

# North Dakota Academy of Science

---

Proceedings  
*of the*  
97th Annual Meeting

April 2005  
Volume 59



Proceedings of the North Dakota Academy of Science (ISBN 0096-9214)

---

Correspondence concerning subscriptions (standing orders), back issues, licensing, as well as instructions for authors and other related matters should be directed to:

Office of the Secretary-Treasurer  
North Dakota Academy of Science  
P.O. Box 7081  
Grand Forks, ND 58202-7081  
USA

Copyright © 2005 by the North Dakota Academy of Science

Typeset by *Terrifying Typesetting*, Merrifield, ND

Printed in the USA

# PROCEEDINGS OF THE NORTH DAKOTA ACADEMY OF SCIENCE

Volume 59

April 2005

---

NORTH DAKOTA ACADEMY OF SCIENCE  
*(Official State Academy; Founded: December 1908)*

2004-2005

## OFFICERS AND MEMBERS OF THE EXECUTIVE COMMITTEE

President ..... Holly Brown-Borg, University of North Dakota  
President-Elect ..... Andre Delorme, Valley City State University  
Past President ..... Anna T. Grazul-Bilska, North Dakota State University  
Secretary-Treasurer..... Jon A. Jackson, University of North Dakota  
Councilors ..... Chris Keller, Minot State University  
Siegfried Detke, University of North Dakota

## EDITORS

Jon A. Jackson ..... University of North Dakota School of Medicine  
Siegfried Detke ..... University of North Dakota School of Medicine

97th Annual Meeting

April 28-29, 2005

Grand Forks, North Dakota

## 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 8– x 11-inch format. Produced from camera-ready copy submitted by authors, it was distributed at the annual meeting. Commencing with Vol. 51 submissions were on computer disk; the *Proceedings* was then assembled with desktop publishing software. This approach allows the Editor to format papers so as to assure the *Proceedings* a consistent look. This methods also produces an electronic copy of the *Proceedings*; the Secretary-Treasurer has the capability to generate electronic copies of past issues.

## VOLUME 59 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 91.

## Symposia Communications

Commencing with the 88th Annual Meeting [Vol. 50], Symposia presenters at annual meetings have had opportunity to contribute full-length articles or multiple-page contributions, thus providing much greater depth and coverage than that ordinarily possible. Speakers have presented educationally-oriented lectures and workshop discussions, and have still provided rigorous, more technical professional papers to the *Proceedings*.

## Collegiate and Professional Communications

Each Collegiate and Professional presentation at the annual meeting is represented by a Communication. Designed as more than a typical abstract but less than a full paper, Communications report results and conclusions, and permit sharing of important data and conclusions. Crucially, they provide for timeliness and ease of production.

## Constitution and Bylaws

This issue of the *Proceedings* also contains the Constitution and Bylaws of the Academy, a list of officers and committee members. We're working on developing a NEW list of dues-paying members of the Academy (we'd appreciate your help in building and adding to this list with names of new and prospective members), a listing of past presidents of the Academy, and an index of presenters and paper authors. Copies of the financial statement and the unapproved minutes from last year's annual business meeting will be available at the meeting as appendices A & B, respectively.

## 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 will accompany 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. The President of the Academy also wishes to sincerely thank Drs. Barbara Shukitt-Hale and Dominic Desiderio, who served as honored guest speakers at this year's meeting.

Holly Brown-Borg  
President

Jon A. Jackson  
Secretary-Treasurer  
*Proceedings* Editor

Communications – Undergraduate ..... 7

Communications – Graduate..... 27

Communications – Professional ..... 61

Constitution of the North Dakota Academy of Science ..... 77

Minutes (Unapproved) of the 2004 Annual Business Meeting ..... 87

Academy Officers and Committees..... 89

Past Presidents and Locations of the Annual Meetings ..... 90

Author Index ..... 91

Statement of Financial Status..... appendix A

UNDERGRADUATE COMMUNICATIONS  
IN THE  
A. RODGER DENISON COMPETITION

## SCHEDULE OF PRESENTATIONS — Badlands and Governors Rooms — UND Memorial Union

**Badlands Room (Session A) — session will be chaired by A. Rodger Denison Competition judges**

- 1:00 Mindy Anderson (VCSU) *COMPARING SEED GERMINATION IN WESTERN PRAIRIE FRINGED ORCHID (PLATANThERA PRAECLARA SHEVIAK AND BOWLES) SEEDS PLANTED IN GRAZED AND UNGRAZED PLOTS*
- 1:20 Shanna Mazurek (MiSU) *HIGH MEMBRANE POTENTIAL SENSITIVITY TO HYDROQUINONE BY ROOT CORTEX CELLS OF COMMON BEAN (PHASEOLUS VULGARIS)*
- 1:40 Michelle Reinholdt (MiSU) *IS TOPOISOMERASE II IMPLICATED IN TRANSLOCATIONS INVOLVING THE MLL GENE?*
- 2:00 Sarah Boese (UND) *ALPHA-2 ADRENERGIC RECEPTOR MODULATION OF HIPPOCAMPAL CA3 NETWORK ACTIVITY*
- 2:20 Wendy Arndt (NDSU) *EFFECTS OF NUTRIENT RESTRICTION AND DIETARY SELENIUM ON CELLULAR PROLIFERATION IN FETAL OVARIAN FOLLICLES OBTAINED FROM SHEEP IN LATE PREGNANCY*
- 2:40 Ashley Zimmer (UND) *SEXUAL BEHAVIOR AND EDUCATION INTERVENTION: ASSESSMENT OF EFFECTIVENESS*
- 3:00 Sarah Dahl (VCSU) *DETERMINING SEED PRODUCTION RATES IN THE FEDERALLY LISTED THREATENED PLANT, THE WESTERN PRAIRIE FRINGED ORCHID (PLATANThERA PRAECLARA SHEVIAK AND BOWLES)*
- 3:20 Shawn Bruce (MiSU) *BURROWING WITH A KINETIC SNOUT IN A SNAKE (ELAPIDAE: ASPIDELAPS SCUTATUS)*
- 3:40 Janel Richter (MiSU) *VARIATION IN METAMORPHIC TIMING CAN BE INDUCED BY VARIATION IN DENSITY WITHOUT DIFFERENCES IN GROWTH RATE IN THE FROG, RANA UTRICULARIA*

**Governors Room (Session B) — session will be chaired by A. Rodger Denison Competition judges**

- 1:00 Shannon Heinle (UND) *CLINOPYROXENE, ORTHOPYROXENE, AND OLIVINE CHEMISTRIES IN ULTRAMAFIC TO MAFIC XENOLITHS*
- 1:20 Carl Jungberg (MaSU) *DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) THROUGH THE APPLICATION OF FLUORESCENT LIFETIME PROBES*
- 1:40 Kayla Fjeldahl (MiSU) *DEPTH PROFILE STUDY OF THE COMPOSITION OF URINARY STONES AND CRYSTALS FROM CANINES AND FELINES USING X-RAY DIFFRACTION*
- 2:00 Cory Mattern (MiSU) *ON THE FORMATION OF BROWN MILLERITE VIA THERMAL ROUTES*
- 2:20 Adam Brayko (MiSU) *MICROABSORPTION OR ABSORPTION CONTRAST IN 'REAL' MATERIALS*
- 2:40 OPEN
- 3:00 Katheryn Junglas (MiSU) *THE ASSESSMENT OF THE NORTHERN LEOPARD FROG (RANA PIPIENS) POPULATION AT UPPER SOURIS WILDLIFE REFUGE*
- 3:20 Kenn Rose (MiSU) *THE EFFECTS OF LIGHT CYCLE ON METAMORPHOSIS IN SALAMANDERS*
- 3:40 Jaime Nett (MiSU) *STRIKE KINEMATICS IN DEATH ADDERS: CONVERGENCE WITH VIPERS?*

COMPARING SEED GERMINATION IN WESTERN PRAIRIE FRINGED ORCHID (*PLATANATHERA PRAECLARA* SHEVIK AND BOWLES) SEEDS PLANTED IN GRAZED AND UNGRAZED PLOTS

Mindy Anderson\*, Bridget O'Brien, and Bonnie Alexander  
Department of Biology, Valley City State University, Valley City

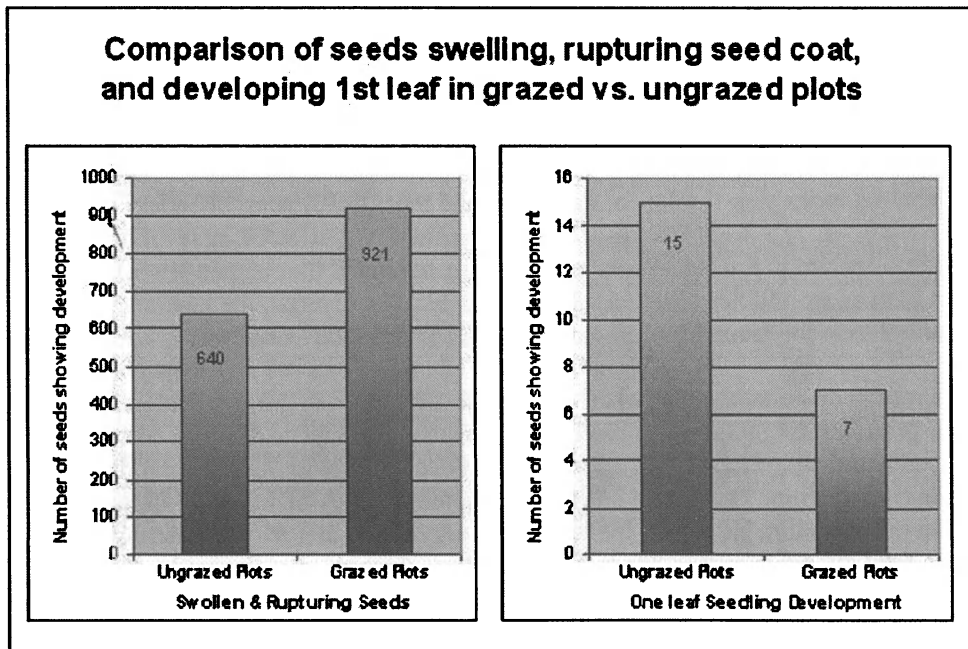
The western prairie fringed orchid (*Platanthera praecularis* Sheviak and Bowles) of the family Orchidaceae has been known to inhabit the Sheyenne National Grasslands in southeastern North Dakota for years. The plant has been federally listed as threatened since 1989 and preserving this remnant population of the orchid is a high priority. The impact of current management regimes, particularly cattle grazing, on the orchid population is unknown.

This study was designed to collect data on the impacts of grazing on the orchid population in the Sheyenne National Grassland. The hypothesis for this study was that cattle grazing in orchid habitat may create disturbances that may improve orchid seed germination (1). Study objectives were to determine stages of seed germination and document numbers of germinating seeds, protocorms, and seedlings in two grazed versus two ungrazed plots.

Seed packets enclosing 0.0005 grams of seeds (about 400-500 seeds) were constructed using plankton netting and plastic slide cases (2). In October of 2003 approximately 10 packets were placed at random in each of two ungrazed swales and another 10 packets in each of two grazed swales, with each packet buried 1 centimeter deep. Seed packets were retrieved in October of 2004 and each packet's contents were transferred to agar plates for examination under a dissecting microscope. Developmental stages leading up to the young seedling stage were assessed and documented.

Results showed 11% of seeds in both grazed and ungrazed plots achieved some development, whereas 89% showed none. Observable differences were evident but z-tests of proportions between the two samples for four developmental categories showed no significance at the 95% confidence level.

Since seed germination is only a small part of the *P. praecularis* lifecycle, additional studies are critical to further understand the impacts of grazing on the orchid population.



1. Bowles M.L. *Natural Areas Journal* 3, 14-37, 1983.

2. Rassmussen HN, Whigham DF. *American Journal of Botany*, 80:1374-1378, 1993.



EFFECTS OF NUTRIENT RESTRICTION AND DIETARY SELENIUM ON CELLULAR  
PROLIFERATION IN FETAL OVARIAN FOLLICLES OBTAINED FROM SHEEP IN  
LATE PREGNANCY

Wendy J. Arndt<sup>1</sup>, Kimberly A. Vonnahme<sup>1,3</sup>, Ewa Borowczyk<sup>1</sup>, Pawel P. Borowicz<sup>1,3</sup>, Marcy Ward<sup>1,3</sup>, Joel S. Caton<sup>1,3</sup>, Dale A. Redmer<sup>1,2,3</sup>, Lawrence P. Reynolds<sup>1,2,3</sup> and Anna T. Grazul-Bilska<sup>1,2,3</sup>

<sup>1</sup>Department of Animal and Range Sciences, <sup>2</sup>Cell Biology Center and <sup>3</sup>Center for Nutrition and Pregnancy, North Dakota State University, Fargo, ND

Hypertrophy and hyperplasia are the major processes for tissue growth and development during fetal growth. The fetal ovaries represent a type of tissue which grows rapidly and express high cellular proliferation rates. During folliculogenesis in the fetus, primordial follicles develop to primary follicles, then secondary and antral follicles. Selenium (Se) is a mineral that has diverse biological functions. Selenium affects cellular proliferation in cancer tissue as well as in digestive tract and placenta. It has been demonstrated that supranutritional levels of selenium and selenium source (organic vs. inorganic) may affect organ and cell growth. Selenium has been shown to reduce cell proliferation and angiogenesis in colon, prostate and lung cancers. Angiogenesis is vital for rapid growth and development of growing tissues.

This experiment was designed to determine if maternal consumption of differing levels of energy and selenium intake impacts fetal ovarian cell proliferation. Sheep (n=36) were fed a maintenance (2.12 Mcal/kg) or an energy restricted (60% of maintenance) diet with high selenium (81.5 µg/kg body weight) or normal selenium (7.4 µg/kg body weight) concentration from 21 days before breeding to day 135 of pregnancy. At slaughter on day 135 of pregnancy fetal ovaries were collected and fixed. Ovaries (n=3-6/nutrition treatment) were sectioned and then stained for the presence of proliferating cell nuclear antigen (PCNA; a marker of proliferating cells). To determine the labeling index (proportion of proliferating cells), digital images of the tissues were taken and analyzed using a computerized image analysis program. The number of proliferating and non-proliferating cells was determined for granulosa and theca cells of secondary (n=99 total) and antral (n=45 total) follicles.

The labeling index was similar for granulosa and theca cells from secondary or antral follicles (overall  $21.0 \pm 0.8\%$  vs.  $21.9 \pm 0.9\%$ , respectively), and was not affected by level of energy or selenium in the diet. High selenium in the maintenance but not the restricted diet tended ( $P < 0.1$ ) to decrease labeling index in ovarian follicles ( $23.0 \pm 1.4\%$  vs.  $20.6 \pm 1.1\%$ ). Overall, the labeling index was greater ( $P < 0.01$ ) for antral than for secondary follicles ( $24.1 \pm 1.1\%$  vs.  $20.2 \pm 0.7\%$ ).

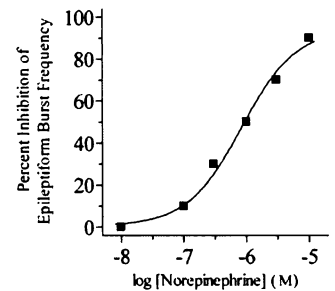
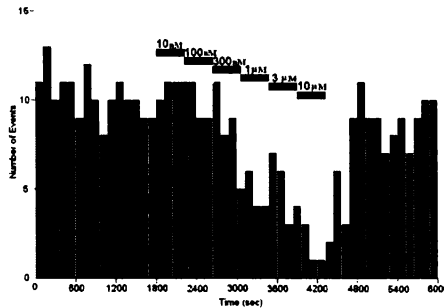
These results demonstrate that when the fetal follicle reaches the antral stage of development, the rate of cellular proliferation increases in theca and granulosa cell layers. The rate of cellular proliferation in secondary and antral follicles was not affected by level of energy in the maternal diet but may be affected by dietary selenium. These data indicate that selenium may be involved in regulation of fetal ovarian cellular proliferation. However, the level of energy in the diet may not be important for cellular proliferation in fetal ovaries, perhaps due to compensatory mechanisms.

## ALPHA-2 ADRENERGIC RECEPTOR MODULATION OF HIPPOCAMPAL CA3 NETWORK ACTIVITY

Sarah J. Boese\*, Jacob D. King, Christopher W.D. Jurgens, Sally J. Pyle<sup>1</sup>, James E. Porter, Van A. Doze  
 Department of Pharmacology, Physiology & Therapeutics and <sup>1</sup>Department of Biology  
 University of North Dakota, Grand Forks, ND 58202-9037, USA

Norepinephrine (NE) has demonstrated excitatory and inhibitory properties; however, the specific pharmacology of these actions of NE has not been clearly established. To address this, we studied the effect of NE on the frequency of hippocampal CA3 network burst activity in rat brain slices. Frequency changes of burst discharges in response to NE were biphasic; low concentrations increased the number of bursts, while higher concentrations reduced their frequency (data not shown), suggesting the involvement of multiple adrenergic receptors (ARs). This hypothesis was confirmed when, in the presence of  $\alpha$ -AR blockade, increasing concentrations of NE caused a monophasic decrease in frequency of these events (see frequency histogram right). Concentration-response curves constructed from this data revealed a

sigmoidal curve that was best-fit by a one-site model, suggesting that perhaps only a single AR type was involved (see curve right). To further determine which ARs were involved, we first examined the effects of a single concentration of NE when applied alone or in the presence of either  $\gamma$  or  $\alpha$ -AR blockade (see Table). NE caused a modest



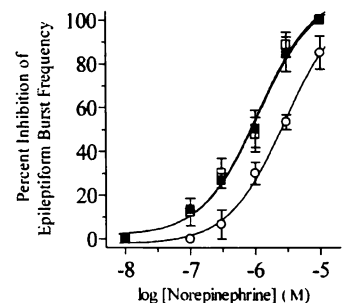
reduction in the frequency of burst discharges. However, the inhibitory effect of NE was significantly more effective following pretreatment with pindolol, a  $\alpha$ -AR blocker (-87.5% vs. -35.4%,  $P < 0.01$ ). In contrast, in  $\gamma$  AR blockade with phentolamine, a selective  $\gamma$  AR antagonist, NE significantly increased activity. These results suggest that the excitatory and inhibitory effects of NE on network activity were mediated via  $\alpha$  and  $\gamma$  ARs, respectively. Antagonists selective for  $\gamma_1$  or  $\gamma_2$  ARs were then used to determine which type of  $\gamma$  AR was involved. While the  $\gamma_1$  AR antagonists prazosin and terazosin did not alter the NE concentration-response curve, the  $\gamma_2$  AR antagonists RS79948 and RX821002 inhibited NE's inhibitory effects (see figure right: NE concentration-response curves in

Adrenergic Receptor Agonists & Antagonists	Control Frequency (Hz)	Treatment Frequency (Hz)	Maximal % Change (statistical significance)
Norepinephrine (10 $\mu$ M)	0.079 $\pm$ 0.006 (n = 10)	0.055 $\pm$ 0.012 (n = 10)	- 35.4 $\pm$ 13.8 (P < 0.05)
Norepinephrine (10 $\mu$ M) + Pindolol (3 $\mu$ M)	0.068 $\pm$ 0.008 (n = 7)	0.009 $\pm$ 0.003 (n = 7)	- 87.5 $\pm$ 4.0 (P < 0.005)
Norepinephrine (10 $\mu$ M) + Phentolamine (10 $\mu$ M)	0.079 $\pm$ 0.008 (n = 7)	0.118 $\pm$ 0.011 (n = 7)	+ 55.6 $\pm$ 13.5 (P < 0.0005)
Norepinephrine (10 $\mu$ M) + Pindolol (3 $\mu$ M) + CGP55845 (10 $\mu$ M)	0.067 $\pm$ 0.007 (n = 5)	0.012 $\pm$ 0.007 (n = 5)	- 84.0 $\pm$ 10.1 (P < 0.005)

control (-), with prazosin (-), or in both RS79948 (-) and prazosin). Furthermore, NE's inhibitory action persisted when all GABA-mediated inhibition was blocked. This data indicates that, under conditions of impaired GABAergic inhibition, the excitatory and inhibitory effects of NE on hippocampal CA3 network activity is mediated via  $\alpha$  and  $\gamma_2$  ARs, respectively. These results further suggest that the mechanism for the  $\gamma_2$  AR-mediated inhibitory effects does not involve the GABAergic system.

