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Volume 67 April 2013

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OFFICERS AND MEMBERS OF THE EXECUTIVE COMMITTEE

EDITOR

105th Annual Meeting

April 26, 2013

Grand Forks, North Dakota

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EDITOR'S NOTES

HISTORY

The *Proceedings of the North Dakota Academy of Science* (NDAS) was first published in 1948, with Volume I reporting the business and scientific papers presented for the 40th annual meeting, May 2-3, 1947. Through Volume XXI**,** the single yearly issue of the *Proceedings* included both abstracts and full papers. Commencing with Volume XXII, the *Proceedings* was published in two parts: A, published prior to the annual meeting, contained an abstract of each paper to be presented at the meeting, and B, published later, contained full papers by some of the presenters.

In 1979 (Vol. 33) the *Proceedings* changed to an 8½ x 11-inch format. Produced from camera-ready copy submitted by authors, it was distributed at the annual meeting. As desktop computing became more prevalent vol. 51-vol. 64 of the *Proceedings* were assembled with desktop publishing software from submitted computer disks. The current volume was assembled from electronic submission of abstracts via email and the *Proceedings* archived online as pdfs.

VOLUME 67 ORGANIZATION

In 2003 the NDAS council voted to accept all abstracts scheduled for presentation at the Annual Meeting. Thus, communications in volumes 58 to present haven't undergone a "typical" peer review. Rather, they provide an accurate reflection of the material presented before the NDAS membership at the Annual Meeting. The presentations in this year's *Proceedings* are presented in three major sections. The first contains the undergraduate communications presented as part of the A. Rodger Denison Student Research Competition. The second section comprises the graduate Denison Competition papers, and the final section comprises professional communications presented by faculty members of the Academy. Readers may locate communications by looking within the major sections of these *Proceedings (see table of contents)* or by referring to the author index on page 62.

IN APPRECIATION

The Academy wishes to acknowledge current and emeritus members of the Academy who continue to support the mission of the North Dakota Academy of Science Research Foundation through their special gifts. A listing of these supporters accompanies the Financial Report. The Academy also wishes to express its thanks to the presenters of papers at the Annual meeting, the session chairs, as well as all who have helped in organizing spaces and places, soliciting manuscripts, and compiling of this year's communications.

Keith Henry, President Paul Lepp, Secretary-Treasurer

SCHEDULE

8:00 AM to 8:50 AM Breakfast and Registration – Alerus Center Ballroom
8:50 AM to 9:00 AM Welcome - Alerus Center Ballroom

Time		Eagle	Meadowlark	Hummingbird
9:00 AM	9:20 AM	Zerr	Fergel	Wax
9:20 AM	9:40 AM	Rosin	Madurapperum	Wu
9:40 AM	10:00 AM	Collette	Goldenstein	Irsfeld
10:00 AM	10:20 AM	Samanta	Ssemadaal	Strating
10:20 AM	10:40 AM	---------- BREAK ----------		
10:40 AM	11:00 AM	Kjelland	Wagner	Wilson
11:00 AM	11:20 AM	Chen	Sandquist	Acharya
11:20 AM	11:40 AM	Stone	J. Crowell	ChallaSivanKanaka
11:40 AM	12:00 PM	Vegi	Bilski	Tian
12:00 PM	1:00 PM	----------LUNCH----------		
1:00 PM	1:20 PM	Mok	Gonzalez	Dintzner
1:20 PM	1:40 PM	Armstrong	Pinsonneault	Felts
1:40 PM	2:00 PM	Gaultney	Isiko	Tendo
2:00 PM	2:20 PM	Kurada	Gbolo	Freda
2:20 PM	2:40 PM	Rastedt	A. Crowell	Welker
2:40 PM	3:00 PM	Shipunov	Bala	Beachy
3:00 PM	3:20 PM	---------- BREAK ----------		

3:30 PM to 4:30 PM Business Meeting – Eagle Room
(all NDAS members are encouraged to participate)

5:00 PM to 6:00 PM Happy Hour - Alerus Center Ballroom
(cash bar)

6:00 PM to 7:00 PM Dinner - Alerus Center Ballroom

7:00 PM to 8:00 PM Keynote Address by Dr. James Giordano
"Neuroscience: The Good, the Bad, the Ugly...and the Need for Neuroethics."

EAGLE ROOM SCHEDULE OF PRESENTATIONS

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9:00 AM FURTHER INVESTIGATION OF A POSSIBLE ROLE FOR ABSCISIC ACID IN CONTROLING THE GROWTH EFFECTS OF INDOLE-3-ACETIC ACID IN EXPANDING *ARABIDOPSIS* LEAVES? Jakob R. Zerr*, Samuel L. Wagner, Amanda M. Roise, Jo Heuschele, Jerry D. Cohen and Christopher P. Keller

9:20 AM DETERMINING THE IMPORTANCE OF MLL-AF9 SPLICE VARIANTS ON CELL PROLIFERATION IN THE MONO MAC 6 ACUTE LEUKEMIA CELL LINE. Haley McClure, Jessica Rosin*, Emily Wheeling, and Heidi Super

9:40 AM α_{1A} ADRENERGIC RECEPTOR INFLUENCES ON PROGENITOR CELL FATE IN THE ADULT HIPPOCAMPUS. Katie Collette*, Amber Nielsen, Dianne Perez, Van Doze

10:00 AM THE TEMPORAL AND SPATIAL EXPRESSION OF OMPR CORRELATES INVERSELY WITH THAT OF FLHD. Priyankar Samanta*, Shelley M. Horne, Birgit M. Prüβ

10:20 AM Break

10:40 AM NORTH DAKOTA GENETIC ANCESTRY: A PILOT STUDY (OF MITOCHONDRIAL DNA DIVERSITY). Katelyn Kjelland*, Igor V. Ovchinnikov

11:00 AM Au-MODIFIED SILICA NANOWIRES: SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS AS HYPERTHERMIA AGENTS FOR PHOTOTHERMAL THERAPY. Jiao Chen*, Xuefeng Li, Xu Wu, Nenny Fahruddin, Min Wu, Julia Xiaojun Zhao

11:20 AM *BORRELIA BURGDORFERI* ISOLATED FROM *PEROMYSCUS MANICULATUS* AND *MYODES GAPPERI* IN EASTERN NORTH DAKOTA. Brandee L. Stone*, Angela M. Floden, and Catherine A. Brissette

11:40 AM *IN VITRO* REPLICATION OF SWINE TORQUE TENO VIRUS 1 (TTV1). Anuradha Vegi*, Sheela Ramamoorthy

12:00 AM Lunch

AFTERNOON SESSION

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1:20 PM GROWTH HORMONE KNOCKOUT MICE DISPLAY DIFFERENCES IN HYPOMETHYLATION AND TRANCRIPTION OF DNA METHYLTRANSFERASES AND INTERSPERSED REPEATS. Vanessa Armstrong*, Sharlene Rakoczy, and Holly Brown-Borg

1:40 PM A LYME DISEASE SPIROCHETE PROTEIN BINDS HUMAN FIBRONECTIN THROUGH MULTIPLE INDEPENDENT DOMAINS. Robert A Gaultney*, Tammy Gonzalez, Angela M Floden, and Catherine A Brissette

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2:20 PM IDENTIFICATION OF DOPAMINE TRANSPORTER PALMITOYL ACYLTRANSFERASES. Danielle E. Rastedt*, James D. Foster, and Roxanne A. Vaughan

2:40 PM THE ANALYSIS OF THE FLORA OF NORTH DAKOTA. Liudmila Abramova, Joshua Beaudoin, Dallas Fry, Jared Schumaier, Jason Theodore, Alexey Shipunov*

MEADOWLARK ROOM SCHEDULE OF PRESENTATIONS

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10:20 AM	Break
10:40 AM	H^+ ION FLUX COMPARISON NEAR ROOTS OF *ARABIDOPSIS THALIANA* AND *ARABIDOPSIS HALERI*. Samuel L. Wagner*, and Christopher P. Keller
11:00 AM	N-CADHERIN UPREGULATION IN ARSENIC-TRANSFORMED UROTHELIAL CELLS. Elizabeth Sandquist*, Seema Somji, Don Sens and Scott Garrett.
11:20 AM	USING TEMPORAL AND PALEOGEOGRAPHIC MAPPING TO IDENTIFY UNDERREPRESENTED REGIONS AND TIME INTERVALS TO AID IN SELECTING NEW SEARCH LOCATIONS. James "Josh" Crowell*, Joseph H. Hartman, Anna M. Crowell
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AFTERNOON SESSION

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Hummingbird Room Schedule of Presentations

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9:40 AM	GENETIC MUTATIONS AFFECT STABILITY OF BACTERIAL BIOFILMS. Meredith Irsfeld*, Justin W. Daniels, Shelley M. Horne, Shane J. Stafslien , Birgit M. Prüß
10:00 AM	OPTIMIZATION OF FLUORESCENCE ENHANCEMENT OF SILICON NANOWIRES COATED WITH GOLD NANOPARTICLES. Shaina Strating*, Dr. Fei Tian, Dr. Julia Xiaojun Zhao
10:20 AM	Break
10:40 AM	DIFFERENTIAL CADMIUM INTEGRATION IN SEVERAL ORGANS DURING SUBLETHAL EXPOSURE IN THE AXOLOTL, *AMBYSTOMA MEXICANUM*. Markus Wilson, Amanda Kraft, Naomi R. Winburn, Ryan Winburn, and Christopher Beachy
11:00 AM	IRREVERSIBLE COCAINE ANALOGS ATTACH NEAR THE DOPAMINE TRANSPORTER ACTIVE SITE. Rejwi Acharya*, Pramod Akula-Bala, Babita Sharma, Mu-Fa Zou, Jianjing Cao, John R. Lever, Amy H. Newman, James D. Foster, Keith Henry & Roxanne A. Vaughan
11:20 AM	ROLE OF PROLINE DIRECTED PHOSHORYLATION SITE ON DOPAMINE TRANSPORTER. Sathyavathi ChallaSivaKanaka, James D. Foster, Roxanne A. Vaughan
11:40 AM	THE SILICON NANOWIRE @SILVER TETRACYANOQUINODIMETHANE HYBRID STRUCTURE: SYNTHESIS AND CHARACTERIZATIONS. Fei Tian*, Julia Xiao Jun Zhao
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UNDERGRADUATE COMMUNICATIONS

IN THE

A. ROGER DENISON COMPETITION

(communications are listed alphabetically by the last name of the presenting author)

Quantitative Structure Activity Relationship Studies Using Mutants of the Human Serotonin Transporter and Analogs of the Antidepressant Citalopram Support the Presence of a Second Low-Affinity Binding Site.

Tyler Dintzner*, Isaac Zoller, Patrick Lamb, Pramod Akula Bala and L. Keith Henry

Department of Pharmacology, Physiology and Therapeutics, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58203

Citalopram (CIT) is a widely prescribed anti-depressant that elicits its effects by interaction with the human transporter (hSERT). In this study, we utilized quantitative structure activity relationship studies (QSAR) by comparing potencies of a series of CIT analogs in hSERT wild-type and Y95F, I172M and S438T mutant backgrounds with [^{3}H] serotonin (5-HT) competitive uptake assays. The QSAR data reveal that alterations to the R groups of CIT almost always result in loss of potency in the wild-type and Y95F backgrounds whereas in I172M and S438T mutant backgrounds the changes to CIT often result in increased potency or no change at all. These data suggest that CIT's binding site in I172M and Y95F backgrounds may be distinct from that of native SERT and support the existence of a second low-affinity CIT binding pocket. Computational induced-fit docking (IFD) studies with comparative hSERT models support this hypothesis. Efforts are underway to verify the location of the second site by site-directed mutagenesis and the substituted cysteine accessibility method (SCAM).

Sponsor: This project was funded in part by an EPSCoR Faculty Startup Grant to LKH

PRELIMINARY STUDY OF THE PHYTOREMEDIATORY POTENTIAL OF BARLEY GROWN ON COAL FLY ASH (FA) BASED MEDIA

Audrey Fergel[1*], Donna Jacob[2], Candace Kraft[1], Jeff Berens[1], Emma Nelson[1], Brandon Meyer[1], Ashley Farnsworth[1], Cody Hoggarth and Jerzy J. Bilski[1]

[1]Department of Biology, Valley City State University, Valley City, ND 58072,
[2]Department of Biology, Wet Ecosystem Research Group, North Dakota State University, Fargo, ND 58102

The utilization of coal fly ash (FA) as a soil amendment is one of the most intensively studied FA reutilization option. Despite the presence of toxic metals, elevated salinity levels, and high pH, up to date research have shown that FA may be used to support plant growth as a component of plant growth media. We investigated barley response to two different types of FA, one obtained by burning semi-bituminous coal (NDSU FA, collected from NDSU power plant), and another obtained by burning lignite coal (VCSU FA, collected from VCSU power plant).

We examined plants germination, growth, and the uptake by plants of selected, potentially toxic elements, such as Al, As, Ba, Be, Cd, Cr, Hg, and Pb. The growth media were composed of a soil as a control, and two mentioned above ashes in the following combinations: 100% soil (the control), 100% NDSU fly ash, 50% NDSU fly ash/50% Peat Moss, 33% NDSU fly ash/33% Peat Moss/33% Soil, 100% VCSU fly ash, 50% VCSU fly ash/50% Peat Moss, 33%VCSU fly ash/33% Peat Moss/33% Soil.

Barley *(Hordeum vulgare)*, has been grown in Petri dishes for 14-21 days in listed above growth media, then harvested and dried. . Plant samples were wet-digested in a nitric-perchloric acid mixture prior to analysis of elements. Chemical analysis was performed using inductively coupled plasma (ICP) emission spectrophotometry. The data were analyzed statistically using ANOVA and Statistical Analysis System (SAS, 2010).

Results indicated that plant growth was greater on media composed of soil and FA when compared to the FA alone. Plants did not grow on the VCSU FA media. Concentration of the most elements in plants grown on the soil control was similar to levels in the growth media containing FA. Barley appeared to be very viable plant, able to tolerate both relatively high amounts of toxic metals and poor growth conditions, such as growth media containing FA. It also has a root system able to stabilize coal FA piles. We concluded that the mineral stress caused by the presence of FA in growth media was tolerated very well by barley. There were noticeable differences in seedlings growth, depending on the type and source of coal FA used.

Large scale implementation of plant cover over coal FA landfills will require conducting in-depth and large scale research. Plants should be grown till reaching maturity and results of such experiments would provide data for large-scale application of "green technology" to establish the growth of selected plant species on coal FA. Our results clearly demonstrated that plants are able to grow in such adverse conditions, as on coal FA media. In addition, our results have shown that the transfer of heavy metals present in FA to plants is limited. Thus, heavy metals transmission to a food chain is unlikely, and therefore, application of FA to plant growth media would not be dangerous from environmental health perspective.

This project was supported by grants from the National Center for Research Resources (5P20RR016471-12) and the National Institute of General Medical Sciences (8 P20 GM103442-12) from the National Institutes of Health.

CHARACTERIZATION OF *ESCHERICHIA COLI* AS A PLASMINOGEN-BINDING PROTEIN

Tammy Gonzalez*, Robert A. Gaultney, Angela M. Floden, Catherine A. Brissette

Department of Microbiology and Immunology, University of North Dakota, Grand Forks, ND

Purpose:

The well-characterized lipoprotein Lpp from *Escherichia coli* is present in two different orientations within the bacterial outer membrane: either exposed to the periplasm or the bacterial surface[1]. The functions of this surface-exposed Lpp have not been determined; however, the C-terminus is lysine rich, and other bacterial proteins with C-terminal lysines have been shown to bind plasminogen[2]. Plasminogen is a pro-enzyme found in all mammals, that when activated into plasmin, is a protease that can degrade fibrin clots as well as the extracellular matrix[3]. Many pathogenic bacteria have been shown to bind host plasmin to disseminate and cause disease, and we hypothesize that *Escherichia coli* surface-exposed Lpp can bind plasminogen.

Methods:

We expressed stable, recombinant Lpp and produced Lpp-specific antibodies in Balb/C mice. The ability of Lpp to bind to plasminogen was tested by ELISA, and urokinase-plasminogen activation assays were done to confirm the conversion of Lpp-bound plasminogen to plasmin. The role of ionic interactions and lysine residues in the Lpp-Plasminogen interaction was tested by ELISA in the presence of excess NaCl, heparin, or aminocaproic acid (lysine analog). Lpp truncations at 10-30 residues upstream of the C-terminus were also created to isolate the residues that are responsible for the binding of surface-exposed Lpp to plasminogen.

