commonly studied transition metal dichalcogenide nanomaterial next to graphene. Molybdenum disulfide has a band gap of 1.2eV in bulk and 1.8eV in monolayer. Because of its bandgap, MoS_2 is being studied for potential application in electronics and transistors.

In this study, we present our research towards development of efficient large-scale synthesis of o MoS_2 films and nanomaterials. We employed the lithium intercalation method to give exfoliated MoS_2 materials which were then deposited on silicon wafers. Lithium intercalation was achieved by adding n-butyl lithium to MoS_2 powder at room temperature. The deposited MoS_2 films were then analyzed using Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and profilometer. The EDS analysis showed the presence of both MoS_2on silicon wafer (1:2 Mo to S ratio). The SEM and profilometer analysis showed that varying layers and thickness of MoS_2 deposited on the silicon wafers.

This work is supported by the National Science Foundation grant (NSF# 1205608). The analysis of MoS_2 wafers was carried out at Howard University at the Howard Nanofabrication Facility (HNF) while most of the synthesis was done at Gallaudet University.

Poster 176 - Cancelled

INFLUENCE OF TEMPERATURE ON THE ANTIOXIDANT CAPACITY OF ARONIA MITSCHURINII

Courtney Rhoades[1], Blessing Aroh[1], Kelsey Chandler[2], and Victoria Volkis[1*]
[1]Department of Natural Sciences, University of Maryland Eastern Shore,
1 Backbone Road, Princess Anne, MD 21853
[2]ACS Project SEED students, summer 2014

Aronia Mitschurinii, also referred to as the black chokeberry, is a fruit-bearing shrub which is native to Maryland. The aronia berry has a dark purple color which can be attributed to the berry's extremely high content of anthocyanins. Antioxidants are an important nutrient needed for capturing naturally formed free radicals in living organisms, and prevention of oxidation and cancer formation. Aronia's reputation of being a super berry entices small farms to use it as a perspective specialty crop. The berry's high content of polyphenols also makes it a likely ingredient in several new products such as, organic teas and vitamin supplements. All these and classic food applications require high temperature pasteurization as a major step during the fruit processing. There are three major effects higher temperatures can have on antioxidants; isomerization, decomposition or the loss of water.

Here we present the data for the antioxidant content of *Aronia Mitschurinii* as a function of the variation in temperature and the time exposed to these temperatures. Detailed measurements and analysis of anthocyanin, flavonoids, polyphenol content and ORAC will be presented and discussed. The aim of this project is to determine the thermal process that would avoid significant decomposition of antioxidants in aronia.

The project is supported by the MAES 2013-2014 SEED grant and Thurgood Marshall Undergraduate Research to Retain and Graduate Students in STEAM Grant.

A G4·K+ HYDROGEL STABILIZED BY AN ANION

Luke P. Skala, Gretchen Marie Peters, and Jeffery T. Davis
Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742

Supramolecular hydrogels derived from natural products have promising applications in diagnostics, drug delivery, and tissue engineering. We studied the formation of a long-lived hydrogel made by mixing guanosine (G, 1) with 0.5 equiv of KB(OH)4. This ratio of borate anion to ligand is crucial for gelation as it links two molecules of 1, which facilitates cation-templated assembly of G4·K+ quartets. The guanosine–borate (GB) hydrogel, which was characterized by cryogenic transmission electron microscopy and circular dichroism and 11B magic-angle-spinning NMR spectroscopy, is stable in water that contains physiologically relevant concentrations of K+. Furthermore, non-covalent interactions, such as electrostatics, π-stacking, and hydrogen bonding, enable the incorporation of a cationic dye and nucleosides into the GB hydrogel.

This work was supported by the US DOE [DE-FG01-98ER14888] and EPSRC (EP/K003674/1). GP thanks the Dept. of Education for a GAANN fellowship.

Afternoon Poster Session
Group AA – Chemical Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

179. **USING POLYMERIC RESINS FOR THE EXTRACTION OF PURE ANTHOCYANIN FROM ARONIA MITSCHURINII BERRIES**

Heather Goldsborough[1], Taiwo Ola[1], Andrew Ristvey[2], and Victoria Volkis[1]
[1]Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21835
[2]University of Maryland Extension, Wye Research & Education Center, P.O. Box 169, Queenstown, MD 21658-0169

180. **ANALYSIS OF THE CATALYTIC ACTIVITY OF IMMOBILIZED PALLADIUM PVP-CAPPED NANOCATALYSTS SUPPORTED ON SILICA MICROSPHERES BY THE HYDROGENATION OF PHENOL**

Joshua E. Kauffman, Nicholas D. Muench, Jason M. Shay, Kyle M. Gross, and Dr. Anderson L. Marsh
Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

181. **GROWTH OF MOLYBDENUM DISULFIDE FILMS ON SILICON WAFERS**

Christopher Mbochwa, Amelework Habtemichael, and Paul Sabila
Chemistry & Physics Program, Science, Technology, and Mathematics Department (DSTM), Gallaudet University, 800 Florida Avenue N.E., Washington DC, 20002

182. **VISIBLE-LIGHT MEDIATED SYNTHESIS OF CONSTRAINED CYCLIC-PEPTIDES FROM PHENACYL PROTECTED CYSTEINE RESIDUES**

Rory C. McAtee[1], Timothy M. Monos[2], and Corey R. J. Stephenson[2]
[1]Department of Chemistry, Lycoming College, Williamsport, PA 17701
[2]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

183. **EEM CHARACTERIZATION OF SURFACE WATERS ALONG A RURAL-TO-URBAN GRADIENT IN BALTIMORE**

Nicholas Rogers, and Lee Blaney
Department of Chemical, Biochemical and Environmental Engineering, UMBC, 1000 Hilltop Circle ECS 314, Baltimore, MD 21250

184. **NEW STRATEGIES AGAINST SUPERBUGS**

Asya Tucker[1], Nick Batista[2], and Susan Gillmor[3]
[1]Department of Chemistry and Biochemistry, Trinity Washington University, 125 Michigan Avenue NE, Washington, DC 20017
[2]Department of Engineering, George Washington University, 725 23rd Street NW, Washington, DC 20052
[3]Department of Chemistry, George Washington University, 725 21st Street NW, Washington, DC 20052

185. **FREE ENERGY SIMULATIONS FOR THE PROTON TRANSFER REACTION BETWEEN THE CATALYTIC RESIDUES IN APO FORM OF HUMAN T-CELL LEUKEMIA VIRUS TYPE I (HTLV-I) PROTEASE**

Kimberly Vogt and Shuhua Ma
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

USING POLYMERIC RESINS FOR THE EXTRACTION OF PURE ANTHOCYANIN FROM ARONIA MITSCHURINII BERRIES

Heather Goldsborough[1], Taiwo Ola[1], Andrew Ristvey[2], and Victoria Volkis[1]
[1]Department of Natural Sciences, University of Maryland Eastern Shore,
Princess Anne, MD 21835
[2]University of Maryland Extension, Wye Research & Education Center,
P.O. Box 169, Queenstown, MD 21658-0169

Aronia Mitschurinii, commonly known as the Black Chokeberry, is a fruiting bush native to the East Coast of the US and cultivated as a specialty food crop in Eastern Europe. A close relative of the apple, it produces small, round berries with deep purple skin and flesh. Aronia berries contain a cocktail of antioxidants in significantly higher concentrations than Acai berries. The most notable of these compounds is anthocyanin. Responsible for giving the berries their deep red color and acting as a potent free radical scavenger, anthocyanin exists in the juice as well as the pulp. It is a valuable nutrient with some technical applications in addition to nutritional ones.

Recently, it has been reported that polymer resins can be used to extract additional value from grape pumice in the form of powdered antioxidants. Extracted antioxidants can be used in health supplements, food products, and possibly in the future as medication ingredients.

This project aims to test current resin techniques, such as those used with grapes, on the extraction of anthocyanin from both Aronia juice and pulp. The resin beads are soaked in aronia juice or a pumice extract and alcohol solution. Once the anthocyanin molecules adsorb to the vast internal surface area of the beads, the beads are removed from the solution and exposed to a solvent where anthocyanin is released from the resin. The solvent is then evaporated off, leaving antioxidant behind. Antioxidant profile before and after the resin treatment, comparison of different resins, as well as purity analysis of isolated products will be presented.

This project is funded by Undergraduate Research to Retain and Graduate Students in STEAM grant. Ms. Goldsborough would also like to thank the UMES Honors Program for support.

ANALYSIS OF THE CATALYTIC ACTIVITY OF IMMOBILIZED PALLADIUM PVP-CAPPED NANOCATALYSTS SUPPORTED ON SILICA MICROSPHERES BY THE HYDROGENATION OF PHENOL

Joshua E. Kauffman, Nicholas D. Muench, Jason M. Shay,
Kyle M. Gross, and Dr. Anderson L. Marsh
Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

Palladium nanoparticles were synthesized using a number of different masses of polyvinylpyrrolidone (PVP) capping agents. Alcohol reduction and seeded growth methods were used to obtain various nanoparticle sizes. SiO_2 microspheres were used to support the nanocatalysts. The supported Pd nanocatalysts were then analyzed using Flame Atomic Absorption Spectroscopy to determine the percent mass of Pd. The nanoparticles and supported nanoparticles were characterized using transmission electron microscopy to obtain the average particle size. In order to determine catalytic properties, the nanocatalysts were used in the hydrogenation of phenol using a benchtop reactor. The reactor allows for variations in pressure, temperature, and spin. Gas chromatography/mass spectrometry was used to identify abundance of the products. The catalytic ability of the various sizes of unsupported nanocatalysts was compared, along with the variations in catalytic ability of the supported nanocatalysts. This was done by solving for the turnover frequency in each reaction and comparing the formation of cyclohexanone.

The authors would like to acknowledge support of the Donors of the American Chemical Society Petroleum Research Fund.

GROWTH OF MOLYBDENUM DISULFIDE FILMS ON SILICON WAFERS

Christopher Mbochwa, Amelework Habtemichael and Paul Sabila
Chemistry & Physics Program, Science, Technology, and Mathematics Department (DSTM),
Gallaudet University, 800 Florida Avenue N.E., Washington DC, 20002

Nanotechnology is science, engineering, and technology conducted at the nanoscale level, which is about 1 to 100 nanometers. A nanometer is about 1 billion times smaller than a meter. Nanotechnology enables the study and applications of extremely small sizes that can be used across many disciples in chemistry, biology, physics, materials science, medicine, environmental protection and engineering.

Our research is interested in electronic properties of molybdenum disulfide (MoS_2) nanomaterials. The overall goal is to develop a process for large-scale synthesis of MoS_2 wafers. The use of silicon-based semiconductors have transformed the computer and electronic industry. However, the current computer processors based on silicon technology is reaching its limit. To counter this, new semiconductor materials need to be developed. MoS_2 paves the way for the development of totally new domain of electronic devices and materials to replace silicon based semiconductors. The band gap of MoS_2 allows for their usage in fabricating transistors. MoS_2 together with other 2D and thin-film materials are applied in flat light-emitting devices and also in transparent electrodes found in large-screen displays such as television sets and computer monitors.

Our research group is interested in developing strategies for the large-scale synthesis of MoS_2 films on silicon and silicon oxide wafers. Our current studies involve the preparation of exfoliated MoS_2 from bulk MoS_2 by using *n*-butyl lithium. The exfoliated MoS_2 films were deposited on wafers and then analyzed by Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS). SEM analysis showed the MoS_2 films deposited on silicon wafer. EDS confirmed that MoS_2 was actually deposited with ratio of Mo:S roughly equals 1:2.

This work is supported by the National Science Foundation grant (NSF DMR 1231319) as part of the Center for Integrated Quantum Materials (CIQM). The analysis of MoS_2 wafers was carried out at Howard University at the Howard Nanofabrication Facility (HNF) while most of the synthesis was done at Gallaudet University.

VISIBLE-LIGHT MEDIATED SYNTHESIS OF CONSTRAINED CYCLIC-PEPTIDES FROM PHENACYL PROTECTED CYSTEINE RESIDUES

Rory C. McAtee[1], Timothy M. Monos[2], and Corey R. J. Stephenson[2]
[1]Department of Chemistry, Lycoming College, Williamsport, PA 17701
[2]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

Online access of this abstract is restricted at the request of the Principal Investigator.

EEM CHARACTERIZATION OF SURFACE WATERS ALONG A RURAL-TO-URBAN GRADIENT IN BALTIMORE

Nicholas Rogers and Lee Blaney
Department of Chemical, Biochemical and Environmental Engineering, UMBC,
1000 Hilltop Circle ECS 314, Baltimore, MD 21250

Dissolved organic matter (DOM) is a broad category of compounds that contains thousands of complex molecules, including materials formed from the decay of plant and animal matter and organic molecules discharged from wastewater treatment plants. DOM is present in every water source and may be an indicator of land use inputs to surface water. Recently, fluorescence spectroscopy has been used to qualitatively measure and characterize DOM from wastewater, water basins, and lake water, among others. This technique allows samples to be quickly, reproducibly and inexpensively analyzed. We propose that fluorescence spectroscopy can be used to determine whether urban waters are compromised by leaking wastewater infrastructure. The hypothesis of this project is that the natural DOM matrix of streams differs from that of raw wastewater. By recording the fluorescence excitation-emission matrices (EEMs) for these waters, the associated signature may indicate the presence of raw wastewater in streams. Surface waters samples were collected from nine sites along a rural-to-urban gradient in Baltimore, Maryland; wastewater samples were collected from an anonymous wastewater treatment plant near Baltimore. EEMs were generated for the surface water and wastewater samples. Analysis of the EEMs indicated that raw wastewater may be leaking into Gwynns Run, which discharges to Gwynns Falls in Southwest Baltimore City. Additionally, the wastewater signature was not observed in more rural streams like the Gwynns Falls at Glyndon (Baltimore County). Ratios of the fluorescence signal at select wavelengths were used to quantitatively describe the DOM matrix along the rural-to-urban gradient. Additionally, inner filter corrections were implemented to adjust the generated data for fluctuations in the fluorescence of the samples. These results indicated that trends in land use (*i.e.*, more rural vs. more urban) are correlated to the fluorescence signature. Upon further validation, we propose using FEEMs to detect leaking wastewater infrastructure in urban settings.

This research was supported in part by a grant to UMBC from the Howard Hughes Medical Institute through the Precollege and Undergraduate Science Education Program, an Undergraduate Research Award from the UMBC Office of Undergraduate Education and the MARC U*STAR Program at UMBC through the National Institute of General Medical Sciences. The authors also acknowledge Mr. Dan Dillon and Dr. Stuart Schwartz for sample collection.

NEW STRATEGIES AGAINST SUPERBUGS

Asya Tucker[1], Nick Batista[2], and Susan Gillmor[3]
[1]Department of Chemistry and Biochemistry, Trinity Washington University, 125 Michigan Avenue NE, Washington, DC 20017
[2]Department of Engineering, George Washington University, 725 23rd Street NW, Washington, DC 20052
[3]Department of Chemistry, George Washington University, 725 21st Street NW, Washington, DC 20052

Superbugs are problematic; antibiotics are less potent as antibiotic resistant bacteria can actively retaliate against conventional treatment methods. We are optimizing drug cocktails of traditional antibiotics with CAMPs (cationic antimicrobial peptides) against Acinetobaacter Baumannii a multi drug resistant (MDR) bacteria and its dormant states. Our lab-on-a-chip micro device forms micro-environments to provide a more effective mimic of the heterogeneous infectious population. Because lab-on-a-chip micro devices are too large for conventional microscopy, I built an inexpensive imaging device that captures digital images of the entire micro device. Combined with fluorescent tags on the bacteria, this customized imaging system will provide the capabilities to screen for the most effective combinations of CAMPs and antibiotics. This will accelerate the screening process and help reach optimal drug combinations more quickly.