#### Acknowledgements

We would like to thank Karen L. Cisek for help with the experiments. Supported in part by North Dakota EPSCoR through NSF grant EPS-0132289 (VAD), NSF CAREER award 0347259 (VAD), and NIH grant 5P20RR017699 from the COBRE program (JEP, VAD).



## MICROABSORPTION OR ABSORPTION CONTRAST IN 'REAL' MATERIALS

Adam Brayko\* and Ryan S. Winburn

Division of Science, Minot State University, 500 University Ave. W., Minot, ND 8707

In using X-ray diffraction and the Rietveld method, there are a number of factors that can influence the results. One such factor is what has historically been termed microabsorption. When accounted for, microabsorption has typically been corrected for using the Brindley correction. Previous studies have shown that the Brindley correction may not be representing what is actually happening in materials. This study examined the potential for microabsorption in 'real' samples, such as coal fly ash.

Mixtures resembling Class F fly ashes were prepared. Each mixture contained varying amounts of quartz ( $\text{SiO}_2$ ), mullite ( $\text{Al}_6\text{Si}_2\text{O}_{11}$ ), hematite ( $\text{Fe}_2\text{O}_3$ ) and magnetite ( $\text{Fe}_3\text{O}_4$ ). The individual components were analyzed for crystallinity using conventional diffraction with SRM 676 as an internal standard. Those materials with absorption coefficients sufficiently different than SRM 676 were also analyzed using an alternate internal standard ( $\text{Cr}_2\text{O}_3$  for the iron containing phases and  $\text{ZnO}$  for the mullite). The mixtures were designed to have a variance in average linear absorption coefficient. Initially four mixtures were analyzed and the relative error for each phase was examined as a function of the average linear absorption coefficient of the mixture. The mixtures were analyzed as prepared and also mixed with silica gel to simulate the amorphous component found in fly ashes and analyzed.

Each mixture was combined with three different internal standards; SRM 676, a small particle size rutile (~0.4mm) and a larger particle size rutile (~1.0mm). This combination of internal standards were chosen because (a) SRM 676 is the 'standard' internal standard and is being certified for crystallinity, (b) the rutile internal standards have an absorption coefficient between that of the highest (magnetite) and lowest (quartz) absorbing materials in the samples and (c) different particle sizes may yield information of particle size effects on microabsorption.

Data were collected using a PANalytical X'Pert MPD system with X'Cellerator detector and graphite monochromator tuned for Cu radiation. The scans were taken from 20-80° 2 $\theta$  using a 0.0167° step size and counting for the equivalent of 200 seconds at each 'step' for a conventional detector (actual scan time was 1 hour 38 minutes). The quantitative analyses were performed using the Rietveld method as implemented in GSAS.

DETERMINING SEED PRODUCTION RATES IN THE FEDERALLY LISTED THREATENED PLANT, THE WESTERN PRAIRIE FRINGED ORCHID (*PLATANATHERA PRAECLARA* SHEVIK AND BOWLES)

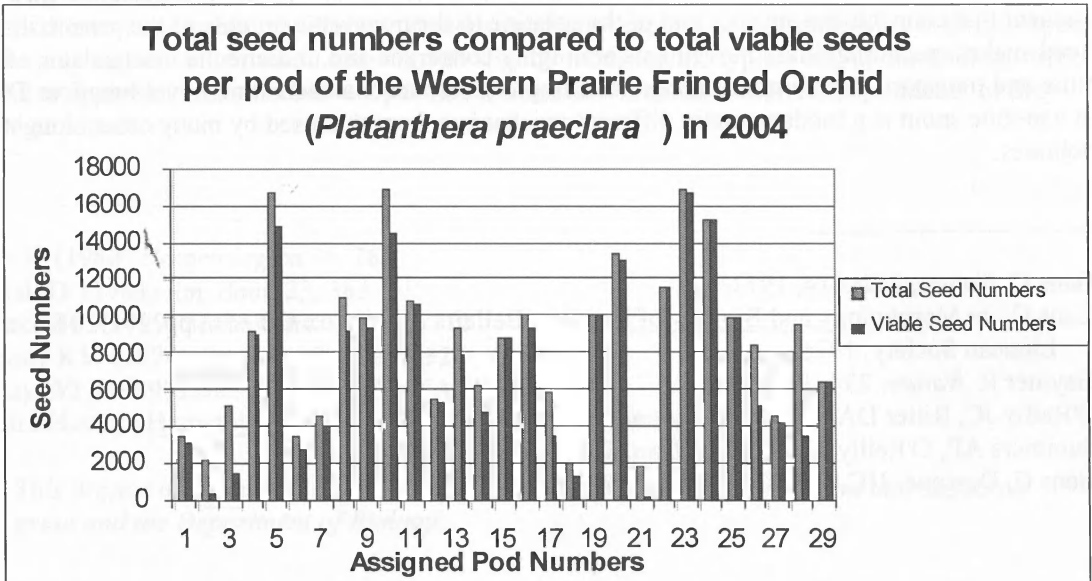
Sarah Dahl\*, Heather Jorissen, and Bonnie Alexander  
Department of Biology, Valley City State University, Valley City

The federal government listed the western prairie fringed orchid (*Platanthera praeclara* Sheviak and Bowles) as a threatened species in 1989. It is North Dakota's only federally listed plant species. There is little knowledge about the number of seeds produced by the orchid and the number of viable seeds produced per pod. The production of viable seeds is of importance in the survival and regeneration of the western prairie fringed orchid population in North Dakota (1). The number of seeds produced may influence the stability of the orchid population and the establishment of new orchid plants in the Sheyenne National Grassland (2). Ninety percent of the North Dakota orchid population grows in the Sheyenne National Grassland in Richland and Ransom counties.

This study examines data collected on the total number of seeds produced per pod and the number of viable seeds per pod. Data from this study can be used by managers to preserve the orchid population and by researchers in planning research and using population viability models.

Under a special permit, 29 mature orchid seed pods were collected in August and September of 2004. Seeds were examined under dissecting scopes at 30 X magnification. Seeds from each pod were counted and examined for viability (the presence of an embryo). The number of embryo-containing seeds and seeds with no embryos were recorded.

The total number of seeds per pod ranged from 1,938 to 17,009, with a mean of 8,643 (sd 4,452). The percent of seeds containing embryos ranged from 11% to 100%, with a mean of 80%. The total number of seeds found in pods in this study differed from previous estimations (Sieg, pers. com. 2004, Erickson, pers. com. 2004). The number of viable seeds per pod differed from previous research data as well (3, 4). It is possible that not only do seed numbers vary between pods but probably vary extensively from season to season and location to location.



1. Armstrong K, Fritz Dm, Miller P, Beyers O (Eds.) 1997. Population and habitat viability assessment for the western prairie fringed orchid at Eugene Mahoney State Park. Final report CBSG, Apple Valley, MN 128 p.
2. Sieg CH, King RM. *American Midland Naturalist* 134(2):307-323, 1995.
3. Sharma J, *Ph.D. thesis*, University of Missouri-Columbia. 2002.
4. Erickson AM, *Master of Science thesis*; North Dakota State University, 2003.

BURROWING WITH A KINETIC SNOOT IN A SNAKE (ELAPIDAE: *ASPIDELAPS SCUTATUS*)

Alexandra Deufel and Shawn D. Bruce\*

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

Few elongate, fossorial vertebrates have been examined for their mechanics of burrowing. Of those that have, all were found to use an akinetic, reinforced skull to push into the soil, powered mostly by contractions of trunk muscles (1-6). In contrast, we have found that the shield-nosed cobra (*Aspidelaps scutatus*, a snake of the family Elapidae) burrows using a kinetic snout that is independently mobile with respect to the rest of the skull. In shield-nosed cobras the head is short, broad, and bears a greatly enlarged rostral scale, which has protruding, vertically-oriented, shovel-shaped lateral edges.

Burrowing mechanics have been studied in few vertebrates because burrowing occurs underground and is difficult to see. We used an aquarium mounted over a mirror to get an inferior view of burrowing. The substrate was washed sand, a friable mixture of particle sizes ranging from 5mm to less than 1mm in diameter with a low content of organics and a water content varying between 2.6 and 5.2 percent. Burrowing was recorded with a SONY Digital8 Handycam and analyzed frame by frame.

Two mechanisms of burrowing are used: 1) anteriorly directed head thrusts during which the body is thrown into a number of loose curves, pushed against the walls of the tunnel to create friction, and the head is extended from a gathered position of the anterior trunk, and 2) side-to-side shovelling using the rostral scale during which the rostral scale and snout are first passively deflected by the material being shovelled aside and the snout then returns to its rest position before continuing to move in the same direction as the head. The premaxilla, to which the rostral scale is attached, lacks any direct muscle attachments. Rostral scale movements are powered by retractions of the palato-ptyergoid bar mediated by a ligament that connects the anterior end of the palatine to the transverse process of the premaxilla. In derived snakes, palatamaxillary movements are highly conserved and underlie the mechanisms of prey capture and transport. *Aspidelaps scutatus* has co-opted these mechanisms for a novel function. Digging with a mobile snout is a fundamentally different mechanism from that used by many other elongate vertebrates.

---

1) Gans C *Nature*, 242: 414, 1973.

2) Gans C, in Morphology and Biology of Reptiles, Bellairs A and Cox CB eds., pp. 191-204. London: Linnean Society, 1976.

3) Gaymer R *Nature*, 234: 150, 1971.

4) O'Reilly JC, Ritter DA, Carrier DR *Nature*, 386: 269, 1997.

5) Summers AP, O'Reilly JC *Zool. J. Linn. Soc.* 121; 65, 1997

6) Gans C, Dessauer HC, Baic D *Science*, 199: 189, 1978.

## STRIKE KINEMATICS IN DEATH ADDERS: CONVERGENCE WITH VIPERS?

Alexandra Deufel and Jaime L. Nett\*

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

Death adders (*Acanthophis*) are terrestrial elapids who superficially resemble vipers. They are ambush hunters with large, triangular heads, slender necks, and stout bodies (1). Elapids and vipers differ significantly in cranial musculoskeletal morphology. Differences relating to prey capture kinematics exist mainly in maxillary morphology and relationships, palato-pterygoid bar connections, and the dorsal constrictor muscles (2-5). Vipers, even though they are basal colubroids, are considered to have the most optimized prey capture apparatus, whereas elapids are less modified compared to non-venomous snakes and achieve their greatest diversity where vipers are absent (6). We examined prey capture kinematics and their morphological correlates in *Acanthophis antarcticus* and compared them to published reports of terrestrial vipers.

Strikes to live prey were recorded with a video camera at 30 frames/sec as well as 250 frames/sec and analyzed frame by frame. Analysis included measurements of distance from prey at strike initiation in snake head lengths, and several angle measurements: snout-braincase, fang axis-braincase, braincase-neck, and gape.

Prey capture in *Acanthophis* only superficially resembles vipers. Prey capture differs from vipers in that *Acanthophis* initiates strikes when prey is very close, almost never releases prey after the strike, and has lesser maxillary mobility. Like in vipers and other hydrophiine elapids, but not elapines, the palatine of *Acanthophis* has no connections to the maxilla, choanal passage, or snout, potentially allowing greater excursions of the jaw apparatus. The origin of the protractor pterygoideus is relatively anterior compared to other elapids but does not approach the extreme elongation seen in some vipers. Anterior origin of the muscle facilitates large palatomaxillary protraction, and may explain how *Acanthophis* begins to approach viper-like prey capture kinematics. *Acanthophis* slightly modified the typical elapid morphology which allowed it to approach but not achieve viper-like kinematics. Elapids remain less optimized for prey capture than vipers.

---

1) Shine R (1980) *Herpetologica* 36, 281

2) Cundall D (1983) *Am. Zool.* 23, 383

3) Fairley NH (1929) *Med. J. Aust.* March, 313

4) Kardong KV (1974) *Forma et functio* 7, 327

5) McKay WJ (1989) *Proc. Linn. Soc. NSW* 4, 893

6) Pough FH *et al.* *Herpetology*; Upper Saddle River, NJ: Prentice Hall, 2001.

*This project was funded by a Minot State University Faculty Development and Research grant and the Department of Biology.*

## DEPTH PROFILE STUDY OF THE COMPOSITION OF URINARY STONES AND CRYSTALS FROM CANINES AND FELINES USING X-RAY DIFFRACTION

Kayla Fjeldahl\* and Ryan S. Winburn

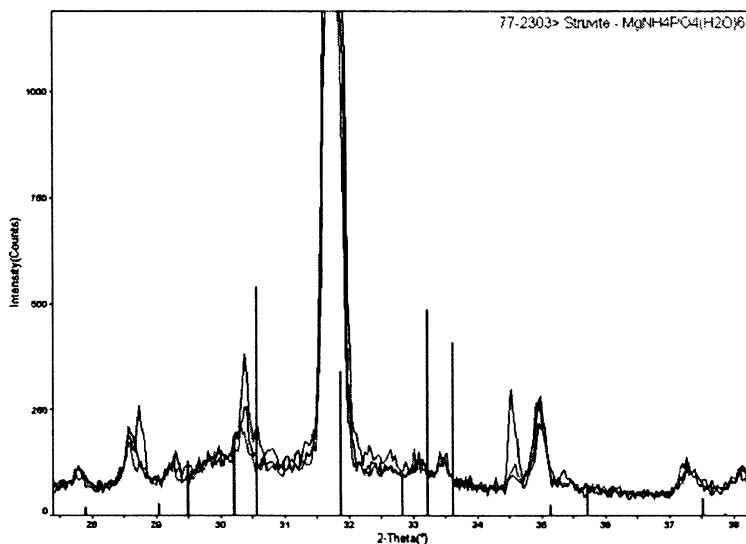
Division of Science, Minot State University, 500 University Ave. W., Minot, ND 58707

Typically, X-ray diffraction (XRD) analyses are conducted on finely crushed powders rather than whole objects. Using XRD, we have attempted to conduct a depth profile study on spherical or irregularly shaped bladder stones taken from canines and felines. Canines and felines share many of the more common medical problems faced by humans. While their bodies are more vulnerable to slight changes of diet, environmental and activity, many of their diseases mimic those found in humans. Mainly due to pH, urinary crystals may arise and cause uncomfortable side effects when urinating. If urinary infections are allowed to progress, the accumulation of these crystals may result in the formation of a stone residing in the bladder, which are typically removed through surgical means.

Urinary stones were analyzed by two methods. First, for samples containing multiple stones, a powdered specimen was prepared by grinding either a whole stone or portion of it. The powder was then analyzed by conventional XRD. Second, depth profiling was conducted on whole stones. To accomplish this, the stone was first placed in an inverted sample cup and the stone height was adjusted using a vernier caliper, such that the stone's surface was sitting at the height of the goniometer center. The depth profiling was then undertaken using a series of omega scans (the angle of the incident X-ray to the sample surface is held constant and the detector is scanned through the appropriate  $2\theta$  range). Each subsequently higher angle penetrates deeper into the stone, giving an 'image' of the depth profile of the stone.

The stones were analyzed for phase composition using Jade 6.5 and the Powder diffraction file database. Analysis of the powdered specimens yielded the major phase(s) of the stones, which was typically struvite ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ). The analysis of the powdered specimen was typically necessary to provide the starting point for the analysis of the stones, as they tended to approach single crystals in the area being analyzed. This caused the peaks to show severe preferred orientation, sometimes at the complete exclusion of some diffracting planes. The series of scans for a given stone were analyzed simultaneously by eye, determining which peaks could be assigned to the major phase and which peaks were unaccounted for. A typical set of overlays is shown in Figure 1.

Figure 1. Overlays of sequential scans of a canine urinary tract stone. Vertical lines indicate peaks due to struvite. Note the peaks at  $28.7^\circ$  and  $35.4^\circ$ , which are unaccounted for in one scan.



Specimens with multiple phases were analyzed using the Rietveld method to determine relative weight percentages of each.

## CLINOPYROXENE, ORTHOPYROXENE, AND OLIVINE CHEMISTRIES IN ULTRAMAFIC TO MAFIC XENOLITHS

Shannon Heinle\* and Dexter Perkins

Department of Geology, University of North Dakota, Grand Forks, ND 58202

Xenoliths are rock fragments or inclusions that are torn from the sides of lava tubes and caught up in magma as it moves through Earth. Xenoliths provide samples of the deep parts of the lower crust and upper mantle. We analyzed several xenoliths from two sites, Kilbourne Hole, New Mexico, and Cima Volcanic Field in the Mojave Desert, California. Steam explosions due to heating of water-saturated sand and silt strata from rising basaltic magma formed Kilbourne Hole. Approximately 40 cinder cones characterize Cima Volcanic Field, where the magma is also of basaltic composition. A fundamental assumption of this study is that the Cima eruptions were not as rapid or dynamic as the Kilbourne Maar Formation. Therefore, Kilbourne xenoliths were brought to the surface more rapidly, whereas Cima xenoliths ascended at a slower pace.

Objectives of this study include: 1) determining mineral composition of olivine, clinopyroxene, and orthopyroxene in the xenoliths; 2) examining individual chemical zoning characteristics of orthopyroxene, clinopyroxene, and olivine; 3) comparing mineral chemistry and zoning differences between xenoliths from different eruption styles; and 4) use xenolith compositions to determine lithospheric mantle pressure and temperature.

Kilbourne xenoliths in this study consisted of pyroxenites, lherzolites and mafic granulites. Cima xenoliths were very similar lherzolites and pyroxenites. The most abundant minerals found were olivine, clinopyroxene, and orthopyroxene. Spinel, a minor component, was either Cr-rich or Cr-poor.

Thin section examination of the xenoliths revealed pyroxenes intermix with olivine. Olivines typically have high interference colors. The pyroxenes were generally large grained with clinopyroxene and showed higher interference colors. Some xenolith showed equigranular texture with almost uniform small grains. Spinel is present in small amounts and shows a deep coloration in plane polarized light. Cr-rich spinel did not appear any different from the Cr-poor spinel in thin section.

Microprobe analysis was centered on olivine, clinopyroxene, orthopyroxene and spinel. Analysis of clinopyroxene of xenoliths from both Kilbourne and Cima showed similarities among a majority of the xenoliths. A few individual xenoliths from both Cima and Kilbourne showed unique values. Orthopyroxene analysis revealed several high aluminum oxide grains in Kilbourne xenoliths, particularly samples 3KB70, 3KB18 and 3KB43. General comparison between Cima and Kilbourne, excluding the high aluminum samples, showed Kilbourne orthopyroxene with higher percentages of iron oxides and Cima samples with a higher percentage in magnesium oxide. Olivine also showed a distinction in regards to iron oxides and magnesium oxide content. Cima xenoliths showed a higher iron oxide content and Kilbourne showed a higher magnesium oxide component. All analyzed spinel showed some chrome content with the Cr-rich samples exhibiting as high as 20% chrome.

Mineral chemical zonation is generally a helpful way to determine the rate of equilibrium. On the bases of different ascent rates of the xenoliths, we hoped to find differences in zonation patterns. Unfortunately, we found very little compositional zoning in xenoliths from either site. Some zoning was found in clinopyroxene grains from Kilbourne samples 3KB59 and 3KB48. This zoning exhibited higher sodium oxide amounts in the core of the grain versus the rim.

Mineral assemblages provide a natural thermometer, a way to measure temperature. With the use of geothermal equations, Kilbourne xenoliths indicate a temperature between 950° C and 1050° C. Pressure estimates from the mineral assemblage and the presence of spinel place the xenoliths anywhere from 8 Kb-24Kb due to the lack of garnet found in the samples. Cima xenoliths are of a similar nature.