Results:

We found that *E. coli* Lpp is readily expressed and is antigenic, and binds effectively to plasminogen as determined by ELISA. Plasminogen-bound Lpp can be converted to active plasmin by the enzyme uPA. The binding of Lpp and plasminogen was not hindered by the lysine analog, but the complex formation was disrupted with NaCl and heparin, thereby suggesting that the interaction is ionic.

Conclusions:

Our experiments with Lpp from *E. coli* show that this protein may be important in the recognition of host molecules by the bacterium. Because *E. coli* is a paradigmatic organism, these results may be useful for other pathogenic bacteria.

Funding: ND EPSCoR

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[1] Cowles C, Yongfeng L, Semmelhack M, Cristea I, Silhavy T. (2011) Molecular Microbiology, 1168-1181.

[2] Brissette C, Haupt K, Barthel D, Cooley A, Bowman A, Skerka C, Wallich R, Zipfel P, Kraiczy P, Stevenson B. (2009) Infection and Immunity, 300-306.

[3] Lahteenmaki K, Kuusela P, Korhonen T. (2001) FEMS Microbiology Reviews, 531-552.

North Dakota Genetic Ancestry: a pilot study (of mitochondrial DNA diversity)

Katelyn Kjelland*, Igor V. Ovchinnikov

Laboratory of Human and Forensic Genetics, Dept. of Biology and Forensic Science Program, University of North Dakota, Grand Forks, ND 58202

Globally, many ethnic groups have been subject to studies of genetic stratification; however, few populations in the United States have undergone examination of itinerant colonization and current ethnic miscellany. In addition, the majority of information gathered from these studies only goes as far as to indicate frequency of particular alleles in continental supergroups when in actuality such groups consist of numerous smaller, diverse populations.

Genetic markers provide information about ethnic background of individuals and, therefore, reveal their immigration history and present-day admixture in the region. To survey the genetic variation among the ND residents and consequences of immigration on genetic diversity in source European populations, the North Dakota Genetic Ancestry Project has been approved by UND's IRB and launched in the Ovchinnikov Lab.

For this pilot study, saliva specimens were collected from 54 unrelated residents of European descent in eastern North Dakota. DNA sequencing of hypervariable regions I and II of mtDNA identified 54 different haplotypes defined by 88 transitions and 4 transversions in 92 variable sites. The population sample demonstrated nucleotide diversity of 0.0128 ± 0.0066 and mean pairwise differences of 9.6 ± 4.47. All main European haplogroups were observed including HV, I, J, K, N, T, W, X, and the most commonly observed H and U.

Demographic parameter tests performed on the sample group suggest population expansion. Further research could afford a better understanding of the genetic landscape and offer more informative platforms for a variety of regional studies such as forensic identification or patterns of immigrant distribution.

"INACTUATION" OF RNA VIRUSES VIA GAMMA-IRRADIATION FOR VACCINE DEVELOPMENT

Kathy Mok [1*], Anuradha Vegi[1] and Sheela Ramamoorthy[1]

[1]Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND 58108

Contemporary vaccines against Porcine Reproductive and Respiratory Disease Syndrome viruses (PRRSV) and Swine Influenza viruses (SIV) are composed of either live attenuated viruses that risk frequent recombination between viral and vaccine strains and reversion to virulence or inactivated viruses that confer short term immunity. In a bid to produce safe and immunologically active vaccines, the development of an "inactuated" vaccine by gamma irradiation whereby viral replication capability is lost yet, the structural integrity and transcriptional activity of the irradiated pathogen is retained to result in intracellular antigen presentation is proposed. Methods used include maintenance of a continuous cell culture of Marc145 swine cells and MDCK cells for viral infections as well as γ-irradiation of the viruses. A kill curve is constructed to determine the optimum irradiation dosage for each virus by immunofluorescent assays. Polymerase chain reaction analysis (PCR) will be used to determine the absence of recombination or reassortment to test our hypothesis that γ-irradiation is able to produce viruses that can induce live-virus-like immunological responses, remain transcriptionally active but unable to undergo replication or recombination.

Determining the Importance of *MLL-AF9* Splice Variants on Cell Proliferation in the Mono Mac 6 Acute Leukemia Cell Line

Haley McClure, Jessica Rosin*, Emily Wheeling, Heidi Super, Ph. D.

Department of Biology, Minot State University, Minot, ND 58707

Several subtypes of human acute leukemia are associated with reciprocal translocations of the Myeloid Lymphoid Leukemia (*MLL*) gene which fuses to more than 50 different loci. We focused on the *MLL-AF9* fusion that results from a translocation between chromosome 9 and chromosome 11. This is one of the most common *MLL* gene fusions observed in human acute leukemia. This fusion is maintained in multiple cell lines including the Mono Mac 6 (MM6) cell line. The Mono Mac 6 cell line persistently produces two splice variants of the *MLL-AF9* fusion mRNA. One of the fusion transcripts contains *MLL* exon 8 while the other does not. It is unknown whether one or both of the splice variants contributes to the leukemia phenotype in MM6 cells. The focus of this study was to determine whether knockdown of one or both of the splice variants affected cell growth and viability. Short-interfering RNAs (siRNAs) were designed to target the translocation junctions of both *MLL-AF9* mRNA splice variants. Electroporation was used to introduce siRNAs into the MM6 cell line. qPCR was used to determine the level of knockdown of the fusion mRNAs and to confirm specificity for the 2 different fusion transcripts. Growth and viability of the cells were tracked over a period of five to seven days to determine if the siRNAs had an effect on the proliferation of the cells. An siRNA targeting the smaller splice variant consistently inhibited growth of MM6 cells. A significantly greater effect was seen when siRNAs targeting both splice variants were used in combination. Our study has shown that *both MLL-AF9* mRNA splice variants likely contribute to the leukemia phenotype. Moreover, the response of MM6 cells to siRNA treatment, suggests RNA interference may be a viable treatment approach for *MLL*-related leukemia. This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM12345.

H^+ ION FLUX COMPARISON NEAR ROOTS OF *ARABIDOPSIS THALIANA* AND *ARABIDOPSIS HALERI*

Samuel L. Wagner*, and Christopher P. Keller Ph.D.
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Cadmium (Cd^{++}) is released into the environment by some industrial and agricultural practices. In humans Cd^{++} exposure can cause lung, kidney and bone damage. An effective way to remove Cd^{++} from the environment is phytoremediation (i.e. soil decontamination by heavy metal-hyperaccumulating plants). As heavy metal hyperaccululating plants tend to be small and slow growing, however, truly effective phytoremediation will require a thorough understanding of the molecular mechanisms employed by hyperaccumulators and, ultimately, incorporation of the underlying genetics into more useful (larger, fast-growing) plant species. In the genus *Arabidopsis*, the model plant species, *Arabidopsis thaliana*, is intolerant of heavy metals while *Arabidopsis hallari* hyperaccumulates both cadmium and zinc. *A. halleri* responds to the presence of Cd^{++} (and Zn^{++}) in soil water by actively uptaking these heavy metals, moving them via xylem out of the roots, and sequestering them in the vacuoles of leaf mesophyll cells. This strategy of compartmentalizing heavy metals allows plants like *A. halleri* to thrive in high concentrations of heavy metals, whereas *A. thaliana* quickly perishes in high heavy metal environments. Though the basic strategy employed by *A. halleri* to survive high Cd^{++} environments is understood, whether the root cells of *A. halleri* differ from those of A. thaliana in Cd^{++} uptake transporter function is not known. In the study described here, we have chosen to attempt to determine if hyperacumulation by *A. halleri* roots is associated with altered heavy metal transporter activity relative to the uptake activity of *A. thaliana* roots. Relying on the well established understanding that Cd^{++} and Zn^{++} uptake by plants involves the same transporters, we are focused on characterizing root ion-flux responses to high Zn^{++} exposure to also understand how *A. halleri* and *A. thaliana* roots might differ in their responses to Cd^{++} exposure. In plant membrane transport, active transport of H^+ forms the driving force for other ion fluxes (including Zn^{++} and Cd^{++}), therefore, in this initial report of our on-going study, we describe a comparison of H^+ fluxes associated with roots of *A. halleri* and *A. thaliana* when treated with a high concentration of Zn^{++}.

Our investigation employs a self-referencing ion selective electrode (SRISE) system to estimate specific ion fluxes associated with individual roots. With SRISE, an ionophore-loaded measuring electrode slowly oscillates (0.1 Hz) between a near position immediately adjacent to the root and a second far position several microns away while the electropotential difference (voltage) between the measuring electrode and a distant reference electrode is monitored. Differences in voltage between the near and far locations indicate ion concentration gradients and can be used to calculate associated ion-fluxes into or out of the adjacent root region. Several investigators have used SRISE to measure H^+ fluxes in root systems. The study of calcium ion and pH oscillation in both pollen tubes and root hairs has led to a greater understanding of what regulates root tip growth.[4] Also the strong oscillatory behavior of the H^+ pump studied in corn roots is thought to be characteristic of a feedback system, as may be expected for homeostasis of actively growing cells[5]

For our experiments, *A. hallari* and *A. thaliana* were grown in a 3% agar medium consisting of Murashige and Skoog mixture for a week. Individual intact plants were placed into a petri dish containing 5.0 mM $ZnCl_2$. Ion flux was measured at the root tip, elongation zone, and maturation zone of the main root for 15 minute periods and total flux computed. In *A. thaliana* there was no statistical difference between the mean fluxes measured at each zone. However, in *A. halleri* there was a statistical difference ($p = 0.445$) between the mean fluxes at the maturation zone (0.130 $nmolm^{-2}s^{-1}$) and the elongation zone (0.214 $nmolm^{-2}s^{-1}$). Across all three zones mean H^+ flux was 0.311 $nmolm^{-2}s^{-1}$ for A. *halleri* and 0.670 $nmolm^{-2}s^{-1}$ average in A. *thaliana*. Preliminary Zn^{++} flux measurements between the same plants show similar trends.

These results hint that the mechanism for hyperaccumulating plants like *A. halleri* begins paradoxically with slower and more controlled H^+ efflux / Zn^{++} influx. Slower initial heavy metal uptake may then allow A. *halleri* to maintain homeostasis and growth at concentrations that are toxic to A. *thaliana*.

This project is supported by grants from the National Center for Research Resources (P20RR016471) and the National Institute of General Medical Sciences (P20 GM103442) from the National Institutes of Health.

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DIFFERENTIAL CADMIUM INTEGRATION IN SEVERAL ORGANS DURING SUBLETHAL EXPOSURE IN THE AXOLOTL, *AMBYSTOMA MEXICANUM*

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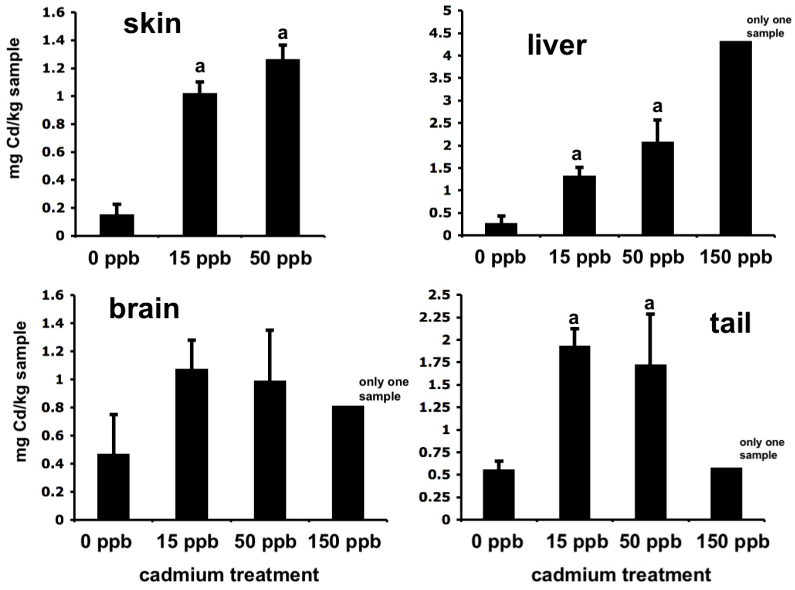
Because amphibians have permeable skin and can be highly aquatic, they exhibit high sensitivity to very low levels of several heavy metals, e.g. cadmium, mercury, and arsenic. Wild amphibian species in North Dakota bioaccumulate cadmium and maintain higher cadmium loads than the habitats they live in. What has not been explored is if there is differential bioaccumulation in different organ system. We tested the hypothesis that the laboratory model salamander *Ambystoma mexicanum* (= axolotl) would differentially accumulate cadmium during different concentrations. Cadmium integration in four axolotl tissues (skin [taken from the head], brain, liver and tail) was tested using graphite furnace atomic absorption spectrophotometry. Given that the LC50 value for axolotl immersed in $CdCl_2$ solution is 102 ppb, we exposed to 75 day old axololts to a 96 hour exposure to 0, 15, 50, and 150 ppb $CdCl_2$. We tested integration only using surviving axololts. There was only one survivor at 150 ppb, and it had higher cadmium content than the other axolotls. We used a two-factor ANOVA to test for the effects of cadmium dosage and tissue type on differences in tissue cadmium content. All tissues except brain had higher cadmium loads at 15 and 50 ppb (compared to controls). In addition, tissues differed in cadmium content. The pattern of tissue accumulation suggests a model for transport into and within the axolotl.



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INTEGRATING SELECT INDUSTRY INTO NEW UBRANISM

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The modern metropolis is faced by an increasely diverse yet interconnected array of challenges including questions of sustainability, environmental impact and quality, community, and transportation needs. Conventional development practices have failed to produce a built environment which meets these challenges. New Urbanism has been offered as an alternative theory and method of planning to more adequately grapple with these problems. Thus far, New Urbanism has been more successful than conventional approaches, however, in spite of its progress so far, the theory must be expanded and refined to more fully reach its own stated goals of better living in a better built environment. I propose the integration of carefully chosen industrial land uses into New Urbanist planning as such an expansion, allowing for greater diversity, decreased envionmental impact, and significant economic benefits not directly addressed by New Urbanist theory.

FURTHER INVESTIGATION OF A POSSIBLE ROLE FOR ABSCISIC ACID IN CONTROLING THE GROWTH EFFECTS OF INDOLE-3-ACETIC ACID IN EXPANDING *ARABIDOPSIS* LEAVES?

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Indole-3-acetic acid (IAA), a plant morphogenic hormone, controls multiple aspects of plant growth including vascular development and root and leaf initiation. Earlier we have examined how IAA impacts leaf expansion in the model plant, *Arabidopsis*. Increasing the IAA content of young expanding intact attached, intact detached, and wounded attached leaves resulted in inhibition of growth, while IAA treatment of excised leaf strips or wounded detached leaves stimulated rather than inhibited growth.[1] These results suggested that leaf tissue must be both wounded and detached from the plant for IAA to induce increased growth.

The current study addresses the requirement for detachment from the plant for IAA-induced growth. Implied is that an entity, continuously supplied by the rest of the plant, somehow interacts with IAA or auxin signal transduction in leaf blades of the intact plant to inhibit growth and preventing IAA-induced growth. A chemical signal seems most likely. Given the root to shoot movement of xylem transport, the chemical signal would seem most likely to originate from the root. Several candidate root derived chemical signals are known but the isoprenoid compound abscisic acid (ABA; 5-(1-Hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl-2,4-pentanedienoic acid) seems a particularly strong candidate as it is a known plant growth inhibitor. At this venue, we have already reported that we have found ABA treatment to be a potent inhibitor of leaf growth in *Arabidopsis* and that ABA and IAA treated leaf strips grew less than IAA treated strips while ABA and IAA treated strips grew more than strips treated with ABA alone.

As for previous experiments for this ongoing project, seedlings of *Arabidopsis* were grown in moist potting soil in a growth chamber at 19°C, with continuous illumination. After 10-14 days, plants were selected with both the first two true leaves 2.8-3.2 mm in diameter and rapidly expanding. One of these first two leaves from each plant served as the experimental leaf and the other leaf served as a paired control. For most experiments, scaled digital images of the individual leaves were prepared for subsequent determination of initial leaf area; for others initial images of excised leaf strips (0.7 mm wide cut transversely across the midpoint of the leaves) were prepared. Either leaf strips or intact attached or intact detached leaves were treated 24 hours with full strength Murashige and Skoog media (with 10 mM KCl and 0.1 mM Mes/Btp (pH 6.0)) or the same +/- 50 μM IAA and/or 10 μM ABA. After 24 hours increased area of leaves and strips was determined from digital images. The effect of IAA treatment on endogenous leaf ABA content in intact attached and in excised strips was determined using a new high-sensitivity high-throughput assay.