FREE ENERGY SIMULATIONS FOR THE PROTON TRANSFER REACTION BETWEEN THE CATALYTIC RESIDUES IN APO FORM OF HUMAN T-CELL LEUKEMIA VIRUS TYPE I (HTLV-I) PROTEASE

Kimberly Vogt and Shuhua Ma
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

Online access of this abstract is restricted at the request of the Principal Investigator.

Afternoon Poster Session
Group BB – Chemical Sciences

Poster #	Title, Author(s) & Affiliation(s)
186.	**BREAKING DOWN THE MILKY WHEY** Jacqueline Clarizio[1], Alenka Hlousek-Radojcic[2], Jacqueline Fajardo[1], and Mark Baillie[1] [1]Department of Biochemistry and Chemistry, University of Delaware, 102 Brown Laboratory, Newark, DE 19716 [2]Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19716
187.	**DETERMINING THE MECHANISM OF OCTYL CHLOROFORMATE USING ACID- BASE TITRATIONS** Megan Durrant and Dr. Malcolm J. D'Souza Department of Chemistry, Wesley College, 120 N. State Street, Dover, DE 19901
188.	**CU(I)-COMPLEXES AS PHOTOREDOX CATALYSTS: AN ALTERNATIVE TO RU(BPY)$_3^{2+}$** Matthew Maple, Daniel Hockersmith, and Dr. Timothy Peelen Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003
189.	**THE SYNTHESIS, CHARACTERIZATION, AND POLYMERIZATION OF THIENYL PHOSPHINE DERIVATIVE PALLADIUM(II) COMPLEXES** Jessica L. Shott, Brian J. Reeves, and Brycelyn M. Boardman Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807
190.	**SYNTHESIS OF GOLD MICELLES** Dylan Soller and Dr. Patricia Kreke Department of Science, Mount St. Mary's University, 16300 Old Emmitsburg Road, Emmitsburg, MD 21727
191.	**SYNTHETIC FLUORESCEIN-BASED CHEMOSENSORS FOR PALLADIUM AND HUMAN SERUM ALBUMIN QUANTIFICATION AND THE DISCOVERY OF AN UNPRECEDENTED RED SHIFT** Jessica M. Williams and Kazunori Koide Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, PA 15260

BREAKING DOWN THE MILKY WHEY

Jacqueline Clarizio[1], Alenka Hlousek-Radojcic[2], Jacqueline Fajardo[1], and Mark Baillie[1]
[1]Department of Biochemistry and Chemistry, University of Delaware,
102 Brown Laboratory, Newark, DE 19716
[2]Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19716

The focus of this HHMI-supported summer research was to develop an integrated laboratory module with emphasis on integration of related concepts of both biology and chemistry. One goal of integration is to connect student's "real world" interests with laboratory techniques and skills. Here, a separations laboratory focusing on health and nutrition was developed. A complex mixture known commonly as "milk" served as the basis for this project. Percent composition of fat, protein, and carbohydrate were determined within the separated fractions. An analysis of various types of milk, including whole, half and half, 2%, and skim, was performed to compare the content. This unique experience for students would allow them not only to gain an understanding of extraction techniques commonly used in both biology and chemistry, but also make a connection to a nutritional aspect.

I would like to thank Howard Hughes Medical Institute for funding towards my research, and my amazing research mentors: Jacqueline Fajardo, Mark Baillie, Alenka Hlousek-Radojcic, and Hal White.

DETERMINING THE MECHANISM OF OCTYL CHLOROFORMATE USING ACID- BASE TITRATIONS

Megan Durrant and Dr. Malcolm J. D'Souza
Department of Chemistry, Wesley College, 120 N. State Street, Dover, DE 19901

Octyl chloroformate (OctOCOCl) is used as a precursor for drugs that inhibit multiple enzymatic pathways to control obesity. The drug inhibits lipase which stops your body from breaking down and absorbing fat. Alkyl chloroformates such as OctOCOCl are important precursors for many products, and, determining the mechanism of reaction for such subtrates is extensive and tedious work. Hence, this undergraduate research project is also funded through the DE-INBRE program as it trains students in the basic biomedical methodologies used to develop more effective drugs.

In this project, the acid-base titration method has been used to study the specific rates of OctOCOCl in different concentrations of aqueous acetone, aqueous ethanol (EtOH), aqueous methanol (MeOH), aqueous 1,1,1,3,3,3-Hexafluoroisoproponal (HFIP), aqueous 2,2,2, trifluoroethanol (TFE), and in mixtures of TFE-EtOH at 25.0 ^{o}C.

The reaction rates of the substrate (OctOCOCl) increased with an increase in the concentration of water in the reaction solvent. The initial trends observed are very similar in nature to those seen when shorter chain alkyl chloroformates were reacted in a similar set of solvents.

This research project is funded by the INBRE grant (8 P20 GM103446-13) sponsored by the National institute for General Medical Sciences and the National Institute for Health, and a second grant from the National Science Foundation (NSF) under EPSCoR Grant No. IIA-1301765 (DE-EPSCoR RII-3).

CU(I)-COMPLEXES AS PHOTOREDOX CATALYSTS: AN ALTERNATIVE TO RU(BPY)$_3^{2+}$

Matthew Maple, Daniel Hockersmith and Dr. Timothy Peelen
Department of Chemistry, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003

The field of organic photochemistry has witnessed a recent expansion with the development of Ru(bpy)$_3^{2+}$-catalyzed photoredox reactions. This complex absorbs light and undergoes metal-to-ligand-charge-transfer (MLCT), generating a charge-separated excited state intermediate that can act as a single-electron transfer (SET) catalyst in redox reactions. Ru(bpy)$_3^{2+}$ and its analogs have been applied to a wide variety of redox reactions in organic syntheses. However, the Ru(bpy)$_3^{2+}$ complex is not without its drawbacks. Ruthenium and iridium are precious metals, which dramatically increases the price of the complexes. This research project focuses on Cu(I)-complexes as cheaper, safer, and more environmentally friendly alternatives to Ru(bpy)$_3^{2+}$ in photoredox reactions. A library of homoleptic and heteroleptic Cu(I)-complexes using substituted-phenanthroline and phosphine ligands has been synthesized and screened in a model reaction, the reduction of 2-bromoacetophenone to acetophenone.

THE SYNTHESIS, CHARACTERIZATION, AND POLYMERIZATION OF THIENYL PHOSPHINE DERIVATIVE PALLADIUM(II) COMPLEXES

Jessica L. Shott, Brian J. Reeves, and Brycelyn M. Boardman
Department of Chemistry and Biochemistry, James Madison University,
Harrisonburg, VA 22807

The use of inorganic crystalline silicon in shipped photovoltaic units offer efficiencies as high as 22.7%. However, this efficiency requires large absorbing layers that rely on expensive substrates and harsh processing conditions. These methods make this technology 5-10 times too expensive to be a cost competitive alternative to conventional electricity production. Recently, organic-inorganic hybrid structures have been developed, which incorporate nanoparticle-polymer blends into the active layer. While these structures offer improved efficiency, phase separation between the inorganic and organic materials can lead to poor charge separation and recombination. A possible solution to this is to covalently link well-defined cobalt chalcogenide clusters to conjugated polymers via polymerizable phosphine ligands. This method will yield highly ordered structures that could become difficult to characterize. To understand the fundamental interactions between the polymer chains and the inorganic core, a simplified model complex will be synthesized. The complex consists of a palladium atom core covalently attached to the functionalized phosphine ligands.

Due to the complicated nature of these structures, characterization has proven to be a difficult endeavor. To gain a better understanding of the characterization data, palladium(II) complexes with functionalized thienyl phosphine ligands have been synthesized and polymerized. The synthesis of brominated thiophene ligands was performed by allowing 2,5-dibromothiophene to react with n-butyllithium, followed by the addition of chlorodiphenylphosphine or chlorodiethylphosphine to produce 2-bromo-5-diphenylphosphinothiophene (**1**) and 2-bromo-5-diethylphosphinothiophene (**2**). Compounds **1** and **2** were allowed to react with dichloro(1,5-cyclooctadiene)palladium (II) to form either bis(2-bromo-5-diphenylphosphinothiophene)palladium(II) (**3**) or bis(2-bromo-5-diethhylphosphinothiophene)palladium(II) (**4**). Polymerizations of **3** and **4** were accomplished using 3-hexylthiophene, trimethyl stannylated thiophene, and catalytic amounts of tetrakis(triphenylphosphine)palladium(0). ^{1}H, ^{13}C, and ^{31}P NMR, ultraviolet-visible spectroscopy, and fluorescence spectroscopy were used to characterize the phosphine ligands, palladium complexes, and polymerizerization attempts. Data indicates that polymerization attempts resulted in potential covalent attachment to the palladium complexes.

The researchers would like to the Research Corporation Cotterell College Science Award for funding this project.

SYNTHESIS OF GOLD MICELLES

Dylan Soller and Dr. Patricia Kreke
Department of Science, Mount St. Mary's University, 16300 Old Emmitsburg Road, Emmitsburg, MD 21727

Micelles are chemical compounds that contain hydrophobic and hydrophilic-components. When exposed to an aqueous environment, the micelles orient their hydrophilic components outwards towards the polar solvent, isolating the hydrophobic tails in the core. Recently synthesized gold nanoparticles will be modified by adding mercaptoundecanol (MUD) to make "Gold Micelles." Once synthesized, the micelles can then store and protect various hydrophobic cancer drugs, which can be released via degradation of the micelle through heat.

I'd like the thank Dr. Dave Nikles from the University of Alabama Tuscaloosa, as well as Dr. Jackie Nikles from the University of Alabama, Birmingham for providing the necessary chemicals for the synthesis, as well as aiding in the characterization of the particles.

SYNTHETIC FLUORESCEIN-BASED CHEMOSENSORS FOR PALLADIUM AND HUMAN SERUM ALBUMIN QUANTIFICATION AND THE DISCOVERY OF AN UNPRECEDENTED RED SHIFT

Jessica M. Williams and Kazunori Koide
Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, PA 15260

Pittsburgh Green and its derivatives provide a platform for quantifying various analytes, including precious metals, reactive oxygen species, and human serum albumin (HSA). Previously, allyl Pittsburgh Green ether (APE) was shown to quantify palladium species in solution through palladium-catalyzed deallylation. The rate of deallylation, and therefore increasing fluorescence over time, is linearly dependent on palladium concentration. This was not initially used to quantify palladium in solid phase, since such heterogenous conditions were considered non-quantitative. Herein we prove this assumption incorrect; this poster describes the high throughput and quantitative fluorometric or colorimetric method for palladium in solid-ore geological samples with success rivaling conventional analytical methods. With Pittsburgh Green II**,** fluorescence was quenched in the presence of HSA in a reproducible and time-independent manner. Here, we report that Pittsburgh Green II underwent an unprecedented absorbance red shift, suggesting the possibility for colorimetric clinical detection and quantification of albumin in human fluids. The quantification methods of HSA and of palladium by these novel chemosensors are simple, efficient, and show expanding potential for real-world applications.

Afternoon Poster Session
Group CC – Chemical Sciences

Poster #	Title, Author(s) & Affiliation(s)
192.	**THE EFFECT OF SAMPLING METHOD ON THE CONCENTRATION OF METHANE IN SOILS** Kelli Kearns[1] and Angelia Seyfferth[2] [1]Department of Civil and Environmental Engineering, University of Delaware, 301 Du Pont Hall, Newark, DE 19716 [2]Department of Plant and Soil Sciences, University of Delaware, 531 South College Avenue, Newark, DE 19716
193.	**IMPROVED METHODOLGY FOR ION PROFILING IN AQUARIUM WATER** Margaret E. LaCourse, Ian W. Shaffer, Joshua A. Wilhide, William R. LaCourse Molecular Characterization and Analysis Complex, UMBC, 1000 Hilltop Circle, Baltimore MD 21250
194.	**USING TRACE ELEMENT SIGNATURES TO DETERMINE COCOA LIQUOR PROVENANCE** Ravleenkaur Khalsa, Sara Maloney, Dr. Shannon E. Stitzel, and Dr. Ryan E. Sours Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252
195.	**EXPLORING ANIONIC UREAS AS REACTION ACTIVATORS IN SAMARIUM (II) IODIDE REDUCTIONS** Christopher C. McAtee, Joe R. Mauck, Erin M. Hale, and Chriss E. McDonald, Ph.D. Department of Chemistry, Lycoming College, 700 College Place, Williamsport, PA 17701
196.	**QUANTIFICATION OF NEUROTRANSMITTERS USING HILIC LC-MS/MS** Timothy R. Monko[1], Emily N. Spurlin[1], Timothy J. Peelen[2], and Erica L. Unger[1] [1]Department of Biology Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003 [2]Department of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003
197.	**SINGLE-CELL METABOLIC INVESTIGATION OF XENOPUS EMBRYOS BY MASS SPECTROMETRY** Sydney Morris, Rosemary M. Onjiko, and Peter Nemes Department of Chemistry, George Washington University, 725 21st Street NW, Washington, DC 20052
198.	**STRUCTURAL ACTIVITY RELATIONSHIP (SAR) STUDIES OF NOVEL ENAMINONE COMPOUNDS AS POTENTIAL ANTI-SEIZURE AGENTS FOR PHARMACO-RESISTANT PARTIAL EPILEPSY** Winfield Whittington[1], Tiffanie Taylor[2], Mohamed Sackor[2], Nnaemeka Emenari[2], and Patrice Jackson-Ayotunde, PhD[2] [1]Department of Natural Sciences, University of Maryland Eastern Shore, One Backbone Road, Princess Anne, MD 21853 [2]Department of Pharmaceutical Sciences, University of Maryland Eastern Shore, School of Pharmacy, One Backbone Road, Princess Anne, MD 21853

THE EFFECT OF SAMPLING METHOD ON THE CONCENTRATION OF METHANE IN SOILS

Kelli Kearns[1] and Angelia Seyfferth[2]
[1]Department of Civil and Environmental Engineering, University of Delaware, 301 Du Pont Hall, Newark, DE 19716
[2]Department of Plant and Soil Sciences, University of Delaware, 531 South College Avenue, Newark, DE 19716

Methane is an important greenhouse gas that is produced when organic material decomposes in the absence of oxygen. Cultivation techniques for various crops such as rice can often influence the amount of methane produced by an area of land, and thus it is important to accurately and precisely measure methane concentrations in these systems.

Here, we compared two different methods to measure methane concentrations in soils treated with or without an added rice husk amendment. The first method was pore water sampling with subsequent headspace equilibration technique, and the second method was a gas-permeable, silicone tubing device fitted to a gas-tight metal tubing and valve system that was connected with compression fittings. The two methods were comparatively tested under the hypothesis that there would be no difference in the two different sampling methods. This hypothesis was disproved by the data collected; the silicone tubing device showed significantly higher concentrations of methane for all soil treatments and at both early and late sampling dates. The data also showed a spike in the methane concentrations of all soil treatments approximately 32 days after flooding the soils with 1.25 Liters of water, and a drop-off in the methane concentrations with all of these treatments was noticed 35 days after flooding. This observation should be considered for timing of sampling in future experiments.

The silicone tubing device showed higher concentrations of methane than the pore water sampling device, which may imply that it is a more effective measurement mechanism, particularly for larger-scale applications and field work. This may be because the greater surface area of the device within the soil allows it to better capture highly concentrated areas of methane. Future research should comparatively test these devices with the addition of rice plants to the testing soils.

This work was made possible by the National Science Foundation (NSF) Grant No. 1338389, NSF EPSCoR Grant No. 11A-1301765, and the State of Delaware.