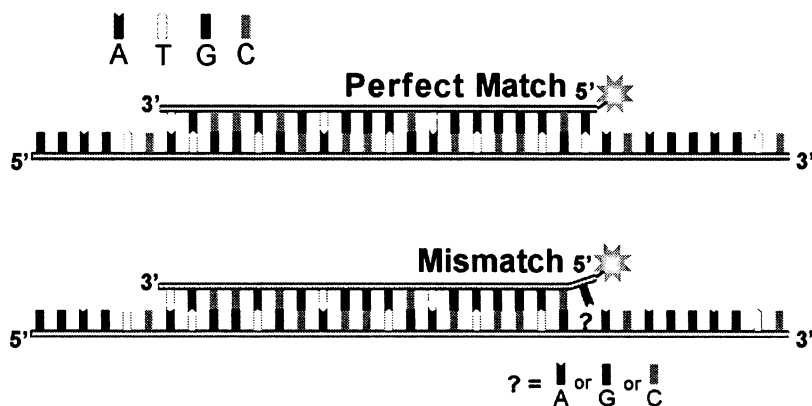
## DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) THROUGH THE APPLICATION OF FLUORESCENT LIFETIME PROBES

Carl E. Jungberg\* and Thomas P. Gonnella

Department of Chemistry, Mayville State University, Mayville, ND 58257

Our research group has implemented novel fluorescence measurement technology developed by our collaborators at Dakota Technologies, Inc. Fluorescence emission is excited by short pulses of light from a microchip laser and complete fluorescence decay waveforms are recorded for every excitation pulse. A wavelength-time matrix (WTM) consists of a series of fluorescence decay waveforms at multiple emission wavelengths. The information contained within the WTMs is relevant to many biomedical research applications, including DNA sequencing, SNP detection, multiplex PCR, and drug-target binding.

Our current research program is aimed at demonstrating that fluorescence lifetime methodology can dramatically simplify and improve current SNP detection techniques as well as allow for multiplexed SNP detection. Initial studies into developing a series of fluorescent SNP detection probes have been performed using the point mutation of the human  $\beta$ -globin gene known to cause sickle cell anemia as a model. These results will be presented and discussed.



*This research was supported by the North Dakota INBRE grant.*

THE ASSESSMENT OF THE NORTHERN LEOPARD FROG (*RANA PIFIENS*) POPULATION AT UPPER SOURIS WILDLIFE REFUGE

Katheryn M. Junglas\*

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

The *Rana pipiens* population has been decreasing in recent years throughout its range (1). It is important to determine the population status of *Rana pipiens* in North Dakota and to note any abnormalities. During the Summer 2004 I tried to find out if any Northern Leopard Frogs exhibited malformations similar to those observed in Minnesota, *e.g.*, extra/missing limbs, extra/missing digits, eyes (1). I also looked for evidence of feminized testes (2) and disease, *e.g.*, chytrid fungi (3), and evaluated sexual size dimorphism and sex ratios.

We evaluated areas that would be potential for holding frogs at Upper Souris National Wildlife Refuge (USNWF), by listening to frog calls. Two types of frogs were confirmed during the calling surveys; Boreal Chorus Frogs (*Pseudacris maculata*), and Northern Leopard Frogs (*R. pipiens*). Ten sites were originally scouted; yet only two ponds had prominent frog choruses. To examine the sites further, nitrate, pH, and phosphate samples were analyzed at the two main sites, there were very few tadpoles found, only two tadpoles were found, in the crossroads site; both fully metamorphosed into healthy juvenile frogs. We caught a total of 27 juveniles from the crossroads site, and 4 frogs from the inlet site (their sizes are comparable to juvenile sizes of metamorphic data (4) on *R. pipiens* from Quebec).

The frogs were euthanized, and underwent the clearing and staining process to assess their bone structure development. The cleared and stained animals were osteologically normal. The external morphology of the gonads appeared to be normal and was sufficient. Thirteen males and 20 females were found, which is not a significant deviation from a 1:1 ratio in metamorphosing tadpoles. The T-Test failed to indicate a significant sex variation in size (mm svl), and in site variation in metamorphic size.

Preliminary results indicate that the frog population in North Dakota exhibits a well-balanced sex ratio, healthy development of gonads, no malformations in the physical appearance or the bone structure, and a healthy snout to urostyle length.

- 
- 1) Lannoo M, *et al.* *Status and Conservation of Midwestern Amphibians*. Iowa City: Univ. Iowa Press, 1998.
  - 2) Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, Vonk A. *PNAS*, 99:5476-80, 2002.
  - 3) Lipps K *Conservation Biology*, 13:117-125, 1999.
  - 4) Gilbert M, Leclair R, Fortin R *J. Herpetology*, 28 (4), 465-470, 1994.

## ON THE FORMATION OF BROWNMILLERITE VIA THERMAL ROUTES

Cory Mattern\*, Sheri Y. Nelson, Gary McDaniel, and Ryan S. Winburn  
 Division of Science, Minot State University, 500 University Ave. W., Minot, ND 58707

The Rietveld method is a quantitative analysis tool for X-ray diffraction (XRD). It relies on known structures in order to quantify the weight percentage of diffracting materials. Solid solution phases are common in 'real life' materials; including fly ash and geologic samples. The fact that the exact composition/structure of a solid solution may not be known may potentially introduce error into the Rietveld refinement. In order to examine this, a series of mixtures containing the synthesized solid solution material brownmillerite,  $\text{Ca}_2(\text{Al,Fe})_2\text{O}_5$ , was to be made. However, contrary to literature reports, the synthesis of the brownmillerite phase was not straight forward. A systematic investigation of the optimal synthetic route is presented here.

Following the procedure of Neubauer *et al.*, (1), calcium carbonate ( $\text{CaCO}_3$ ), gamma-aluminum oxide ( $\gamma\text{-Al}_2\text{O}_3$ ) and iron (III) oxide ( $\text{Fe}_2\text{O}_3$ ) were thoroughly mixed using a mortar and pestle. The mixture was then placed in a furnace at 1150 °C for fifteen hours, grinding the sample every five hours. The sample was then analyzed for completion using XRD. The resulting material was not brownmillerite. The XRD pattern indicated starting material was still present and a compound with peaks in the general positions for brownmillerite. However, the peaks were shifted to slightly lower angles from those of brownmillerite. Qualitative search/match was able to provide a match for the new peak position, srebrodolskite,  $\text{Ca}_2\text{Fe}_2\text{O}_5$ . In light of this, a new synthesis was undertaken at 1200 °C, with specimens taken from the reaction vessel at various points over the course of the synthesis (0.5 hr, 1 hr, 2 hr, 3 hr, 4 hr, 24 hr, 48 hr, etc.) until the only material found by XRD was brownmillerite. The reaction quickly produced srebrodolskite (< 0.5 hr), with calcium aluminum oxide phases forming much more slowly (between 24-48 hours). After 48 hours it appears that the srebrodolskite phase begins to incorporate aluminum as evidenced by a shift in the peak positions towards those of brownmillerite. The calcium aluminum oxide phases slowly disappear over the next 72 hours until brownmillerite was the only product. The relative weight percentages as determined by Rietveld refinement for various times within the reaction period are shown in Table 1.

Table 1. Relative Weight Percentages of Phases During Synthesis of Brownmillerite at 1200 °C

Time Phase	hour	12 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Brownmillerite	4.7	20.8	26.8	38.0	54.4	68.5	100.0
$\text{Ca}_2\text{Fe}_2\text{O}_5$	62.7	48.3	41.2	38.8	29.2	24.4	-
$\text{Ca}_9\text{Al}_6\text{O}_{18}$	4.0	14.6	16.6	12.5	7.0	2.6	-
Mayenite	3.0	11.9	14.0	10.7	9.2	4.2	-
Lime	25.2	4.4	1.4	-	-	-	-
Hematite	0.3	3.2	-	-	-	-	-

The brownmillerite phase was then analyzed for crystallinity using the Rietveld method. Unfortunately, the crystallinity was only on the order of 30% for the various syntheses. In light of this, a systematic study of the thermal synthesis of brownmillerite was undertaken. The first part of the study, which is reported here, focuses on the effect of temperature and reaction mass on the (a) synthesis time and (b) crystallinity of the resulting brownmillerite.

1) Neubauer J, Sieber R, Kuzel H-J, Ecker M. *Cem. And Conc. Res.*, 26, 77-82, 1996.

HIGH MEMBRANE POTENTIAL SENSITIVITY TO HYDROQUINONE BY ROOT CORTEX CELLS OF COMMON BEAN (*PHASEOLUS VULGARIS*)

Shanna A. Mazurek\*, Morgan L. Grundstad, Joshua E. Seil, Richard R. Barkosky, Christopher P. Keller  
Department of Biology, Minot State University, Minot, North Dakota 58707

Many plant-derived phenolic compounds elicit phytotoxic responses in a broad range of plants (Einhellig, 2004). Earlier laboratory and greenhouse experimentation into the mechanisms of action of hydroquinone (HQ) has demonstrated that plants exposed to millimolar concentrations exhibit changes in plant-water balance, photosynthesis, and ultimately plant growth (Barkosky et al., 1999). Rhizosphere concentrations of HQ that are clearly injurious to plants in the field, however, are often much lower; in the micromolar range. It is reasonable to suspect that the concentration-dependant toxic effects of HQ result from alterations to the root plasma membrane in direct rhizosphere contact. Our hypothesis is that HQ-induced changes in physiology and growth are initiated at the root/soil water interface and should be evident as perturbations of root membrane potentials. To test this hypothesis, we monitored membrane potentials across root membranes of the common bean, *Phaseolus vulgaris*, exposed to acute applications of a range of HQ concentrations (0.1–M – 0.25mM) and, as a control, 10–M arbutin. Arbutin is the naturally occurring non-toxic monoglucoside of HQ.

Bean root tips from 6-day old seedlings were pre-incubated in 0.1 mM KCl, 1 mM CaCl<sub>2</sub>, and 1M Mes/BTP pH 6.0 for 1-8 hours, then mounted in a chamber, and perfused with the same medium. A microelectrode filled with 1 M KCl and connected to an electrometer (Electro 705; World Precision Instruments, Sarasota FL) and a chart recorder (or computerized recorder) was then used to impale a root cortical cell. Once a stable membrane potential was recorded at -100mV or lower for 10 minutes, the incubation medium flow was switched to include HQ.

Root membrane potentials typically responded immediately to exposure to the HQ solution. An initial small hyperpolarization (0-15 mV), generally reaching a minimum in 2-6 minutes, was followed by a larger (4-50 mV) sustained (>20 min) depolarization. In most cases the membrane potential showed little sign of recovering from the depolarization before the recording was lost (after up to 1 hour). The electrical response to HQ was dose responsive, with the magnitude of both the hyperpolarization and depolarization being smaller with lower concentrations. Both responses were still evident, however, even at 0.1 –M; the lowest concentration tested. The dose-response results correlate well with the chronic inhibitory effects of the various HQ concentrations on the bean plant growth (Mazurek et al., 2004) and therefore suggest that HQ-induced changes in root cell membrane ion transport have a causal relationship to long term plant injury.

---

Barkosky RR, Butler JL, Einhellig FA *J. Chem. Ecol.* 25(7): 1611-1621, 1999.

Einhellig FA. *in Allelopathy: Chemistry and Mode of Action of Allelochemicals*, 2004.

Mazurek SA, Barkosky RR, Keller CP *Proc. North Dakota Acad. Sci.* 58: 41, 2004.

IS TOPOISOMERASE II IMPLICATED IN TRANSLOCATIONS INVOLVING THE *MLL* GENE?

Michelle L. Reinholdt\*, Jessica A. Townsend, and Heidi J. Super  
Department of Biology, Minot State University, Minot, ND 58707

The Myeloid Lymphoid Leukemia (*MLL*) breakpoint cluster region is located on the long arm of chromosome 11. In this 8.3 kilobase (kb) section, specific, reciprocal translocations are common. A translocation occurs when two non-homologous chromosomes exchange DNA with one another. *MLL* translocations occur with more than 40 other chromosomal regions and often result in alteration of gene regulation. *MLL* translocations are also detected in some secondary leukemias developing from treatment of a primary tumor with topoisomerase II (topo II) inhibiting drugs.

The mechanism of breakage within the *MLL* region and partner genes is enigmatic. Several *in vitro* studies indicate that the region is highly susceptible to cleavage by topo II (1-3). Topo II, a nuclear protein that is essential for normal cellular processes such as replication, binds to double-stranded DNA cutting it to relieve supercoiling. The enzyme also reanneals the DNA to return it to normal structure and function. The preliminary hypothesis for this project is that topo II causes breaks in the *MLL* translocation breakpoint region and in *MLL* partner genes, resulting in leukemogenic translocations. Although one *MLL* translocation partner gene has shown susceptibility to breakage with topo II inhibiting drugs, such as etoposide, others remain to be tested. Our study focuses on the most frequent *MLL* partner, *AF4*.

We tested this hypothesis using a plasmid containing the *MLL* breakpoint region (BB9.0). It was grown in *Escherichia coli* (*E. coli*) cultures on Luria Bertani media containing ampicillin. The plasmid DNA was extracted and treated with etoposide, a topo II inhibiting agent. It was then digested with restriction enzymes, separated using gel electrophoresis, and transferred to a nitrocellulose membrane by a Southern blotting mechanism. The *MLL* breakpoint probe was selected and amplified using Polymerase Chain Reaction (PCR). The membrane was hybridized with the labeled probe and analyzed for DNA breakage using chemiluminescent detection. Three bacterial artificial chromosomes (BAC-E7, BAC-22, BAC-C8) located in a breakpoint fragment from AF4 (on chromosome 4) were investigated in a similar manner. BAC DNA was grown in *E. coli* cultures on Luria Bertani containing chloramphenicol and extracted. After treatment with etoposide it was digested with *Bam*HI, separated by electrophoresis, transferred to a nitrocellulose membrane, hybridized, and analyzed using chemiluminescent detection. We expect that etoposide treatment will lead to cleavage of the DNA. A germline band will appear in addition to shifted bands of different size fragments correlating with breakage.

- 
- 1) Aplan PD, Chervinsky DS, Stanulla M, Burhans C (1996) *Blood*, 87: 2649-2658, 1996.
  - 2) Rowley JD *Cancer Biology*, 4:377-385, 1993.
  - 3) Rubnitz JE, Behm FG, Downing JR *Leukemia*, 10: 74-82, 1996.

VARIATION IN METAMORPHIC TIMING CAN BE INDUCED BY VARIATION IN DENSITY WITHOUT DIFFERENCES IN GROWTH RATE IN THE FROG, *RANA UTRICULARIA*Janel Richter<sup>1</sup>, Lincoln Martin<sup>2</sup>, and Christopher K. Beachy<sup>1</sup><sup>1</sup>Department of Biology, Minot State University, Minot, ND 58707; <sup>2</sup>Sawyer School, Sawyer, ND 58781

The standard model for understanding the timing of amphibian metamorphosis provided by Wilbur and Collins predicts that a growing larva will delay metamorphosis in order to take advantage of a good growth opportunity; if a larval habitat deteriorates (e.g., arrival of predators, pond desiccation), then a reduction in growth rate should be transduced to an endocrinological response, i.e., the initiation of metamorphosis (1). While this optimality model can provide an intuitive understanding of when metamorphosis can occur, there appears to be little empirical support for it. Most field studies use this optimality model to provide an interpretation of how geographic variation in larval density results in variation in metamorphic timing (2). However, there are no field studies that have information on growth rates of individuals; an adequate test of the model requires these growth rate estimates.

We tested the efficacy of the Wilbur-Collins model by growing tadpoles of the southern leopard frog, *Rana utricularia*, in different densities (1, 2, 4, 8, 10, and 20 tadpoles) in equivalent water volumes and with equivalent per capita food amounts. In the context of the Wilbur-Collins model, we predicted that variation in density that is not accompanied by variation in growth rate will fail to induce treatment differences in metamorphic timing.

We obtained embryos of larval *R. utricularia* from Sullivan Supply Company (Middleboro, Tennessee) in October 2003. Upon hatching, tadpoles were haphazardly assorted into 11 X 5.5 X 3.5 inch containers filled with 2600 ml of reverse-osmosis [RO] water that held 1, 2, 4, 8, 10, or 20 tadpoles. Every 3 days, tadpoles were removed from containers, which were cleaned, refilled with 2600 ml of fresh RO water, and tadpoles were replaced into the containers. Feedings occurred immediately after water changes (i.e., every 3 days). Feedings consisted of a per capita 25 mg aliquot of a finely ground 1:1 mixture of rabbit chow and fish food flakes, i.e., the 1-tadpole treatment received a 25 mg aliquot whereas the 10-tadpole treatment received a 250 mg aliquot at each feeding. After 3 months, the aliquots were tripled. Three months after the food increases, one-half of the treatments were subjected to a food reduction that returned these treatments to a per capita 25 mg aliquot. This food reduction occurred at approximately the same time as natural metamorphic timing from the population where these tadpoles were drawn. Thus we used a full-factorial two-factor design manipulating density (6 levels) and food reduction (2 levels) for total of 12 treatments each replicated six times (for a total of 450 tadpoles in 72 containers). We analyzed metamorphic timing and mass of all transforming tadpoles with a two-way MANOVA with density and food as treatment effects. We used  $\alpha = 0.05$  as significance criterion, and Wilks'  $\lambda$  was used as our multivariate test statistic.

Inspection of growth curves and analysis of mean tadpole mass resulted in no significant differences in growth, as intended by the equivalent per capita food treatments. Mean survival to metamorphosis was 8% in the 1-tadpole treatment and 25% in the 2-tadpole; these treatments were subsequently excluded from analysis. Mean survival varied from 38-41% in the remaining treatments. Food-reduction failed to induce differences in metamorphic timing. Tadpole metamorphic timing differed significantly when comparing the 4-tadpole with 20-tadpole treatments. This means that tadpole density can affect metamorphic timing without requiring a growth-rate transduction of tadpole density. This finding stands in contrast to the Wilbur-Collins model.

---

1) Wilbur HM, Collins JP *Science*, 182:1305-1314, 1973.

2) Scott DE *Ecology*, 71:296-306, 1990.

## THE EFFECTS OF LIGHT CYCLE ON METAMORPHOSIS IN SALAMANDERS

Kenn Rose<sup>1,2</sup> and Christopher K. Beachy<sup>1</sup><sup>1</sup>Department of Biology and <sup>2</sup>Department of Psychology, Minot State University, Minot, ND 58707

Immersion of frog tail tissue in thyroid hormone in the absence of light fails to initiate metamorphosis, evidenced as tail resorption (1). The observation, coupled with (a) the fact that almost all obligate cave dwelling salamanders are paedomorphic, *i.e.*, become sexually mature without undergoing a metamorphosis (2), and (b) precocious metamorphosis of plethodontid salamanders that appeared to be correlated with the change in ambient light cycle that occurs in late winter (C. Beachy, unpublished data) suggests an important potential role for light and the light:dark cycle for influencing metamorphic timing.

We tested the hypothesis that variation in light cycle will result in differences in metamorphic timing by exposing groups of larval salamanders (*Ambystoma tigrinum* and *A. maculatum*) to different light:dark (LD) cycles. Four treatments of LD were initiated: constant long day (16:8 LD), long-to-short day (16:8 switched to 12:12), constant short day (8:16), and short-to-long (8:18 switched to 12:12). In two separate experiments, animals were randomly assigned in groups of 4 (*A. tigrinum*) or 10 (*A. maculatum*) animals to 10 gallon aquaria filled with 11 liters of reverse osmosis water. Larvae were fed brine shrimp nauplii and tubificid worms *ad libitum*, weighed every 10 days to confirm no growth variation among individuals in the same aquarium, and weighed upon metamorphosis (when duration of larval period was also recorded). Mass every 10 days, metamorphic mass, and larval period were analyzed in a one-factor MANOVA that evaluated the LD regimes as treatment effect. Significance was evaluated at  $\alpha = 0.05$ , and LSD tests were used for *post hoc* comparisons when significant treatment effects were indicated.