Here we report first that ABA was found to inhibit leaf strip expansion of control and of IAA treated tissue. Mutant plants (CS5736: Arabidopsis Biological Resource Center), deficient in ABA synthesis, produced leaves with more robust growth detached from the plant which were not growth-inhibited by IAA. Attached mutant leaves also were not growth inhibited by IAA. We are also testing the effect of abamineSG (a new potent ABA synthesis inhibitor) on the growth of IAA treated detached leaves. An initial pilot study (n=24) found a statistically insignificant trend towards IAA-induced leaf growth in the presence of 30 μM abamineSG. These results are consistent with an hypothesis that IAA treatment of Arabidopsis leaves induces ABA synthesis in roots and intact leaf tissue leading to slower growth. Conflicting with the results described above, however, so-far limited assays of endogenous leaf tissue ABA content found ABA levels not to differ between IAA-treated and untreated intact detached leaves or strips.

This project is supported by grants from the National Center for Research Resources (P20RR016471) and the National Institute of General Medical Sciences (P20 GM103442) from the National Institutes of Health.

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GRADUATE COMMUNICATIONS

IN THE

A. ROGER DENISON COMPETITION

(communications are listed alphabetically by the last name of the presenting author)

IRREVERSIBLE COCAINE ANALOGS ATTACH NEAR THE DOPAMINE TRANSPORTER ACTIVE SITE

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The dopamine transporter (DAT) is an integral membrane protein that is responsible for the reuptake of extraneuronal dopamine (DA) and thus, for terminating dopaminergic neurotransmission. DA levels are tightly regulated, as high levels are associated with psychological disorders and drug addiction, while low levels are associated with depression and loss of motor function. The DAT consists of 12 transmembrane (TM) domains with N- and C-termini extending into the cytoplasm. Although the DAT has not been crystallized, the crystal structure of the homologous bacterial leucine transporter, $LeuT_{Aa,}$ has provided insights into the DAT's tertiary structure (1). Based upon $LeuT_{Aa}$, the active site of DAT is predicted to be comprised of residues from TMs 1, 3, 6, and 8 (2). The DAT is also a major site of action for the psychostimulant cocaine, which binds the DAT and inhibits DA uptake. Although empirically based molecular models predicting cocaine analog binding poses, based on the $LeuT_{Aa}$ crystal structure, have been reported (2), the actual protein-drug interactions that confer high affinity cocaine analog binding and inhibition of dopamine transport have not been experimentally determined.

To elucidate how cocaine binds to the DAT, we are mapping the attachment site of the irreversible cocaine analogs, *N*-[4-(4-azido-3-^{125}I-iodophenyl)-butyl]-2-β-carbomethoxy-3-β-(4-chlorophenyl) tropane (^{125}I-MFZ 2-24) and 3–β-(4-chlorophenyl) tropane-2–β-carboxylic acid, 4-azido-3-^{125}I-iodophenylester, (^{125}I-RTI 82). These compounds have similar core structure but differ in the position of the reactive azido (N_3) group. Previous studies have shown that ^{125}I-MFZ 2-24 and ^{125}I-RTI-82 bind irreversibly to the DAT protein in TM domains 1 and 6, respectively (3, 4). To further narrow the adduction site, we created several methionine substitution mutants across TMs 1 and 6 to generate custom cyanogen bromide cleavage sites. The results obtained have narrowed the site of covalent adduction of ^{125}I-MFZ 2-24 to either Asp 79 or Leu 80 in TM 1 and the site of covalent adduction of ^{125}I-RTI 82 to Phe 320 r in TM 6. This is the shortest DAT sequence ever identified with photolabeling and peptide mapping techniques. Our data strongly indicate that the cocaine analogs attach to the DAT near the DA active site. Furthermore, these biochemical data are in agreement with the best poses obtained from computational docking analyses. Together these findings provide experimentally-derived evidence supporting the site and orientation of a cocaine analog binding to the DAT.

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GROWTH HORMONE KNOCKOUT MICE DISPLAY DIFFERENCES IN HYPOMETHYLATION AND TRANCRIPTION OF DNA METHYLTRANSFERASES AND INTERSPERSED REPEATS

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Methylation reactions are important for the establishment and maintenance of epigenetic methylation tags on DNA and histone molecules critical for the development and life-long function of an organism. Global DNA hypomethylation and hypermethylation of GC rich regions are correlated with aging and in age-related diseases such as cancer (1). Pathologies associated with aging are theorized to be a gradual process resulting from epigenetic dysregulation, including alterations in the methylation patterns of repetitive elements known as interspersed repeats (IRs), which are normally highly methylated (2). DNA methylation is linked to thiol metabolism by glycine-*N*-methyltransferase (GNMT), and it's catalyzation of *S*-adenosylmethionine (SAM) to *S*-adenosylhomocysteine (SAH). SAM is the major methyl donor for DNA methylation and increased or decreased GNMT activity has been shown to affect DNA methylation in the livers of rats and human hepatic carcinoma cultures (3, 4). Alterations of methionine through diet or cell treatment are known to affect the activity of GNMT and DNA methyltransferases (DNMTs), the enzyme class responsible for the maintenance and *de novo* catalyzation of methyl groups onto genomic DNA (3, 5, 6). Growth hormone (GH) is a regulator of GNMT activity (7). Mice deficient in GH signaling (Ames dwarf) live longer than their wild-type counterparts and exhibit higher liver GNMT activity and GH treatment of dwarf mice decreases GNMT activity (8, 9). Alternatively, GH transgenic (GHtg) mice exhibit premature aging and have shortened lifespans (10). We hypothesize that DNMT expression and patterns of methylation on IRs, differ between mice with altered growth hormone signaling compared to wild-type mice during aging.

Our previous work has shown the DNA methyltransferases (DNMT1, DNMT3a, DNMT3b) are differentially expressed in the Ames dwarf and GHtg murine liver compared to their wild-type counterparts suggesting that GH status may be involved. Differences in the hypomethylation of IRs in Ames dwarf and GHtg mice were also observed. Growth hormone binding protein knockout (GHRKO/BP) mice are deficient in GH signaling, contributing to their small size and increased longevity. Using McrPCR techniques and RT-PCR, we studied the hypomethylation and transcription of well-known IRs (LINE1, SINE B1, SINE B2, and IAP-LTR) at three age groups (3, 6 and 12 months). GHRKO/BP mice displayed age related differences in hypomethylation of SINE B2, LINE1, and IAP-LTR ($p=0.0012$, $p<0.0001$, and $p=0.0691$ respectively). Genotype was a factor only for the hypomethylation of LINE1 ($p=0.0026$). In GHRKO/BP and GHtg mice, genotype transcriptional differences were observed in IAP-LTR and LINE1 (GHRKO: $p=0.0273$,$p=0.0059$, GHtg: $p=0.0672$,$p=0.0113$ respectively). In addition, transcriptional expression of all three catalytically active DNMTs (1,3a, and 3b) was studied in the GHRKO/BP mouse at 3, 12 and 24 months of age. Age was a factor in the expression of DNMT1 ($p=0.003$), DNMT3a ($p=0.0001$), and DNMT3b ($p<0.0001$).Genotype was a major factor in the expression of DNMT1 and DNMT3a ($p=0.0079$ and $p=0.0085$ respectively). DNMT1 expression exhibited a significant interaction between the two factors ($p=0.0473$.) These studies contribute to growing evidence of the importance of growth hormone on DNA methylation which we hypothesize may play an important role in the longer life span of mice deficient in GH signaling.

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ROLE OF PROLINE DIRECTED PHOSHORYLATION SITE ON DOPAMINE TRANSPORTER

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The dopamine transporter (DAT) clears extraneuronal dopamine (DA) and thus controls the spatio-temporal dynamics of dopaminergic neurotransmission. DAT is the major target for psychostimulants such as cocaine (COC) and amphetamine (AMPH). DAT has a serine cluster on the distal N-terminus that is phosphorylated in a protein kinase C-dependent manner and a membrane proximal proline-directed site, Thr(T) 53, that is phosphorylated *in vitro* by Mitogen activated protein kinases. COC and AMPH impact DAT regulatory properties including uptake activity and surface expression. Although, the mechanism of drug action on DAT is unclear, DAT phosphorylation is associated with altered surface expression and DAT activity. In this study, we examined the effect of DA and several psychostimulant drugs on the phosphorylation of rat DAT (rDAT) using a newly developed phospho-specific antibody that detects phosphorylated T53 residue on rDAT. Immunoblotting revealed that DAT substrates led to increased DAT T53 phosphorylation. This increase was transport or binding dependent as COC blocked the AMPH effect. In rat striatal synaptosomes METH-stimulated DAT T53 phosphorylation was rapid, occurring within 60 sec. In contrast, uptake blockers did not influence T53 phosphorylation. Because phosphorylation at proline-directed sites causes cis-isomerization of the protein backbone, we tested this effect on DAT T53 by inhibiting Pin1, a peptidyl prolyl isomerase which catalyzes the isomerization of pThr/pSer backbones using a small molecule inhibitor, juglone. Juglone treatment resulted in the accumulation of pThr53 suggesting a role for Pin1 in regulating rDAT T53 phosphorylation and thereby its function. Our findings indicate that translocation or binding of substrates stimulate T53 phosphorylation which is associated with major structural rearrangements of the membrane-proximal N-terminus. We hypothesize that substrate-mediated phosphorylation-dependent structural rearrangement resulting from prolyl cis-isomerization could thus act as a mechanism affecting DAT function.

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Au-MODIFIED SILICA NANOWIRES: SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS AS HYPERTHERMIA AGENTS FOR PHOTOTHERMAL THERAPY

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Metal nanoshells are of great interests, especially gold nanoshells, due to their tunable optical resonances, inertness in biological medium, good biocompatibility, and ready bioconjugation. In this study, we described the synthesis, characterization, and application of a novel nanocomposite made of silica nanowires (SiNWs) covered with a gold shell of tunable thickness. With the formation of gold shell on the surface, the Au-modified SiNWs gained a strong surface plasmon resonance absorption in the near infrared (NIR) region. They can strongly absorb NIR light and convert it into cytotoxic heat upon NIR laser irradiation. This new nanocomposite showed excellent *in vitro* biocompatibility and improved photothermal cancer cell destroying efficacy. Notable, most photothermolysis studies require high laser power irradiation (1.5 – 48.6 W/cm^2), which is higher than the maximal permissible exposure (MPE) of skin per ANSI (American National Standard for Safe Use of Lasers) regulation (e.g., 0.4 W/cm^2 at 850 nm), to destroy cancer cells. Remarkably, a much lower laser power irradiation (0.3 W/cm^2, lower than the MPE skin) is required for photodestructuion of cancer cells in vitro by applying this newly developed Au-modified SiNWs. They may thus be a promising candidate as a hyperthermia agent.

Support: The work was supported by the National Science Foundation (NSF)-CHE0947043.

α_{1A} ADRENERGIC RECEPTOR INFLUENCES ON PROGENITOR CELL FATE IN THE ADULT HIPPOCAMPUS

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Long-term stimulation of α_{1A} adrenergic receptors (α_{1A}ARs) increases the number of proliferating cells in the subgranular zone (SGZ) of the adult dentate gyrus and enhances learning and memory in the mouse. The creation of new neurons in the SGZ of the adult brain has been linked with improved learning and memory in mouse, rat, and primate models. The role of the new neurons in learning and memory has not been fully examined though some studies show that the new cells play different roles as immature and mature cells. We chronically treated mice with cirazoline, an α_{1A}AR agonist, to determine the effect of α_{1A}AR activation on differentiation and survival of the adult-generated cells in the hippocampus. Animals were injected with BrdU after 4 weeks of treatment to label dividing cells and perfused 4 weeks later to allow time for the new cells to differentiate. Immunohistochemical markers were used to identify the new cells which included excitatory granule cells, astrocytes, and a very small number of the interneurons.

Supported by North Dakota EPSCoR IIP Seed Award Grant EPS-081442, National Center for Research Resources Grant P20RR016741 and National Institute of General Medical Sciences Grant P20GM103442 from the NIH, NHLBI Grant RO1HL61438, NSF Graduate Research Fellowship Program, and NSF MRI Grant 0619688.

USING THE GEOTHERMAL GRADIENT FROM OIL AND GAS BHTS AS A DIRECT INDICATOR FOR SUBSURFACE STRUCTURE AND GEOTHERMAL POTENTIAL: NEBRASKA

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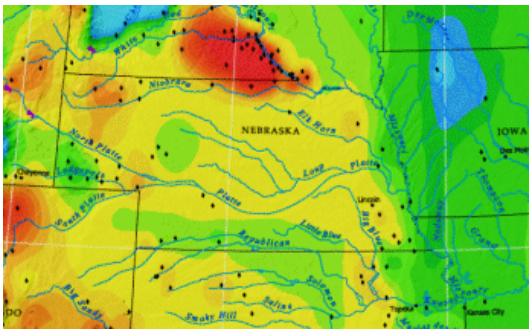
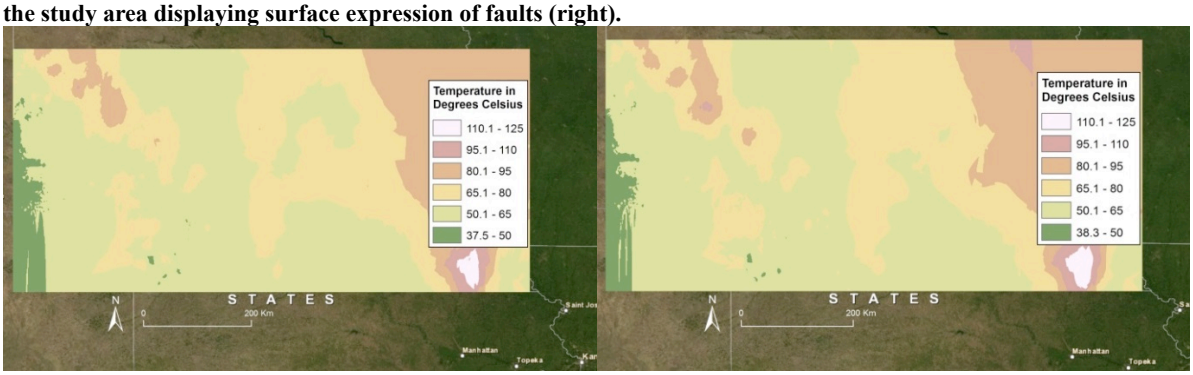
Indirect methods, such as geothermometry and analysis of satellite imagery, have been used to identify parts of northwestern Nebraska that have high geothermal potential. Earlier publications have shown that direct geophysical methods should be used to validate these findings; however, such methods of wide-scale subsurface detection can be prohibitively expensive. We have reproduced the earlier findings of subsurface structure and geothermal potential using bottom-hole temperature (BHT) measurements from oil and gas well data obtained from the new National Geothermal Data System (NGDS) website. Geothermal gradients calculated from BHT data were interpolated using the Kriging method to assess temperature distribution in the subsurface. A temperature anomaly was discovered that could not be accounted for with erroneous data recording; therefore, we have concluded that the anomaly exists and may be an expression of the Chadron Fault. We propose this direct indication method as an inexpensive alternative to finding subsurface structures and geothermal potential.



Figures 1 and 2: Cropped Geothermal Map of North America showing Nebraska heat flow (left) and satellite imagery of the study area displaying surface expression of faults (right).



Figures 3 and 4: Interpolation by Kriging method of the geothermal gradient (left). The image on the right is an interpolation once common data entry errors are removed. Only a minor difference can be seen between the images, indicating existence of the temperature anomaly.

This material is based upon work supported by the Department of Energy Geothermal Technologies Program under Award Numbers DE-EE0002854 and DE-EE0002731, and the North Dakota Department of Commerce Centers of Excellence Program.

USING TEMPORAL AND PALEOGEOGRAPHIC MAPPING TO IDENTIFY UNDERREPRESENTED REGIONS AND TIME INTERVALS TO AID IN SELECTING NEW SEARCH LOCATIONS

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Temporal mapping of known continental molluscan type specimens can highlight periods in time that are still under-sampled for fossil continental mollusks, allowing for targeted searches to try and fill these gaps in the biostratigraphic records. Geographic mapping of known type localities can show regions that have been under-sampled, and thus possibly underrepresented, and can also allow for targeted searches.