IMPROVED METHODOLGY FOR ION PROFILING IN AQUARIUM WATER

Margaret E. LaCourse, Ian W. Shaffer, Joshua A. Wilhide, William R. LaCourse
Molecular Characterization and Analysis Complex, UMBC,
1000 Hilltop Circle, Baltimore MD 21250

Aquariums are an important part of modern society, providing both a way to explore the wonders of the ocean and a source of entertainment. Assaying artificial seawater is essential for the proper maintenance of aquariums in order to ensure that its composition is similar to that of natural seawater, providing aquatic life with the healthy environment it needs to thrive. Therefore, it is of critical importance to determine the profile of cations and anions present in the water from various dissolved salts. Current methods typically employ ion chromatography, but these methods are lacking, requiring long run times and/or providing insufficient data. The goal of this project is to improve, optimize, and validate an ion chromatographic method for the determination of ions in aquarium water. Ion chromatography works by separating various charged components in solution using an ion-exchange column followed by conductivity detection, a type of electrochemical detection. A test solution containing standard ions in the ratio of natural seawater was analyzed using the method currently in use. Aqueous ion standards (i.e., Li^+, Na^+, NH_4^+, Mg^{2+}, K^+, Ca^{2+}, Rb^+, Sr^{2+}) were run to determine elution time, peak height, peak area, peak shape, and other analytical figures of merit. Variables such as column temperature, cell temperature, and mobile phase composition were studied. As a consequence, column temperature and the molarity of the mobile phase were optimized to achieve better peak characteristics in a shorter overall run time. Additionally, spiking experiments were performed to confirm the identity of each peak in the chromatographic separation. Current research focuses on optimizing the following: injection loop size, suppressor voltages, sample dilutions, and sample storage as well as evaluating analytical figures of merit and validating the optimized method for cation analysis. Future research will focus on optimization of a separation and detection method for anions in seawater.

We would like to thank the National Aquarium in Baltimore, especially Jill Arnold and Kim Gaeta for their continued support, insight, and valuable discussions.

USING TRACE ELEMENT SIGNATURES TO DETERMINE COCOA LIQUOR PROVENANCE

Ravleenkaur Khalsa, Sara Maloney, Dr. Shannon E. Stitzel, and Dr. Ryan E. Sours
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

Both consumers and manufacturers have recently become more concerned with food product provenance for a variety of reasons, including production ethics, product quality, and health benefits. An example is chocolate, which is a mixture of cocoa liquor, cocoa butter, milk, and sugar. The flavor and quality of chocolate is heavily influenced by the quality of the cocoa liquor used in its production. Cocoa liquor is derived from cocoa beans, which have been roasted and ground, to a liquid. This project focused on single-origin cocoa liquor samples to determine if elemental signatures in the liquor could be correlated to the country of origin. Elemental analysis was performed using ICP-MS and the resulting data was analyzed by chemometric approaches. Three main questions were investigated: 1) Could cocoa liquor samples from four or more countries be discriminated from each other? 2) Would liquor samples from the same country but undergoing different processing treatments have the same elemental signature? 3) Which elements were important for defining the elemental signature for provenance determination?

Initial results indicate that single-origin cocoa liquors from four countries can be discriminated from each other based on their elemental signatures. In addition, the cocoa liquor samples that were produced from different treatment processes (temp, roast time, etc), while having some differences in their signatures, were still correctly classified by country of origin. Future work will examine additional single-source origin cocoa liquors and will aim to determine which elements are most significant in defining a signature that will allow the determination of provenance.

The authors would like to thank Dr. Ed Seguine for the donation of the cocoa liquor samples and many helpful discussions about cocoa and chocolate production.

EXPLORING ANIONIC UREAS AS REACTION ACTIVATORS IN SAMARIUM (II) IODIDE REDUCTIONS

Christopher C. McAtee, Joe R. Mauck, Erin M. Hale, and Chriss E. McDonald, Ph.D.
Department of Chemistry, Lycoming College, 700 College Place, Williamsport, PA 17701

Online access of this abstract is restricted at the request of the Principal Investigator.

QUANTIFICATION OF NEUROTRANSMITTERS USING HILIC LC-MS/MS

Timothy R. Monko[1], Emily N. Spurlin[1], Timothy J. Peelen[2], and Erica L. Unger[1]
[1]Department of Biology Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003
[2]Department of Chemistry, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003

Quantifying small neurotransmitters from biological samples presents a challenge due to relatively low concentration and low sample volume. However, liquid chromatography tandem mass spectrometry (LC-MS/MS) provides specific and sensitive detection of analytes at very low concentrations and requires little sample. Traditional reverse phase liquid chromatography (RPLC) suffers from poor retention time for accurate analysis of brain amino acid and amine neurotransmitters. Instead, the usage of hydrophilic interaction liquid chromatography (HILIC) columns is a viable alternative for measuring polar analytes. HILIC uses a polar stationary phase, and a combination of two polar eluents that form a liquid-liquid separation layer. The more polar eluent and most polar analytes associate more closely with the column; increasing the concentration of the most polar eluent, usually water, will elute polar compounds. Therefore, HILIC columns provide excellent selectivity and sensitivity for quantification of amino acid and amide neurotransmitters. A fast and reliable method using LC-MS/MS has been developed to simultaneously quantify multiple neurotransmitters in rodent brain tissue and in cell cultures.

This research was funded by the Paul Wolfe Research Fund and Lebanon Valley College.

SINGLE-CELL METABOLIC INVESTIGATION OF XENOPUS EMBRYOS BY MASS SPECTROMETRY

Sydney Morris, Rosemary M. Onjiko, and Peter Nemes
Department of Chemistry, George Washington University, 725 21st Street NW, Washington, DC 20052

The ability to characterize the cellular metabolome, the full suite of metabolites, is required to enhance understanding of basic biochemistry in embryo development, but this is critically dependent on the availability of new analytical technologies and protocols. Although mass spectrometry (MS) has become the method of choice for the measurement of metabolites, it traditionally averages out millions of cells, losing information characteristic of single cells. There is a high, yet unmet need for analytical techniques that can address the enormous diversity and broad concentration range of metabolites (e.g., millimolar to picomolar) anticipated in embryonic cells (blastomeres).

Chemical separation by capillary electrophoresis (CE) has a demonstrated ability to address the metabolomic complexity of biological systems; CE delivers high separation efficiency and is compatible with small sample volumes. As a result, the combination of CE and MS presents an opportunity to measure an array of small compounds including amino acids, neurotransmitters, energy carriers, and oligonucleotides in single blastomeres.

We present an analytical workflow based on CE electrospray ionization (ESI) MS to identify metabolites in single blastomeres in the South African clawed frog, *Xenopus laevis*, the favorite model in cell and developmental biology. We demonstrated that our CE-ESI-MS platform is compatible with less than 10 nL sample volume with a detection limit of 300 fmol for amino acids. Then, we applied the platform to measure metabolites in single blastomeres isolated from 16-cell *Xenopus embryos*.

In conclusion, our data demonstrates the utility of single-cell CE-ESI-MS for cell/developmental biology. High-efficiency separation by CE in combination with tandem MS facilitated the identification of a number of endogenous metabolites. These results should enable a better understanding of the model for its use in the study of human conditions and diseases. Our current efforts are aimed at applying the technique to elucidate metabolomic changes linked to embryonic development.

This work was supported by the George Washington Department of Chemistry Start-Up Funds (PN) and the GW Undergraduate Research Assistantship Funds (PN). The authors thank William F. Rutkowski of the Department of Mechanical and Aerospace Engineering (GW) for machining the sample-loading component of the CE-ESI-MS system.

STRUCTURAL ACTIVITY RELATIONSHIP (SAR) STUDIES OF NOVEL ENAMINONE COMPOUNDS AS POTENTIAL ANTI-SEIZURE AGENTS FOR PHARMACO-RESISTANT PARTIAL EPILEPSY

Winfield Whittington[1], Tiffanie Taylor[2], Mohamed Sackor[2],
Nnaemeka Emenari[2], and Patrice Jackson-Ayotunde, PhD[2]
[1]Department of Natural Sciences, University of Maryland Eastern Shore, One Backbone Road, Princess Anne, MD 21853
[2]Department of Pharmaceutical Sciences, University of Maryland Eastern Shore, School of Pharmacy, One Backbone Road, Princess Anne, MD 21853

Online access of this abstract is restricted at the request of the Principal Investigator.

Afternoon Poster Session
Group DD – Chemical Sciences

Poster # | **Title, Author(s) & Affiliation(s)**

199. **FUELING THE INTERDISCIPLANRY FLAME: EXPLORING PLANT-BASED ALTERNATIVE FUELS IN THE UNDERGRADUATE LABORATORY**

Gabriel Gregorzak[1], Mark Baillie[1], Alenka Hlousek-Radojcic[2], and Jacqueline Fajardo[1]
[1]Department of Chemistry & Biochemistry, University of Delaware,
102 Brown Laboratory, Newark, DE 19717
[2]Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19717

200. **ELECTRONIC CIGARETTE AEROSOL ANALYSIS: INSTUMENTATION AND METHOD DEVELOPMENT**

Jonathan P. Jopse and Ellen Hondrogiannis Ph.D.
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

201. **VITELLOGENIN (VTG) AS A BIOMARKER FOR CONTAMINANTS OF EMERGING CONCERN (CECs) IN MARYLAND COASTAL BAYS (MCBs)**

Ijeoma Ngoka[1], Chelsea Richardson[1], Rehab Abass[1], Roman Jesien[2], and Ali Ishaque[1]
[1]Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853
[2]Maryland Coastal Bays Foundation, 8219 Stephen Decatur Highway, Berlin, MD 21811

202. **CHARACTERIZATON OF A NEAR-INFRARED FLUROIMMUNOASSAY PERFORMED ON AN EVANESCENT WAVE FLUORESCENCE BIOSENSOR**

Olumayokun Odukale[1], Dr. Angela Winstead[2] and Dr. Richard Williams[2].
[1]Department of Civil Engineering. Morgan State University,
1700 E. Cold Spring Lane, Baltimore MD 21251
[2]Department of Chemistry, Morgan State University,
1700 E. Cold Spring Lane, Baltimore, MD 21251

203. **TANNIN CONTENT OF TEAS**

Sarah Toone, Bradley Brown, Monica Ahir, and Dr. Richard Foust
Department of Chemistry and Biochemistry, James Madison University,
901 Main Street, Harrisonburg, VA 22807

204. **MULTIVARIATE, LINEAR SOLVATION ENERGY RELATIONSHIP (LSER), AND K–K ANALYSIS OF ANALYTE RETENTION IN REVERSED PHASE LIQUID CHROMATOGRAPHY**

Peter Willard and Eric Williamsen
Department of Chemistry, Ursinus College, 601 E. Main Street, Collegeville, PA 19426

205. **NEW EXPERIMENTAL AND KINETIC DATA FOR 2-ETHYL HEXYL CHLOROFORMATE**

Alora Wilson, Katelyn Null, and Dr. Malcolm J. D'Souza
Department of Biochemistry, Wesley College, 120 N. State Street, Dover, DE 19901

FUELING THE INTERDISCIPLANRY FLAME: EXPLORING PLANT-BASED ALTERNATIVE FUELS IN THE UNDERGRADUATE LABORATORY

Gabriel Gregorzak[1], Mark Baillie[1], Alenka Hlousek-Radojcic[2], and Jacqueline Fajardo[1]
[1]Department of Chemistry & Biochemistry, University of Delaware,
102 Brown Laboratory, Newark, DE 19717
[2]Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19717

A laboratory module intended for an integrated course was developed to highlight relevant and interrelated concepts described in both introductory biology and general chemistry with an enrollment of nearly 500 students. The ever-present depletion of petroleum energy reserves is an ongoing societal concern. Concomitantly, there is growing interest in identifying natural sources of alternative fuels and optimizing the efficiency of their use. To enhance student awareness of alternative fuel availability from natural resources, we have developed a lab centered on the production of biofuels produced from seed-oil extracts. Oil was physically extracted from a variety of seed types including pumpkin, sunflower, walnut, & flax, and utilized an efficient, small-scale, and green approach. The physical extraction technique replaced commonly used organic solvent extraction methods used in many academic and educational labs. Extracted oil was then subject to transesterification to yield the biofuel product. The energy content of this biofuel was measured and compared to ethanol using calorimetric analysis. This distinctive laboratory experience will allow large numbers of freshmen students to recognize the broader implications of their curriculum beyond the boundaries of the classroom.

This project was made possible through funding by the Howard Hughes Medical Institute.

ELECTRONIC CIGARETTE AEROSOL ANALYSIS: INSTUMENTATION AND METHOD DEVELOPMENT

Jonathan P. Jopse and Ellen Hondrogiannis Ph.D.
Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

The purpose of this work was to develop an open source platform for the testing of nicotine levels in aerosols generated from electronic cigarettes (e-cigs). Existing studies that employed smoking devices for the quantification of nicotine delivery have often been ad hoc assemblies or made use of specialized components. Concerns over the safety of e-cigs and regulators' desires for additional information warrant the development of an accessible and extensible platform for e-cig testing. We have constructed a smoking device using open source electronics and readily available components that is cost effective and capable of mimicking the smoking of an e-cig over a wide range of conditions. A Arduino MCU provided control of electrically operated valves and relays and returned data during testing to a host computer. A medical vacuum regulator was used to control puff pressure and simple solvent extractors were employed to capture aerosols. Calibration of our smoking device was accomplished using the V2 brand of e-cig and an e-liquid of known composition. We present these results along with the GC/MS method we developed for the quantification of the nicotine delivered from the e-cigarette.

Funding for this work was provided by the Jess & Mildred Fisher College of Science and Mathematics at Towson University.

VITELLOGENIN (VTG) AS A BIOMARKER FOR CONTAMINANTS OF EMERGING CONCERN (CECs) IN MARYLAND COASTAL BAYS (MCBs)

Ijeoma Ngoka[1], Chelsea Richardson[1], Rehab Abass[1], Roman Jesien[2], and Ali Ishaque[1]
[1]Department of Natural Sciences, University of Maryland Eastern Shore,
Princess Anne, MD 21853
[2]Maryland Coastal Bays Foundation, 8219 Stephen Decatur Highway, Berlin, MD 21811

Contaminants of Emerging Concern (CECs) have received wide interest for the past decade. Some of these contaminants are endocrine disrupting chemicals (EDCs). Some CECs get into aquatic environments s from waste water treatment plants, recreational activities, and urban settlements. EDCs threaten the reproductive success and long term survival of aquatic species. For example, the release of EDCs can induce the expression of VTG in male fish, leading to reproductive failure, intersex characteristics, and a collapse of sensitive fish population. The presence of VTG in male and juvenile fish is commonly used as a biomarker of endocrine disruption.

Mummichog (*Fundulus heteroclitus),* was chosen as the study species due to its abundance in shallow water close to the shore and their non-migratory behavior, making them very good bio-indicators for spatial contamination. Samples were collected from two sites in the MCBs (Public Landing and Lewis Road). Enzyme-linked immunosorbent assay (ELISA) was used to study the presence of VTG in male fish. VTG concentration in samples from Public landing ranged from 0.7µg/ml - 61.06µg/ml and 0.2µg/ml – 152.3µg/ml for samples from Lewis road. Results from this study suggested the presence of some EDCs in that environment and could be affecting reproduction in fish populations. Chemical analysis will be done to identify the possible CECs responsible for the VTG production in the male fish.

We acknowledge with thanks, the funding support from UMES CREST grant funded by NSF.