At time of this submission, the experiment using *A. tigrinum* confirmed that LD had a significant effect on metamorphic timing. Larvae from the 16:8 treatments (constant and the long-to-short) metamorphosed earlier than short day larvae. However, temperature data indicate a correlated and confounding thermal effect. Long day aquaria were consistently 0.5-1.0°C warmer than short day aquaria. All temperature experiments on amphibian metamorphosis demonstrate that warmer temperature results in early metamorphosis (3). While this temperature difference could be considered minimal, we are repeating this experiment with *A. maculatum* in a different experimental setup in order to delete this source of temperature variation.

- 
- 1) Wright ML, Pathammavong N, Basso, CA *Gen. Comp. Endocrinol.*, 79, 89-94, 1990.
  - 2) Petranka, J. Salamanders of the United States and Canada. Washington, DC: Smithsonian Press, 1998.
  - 3) Hickerson CA, Barker E, Beachy CK *Southeastern Naturalist*, 5, *in press*.

## SEXUAL BEHAVIOR AND EDUCATION INTERVENTION: ASSESSMENT OF EFFECTIVENESS

Ashley Zimmer\*

Honors Program, University of North Dakota, Grand Forks, ND 58202

The Popular Opinion Leader (POL) model was used to assess sexual behaviors and effectiveness of an education intervention in college aged (18-22) women at UND. The perceived level of risk was compared to actual risk behaviors before and after opinion leader-led intervention conversations. Preliminary results exhibit initial high levels of risk behaviors including: negative attitudes towards and inconsistent condom use, multiple sexual partners, perceived demographic immunity, and deficient knowledge regarding HIV and other Sexually Transmitted Diseases (STDs). The intervention model worked to decrease negative attitudes regarding condoms and increase the use of protection against STDs and unintended pregnancy at every intercourse. Identifying the Midwest, college, female demographic as a risk group confirms the need for future study, and increased awareness and education regarding sexual risk behaviors.



GRADUATE COMMUNICATIONS  
IN THE  
A. RODGER DENISON COMPETITION

## SCHEDULE OF PRESENTATIONS — Lecture Bowl and River Valley Room — UND Memorial Union

**Lecture Bowl (Session A) — session will be chaired by A. Rodger Denison Competition judges**

- 1:00 Laura Parnas (UND): *DOPAMINE TRANSPORTER LIGAND BINDING DOMAINS ANALYZED THROUGH PHOTOAFFINITY LABELING*
- 1:20 J.A. Sattler (UND): *WHOLE-BODY VERSUS SKELETAL CALCIUM RETENTION IN RATS: SHORT- AND LONG-TERM COMPARISONS USING <sup>47</sup>CA and <sup>41</sup>CA TRACERS*
- 1:40 Pawel Borowicz (NDSU): *EXPRESSION OF THE MAJOR ANGIOGENIC FACTORS IN THE PLACENTA OF ADOLESCENT EWES*
- 2:00 Deanna O'Bryant (UND): *HLA-DQ8ab TRANSGENIC MICE DEMONSTRATE AN INCREASED RESISTANCE TO THE PLAGUE*
- 2:20 Sara Simmers (UM/St. Paul): *THE INFLUENCE AND EFFECTIVENESS OF RECLAMATION SEEDING ON GRASSLAND RECOVERY*
- 2:40 Mark Cervinski (UND): *SYNTAXIN 1A REGULATES DOPAMINE TRANSPORTER PHOSPHORYLATION AND ACTIVITY*
- 3:00 Ewa Borowczyk (NDSU): *GAP JUNCTIONAL PROTEINS CONNEXINS (Cx) 26, 32, 37 AND 43 mRNA EXPRESSION IN THE OVINE OVARIAN FOLLICLES DURING THE PERIOVULATORY PERIOD*
- 3:20 Katie Rau (UND): *A<sub>1</sub>-ADRENERGIC-MEDIATED ENHANCEMENT OF HIPPOCAMPAL CA<sub>3</sub> NETWORK ACTIVITY*
- 3:40 Balachandra Gorentla (UND): *ACUTE EFFECTS OF DOPAMINE AND TRANSPORT BLOCKERS ON DOPAMINE TRANSPORTER PHOSPHORYLATION AND REGULATION*
- 4:00 Jessica Evoniuk (NDSU): *AN EPIDEMIOLOGICAL REVIEW OF A FATAL NEURODEGENERATIVE DISEASE OUTBREAK*
- 4:20 Kelly Durick (UND): *CHARACTERIZATION OF BORON'S ANTI-INFLAMMATORY EFFECT: DOWN-REGULATION THROUGH THE NF- $\kappa$ B PATHWAY*
- 4:40 Surajan Mamidi (NDSU): *NUCLEOTIDE DIVERSITY OF CHALONE ISOMERASE IN COMMON BEAN (P. VULGARIS)*
- 5:00 Sunitha Bollimuntha (UND): *TRPC3-VAMP2 INTERACTION DICTATES PLASMA MEMBRANE LOCALIZATION AND FUNCTION OF TRPC3 IN NEURONAL AND NON-NEURONAL CELLS*
- 5:20 MR O'Neil (NDSU): *EFFECTS OF ESTRADIOL (E<sub>2</sub>) AND LINSEED MEAL (LSM) ON ORGAN WEIGHTS IN OVARIECTOMIZED EWES*

## SCHEDULE OF PRESENTATIONS — Lecture Bowl and River Valley Room — UND Memorial Union

**River Valley Room (Session B) — session will be chaired by A. Rodger Denison Competition judges**

- 1:00 Victor Waingeh (UND): *GLYCOLYTIC ENZYME INTERACTIONS WITH F-ACTIN: COMPARING RABBIT AND YEAST*
- 1:20 Lei Ding (UND): *A MODEL OF OSTEOARTHRITIC CARTILAGE DAMAGE: ACTION OF FIBRONECTIN FRAGMENTS*
- 1:40 Yuhui Jin (UND): *TOXICITY OF NANOMATERIALS TO LIVING CELLS*
- 2:00 Shibichakravarthy Kannan (UND): *MODULATION OF SRC-LIKE TYROSINE KINASE LYN PLAYS A ROLE IN PSEUDOMONAS AERUGINOSA INFECTION IN LUNG CELLS*
- 2:20 Shamus Funk (NDSU): *INDICATIONS FOR STRONG METAL SUPPORT INTERACTIONS: THE CASE OF CO<sub>2</sub> ADSORPTION ON Cu NANO PARTICLES SUPPORTED ON ZnO(0001)*
- 2:40 Alexander Azenkeng (UND): *THEORETICAL STUDIES OF Ti<sub>2</sub>H<sub>5</sub><sup>+</sup> AND RELATED MOLECULES*
- 3:00 Shankar Karki (UND): *SUBSTITUTION AND PRICE EFFECTS OF CARBON EMISSION PENALTIES ON CO<sub>2</sub> EMISSIONS REDUCTION FROM ELECTRIC POWER PLANTS*
- 3:20 Promise Yong (UND): *PHOTOCHEMISTRY OF 2-NITROBENZYL ENOL ETHERS: OXIDATIVE C=C BOND SCISSION*
- 3:40 Julius Ngwendson (UND): *AROMATIC TOSYLATE ESTERS AS LIGANDS FOR METAL IONS*
- 4:00 Sarita Pachhai (UND): *USGS 30-M AND LIDAR 1-M DEMs TO ASSESS THE ACCURACY OF ELEVATIONS IN THE RED RIVER BASIN OF THE NORTH*
- 4:20 Song Liang (UND): *FLUORESCENT NANOSENSOR FOR TARGETING SELENIUM IN A MIXTURE*
- 4:40 Eric Njabon (UND): *COMPUTER MODELING OF ELECTROSTATIC INTERACTIONS BETWEEN LDH AND ACTIN*
- 5:00 Neville Forlemu (UND): *BROWNIAN DYNAMICS SIMULATIONS OF INTERACTIONS BETWEEN FISH ALDOLASE AND G-OR F-ACTIN*
- 5:20 Brent Pringle (UND): *USING HIGH SPATIAL RESOLUTION SATELLITE IMAGERY TO MAP LEAFY SPURGE (EUPHORBIA ESULA) AND CANADA THISTLE (CIRSIIUM ARVENSE) IN THE NORTH UNIT OF THEODORE ROOSEVELT NATIONAL PARK*
- 5:40 Divine Dugah (UND): *LANTHANIDE METAL CHEMISTRY: CATALYSIS OF BIODEGRADABLE AND BIOFRIENDLY POLYMERS*

THEORETICAL STUDIES OF  $Ti_2H_5^+$  AND RELATED MOLECULES

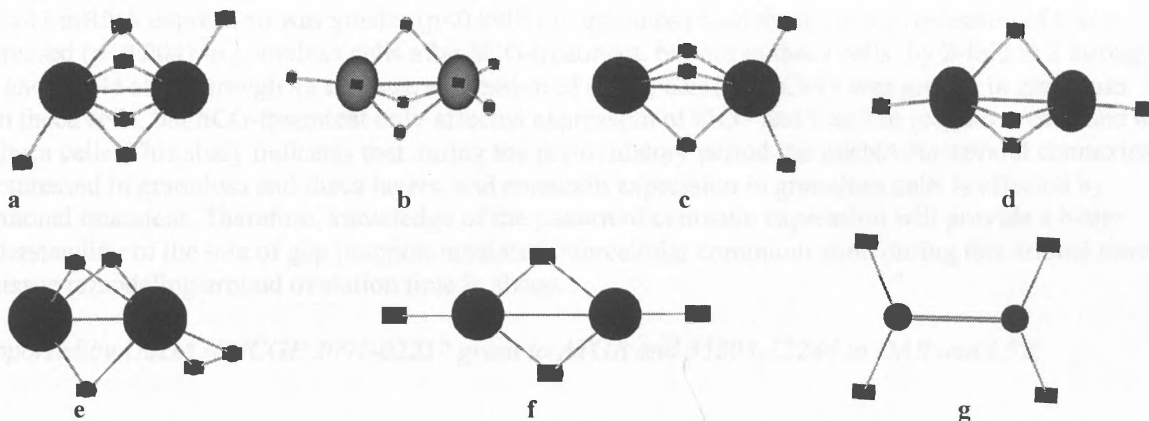
Alexander Azenkeng\* and Mark R. Hoffmann

Department of Chemistry, University of North Dakota, Grand Forks, ND 58202

Titanium hydrides are well known for their catalytic activity in many reactions including polymerization of olefins and nitrogen fixation (1). Ditungsten hydrides ( $Ti_2H_6$ ) have been studied previously in detail (2) and are known to possess interesting magnetic properties (3), but none of the ions have been studied yet. In the case of the related diruthenium complexes (4), it has been noted that the bridging hydrogens exhibit some acid/base properties. The goal of the present study is to obtain optimized geometries for  $Ti_2H_5^+$ , with corroborating studies of  $Ti_2H_4$  and  $Ti_2H_6$ , with the aim to understand better structural relationships on their ground and low-lying singlet states potential energy surfaces. Similar molecules and ions could have potential applications in areas such as catalysis and the fabrication of magnetic switches; in addition, they are expected to exhibit interesting gas-phase acidity and/or basicity properties. These molecules could also provide insight into modeling cluster relevant to the study of the transportation of pollutants in ground water aquifers.

Calculations were performed using the triply split 6-311G\*\* basis set at various levels of theory including Hartree-Fock (HF), multiconfigurational self-consistent field (MCSCF), multiconfigurational quasidegenerate perturbation theory (MCQDPT) and density-functional theory (DFT). MCSCF properly accounts for nondynamic electron correlation, while MCQDPT optimizations ensure adequate treatment of both nondynamic and dynamic electron correlation, which was essential for these systems. Preliminary DFT calculations were attempted as a cheap alternative to MCQDPT, but it proved to fail in this case since it produced results that were qualitatively different from those obtained from MCSCF. The reason for such disagreement is thought to be due to improper description of spin properties of the system by DFT.

Two low-lying open-shell singlet states ( $^1A'$  and  $^1A''$ ) with  $C_s$  symmetry were optimized for  $Ti_2H_6$  and several others for  $Ti_2H_5^+$  in  $C_1$ ,  $C_2$  and  $C_s$  symmetry point groups. For  $Ti_2H_4$ , a doubly hydrogen bridged ground state closed shell singlet structure with  $D_{2h}$  symmetry was found at the MCSCF level. In all the optimized structures, no significant bonding was observed between the titanium atoms in the case of open shell singlets. However, in the case of a closed shell structure, correlated calculations may support up to a double bond. MCSCF vibrational frequencies have also been computed for  $Ti_2H_6$ ,  $Ti_2H_5^+$  and  $Ti_2H_4$ . All these data is available and because of limited space they have not been included here. However, the optimized structures are shown below for:  $Ti_2H_6$  a) MCQDPT b) DFT/B3LYP,  $Ti_2H_5^+$  (c, d) MCQDPT (e) DFT/B3LYP,  $Ti_2H_4$  (f) MCSCF bridged isomer and (g) RHF unbridged isomer



We have obtained equilibrium geometries for  $Ti_2H_5^+$ ,  $Ti_2H_6$  and  $Ti_2H_4$ , most of which indicate that a predominantly triply hydrogen bridged structure is the most stable. On the basis of preliminary calculations, these molecules are also likely to show some gas-phase acidity and/or basicity.

1) Toogood GE, Wallbridge MGH *Adv. Inorg. Chem. Radiochem.*, **25**, 267, 1982.

2) Webb SP, Gordon MS *J. Am. Chem. Soc.*, **120**, 3846, 1998.

3) Webb SP, Gordon MS *J. Chem. Phys.*, **109**, 919, 1998.

4) Hans-Christian B, Marion G, Kurt M, Christoph WJ *J. Organomet. Chem.* **628**, 144, 2001.

## TRPC3-VAMP2 INTERACTION DICTATES PLASMA MEMBRANE LOCALIZATION AND FUNCTION OF TRPC3 IN NEURONAL AND NON-NEURONAL CELLS

Sunitha Bollimuntha\* and Brij B. Singh

Department of Biochemistry and Molecular Biology, School of Medicine and Health Sciences,  
University of North Dakota, Grand Forks, ND 58201

Mammalian homologues of the *Drosophila* Transient Receptor Potential (TRP) channels are the putative calcium ion channels, which function through receptor-operated calcium entry mechanism. Our results indicate that TRPC3 channel are present in the vesicles and get fused to the plasma membrane upon agonist mediated receptor activation. Both TRPC3 and VAMP2 antibodies could co-immunoprecipitate endogenous TRPC3, VAMP2 and other SNARE complex proteins in neuronal and non-neuronal cells. Confocal microscopic studies revealed that GFP-TRPC3 co-localizes with CFP-VAMP2, which showed a punctate staining on the plasma membrane and in mobile intracellular vesicles. On the contrary expression of a dominant negative VAMP2 (DnVAMP2) showed mislocalization of the TRPC3 protein, which suggests that their interaction is crucial for membrane localization of the TRPC3 protein. Fluorescence recovery after photo bleaching (FRAP) further confirmed the vesicle fusion hypothesis. Functional studies performed using fura2, confirmed that TRPC3 mediated calcium influx was inhibited in DnVAMP2 expressing cells. The minimal TRPC3-VAMP2 interacting domain was identified and both immunoprecipitation and confocal microscopic studies showed that VAMP2 interacts with TRPC3 at the coiled-coil domain of the N-terminus. In aggregate our results suggest that the interaction between TRPC3 and VAMP2 is important for the localization of TRPC3 and causes increased  $\text{Ca}^{2+}$  influx upon stimulation.

## GAP JUNCTIONAL PROTEINS CONNEXINS (Cx) 26, 32, 37 AND 43 mRNA EXPRESSION IN THE OVINE OVARIAN FOLLICLES DURING THE PERIOVULATORY PERIOD

<sup>1</sup>Ewa Borowczyk\*, <sup>1,2</sup>Mary Lynn Johnson, <sup>1,2</sup>Dale A Redmer, <sup>1,2</sup>Lawrence P Reynolds,

<sup>1</sup>Chainarong Navanukraw and <sup>1,2</sup>Anna T Grazul-Bilska,

<sup>1</sup>Department of Animal & Range Sciences, and <sup>2</sup>Cell Biology Center, <sup>1,2</sup>North Dakota State University, Fargo, ND USA 58105

The transition of the preovulatory follicle into the corpus luteum is very critical event that involves structural and functional changes in nonvascular granulosa and highly vascular theca layers of the preovulatory follicle. However, this complex process initiated by ovulation and followed by luteinization, similar to wound healing or tumor formation, needs to be highly regulated and coordinated. Gap junction-mediated intercellular communication seems to play an important role in tissue growth, differentiation and remodeling. Gap junctions are encoded by a multi-gene family known as the connexins. Each connexin exhibits a unique pattern of expression that can be metabolically, hormonally or developmentally regulated. To evaluate the expression of Cx26, Cx32, Cx37 and Cx43 during the periovulatory period, ovaries were collected from ewes ( $n = 5-7/\text{group}$ ) injected twice daily with follicle stimulating hormone (FSH) on days 13 and 14 of the estrous cycle, and with FSH and human chorionic gonadotropin (hCG; ovulatory dose) on the morning of day 15 of the estrous cycle. Total cellular RNA (tcRNA) was isolated from snap-frozen samples of granulosa and theca cells from preovulatory (-4 mm) follicles that were obtained at 0, 2, 4, 8, 12, 24, and 48 h after hCG-treatment. The quantity and quality of the tcRNA were evaluated using an Agilent 2100 bioanalyzer (Agilent Technologies, Wilmington, DE) and mRNA expression for connexins was quantified and normalized to expression of the 18s ribosomal gene by real time RT-PCR using the ABI Prism 7000 (Applied Biosystems, Foster City, CA). The mRNA levels of Cx26 were similar for granulosa and theca cells and remained unchanged after hCG-treatment. Expression of Cx32 was 10-fold greater ( $p < 0.001$ ) for granulosa than for theca cells and did not change after hCG-treatment. Cx37 mRNA expression was greater ( $p < 0.01$ ) for granulosa than for theca cells and hCG-treatment tended to increase ( $p < 0.08$ ) Cx37 expression during periovulatory period but only in granulosa cells. Cx37 expression increased ( $p < 0.06$ ) 2-fold at 12 h after hCG-treatment compared with 0 h. Cx43 mRNA expression was greater ( $p < 0.0001$ ) in granulosa than theca cells. Expression of Cx43 decreased ( $p < 0.004$ ) in granulosa cells after hCG-treatment, but not in theca cells, by 2-fold at 2 through 8 h and 3-fold at 24 through 48 h. Thus, expression of Cx32, Cx37 and Cx43 was greater in granulosa than theca cells, but hCG-treatment only affected expression of Cx37 and Cx43 in granulosa cells and not in theca cells. This study indicates that during the periovulatory period the mRNA for several connexins is expressed in granulosa and theca layers, and connexin expression in granulosa cells is affected by hormonal treatment. Therefore, knowledge of the pattern of connexin expression will provide a better understanding of the role of gap junction-mediated intercellular communication during this critical time of tissue remodeling around ovulation time in sheep.

*Supported by USDA NRICGP 2001-02257 grant to ATGB and 35203-12246 to DAR and LPR.*

## EXPRESSION OF THE MAJOR ANGIOGENIC FACTORS IN THE PLACENTA OF ADOLESCENT EWES

Pawel P. Borowicz\*, Mary Lynn Johnson, Ewa Borowczyk, Kimberly A. Vonnahme,  
Anna T. Grazul-Bilska, Dale A. Redmer, Lawrence P. Reynolds

Center for Nutrition and Pregnancy, Department of Animal and Range Sciences, North Dakota State University, Fargo, ND 58105-5727, USA.