The creation of such maps requires three things; an extensive database of fossil molluscan taxa, paleogeographic maps, and geologic maps. An existing database (developed by Hartman) was expanded and placed in a fully relational database management system in the current project. North American base maps by Ron Blakey showing both the suggested paleolandscape at various times throughout the Cretaceous as well as showing modern political boundaries were used. (1).

ArcGIS was used to georeference Blakey's maps. Type localities were divided into groups based on their known ages (e.g., a 65mya to 70mya group). Each type locality age bracket was then plotted on the appropriate paleogeographic maps (e.g., the 65mya to 70mya set was plotted on both the 67mya and 70mya paleogeographic maps), thus registering fossil localities in the suggested paleolandscapes. Type localities, and thus type specimens, were now shown in their approximate locations relative to the Western Interior Seaway. The coastlines of the seaway were traced in ArcGIS for each of the paleogeographic maps. The fossil localities and the seaway traces were superimposed on the USGS Tapestry of Time and Terrain map (2).

Plotting the type locality data on the paleogeographic base maps showed strong temporal and geographical biases. Maps were created by plotting the traces of the seaway from various times in the Cretaceous and plotting the type localities for the same times on the Tapestry map, which showed areas that have been heavily sampled. The maps created from these plots also show the bedrock in each age bracket that likely had the paleoenvironmental conditions for continental mollusks to exist (i.e., freshwater) which have no type localities present. Future work will include the search for outcrops and fossils in the areas identified as appropriate and under-represented on the maps. Searches can be conducted for fossils of specific ages using this mapping method to identify areas of high interest. Work must also include examining the nature of the geographic and temporal distribution biases to determine if they are due to geologic reasons, or due to humans (e.g., collecting or naming trends).

One extra benefit of this work is verifying accuracy of the paleogeographic maps within the known age ranges of the type localities. In all cases except one, continental (fluvial) mollusk localities plot on land, mostly along noted rivers leading to Western Interior Seaway coastlines. One locality plotted in the middle of the seaway on each paleogeographic map from the Cretaceous which overlapped the localities current age assignment. This record must be examined to determine the source of the discrepancy.

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HARNESSING CHAOS: ELUCIDATING THE ION-COUPLING MECHANISM OF NEUROTRANSMITTER TRANSPORTERS

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Chemical neurotransmission in the central nervous system is involves a collection of highly regulated processes. The SLC6 family of proteins plays an important role by exploiting energetically-favorable Na^+ gradients for the thermodynamically-uphill transport of released neurotransmitters back into the pre-synaptic terminals. This has a critical influence on synaptic transmission by both terminating receptor-mediated signaling and replenishing vesicular neurotransmitter pools. Whereas SLC6 transporters are found from bacteria to man, the vast majority of information concerning the SLC6 family comes from studies of the glycine, serotonin (5-HT), norepinephrine, γ-aminobutyric acid and dopamine transporters (GLYT, SERT, NET, GAT and DAT, respectively), due to their importance in health and addiction (1). For instance, hSERT has importance in human health, as it is a target for a number of therapeutic and illicit drugs, including serotonin reuptake inhibitors (SSRI), cocaine and 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") (1).

Recently, a number of solute transporters from different protein families have been crystallized, providing critical details about the structural features of these carriers (2, 3). Surprisingly these transporters all share a similar structural arrangement of transmembrane helices despite possessing little to no sequence homology (3, 4). This structural scaffold termed the LeuT-fold, after the initial solving of the LeuT transporter from the SLC6 family, must possess structural aspects conducive to solute transport. Importantly, crystal structures representing the "open-to-out", "occluded" and "open-to-in" conformations of LeuT have been solved, suggesting transport occurs primarily by an alternating access mechanism (2, 5). However, these static poses of dynamic proteins fall short of explaining how ion binding is mechanistically and energetically coupled to the transport of substrate.

SERT and LeuT share >55% identity within the centrally located substrate and ion binding regions suggesting the two Na^+ binding sites found in LeuT (Na1 and Na2) also exist in hSERT (6). Interestingly, biochemical data indicate only one Na^+ is translocated per 5-HT transport cycle (7). This leads to the hypothesis that the Na1 and Na2 sites have specialized roles, an idea supported by computational analysis of the ion binding sites and recent crystal structures (5,8,9). However, the distinct roles of the Na1 and Na2 sites in hSERT remain largely uncharacterized. To understand the roles the Na1 and Na2 sites perform in hSERT, we used site-directed mutagenesis combined with biochemical and electrophysiological analyses to determine how alterations of the Na^+ sites affect ion dependency, selectivity as well as ion and 5-HT cotransport.

Substitution at a single, highly conserved asparagine (Asn101 to Ala or Cys), that is predicted to coordinate Na^+ at the Na1 site, radically alters the ionic coupling mechanism. This mutation eliminates the need for extracellular Cl^- and alters the cation selectivity in hSERT allowing for Ca^{2+} to functionally replace Na^+ during transport. These findings are supported by two-electrode voltage clamp studies in *Xenopus* oocytes where 5-HT induces significant current in the mutants with a Ca^{2+} buffer lacking Na^+. However, I/V analysis suggests that Ca^{2+} does not permeate the membrane during 5-HT transport. Taken together, these data show that, in the hSERT N101 mutant background, Ca^{2+} binding is sufficient to generate substrate translocation but does not contribute to concentrative uptake. Moreover, the diminished uphill transport by these mutants reveals an uncoupling between the Na1 and Na2 sites in the translocation process, suggesting a mechanistic role for Na^+ coordination at Na1, and highlighting the critical interplay between the substrate and ion binding sites during substrate transport.

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AWARENESS OF BOVINE TUBERCULOSIS AND RELATED PUBLIC HEALTH PROBLEMS AMONG PASTORAL COMMUNITIES: A SURVEY OF KAABONG DISTRICT, UGANDA

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Background: Bovine tuberculosis is an important public health concern because of its zoonotic potential. *Mycobacterium bovis,* the causative agent of bovine tuberculosis has been isolated from pastoral communities and cattle in Karamoja region. This study assessed the level of knowledge of cattle owners on bovine tuberculosis in Kaabong district, Karamoja region in Uganda.

Methods: A cross-sectional survey of 60 cattle owners from three sub-counties of Kaabong district was conducted using Interviewer-administered questionnaires. Awareness on several factors was evaluated including: clinical signs and post mortem lesions in cattle, transmission to humans and vice versa, transmission routes in humans, symptoms in humans, level of interaction between wildlife and cattle, and sharing of water points by cattle and wild life.

Results: Of the 60 respondents, 88% (53/60) reported emaciation as a symptom of bovine tuberculosis, followed by moist sounding cough 80% (48/60) and fever 80% (48/60). The most reported postmortem lesions included tubercles in lungs 95% (57/60) and abscesses in lymph nodes 78% (47/60)). 93% (56/60) were aware that bovine tuberculosis can be transmitted to humans and vice versa, and reported routes of transmission included raw meat 90% (54/60) and cough 80% (48/60), and sharing cups 48% (29/60). Cattle contact with wild life was reported by 65% (39/60) of respondents.

Conclusion: This study revealed a high level of awareness among cattle owners about bovine tuberculosis and related public health concerns, as well as a strong interaction between wildlife, domestic animals, and humans. This knowledge can be utilized by the government to support public health interventions.

A LYME DISEASE SPIROCHETE PROTEIN BINDS HUMAN FIBRONECTIN THROUGH MULTIPLE INDEPENDENT DOMAINS

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The eukaryotic protein fibronectin (Fn) is a common target for bacterial pathogens; including staphylococcal and streptococcal species important in causing common infections. The Lyme disease spirochete, *Borrelia burgdorferi*, has several known fibronectin-binding proteins, thereby suggesting importance of this host factor in a Lyme disease infection. Recently, another borrelial protein, BB0347, was identified as having sequence identity with other known fibronectin-binding proteins, as determined by a BLAST search. Herein, we characterize the interaction between Fn and this new bacterial protein.

To analyze the potential for this protein in a mammalian infection, we created recombinant BB0347 and analyzed its binding to fibronectin *in vitro*. We were also able to determine the expression of this protein by *B. burgdorferi* in culture conditions via RT/QRT-PCR and BB0347-specific Western blotting. Additionally, we cloned peptides from the full-length protein into expression vectors to determine the location and prevalence of potential Fn-binding sites. By using a fragment of Fn, we also examined the sub-protein location of the BB0347-Fn interaction, and various inhibitors allowed us to further characterize the molecular interactions responsible. The potential importance of BB0347 was also examined by 1) determining the sub-cellular location of the protein by protease protection assays and immunofluorescence 2) analyzing the known cell-binding domain of fibronectin for stearic hindrance upon treatment with BB0347, and 3) examining the immunogenicity of the bacterial protein in a mouse infection.

BB0347 binds Fn in vitro via the heparin binding domain of the host protein, and multiple, independent sites within the bacterial molecule. Increasing the ionic strength of the interaction buffer inhibited the binding, thereby implicating charged residues; however, no role was found for the charged amino acid lysine. Additionally, the *bb0347* gene is expressed by the bacterium in culture, and this phenomenon is at least partially dependent on temperature, as with other known borrelial pathogenic genes. BB0347 was also determined to be located on the outer membrane of *B. burgdorferi* and did not interfere with the binding of fibronectin-CBD-specific antibodies to the host protein. Lastly, mice injected with live *B. burgdorferi* formed antibodies against BB0347, suggesting a potential for therapies that target this protein in a Lyme disease patient.

Our data suggest as possible role for BB0347 in Lyme disease pathogenesis, as it shares characteristics with other proteins, such as those from the opportunistic pathogen *Staphylococcus aureus*, which are known to be crucial for disease progression. Further work is being performed to elucidate the specific role for BB0347 in a murine infection. Finally, due to its specific properties, we have decided to name the protein **B**orrelial **O**uter-membrane **B**inding protein **A** (BobA).

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EFFECT OF ABLATED α_{1A}-ADRENERGIC RECEPTOR STIMULATED NEUROGENESIS ON COGNITIVE FUNCTION

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It is unclear what role α_{1A}-adrenergic receptors (ARs) have on neurogenesis, cognition and mood. The goal of this study is to examine how the α_{1A}-ARs relationship to neurogenesis influences cognition and mood. Mice over-expressing the α_{1A}-AR showed increased cognition and neurogenesis over normal mice. We hypothesize activation of the α_{1A}-AR alone is not responsible for improvements in cognition and mood, but it is the receptor-stimulated neurogenesis. To test this hypothesis, mice are treated with selective α_{1A}-AR agonist, cirazoline (CRZ). A cannula and osmotic pump is surgically inserted into mice with either artificial cerebral spinal fluid or anti-mitotic agent cytosine arabinoside (AraC). Mice undergo behavioral testing (Morris water maze, novel object recognition, forced swim, tail suspension, and open field), are sacrificed, brains fixed, sectioned and stained with cell markers for immature and dividing neurons. Stereology was used to estimate cell populations. Preliminary results show AraC mice are significantly slower in solving the Morris Water Maze than AraC-CRZ mice. The results of this study may lead to development of therapeutic strategies for neurodegenerative diseases.

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GENETIC MUTATIONS AFFECT STABILITY OF BACTERIAL BIOFILMS

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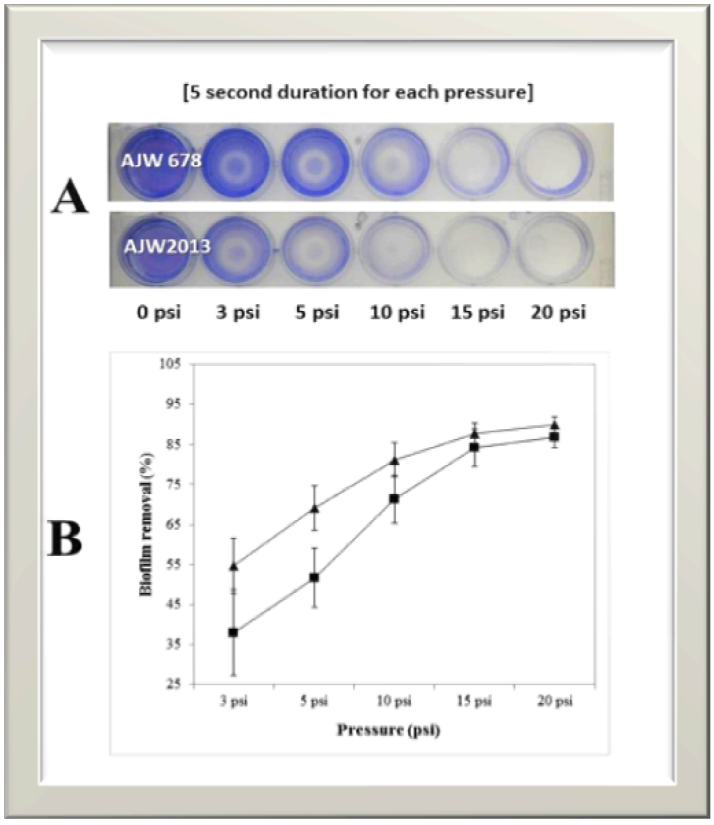
Previous research by this lab has shown that the amount and the three dimensional structures of biofilm can vary largely in different genetic backgrounds of *Escherichia coli*. With this research, we wanted to see whether changes in structures were paralleled by differences in stability of the biofilm.

The Center for Nanoscale Science and Engineering's automated Water jet Apparatus was designed to allow for large scale testing of adhesion strength of biofilms to many different coating surfaces. The custom software allows for control of experimental parameters, specifically the pressure and duration of the off-center spin jet. To optimize this system for the performance of our experiment, 24-well plates were inoculated with cultures of an *E. coli* K-12 strain and subjected to water jet treatment. This was done at different pressures at a set time of 5 s. After water jetting, crystal violet staining was used to quantify the amount of biofilm formation left behind, and absorbance values can be read after extraction of the crystal violet (CV) with acetic acid. An example of an output of this experiment is shown in the figure to the right. Panel A shows the CV stain of the wells, Panel B the quantitative CV data.



We then tested a series of genetic mutations that affected biofilm associated cell surface organelles. The fimbriae mutant had the largest negative effect on biofilm amounts. The *ackA pta* mutant (AJW2013) which was unable to synthesize acetate had the largest effect on biofilm stability. Altogether, we were able to quantify the stability of the biofilms, which can now be used to screen a larger set of mutants, as well as small chemicals that would inhibit biofilm.

Bacterial strains were provided by Dr. Alan J. Wolfe (Loyola University Chicago, Maywood IL). The work was funded by grant 1R15AI089404 from the NIH/NIAID. MI was funded by the ND Experiment Station. The Center for Nanoscale Science and Engineering was funded by Army Research Office Contract Number W911NF-10-1-0519.

MOLECULAR EPIDEMIOLOGY OF NON- 0157 SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* ISOLATES FROM CATTLE

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Shiga Toxin Producing *Escherichia coli* (STEC) are harbored by ruminants especially cattle which shed the bacteria in feces. STEC strains find their way to human foods through contamination by feces from infected animals. STEC strains are reported to be frequent contaminants of food including beef, milk and fresh produce causing severe human illnesses. Non-O157 STEC, such as O26, O111, O103, O121, O45 and O145, are reported to cause severe human illnesses in the United States with STEC O111 associated with about 40% of the non-O157 STEC outbreaks. While subtypying of STEC isolates using nucleic acid based methods have been used to identify clonal groups and phylogenetic relationships among isolates, these efforts have mainly focused on human clinical isolates. Therefore, there is a need to study STEC isolates from animals. The goal of this study was to characterize, and establish relationship among, STEC isolates from cattle using multi-locus sequences typing (MLST). DNA was isolated from 43 non-O157 STEC strains, and 7 housekeeping genes were sequenced from each strain. Genius version 6.0.6 software was used to assemble and analyze sequences. Sequences were compared to those in the STEC Center MLST database to determine the sequences type of each strain. Sequences will also be used to determine phylogenetic relationships among the cattle STEC isolates and compared to human STEC isolates. This study will provide valuable data on STEC subtypes that are commonly found in cattle, as well as the clonal relationships among cattle and human STEC isolates, information that is crucial for food safety policy development.

CORTICOTROPIN-RELEASING FACTOR FACILITATES EPILEPTIFORM ACTIVITY IN THE ENTORHINAL CORTEX VIA ACTIVATION OF CRF_2 RECEPTORS AND H-CHANNELS

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PURPOSE: The entorhinal cortex (EC) is involved in initiation and maintenance of temporal lobe epilepsy and corticotropin-releasing factor (CRF) is a potent epileptogenic neuropeptide. The role of CRF in EC is not known. The purpose of the project was to determine the role and the mechanisms of CRF in facilitating epileptiform activity in the EC.