CHARACTERIZATON OF A NEAR-INFRARED FLUROIMMUNOASSAY PERFORMED ON AN EVANESCENT WAVE FLUORESCENCE BIOSENSOR

Olumayokun Odukale[1], Dr. Angela Winstead[2] and Dr. Richard Williams[2].
[1]Department of Civil Engineering. Morgan State University,
1700 E. Cold Spring Lane, Baltimore MD 21251
[2]Department of Chemistry, Morgan State University,
1700 E. Cold Spring Lane, Baltimore, MD 21251

The potential of evanescent wave fiber optic biosensors to detect a variety of analytes at the fiber's surface at ultrasensitive levels with minimal interference has been widely discussed in literature. The RAPTOR is a commercially available analytical instrument that uses a fiber optic biosensor platform to detect analytes using multiple channels. The advantages of analyte detection in the near-infrared region (650 – 1000 nm) have also been reported. We recently modified the RAPTOR to detect fluorescence using an excitation source near 780 nm. This wavelength corresponds with a family of heptamethine cyanine fluorophores that possess fluorescence properties that are in the near-IR as opposed to the current fluorophores used with the RAPTOR, which are excited at 635 nm. Utilizing our near-IR wavelength results in significant reduction of interference from biomolecules that fluoresce in the 635-650 nm region.

In this study, we report the results of a fluoroimmunoassay performed on the modified RAPTOR where different levels of goat anti-human IgG (GAHG) are detected in a sandwich immunoassay format. We were capable of detecting GAHG using the modified RAPTOR at concentrations as low as 100ng/mL. Future work includes improving binding of capture antibody to wave guide surfaces to reduce non-specific binding and optimizing concentration relationship between surface antigen and labeled antibody reagent.

We acknowledge the support of the Department of Defense through Department of Defense Grant W911NF-11-1-0157.

TANNIN CONTENT OF TEAS

Sarah Toone, Bradley Brown, Monica Ahir, and Dr. Richard Foust
Department of Chemistry and Biochemistry, James Madison University,
901 Main Street, Harrisonburg, VA 22807

Tannins are polyphenolic substances, present in the leaves of most plants and an important component in many foods. They contribute to the flavor and taste of food, and add bitterness to red wine. Tannin content is also one of the five basic characteristics used to classify wines.

Tannins cause body tissues to shrink or contract, a quality that is called "astringency." Astringency is responsible for that familiar dry taste behind after a cup of tea, and also causes blood clots to form when wet tea bags are placed on an open wound. This particular trait has led many oral surgeons to recommend that their patients gently bite teabags after surgery to help speed the healing process.

To provide the best recommendation for patients, surgeons need to know which variety of tea contains the highest percentage of tannins, and would therefore stop bleeding most effectively. To test this hypothesis, ten readily available teas (National Cup, Lipton English Breakfast, Luzianne, Lipton Natural Energy, Red Rose, Tetley British Blend, Great Value Black Tea, Twinings English Breakfast, Great Value Green Tea, and Lipton Pure Green Tea) were treated with a 50% water/acetone mixture in a Soxhlet extractor to find the tannin content.

National Cup, a variety of orange pekoe tea, contains the highest tannin content (47.4%). Another brand of orange pekoe tea, Red Rose, was next highest with a difference between the two of just 1.21%. This would suggest that, out of the types tested, orange pekoe teas have the highest percentage of tannins and would be best recommended to oral surgery patients.
We are currently analyzing the tannin extracts by HPLC/MS to identify the molecular composition of the tannins extracted from this group of 10 teas.

We thank Dr. Philip Pandolfi, DMD, for suggesting this problem and the James Madison University Department of Chemistry and Biochemistry for their generous support.

MULTIVARIATE, LINEAR SOLVATION ENERGY RELATIONSHIP (LSER), AND K–K ANALYSIS OF ANALYTE RETENTION IN REVERSED PHASE LIQUID CHROMATOGRAPHY

Peter Willard and Eric Williamsen
Department of Chemistry, Ursinus College, 601 E. Main Street, Collegeville, PA 19426

High-Performance Liquid Chromatography (HPLC) is one of the most widely used separation techniques and represents a critical step in the process of drug discovery and medical diagnostics. HPLC relies upon the differing intermolecular forces between compounds with the stationary phase of the column to separate analytes. These interactions change depending upon the solvent, temperature, pH, and other factors; by altering these conditions, the amount of time a compound is retained in the column can be changed. However, a detailed molecular understanding of how these differences alter the intermolecular forces and compound retention is not well characterized. A deeper understanding of these forces will allow for better separations and analyte-specific separation techniques.

To investigate this, we have obtained the retention times for 40 analytes at eight temperatures ranging from 10-80°C, in seven different methanol and acetonitrile mobile phase compositions, and two types of alkyl and fluoroalkyl columns. Due to the multivariate nature of the data, it has been analyzed using several techniques, including multivariate (cluster, principle component, etc.) and k-k analysis to reveal trends in analyte retention with condition changes. Linear Solvation Energy Relationship (LSER) analysis was also applied to investigate the importance and change in specific intermolecular interactions to characterize the forces governing the separation process in alkyl and fluoroalkyl columns.

NEW EXPERIMENTAL AND KINETIC DATA FOR 2-ETHYL HEXYL CHLOROFORMATE

Alora Wilson, Katelyn Null, and Dr. Malcolm J. D'Souza
Department of Biochemistry, Wesley College, 120 N. State Street, Dover, DE 19901

2-Ethylhexyl chloroformte is important in the Quantitative High Throughout Screening (qHTS) assay of hormone receptor inhibitors and activators since it identifies the hormones that catalyze multiple enzymatic pathways. Additionally, 2-ethylhexyl chloroformate is important for the qHTS assay for small molecule activators of the heat shock response signaling pathway (PubChem).

Utilizing lacmoid in acetone as the indicator and sodium methoxide as the base in acid-base titrations, the specific rates of reaction are studied in various aqueous acetone, and aqueous fluoroalcohol concentrations. Here, 2-ethylhexyl chloroformates is studied in aqueous acetone, aqueous 2,2,2-trifluoroethanol (TFE), and aqueous 1,1,1,3,3,3-hexafluoro-2-propanol mixtures. Rates are determined using aqueous fluoroalcohol solvents at 25.0°C.

Initial results suggest that compound reactivity is strongly dependent on type of solvent used. In aqueous TFE, the more water present, the faster the reaction rate. In TFE-Ethanol mixtures, the reaction rate is faster when more ethanol is present. In HFIP, the more water present, the slower the reaction is. Since this trend is observed in the highly ionizing fluoroalcohols, it suggests that the mechanism of reaction is strongly dependent on solvent nucleophilicity.

This research is funded by the National Institute of General Medical Sciences - NIGMS (8 P20 GM103446-13) from the National Institutes of Health (DE-INBRE)

"2-Ethylhexyl Chloroformate." **- PubChem**. National Center for Biotechnology Information, n.d. Web. 25 June 2014. <http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=62783#x299>.

Afternoon Poster Session
Group EE – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

206. **UNDERSTANDING THE ALLOSTERIC INHIBITION OF PTP1B**
Kelly Bird[1,2], Sean Whittier[2], Dr. J. Patrick Loria[2], and Dr. James Lipchock[1,2]
[1]Department of Chemistry, Toll Science Center, Washington College, Chestertown MD 21620
[2]Department of Chemistry, Yale University, New Haven CT 06511

207. **REGULATION OF MESOTHELIN AND KALLIKIREIN-RELATED PEPTIDASE 5 IN HUMAN ESOPHAGEAL KERATINOCYTES BY THE ZINC-FINGER TRANSCRIPTION FACTOR KRÜPPEL-LIKE FACTOR 4**
Michael Fagiola[1], Taicheng Zhou[2], Yizeng Yang[2], and Jonathan Katz[2]
[1]Department of Chemistry, Shepherd University, Shepherdstown, WV 25443
[2]NIH Center for Molecular Studies in Digestive and Liver Diseases & the Division of Gastroenterology; Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104

208. **A PATHWAY TO UNDERSTANDING SCHIZOPHRENIA: IDENTIFICATION OF SUBSTRATES OF THE E3 LIGASE, UFL1**
Brenda Gutierrez[1], Lydia Emerson[2], Ellen Vieux[3], and Jon M. Madison[4]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2, 3, 4] Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, 75 Ames Street, Cambridge, MA 02142

209. **CHARACTERIZATION OF RUBRERYTHRIN AND SYMERYTHRIN MODEL PROTEINS**
Jenna Pellegrino, Rachel Z. Polinski, Sabrina N. Cimerol, and Dr. Amanda J. Reig
Department of Chemistry, Ursinus College, 601 E. Main Street, Collegeville, PA 19426

210. **EXPRESSION OF NEURONAL NITRIC OXIDE SYNTHASE (NNOS) IN THE EXTRATESTICULAR PATHWAY AND ITS ROLE IN MURINE SPERM MATURATION**
Rebecca Pollak and Patricia A. Martin-DeLeon
Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19716

211. **EXAMINATION OF A CYSTEINE-RICH MOTIF'S METAL BINDING CAPABILITIES IN AN UNCHARACTERIZED PROTEIN**
Derek Shirey and Kristina O. Pazehoski
Department of Biology, Grove City College, 100 Campus Drive,Grove City, PA 16127

212. **INVESTIGATING THE ENZYMATIC ACETYL TRANSFER REACTION PATHWAY**
Byron H. Young and Dr. Christopher Berndsen
Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, VA 22801

UNDERSTANDING THE ALLOSTERIC INHIBITION OF PTP1B

Kelly Bird[1,2], Sean Whittier[2], Dr. J. Patrick Loria[2], and Dr. James Lipchock[1,2]
[1]Department of Chemistry, Toll Science Center, Washington College, Chestertown MD 21620
[2]Department of Chemistry, Yale University, New Haven CT 06511

Online access of this abstract is restricted at the request of the Principal Investigator.

REGULATION OF MESOTHELIN AND KALLIKIREIN-RELATED PEPTIDASE 5 IN HUMAN ESOPHAGEAL KERATINOCYTES BY THE ZINC-FINGER TRANSCRIPTION FACTOR KRÜPPEL-LIKE FACTOR 4

Michael Fagiola[1], Taicheng Zhou[2], Yizeng Yang[2], and Jonathan Katz[2]
[1]Department of Chemistry, Shepherd University, Shepherdstown, WV 25443
[2]NIH Center for Molecular Studies in Digestive and Liver Diseases & the Division of Gastroenterology; Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104

Krüppel-like factor 4 (KLF4) is a key transcriptional regulator that functions in a number of cellular processes such as proliferation, differentiation, migration, development, and carcinogenesis. In the esophagus, KLF4 is typically induced in differentiating keratinocytes and plays a role in esophageal keratinocyte proliferation, migration and differentiation functions. *Klf4* deletion in esophageal epithelia of mice alters keratinocyte morphology and delays keratinocyte differentiation at 5 weeks of age and 3 months of age; these mice eventually developed a premalignant condition known as squamous cell dysplasia. To determine the targets of KLF4 in esophageal epithelial cells, we performed gene expression analyses on esophageal epithelia of mice at 3 months of age. Among the most differentially regulated genes were *MSLN* and *KLK5*, two genes implicated in squamous cell differentiation and carcinogenesis. We hypothesized that KLF4 was a direct transcriptional regulator of *MSLN* and *KLK5* during proliferation and/or differentiation in human esophageal keratinocytes. In studies using human primary esophageal keratinocytes in culture, we found that KLF4 was induced during keratinocyte differentiation and that KLF4 loss altered differentiation. Taken together, these findings are consistent with an important role for KLF4 in human esophageal keratinocyte differentiation. By quantitative real-time PCR (qPCR) and Western Blot, respectively, KLF4 loss in human esophageal keratinocytes also resulted in decreased mRNA and protein levels for *MSLN* and *KLK5*. Further, by chromatin immunoprecipitation (ChIP), we found that the *MSLN* and *KLK5* gene promoters were directly bound by KLF4. Thus, KLF4 is important for human esophageal keratinocyte differentiation and directly regulates *MSLN* and *KLK5*.

The authors acknowledge support from US National Institutes of Health (NIH) NIDDK R01-DK069984 (to J.P.K.), and by the Undergraduate Student Scholars Program at the University of Pennsylvania Center for Molecular Studies in Digestive and Liver Diseases (NIH R25-DK066028).

A PATHWAY TO UNDERSTANDING SCHIZOPHRENIA: IDENTIFICATION OF SUBSTRATES OF THE E3 LIGASE, UFL1

Brenda Gutierrez[1], Lydia Emerson[2], Ellen Vieux[3] and Jon M. Madison[4]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2, 3, 4]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard,
75 Ames Street, Cambridge, MA 02142

Schizophrenia is a common, heritable mental illness that is costly for individuals, families and society. Recently, many seemingly unrelated genes have been genetically associated with schizophrenia (Scz). A major objective is to identify biochemical pathways that connect these seemingly unrelated Scz-associated genes. Further study of these biochemical pathways might help us gain a better understanding of the molecular basis of Scz and provide us with potential therapeutic targets. The biochemical pathways we chose to focus on involve several E3 ligases implicated in Scz. E3 ligases are enzymes that regulate the stability and function of proteins by catalyzing the transfer of ubiquitin or ubiquitin-like molecules to these proteins. We hypothesized that Scz-associated E3 ligases target other Scz genetically-associated proteins in neurons – the E3 ligases and their substrates would comprise a biochemical pathway. To identify E3 ligase substrates, we are developing biochemical tools that utilize affinity tagging in cells to identify substrates and binding partners of a specific E3 ligase identified by exome sequencing of schizophrenia patients called UFL1. UFL1 catalyzes the transfer of the ubiquitin-like protein UFM1 to substrates. We created an Avitagged-UFM1 by genetically encoding an affinity tag called Avi that is expressed at the N-terminus of UFM1. To identify UFL1 substrates, we used biotinylation to recognize the Avitagged-UFM1 and capture UFM1-tagged proteins. Once UFL1 substrates are identified, we will compare them to products of Scz-implicated genes and search for an overlap. Finding an overlapping subset of UFL1 substrates and genes genetically associated with schizophrenia would represent a key step toward identifying biochemical pathways for further study and for eventual therapeutic intervention.

CHARACTERIZATION OF RUBRERYTHRIN AND SYMERYTHRIN MODEL PROTEINS

Jenna Pellegrino, Rachel Z. Polinski, Sabrina N. Cimerol, and Dr. Amanda J. Reig
Department of Chemistry, Ursinus College, 601 E. Main Street, Collegeville, PA 19426

The ferritin-like superfamily (FLSF) is a class of proteins that contain a diiron active site and participate in important biochemical pathways, including fatty-acid desaturation and radical generation. The canonical FLSF sequence contains four carboxylate and two histidine metal-binding ligands in the active site. Interestingly, rubrerythrin (Rbr) and symerythrin (Sym) have one and two additional carboxylate residues, respectively, and exhibit unique reactivity with hydrogen peroxide. The correlation of these proteins possessing additional carboxylate residues and having enhanced peroxidase function is currently not well understood. The purpose of our research is to understand the role of the active site structure in diiron protein function, with a specific focus on the naturally occurring proteins rubrerythrin and symerythrin.

Model proteins of Rbr and Sym were created based on the *de novo*-designed G4DFsc protein, which is a 4-helix mimic of the 2-His/4-carboxylate structure. Carboxylate residues, both aspartate (D) and glutamate (E), were introduced at positions G14 and/or G47 to generate Rbr and Sym models of varying carboxylate combination. Prior work has characterized the metal binding and reactivity with the Rbr model proteins G14D and G14E, both through iron and cobalt binding assays. Our current work has focused on the metal binding activity of the Sym proteins G14E/G47D and G14D/G47E with iron and cobalt. These studies will give us insight into how systematic changes to structural features of a protein affect its metal-binding folding and reactivity.

Financial support for this work was provided by Ursinus College, the Howard Hughes Medical Institute Undergraduate Science Education Program, and the NIH (GM110657).