Establishment of functional fetal and placental circulations is some of the earliest events during embryonic/placental development. It has been shown that the large increase in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on the dramatic growth of the placental vascular beds and the resultant large increases in uterine and umbilical blood flows. Additionally, factors that affect fetal growth, such as maternal genotype, increased numbers of fetuses, maternal undernutrition, maternal age, parity, or environmental heat or cold stress, typically have similar effects on placental size, and also are associated with reduced rates of fetal oxygen and nutrient uptakes and placental blood flow. Adolescent mothers have an increased risk of preterm delivery of low birth weight infants who exhibit high mortality rates within the first year of life. Placental growth and development precede that of the fetus, and there is a strong correlation between placental mass and fetal size, and also with neonatal outcome. The large increase in fetal size during the last half of gestation is supported by the dramatic growth of the placental vascular beds, resulting in large increases in uterine and umbilical blood flows. We proposed that the effect of maternal age can be explained by reduced placental expression of the major angiogenic factors and(or) their receptors in association with reduced placental vascular development. The experimental group comprised 6 donor Columbia ewes, 6 donor Romanov ewes, 20 virgin Columbia recipients (10 lambs [peri-pubertal, PP]; 10 yearlings, [early adulthood, EA]) and 20 virgin Romanov recipients (10 PP and 10 EA). Pregnancy was established by embryo transfer to ensure that singleton pregnancies were established and that within a breed, PP and EA recipients received embryos from a consistent source. Breeds as well as maternal age groups were penned separately and received the same diet. To evaluate placental expression of the major angiogenic factors, gravid uteri were obtained on day 135 of gestation. Samples of the maternal caruncular (CAR) and fetal cotyledonary (COT) portions of the placenta were snap frozen and later analyzed for vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor-1 (VEGFR-1), vascular endothelial growth factor receptor-2 (VEGFR-2), angiopoietin-1 (ANG-1), angiopoietin-2 (ANG-2), receptor for both Ang-1 and Ang-2 Tie-2, fibroblast growth factor (FGF), endothelial nitric oxide synthase (eNOS), and soluble guanylate cyclase (sGC). Determination of mRNA concentrations was by real-time RT-PCR (ABI Prism 7000). We also determined the weight of the fetus, fetal membranes and CAR and COT. For COT, concentrations of mRNA were less in PP vs. EA for VEGF ( $P<0.05$ ), Ang-1 ( $P<0.04$ ), Tie-2 ( $P<0.05$ ), eNOS ( $P<0.0001$ ), and sGC ( $P<0.03$ ). For CAR, there were no differences in mRNA concentrations for angiogenic factors in PP vs. EA. Reduced expression of angiogenic factors in COT but not CAR of PP vs. EA was reflected by reduced COT ( $P<0.06$ ) but not CAR weight. Both fetal membrane weight ( $P<0.002$ ) and fetal weight ( $P<0.09$ ) also were reduced in PP vs. EA groups. These data indicate that maternal age (peri-pubertal vs. early adulthood) affects the levels of fetal placental expression of mRNA for major angiogenic factors, which may result in reduced fetal size and low birth weights.

*Supported by NIH grant HL64141 to LPR and DAR.*

## SYNTAXIN 1A REGULATES DOPAMINE TRANSPORTER PHOSPHORYLATION AND ACTIVITY

Mark A. Cervinski\* and Roxanne Vaughan

Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND

The dopamine transporter (DAT) is an integral membrane protein located in dopamine-producing neurons and is responsible for clearing the synapse of secreted dopamine (DA) following neural depolarization and synaptic release. As well as regulating synaptic levels of DA, DAT is also the target for many psychostimulant and therapeutic drugs such as cocaine, amphetamines, the antidepressant bupropion (Wellbutrin™) and the ADHD treatment methylphenidate (Ritalin™). Increasing evidence suggests that multiple signals and interacting proteins mediate the phosphorylation, regulation and subcellular distribution of DAT. The related neurotransmitter transporters GAT1, NET, and SERT interact with the SNARE protein syntaxin 1A, and this interaction plays a role in their subcellular localization and functional regulation. The GAT1 and NET interactions with syntaxin are regulated by PKC which further suggests complex dynamic relationships between these proteins.

We have explored the potential interaction between syntaxin 1A and DAT in rat striatal neurons by examining the ability of botulinum toxin C (BoNT/C), which cleaves syntaxin 1A, to affect DAT phosphorylation and transport activity.

Phosphorylation: Rat striatal slices were metabolically labeled with  $^{32}\text{PO}_4$ , treated with or without the indicated dose of BoNT/C for the times indicated. The tissue was then solubilized with 0.5% SDS and DATs were subjected to immunoprecipitation and autoradiography.

DAT transport activity: Rat striatal slices were treated with BoNT/C as above and were homogenized with a Teflon-glass homogenizer and centrifuged to produce a synaptosomal pellet. For analysis of [ $^3\text{H}$ ]DA uptake the pellet was resuspended in SP and the resuspended synaptosomes were added to tubes containing [ $^3\text{H}$ ]DA. The incorporated radioactivity was then quantified in a liquid scintillation counter.

The DATs from tissue treated with BoNT/C showed a time dependent reduction in phosphorylation to 81% of the basal level by 20 min., while longer treatment times caused decreases to 69% of basal. BoNT/C also demonstrated a dose dependent reduction of DAT phosphorylation to 84% of the basal level with a 10 ng/ml dose of BoNT/C, while increased doses further reduced DAT phosphorylation to 70% of basal. DAT transport activity was also affected by BoNT/C. Rat striatal synaptosomes treated with BoNT/C produced a time dependent increase in [ $^3\text{H}$ ]DA uptake that was 123% of control by 20 min, while longer treatment times caused increases of 153% of control. The BoNT/C effect on [ $^3\text{H}$ ]DA uptake was also dose dependent as a dose of 10ng/ml BoNT/C produced an increase of 115%, while increased doses further increased uptake to 133% over the control value. Treatment of tissue with BoNT/C did not affect the total level of DAT but decreased the level syntaxin 1A that was detectable in the striatal tissue. The BoNT/C mediated decrease in transporter phosphorylation but not transporter number implicates an interaction between DAT and syntaxin 1A that affects the basal level of transporter phosphorylation.

These results demonstrate a functional interaction between DAT and the SNARE protein syntaxin 1A. This interaction may therefore represent part of a mechanism controlling transporter localization and regulation that may modulate neurotransmitter clearance and synaptic transmission.

*Supported by: NRSA F31 DA017520-01(MAC) and DA 13147(RAV).*



## A MODEL OF OSTEOARTHRITIC CARTILAGE DAMAGE: ACTION OF FIBRONECTIN FRAGMENTS

Lei Ding\*, D.P. Guo, B. Singh, and G.A. Homandberg

Department of Biochemistry and Molecular Biology, School of Medicine and Health Sciences,  
University of North Dakota, Grand Forks, ND, USA.

Osteoarthritis (OA) is a degenerative joint disease in which the cartilage that covers the ends of bones in the joint deteriorates, causing pain and loss of movement as bone begins to rub against bone. Previous studies from our laboratory showed that Fibronectin Fragments (Fn-fs), the degradation products of a ubiquitous extracellular matrix protein-Fibronectin (Fn), greatly augment cartilage destruction through upregulation of catabolic cytokines and matrix metalloproteinases (MMPs). This leads to severe cartilage matrix damage as observed in OA. The precursor, native Fn, is inactive. How Fn-fs cause changes in signal transduction that lead to enhanced chondrolytic activities is unknown. Since receptor-binding Fn peptides decrease focal contacts, disturb cytoskeletal elements such as actin and decrease Fn receptor aggregation in fibroblasts, we have proposed that Fn-fs have similar effects in chondrocytes and that these changes in receptor and cytoskeletal elements alter the location of upstream kinases near receptors which leads to altered signal transduction and emergence of inflammatory MAP kinase pathways. Our first objective was to compare Fn-fs to Fn in terms of activation of kinases that are involved in integrin signaling, catabolic cytokine upregulation and MMP expression. The second objective was to investigate if blocking of some of these kinases leads to blocking of MMP expression. The third objective was to investigate whether the most cartilage-damaging N-terminal 29-kDa Fn-f alters actin structure and disrupts the Fn receptor,  $\alpha_5\beta_1$ , as has been observed upon addition of small tetrapeptides to fibroblasts. The fourth objective is to ultimately determine whether these changes in cytoskeletal structure might explain changes in kinase location, which in turn, turn on/off kinase activity.

Chondrocyte monolayer cultures were derived from the cartilage of bovine metacarpophalangeal joints. Changes in intracellular kinase activity were examined by Western Blotting by use of antibodies specific to total and phosphorylated kinase forms. The targets included focal adhesion kinase (FAK), Pyk2 (a soluble form of FAK), c-Src which can phosphorylate FAK, and the MAP kinases ERK1/2, p38 and JNK. The transcription factor, NF- $\kappa$ B, (involved in catabolic cytokine upregulation) was also investigated. The effects of Fn and Fn-fs on kinetics and effects as a function of concentration were examined. The effects of several kinase inhibitors on downregulation of MMPs were also tested. Fluorescent confocal microscopy was used to visualize changes caused by added Fn-f or Fn. Bovine chondrocyte monolayer cultures were treated with unlabeled or with FITC-labeled Fn or Fn-fs for 4 hrs or with BSA as a negative control. Cells were then fixed by 3% paraformaldehyde, permeabilized and probed with antibody against  $\alpha_5$  integrin subunit to test for changes in Fn receptor or with rhodamine phalloidin to test for changes in actin. FITC- or TRITC labeled phospho-specific antibody against Pyk2, FAK, c-Src or JNK were used to target the location of activated kinases.

Fibronectin fragments activated c-Src, Pyk2, ERK1/2, p38 and JNK and NF- $\kappa$ B while native Fn only enhanced activation of FAK and ERK1/2. For c-src, Fn appeared to enhance phosphorylation at an inactivation site, while the 29-kDa Fn-f instead, weakly enhanced phosphorylation at an activation site. Inhibitors of PYK2, MEK, JNK and PKC- $\delta$  blocked Fn-f mediated upregulation of MMP-3 and MMP-13. N-terminal 29-kDa Fn-f disrupted Fn receptor ( $\alpha_5\beta_1$ ) aggregation and actin structure, the latter of which was associated with movement of c-Src away from the plasma membrane. The movements of activated Pyk2, FAK or JNK are under investigation.

We propose a tentative model of cartilage damage by Fn-fs: after Fn-fs bind to receptor, receptor aggregation is disrupted and actin distribution is altered. This is associated with movement of c-Src or other factors in such a fashion that Pyk2 activity is enhanced (Movement of other upstream kinases or factors is under investigation). This leads to activation of MAP kinases (p38, JNK and ERK 1/2) and transcription factor NF- $\kappa$ B. This would lead to NF- $\kappa$ B translocation into the nucleus, upregulation of catabolic cytokines and MMPs and consequent matrix proteoglycan (PG) degradation. In contrast, binding of native Fn does not alter receptor or actin, does not alter c-Src distribution and Pyk2 is not activated. Thus, the inflammatory MAP kinases-p38 and JNK are not activated and cartilage damage does not occur. Since the Fn-f system is a model of OA and represents a unique dysregulation of integrin signaling that may occur in other pathologies as well, further knowledge of the mechanism may be applicable to therapeutic intervention. Future work will more precisely delineate the mechanism and test additional kinase inhibitors for their interventive potential.

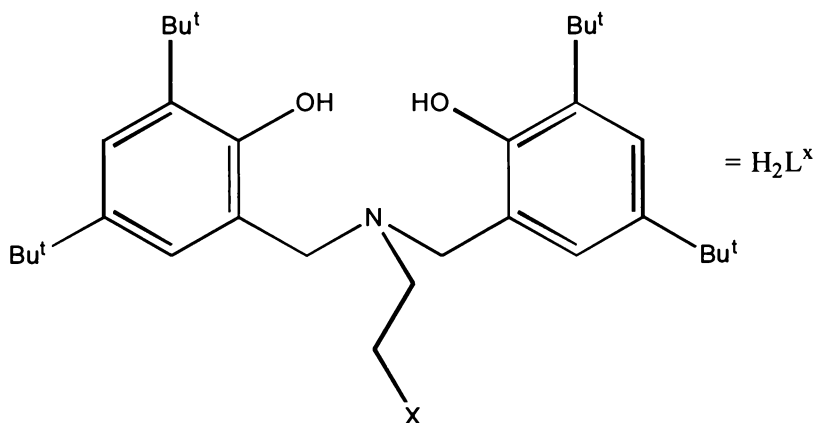
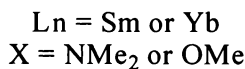
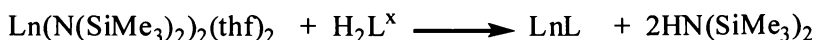
LANTHANIDE METAL CHEMISTRY: CATALYSIS OF BIODEGRADABLE AND BIOFRIENDLY POLYMERS

Pascal Binda\*, Divine Dugah\*, Chassidy Nelson, Ewan Delbridge  
Department of Chemistry, University of North Dakota, Grand Forks ND 58202

As the rapid depletion of petrochemical feedstock continues, the production of new, useful and environmental benign polymers is of great importance. Such polymers have been found to be biodegradable and bioassimilable and are derived from renewable resource such as cornstarch and beets.

As a result of their intriguing properties of biodegradability and bioassimilability, these polymers have found a multitude of applications ranging from packaging materials and clothing to biomedical uses (*e.g.*, drug delivery agents, tissue engineering and polymer splints for orthopedic repairs).

Poly lactide (PLA), a polyester obtained from the monomer lactide (LA), is an example of a biodegradable polymer to receive much attention. Cognizant of the fact that the properties of PLA can be immensely influenced by its microstructures, catalyst design is crucial in influencing the polymerization process. Although several LA polymerizing catalysts are known, little is understood of the polymerization mechanism. To address this deficiency, a series of new divalent bisphenolate lanthanide complexes  $[LnL^{NMe_2}]$  and  $[LnL^{OMe}]$ , ( $Ln = Sm, Yb$ ) have been synthesized as potential precatalysts for the ring opening polymerization of LA. These complexes were prepared by the transamination utilizing  $Ln(N(SiMe_3)_2)(thf)_2$  with one equivalent of the ligands  $H_2L^{NMe_2}$  and  $H_2L^{OMe}$ .



## CHARACTERIZATION OF BORON'S ANTI-INFLAMMATORY EFFECT: DOWN-REGULATION THROUGH THE NF-kappaB PATHWAY

Kelly A. Durick\*<sup>1</sup>, Michiyo Tomita<sup>2</sup>, Curtiss D. Hunt<sup>3</sup>, and David S. Bradley<sup>1</sup>

Departments of <sup>1</sup>Microbiology and Immunology, and <sup>2</sup>Medicine, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND; and <sup>3</sup>USDA ARS Grand Forks Human Research Nutrition Center, Grand Forks, ND

We have previously demonstrated that dietary boron (B) supplementation was able to prevent the onset of, and ameliorate ongoing, collagen-induced arthritis, as well as significantly reduce the incidence of experimental autoimmune encephalomyelitis, suggesting that Boron has anti-inflammatory properties. However, little is known about the mechanism of Boron in immunoregulation despite evidence that boric acid or substituted boric acid compounds competitively inhibit the *in vitro* activities of NAD<sup>+</sup>, NADP- and FAD-requiring oxidoreductase enzymes and serine proteases, each with important regulatory functions in inflammation. We provide evidence here that Boron is able to prevent the activation of pro-inflammatory cytokine and transcription factor genes *in vitro*. A murine macrophage line, J774-A1, was grown in the presence or absence of supplemental Boron (– 5 days), stimulated with LPS, and the activation of inflammation-related genes was quantified by RT-PCR. The LPS-stimulated J774 cells grown in 10 or 100 –mol supplemental Boron displayed decreased levels of TNF–, IL-1–, MIP-1–, and iNOS expression, compared to the LPS-activated Boron minus (B<sup>–</sup>) controls. Each of these factors is under NF- $\gamma$ B control. SOD transcription rate (not under NF- $\gamma$ B regulation) did not change with Boron supplementation of the media. These data suggest that Boron may down-regulate inflammation at a site upstream of cytokine gene activation in the NF- $\gamma$ B regulated pathway.

AN EPIDEMIOLOGICAL REVIEW OF A FATAL NEURODEGENERATIVE DISEASE  
OUTBREAK: QUANTIFICATION OF GENETIC RISK AND THE IMPACT OF LATERAL  
TRANSMISSION IN THE OUTBREAK

Jessica M. Evoniuk<sup>1</sup>, Charles L. Stoltenow<sup>1</sup>, Katherine I. O'Rourke<sup>2</sup>, Dale A. Redmer<sup>1</sup>, Bert L. Moore<sup>1</sup>

<sup>1</sup>Department of Animal and Range Sciences, North Dakota State University, Fargo, ND 58105-5727

<sup>2</sup>USDA Agricultural Research Service, Animal Disease Research Unit, Pullman, WA 99164-6630

Scrapie in sheep and goats, a fatal neurodegenerative disease, is a member of the family of transmissible spongiform encephalopathies (TSEs), which includes bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease in deer and elk, and Creutzfeldt-Jakob disease in humans. Common to all TSE diseases is the accumulation of the abnormal PrP<sup>Sc</sup> form of the normal prion protein PrP<sup>C</sup>, predominantly in the brain and nervous tissue in late stage disease. This infectious protein, PrP<sup>Sc</sup>, is thought to be the causative agent of TSE.

Susceptibility to scrapie is primarily controlled by polymorphisms in the prion protein gene (PRNP). Three of these polymorphisms are strongly linked to the occurrence of natural and experimental scrapie. These are valine (V) or alanine (A) at codon 136, arginine (R) or histidine (H) at codon 154, and glutamine (Q), arginine (R), or histidine (H) at codon 171. In the United States, diploid genotypes are determined by a number of commercially available assays and typically shown as the results at codon 136 and 171 (AAQQ, AAQR, AARR, VVQQ, AVQQ or AVQR). Relative susceptibility and incubation time are a function of PRNP genotype and scrapie strain.

The objective of this retrospective study was to characterize an outbreak of valine-associated scrapie in a United States flock, quantify the risk of scrapie infection based on allele frequencies at codon 136 and 171 and provide evidence of lateral transmission.

One thousand six sheep, with genotypes at codon 171 (n=164) or codons 136 and 171 (n=842) and scrapie diagnostic testing (n=190), with 44 sheep considered positive for the disease were studied. Genotype was determined using commercial testing, with presence or absence of PrP<sup>Sc</sup> through immunohistochemical localization in either neural or lymphatic tissue used to determine scrapie status.

Animals with at least one V<sub>136</sub> (n=160) allele were 52.3 times more likely to be positive than sheep homozygous for A<sub>136</sub>. Homozygous V<sub>136</sub> sheep (n=72) were 1.7 times more likely to be positive than heterozygotes. For homozygous Q<sub>171</sub> animals (n=127), those at least heterozygous for V<sub>136</sub> were 36.4 times more likely to be positive than those homozygous for A<sub>136</sub>. Of animals (72) with at least one V<sub>136</sub>, AVQR sheep were 1.96 times more likely to be negative than AVQQ and/or VVQQ sheep, despite the V<sub>136</sub> at codon 136. Exposure route was estimated in several cases based on average incubation times for AVQQ and VVQQ sheep. Using the date of transmission, which is based on the death date, we provide evidence of lateral transmission, (transmission via the environment or by close contact with infected individuals).

In conclusion, this flock had an outbreak of valine-associated scrapie, associated with a relatively high frequency of sheep with valine at codon 136, introduction of a valine-dependent scrapie strain, and the occurrence of lateral transmission. Our findings are uncharacteristic of most scrapie outbreaks in the United States, in which the alanine-associated scrapie is most prevalent. Further study of this rare form of this disease will provide important information regarding the transmission and etiology of scrapie in sheep and TSE diseases in general.