MATERIALS AND METHODS: Slice preparation: Horizontal brain slices including the EC, subiculum and hippocampus obtained from 13-18-day-old Sprague Dawley rats in ice cold saline and incubated in the same solution at 35°C for 40 min and at room temperature (~24°C) until use.
Epileptiform activity recordings: Electrode containing the extracellular solution used to record epileptiform activity from layer III of the EC. External solution including the $GABA_A$ receptor blocker picrotoxin (100 μM), and (in mM) 130 NaCl, 24 $NaHCO_3$, 5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.5 $MgCl_2$ and 10 glucose, saturated with 95% O_2 and 5% CO_2 (pH 7.4) was bath applied. Drugs were bath applied after stable control.
Perforated-patch recordings: Ih recorded from layer II stellate neurons of the EC. Extracellular solution comprise (in mM) 130 NaCl, 24 $NaHCO_3$, 3.5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.5 $MgCl_2$ and 10 glucose, saturated with 95% O_2 and 5% CO_2 (pH 7.4), and Tetradotoxin (0.5 μM). Recording pipettes tip-filled with the intracellular solution comprising 100 K^+-gluconate, 0.6 EGTA, 5 $MgCl_2$, 8 NaCl, 2 ATP_2Na, 0.3 GTPNa and 40 HEPES (pH 7.3) and then back-filled with freshly prepared K^+-gluconate intracellular solution containing amphotericin B (200 μg/ml).
Immunocytochemistry: Rat brains postfixed, cryoprotected, treated with H_2O_2, incubated in normal donkey serum and then with the primary antibodies and biotinylated donkey anti-goat IgG, finally with avidin-biocytin complex. Positive signals detected by Diaminobenzidine. Slides visualized and photographed with a Leica microscope.
Western blot: Medial EC is lysed and protein concentrations determined using Bradford. Equivalent amount of total protein loaded to each well, separated by 12 % SDS–PAGE, and transferred to the polyvinylidene difluoride membranes. Blots incubated with individual primary antibodies followed by secondary antibody. Immunoreactive bands visualized by SuperSignal West Pico Chemiluminescent Substrate and detected by a Biospectrum Imagining System.

RESULTS: The EC expressed high levels of CRF and CRF_2 receptors and bath application of CRF increased the frequency of picrotoxin-induced epileptiform activity recorded from layer III of the EC in slices, via CRF_2 receptors and cyclic AMP, whereas protein kinase A was partially involved. Application of ZD 7288, a blocker of the hyperpolarization-activated channels (H-channels), significantly reduced the frequency of epileptiform activity but increased the numbers of the synchronizing events within single epileptiform activity and the duration of individual epileptiform activity. In the presence of ZD 7288, CRF failed to increase the frequency of epileptiform activity but still augmented the numbers of the synchronizing events in an epileptiform activity and the duration of epileptiform activity suggesting that part of the effects of CRF on epileptiform activity is mediated via H-channels. Furthermore, CRF increased H-channel currents recorded from layer II stellate neurons via activation of CRF_2 receptors. Cyclic AMP not protein kinase A was responsible for CRF-mediated facilitation of H-channel currents.

CONCLUSIONS: CRF facilitates epileptiform activity in the EC via activation of CRF_2 receptors. Functions of cAMP and partially PKA are required for CRF-mediated augmentation of epileptiform activity. CRF increases Ih in the EC via cAMP but not PKA and may play a role for CRF-mediated enhancement of epileptiform activity.

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TURNOVER RATES OF SOIL ORGANIC CARBON FRACTIONS DETERMINE CARBON SEQUESTRATION POTENTIAL OF TROPICAL FORESTS IN SRI LANKA

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Long term deposition of atmospheric C as lithosphere formation is known as carbon sequestration (CS), the main remedial measures for global climatic change. Most studies on C budgeting have considered soil organic carbon (SOC) as a whole without taking into account its constituent fractions. In the present study therefore, an attempt was made to understand variability of the SOC fractions of two selected dry and wet zone forests of Sri Lanka. Monthly soil core samples were collected up to 30 cm depth from permanent sampling plots of two wet and dry zone forests. Loss on ignition, a thermal oxidation method, was used to recognize the different SOC fractions. The study fractionated SOC as free litter fraction (active pool), fulvic fraction (inter mediate pool) and humic fraction (passive pool), as recognized by weight loss from 150-200 ^{o}C, 200-400 ^{o}C and 400-550 ^{o}C, respectively.

The analysis revealed that free soil litter, fulvic and humic fractions constituted 16%, 53% and 31%, respectively of the SOC of the two forests. The SOC sequestered in the wet and dry zone forests were 108 t/ha and 94 t/ha, respectively. The difference was mainly attributed to climatic parameters. Floristic composition was not that important in soil CS. It was apparent that there was a transfer of C from free litter fraction to fulvic fraction, as was reflected by simultaneous change of their pool sizes. In the absence of this transfer, there was an accumulation of the free litter fraction, while declining the fulvic fraction, possibly due to decomposition. There was a considerable decrease of the humic fraction, which could be due to fresh C supply as dissolved organic C from the floor litter layer to the soil, which helped decompose rapidly the humic fractions by the rapidly growing microbial biomass after the rains. In the wet zone forest, there was a similar changing pattern of the SOC fractions as of the dry zone. These results suggest that it is important to consider turnover rates of the SOC fractions in evaluating soil CS potential in the tropical forest ecosystems.

SITE-DIRECTED MUTAGENESIS OF PHE319 ON TRANSMEMBRANE DOMAIN 6 OF THE DOPAMINE TRANSPORTER DOES NOT ALTER AFFINITY OF THE COCAINE ANALOG RTI-82.

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The dopamine transporter (DAT) is a member of the SLC6A family of secondary-active Na^+/Cl^- dependent neurotransmitter transporters and functions to clear dopamine (DA) from the synapse by DA reuptake into the presynaptic terminal. The drug of abuse cocaine binds to DAT inhibiting DA reuptake. Importantly, details regarding the mechanism by which cocaine inhibits transport remain unclear. Recent studies have yielded confounding results concerning the number of cocaine binding sites and whether it inhibits DA binding to DAT in a competitive or non-competitive manner. We have developed computational models and performed small molecule docking of a cocaine-based photoaffinity analog RTI-82 (Figure 1) in conjunction with biochemical peptide mapping analyses of crosslinked RTI-82/DAT complexes. The computational modeling places the photoactive phenyl azido moiety of RTI-82 proximal to Phe319 in DAT. This finding has been confirmed by peptide digestion mapping of the crosslinked products. These results suggest that mutations at Phe319 should have a direct impact on RTI-82 binding affinity to DAT. Therefore, we are using site-directed mutagenesis to introduce mutations at Phe319 and analyze their impact using competitive uptake and binding assays. Aromatic residues at this position appear to be important for function as Tyr319 and Trp319 mutants were well tolerated. Substitution with the charged or polar amino acids Asp and Cys resulted in loss of transporter function while Met319 retained partial activity. These findings suggest that hydrophobic and aromatic side chains at this position are necessary for DAT function. Despite the loss of transport activity in the Asp319 and Cys319 mutants, RTI-82 could inhibit binding of the cocaine analog [^{3}H]CFT in a dose dependent manner indicating that the RTI-82 binding site was preserved. We observed that the potency of RTI-82 did not change significantly (<3-fold) when Phe319 was mutated to Tyr, Trp or Met. Given the flexible nature of the phenyl azido arm and the proximity of Phe319 to the more sterically open vestibule it is possible that the phenyl azido group can be accommodated by other interactions and that the major contribution to binding affinity of RTI-82 comes from the tropane ring core structure of the molecule. Recent findings from several groups have suggested that there may be two antagonist binding sites (S1 and S2) on monoamine transporters. We are currently using the substituted cysteine accessibility method (SCAM) in conjunction with site-directed cysteine mutants to determine if the S1 and/or S2 sites accommodate the core structure of RTI-82.

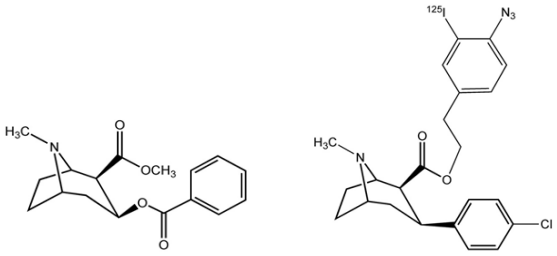

Figure 1: Cocaine (left) and RTI-82 (right) share essential components of the cocaine pharmacophore including the tropane nitrogen and phenyl ring. RTI-82 differs primarily in the addition of a photoactive phenyl azido moiety.

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IDENTIFICATION OF DOPAMINE TRANSPORTER PALMITOYL ACYLTRANSFERASES

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The dopamine transporter (DAT) is an integral membrane protein that mediates the reuptake of dopamine (DA) from the synaptic space, thus regulating dopaminergic neurotransmission. Our laboratory recently found that DAT undergoes *S*-palmitoylation, in which a saturated 16-carbon palmitate group is added to a cysteine via a thioester bond. Protein palmitoylation is known to regulate diverse aspects of neuronal protein trafficking and function, but its role in DAT is poorly understood. More specifically, *S*-palmitoylation is a reversible lipid modification, in which the protein undergoes cycles of palmitoylation and depalmitoylation, catalyzed by palmitoyl acyltransferases (PATs) and palmitoyl-protein thioesterases (PPTs). Recent studies identified a 23 member family of DHHC (Asp-His-His-Cys) proteins in mammalian cells as PATs; however these enzymes remain poorly characterized. The identity of the PATs involved in the regulation of DAT palmitoylation is unknown and therefore the overall goal of this study is to identify which enzymes impact DAT palmitoylation. This will be done by co-expressing rDAT individually with each of the 23 PAT enzymes, followed by assessment of DAT palmitoylation. Primary results indicate that enzyme DHHC2, DHHC3, DHHC8, and DHHC15 increases palmitoylation of DAT, while DHHC11 and DHHC7 have no effect when compared to control levels in $LLCPK_1$ cells. Palmitoylation of DAT by PAT enzymes will be confirmed by site-directed mutagenesis of DHHC enzymes to determine the specificity, followed by analysis of palmitoylation effects on DAT function.

THE TEMPORAL AND SPATIAL EXPRESSION OF *OMPR* CORRELATES INVERSELY WITH THAT OF *FLHD*

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Biofilms can be defined as a complex aggregation of single or multi-species bacterial communities that have several thousand times more antibiotic resistance than single planktonic bacteria. To identity novel targets for the development of biofilm prevention and treatment techniques, our long term goal is to determine genes that get expressed early in biofilm development (prevention targets) and genes that get expressed late and in the outer layer of the biofilm (treatment targets). Biofilm formation is regulated by numerous regulators, including the two-component osmoregulator EnvZ/OmpR and the global regulator FlhD/FlhC. With this study, we determined the temporal and spatial expression of *flhD* and *ompR* in *E. coli* biofilm.

Promoter fusion plasmids of *flhD* and *ompR* to GFP were used. The *E. coli* K-12 strain AJW678 and its isogenic *ompR* mutant strain were transformed with the *flhD*::GFP plasmid, the parental strain was also transformed with the *ompR*::GFP plasmid. Biofilm was formed in flow cells at room temperature in TB for a maximum of 60 h. Fluorescence was detected with a Zeiss Axio Observer Z2 upright microscope. Fluorescence intensity of the bacteria was calculated across the images with Image-Pro Plus software and plotted against time (temporal) or distance from the surface (spatial) of biofilms.

In the temporal experiment (Fig. 1), *flhD* expression in the parent (orange) was high at two time points: early stage (12 h) and late stage (51 h), whereas the highest expression of *ompR* (black) was in between these two time points at 35 h. The expression of *flhD* in the *ompR* mutant (green) increased over the first 12 h and then was not growth phase dependent anymore. Spatially, the expression of *flhD* was almost limited to the top layer of the 12 h and 51 h biofilm. In contrast, expression of *ompR* was highest towards the surface of the 35 h biofilm.

We conclude that *flhD* expression was high whenever *ompR* expression was low with respect to both, temporal and spatial expression. This could be attributed to the fact that phosphorylated OmpR is a repressor of *flhD* expression (1). The lack of a growth phase dependence of *flhD* expression in biofilm of the *ompR* mutant (beyond 12h) further supports this notion.

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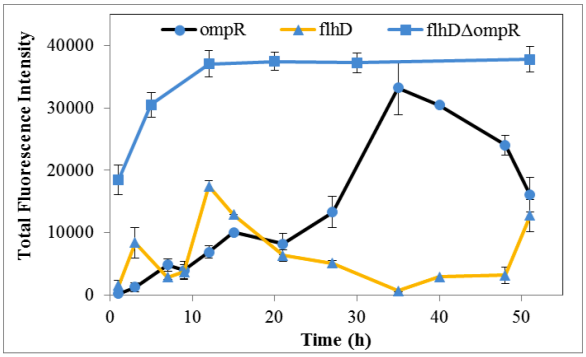

Fig. 1:

Temporal expression of *flhD* and *ompR*

N-CADHERIN UPREGULATION IN ARSENIC-TRANSFORMED UROTHELIAL CELLS

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The switch from E-cadherin to N-cadherin is a well-known indicator of the epithelial-to-mesenchymal transition (EMT) occurring in bladder cancer. N-cadherin upregulation is correlated with decreased survival and poor prognosis in patients, and its inhibition is antitumorigenic. While the factors mediating the decrease in E-cadherin expression are well-established, little is known of the factors regulating the increase in N-cadherin expression. Heavy metals are known carcinogens and implicated in the initiation of bladder cancer. Of all the commonly induced genes in five independently transformed human urothelial (UROtsa) cell lines, N-cadherin exhibits the highest induction. It is hypothesized that arsenic directly regulates the increase of N-cadherin in UROtsa cells in contrast to a transformation-mediated induction. To test this hypothesis, UROtsa cells were exposed to arsenic at varying levels and the expression of N-cadherin mRNA compared to untreated cells. Western blots were also performed to confirm increased protein levels in UROtsa cells treated with arsenic. In future experiments, expression of putative transcription factors for N-cadherin will be measured in an effort to elucidate the regulatory mechanisms of N-cadherin expression after arsenic exposure.

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R25 ES016250 from National Institute of Environmental Sciences

ANTIBODY RESPONSES TO THE NON-STRUCTURAL PROTEINS OF PORCINE CICROVIRUS STRAIN 2 (PCV2)

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Porcine circovirus strain 2 (PCV2) is the cause of porcine circovirus associated diseases (PCVAD) in pigs, a disease that has caused great economic losses in the pig industry in the US. Four open reading frames (ORF) or genes of the PCV2 genome are currently known i.e. Cap (ORF2) gene, Rep (ORF1) gene, ORF3 gene and ORF4 gene. All of the current commercial vaccines are directed towards the immunogenic capsid gene (ORF2) of PCV2. Very little is known about the immunogenicity of the other proteins or their role in protection against PCV2. Knowledge of whether the non-structural proteins are immunogenic also has important implications on the diagnosis of PCVAD. Therefore, the methodology for this study is focused on recombinant protein expression and immunological detection of the PCV2 non-structural proteins. The PCV2 ORF1, 2 and 3 were amplified from the PCV2 genome using specific primers for ligation into the *pRSETA* (bacterial expression), pBacHT (insect cell expression) or V5HisTOPO (mammalian expression) plasmid vectors, transformation into chemically competent BL21 (DE3) *E. coli* cells or SF9 insect cells followed by PCV2 specific swine antibody detection of recombinant protein expression in SDS-PAGE, western blotting or immunofluorescence. Subsequent medium term goals include protein purification by affinity chromatography, PCV2-specific ELISA development and the assessment of protective immune responses. Therefore, our study is important in advancing comprehensive knowledge regarding the host antibody responses to PCV2.