EXPRESSION OF NEURONAL NITRIC OXIDE SYNTHASE (NNOS) IN THE EXTRATESTICULAR PATHWAY AND ITS ROLE IN MURINE SPERM MATURATION

Rebecca Pollak and Patricia A. Martin-DeLeon
Department of Biological Sciences, University of Delaware, 118 Wolf Hall, Newark, DE 19716

Neuronal nitric oxide synthase (nNOS) is one of two constitutive enzyme variants responsible for the production of nitric oxide (NO) from L-arginine in mammalian cells. In somatic cells, this membrane-associated protein has been shown to be activated by Ca^{2+} and to interact with Plasma Membrane Calcium ATPase 4 (PMCA4), the major calcium efflux pump in murine sperm (Wennemuth et. al. 2003), which negatively regulates it. NO is an important second messenger, and is required for a variety of sperm functions, including motility and fertilizing ability (Ramya et. al. 2011). Recently, the DeLeon lab has shown the expression of PMCA4 in the murine epididymis. However, no work has been done on the expression of nNOS in the extratesticular pathway of any mammalian species. Therefore, we set out to investigate the expression pattern of nNOS in the post-testicular pathway and its role in sperm maturation. Here, we show the presence of nNOS in all three regions of the murine epididymis (caput, corpus, and cauda), including the basal and apical regions of the cells, via immunofluorescence for the first time. Western blotting results confirmed the expression of nNOS throughout the epididymis. Furthermore, preliminary immunofluorescence results suggest that the process of capacitation, in which the sperm experience a major influx of calcium, may influence the localization of nNOS.

This work is supported by NIH-R03HD073523 to P. A. M.-D.

EXAMINATION OF A CYSTEINE-RICH MOTIF'S METAL BINDING CAPABILITIES IN AN UNCHARACTERIZED PROTEIN

Derek Shirey and Kristina O. Pazehoski
Department of Biology, Grove City College, 100 Campus Drive, Grove City, PA 16127

Regulating copper homeostasis in the cell, CopY utilizes a cysteine-rich motif, CxC(x_{4-6})CxC (x = any other amino acid), to target and bind metals in *Enterococcus hirae*. The CopY repressor protein utilizes this metal binding site by forming a complex with one zinc(II) ion or two copper(I) ions causing an alteration to the function of the protein. The change in function is most likely due to a conformational change in the quaternary structure of the protein upon metal exchange. Found in a broad array of organisms, the motif is specifically being studied in a methyltransferase expressed by *Desulfvibrio alaskensis*. To further characterize the ability of this protein to bind metals, we induce the expression of a pET-14b expression vector containing the gene of interest. With a pure sample of the uncharacterized protein of *D. alaskensis*, the goal of this research is to determine if the protein containing the cysteine motif has the ability to bind metals. Subsequently, to determine why the metal is bound, analyzing adaptations to the quaternary structure of the protein may suggest that the bound metals affect the function of the protein.

We would like to acknowledge the generous financial support provided for this project by the Grove City College Swezey Fund.

INVESTIGATING THE ENZYMATIC ACETYL TRANSFER REACTION PATHWAY

Byron H. Young and Dr. Christopher Berndsen
Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, Harrisonburg, VA 22801

Enzyme catalyzed protein acetylation, which involves the transfer of an acetyl group to a lysine side chain, serves many vital cellular purposes. Examples of this include the influencing of gene expression and the regulation of many cellular pathways. Defects in enzymatic acetylation have been linked to many common diseases including cancer, insomnia, and anemia. Despite decades of research into the biological function of protein acetylation, the enzymatic mechanism of acetyl transfer is unknown. We aim to investigate this using the yeast protein acetyltransferase GCN5 with the initial goal of studying proton transfer during the reaction. This information is crucial for building the transition state of the reaction and therefore is integral to determining its mechanism. We have been the first to investigate the solvent isotope effects on the catalysis of acetyl transfer utilizing deuterium oxide. Our data show a normal deuterium solvent isotope effect for this reaction, suggesting that proton transfer occurs during the acetyl transfer reaction. Future experiments will determine the number of protons being transferred and will measure the kinetic isotope effects on the GCN5 acetyl transfer reaction. The collection of these results will allow us to determine the mechanism of catalysis, and thereby set the groundwork for further investigation into enzymatic acetyl transfer.

Afternoon Poster Session
Group FF – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

213. **CHARACTERIZING THE MID1 FIBRONECTIN TYPE III DOMAIN THROUGH NMR STUDIES**

Daniel Bernstein, Haijuan Du, and Michael Massiah
Department of Chemistry, George Washington University, 725 21st Street NW, Washington, DC 20052

214. **PROTEIN-PROTEIN INTERACTIONS BETWEEN THE LECTIN-LIKE DOMAIN OF THROMBOMODULIN AND COMPLEMENT COMPONENT 3**

Nathan Fritzinger, Dan DeHelian, and Julia Koeppe
Department of BioChemistry, Ursinus College, 601 E. Main Street, Collegeville, PA 19426

215. **REV-ING UP THE NMR**

Lindsay Glang[1], Roald Teuben[1], Andy DeLaitsch[2], Aron Winton[3], Jan Marchant[1], Yuanyuan Liu[1], and Michael F. Summers[1]
[1]Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706
[3]Summer Biomedical Training Program, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

216. **STRUCTURAL CHARACTERIZATION OF THE CHEMOKINE RECEPTOR CXCR4 IN COMPLEX WITH SDF-1**

Joshua Temple[1], Lauren Holden[2], and Tracy Handel[2]
[1]Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, VA 22807
[2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92092

217. **USING NMR SPECTROSCOPY TO RESOLVE THE STRUCTURE OF HIV-1 RRE**

Roald Teuben[1], Lindsay Glang[1], Andy DeLaitsch[2], Yuanyuan Liu[1], Jan Marchant[1], and Michael F. Summers[1]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706

218. **UNDERSTANDING E2 MECHANISM USING NMR**

Emily Todd, Reid Putney, Dr. Christopher Berndsen, and Dr. Nathan Wright
Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, VA 22801

CHARACTERIZING THE MID1 FIBRONECTIN TYPE III DOMAIN THROUGH NMR STUDIES

Daniel Bernstein, Haijuan Du, and Michael Massiah
Department of Chemistry, George Washington University,
725 21st Street NW, Washington, DC 20052

MID1 is a microtubule-associated protein that regulates the cellular concentration of Protein Phosphatase 2A (PP2A) and Alpha-4 through the ubiquitination pathway. Mutations of MID1 are linked to X-Linked Opitz G Syndrome (XLOS), a developmental disorder characterized by abnormalities along the midline of the body that include cleft lip, agenesis of the corpus callosum, and various organ complications. A comprehensive understanding of the structural components of MID1 and its various domains can yield better insight into the mechanism of function of MID1 in its role in causing XLOS.

The Fibronectin Type III (FNIII) domain is located within the C-terminal half of MID1 and its structure and function, as part of MID1, are unknown. Three mutations within this domain (P441L, G452S, and V463F) were found in XLOS patients. My studies have focused on optimizing conditions to improve the stability of the FNIII domain at high concentration for structural and functional studies. Here I compare the solubility of two constructs of FNIII with different lengths. I investigated the effect of pH on the wild-type FNIII structure using two-dimensional Nuclear Magnetic Resonance (NMR) spectroscopy, and compared the structural effect of one of the mutations. This work will serve as the foundation for determining the structure of the wild-type FNIII and for understanding the effect of the mutations on structure.

PROTEIN-PROTEIN INTERACTIONS BETWEEN THE LECTIN-LIKE DOMAIN OF THROMBOMODULIN AND COMPLEMENT COMPONENT 3

Nathan Fritzinger, Dan DeHelian, and Julia Koeppe
Department of BioChemistry, Ursinus College, 601 E. Main Street, Collegeville, PA 19426

Protein-protein interactions are vital to the proper functioning of numerous biological systems. Thrombomodulin (TM) is a protein that is involved in the down-regulation of coagulation induced by the clotting protein thrombin. Complement component 3 (C3) is a vital component of the complement system, which is involved in innate immunity against bacteria and viruses. However, dysregulation of C3 can lead to the degradation of host cells.

Evidence suggests that the lectin-like domain of TM, which protrudes into the bloodstream from the host's epithelial cells, may interact with active C3 (C3b) to inactivate it, thus preventing host cell degradation. The research conducted herein required the expression and purification of the lectin-like domain of TM, called TM680, in yeast cells and the isolation and purification of C3 from bovine blood plasma.

To aid in specific immobilization of the TM680, two lysine residues were converted to methionine by site-directed mutagenesis via polymerase chain reaction, although only one mutation (K147M) was achieved in time for further testing. A pull down assay was then performed with the wild-type and mutant proteins to determine protein-protein interactions but the results were inconclusive. Once interactions are confirmed, hydrogen/deuterium exchange followed by matrix assisted laser desorption and ionization time of flight mass spectroscopy (MALDI-TOF MS) will be performed to determine which amino acids are involved in binding.

REV-ING UP THE NMR

Lindsay Glang[1], Roald Teuben[1], Andy DeLaitsch[2], Aron Winton[3], Jan Marchant[1], Yuanyuan Liu[1], and Michael F. Summers[1]
[1]Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706
[3]Summer Biomedical Training Program, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

The Rev-response element (RRE) is a region of the HIV-1 genome that is implicated in the export of viral RNA. It provides a scaffold where several copies of the accessory protein Rev bind. This Rev-RRE complex is recognized by the host nuclear export machinery, and provides a means for export of the full HIV-1 genome.

Using nuclear magnetic resonance (NMR) spectroscopy, our group has been working to calculate the solution structure of the RRE. NMR spectra of large RNAs are difficult to analyze, predominantly because of poor chemical shift dispersion leading to severe overlap. To combat this we have produced RNA molecules containing partially deuterated nucleotides, which do not give peaks in our NMR spectra, thus greatly simplifying them. Further aiding our analysis we produced an array of fragments corresponding to different regions of the full-length RRE. By comparison of the fragment and full-length spectra we have begun to map out the RRE secondary structure, a vital step in 3D structure determination. In addition to designing fragments, we made use of long-range probing by Adenosine Interaction Detection (lr-AID). In this method, mutations are made which will give rise to a distinct signature peak that only appears if specific base-pairings occur.

Through obtaining the structure of the RRE, a better understanding of the mechanisms by which it operates will arise.

I would like to acknowledge the NIH/NIGMS # P50GM103297, and the Howard Hughes Medical Institute.

STRUCTURAL CHARACTERIZATION OF THE CHEMOKINE RECEPTOR CXCR4 IN COMPLEX WITH SDF-1

Joshua Temple[1], Lauren Holden[2], and Tracy Handel[2]
[1]Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, Harrisonburg, VA 22807
[2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego,
9500 Gilman Drive, La Jolla, CA 92092

Online access of this abstract is restricted at the request of the Principal Investigator.

USING NMR SPECTROSCOPY TO RESOLVE THE STRUCTURE OF HIV-1 RRE

Roald Teuben[1], Lindsay Glang[1], Andy DeLaitsch[2], Yuanyuan Liu[1],
Jan Marchant[1], and Michael F. Summers[1]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biochemistry, University of Wisconsin-Madison,
433 Babcock Drive, Madison, WI 53706

HIV-1 is a retrovirus that reproduces by inserting its viral genome into a host cell, specifically targeting CD4+ T cells. A critical step in the late phase of the viral lifecycle is the export of viral mRNA transcripts that have not gone through normal host cell splicing. The RRE (Rev Response Element) is a region of the HIV-1 RNA genome that mediates the nuclear export of unspliced and partially spliced mRNA. Export is initiated by viral Rev proteins forming a complex with an RRE, whereupon the Rev-RRE complex initiates recruitment of host cell nuclear export machinery.

The role of the RRE in the viral lifecycle makes it an attractive area of research, so we are attempting to resolve the structure of the RRE using NMR techniques. Our work involves designing RRE derivatives that allow us to distinguish and build upon computationally predicted structures. Our investigations into the structure of the RRE involve the synthesis of smaller RNA fragments that are more easily analyzed via NMR spectroscopy. We also design mutated versions of the RRE that employ the "lr-AID" technique (long-range probing by adenosine interaction detection) which simplify analysis of NMR spectra. Furthermore, we selectively incorporate isotope-labeled nucleotides into our RNA that are less visible in our NMR experiments, which further complement efforts toward structure determination of the RRE via NMR.

Other studies have made progress in developing structural models for the RRE using various spectroscopic techniques such as SAXS (small angle X-ray scattering), as well as in characterizing Rev-RRE interactions. Our research aims to enrich this body of knowledge.

This work is supported by the NIH/NIGMS grant #P50GM103297 and the Howard Hughes Medical Institute. We would like to thank SBTP participants Andy DeLaitsch and Aron Winton for contributing to this study. We would also like to thank Michael Summers for making our work possible, and Jan Marchant for his guidance.

UNDERSTANDING E2 MECHANISM USING NMR

Emily Todd, Reid Putney, Dr. Christopher Berndsen, and Dr. Nathan Wright
Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, Harrisonburg, VA 22801

The ubiquitination pathway controls human cellular processes such as cell cycle division, DNA transcription and repair, and immune responses. In these processes, ubiquitin is covalently attached to a substrate protein by the combined efforts of an E1 activating enzyme, an E2 conjugating enzyme, and an E3 ubiquitin ligase. We are concerned with investigating the catalytic mechanism of ubiquitin transfer onto the substrate protein. We specifically want to understand how the structure of Ubc13 helps with catalytic activity. The structure of yeast Ubc13, an E2 conjugating enzyme, has been solved using nuclear magnetic resonance (NMR). The study of the structure of Ubc13 compared to that of Ubc13 bound to ubiquitin in the active site will show the changes in the active site upon binding. Through the use of transition state mimics of ubiquitin conjugating reaction we will completely describe how Ubc13 stabilizes the ubiquitination conjugation pathway.

Afternoon Poster Session
Group GG – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

219. **IDENTIFICATION OF PREVIOUSLY UNSEQUENCED VIRUSES FROM WILD-CAUGHT MOSQUITOES BY METAGENOMIC SEQUENCING**

Tara Biser[1,2], Kenneth G. Frey[2,3], Cassie L. Redden[2,3], Joseph J. Anderson[2,4] Jens C. Andersen[2,5], Kevin L. Schully[2,3], Regina Z. Cer[2,3], Vishwesh P. Mokashi[2], and Kimberly A. Bishop-Lilly[2,3]
[1]Hood College, 401 Rosemont Avenue, Frederick, MD 21701
[2]NMRC-Frederick, 8400 Research Plaza, Fort Detrick, MD 21701
[3]Henry M. Jackson Foundation, 6720 Rockledge Drive, Bethesda, MD 20817
[4]Defense Threat Reduction Agency, 8725 John J Kingman Road, Fort Belvoir, VA 22060
[5]The Informatics Applications Group, Inc., 1760 Reston Parkway, Reston, VA 20190

220. **STRUCTURE OF THE CORE ENCAPSIDATION SIGNAL OF HIV-1**

Gregory Campbell Carter, Alyssa T. Florwick, Justin Leonel C. Santos, Sarah C. Keane, and Michael F. Summers
Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

221. **AG6: MECHANISMS AND RESULTS OF ALLOSTERIC INHIBITION IN HEPATITIS C VIRAL POLYMERASE**

Daniel Dagenhart, Marie Espiritu, Ester Sesmero, and Dr. Ian Thorpe
Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

222. **STRUCTURAL ANALYSIS OF THE ECTODOMAIN OF THE ANTI-VIRAL PROTEIN BST-2**

Kelly Du Pont, Aidan McKenzie, Samantha Chinn, Dr. Christopher Berndsen
Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807

223. **STRUCTURAL BASIS OF HIV-1 GENOME RECOGNITION VIA VIRAL RNA-PROTEIN INTERACTION**

Alyssa Florwick, Greg Carter, Justin Santos, Sarah Keane, and Michael Summers
Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

224. **THE INTERACTION BETWEEN SET5 AND GLC7**

Mitchell L. Kelly and Erin M Green
Department of Biology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

IDENTIFICATION OF PREVIOUSLY UNSEQUENCED VIRUSES FROM WILD-CAUGHT MOSQUITOES BY METAGENOMIC SEQUENCING

Tara Biser[1,2], Kenneth G. Frey[2,3], Cassie L. Redden[2,3], Joseph J. Anderson[2,4] Jens C. Andersen[2,5], Kevin L. Schully[2,3], Regina Z. Cer[2,3], Vishwesh P. Mokashi[2], and Kimberly A. Bishop-Lilly[2,3]
[1]Hood College, 401 Rosemont Avenue, Frederick, MD 21701
[2]NMRC-Frederick, 8400 Research Plaza, Fort Detrick, MD 21701
[3]Henry M. Jackson Foundation, 6720 Rockledge Drive, Bethesda, MD 20817
[4]Defense Threat Reduction Agency, 8725 John J Kingman Road, Fort Belvoir, VA 22060
[5]The Informatics Applications Group, Inc., 1760 Reston Parkway, Reston, VA 20190

Metagenomic sequencing (unbiased, high-throughput sequencing directly from complex matrices) can be used to analyze the genetic material contained in a taxonomically diverse sample. This method has the potential to be used for several applications, including diagnostics and biosurveillance. Mosquitoes, which feed on a variety of organisms within their environment, are ideal subjects for this type of analysis.