*Supported in part by USDA, ARS, ADRU.*

BROWNIAN DYNAMICS SIMULATIONS OF INTERACTIONS BETWEEN FISH ALDOLASE AND G-OR F-ACTIN

Neville Y. Forlemu\*, Victor F. Waingeh, Steve L. Lowe and Kathryn A. Thomasson  
Department of Chemistry, University of North Dakota, Grand Forks, North Dakota 58202-9024

The intracellular milieu is not a simple, homogenous, aqueous state. Protein – protein interactions play an essential role in cellular organization of metabolic pathways. Actin filaments may play a vital role in compartmentation and substrate channeling in glycolysis (1). Previous computational and experimental studies have shown strong interactions between certain glycolytic enzymes and the cytoskeletal protein actin in a variety of species (2-4). This enzyme-F-actin equilibrium is not only influenced by pH and ionic strength, but by metabolites as well, and is postulated to be electrostatic in nature (5). Brownian dynamics (BD) simulations of the binding of the muscle form of fish fructose-1,6-bisphosphate aldolase (aldolase) to F-or G-actin confirm the strong interactions previously demonstrated in rabbit (3).

To create the three-dimensional structures needed for BD simulations, the Homology module of the Insight@II modeling package (Accelrys, San Diego, CA) was used to build the tertiary structures of fish aldolase and actin. Formal charges were then assigned to each protein in modeling package MacroDox (6). The electrostatic potential about each protein was determined by solving the linearized Poisson-Boltzmann equation at pH = 7.0, ionic strength I = 0.05 M, and temperature = 298.15 K. Multiple trajectory runs for both G-actin and F-actin with aldolase were carried out to investigate the nature of interactions and determine docking modes. Single trajectory runs between F-actin and aldolase were made to study the energetics of this type of interaction.

The aldolase grooves (between the A&D and B&C subunits) comprised positively charged residues (Lys's 341, 348, 13, 347, 27 and Arg 293) that are critical to binding with the negatively charged amino terminus of actin (residues Asp 1, 2, 3 and Glu 4) and other exposed patches of actin subdomain 1 (Asp's 24, 25, 99, 100, 364). Like rabbit, the presence of the exposed positive residues in the aldolase grooves is critical for binding to the negative subdomain 1 of actin. Table 1 lists the salt bridges found in a typical complex formed between aldolase and F-actin. BD results show a large Boltzmann population of similar complexes occurring around the aldolase grooves, and reaffirm the idea that the quaternary structure of the proteins is critical for these reversible interactions.

**Table 1: Salt Bridges in a F-actin/Aldolase Complex (Energy -12.1 kcal/mol).**

Aldolase		F-actin		Distance (Å)
Residue	Atom	Residue	Atom	
Lys 341C	NZ	Asp 1 D	OD2	2.39
Lys 341C	NZ	Asp 1 D	OD1	3.15
Lys 341C	NZ	Glu 364D	OE1	4.35
Arg 293C	NH2	Glu 100D	OE1	4.64
Arg 293C	NH2	Glu 100D	OE2	5.09
Lys 341B	NZ	Asp 363D	OD1	5.34
Lys 341B	NZ	Asp 363D	OD2	5.45
Lys 139B	NZ	Asp 2 D	OD1	6.25

*Project support provided by NIH/NIGMS Grant No. 2 R15 GM055929-03 and the North Dakota CCBN.*

1. Lynch RM, Paul RJ. *Science*, 222, 1344-1346, 1983.
2. Nakagawa T, Nagayama F. *Nippon. Suisan. Gakkaishi*, 55, 165-171, 1989.
3. Ouporov IV, Knull HR, Thomasson KA. *Biophys. J.*, 76, 17-27, 1999.
4. Waingeh FV, Lowe SL, Thomasson KA. *Biopolymers*, 73, 533-541, 2004.
5. Arnold H, Pette D. *Eur. J. Biochem.*, 6, 163-171, 1968
6. Northrup SH, Thomasson KA, Miller CM, Barker PD, Eltis LD, Guillemette JG, Inglis SC, Mauk AG. *Biochem.*, 32, 6613-6623, 1993.

## ACUTE EFFECTS OF DOPAMINE AND TRANSPORT BLOCKERS ON DOPAMINE TRANSPORTER PHOSPHORYLATION AND REGULATION

Balachandra K. Gorentla\* and Roxanne Vaughan

Department of Biochemistry & Molecular Biology, University of North Dakota, Grand Forks, ND 58202

The dopamine transporter (DAT) is a synaptic transmembrane protein found on dopaminergic neurons. It maintains homeostasis in synaptic neurotransmission by regulated dopamine uptake. In addition, DAT act as the target for psychostimulants like cocaine and amphetamine, and for therapeutic drugs like Ritalin. In rat striatal tissues and model cell lines, it has been demonstrated that the activation of PKC leads to dose dependent increase of DAT phosphorylation and correlative decrease in transport activity. It has also shown in previous studies that dopamine induces down regulation of DAT and cocaine induces up regulation of DAT by changing the surface level expression. It is not known if dopamine or transport blockers such as cocaine exert their effects by modulating intracellular signaling mechanisms that may then affect the DAT activity.

In this study we investigated if PKC-dependent DAT phosphorylation and transport activity were affected by acute exposure of dopamine or the transport blockers such as cocaine, mazindol, GBR12909, or the cocaine analog CFT. This study was done using in vivo <sup>32</sup>P-orthophosphate labeling in the presence of dopamine and various transport blockers in LLC-PK<sub>1</sub> cells expressing rDAT. The DAT phosphorylation was analyzed using immunoprecipitation, SDS-PAGE followed by autoradiography. Uptake assays were performed after pretreatment of dopamine or various transport blockers to study the transport activity.

Our study has shown that cocaine, CFT and mazindol did not affect the transport activity or basal or PKC stimulated phosphorylation. These results suggest that acute exposure of cocaine, CFT, or mazindol do not affect the signaling process related to DAT phosphorylation. However the GBR12909 significantly attenuated PKC stimulated DAT phosphorylation, suggesting that acute exposure of different classes of transport blockers may have differential effects on processes related to DAT phosphorylation. Dopamine alone increased DAT phosphorylation and correlatively decreased transport activity. Dopamine pretreatment was not additive with PKC stimulated DAT phosphorylation or down regulation of transport activity, suggesting dopamine induced DAT down regulation may be PKC dependent.

*Supported by NIH DA 13147*

## TOXICITY OF NANOMATERIALS TO LIVING CELLS

Yuhui Jin\*<sup>1</sup>, Yanfu Huan<sup>1</sup>, Julia Xiaojun Zhao<sup>1</sup>, Min Wu<sup>2</sup>, Shibichakravarthy Kannan<sup>2</sup>  
Departments of <sup>1</sup>Chemistry, and <sup>2</sup>Biochemistry and Molecular Biology, University of North Dakota,  
Grand Forks, ND 58202

We have investigated the toxicity of nanomaterials to living cells at the cellular and molecular levels. Recently, nanomaterials have being rapidly developed and shown great potential for use in various bioapplications due to their unique optical properties and high surface-to-volume ratio. Among them, one of the most attractive potential applications is the use of nanomaterials in cancer therapy. However, current researches have not demonstrated whether nanomaterials are nontoxic to living system. Therefore, investigation of toxicity of nanomaterials to living cells is critically needed.

We have studied the toxicity of nanomaterials to living cells from whole cell to molecular levels, such as the effects of nanomaterials on DNA damage, DNA repair activity, apoptosis, cell proliferation and death. Preliminary results have shown that nanomaterials have no apparent effects on survival in several types of living cells including lung epithelial cells A549 and Mardin-Darby canine kidney cells up to 72 hours. Our data also further demonstrate that nanomaterials do not cause significant DNA damage on specific bases or strand breaks against controls. We expect that this study will provide critical information for biotechnological and biomedical applications of nanomaterials.

MODULATION OF SRC-LIKE TYROSINE KINASE LYN PLAYS A ROLE IN *PSEUDOMONAS AERUGINOSA* INFECTION IN LUNG CELLS

Shibichakravarthy Kannan\*, Daniel C Foster, Jessica Knittel, and Min Wu

Department of Biochemistry and Molecular Biology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND

*Pseudomonas aeruginosa* (PA) can cause severe infection in immunodeficient individuals, such as Cystic Fibrosis (CF) patients. The initial contact and subsequent invasion of alveolar epithelium by the bacterium involves a series of proteins and lipids and these signaling events influence the outcome of the invasion process. Here, we investigated the mechanism of invasion of PA into alveolar epithelial cells. We found that PA could invade alveolar epithelial type II cells, and it was mediated by lipid raft activation. We demonstrate that Src-like tyrosine kinase Lyn p53/p56 which is associated with rafts was induced by PA infection in airway and lung epithelial cells. PA was found to actively recruit Lyn into rafts. Using transwell apparatus, Lyn was shown to move to apical regions of the pseudo epithelium upon PA infection by immunofluorescence staining. Lyn activation was prevented by polyunsaturated fatty acids like docosahexaenoic acid (DHA). DHA treatment prior to infection also significantly decreased PA internalization in epithelial cells as compared to controls. In addition, transfection of alveolar epithelial A549 cells with Lyn dominant negative construct and use of Src p56 specific inhibitor PP2 drastically reduced bacterial internalization. Changes in Lyn levels corresponded to changes in actin cytoskeleton. Thus we conclude that PA utilizes Lyn to rearrange cytoskeleton for invasion and blocking this pathway prevents its internalization. Hence we propose that Lyn may play a crucial role in the pathogenesis of PA induced lung damage in CF.



SUBSTITUTION AND PRICE EFFECTS OF CARBON EMISSION PENALTIES ON CO<sub>2</sub>  
EMISSIONS REDUCTION FROM ELECTRIC POWER PLANTS

Shankar Karki\*<sup>1</sup>, Michael D. Mann<sup>2</sup>, and Hossein Salehfar<sup>3</sup>

<sup>1</sup>School of Engineering and Mines, and the Departments of <sup>2</sup>Chemical Engineering, and <sup>3</sup>Electrical Engineering, University of North Dakota, Grand Forks, North Dakota 58202-9020

Eight years after its inception, the Kyoto Protocol finally entered into force on February 16, 2005. In this respect, it is imperative to explore different greenhouse gas (GHGs) emissions reduction options in developing countries under the Kyoto Protocol. A number of analyses reveal that half of the global emission reductions can be achieved by substituting fossil fuel by non-fossil fuel (*e.g.*, nuclear and renewable energy) sources for electric power generation. Pricing policies are more likely to achieve such a substitution resulting in a significant CO<sub>2</sub> emissions reduction. For this, a carbon emission penalty on fossil fuel based generation is one of the mechanisms of pricing carbon emissions. Imposing a carbon emission penalty on the fossil fuel generation enhances renewable based energy generation to substitute carbon intensive power generation. This effect is called the “substitution effect”. The corollary effect of imposing emission penalties on fossil fuel generating plants will be an increase in the average electricity price. This, in turn, will decrease the energy demand from customers due to the negative price elasticity of electricity demand. This effect is called the “price effect”. This paper examines the substitution and price effect of carbon emission penalties on fossil fuel generation in mitigating CO<sub>2</sub> emissions from the electric power sector in developing countries. Also discussed will be the costs and benefits of emission penalties in the electricity sector.

## FLUORESCENT NANOSENSOR FOR TARGETING SELENIUM IN A MIXTURE

Song Liang\*, Yanfu Huan, David T. Pierce, and Julia Xiaojun Zhao  
Department of Chemistry, University of North Dakota, Grand Forks, ND 58202

We have developed a novel nanosensor method for the highly sensitive determination of selenium in predigested organic samples. Recently, human studies have identified that trace amount of selenium provides a strong protecting effect against certain forms of cancer. Meanwhile, selenium deficiency could increase the risks of prostate colon cancers; cardiovascular disease as well as other health problems. Because of these potential health benefits, Se-enriched products are currently being developed for marketing. To ensure that the amount of selenium in these products meets current standards, a sensitive, rapid and simple determination of selenium in biological samples is critically needed.

The developed nanosensor method provides reproducible detection of trace amounts of inorganic selenium from aqueous biological samples. Optimal conditions of selenium detection have been investigated and the analytical characteristics of the nanosensor have been explored. Preliminary results have shown the nanosensor method was capable of detecting inorganic selenium in the range of 20 ng/L to 20  $\mu$ g/L using a variety of biological materials. The nanosensor method is convenient, fast, inexpensive, highly sensitive and also selective for trace concentrations of inorganic selenium.

NUCLEOTIDE DIVERSITY OF CHALONE ISOMERASE IN COMMON BEAN (*P. VULGARIS*)

Sujan Mamidi\*, Rian K. Lee, Phillip E. McClean

Department of Plant Sciences, North Dakota State University, Fargo, ND, USA

Evolution is a complex process in which several changes take place at the nucleotide level which are mainly indels and SNP (Single nucleotide polymorphisms). These changes are responsible for changes in translated proteins producing different morphological and/or physiological changes. Sequence comparisons provide an understanding of the evolution of gene families, describe molecular basis of adaptation and elucidate patterns of gene regulation and protein evolution.

Chalcone isomerase (CHI) is a gene that converts chalcone to naringenin. It is part of the flavonoid biosynthetic pathway in legumes, produces flavonoids and isoflavonoids. The objective of this project is to compare the patterns of sequence diversity at CHI and several loci in common bean, to investigate the utilization of intron sequence data for both phylogenetic and population genetic studies by analyzing both closely and distantly related common bean clades. This research permits the broadening of current germplasm base allowing the development of new cultivars, designing SNPs and other allele-specific primers to benefit breeders, establish the utilization of intron sequences as a tool for phylogenetic and population genetic studies.

Landraces, which represent the domesticated diversity in common bean, are being used for initial analysis. The approach is to sequence gene fragments by using primers based on published Tendergreen locus (2.1 Kb in length with four exons and three introns), and use phylogenetic analysis to study the relationship between these landraces. Based on sequence polymorphisms, SNP based primers will be designed and will be used to study polymorphism in the cultivars. Then population genetic parameters like nucleotide polymorphism, nucleotide diversity,  $F_u$  and  $L_i$   $D^*$  and  $F^*$  parameters, Tajima  $D$  parameter will be calculated. The results along with the statistics will be presented.

## AROMATIC TOSYLATE ESTERS AS LIGANDS FOR METAL IONS

Julius N. Ngwendson\*, Angie C. Olson, and Anamitro Banerjee  
Department of Chemistry, University of North Dakota, Grand Forks, ND 58202

We are investigating aromatic EDTA analogs as ligands for metal cations. X-ray crystallographic data and chemical reactivity of some of the tosylate ester intermediates indicate that the two aromatic rings face each other suggesting a  $\pi$ - $\pi$  interaction. This is consistent with the geometry of similar compounds reported in the literature.

We are investigating the possibility of using these compounds as ligands for metal cations forming metallocene type molecules (except that the rings are at an angle to each other). Compounds of this type have not been reported yet. If successful, the carboxylic acid group in the molecule may be used to connect it to a peptide chain. These compounds have potential applications as catalysts or as metal cation scavengers.

## COMPUTER MODELING OF ELECTROSTATIC INTERACTIONS BETWEEN LDH AND ACTIN

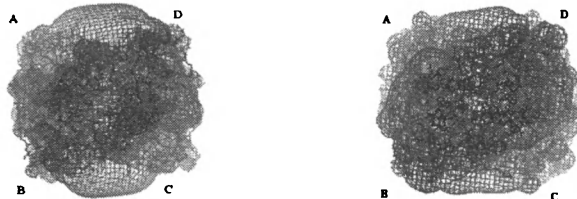
Eric N. Njabon\*, Stephen L. Lowe, Victor F. Waingeh, Kathryn A. Thomasson  
Department of Chemistry University of North Dakota, Grand Forks, ND 58202-9024

F-actin may play an important role in cellular metabolism by providing the conditions for high specificity for reactions within various metabolic pathways (1). The interaction of F-actin and glycolytic enzymes is one mechanism by which glycolytic enzymes can compartment (2). Experimental studies have shown that many glycolytic enzymes bind cytoskeletal proteins reversibly including lactate dehydrogenase (LDH), fructose-1,6-bisphosphate aldolase (aldolase), glyceraldehydes 3-phosphate dehydrogenase (GAPDH), 3-phosphoglycerate kinase, glucose isomerase, phosphofructokinase, and pyruvate kinase (3,4) and that the interaction may be electrostatic in nature (3). LDH is particularly interesting because different isoforms have different binding affinities (5). Herein, Brownian dynamics computer simulations examine the interactions of LDH isoforms with G- and F-actin.

Computational models of rabbit muscle and heart lactate dehydrogenase (LDHA and LDHB) were built by homology modeling from the crystal structures of human and pig LDHA obtained from RCSB Protein Data Bank. The LDH models were assigned charges using the Tanford-Kirkwood method in the molecular modeling package MacroDox. The electrostatic potential around each protein model was calculated at pH = 7, ionic strength  $I = 0.05$  M, and temperature = 298.15 K. Brownian dynamics (BD) simulations between LDH with previously built models of F-actin and G-actin (2) were performed.

Strong electrostatic interactions were observed between LDHA with F- or G-actin for both rabbit and human proteins. The important rabbit LDHA residues interacting with G-actin were Lys 89, Glu 15, Arg156. The important rabbit LDHA residues interacting with F-actin were Lys 277, Lys 13, Lys 221. The important actin residues identified were Glu 2, Asp 1, Asp 25, Glu 364, Glu 99, Glu 4, and Asp 363. Similar residues were observed for the interaction between human LDHA and actin. The residues identified as critical for binding with actin are located in the positive groove of the electrostatic field of LDH (Figure 1). The interaction of both species of LDHB with actin was much weaker because the electrostatic field about LDHB is very different from LDHA (Figure 1). BD simulations reveal strong interactions between the muscle form of LDH (LDHA) and F-actin, but like experiment the interactions of the heart form of LDH (LDHB) with F-actin are considerably weaker.

**Figure 1.** The electrostatic potential around LDH. Left LDHA Right LDHB. Light grey represents isopotential surfaces with a charge of +0.5 kcal/mol, the dark grey corresponds to isopotential surfaces of -0.5 kcal/mol.



Support for this project was provided by NIH/GMS 2R15 GM55929-03 and the ND CCBN.

1. Lakatos S, Minton AJ *Biol. Chem.*, 266, 18707-18713, 1991.
2. Ouporov IV, Knull HR, Thomasson KA *Biophys. J.*, 76, 17-27, 1999.
3. Knull HR, Walsh J *Curr. Top. Cell. Regul.*, 33, 15-30, 1992.
4. Ovadi J, Orosz F New Concepts for Control of Glycolysis, *in Channeling in Intermediary Metabolism*, Agius L & Sheratt H, eds. London: Portland Press, pp 237-268, 2001.
5. Berggren T, Arner A, Uveltis B *Urol. Res.*, 23, 595-9, 1995.

## HLA-DQ8ab TRANSGENIC MICE DEMONSTRATE AN INCREASED RESISTANCE TO THE PLAGUE

Deanna M. O'Bryant\*, Matthew L. Nilles, and David S. Bradley

Department of Microbiology and Immunology, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58202

The number of different antigenic peptides that can be presented by a Major Histocompatibility Complex (MHC) Class II molecule varies greatly from allele to allele. The more restricted the presentation, the less likely that molecule is to present self-peptides inducing an autoimmune response. However, those molecules are also less likely to present pathogenic antigens, leaving an individual potentially compromised in being able to clear an infection. Conversely, presentation of a large number of antigens, termed "promiscuity," may provide increased protection against pathogens, but also increased risk to develop an autoimmune disease. The human HLA-DQ8 allele, linked to increased susceptibility for rheumatoid arthritis (RA), and insulin-dependant diabetes mellitus, is promiscuous, *i.e.*, it allows for the presentation of a greater number of antigenic peptides, which has been postulated to allow thymic selection of self-reactive T cells that are activated in the periphery when encountering self-antigens. The promiscuity of the DQ8 molecule, while undesirable in predisposing an individual to autoimmune diseases, may be evolutionarily advantageous in protection from pathogens. To better understand promiscuity in antigen presentation we compared the susceptibility to *Yersinia pestis* of three different strains of mice: HLA-DQ8ab transgenic (tg) Ab0 mice, B10.T(6R), and out bred Swiss Webster mice expressing endogenous mouse MHC class II molecules. The amount of IgM and total IgG produced in HLA-DQ8abt<sub>g</sub> and out bred Swiss Webster mice was determined by ELISA.