BORRELIA BURGDORFERI ISOLATED FROM *PEROMYSCUS MANICULATUS* AND *MYODES GAPPERI* IN EASTERN NORTH DAKOTA

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Eastern North Dakota is traditionally viewed as a non-endemic area for Lyme disease, though the emergence of confirmed Lyme disease cases among residents suggests eastern North Dakota is minimally a transition zone for *Borrelia burgdorferi* and *Ixodes scapularis*. Indeed, based upon the isolation of infected nymphal *Ixodes scapularis* ticks, Diuk-Wasser *et al*. (1), described eastern North Dakota as a transitional zone. In our study, five spirochetes, based on cell morphology, were isolated from the hearts of *Peromyscus maniculatus* and *Myodes gapperi* around the Turtle River and Forest River areas of Grand Forks, ND. The spirochetes underwent no more than six passages *in vitro* before genetic analyses were performed. Sequence analysis of 16S, *flaB, ospA, ospC, p66,* and the 16S-23S intergenic spacer region (IGS) indicates the spirochetes are *B. burgdorferi*. 16S sequences (ca. 136 nucleotides) aligned to multiple *Borrelia* spp. using the Ribosomal Database Project website. BLAST results and alignments of ca. 40 residues of *flaB* showed 100% identity to multiple *B. burgdorferi* strains. *p66* showed 100% sequence identity across 239 residues to *B. burgdorferi* B31. Two of the five spirochetes were negative for *ospA* despite multiplex PCR results showing all possessed the plasmid carrying *ospA*. Mutiplex PCR for twelve linear and nine circular plasmids revealed a unique complement of linear and circular plasmids among the isolates, suggesting either the arrival of multiple strains of *B. burgdorferi* or rapid divergence of a single strain. All five isolates are missing linear plasmid 25, suggesting they have a low-infectivity phenotype. Future work includes: alignment of our eastern North Dakota *B. burgdorferi* sequences with *B. burgdorferi* from western Minnesota to determine directionality and evolutionary history of *B. burgdorferi* in eastern North Dakota; collection of *Ixodes* ticks and comparisons of *B. burgdorferi* to those found in local small mammals; and infection studies to determine the infectivity phenotype of the local isolates. In total, our results confirm *B. burgdorferi* is present in the small mammal population of eastern North Dakota, further corroborating the eastern progression of *B. burgdorferi*.

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OPTIMIZATION OF FLUORESCENCE ENHANCEMENT OF SILICON NANOWIRES COATED WITH GOLD NANOPARTICLES

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Surface enhanced fluorescence (SEF) is an important topic in medical and microscopy imaging. Increasing fluorescence efficiency leads to better medical imaging with fewer fluorescence materials needed. Nano-scale objects lend their unique optical and electronic properties to make ideal SEF structures. Specifically, silicon nanowires and gold nanoparticles are used to construct a novel SEF structure.

Both silicon and gold are nontoxic, making them ideal for imaging in living organisms. A nanostructure of silicon nanowires coated with gold nanoparticles and a silica layer doped with the fluorescent dye, tris(bipyridine) ruthenium(II) chloride, Ru(bpy) was created. The localized surface plasmon resonance of gold nanoparticles is an important property for fluorescence enhancement. When gold nanoparticles are coupled with a fluorescent dye, the fluorescence signal is increased several times compared to dye that is not coupled with gold nanoparticles.

A series of experiments has been conducted to create the novel SiNW@AuNP SEF structure and determine the parameters for its optimum fluorescence enhancement. Specifically, the effect of different diameters of gold nanoparticles was studied. Analysis conducted with scanning electron microscopy and fluorescence spectroscopy determined fluorescence enhancement.

TEMPORAL AND SPATIAL DISTRIBUTION OF ANTHRAX OUTBREAKS IN CATTLE AND WILD ANIMALS IN UGANDA, 1956 TO 2010

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Anthrax is a highly fatal zoonotic bacterial infection that can lead to significant public health consequences. This study was conducted to assess the spatial and temporal distribution of anthrax outbreaks in domestic and wild animals in Uganda for the period 1956-2010. Data on anthrax outbreaks were obtained from the monthly and annual reports of the National Animal Disease Diagnostics and Epidemiology Center and the National Animal Livestock Resources Research Institute in Uganda. Also, weather data (rainfall) were obtained from the Zoology Department, Makerere University. Data were analyzed in relation to temporal and spatial factors. A total of 17 anthrax outbreaks occurred during the study period; 10 outbreaks in the rainy season and 7 in the dry season. There was no significant association between season and the occurrence of anthrax($X2=0.42$, $P<0.838$, OR=1.12, 95% CI: 0.38, 3.30); the probably of anthrax occurrence in both seasons was similar. The highest number of outbreaks (n=6) were recorded during 2000-2010. The mean annual number of anthrax outbreaks increased from 0.5 during the period of 1956-1966 to 0.6 for the period 2000-2010. Similarly, the results demonstrated a spatial increase in the number of districts affected by anthrax outbreaks between 1978-2010 with the majority of cases (53%) reported in Kasese district where Queen Elizabeth National Park (QENP) is located. Animal anthrax outbreaks are on an increase in Uganda occurring mainly in QENP in June and November which are dry and wet months, respectively. There is need for the government through its agencies like MAAIF to adopt a strong and systematic quality control program of anthrax vaccination campaigns, make anthrax a public good disease and ensure that all the susceptible animals mainly cattle in the areas surrounding QENP are vaccinated annually. These data contribute significantly to increasing public awareness of the association between anthrax outbreaks and weather conditions and evaluation of the current control and preventive measures for anthrax outbreaks in Uganda.

COMPARISON BETWEEN THE PRESENCE OF THE CRONOBACTER PLAMINOGEN ACTIVATOR PROTEIN, CPA, AND THE ABILITY OF CRONOBACTER SAKAZAKII ISOALTES TO RESIST COMPLEMENT

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Cronobacter sakazakii is an emerging opportunistic pathogen of the family Enterobacteriaceae responsible for outbreaks of neonatal meningitis and necrotizing enterocolitis in the immunocompromised. However, virulence mechanisms of this infectious agent are not well understood. Surviving in blood serum despite the presence of active complement proteins is well known as a virulence factor among isolates of *Escherichia coli* and other members of the Enterobacteriaceae. Recently, a potential virulence protein has been highlighted in the forefront of research into the virulence of *Cronobacter* sakazakii. The outer membrane protease (Cpa), a plasminogen activator, is capable of inhibiting the complement cascade of the nonspecific host immune system (1). In this study, 36 *Cronobacter sakazakii* isolates were investigated for the presence of the *cpa* gene via polymerase chain reaction and challenged in a complement resistance assay using serum with active complement. The objective of this study was to determine the relationship between the presence of *cpa* and *Cronobacter sakazakii's* ability to survive and grow in the presence of complement proteins.

Methods. *Cronobacter sakazakii* isolates CT-2, N52, N72, BAA-984, ATCC #29544, ATCC #29004 and 30 other isolates obtained from Cornell University were used in this study. Controls included; positive control *Escherichia coli* V1, known to be resistant to the effects of complement, and negative control *Escherichia coli* A1, which is susceptible to complement activity (2). Isolates were inoculated into Luria Bertani (LB) broth (EMD Chemicals, Gobbstown, NJ) and incubated at 37°C shaking 200 rpm for 30 minutes in order to reach early log phase. Each isolate was pelleted by centrifugation and washed with phosphate buffered saline pH 7.0. The pellets were re-suspended in 50% chicken serum/50% PBS or 50% heat inactivated serum/50% PBS. The experiments were incubated statically at 37°C and colony counts were performed on LB agar at zero hour and then three subsequent two hour time points for a total of six hours.

Results. The positive control isolate was not inhibited by the active complement, whereas the negative control ceased to grow in the presence of active complement. Both the positive and negative controls were not inhibited by the heat inactivated serum, as was expected. Of the *Cronobacter sakazakii* isolates tested, there was one found to possess *cpa* that was resistant to the active complement and nine that possessed the gene that were inhibited by the active complement.

Discussion. A study performed by Franco et. al, suggested that the *cpa* may be a virulence factor involved in serum resistance and attribute to the pathogen's ability to cause meningitis and necrotizing enterocolitis (1). Although limited by the small number of isolates and using chicken serum as opposed to human serum, this study did not demonstrate that the presence of *cpa* in *Cronobacter sakazakii* was associated with the ability to survive in the presence of active complement. Due to the limitations of the study, further investigation is required to determine if these results hold true with additional isolates and using human serum as the source of complement.

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"Bottom-Up" Graphene Quantum Dots for Fluorescence *In Vivo* Imaging and Colorimetric Sensing

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Abstract

A facial bottom-up method for the synthesis of fluorescent graphene quantum dots (GQDs) was developed by one-step pyrolysis of glutamic acid. The GQDs showed relatively strong blue, green and red photoluminescent (PL) under the irradiation of ultra-violet, blue and green light, respectively. Interestingly, the GQDs emit NIR fluorescence in the range of 800-850 nm with the excitation dependent manner. The quantum yield of GQDs in the blue range was 66.7% when they were irradiated at 360 nm. And the quantum yield of GQDs when they were irradiated at 780 nm was 1.6%, which demonstrated the upconversion fluorescence properties. For both the regular fluorescence and upconversion fluorescence of GQDs, they both showed the well-known excitation-dependent PL behavior. Furthermore, the feasibility of using for the fluorescence in vivo imaging in mice was investigated, which showed their potential in the bioimaging applications because of their great biocompatibility and low cytotoxicity. In addition, the GQDs show the intrinsic peroxidase-like catalytic activity, which is similar to graphene sheet and carbon nanotubes. Coupled with ABTS, the GQDs can be used for the detection of hydrogen peroxide with the limit of detection of 20 μM.

PROFESSIONAL COMMUNICATIONS

(communications are listed alphabetically by the last name of the presenting author)

BINDING INTERACTIONS OF RTI-82, A COCAINE-LIKE PHOTOAFFINITY LIGAND, TO THE DOPAMINE TRANSPORTER

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The dopamine transporter (DAT) is the principal target for drugs of abuse such as cocaine and methamphetamine. However, the molecular interactions of these compounds with DAT are poorly understood. Crystal structures for a homologous protein, the Leucine transporter (LeuT) from Aquifex aeolicus (1, 2), have been obtained and provide a template to develop comparative structural models and perform computational analyses to facilitate unraveling of the molecular basis of these interactions. In this study, comparative models of rat DAT (rDAT) were constructed based on the LeuT 'occluded' and 'open-to-out' structures (PDBID: 2A65 and 3F3A, respectively) followed by iterative model refinement and energy minimization in the molecular modeling package Rosetta 3.1 (3), in the absence of Na^+ and Cl^- ions. The twenty best rDAT structures obtained (10 each from 2A65 and 3F3A) were carried forward for flexible ensemble docking of the cocaine-like photo affinity ligand, RTI-82, using RosettaLigand. In addition, Na^+ and Cl^- were placed in their putative binding sites in 3F3A and 2A65-based Rosetta 3.1 models and analyzed by induced fit docking (IFD). Molecular dynamic simulations of the docked complexes moved the inhibitor deeper into rDAT central binding pocket establishing better RTI-82 transporter interactions. The core tropane ring substructure of RTI-82 was located in an area surrounded by TMs 1, 3, 6 and 8 proximal to the ion binding sites. Whereas, the phenylarylazide substituent on the 2beta position of the tropane ring traversed into the external binding pocket interacting with the F319 residue on TM6 through π-π interactions supporting the recent cross-linking data demonstrating adduction of aryl azido group to F320 in hDAT (F319 in rDAT). The identification of F319 as a contact residue for RTI-82 independently by biochemical crosslinking and computational modeling strongly supports that our comparative models and docking methodologies have predictive value for small molecule binding to DAT.

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EFFECT OF TEMPERATURE, SEX, AND MATURATION STATUS ON METAMORPHOSIS IN THE WESTERN TIGER SALAMANDER, *AMBYSTOMA MAVORTIUM*

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The western tiger salamander (*Ambystoma mavortium*) exhibits "facultative paedomorphosis" throughout many parts of its range (western North America). Facultative paedomorphosis refers to among-individual variation in metamorphosis: some individuals experience the typical metamorphosis as a juvenile whereas others become sexual mature and forego metamorphosis (i.e., become "larval adults"). While evolutionary theory typically considers the paedomorphic status as fixed once attained (e.g., Gould, 1977), more recent observations indicate that paedomorphic salamanders are still capable of metamorphosis (Winne and Ryan, 2001).

Like other western tiger salamander populations, growth is very rapid and individuals can attain large sizes (>100 mm SVL) in their first summer of life. In North Dakota, populations are completely metamorphic when ponds are ephemeral (and can reach high temperatures), completely paedomorphic in deep cattle ponds that always have water and do not freeze completely (and are always cool), and are mixed metamorphic/paedomorphic in larger shallow lakes that dry periodically (e.g., once every 10-20 years).

We hypothesized that temperature variation among these types of habitats is a significant cause of life cycle variation. In order to test this hypothesis, we collected 77 salamanders using minnow traps from the flooded Agsite Pond/Swalls Lake in Ward County in northwestern North Dakota. This site is characterized by a mixed population of paedomorphic and metamorphic salamanders. We tested three hypotheses: (1) metamorphosis is temperature dependent; (2) metamorphosis is dependent on maturation status; and (3) metamorphosis is dependent on sex. We placed large (>85 mm SVL) larvae in individual boxes and placed 40 larvae in a cooler at 14 degrees C and 37 larvae in a cooler at 20 degrees C. We used a 3-way log-linear analysis to evaluate the categorical response of metamorphosis (yes or no) and how this response differed among temperature treatments and across sexes and maturation status (sexually mature or juvenile). As expected, larvae are more likely to metamorphose at high temperature. However, this effect is influenced in a complex way by sex and maturation. Females always showed a propensity to metamorphose, although this was less strong in adult females. In stark contrast, all juvenile males at high temperature metamorphosed and no adult males metamorphosed under any condition. Approximately 10% of all species of salamander express either obligate or facultative paedomorphosis (Lanoo, 2005). These complex interactions suggest that life cycle evolution in facultatively paedomorphic salamanders is influenced by the variation in life history pressures experienced by males and females.

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LEACHING OF SELECTED MICRONUTRIENTS FROM COAL FLY ASH (FA).

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The potential for environmental contamination by major coal combustion residue, fly ash (FA) is one of the main areas of concern regarding proper disposal of these coal combustion by-products.

Fly ash contains several toxic elements, such as Arsenic (As), cadmium (Cd), cobalt (Co),Mercury (Hg) lead (Pb), zinc (Zn), and other heavy metals, which can leach out and contaminate soils as well as surface water and groundwater. When fly ash is not properly disposed, and if leached, these elements cause the severe contamination of subsurface water. Consequently, these elements may become a hazard to the environment because of their contributionto the formation of toxic compounds. This contamination could lead to health,environmental and land-use problems .

Specific Aim of our leaching experiments is to determine the environmental safety of different growth media composed of FA and soil. In this study we investigated the leaching of selected elements belonging to Group 1 (Cs and Li) and from Group 2 (Be, Sr, and Ba) from the soil control and plant growth substrate composed of coal fly ashes from two sources, and ashes mixed with soil and with the soil and sphagnum peat moss (SPM).

Plexiglas columns (30.4 -cm long, 5-cm inner diameter) have been employed to study the transport and leaching of cations and heavy metals from a FA amended soil. A Fargo-Ryan soil (pH=6.1, organic matter =8%) has be sampled and used as a control treatment.

This study investigated the leaching of selected trace elements (Cs, Li, Be, Sr and Ba) from plant growth media made of two coal fly ashes (one from semi-bituminous coal and one from lignite), and from these ashes combined with the soil and with the soil and sphagnum peat moss. Leachate fractions has been collected at each ½ pore volume for a total of five pore volumes. Concentrations of mentioned above trace elements in plant growth media and in leachate has been determined using inductively coupled plasma (ICP) emission spectrophotometry.

The presence of sphagnum peat moss and soil in coal ash based plant growth media expressed ameliorative role reducing the presence of trace elements in the leachate. Elevated concentrations of Li, Sr and Ba in the leachate may cause some en environmental health concerns and require further investigations.

This project was supported by grants from the National Center for Research Resources (5P20RR016471-12) and the National Institute of General Medical Sciences (8 P20 GM103442-12) from the National Institutes of Health.

THE ANALYSIS OF THE FLORA OF NORTH DAKOTA

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North Dakota has never been surveyed in full for plant diversity. Before 2011, more than 40% of area were still awaiting botanical research. In 2011, we started the systematic investigation of the state flora with a top priority to fill "botanical white spots" and normalize the sampling level for counties under-represented in herbarium collections. To date, most of the territory is covered with sampling of approximately 30 by 30 miles density. We collected more then 3,100 herbarium samples (all geo-referenced and photographed) and deposited them into Minot State University herbarium collection (now internationally recognized as MISU). As a result, the size of collection was doubled (from 2,786 samples in 2010 to 5,920). All samples are represented in the on-line electronic database. 16 new plant species were found in our state and 7 species for the first time were tied to the proper location.