Viral genomes from wild-caught mosquitoes in the contiguous United States (CONUS) have not been fully characterized in public databases. Research in this area has primarily focused on only viruses with DNA genomes. This suggests that we have very little knowledge of the microbiota of an important disease vector on our own soil, and likely even less knowledge of the microbiota of disease vectors where our troops are deployed overseas. Small distances between collection locations can result in completely distinct mosquito viromes, thus illustrating the extent of diversity that exists within these tiny vectors.

The aim of the current study is to determine if samples of mosquitoes collected from different sites (CONUS, east coast) could be differentiated using metagenomic analysis of the RNA fraction. Mosquito samples were collected from several locations, sequenced, and analyzed. Both total and viral-enriched RNA samples were created from each site. Preliminary data suggest the presence of multiple previously unsequenced viruses within these samples. Genome characterization of these apparently novel viruses is currently in progress.

Funding was provided by the American Society for Engineering Education through the Naval Research Enterprise Internship Program.

STRUCTURE OF THE CORE ENCAPSIDATION SIGNAL OF HIV-1

Gregory Campbell Carter, Alyssa T. Florwick, Justin Leonel C. Santos,
Sarah C. Keane, and Michael F. Summers
Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, UMBC,
1000 Hilltop Circle, Baltimore, MD 21250

An estimated 35 million people worldwide currently live with Human Immunodeficiency Virus (HIV-1), with an estimated 1.7 million associated deaths a year. While many retroviral drugs for the disease exist, these function merely as treatments, and there is currently no cure. Further, current treatments are reduced in efficacy due to high cost and poor compliance, allowing the virus to evolve drug-resistant strains. Current retroviral treatments approved by the FDA target entry of infectious virion, reverse transcription, or virion maturation, but none currently target the genome recognition step. Our group focused on the structure of a portion of the HIV-1 genome within the 5' leader which is necessary for packaging, an RNA known as the Core Encapsidation Signal (CES). Using various labeling, fragmentation, and Long Range Adenosine Interaction Detection (lr-AID) schemes in NMR experiments, our group has identified both the secondary and tertiary structure of CES. With this knowledge, drug development targeting genome packaging becomes a possibility.

Funded by NIH/NIGMS grant 1P50GM103297 and was conducted at the Howard Hughes Medical Institute at UMBC. I would also like to acknowledge Sayo McCowin, Seung Ho Choi, Briaunna Minor, Holly Summers,

AG6: MECHANISMS AND RESULTS OF ALLOSTERIC INHIBITION IN HEPATITIS C VIRAL POLYMERASE

Daniel Dagenhart, Marie Espiritu, Ester Sesmero, and Dr. Ian Thorpe
Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

About 150-200 million people in the world are infected with Hepatitis C (HCV), a virus that leads to cirrhosis of the liver as well as hepatocellular carcinoma. In the US, an estimated 3.2 million people are infected with this virus; there is no vaccine currently available, and current treatments are not completely effective. The RNA-dependent RNA-polymerase of HCV, NS5B, has a "right-handed", globular shape that includes finger, thumb, and palm domains. The conformations of this polymerase are known to be key to the replicative efficacy of the virus. Previous research literature identified two distinct conformations adopted by the polymerase, namely "closed" and "open"; these two forms are associated with the initiation and elongation stages of replication respectively. Observed structural changes between these conformations are movements of the Δ1 loop, the β-flap, as well as the entire thumb subdomains.

Our goal is to examine the effects and mechanisms of utilizing ligand AG6 as an allosteric inhibitor of HCV. Through its allosteric function, AG6 can bind to the finger domain of the polymerase where it performs its inhibitory activity. To accomplish this, we use Molecular Dynamics (MD) simulations with explicit solvent to characterize the interactions of the NS5B polymerase with ligand AG6. We expect to elucidate the specific structural or dynamic changes caused by AG6 that affect replication efforts. The new insights obtained may lead to the development of enhanced and novel anti-viral treatments for HCV.

STRUCTURAL ANALYSIS OF THE ECTODOMAIN OF THE ANTI-VIRAL PROTEIN BST-2

Kelly Du Pont, Aidan McKenzie, Samantha Chinn, Dr. Christopher Berndsen
Department of Chemistry and Biochemistry, James Madison University,
901 Carrier Drive, MSC 4501, Harrisonburg, VA 22807

Human BST-2/tetherin is a host factor that inhibits release of HIV-1, HIV-2, and SIV from the cell surface. Viruses can evade this inhibition through antagonistic viral protein interactions with BST-2. Structurally, full-length BST-2 consists of an N-terminal cytoplasmic domain, a transmembrane domain, an ectodomain, and a C-terminal membrane anchor. The N-terminal side of the ectodomain contains three cysteine residues; each can contribute to the formation of cysteine-linked dimers. For simplicity, we explored the ectodomain of BST-2 to further understand the flexibility of the protein. Recent cellular studies suggest BST-2 is flexible with regards to the dimerization and ability to function properly. However, x-ray crystallography suggests the ectodomain is rigid. Through limited proteolysis, molecular dynamics and small-angle x-ray scattering, we showed that the ectodomain of BST-2 is flexible. These studies will show how the innate immune system protein, BST2, interferes with viral budding.

STRUCTURAL BASIS OF HIV-1 GENOME RECOGNITION VIA VIRAL RNA-PROTEIN INTERACTION

Alyssa Florwick, Greg Carter, Justin Santos, Sarah Keane, and Michael Summers
Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, UMBC,
1000 Hilltop Circle, Baltimore, MD 21250

According to the World Health Organization, more than 35 million people worldwide are currently living with Human Immunodeficiency Virus (HIV). There are 31 antiretroviral drugs (ARVs) to date approved for treatment, yet there is no cure for infection. To elucidate new opportunities for drug development, we study the mechanism of genome packaging, a crucial step in viral proliferation. Packaging is mediated via the highly specific selection of the dimeric genome by the nucleocapsid (NC) domain of viral Gag polyprotein. Previous studies have identified the Core Encapsidation Signal (CES), the minimal region of the 5' untranslated region required to direct genome packaging. Using NMR spectroscopy, we have determined the 3-D structure of CES. This structure contains tandem three-way junctions that sequester the splice donor site. We propose that the tandem three-way junctions, along with the psi hairpin, both of which contain a number of exposed or weakly base-paired guanosines, have a large number of NC binding sites. In order to identify NC binding sites, we used site-directed mutagenesis to mutate specific guanosines (G) to adenosines (A), which should inhibit NC binding. Using isothermal titration calorimetry, we carried out binding studies with RNA constructs containing clusters of G to A mutations at the Psi hairpin, and both three-way junctions. These data confirm the existence of NC binding sites at junction and hairpin guanosines. Understanding the determinants of this RNA-protein interaction between CES and NC provides more information on the mechanism of genome selection. Better understanding of the structural mechanism of HIV-1 genome recognition provides new possibilities for late-phase viral inhibition.

This research was funded by NIH/NIGMS grant *1P50GM103297* and was conducted at the Howard Hughes Medical Institute at UMBC. We would also like to acknowledge members of our research team – Sayo McCowin[1], and Seung Ho Choi[1].

THE INTERACTION BETWEEN SET5 AND GLC7

Mitchell L.u Kelly and Erin M Green
Department of Biology, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Set5 is an evolutionary conserved lysine methyltransferase in budding yeast cells that is shown to play an important role in chromatin regulation. However, very little is known about Set5, including how its activity is regulated and whether it functions in other cellular pathways. Using co-immunoprecipitation experiments, our work shows that Set5 interacts with Glc7, the single gene in budding yeast that codes for protein phosphatase-1, an important regulator of the cell cycle. Further study of this interaction revealed that the process is phosphorylation independent. In addition, we are performing genetic tests between mutants of Glc7, the Ipl1 kinase and Set5 to determine how this phosphatase-kinase pathway interacts with the biochemical pathway of Set5. This research will uncover important new aspects of the relationship between chromatin regulation and the cell cycle of eukaryotes dependent on the Set5 methyltransferase.

Afternoon Poster Session
Group HH – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

225. **INVESTIGATION OF SYNERGIC EFFECT OF TRADITIONAL ANTIBACTERIAL AGENTS ON *B. SUBTILIS* AND *E. COLI***

Lamarque Coke, Phuong Le, Ellis Benjamin, and Earl Benjamin III
Department of Chemistry, The Richard Stockton College,
101 Vera King Farris Drive, Galloway, NJ 08205-9441

226. **RUTHENIUM (II) TRIS-BYPYRIDINE- AND COPPER (II)-MEDIATED CROSS-LINKING FOR THE ELUDICATION OF DOMAIN INTERACTIONS IN E. COLI GMP SYNTHETASE**

Laura Misiewicz[1] and Walter Patton[1,2]
[1]Program in Biochemistry and Molecular Biology, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003
[2]Departement of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

227. **DETERMINATION OF METAL BINDING SIGNIFICANCE IN METHYLTRANSFERASE CYSTEINE-RICH MOTIF**

Madalyn Plessinger and Kristina O. Pazehoski
Department of Biology, Grove City College, 100 Campus Drive, Grove City, PA 16127

228. **DEVELOPMENT AND REFINEMENT OF THE CONDITIONS FOR THE CRYSTALLIZATION *E. COLI* GMP SYNTHETASE**

Alyssa Shultz[1], Amelia Capuano[1] and Walter Patton[1,2]
[1]Program in Biochemistry and Molecular Biology, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003
[2]Departement of Chemistry, Lebanon Valley College, 101 N. College Avenue, Annville, PA 17003

229. **IDENTIFYING INTERACTION PARTNERS AMONG MEMBRANE-ASSOCIATED PROTEINS OF THE BMP SIGNALING PATHWAY IN *CAENORHABDITIS ELEGANS***

Neta Shwartz[1], Herong Shi[2], and Jun Kelly Liu[2]
[1] Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252
[2] Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853

230. **STUDYING THE INTOXICATION PATHWAY OF RECOMBINANT IMMUNOTOXINS BASED ON *PSEUDOMONAS* EXOTOXIN A USING INHIBITORS OF ELONGATION FACTOR 2 KINASE AND FURIN**

Jamy Therres[1] and John E. Weldon, Ph.D.[1,2]
[1]Molecular Biology, Biochemistry, and Bioinformatics Program Towson University,
8000 York Road, Towson, MD 21252
[2]Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252

231. **PROTEIN-PROTEIN INTERACTIONS OF HISTONE METHYLTRANSFERASE SET5 IN BUDDING YEAST**

Rashi Turniansky and Erin Green
Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

INVESTIGATION OF SYNERGIC EFFECT OF TRADITIONAL ANTIBACTERIAL AGENTS ON *B. SUBTILIS* AND *E. COLI*

Lamarque Coke, Phuong Le, Ellis Benjamin, and Earl Benjamin III
Department of Chemistry, The Richard Stockton College, 101 Vera King Farris Drive, Galloway, NJ 08205-9441

Antibiotic resistance is a problem that has far reaching implications. When antibiotic resistance is coupled to pathogenic conditions, patient outcome is severely diminished. Antibiotic resistance has been found in bacteria including but not limited to *E. coli*, *P. aeruginosa*, *S. aureus*, *V. cholera*, *K. oxytoca*, *C. koseri*, *P. stuartii*, and *M. morganii*. In order to better understand antibiotic resistance several established antibiotic agents (Chloromycetin, Erythromycin, Penicillin, Streptomycin, Tetracycline, Chloromycetin, Furadantin, Kanamycin, Nalidixic Acid, and Triple Sulfa) were combined and tested to via the disc diffusion method using *E. coli* and *B. subtilis* to determine the combined effects of each antibiotic combination. The results showed varied disinfection responses for the antibiotic combinations. These results will allow for the development of mechanistic models for these antibiotic combinations.

RUTHENIUM (II) TRIS-BYPYRIDINE- AND COPPER (II)-MEDIATED CROSS-LINKING FOR THE ELUDICATION OF DOMAIN INTERACTIONS IN E. COLI GMP SYNTHETASE

Laura Misiewicz[1] and Walter Patton[1,2]
[1]Program in Biochemistry and Molecular Biology, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003
[2]Departement of Chemistry, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003

Online access of this abstract is restricted at the request of the Principal Investigator.

DETERMINATION OF METAL BINDING SIGNIFICANCE IN METHYLTRANSFERASE CYSTEINE-RICH MOTIF

Madalyn Plessinger and Kristina O. Pazehoski
Department of Biology, Grove City College, 100 Campus Drive, Grove City, PA 16127

A broad collection of organisms synthesize proteins that function for the regulation of metal ions within a cell. The amino acid sequence of study, $CxC(x_{4-6})CxC$, was originally identified within the CopY protein found in the model bacteria *Enterococcus hirae.* CopY has the ability to bind either one zinc(II) ion or two copper(I) ions. When the protein is bound to a copper ion, it undergoes a conformational change that alters the quaternary structure and function of the protein. Metal-activated proteins may have several roles in biological systems. Specifically, CopY acts as a transcriptional repressor protein that controls the Cop operon. Another abundant protein, produced by *Campylobacter jejuni*, acts as a methyltransferase. This protein contains the aforementioned cysteine motif. A pET-14b vector containing the gene of interest was inserted into *E. coli* cells and grown in culture to isolate a pure sample of the *C. jejuni* methyltransferase. The sample was used to determine if the amino acid motif is sufficient for the protein to bind metals. Following the confirmation of metal binding, changes in quaternary structure will be analyzed in hopes of explaining the connection between metal binding and change of protein structure and function.

We would like to acknowledge the generous financial support provided for this project by the Grove City College Swezey Fund.