Strain	Male	Female
	LD <sub>50</sub>	
DQ8ab(tg)	1.0 X 10 <sup>3</sup> CFU	3.2 X 10 <sup>4</sup> CFU
B10.T(6R)	3.0 X 10 <sup>2</sup> CFU	1.4 X 10 <sup>4</sup> CFU
Out bred	2.1 X 10 <sup>1</sup> CFU	2.2 X 10 <sup>1</sup> CFU
Mean Time to Death (Days)		
DQ8ab(tg)	7	7
B10.T(6R)	7	8
Out bred	7	7

Resistance against a *Y. pestis* infection is increased in female tg and inbred B10 mice, where no sex difference was observed with out bred Swiss Webster mice. Total IgG antibody production analysis demonstrated that the DQ8ab tg mice produced an IgG response to *Y. pestis* that increased over the entire course of the infection, compared to the out bred mice, which were unable to mount an antibody response against *Y. pestis*. IgM analysis revealed that out bred males have an impaired ability to elicit an IgM antibody response.

EFFECTS OF ESTRADIOL (E<sub>2</sub>) AND LINSEED MEAL (LSM) ON ORGAN WEIGHTS IN OVARECTOMIZED EWES

M. R. O'Neil\*, G.P. Lardy, L. Reynolds, J.S. Caton, K. Vonnahme  
Department of Animal and Range Science, North Dakota State University

Flaxseed contains secoisolariciresinol diglycoside (SDG), a phytoestrogen (PE) proposed to have both estrogenic and anti-estrogenic properties. The objective of the current study was to determine the estrogenic properties of SDG in OVX ewes. OVX ewes (n = 48) were fed a PE-free diet for four weeks (d -28 to d 0) following OVX to ensure the absence of any circulating endogenous estrogen or dietary PE. On d 0, OVX ewes were assigned randomly to a control group (PE-free diet; CON; n=12) or a 12.5% FSM for 1, 7, or 14 d (n = 12/group). Diets were based on beet pulp and formulated to provide similar amounts of energy (2.7 Mcal/kg) and CP (13.6%). On the last day of FSM feeding, OVX ewes were implanted with a subcutaneous E<sub>2</sub> implant (100 mg) for 0, 6, or 24 h. At necropsy, uteri, liver, and duodenum were weighed. Tissue weights are expressed as % empty body weight (live weight minus blood and digesta weight). The effects of E<sub>2</sub> on uterine weight depended upon hours exposed to E<sub>2</sub> and days fed FSM (P < 0.05). Uterine weight increased (P < 0.05) from 0 h to 24 h E<sub>2</sub> treatment in CON and 1 d FSM fed ewes. However, in ewes fed FSM for 7 or 14 d, E<sub>2</sub> exposure had no effect on uterine weight. Similarly, liver and duodenal weights for CON ewes increased with increased E<sub>2</sub> exposure (P < 0.05), but the increase in liver weight was ablated in ewes fed FSM for 1, 7, or 14 d. Furthermore, ewes fed FSM for 14 d and 24 h E<sub>2</sub> exposure had decreased (P<0.05) duodenal weight compared to ewes fed 14 d FSM and 0 h E<sub>2</sub> exposure. PE compounds in FSM influence the effects of exogenous E<sub>2</sub> on organ mass in OVX ewes. These effects warrant further investigation at a mechanistic level.

**Table 1.** Effect of dietary FSM and exogenous E<sub>2</sub> on tissue weights in OVX ewes (% body weight)

	Hours Post E <sub>2</sub>	Days Fed LSM				Pooled SEM
		0	1	7	14	
Uterus	0	0.05 <sup>a,b*</sup>	0.05 <sup>a</sup>	0.06 <sup>a,b*</sup>	0.06 <sup>a,b</sup>	0.01
	6	0.07 <sup>a,b</sup>	0.07 <sup>b,c,d</sup>	0.07 <sup>a,b,c</sup>	0.06 <sup>a,b</sup>	0.01
	24	0.10 <sup>c</sup>	0.13 <sup>f</sup>	0.09 <sup>c,d,e</sup>	0.09 <sup>d,e</sup>	0.01
Liver	0	1.34 <sup>a*</sup>	1.45 <sup>a,b</sup>	1.54 <sup>a,b,c*</sup>	1.48 <sup>a,b,c</sup>	0.04
	6	1.52 <sup>a,b,c*</sup>	1.51 <sup>a,b,c</sup>	1.50 <sup>a,b,c</sup>	1.38 <sup>a</sup>	0.06
	24	1.63 <sup>c</sup>	1.59 <sup>b,c</sup>	1.63 <sup>c</sup>	1.39 <sup>a</sup>	0.06
Duodenum	0	0.12 <sup>a*</sup>	0.19 <sup>b,c</sup>	0.20 <sup>b,c*</sup>	0.21 <sup>c</sup>	0.02
	6	0.16 <sup>a,b,c</sup>	0.19 <sup>b,c</sup>	0.20 <sup>b,c</sup>	0.12 <sup>a</sup>	0.02
	24	0.19 <sup>b,c</sup>	0.16 <sup>a,b,c</sup>	0.14 <sup>a,b</sup>	0.15 <sup>a,b</sup>	0.01

<sup>a,b,c,d,e,f</sup> Means ± SEM within tissue differ, P<0.05; \* n=3 ewes

USGS 30-M AND LIDAR 1-M DEMs TO ASSESS THE ACCURACY OF ELEVATIONS IN  
THE RED RIVER BASIN OF THE NORTH

Sarita Pachhai<sup>1</sup>, Bradley C. Rundquist<sup>1</sup>, Wesley D. Peck<sup>2</sup> and Bethany A. Bolles<sup>2</sup>

<sup>1</sup>Department of Geography, and <sup>2</sup>Energy & Environment Research Center,  
University of North Dakota, Grand Forks, ND 58202-9018

Digital elevation models (DEMs) are important data sources for land elevation information, and are increasingly used at a variety of mapping scales for a range of applications such as telecommunications, forestry, agriculture, urban planning, hydrological, and flood modelling. Elevation accuracy is one of the critical factors that affects the usefulness of DEMs. The study evaluates the accuracy of the topographic elevations presented in both 30-m National Elevation and a 1-m Light Detection And Ranging (LiDAR) datasets for the Forest River watershed of the Red River of the North, where both types of DEM datasets are available. We use a Geographic Information System (GIS) to analyze and visualize phenomena related to topographic elevation. The results will provide an evaluation of the discrepancies in modeled elevation, which depend on the spatial resolution of DEM data over the flat terrain of the basin.



## DOPAMINE TRANSPORTER LIGAND BINDING DOMAINS ANALYZED THROUGH PHOTOAFFINITY LABELING

M. Laura Parnas<sup>1\*</sup>, Jon D. Gaffaney<sup>1</sup>, Amy H. Newman<sup>2</sup>, Mu-Fa Zou<sup>2</sup>, John R. Lever<sup>3</sup>, Alope K. Dutta<sup>4</sup>, Rohit Kolhatkar<sup>4</sup>, and Roxanne A. Vaughan<sup>1</sup>

<sup>1</sup>University of North Dakota School of Medicine, Grand Forks, ND; <sup>2</sup>Medicinal Chemistry Section, NIDA-IRP, Baltimore, MD; <sup>3</sup>Department of Radiology, University of Missouri, Columbia, MO;

<sup>4</sup>Department of Pharmaceutical Sciences, Wayne State University, Detroit, MI

Dopamine neurotransmission in the brain is terminated by the dopamine transporter (DAT), a presynaptic transmembrane protein. This process regulates synaptic dopamine concentration and accessibility. A wide range of structurally distinct compounds such as the psychostimulant cocaine and GBR 12909, a piperazine derivative, bind to DAT and block its transport activity. The localization of the binding site for these uptake blockers and their relationship to the dopamine translocation pathway is unknown. The present studies examine the incorporation site on DAT of a novel cocaine photoaffinity label, [<sup>125</sup>I]MFZ-2-24 and a novel GBR 12909 photoaffinity label, [<sup>125</sup>I]D-147 in comparison to known binding sites of other DAT inhibitors.

Rat striatal dopamine transporters were photoaffinity labeled with [<sup>125</sup>I]MFZ-2-24 or [<sup>125</sup>I]D-147 and analyzed by trypsin proteolysis followed by epitope-specific immunoprecipitation using antisera 16 and 5, originated to N-terminal and extracellular loop 2 (EL2) amino acids respectively. Samples were electrophoresed using SDS-PAGE and autoradiography was obtained.

These approaches generated DAT [<sup>125</sup>I]MFZ-2-24 photolabeled fragments corresponding to N-terminal transmembrane domains (TMs) 1 and 2, and [<sup>125</sup>I]D-147 photolabeled fragments corresponding to both N-terminal TMs 1-2 and TMs 4-6. The fragments showed pharmacological and immunological specificity of antiserum recognition through peptide block analysis of antiserum recognition and photolabel displacement by known DAT substrates and blockers. The TM 1-2 region has been previously identified as a site for binding of other piperazine photoaffinity label [<sup>125</sup>I]DEEP, whereas a closely related cocaine analog, [<sup>125</sup>I]RTI-82, is known to bind to TMs 4-6.

The three-dimensional structure of DAT is not known, but the current findings suggest that TMs 1-2 and 4-6 are in close proximity and encompass at least part of the binding site for these two classes of uptake blockers. The incorporation pattern obtained for these photolabels suggests their spatial orientation within the binding pocket. More precise identification of the binding sites is currently being investigated by subjecting labeled peptide fragments to analysis by HPLC and mass spectrometry.

*Supported by NIDA DA 15175, and NIDA-IRP.*

USING HIGH SPATIAL RESOLUTION SATELLITE IMAGERY TO MAP LEAFY SPURGE  
(*EUPHORBIA ESULA*) AND CANADA THISTLE (*CIRSIUM ARVENSE*) IN THE NORTH UNIT OF  
THEODORE ROOSEVELT NATIONAL PARK

Brent E. Pringle\* and Bradley C. Rundquist  
Department of Geography, University of North Dakota, Grand Forks, ND 58202

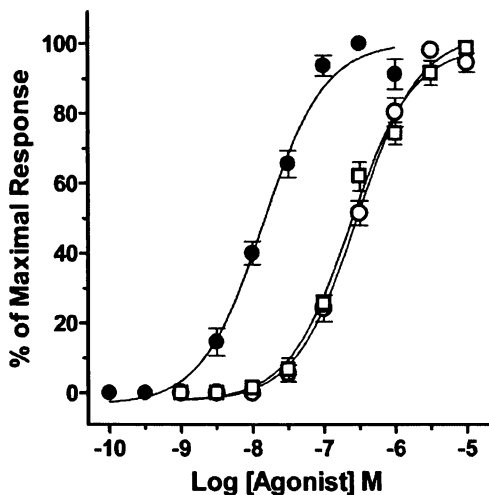
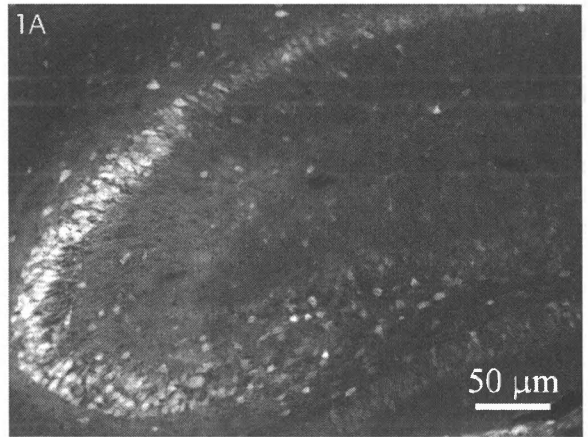
The aggressive invasions of both leafy spurge and Canada thistle into Theodore Roosevelt National Park (THRO) have gained much attention because of their ability to severely degrade large areas of land and displace native flora. The North Unit of THRO allows for minimal access, due to rugged terrain, for park managers to map infestations on the ground. Therefore, the use of geospatial technologies such as remote sensing and Geographical Information Systems (GIS) to help locate and map the extent of these plants has been sought after by park managers. IKONOS 4 x 4 m spatial resolution imagery was collected along with Global Positioning System (GPS) points of known infestations. ERDAS image processing software was used to find spectral signatures and results were put into a model, which was used to classify the image for both plant species. Of the North Unit's 9,754 ha land area, 12.8 ha was classified as Canada thistle and 12.7 ha was classified as leafy spurge.

## $\alpha_1$ -ADRENERGIC-MEDIATED ENHANCEMENT OF HIPPOCAMPAL CA3 NETWORK ACTIVITY

Katie E. Rau\*<sup>1</sup>, C.W.D. Jurgens<sup>1</sup>, C.A. Knudson<sup>2</sup>, J.D. King<sup>1</sup>, P.A. Carr<sup>2</sup>, J.E. Porter<sup>1</sup> and Van A. Doze<sup>1</sup>

<sup>1</sup>Departments of <sup>1</sup>Pharmacology, Physiology & Therapeutics, and <sup>2</sup>Anatomy & Cell Biology, University of North Dakota School of Medicine & Health Sciences, Grand Forks, ND 58202-9037, USA

Norepinephrine (NE), an endogenous neurotransmitter, has been shown to reinforce certain memory processes. The hippocampus, a higher cortical structure that receives extensive adrenergic inputs, is known to be critically involved in learning and memory. In particular, the CA3 region of the hippocampus has been implicated in associative memory recall and rapid memory acquisition. In this study, we investigated the effect of  $\alpha_1$  adrenergic receptor (AR) activation on hippocampal CA3 network activity. AR expression was first examined using immunocytochemistry with antibodies against  $\alpha_1$ ARs, which were found to be exceptionally dense in hippocampal CA3 pyramidal neurons (see figure 1A). Next, single-cell real time RT-PCR was used to determine the mRNA expression pattern of  $\alpha$ -ARs in CA3 pyramidal neurons. All pyramidal neurons expressed mRNA for  $\alpha$ -ARs (data not shown).



Hippocampal CA3 network activity was then examined *in vitro* using electrophysiological recordings in rat brain slices. The selective  $\alpha_1$ -AR agonist isoproterenol caused an enhancement of hippocampal CA3 network activity, as measured by an increase in the frequency of spontaneous burst discharges recorded in the hippocampal CA3 region. In the presence of  $\gamma$  AR blockade, concentration-response curves for isoproterenol (-), NE (-) and epinephrine (-) suggested that a  $\alpha_1$ -AR was involved in this response, as the rank order of potency was isoproterenol > NE = epinephrine (see agonist curves, left). Finally, equilibrium dissociation constants ( $pK_b$ ) of subtype-selective  $\alpha$ -AR antagonists were functionally determined to characterize the AR subtype modulating

hippocampal CA3 activity. The selective  $\alpha_1$ -AR antagonists, atenolol and metoprolol, blocked isoproterenol-induced enhancement with apparent  $K_b$  values of  $85 \pm 36$  and  $3.9 \pm 1.7$  nM, respectively. In contrast, the selective  $\alpha_2$ -AR antagonists, ICI-118,551 and butoxamine, inhibited isoproterenol-mediated enhancement with apparent low affinities ( $K_b = 222 \pm 61$  and  $9,268 \pm 512$  nM, respectively). Together, this pharmacological profile of subtype-selective  $\alpha$ -AR antagonists and the expression pattern of  $\alpha$ -AR in CA3 pyramidal neurons indicate that in this model,  $\alpha_1$ -AR activation enhances hippocampal CA3 activity. Insight into this effect may lead to a greater understanding of how NE modulates hippocampal networks and the role of NE in learning and memory.

### Acknowledgements

We would like to thank Sarah J. Boese and Kristin L. Hillman for help with the experiments. Supported in part by North Dakota EPSCoR, NSF grant EPS-0132289 (VAD), NSF CAREER award 0347259 (VAD), and NIH grant 5P20RR017699 from the COBRE program (PAC, JEP, VAD).

**WHOLE-BODY VERSUS SKELETAL CALCIUM RETENTION IN RATS: SHORT- AND LONG-TERM COMPARISONS USING  $^{47}\text{Ca}$  and  $^{41}\text{Ca}$  TRACERS**

J. A. Sattler\*<sup>1</sup>, M. R. Soule<sup>2</sup>, D. J. Hillegonds<sup>3</sup>, Z.K. Roughead<sup>2</sup> and J. L. Wagner<sup>1</sup>

<sup>1</sup>Department of Physics, University of North Dakota, <sup>2</sup>USDA-ARS Grand Forks Human Nutrition Research Center, Grand Forks, ND, and <sup>3</sup>Center for Accelerator Mass Spectrometry, Lawrence Livermore Laboratory, Livermore, CA

We sought to determine if whole body calcium (Ca) retention reflects skeletal uptake of calcium and to evaluate the feasibility of using urinary  $^{41}\text{Ca}$  for measurement of bone resorption.

Adult, female, Sprague-Dawley rats were injected intra-peritoneally with 0.8 (n=16) or 4  $\mu\text{Ci}$  (n=56) of  $^{47}\text{Ca}$  radiotracer; a subset (n=42) received a 2 nCi dose of  $^{41}\text{Ca}$ . To model the short term kinetics of the tracer in the body, its fractional retention in the whole body, heart, plasma, vertebrae (L5), tibia head and midshaft, muscle, as well as its total elimination in the urine and feces were determined at regular intervals by  $\gamma$ -scintillation counting. The data for the first 15 d are reported (mean  $\pm$  SD). Long-term kinetics ( $^{41}\text{Ca}$  data) were presented at EB.

Fecal excretion was the primary route of elimination of the absorbed calcium and was  $\sim$ 5 times higher than the urinary route. At 48 h, an estimated  $92 \pm 2$  (% dose) was retained in bone and only negligible amounts ( $<0.02\%$ ) were detected in the plasma, heart, and muscle compartments. Based on a single exponential model fit to the retention data, the biological half-life of the tracer was  $98 \pm 18$  d. On d 15, the whole body retention of the tracer was  $85 \pm 3$  (% dose), all of which resided in the bone. During the first 15 d, body calcium retention correlated strongly with skeletal calcium retention ( $R^2 = 0.99$ , n=49). We conclude that whole body calcium retention can be used as an accurate surrogate measure of calcium retention in the skeleton.

## THE INFLUENCE AND EFFECTIVENESS OF RECLAMATION SEEDING ON GRASSLAND RECOVERY

Sara Simmers\* and Susan Galatowitsch

Conservation Biology Program and Dept. of Horticultural Science, University of Minnesota, St. Paul, MN 55108

Research regarding road effects on plant communities has primarily focused on roadside vegetation and its relation to intact roads; these studies have documented compositional changes (1), declines in native species richness and increases in exotics (2, 3, 4). Even when a road is temporary, these changes have potential long-term impacts on plant communities. The Little Missouri National Grasslands (LMNG) of western North Dakota provides an excellent setting to study road impacts on native vegetation since its multiple-use management necessitates the construction of roads for oil and gas development, while simultaneously requiring that native prairie be conserved. The LMNG provides a further opportunity to study the reversibility of these impacts since road reclamation is required after mineral extraction ceases. Here we report on the first season of a two-year field study of vegetation along reclaimed access roads. Our objectives are to compare current species compositions with seed mixes used during reclamation, to assess the effectiveness of seeding particular species, and to determine patterns in compositional changes related to distance from the road. We selected and sampled all reclaimed roads for which there were records of reclamation seed mixes, along with several other criteria to ensure comparable histories. From June to August 2004, we surveyed 35 reclaimed roads throughout the Medora District of the LMNG, dating from the early 1980s to 2001. We placed three to five (depending on total length) 100 m perpendicular transects at random points along each road, centered at the midpoint. From the midpoint of each transect, we sampled a 1 m<sup>2</sup> plot at distances 3, 10, 25 and 50 m using a modified relevé technique by identifying all vascular plant species and assigning cover class estimates (5). Other variables such as elevation, aspect and grazing level were also recorded.