Data collected in 2011-2012 allowed us to update the draft checklist and publish the first full list of North Dakota plants: http://ashipunov.info/shipunov/fnddb. This Web page is a product of complex interactions between more than 40 species lists and 8 "filters" which add information to species names (like common name, name of family etc.). The nomenclature problems were avoided via programmatic normalization of names. More than 2,800 photographs made in the field were attached to plant names. Along with the Web service, we also made the downloadable PDF book (ISBN 978-1-4675-6379-6) with almost the same content (names, sources, maps and photographs). When new name or new list appear, both book and Web page will be updated simultaneously through scripts written for R statistical environment and TeX/LaTeX text formatting system.

Flora of North Dakota consists of 1651 species, 665 genera and 121 families of vascular plants. In addition, approximately 300 species are mentioned for our state without location. The state has typical north temperate grass- and wetland flora with a dominance of aster and grass families, sedges and legumes. The most frequently collected species are water knotweed, asters, horsetails and grasses. Among our counties, Ransom and Richland have the most diverse flora (more than 55% of state species), and Trail, Adams and Towner -- least diverse (less than 25%). North Dakota has 131 "local endemics": plants which do not occur in any neighboring state or province. There are also 43 species which were found in all surrounding territories (but not in our state), they could be found in North Dakota in future. Interestingly, the proportion of plants common between our state and neighboring territories (Fig. 1), is equal (proportion test X-squared = 24.35, df =3, $p << 0.05$). Consequently, North Dakota may be called a *cross-road state* from the view of plant distribution. The cluster analysis of species composition revealed that our flora is most similar to the flora of South Dakota (Fig. 2). However, our state has the biggest number of common unique plants (plants which grow only in two states) with Minnesota (41 species) and then with Montana (29), this probably indicate the current routes of plant distribution.

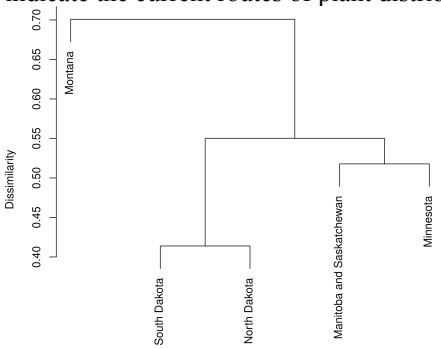

Figure 1. Proportions of flora elements.

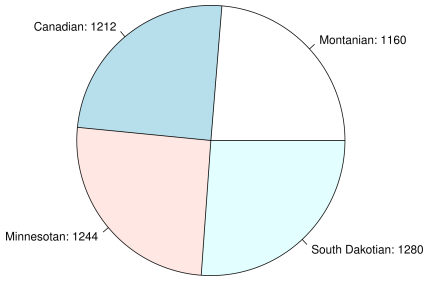

Figure 2. Relatedness of states' floras.

This summer we plan to start the research on two biodiversity hotspots in the state: UND Forest River Biology Area and Little South Pembina River Valley.

Supported by North Dakota INBRE and Great Plains Center of Minot State University. We are grateful to many people, including Shawn DeKeyser (NDSU: NDA Herbarium) and Kathryn Yurkonis (UND: GFND Herbarium) for their invaluable help with collections.

THE SILICON NANOWIRE @SILVER TETRACYANOQUINODIMETHANE HYBRID STRUCTURE: SYNTHESIS AND CHARACTERIZATIONS

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A facial bottom-up method for synthesis Silicon Nanowire@Silver Tetracyanoquinodimeth hybrid nanostructure was developed. The silicon nanowires (SiNWs) array was synthesized by etching silicon wafer using hydrofluoride (HF). The silver nitride was added and reduced into silver nanoparticles (Ag NPs) which can be found on the top of SiNWs. Then the silicon wafer was heated with Tetracyanoquinodimethane (TCNQ) in the bottle. The TCNQ molecular vaporized would react with silver on the surface of NPs and form the needle like anabranches on the top of SiNWs. the morphology of material can be adjusted by changing the reaction parameters, the morphology the SiNWs@AgTCNQ hybrid nanostructure were characterized by scanning electrical microscopy(SEM), Energy-dispersive X-ray spectroscopy (EDS), Fourier transform infrared spectroscopy (FT IR), et al. Due to the good field emission property of SiNWs and AgTCNQ. This hybrid nanostructure is the promising candidate to achieve excellent performance on field emission. .

IN VITRO REPLICATION OF SWINE TORQUE TENO VIRUS 1 (TTV1)

Anuradha Vegi[1]*, Sheela Ramamoorthy[1]

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Purpose of the research: Swine Torque Teno sus viruses (TTSuV or TTV) resemble human TTVs and belong to family Anelloviridae, genus *Iotatorquevirus*. There are two main swine TTV groups – TTV1 and TTV2. Swine TTV1 is non-enveloped, circular, negative sense single stranded DNA virus of 2.8 kb. The TTV1 genome has three open reading frames – ORF1, 2 and 3 [1]. Although, widely distributed in swine populations, currently there is no data to confirm the replication of DNA and growth of viable swine TTV1 virions in animal cell cultures. Moreover, TTV contamination of mammalian cell cultures used for biomedical experimentation may result in unintended experimental consequences.

Methodology: In the current study, DNA was obtained from swine cell lineages – PK15N (NVSL), PK15 (ATCC), ST, 3D4/31, and cell culture media components including trypsin, Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10%)(complete DMEM) and Hank's balanced salt solution (HBSS). Extracted DNA were examined for the presence of TTV1 DNA using polymerase chain reaction (PCR) with TTV1 specific primers. For all the samples that were positive for TTV1 DNA, the presence of TTV1- specific mRNA was examined by reverse transcription PCR targeting the TTV1 ORF3 which is a spliced gene. Further investigation to assess the presence of TTV1 ORF1 capsid protein expression in DNA positive cell cultures via immunofluorescence assay is underway.

Results and conclusions: TTV1 DNA was observed in cell lineages – ST, 3D4/31, as well as complete DMEM and trypsin. However, no viral DNA was found in PK15N, PK15 (ATCC) cells as well as HBSS although the same media was used to culture all cell lines, indicating that the DNA was detected in cells. Further research is being carried out to confirm the mRNA presence and protein expression of TTV1. The presence of the viral DNA highlight that there is a possibility of TTV1contamination in cell cultures and at the same time the evidence may be indicating the actual replication of TTV1 *in vitro*.

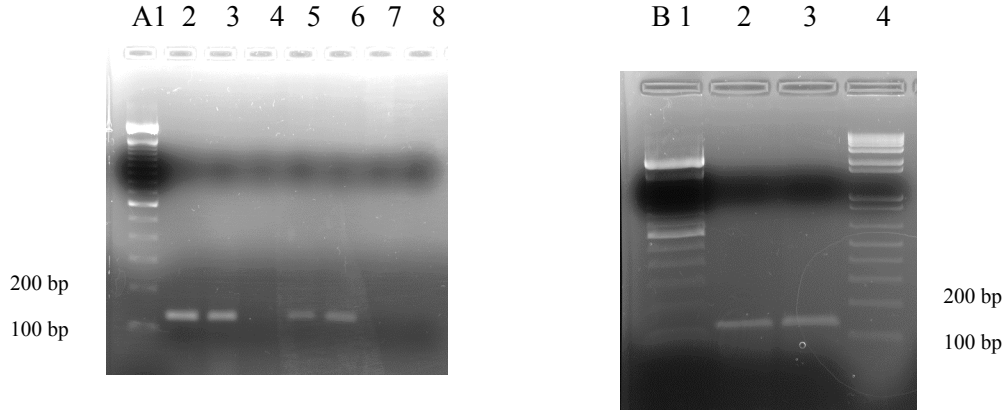

Figure 1 (A and B): PCR amplification of TTV1 DNA (125 bp) in cell lineages and cell culture components A) Lane 1: 100 bp ladder (Invitrogen), lane 2: TTV1 DNA from swine liver (positive control), lane 3: ST cells, Lane 4: PK15N, Lane 5: 3D4/31, Lane 6: complete DMEM, Lane 7: PK15 (ATCC), Lane 8: HBSS; B) Lane 1: 100 bp ladder (Invitrogen), Lane 2: TTV1 DNA from swine liver (positive control), Lane 3: Trypsin and Lane 4: 1 kb plus ladder (Invitrogen).

References:

[1] Niel C, Diniz-Mendes L, Devalle S. (2005) J Gen Virol., 86(5) pp 1343-1347.

CONSTITUTION OF THE NORTH DAKOTA ACADEMY OF SCIENCE

Founded 1908, Official State Academy 1958

ARTICLE I - *Name and Purpose*

Section 1. This association shall be called the NORTH DAKOTA ACADEMY OF SCIENCE.

Section 2. The purpose of this association shall be to promote and conduct scientific research and to diffuse scientific knowledge.

ARTICLE II - *Membership*

Membership in the Academy shall be composed of persons who share the stated purpose of the Academy and who are active or interested in some field of scientific endeavor.

ARTICLE III - *Council*

The officers of the Academy shall be a President, a President-Elect, and a Secretary-Treasurer. The Council, consisting of the officers, the retiring President, and three elected Councilors, shall be responsible for the fulfillment of the scientific and business obligations of the Academy.

ARTICLE V - *Dissolution and Limits of Action*

Section 1. In the event of dissolution of the Academy, any remaining assets shall be distributed to organizations organized and operated exclusively for education and scientific purposes as shall at the time qualify as exempt organizations under Section 501(c) (3) of the Internal Revenue Code of 1954.

Section 2. No substantial part of the activities of the Academy shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Academy shall not participate in or intervene in, any political campaign on behalf of any candidate for public office.

Section 3. No part of any net earnings shall inure to the benefit of, or be distributable to, Academy members or officers, or other private persons, except that the Academy may authorize the payment of reasonable compensation for services rendered.

ARTICLE VI - *Amendments*

Section 1. This Constitution may be amended at any annual Business Meeting of the Academy by a two-thirds vote. Proposed amendments shall be submitted in writing to the Secretary-Treasurer who shall send them to the members at least two weeks before the meeting at which such amendments are to be considered.

Section 2. Bylaws may be adopted or repealed at any regular business meeting by a two-thirds vote.

BYLAWS

BYLAW 1. *Meetings*

Section 1. *Scientific Meetings*. The Academy shall hold at least one annual scientific meeting each year at a time and place determined by the Council. Other scientific meetings, regional, state, or local, may be held at times and places determined by the Council. The Council shall establish regulations governing the presentation of papers at Academy sessions. Such regulations shall be made available to members at least three months before any meeting at which they are to apply.

Section 2. *Business Meetings*. A Business Meeting of the membership shall be scheduled at the regular, annual scientific meeting of the Academy. Ten percent of the active members shall constitute a quorum at the annual business meeting.

Section 3. *Special Meetings*. Special meetings shall be called by the President upon the request of ten percent of the active members and require twenty percent of the active members for a quorum. Notice of the time and place of such meetings shall be sent to all members of the Academy at least four weeks in advance of the meeting. Only matters specified in the call can be transacted at a special meeting.

Section 4. *Procedure*. Parliamentary procedures to be followed in all business meetings shall be those specified in "Standard Code of Parliamentary Procedure" by Alice F. Sturgis.

BYLAW 2. *Financial*

Section 1. *Fiscal year*. The fiscal year shall run concurrently with the calendar year from January 1 to December 31.

Section 2. *Dues and Assessments*. The annual dues and assessments may be changed from time to time by the Council, subject to approval by a two-thirds vote of the members at an annual Business Meeting. These dues are payable by January 31 for the current fiscal year or by the Annual North Dakota Academy of Science Meeting for those registering for the meeting

Section 3. *Supporting Members*. Council shall maintain a program to encourage members to voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.

Section 4. *Sustaining Members*. Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at $50 or greater may be invited by the President or designee to become a Sustaining Associate.

Section 5. *Audit and Reports*. The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year. The Secretary-Treasurer shall report on the financial affairs of the Society, including the results of an annual audit, as may be requested by the Council.

BYLAW 3. *Membership*

Section 1. *Membership Categories*. Classes of membership shall include the following: (a) Regular, (b) Student, (c) Emeritus, (d) Honorary, (e) Supporting, (f) Sustaining, and (g) Lifetime Members.

Section 2. *Eligibility and Procedure for Membership*. Candidates for membership, except Sustaining Member, may be proposed by any regular or emeritus member of the Academy by submitting the candidate's name to the chairman of the Membership Committee.

(a) *Regular Members*. Any person who is active or interested in some field of scientific endeavor shall be eligible for regular membership. A majority vote of Council shall elect to regular membership.

(b) *Student Members*. Any student who is an undergraduate or graduate student in some field of science shall be eligible for student membership. A majority vote of Council shall elect to regular membership.

(c) *Emeritus Members*. Any member in good standing upon formal retirement is eligible for emeritus membership. A majority vote of Council shall elect to emeritus membership.

(d) *Honorary Member*s. The Academy may recognize, by awarding honorary membership, any person (nonmember or member) who has in any way made an outstanding contribution to science. It shall be the responsibility of the Membership Committee to be aware of individuals whom it would be fitting for the Academy to honor in this fashion. A two-thirds vote of members attending the annual business meeting shall elect to honorary membership.

(e) *Supporting Members*. Regular or student members may voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.

(f) *Sustaining Associates*. Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at $50 or greater may be invited by the President or designee to become a Sustaining Associate.

(g) *Lifetime Members*. Any regular member in current good standing for at least one year may become a Lifetime Member by paying an assessment equal to 18 times the current annual dues in one lump sum or in two equal payments over the current and following year.

Section 3. *Privileges of Membership*.

(a) Voting at the annual business meeting is permitted of regular and emeritus members.

(b) Members of all categories may attend business meetings of the Academy.

(c) The Secretary-Treasurer and members of Council must be regular members in good standing.

(d) Regular, student, and emeritus members may submit abstracts or communications for scientific meetings of the Academy.

(e) Emeritus and Honorary Members shall be exempt from payment of dues.

(f) A Sustaining Member is provided a display area at the annual scientific meeting of five linear feet per $50 donation up to a maximum of 20 linear feet.

(g) Every member in good standing shall receive a printed copy or an electronic copy (if available and of equal or lesser cost than the printed copy) of the annual *Proceedings of the North Dakota Academy of Science*, the form to be determined by the member.

(h) Special offices such as Historian may be created by the unanimous vote of the regular members at the annual Business Meeting.

(i) All student research participants shall receive a properly inscribed certificate.

Section 4. *Forfeiture of Membership*.

(a) *Nonpayment of dues*. Members shall be dropped from the active list on 31 November following the nonpayment of dues during the membership year commencing the previous 1 December. A member may return to the active list by paying the current year dues.

(b) *Expulsion for Cause*. Membership may be terminated for conduct injurious to the Academy or contrary to the best interests of the Academy. The accused member shall be given an opportunity for a hearing before the Council. If a majority of the Council votes to expel the member, the action must be ratified by at least two-thirds of the members present at the next annual business meeting of the Academy. An expelled member shall forfeit all paid dues and assessments.

BYLAW 4. *Duties and Responsibilities of the Council and Council Members*

Section 1. *Council*. The Council shall meet, at the call of the President, at least twice a year. The Council shall:

(a) be the governing board of the Academy, responsible only to the membership.

(b) arrange for programs, approve committee appointments, be responsible for the fiscal affairs of the Academy, and transact such business as necessary and desirable for function and growth of the Academy.

(c) determine the location of the annual meeting three years in advance.

(d) annually appoint an Academy representative to the National Association of Academies of Science and to Section X (General) of the American Association for the Advancement of Science.

(e) shall appoint and may compensate a Secretary-Treasurer.

(f) shall appoint and may compensate an Editor of the PROCEEDINGS and other publications.

(g) shall be empowered to charge a publication fee of authors on a per page basis.

(h) shall control all activities of the Academy including grant applications.

Section 2. *President*. The President shall preside at meetings of the Council and over the annual business meeting of the Academy at the close of the regular term office. The President shall vote only to break a tie. Unless otherwise specified, the President shall, with the approval of the Council, appoint members to serve on Standing Committees and *ad hoc* Committees, designate the chair of each Committee, and appoint representatives to other organizations. The President serves as Coordinator of the Local Arrangements Committee for the annual meeting that occurs at the end of the President's term.

Section 3. *President-Elect*. The President-elect shall be considered a vice president and shall serve as such in the absence of the President.

Section 4. *Past-President*. The retiring President shall serve as Past-President and chair of the Nominating Committee. The Past President shall serve ex officio on those committees designated by the President and shall serve in the absence of the President and President-elect.