DEVELOPMENT AND REFINEMENT OF THE CONDITIONS FOR THE CRYSTALLIZATION *E. COLI* GMP SYNTHETASE

Alyssa Shultz[1], Amelia Capuano[1] and Walter Patton[1,2]
[1]Program in Biochemistry and Molecular Biology, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003
[2]Departement of Chemistry, Lebanon Valley College,
101 N. College Avenue, Annville, PA 17003

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IDENTIFYING INTERACTION PARTNERS AMONG MEMBRANE-ASSOCIATED PROTEINS OF THE BMP SIGNALING PATHWAY IN *CAENORHABDITIS ELEGANS*

Neta Shwartz[1], Herong Shi[2], and Jun Kelly Liu[2]
[1] Department of Biological Sciences, Towson University,
8000 York Road, Towson, MD 21252
[2] Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853

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STUDYING THE INTOXICATION PATHWAY OF RECOMBINANT IMMUNOTOXINS BASED ON *PSEUDOMONAS* EXOTOXIN A USING INHIBITORS OF ELONGATION FACTOR 2 KINASE AND FURIN

Jamy Therres[1] and John E. Weldon, Ph.D.[1,2]
[1]Molecular Biology, Biochemistry, and Bioinformatics Program Towson University, 8000 York Road, Towson, MD 21252
[2]Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252

Pseudomonas exotoxin A (PE) is a potent virulence factor secreted by the opportunistic human pathogen *Pseudomonas aeruginosa*. PE utilizes receptor-mediated endocytosis to enter cells, is processed in endosomes, and traffics using a retrograde pathway to the endoplasmic reticulum, where it crosses into the cytosol. Once in the cytosol, PE irreversibly inactivates translation elongation factor 2 (EF2) via ADP-ribosylation. Because of its potency and receptor-mediated pathway, PE has been studied as a potential cancer therapeutic by using an engineered targeting domain to direct it to specific malignant cell types. Several of these PE-based "recombinant immunotoxins" (RITs) are currently in clinical trials as cancer therapeutics. Using our knowledge of the stages of PE intoxication, we have explored how drug intervention at two of the intoxication stages influences the activity of RITs. First, we utilized a small molecule inhibitor of the transmembrane protease furin. Previous work has shown that furin cleaves PE during endosomal trafficking. To evaluate the importance of furin cleavage in the intoxication of PE-based RITs, we treated the CA46 Burkitt's lymphoma cell line with a small molecule inhibitor of furin prior to RIT treatment. Cells treated with the inhibitor were 2-fold less sensitive to the RIT HA22-LR, suggesting that furin has a small but important role in intoxication. Second, we utilized small molecule inhibitors of EF2 kinase (EF2K), which is the only known endogenous regulator of EF2 activity. EF2K normally inactivates EF2 via phosphorylation, and has been considered as a potential target for cancer therapeutics. The convergence of EF2K and PE-based RITs at their mutual target EF2 suggested the possibility of a synergistic relationship between the two treatments. We are currently exploring the effect of treating CA46 cells with EF2K inhibitors prior to RIT treatment. Initial experiments have shown no major change in sensitivity to the RIT HA22-LR.

I wish to thank the Linda Sweeting Summer Undergraduate Research Committee and the Department of Biological Sciences for supporting this research.

PROTEIN-PROTEIN INTERACTIONS OF HISTONE METHYLTRANSFERASE SET5 IN BUDDING YEAST

Rashi Turniansky and Erin Green
Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Histone methylation is a type of post-translational modification, used to regulate the way in which DNA is packaged in chromosomes; when chromatin is more tightly packed, it is called heterochromatin, and cannot transcribed as easily, leading to decreased gene expression, while the less dense euchromatin facilitates transcription. Histone methylation often mediates the transition between heterochromatin and euchromatin. Set5 is a conserved lysine methyltransferase from budding yeast (Saccharomyces cerevisiae) which has been shown to methylate lysine residues on the tail of histone H4.

Although the biochemical activity of Set5 is known, a complete understanding of its function in the cell has yet to be elucidated. Using immunoprecipitation, we have been working to discover each of the proteins that interact with Set5 in cells. In these experiments, we have determined that other proteins likely interact with Set5, and we will be identifying them using mass spectrometry. We expect these results to shed light on the function of Set5 in important cellular process.

Afternoon Poster Session
Group II – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

232. **USING GEL CENTRIFUGATION TO DETERMINE THE MONOMER-DIMER EQUILIBRIUM OF THE HIV-1 5'-LEADER**

Nick Bolden[1], Vern Van[1], Canessa Swanson[2], Jessica Smith[3],
Sarah Monti[1], and Michael Summers[1]
[1]Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, UMBC,
1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biology, Jackson State University, 1400 JR Lynch Street, Jackson, MS 39217
[3]The Seed School of Maryland, 200 Font Hill Avenue, Baltimore, MD 21223

233. **SURVIVAL OF THE FITTEST RNA APTAMER: USING ARTIFICIAL EVOLUTION TO DETECT A METABOLITE**

Joseph Carrara and Jonathan Ouellet
Department of Chemistry and Physics, Monmouth University, 400 Cedar Avenue,
West Long Branch, NJ 07764

234. **EXTRACELLULAR VESICLES AND MICRORNAS IN HIV RESISTANCE**

Grace Hancock, Melissa McAlexander, Aleksandra Olekhnovich, and Kenneth Witwer
Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine,
733 N Broadway, Baltimore, MD 21205

235. **EXPRESSION AND PURIFICATION OF *DICTYOSTELIUM DISCOIDEUM* DDB_G0278957 PROTEIN, A PUTATIVE MRNA DECAPPING ENZYME**

Anna Kokubu, Caitlin Pozmanter, and Susan Parrish
Department of Biology, McDaniel College, 2 College Hill, Westminster, MD 21157

236. **LONG TERM AND ACUTE EFFECT OF ATRAZINE ON SEXUAL DEVELOPMENT OF ZEBRAFISH**

Isabelle Malouf and Dr. Baker
Department of Biology University of Mary Washington, 1701 College Avenue, Fredericksburg, VA 22401

237. **CHARACTERIZATION OF THE MONOMERIC HIV-1 5'-UTR**

Verna Van[1], Nick Bolden[1], Canessa Swanson[2], Sarah Monti[1], and Michael Summers[1]
[1]Department of Chemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biology, Jackson State University, 1400 JR Lynch Street, Jackson, MS 39217

238. **INVESTIGATING THE MECHANISM OF CALCIUM METAL ION CATALYZED RNA PHOSPHODIESTER CLEAVAGE**

Saif Yasin, Kyle Messina, and Dr. Adam Cassano
Department of Biochemistry and Molecular Biology, Drew University,
36 Madison Avenue, Madison, NJ 07005

USING GEL CENTRIFUGATION TO DETERMINE THE MONOMER-DIMER EQUILIBRIUM OF THE HIV-1 5'-LEADER

Nick Bolden[1], Vern Van[1], Canessa Swanson[2], Jessica Smith[3],
Sarah Monti[1], and Michael Summers[1]
[1]Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biology, Jackson State University, 1400 JR Lynch Street, Jackson, MS 39217
[3]The Seed School of Maryland, 200 Font Hill Avenue, Baltimore, MD 21223

An estimated 35 million people worldwide live with Human Immunodeficiency Virus (HIV-1), with approximately 1.7 million associated deaths per year. HIV-1 is a retrovirus that infects the T-cells of the host's immune system. After infection, the virus reverse-transcribes its RNA genome into DNA and integrates this viral DNA into the host DNA, which is eventually transcribed into RNA by the host's cellular machinery. The 5' untranslated region (UTR) of the RNA, is the most conserved region of the genome. It exists in equilibrium between a monomeric form, important for translation, and a dimeric form, which is selectively packaged into new viral particles. We made several point mutations to observe their effect on the monomer-dimer equilibrium. While normally gel electrophoresis is used to determine the equilibrium of monomer and dimer, we have developed an alternative technique that uses gel centrifugation. We are developing this technique as it may allow us to look at the monomer-dimer equilibria of different RNAs, such as HIV-2 and SIV 5' UTRs, which have weaker "kissing" dimer interactions. When gel electrophoresis is used on these weaker dimers, the magnesium that stabilizes the dimer is drawn towards the opposite electrical pole as the RNA, providing inaccurate data by dissociating the dimers and causing a larger percentage of monomer to be visualized. Magnesium containing gels are used to address this, but it is difficult to determine how much magnesium is needed to obtain accurate results. The centrifugation method is also significantly faster than gel electrophoresis, and since no buffer is required, there is less chance of interconversion, which could cause an inaccurate measure of equilibrium. Future work includes testing this method on retroviruses with a "kissing dimer" interactions and eventually determining a method to quantify the amount of monomer and dimer to provide an accurate account of the equilibrium.

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SURVIVAL OF THE FITTEST RNA APTAMER: USING ARTIFICIAL EVOLUTION TO DETECT A METABOLITE

Joseph Carrara and Jonathan Ouellet
Department of Chemistry and Physics, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ 07764

The hammerhead RNA ribozyme is a self-cleaving RNA molecule that breaks at a cleavage site between stem I and stem III. For this project we have created the chimeric molecule where the stem II of the hammerhead has been replaced with 35 random nucleotides. Cleavage occurs under conditions that allow the N35 region to stabilize stem II, thus stabilizing the entire molecule. The rational design of the selection process is done in a way that this region will stabilize exclusively when bound to a ligand. The region of 35 random nucleotides allows for a theoretical possibility of $1x10^{21}$ different RNA ribozymes. This pool of molecules will be subjected to in-vitro evolution, selecting for only the aptamers that create high affinity bonds to the oncometabolite 2-Hydroxyglutarate.

In the common citric acid cycle Isocitrate Dehydrogenase (IDH) produces α-Ketoglutarate . A gene mutation for the enzyme IDH results in a single amino acid substitution, creating mutated enzyme IDH1 which now reduces α-Ketoglutarate to 2-HG. The 2-HG produced is not useful to an organism and is inhibitory to the completion of the citric acid cycle. High levels of 2-HG in cells occur in most gliomas and acute myelogenous leukemia (AML) suggesting a strong correlation between elevated 2-HG levels and cancer.

Self-cleavage of the allosteric ribozyme that occurs exclusively in cells that contain 2-HG creates the ability for the selected aptamer to behave as a biosensor when modified to provide an indication of when binding occurs. This project would provide the ability to target individual cancer cells from healthy cells in-vivo. Furthermore, with the ability to target individual cells the aptamer may be modified to create a deadly protein such as Snake Venom L-Amino Oxidase, thus killing the cancer cell selectively.

We would like to thank The Bristol Meyers Squibb Company for providing a generous stipend for work over the summer and the Monmouth University School of Science and Department of Chemistry and Physics for the necessary funding for the research project.

EXTRACELLULAR VESICLES AND MICRORNAS IN HIV RESISTANCE

Grace Hancock, Melissa McAlexander, Aleksandra Olekhnovich, and Kenneth Witwer
Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine,
733 N Broadway, Baltimore, MD 21205

Background and Research Question. The cervicovaginal compartment is an important site of HIV infection. It contains secretions rich in proteins and other factors that may offer insight into mechanisms of cell communication relating to disease. Extracellular vesicles (EV) that are shed from most cell types contain small non-coding RNA sequences that may regulate gene expression in recipient cells. In a pilot study of cervicovaginal lavage (CVL) samples from participants in a sex worker cohort, EV counts and a number of detected miRNAs were found to be higher in individuals who remained HIV-negative after extended exposure to HIV than in HIV-positive individuals. In this study, we sought to confirm and extend these results in a larger group of samples.

Methods and Experiments. 45 CVL samples were obtained from a cohort of female sex workers in Kenya from three groups: HIV-infected, HIV-uninfected, and HIV-resistant or highly exposed seronegative (HESN). EV were isolated and quantified by ultracentrifugation followed with nanoparticle tracking analysis using NanoSight technology. RNA was isolated and quantified by real-time quantitative PCR assays. Custom microarray assays were designed for miRNA profiling.

Results and conclusions. In comparing the three groups, EV counts were consistent with the pilot study in that the HIV-negative individuals showed higher concentration of EV. Interestingly, HESN had particle counts similar to those of HIV-infected individuals. Additionally, and consistent with the pilot study, miR-223 expression was greater in the HIV-negative individuals. However, the similar levels of this miRNA in HESN and HIV-1 infected individuals suggest this miRNA may not play a major role in protection. Broader miRNA expression evaluation of these groups is needed, since miRNAs responsible for regulation of HIV in resistant individuals may be useful as biomarkers and in potential development of novel HIV prophylaxis.

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EXPRESSION AND PURIFICATION OF *DICTYOSTELIUM DISCOIDEUM* DDB_G0278957 PROTEIN, A PUTATIVE MRNA DECAPPING ENZYME

Anna Kokubu, Caitlin Pozmanter, and Susan Parrish
Department of Biology, McDaniel College, 2 College Hill, Westminster, MD 21157

Dictyostelium discoideum is a social amoeba that aggregates to become a multicellular organism when starved for nutrients, making it a model organism for cell and developmental biology. *D. discoideum* DDB_G0278957 shares sequence similarity with Mimivirus L534, an enzyme previously shown to decap mRNA, a process that may enhance mRNA turnover during infection. To examine if *D. discoideum* DDB_G0278957 (L534) decaps mRNA to modulate gene expression during development, the *D. discoideum* L534 cDNA was cloned and expressed in *E. coli* and subsequently purified by affinity chromatography. To accomplish this goal, first *D. discoideum* mRNA was isolated and reverse transcribed and the resulting L534 cDNA was amplified by PCR. Next, the L534 cDNA was cloned into the pmal-c2x protein expression plasmid and transformed into *E.coli* to produce a maltose binding protein (MBP) L534 (MBP-L534) fusion protein. The resulting MBP-L534 protein was purified by affinity chromatography using amylose resin and analyzed by SDS-PAGE and Western Blotting. Full-length MBP-L534 protein was detected using both methods; however a smaller band corresponding to MBP was also visualized, indicating that the fusion protein was somewhat unstable. To attempt to increase the amount of full-length fusion protein, bacterial growth and induction times will be optimized. In addition, MBP-L534 will be further purified using a second affinity chromatography resin. When more full-length MBP-L534 is obtained, the MBP-L534 protein will be evaluated for mRNA decapping activity *in vitro*.

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LONG TERM AND ACUTE EFFECT OF ATRAZINE ON SEXUAL DEVELOPMENT OF ZEBRAFISH

Isabelle Malouf and Dr. Dianne Baker
Department of Biology University of Mary Washington,
1701 College Avenue, Fredericksburg, VA 22401

Increasing evidence indicates that atrazine, a commonly used agricultural herbicide, acts as an endocrine disrupter. In several vertebrate studies, acute short-term exposure to atrazine has caused feminization. However, it remains unclear how atrazine mediates these effects. This study focuses on the effects of atrazine on gene expression of enzymes that are key to the synthesis of sex steroids: cytochrome p450 aromatase (cyp19), 17β- hydroxysteroid dehydrogenase (17β-HSD), vitellogenin (vtg), and steroidogenic acute regulatory protein (StAR). Cyp19 plays a key role in feminization as it converts testosterone, a masculinizing hormone, to estradiol, a feminizing hormone. 17β- HSD also produces estradiol, by converting estrone, into estradiol. Vtg, an egg-yolk protein precursor, is commonly used in toxicology studies as an indicator of feminization. StAR transcript levels indicate the amount of cholesterol being shuttled into the steroidogenic pathway. To test for effects of atrazine on expression of these genes, triplicate tanks of juvenile zebrafish (*Danio rerio*) were exposed from days 20-35 post-fertilization to either 400µg/L of atrazine, 0.05µg/L estradiol, or control water. After the 15-day treatment, six fish were collected from each replicate tank and anesthetized, and viscera were collected. Total RNA was extracted from each sample and converted into cDNA by reverse transcription and quantitative PCR (qPCR) assays were developed to measure transcript levels of each gene. Differences in transcript levels among treatments are now being assessed. Following the analysis of juveniles, remaining zebrafish from each treatment will be dissected at maturity, gonads and liver will be removed, and RNA will be extracted. Transcripts of vtg will be measured in the liver and transcripts of cyp19, 17β-HSD, and StAR will be measured in the gonads. The results of this study will contribute to the discussion of the safety and continued use of atrazine.