Section 5. *Secretary-Treasurer*. The Secretary-Treasurer shall:

(1) Assist Council in carrying on the functions of the Academy including the receipt and disbursement of funds under the direction of Council.

(2) Manage the Academy Offices under Council's general supervision.

(3) Serve as Managing Editor of the *Proceedings of the North Dakota Academy of Science*.
(4) Prepare a summary of the most recent audit and a report of the Academy's current financial status. This information shall be shared with the membership at the annual business meeting and published in the PROCEEDINGS following the business meeting.
(5) Perform all other duties of the Secretary-Treasurer listed in the Bylaws.
(6) Serve as archivist and be responsible for all official records, archives, and historic material which shall be in reposit with the Secretary-Treasurer.

BYLAW 5. *Appointment, Nomination and Election of Members of Council*

Section 1. *Eligibility for Office*. All candidates for election or appointment to the Council must be regular members in good standing. Nominees for President-elect must be members who reside within easy commuting distance of the site of the annual meeting selected by the Council that occurs when the President-elect serves as President.

Section 2. *Nomination Procedures*. The Nominating Committee shall be responsible for all nominations to elective office, shall determine the eligibility of nominees, shall ascertain that nominees are willing to stand for office, and shall be required to advance to the Secretary-Treasurer at least two names for each open position as needed. Academy members shall have been encouraged to suggest nominees to the committee prior to the Committee submitting its report.

Section 3. *Election Procedures*. Election shall be by secret mail ballot. The Secretary-Treasurer shall prepare a printed ballot that bears all names submitted by the Nominating Committee, that contains a brief biography of each candidate, and that has space for write-in candidates for each office. This ballot is to be mailed to all members no later than 1 November. Each member wishing to vote must return the marked ballot in a sealed signed envelope to the Secretary-Treasurer postmarked not more than thirty days after the ballots were mailed out to members. The President shall appoint tellers, who shall count the ballots that have been received by the Secretary-Treasurer and the tellers shall present the results in writing to the President. A plurality of the votes cast shall be necessary to elect and in the case of a tie vote, the President shall cast the deciding vote. The results of the election shall be announced at the annual Business Meeting.

Section 4. *Term office*. A President-Elect shall be elected annually by the membership and the following years shall succeed automatically to President and Past President to constitute a three-year nonrenewable term. Three Councilors shall be elected by the membership to three-year, non-renewable terms on a rotating basis. All elected Council members shall take office at the end of the next annual Business Meeting following election and shall continue until relieved by their successors. Council is empowered to appoint and compensate a Secretary-Treasurer to successive three-year terms that commence with the beginning of the fiscal year.

Section 5. *Removal from office or position.* If for any reason any elected member of Council is unable to fulfill his/her duties, the Council member may be removed from office by two-thirds vote of Council. If for any reason the Secretary-Treasurer is unable to fulfill his/her duties, the Secretary-Treasurer may be relieved of all duties by a majority vote of Council.

Section 6. *Interim vacancies*. Should a vacancy occur in the Presidency, the Council by a majority vote shall appoint a member of the Academy able to coordinate the next annual meeting to fill the unexpired term. A retiring interim President shall succeed automatically to Past President. Should a vacancy occur in the Presidency-elect, the Council shall reassess and change the location of the coinciding annual meeting as necessary and then call for a special election by mail ballot. An interim vacancy in the Past-Presidency shall be filled by the most recently retired Past-President able to fill the duties of the Past-President. Persons appointed to fill the unexpired term of Secretary-Treasurer are expected to remain in the position for a minimum of three years. A vacancy in the office of Councilor shall be filled by a majority vote of Council until the following election at which time the interim Councilor may stand for a full three year nonrenewable term.

BYLAW 6. *Committees*

Section 1. *Standing Committees*. Standing committees shall include but not be limited to, the following: Editorial, Education, Denison Award, Necrology, Nominating, Resolution, Membership, and Audit Committees. The President shall appoint members of committees other than the Nominating and Audit Committees.

Section 2. *Editorial Committee*. The Editorial Committee shall consist of three regular members appointed to three year terms. The duties are explained in BYLAW 7 (Publications).

Section 3. *Education Committee*. The Education Committee shall consist of five regular members and two high school teachers appointed to five year terms. The Education Committee shall work with high school students and teachers in the state, in visitation programs, Science Talent Search programs, and other programs to stimulate an interest in science by the youth of the state. It shall operate the Junior Academy of Science program and administer the AAAS high school research program.

Section 4. *Denison Awards Committee*. The Denison Awards Committee shall consist of six regular members appointed to three year terms. The Denison Awards Committee shall have as its prime duty the judging of student research and paper competitions, both undergraduate and graduate, and any other similar competitions. The committee shall also maintain the criteria to be used in the judging and selection of papers, such criteria to be circulated to prospective competitors.

Section 5. *Necrology Committee*. The Necrology Committee shall consist of three regular members appointed to three year terms. The Necrology Committee shall report to the annual meeting on those deceased during the preceding year. Obituaries may be included in the minutes of the annual meeting and/or published in the Proceedings.

Section 6. *Nominating Committee*. The Nominating Committee shall consist of the five most recent past-presidents. The major duties of the Nominating Committee are listed in BYLAW 5 (*Appointment, Nomination and Election of Members of Council*). The Nominating Committee will also administer the selection process, develop a separate funding source for a monetary award, and develop, for Executive Committee approval, the criteria for the North Dakota Academy of Science Achievement Award.

Section 7. *Resolution Committee*. The Resolution Committee shall consist of three regular members appointed to three year terms. The Resolution Committee shall prepare such resolutions of recognition and thanks as appropriate for the annual meeting. Further, the Committee shall receive suggested resolutions for the membership and transmit such resolutions and the Committee recommendation to the membership.

Section 8. *Membership Committee*. T he Membership Committee shall consist of unlimited numbers of regular members appointed annually.

Section 9. *Audit Committee*. The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year.

Section 10. *State Science Advisory Committee*. The State Science Advisory Committee (SSAC) shall consist of five regular or emeritus members appointed to four year terms. The SSAC shall serve to direct questions of a scientific nature to the appropriate expert as requested, shall inform regional granting agencies and state and national science policymakers of its expertise and availability and shall counsel those agencies and persons upon their request. The SSAC shall adhere in particular to the guidelines described in Article V, Section 2 of the Constitution.

Section 11. *Ad hoc Committees*. The President may appoint such additional committees as may be needed to carry out the functions of the Academy. Ad hoc committees serve only during the tenure of the president who appointed them. Reports of ad hoc committees shall be presented to Council or to the annual meeting.

BYLAW 7. *Publications*

Section 1. *Editorial Committee*. Three regular members are appointed to the Editorial Committee for renewable three year terms. The Editorial Committee shall develop and recommend the Academy publication program and policies to the Council. It will assist the Editors of each official publication in reviewing manuscripts for those publications that include the *Proceedings*. Chairs of symposia will review manuscripts written for relevant symposia.

Section 2. *Managing Editor*. The Secretary-Treasurer shall serve as the

Section 3. *Editor*. Editors shall serve three year terms. The Editors shall edit all official publications of the

Academy including the *Proceedings*.

BYLAW 8. *Memorial Fund*

The Council of the Academy shall establish a J. Donald Henderson Memorial Fund and administer this fund so that the proceeds will be used to promote science in North Dakota.

BYLAW 9. *Fiscal Year*

The fiscal year of the North Dakota Academy of Science, for the purpose of financial business, shall be 1 January to 31 December.

BYLAW 10. *Achievement Award*

The Academy establishes the North Dakota Academy of Science Achievement Award to be given periodically to an Academy member in recognition of excellence in one or more of the following:
a. Nationally recognized scientific research.
b. Science education.
c. Service to the Academy in advancing its goals.
The Nominating Committee will administer the selection process, will develop a separate funding source for a monetary award, and will develop, for Council approval, the criteria for the award.

BYLAW 11. *Research Foundation*

The **North Dakota Science Research Foundation** is established as an operating arm of the Academy. The purposes of the Foundation are:
(1) to receive funds from grants, gifts, bequests, and contributions from organizations and individuals, and (2) to use the income solely for the making of grants in support of scientific research in the State of North Dakota. Not less than 50% of the eligible monies received shall be placed in an endowment from which only the accrued interest shall be granted.

The foundation shall be responsible for soliciting the funds for the purposes described. The Foundation funds shall be in the custody of the Secretary-Treasurer of the Academy and shall be separately accounted for annually. The Foundation Board of Directors shall be comprised of five members of the Academy, representing different disciplines. Members shall be appointed by the President of staggered five year terms. The chairperson of the Board shall be appointed annually by the President. The Board shall be responsible for developing operating procedures, guidelines for proposals, evaluation criteria, granting policies, monitoring procedures, and reporting requirements, all of which shall be submitted to the Executive Committee for ratification before implementation.

The Foundation shall present a written and oral report to the membership of the Academy at each annual meeting, and the Secretary-Treasurer shall present an accompanying financial report.

BYLAW 12. *Affiliations*

The Academy may affiliate itself with other organizations which have purposes consistent with the purposes of the Academy. Such affiliations must be approved by the Council and by a majority of those attending a regularly scheduled business meeting of the membership.

BYLAW 13. *Indemnification*

Section 1. Every member of the Council or employee of the North Dakota Academy of Science shall be indemnified by the Academy against all expenses and liabilities, including counsel fees, reasonably incurred or imposed upon him/her in connection with any proceedings to which he or she may be made part, or in which he or she may become involved, by reason of being or having been a member of the Council, or employee at the time such expenses are incurred, except in such cases wherein the member of the Council or employee is adjudged guilty of willful misfeasance or malfeasance in the performance of his or her duties. Provided, however, that in the event of a settlement of the indemnification herein shall apply only when the Council approves such settlement and reimbursement as being for the best interests of the Academy. The foregoing right of indemnification shall be in addition to and not exclusive of all other rights to which such members of the Council or employee may be entitled.

ACADEMY OFFICERS AND COMMITTEES

Executive Committee Membership
President
Past-President
President-Elect
Secretary-Treasurer (three-year term)
Councilors (three-year terms)

President
L. Keith Henry
Department of Pharmacology, Physiology & Therapeutics
501 N Columbia Rd, stop 9037
Grand Forks ND 58202
(701) 777-2295
keith.henry@med.und.edu

President-Elect
Jerzy Bilski
Math, Science and Technology
Rhoades Science Center 203
Valley City State University
Valley City, ND 58072
701-845-7453
jerzy.bilski@vcsu.edu

Past-President
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Michael_Bingle-Davis@cameco.com

Secretary-Treasurer
Paul Lepp (2010-2013)
Department of Biology
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Councilors
Christopher Beachy (2010-2013)
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500 University Avenue W
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Ronald Jyring (2010-2013)
Bismarck State College
PO Box 5587
Bismarck, ND 58506
701-224-5459
Ronald.Jyring@bsc.nodak.edu

Vacant

COMMITTEES OF THE NORTH DAKOTA ACADEMY OF SCIENCE

Executive Committee*
Editorial Committee*
Education Committee*
Denison Awards Committee*
Necrology Committee*
Nominating Committee*
Resolution Committee*
Membership Committee*
North Dakota Research Foundation Board of Directors*
Historian*

*indicates available openings

PAST PRESIDENTS AND THE LOCATIONS

OF THE ANNUAL MEETING OF THE NORTH DAKOTA ACADEMY OF SCIENCE

1909 M A Brannon	Grand Forks
1910 M A Brannon	Fargo
1911 C B Waldron	Grand Forks
1912 L B McMullen	Fargo
1913 Louis VanEs	Grand Forks
1914 A G Leonard	Fargo
1915 W B Bell	Grand Forks
1916 Lura Perrine	Fargo
1917 A H Taylor	Grand Forks
1918 R C Doneghue	Fargo
1919 H E French	Grand Forks
1920 J W Ince	Fargo
1921 L R Waldron	Grand Forks
1922 Daniel Freeman	Fargo
1923 Norma Preifer	Grand Forks
1924 O A Stevens	Fargo
1925 David R Jenkins	Grand Forks
1926 E S Reynolds	Fargo
1927 Karl H Fussler	Grand Forks
1928 H L Walster	Fargo
1929 G A Talbert	Grand Forks
1930 R M Dolve	Fargo
1931 H E Simpson	Grand Forks
1932 A D Wheedon	Fargo
1933 G C Wheeler	Grand Forks
1934 C I Nelson	Fargo
1935 E A Baird	Grand Forks
1936 LR Waldron	Fargo
1937 J L Hundley	Grand Forks
1938 P J Olson	Fargo
1939 ED Coon	Grand Forks
1940 J R Dice	Fargo
1941 F C Foley	Grand Forks
1942 F W Christensen	Fargo
1943 Neal Weber	Grand Forks
1944 E A Helgeson	Fargo
1945 W H Moran	Grand Forks
1946 J A Longwell	Fargo
1947 A M Cooley	Grand Forks
1948 R H Harris	Fargo
1949 R B Winner	Grand Forks
1950 R E Dunbar	Fargo
1951 A K Saiki	Grand Forks
1952 Glenn Smith	Fargo
1953 Wilson Laird	Grand Forks
1954 C O Clagett	Fargo
1955 G A Abbott	Grand Forks
1956 H B Hart	Jamestown
1957 W E Comatzer	Grand Forks
1958 W C Whitman	Fargo
1959 Arthur W Koth	Minot
1960 H J Klosterman	Fargo
1961 Vera Facey	Grand Forks
1962 J F Cassel	Fargo
1963 C A Wardner	Grand Forks
1964 Fred H Sands	Fargo
1965 P B Kannowski	Grand Forks
1966 Paul C Sandal	Fargo
1967 F D Holland, Jr	Grand Forks
1968 W E Dinusson	Fargo
1969 Paul D Leiby	Minot
1970 Roland G Severson	Grand Forks
1971 Robert L Burgess	Fargo
1972 John C Thompson	Dickinson
1973 John R Reid	Grand Forks
1974 Richard L Kiesling	Fargo
1975 Arthur W DaFoe	Valley City
1976 Donald R Scoby	Fargo
1977 Om P Madhok	Minot
1978 James A Stewart	Grand Forks
1979 Jerome M Knoblich	Aberdeen, SD
1980 Duane O Erickson	Fargo
1981 Robert G Todd	Dickinson
1982 Eric N Clausen	Bismarck
1983 Virgil I Stenberg	Grand Forks
1984 Gary Clambey	Fargo
1985 Michael Thompson	Minot
1986 Elliot Shubert	Grand Forks
1987 William Barker	Fargo
1988 Bonnie Heidel	Bismarck
1989 Forrest Nielsen	Grand Forks
1990 David Davis	Fargo
1991 Clark Markell	Minot
1992 John Brauner	Grand Forks
1993 John Brauner	Jamestown
1994 Glen Statler	Fargo
1995 Carolyn Godfread	Bismarck
1996 Eileen Starr	Valley City
1997 Curtiss Hunt	Grand Forks
1998 Allen Kihm	Minot
1999 Joseph Hartman	Grand Forks
2000 Mark Sheridan	Moorhead, MN
2001 Ron Jyring	Bismarck
2002 Jody Rada	Grand Forks
2003 Richard Barkosky	Minot
2004 Anna Grazul-Bilska	Fargo
2005 Holly Brown-Borg	Grand Forks
2006 Andre Delorme	Valley City
2007 Chris Keller	Minot
2008 Van Doze	Grand Forks
2009 Birgit M Prüβ,	Fargo
2010 Paul W. Lepp	Minot
2011 Lyle Best	Belcourt
2012 Michael A. Bingle-Davis	Wyoming
2013 Keith Henry	Grand Forks

MINUTES OF THE NORTH DAKOTA ACADEMY OF SCIENCE

ANNUAL BUSINESS MEETING 2012

No formal business meeting was held.

Meeting statistics: 29 Registered attendees
10 Professionals
6 Graduate Students
13 Undergraduate Students
0 Vendors

We had 5 professional talks, 6 Denison graduate student talks and 5 Denison undergraduate student talks. The Denison Awards were presented by President Bingle-Davis. The award winners were:

Denison Undergraduate Award			Denison Graduate Award		
2nd runner-up	Ranelle Ivens	$100	2nd runner-up	Lalitha Kurada	$100
1st runner-up	Misty Hueser	$150	1st runner-up	Ty Lynnes	$150
Winner	Markus Wilson	$200	Winner	Steven Wu	$200

Respectfully submitted,
Paul Lepp, Secretary-Treasurer

Index of Authors

Lifetime Members

F. D. “Bud” Holland
Ron Jyring
Allen Kihm