CHARACTERIZATION OF THE MONOMERIC HIV-1 5'-UTR

Verna Van[1], Nick Bolden[1], Canessa Swanson[2], Sarah Monti[1], and Michael Summers[1]
[1]Department of Chemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Department of Biology, Jackson State University, 1400 JR Lynch Street, Jackson, MS 39217

Acquired Immunodeficiency Syndrome (AIDS) is caused by Human Immunodeficiency Virus (HIV), a retrovirus whichcontains two copies of its RNA genome. Infection of a host organism by HIV results in a compromised immune system vulnerable to opportunistic and secondary infections. Currently, a number of drug therapies exist to treat HIV; however, the virus's high mutation rate combined with noncompliance can lead to drug resistance and the need for new therapeutics. A potential drug target for HIV-1 is the highly conserved 5'-untranslated region (5'-UTR) of its RNA genome. The 5'-UTR exists in an equilibrium between a monomeric conformation, which promotes translation of the viral genome, and a dimeric conformation, which is selectively packaged into new viral particles. Learning about the monomeric and dimeric tertiary structure of the 5'-UTR will allow rational drug design and increase our overall knowledge of HIV.

This study utilizes nuclear magnetic resonance (NMR) to characterize the structure of the monomeric 5'-UTR. NMR studies have allowed identification of an unexpected long-range interaction between residues of the U5 region and the dimerization initiation site (DIS) that stabilizes the monomeric conformation. Further NMR studies suggest the existence of predicted structural elements including the TAR, DIS, Ψ, and splice donor hairpins.

The presence of the splice donor hairpin in the monomeric 5'-UTR is significant because the hairpin has been functionally implicated in splicing the genome, which occurs in the monomeric conformation. Unexpectedly, the splice donor hairpin does not form in the dimeric 5'-UTR. Splice donor is an intriguing example of the extensive shift in secondary structure from the monomeric conformation to the dimeric conformation of the 5'-UTR. Future studies will allow complete characterization of the secondary and tertiary structure of the monomeric 5'-UTR. Additional studies of the dimeric 5'-UTR structure will allow the extent of the conformational rearrangement to be identified.

This study is funded in part by NIGMS-NIH Grant 5R01GM042561 and the Howard Hughes Medical Institute at the University of Maryland, Baltimore County.

INVESTIGATING THE MECHANISM OF CALCIUM METAL ION CATALYZED RNA PHOSPHODIESTER CLEAVAGE

Saif Yasin, Kyle Messina, and Dr. Adam Cassano
Department of Biochemistry and Molecular Biology, Drew University,
36 Madison Avenue, Madison, NJ 07005

Metal ion catalysis is key to many natural enzymes responsible for catalyzing phosphodiester cleavage. Understanding this mechanism can inform the development of artificial nucleases for therapeutic applications. Studies have been conducted to elucidate the mechanism of catalysis when assisted by Zn^{+2} and other transitional metal ions; however, the mechanism for calcium metal ion catalysis is still unknown. Based on prior research, we hypothesized that Ca^{+2} interacts directly with RNA's non-bridging oxygens, though inner sphere coordination. This hypothesis was tested against the possibility that the metal ion is interacting indirectly through outer sphere electrostatic stabilization. Our data suggests inner sphere coordination because Ca^{+2} (39-Fold) is a more effective catalyst than $Co(NH_3)^{+3}$ (2.5-Fold), which cannot participate in inner sphere coordination. This hypothesis was further supported because a rate decrease was shown when a non-bridging oxygen was substituted with a sulfur. Ca^{+2} and Mg^{+2} had thio-effects of about 2.6 and 3.2. In comparison to $Co(NH_3)_6^{+3}$ and Na^{+} with thio-effects of 1.2 and 0.6 respectively, the sulfur is shown to only interrupt the Ca^{+2} and Mg^{+2} interactions. This suggests direct contact with the negatively charged oxygens in the backbone. In addition to these experimental studies we modeled the system computationally to gain thermodynamic insight. Calculations with Density Functional Theory showed an output with a calcium ion interacting with one oxygen and allowing hydrogen bonding to occur from its primary hydration sphere. The input had the calcium ion interacting with both oxygen atoms. These methods give us greater support for the inner sphere coordination hypothesis.

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Afternoon Poster Session
Group JJ – Biochemistry & Molecular Biology

Poster # | **Title, Author(s) & Affiliation(s)**

239. **SEARCH FOR *TERT* PROMOTER MUTATIONS IN PANCREATIC CANCER CELL LINES**

Justine Lottermoser[1], Qi Lan[2], Jason Hoskins[2], Irene Collins[2], Marta Dzyadyk[2], Mickey Emmanuel[2], Jinping Jia[2], and Laufey Amundadottir[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Laboratory of Translational Genomics, DCEG/NCI, National Institutes of Health, 8717 Grovemont Circle, Gaithersburg, MD 20877

240. **TARGETING PANCREATIC CANCER WITH CELLULAR IMMUNITY**

Caitlin Mason[1], Matthew Lauver[2], Glenn Jones[2], John Harms[2], and Lawrence Mylin[2]
[1] Department of Chemistry and Biochemistry, Messiah College, One College Avenue Suite 3030, Mechanicsburg, PA 17055
[2] Department of Biological Sciences, Messiah College, One College Avenue Suite 3030, Mechanicsburg, PA 17055

241. **TURNING OFF THE GPS: THE ROLE OF FURIN IN THE INTOXICATION PATHWAY OF RECOMBINANT IMMUNOTOXINS BASED ON *PSEUDOMONAS* EXOTOXIN A**

Bryan Miller[1], Jamy Therres[1], and John Weldon Ph.D.[1,2]
[1]Molecular Biology, Biochemistry and Bioinformatics Program, Towson University, Towson, MD 21252
[2]Department of Biological Sciences, Towson University, Towson, MD 21252

242. **STRUCTURAL ELUCIDATION OF THE IG59 DOMAIN OF OBSCURIN**

Rachel Policke, Matt Oehler, Tracy Caldwell, Dr. Chris Berndsen, and Dr. Nathan Wright
Department of Chemisty and Biochemistry, James Madison University, 800 South Main Street, Harrisonburg, VA 22801

243. **BIOCHEMICAL CHARACTERIZATION OF NOD1, AN INNATE IMMUNE RECEPTOR**

Thomas Rivas, Mackenzie Lauro, Dr. Catherine Grimes, and Dr. Brian Bahnson
Department of Chemistry and Biochemistry, University of Delaware, 316 Drake Hall, Newark, DE 19716

244. **DEVELOPMENT OF A MONOCLONAL ANTIBODY AGAINST THE CHOLECYSTOKININ-2 RECEPTOR**

Evan Shirey and John F. Harms
Department of Biological Sciences, Messiah College, Mechanicsburg, PA 17055

245. **DIMETHYL FUMARATE INDUCES CYTOPROTECTION AND INHIBITS VASO-OCCLUSION IN TRANSGENIC SICKLE MICE**

Pauline Xu[1], Ping Zhang[2], John Belcher[2], and Gregory Vercellotti[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Division of Hematology, Oncology, and Transplantation, Department of Medicine, University of Minnesota Medical School, 420 Delaware Street SE, Minneapolis, MN 55455

SEARCH FOR *TERT* PROMOTER MUTATIONS IN PANCREATIC CANCER CELL LINES

Justine Lottermoser[1], Qi Lan[2], Jason Hoskins[2], Irene Collins[2], Marta Dzyadyk[2], Mickey Emmanuel[2], Jinping Jia[2], and Laufey Amundadottir[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Laboratory of Translational Genomics, DCEG/NCI, National Institutes of Health, 8717 Grovemont Circle, Gaithersburg, MD 20877

Although pancreatic cancer is the tenth most common cancer in the United States, it is the fourth most common cause of cancer mortality. Like any disease, pancreatic cancer is influenced by a combination of lifestyle and genetic factors, but the full extent of genetic causation is still undergoing much research.

Human *telomerase reverse transcriptase* (*TERT*) encodes the catalytic subunit of telomerase, which takes part in maintaining genomic integrity by elongating telomeres. *TERT* expression is usually inhibited in somatic cells except for cells undergoing self-renewal. However, immortality in some cancer cells has been partially attributed to *TERT* over-expression. Recent research has shown that certain mutations in the *TERT* promoter lead to increased telomerase activity. Such mutations have been found frequently in melanoma, bladder and liver cancer among various other cancer types. Because the promoter is essential for the regulation of *TERT,* we investigated whether pancreatic cancer cells also have mutations in the *TERT* promoter. We sequenced genomic DNA from various pancreatic tumor-derived cell lines. No mutations were detected in the *TERT* promoter in any of the pancreatic tumor-derived cell lines.

This project was funded by the NIH Summer Internship Program.

TARGETING PANCREATIC CANCER WITH CELLULAR IMMUNITY

Caitlin Mason[1], Matthew Lauver[2], Glenn Jones[2], John Harms[2] and Lawrence Mylin[2]
[1] Department of Chemistry and Biochemistry, Messiah College,
One College Avenue Suite 3030, Mechanicsburg, PA 17055
[2] Department of Biological Sciences, Messiah College, One College Avenue Suite 3030,
Mechanicsburg, PA 17055

The goal of this study was to investigate the potential for using T cell responses targeting intron-encoded amino acid sequences of the CCKC pancreatic-cancer-associated form of the cholecystokinin receptor (CCKR) to control tumor growth in mice. A splice variant of the CCKB receptor, known as CCKC (cancer-associated CCK receptor) contains a unique insertion of 69 amino acids encoded by an intron that is usually removed from the normal CCKB receptor mRNA. Preliminary experiments have demonstrated by an IFN-γ ELISPOT that T cell responses could be generated in mice by intradermal immunization with a synthetic 20mer peptide representing a portion of the 69 amino acid intron-encoded sequence. Subsequent experiments utilized Intracellular Cytokine Staining for IFN-γ-expressing T cells to determine whether the responsive cells were of the CD8+ or CD4+ subset(s). While overall responses to the repeat intradermal immunizations were weak, several mice did show evidence of intron peptide-specific CD8+. TriVax is a more recent intravenous peptide immunization method in which synthetic peptide is injected in combination with poly IC and an anti-CD40 antibody and is reputed to generate robust T cell responses. The TriVax method was used to compare responses to a CD4+ SV40 T ag 525-543 peptide and the CCK intron peptide by ELISPOT and ICS. While the T ag peptide induced robust numbers of IFN-g-secreting cells that were of the CD4+ T subset, the CCK intron peptide failed to induce IFN-γ-secreting cells that could be detected by either method. Immunization with irradiated murine pancreatic cancer cell lines engineered to express the CCKB receptor splice variant failed to induce intron-specific T cell responses that could be detected by ELISPOT or ICS. The interpretation of the results of these experiments may require closer analysis of the quality of the intron-peptide stock following extended storage at -20°C.

This research was supported by the Steinbrecher Undergraduate Summer Research Program at Messiah College.

TURNING OFF THE GPS: THE ROLE OF FURIN IN THE INTOXICATION PATHWAY OF RECOMBINANT IMMUNOTOXINS BASED ON *PSEUDOMONAS* EXOTOXIN A

Bryan Miller[1], Jamy Therres[1], and John Weldon Ph.D.[1,2]
[1]Molecular Biology, Biochemistry and Bioinformatics Program,
Towson University, Towson, MD 21252
[2]Department of Biological Sciences, Towson University, Towson, MD 21252

Pseudomonas exotoxin A (PE) is a bacterial toxin that inactivates protein synthesis in eukaryotic cells. Derivatives of PE, called recombinant immunotoxins (RITs), are currently being evaluated for therapeutic use in the treatment of cancer. PE enters the cell through receptor mediated endocytosis, then traffics through the Golgi to the endoplasmic reticulum (ER) where it crosses into the cytosol. In the cytosol, PE catalytically inactivates elongation factor 2 (EF2), through ADP-ribosylation. The inactivation of EF2 halts protein synthesis and kills the cell. Furin is a transmembrane protease that catalytically activates protein precursors in the secretory pathway. Furin is involved in the intoxication pathway of PE through cleavage of the polypeptide backbone, which activates the toxin by separating the cell-binding domain from the catalytic domain. A signal sequence in the cytoplasmic domain of furin directs it through a regulated trafficking itinerary between the cell surface and the Golgi. We propose that furin not only activates PE through cleavage, but also acts as a chaperone to transport PE from the endolysosomal system to the Golgi. We are evaluating the hypothesis that furin acts as a chaperone by utilizing a furin mutant that is unable to properly traffic from the cell surface to the Golgi, but is otherwise unaffected. Preliminary results show that HEK293 cells expressing mutant furin are 5-10X less sensitive to the PE-derived RIT HB21-LR than cells expressing wild type furin. This suggests that furin may have a trafficking role in intoxication in addition to a catalytic role.

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STRUCTURAL ELUCIDATION OF THE IG59 DOMAIN OF OBSCURIN

Rachel Policke, Matt Oehler, Tracy Caldwell, Dr. Chris Berndsen, and Dr. Nathan Wright
Department of Chemisty and Biochemistry, James Madison University,
800 South Main Street, Harrisonburg, VA 22801

Obscurin (700-800kDa) is the giant muscle protein and is the only known link between the contractile apparatus and the surrounding membrane structure within myocites. Specifically, the Ig58 and Ig59 domains of obscurin bind to the Zig9/10 domains of titin; ablation of this interaction has been linked to hypertrophic cardiomyopathy. In order to better understand the molecular underpinning of this disease, we solved the high-resolution structure (1.16 Å)of the Ig59 domain of obscurin. This domain folded into a classic Ig-like domain, consisting of two beta sheets and a well-defined hydrophobic core.

BIOCHEMICAL CHARACTERIZATION OF NOD1, AN INNATE IMMUNE RECEPTOR

Thomas Rivas, Mackenzie Lauro, Dr. Catherine Grimes, and Dr. Brian Bahnson
Department of Chemistry and Biochemistry, University of Delaware,
316 Drake Hall, Newark, DE 19716

Innate immunity is an intricate system employed by humans to regulate the trillions of bacteria that live in our body. One protein integral to the innate immune response is called nucleotide-binding oligomerization domain-containing protein 1 (NOD1). NOD1 is a cytosolic, membrane-associated protein that is linked to irritable bowel syndrome, asthma, diabetes, and obesity. These diseases are thought to arise from improper mRNA splicing of NOD1 transcripts leading to a poorly functioning NOD1 protein receptor that can no longer regulate an overstimulated inflammatory response. Activation occurs when NOD1 binds to a bacterial cell wall component called iE-DAP, which then signals the the NF-κB cascade to produce pro-inflammatory cytokines and chemokines.

Current research is trying to describe the binding of iE-DAP to NOD1, however, these studies almost exclusively use expensive and low-yielding mammalian cells to obtain their active protein. This project aims to develop a bacterial expression system to express wildtype NOD1 and the LRR domain, which is the region thought to be involved in ligand binding. Currently I have produced both constructs in high yield from an *E. coli* system to allow their biochemical characterization through peptidoglycan pull-down experiments and circular dichroism. Future work aims to obtain crystal structures of NOD1 bound to its ligand and perform NMR studies to observe ligand binding events.

Funding for this research is provided by the Delaware INBRE program.

DEVELOPMENT OF A MONOCLONAL ANTIBODY AGAINST THE CHOLECYSTOKININ-2 RECEPTOR

Evan Shirey and John F. Harms
Department of Biological Sciences, Messiah College, Mechanicsburg PA 17055

Online access of this abstract is restricted at the request of the Principal Investigator.

DIMETHYL FUMARATE INDUCES CYTOPROTECTION AND INHIBITS VASO-OCCLUSION IN TRANSGENIC SICKLE MICE

Pauline Xu[1], Ping Zhang[2], John Belcher[2], and Gregory Vercellotti[2]
[1]Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250
[2]Division of Hematology, Oncology, and Transplantation,
Department of Medicine, University of Minnesota Medical School,
420 Delaware Street SE, Minneapolis, MN 55455

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