Most reclamations prior to about 1990 included the seeding of non-native forage species, while most after 1990 use only native species. In either scenario, a typical seed mix consists of about 4-6 species, whereas the undisturbed grassland at our sites typically had 28-34 species present. This may explain why there is a visible compositional difference in the plant community between the physically disturbed area on and along the reclaimed road and the undisturbed area further out, even where native species were seeded. For those species that were listed on seed tag records, 17 were seeded at more than 5 sites and 8 species were seeded at more than 10 sites. For the purposes of exploratory data analysis, we considered species seeded or present at more than 5 sites to be “frequent” enough to assess patterns. Those species that were seeded frequently and present frequently at 3 m at those seeded sites (i.e. “effectively seeded”) include the natives *Agropyron smithii*, *Stipa viridula*, *Calamovilfa longifolia*, *Linum perenne*, and the non-natives *Agropyron intermedium*, *Agropyron cristatum* and *Meliolotus officinalis*. Those species that were seeded frequently, yet present infrequently (“ineffectively seeded”) includes a mixture of eight native grasses and forbs: *Andropogon scoparius*, *Achillea millefolium*, *Agropyron riparium*, *Agropyron caninum*, *Ratibida columnifera*, *Bouteloua gracilis*, *Sporobolus cryptandrus*, and *Festuca ovina*. When frequency data for all sites is considered, species seeded during reclamation dominate the 3 m plots. At 10 m, seeded species make up about half of the most frequent species, dropping to an even lower proportion at 25 and 50 m distances from the reclaimed road. This pattern can be partly explained by the increase in total species as distance from the disturbed roadbed increased, but also by the fact that several seeded species were not present at distances greater than 10 m. Total frequency data also suggests certain species are able to recolonize the disturbed area without reseeding. Species that were not seeded, yet present in more than 15 of 3 m plots include *Artemisia cana*, *Artemisia frigida*, *Distichlis spicata*, *Koeleria pyramidata*, and *Stipa comata*. Seeding practices are affecting plant composition in at least 2 ways 1) non-native forage species used in early reclamations persist within the community and 2) though some native species are able to recolonize without seeding, in general the low diversity seed mixes used have yielded a low diversity, slow to recover zone spanning several meters on either side of the reclaimed road.

1. Angold PG *J Appl Ecol* 34:409-417, 1997.
2. Forcella F & Harvey SJ *Madroño* 30:102-109, 1983.
3. Tyser RW & Worley CA *Cons Biol* 6:253-262, 1992.
4. Gelbard JL & Belnap J *Cons Biol* 17:420-432, 2003.
5. Mueller-Dombois D & Ellenberg H *Aims and Methods of Vegetation Ecology*. New York: J Wiley & Sons, 1974.

## GLYCOLYTIC ENZYME INTERACTIONS WITH F-ACTIN: COMPARING RABBIT AND YEAST

Victor F. Waingeh\*<sup>1</sup>, Stephen L. Lowe<sup>1</sup>, Evgenii I. Kozliak<sup>1</sup>, Carol D. Gustafson<sup>2</sup>,  
Harvey R. Knull<sup>2</sup>, and Kathryn A. Thomasson<sup>1</sup>

Departments of <sup>1</sup>Chemistry & <sup>2</sup>Biochemistry, University of North Dakota, Grand Forks, ND 58202

Filamentous actin (F-actin) is the polymerized form of actin and constitutes the main component of the cytoskeleton. F-actin plays an important role in cellular metabolism by providing conditions for high specificity of reactions in a variety of metabolic pathways. A number of glycolytic enzymes, including fructose-1,6-bisphosphate aldolase (aldolase) and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) have been shown to bind F-actin (1-3) and these interactions have been shown to be electrostatic (1,4). Herein, computational and experimental approaches are employed to examine the enzyme/F-actin interactions in different species (yeast and rabbit). Comparing the trends across species provides a better understanding of the nature and role of these interactions.

Affinity chromatography and ultracentrifugation copelleting were used to study the binding of rabbit muscle and yeast enzymes to rabbit muscle and yeast actin. Falling ball viscometry was employed to demonstrate the possibility of cross-linking between the enzymes and actin. A Scatchard analysis of the copelleting results determined the binding affinities of enzymes to F-actin.

To complement the experiment, the computational method of Brownian dynamics simulated the enzyme-actin interactions and determined interaction free energies and binding modes. The molecular modeling package, MacroDox was used to calculate protein charges, determine electrostatic potential fields around the proteins and run the simulations. Calculations were done at pH = 7.0, ionic strength, I = 0.05 M, and temperature = 298.15 K.

Table 1 shows the binding dissociation constants and free energies for enzymes interacting with F-actin in yeast and rabbit muscle. The results show that rabbit enzymes bind rabbit F-actin with both high and low affinity, whereas yeast enzymes show only low affinity binding to yeast F-actin. These observations are also observed by the BD simulations, which reveal much more negative free energies for rabbit enzymes/F-actin interactions than for yeast enzyme/F-actin interactions. Thus, enzyme/actin interactions do occur in yeast, but to a lesser extent and with much lower affinities.

**Table 1: Dissociation Constants and Calculated Free Energy for Enzyme/Actin Interactions.**

*The "true"  $K_D$  represents the dissociation constant per actin monomer bound.*

Actin	Enzyme	Affinity	$K_D$ (mM)	"True" $K_D$ (mM)	Free Energy (kcal/mol)
Muscle	Muscle Aldolase	high	0.67	0.03±0.08	-1.91±0.08
		low	12.25	3.05±0.19	
Muscle	Muscle GAPDH	high	0.71	0.03±0.01	-0.81±0.10
		low	15.80	3.50±0.20	
Yeast	Yeast aldolase	low	12.90	1.29±0.09	+0.48±0.10
Yeast	Yeast GAPDH	low	8.4	0.84±0.06	-0.51±0.04

We thank the ND CCBN and NIH/NIGMS Grant No. 2 R15 GM055929-03 & ND EPSCoR for support. We also thank Karl Wald and Dr. Katherine Sukalski for their valuable contributions.

1) Knull HR, Walsh J. *Curr. Top. Cell. Regul.*, 33, 15–30, 1992.

2) Ouporov IV, Knull HR, Lowe SL, Thomasson KA. *J. Mol. Recognit.*, 14, 29-41, 2001.

3) Nakagawa T, Nagyama F. *Nippon Suisan Gakkaiski*, 55, 165-171, 1989.

4) Waingeh VF, Lowe SL, Thomasson KA *Biopolymers*, 73, 533-541, 2004.

INDICATIONS FOR STRONG METAL SUPPORT INTERACTIONS: THE CASE OF CO<sub>2</sub> ADSORPTION ON Cu NANO PARTICLES SUPPORTED ON ZnO(0001)

J. Wang, S. Funk\*, and U. Burghaus

Department of Chemistry, Biochemistry and Molecular Biology, North Dakota State University, Fargo, ND 58105

The strong metal support interaction (SMSI)<sup>1,2</sup>, defined as a close chemical and physical interaction between metal deposits (clusters) and a metal oxide support, is of great importance in heterogeneous catalysis. The concept is generally applied to account for changes in catalytic activity and selectivity when metals are supported on metal oxide substrates. In order to gain a deeper understanding in these phenomena, a well defined model catalyst, Cu/ZnO(0001), was employed by means of Cu metal vapor deposition on a ZnO(0001) single crystal support. Furthermore, CO<sub>2</sub> has been used as a probe molecule.

In the present study, we will provide consistent hints for strong metal support interactions in CO<sub>2</sub> adsorption on Cu/ZnO(0001) by a combination of molecular beam scattering techniques with thermal desorption spectroscopy (TDS) measurements<sup>3</sup>. Accordingly, the Cu nano-particles act initially, via an intrinsic precursor state, as the landing site for the gas phase species; however, these populate solely along the perimeter of the Cu deposits. With increasing CO<sub>2</sub> exposure, the Cu cluster become covered as well. The Cu-free areas of the ZnO support, however, remain essentially clean. Therefore, both adsorption dynamics and kinetics contradict those seen on the bare ZnO(0001) support<sup>4</sup>, which indicates synergistic effects such as a strong metal support interaction.

- 
- 1) Tauster SJ, Fung SC, R.T.K. Baker RTK, Horsley JA *Science* 211, 1121-1125, 1981.
  - 2) Goodman DW, *Catalysis Letters* 99: 1-4, 2005.
  - 3) Wang J, Funk S, Burghaus U, *in preparation*.
  - 4) Wang J, Burghaus U *Chemical Physics Letters* 403, 42-46, 2004.

## PHOTOCHEMISTRY OF 2-NITROBENZYL ENOL ETHERS: OXIDATIVE C=C BOND SCISSION

Promise K. Yong\* and Anamitro Banerjee

Department of Chemistry, University of North Dakota, Grand Forks, ND 58202

Enols are important reactive intermediates in organic chemistry. We investigated their photochemical generation from the photolysis of 2-nitrobenzyl enol ethers. Photogeneration of enols will provide a temporal and spatial control over the release under neutral or physiological conditions. When the photolysis of the vinyl ethers were carried out in presence of air in acetonitrile as solvent, a C=C bond scission occurred, that resulted in the formation of the ketone (about 60%), while the expected aldehyde was obtained as a minor product (about 20%). Based on product studies, we speculate that a photoinduced electron transfer, followed by a 1,2-dioxetane formation may be responsible for the unexpected formation of the ketone. While this reaction does not lead to the photogeneration of enols, it gives new insights into the photochemical behavior of vinyl ethers.



**COMMUNICATIONS**  
**PROFESSIONAL**

## SCHEDULE OF PRESENTATIONS — Lecture Bowl and River Valley Room — UND Memorial Union

**Lecture Bowl (Session A) — Glenn Lykken (Univ. North Dakota) session chair**

- 9:00 Carolyn Jordan (UND EERC): *ENVIRONMENTAL IMPLICATIONS OF SELENIUM'S EFFECTS ON MERCURY RETIREMENT*
- 9:20 J. Wang (NDSU): *STRUCTURE ACTIVITY RELATIONSHIP: THE CASE OF CO<sub>2</sub>/ZnO(0001) AND CO/Cu-ZnO(0001)*
- 9:40 Saiprassad Jangiti (UND): *DESIGN OF GAS-SOLID FIXED-BED REACTORS USING COMPUTATIONAL FLUID DYNAMICS*
- 10:00 LeRoy Pazdernik (UND): *NANOTECHNOLOGY: ALIGNING PROTEIN MOLECULES IN MONOLAYER FILMS OF NANOMETER THICKNESS*
- 10:20 Coffee Break
- 10:40 Nick Ralston (UND EERC): *COMPARISON OF DNA DAMAGE RESULTING FROM EXPOSURE TO VARIOUS NICKEL SPECIES*
- 11:00 Glenn Lykken (UND): *THE RADON WE BREATHE: PROGENY IN THE US REFLECTS SEASONAL VARIATION*
- 11:20 Laura Raymond (UND EERC): *PHYSIOLOGICAL IMPLICATIONS OF MERCURY'S EFFECTS ON SELENIUM AVAILABILITY*
- 11:40 Richard Josephs, UND **North Dakota Research Foundation Lecture: A PETROGRAPHIC ANALYSIS OF EXTENDED MIDDLE MISSOURI CERAMICS FROM NORTH DAKOTA**

**Lecture Bowl (Session B) — Siegfried Detke (Univ. North Dakota) session chair**

- 9:00 Cindy Anderson (UND): *INTERGENERATIONAL EFFECTS OF IN-UTERO STRESS: PERPETUATION OF HYPERTENSION AS A RESPONSE TO DEVELOPMENTAL PLASTICITY*
- 9:20 Heidi Super (Minot State): *STUDIES OF CHROMOSOME REARRANGEMENT AND GENE FUSIONS INVOLVING THE HUMAN MYELOID-LYMPHOID LEUKEMIA GENE (MLL)*
- 9:40 Rugao Liu (UND):
- 10:00 Siegfried Detke (UND): *COMPARISON OF THE LEVELS OF THE SPICING FACTOR PSF IN INDIVIDUALS WITH DYSTONIA*
- 10:20 Coffee Break
- 10:40 Chris Keller (Minot State): *GENETIC CONTROL OF LEAF EXPANSION IN ARABIDOPSIS*
- 11:00 Chris Beachy (Minot State): *FUNCTIONAL GENOMICS AND DEVELOPMENT OF A ECOTOXICOLOGICAL MODEL USING SALAMANDER*
- 11:20 Kirill Levine (NDSU): *ELECTRODEPOSITION OF POLYPYRROLE ON ALUMINUM ALLOY 2024-T3 IN THE PRESENCE OF DIFFERENT MEDIATING COMPOUNDS FOR THE PURPOSE OF CORROSION PROTECTION*
- 11:40 Gene Homandberg (UND):

## INTERGENERATIONAL EFFECTS OF IN-UTERO STRESS: PERPETUATION OF HYPERTENSION AS A RESPONSE TO DEVELOPMENTAL PLASTICITY

Cindy M. Anderson\*<sup>2</sup>, Faye Lopez<sup>1</sup>, Ashley Zimmer<sup>1</sup>, Hai-Ying Zhang<sup>1</sup>, Joseph N. Benoit<sup>1</sup>  
 Department of Pharmacology, Physiology and Therapeutics, School of Medicine and Health Sciences<sup>1</sup>  
 Family and Community Nursing, College of Nursing<sup>2</sup>, University of North Dakota, Grand Forks, ND

Several investigators have suggested that fetal stress predisposes the adult to pathologic conditions including insulin resistance, atherosclerosis, and hypertension [1, 2]. Yet, little is known about the mechanism contributing to long-term cardiovascular risk in offspring exposed to in-utero stress. Further, the mechanism contributing to the perpetuation of cardiovascular risk in subsequent generations is unclear [3]. The purpose of this study was to determine the influence of in-utero stress on systolic blood pressure and vascular responsiveness in male and female rats exposed to in-utero stress (first generation), and in the offspring of affected first generation rats (second generation).

Reduced utero-placental perfusion pressure (RUPP) was used as the method for induction of in-utero stress, as it mimics the origins and pathologic findings associated with the human condition, preeclampsia [4]. RUPP was induced by surgical constriction of the uterine arteries and abdominal aortae of pregnant Sprague-Dawley rats (n=16) on day 14 of gestation, with resulting offspring considered first generation (male n=52; female n=61). Control dams (n=14) were bred and completed gestation without intervention, their offspring comprising the control offspring group (male n=89; female n=73). Male and female offspring from the first generation experimental group were bred, comprising the second generation experimental group (male n=30; female n=37).

Dams were allowed to deliver spontaneously. Offspring from control, first and second generation experimental groups were identified and weighed within 12 hours of birth. Weights were measured weekly up to a maximum of 12 weeks of age. Systolic blood pressure was measured weekly beginning at 4 weeks of age using a tail cuff coupled to a photoelectric sensor and dual channel recorder. At 6, 9 and 12 weeks of age, animals were euthanized. Mesenteric arteries were dissected free from surrounding tissue and mounted on a small vessel wire myograph for measurement of isometric tension (n=5/gender/group). Compared to control, first generation litters were smaller (p<0.01) and reduced birth weight was evident only in male offspring (p<0.001). Second generation offspring birth weight was significantly increased over both control and first generation weights among males (p<0.001) and females (p<0.01). Systolic blood pressure was increased in both male and female first (all age points, p<0.001) and in second generation experimental offspring by 5 weeks (p<0.001) and 9 weeks (p<0.001) in males and females respectively, as compared to control. Mesenteric resistance arteries demonstrated increased responsiveness to vasoconstrictor agents phenylephrine and potassium chloride in all experimental offspring. Endothelial-dependent and independent relaxation was not significantly different between groups. Our data suggest that in-utero stress alters developmental processes influencing vascular regulation which are independent of fetal growth restriction and that these alterations persist through subsequent generations, resulting in hypertension.

- 
- 1) Barker DJ, Eriksson JG, Forsen T, Osmond C *Int J Epidemiol.*, 31(6), 1235-9, 2002.
  - 2) Lau C, Rogers JM *Birth Defects Res C Embryo Today*, 72(4), 300-12, 2004.
  - 3) Barker DJ *J Epidemiol Community Health*, 58(2), 114-5, 2004.
  - 4) Anderson CM, Lopez F, Zhang H, Pavlish K, Benoit JN *Biol Reprod.*, 72(3), 762-6, 2005.

*Supported by University of North Dakota Faculty Research Seed Money Council, UND College of Nursing, Eta Upsilon Chapter of Sigma Theta Tau International and NIDDK-51430*

FUNCTIONAL GENOMICS AND DEVELOPMENT OF A ECOTOXICOLOGICAL  
MODEL USING SALAMANDERChristopher K. Beachy\*<sup>1</sup> and S. Randal Voss<sup>2</sup><sup>1</sup>Department of Biology, Minot State University, Minot, ND 58707 and <sup>2</sup>Department of Biology,  
University of Kentucky, Lexington, KY

We are using a functional genomic approach to develop use of salamander (the axolotl, *Ambystoma mexicanum*) as a ecotoxicological model. Our objective is to develop an *in vivo* bioassay to test water borne chemicals for endocrine disruption at the molecular level. In this abstract, we describe our initial project that is underway toward development of salamander as *in vivo* bioassay.

We will define the gene expression profile of erythrocytes collected from TH treated vs TH non-treated larval aquatic salamanders at a known developmental stage. Our experiment will identify specific gene expression differences that can serve as biomarkers to characterize chemically-mediated disruptions of thyroid hormone induced gene expression. In a follow-up experiment we will use our bioassay to test a known aquatic toxicant (atrazine).

Salamanders (*A. mexicanum*) will be grown to a known developmental stage (72 hours after Developmental Stage 55). Two groups of individuals will be established at this time: One group of 12 individuals (Group A) will be treated with TH and another group of 12 individuals (Group B) will not be treated. Prior to TH treatment, 4 subgroups of 3 individuals each will be established within groups (A1-A4; B1-B4). At this time, blood cells will be collected from all individuals and an equivalent number of blood cells from each individual will be pooled to obtain subgroup pools for total RNA isolation. Using this same method, total RNA will be isolated from three subsequent time points (Time 1-3). Thus, we will obtain 4 total RNA isolations for each subgroup, and each will be used separately to make probes for microarray analysis using a custom oligonucleotide chip (Affymetrix). In this experiment, we will process 2 groups x 4 subgroups x 4 time points = 32 chips.

In a follow-up experiment we will examine the effect of Atrazine on TH induced gene expression. Atrazine will be administered at 1 physiologically relevant concentration throughout the larval period: 4 subgroups (3 individuals each) x 4 time points = 16 chips.

## GLUTATHIONE DEPLETION INCREASES OXIDATIVE STRESS AND PROMOTES MOTOR NEURON-LIKE CELL APOPTOSIS

Liying Chi, Yan Ke, Chun Luo, and Rugao Liu\*

Department of Anatomy and Cell Biology, University of North Dakota School of Medicine, School of Medicine and Health Sciences, Grand Forks, ND

The mechanism of selective and age-dependent motor neuron degeneration in human amyotrophic lateral sclerosis (ALS) has not been defined and the role of glutathione (GSH) in association with motor neuron death remains largely unknown. A motor neuron-like cell culture system and a transgenic mouse model were used to study the alteration of cellular GSH in motor neuron cell death. Exposure of NSC34 cells to Ethacrynic Acid (EA) or L-Buthionine Sulfoximine (BSO) dramatically reduced the cellular GSH level, and was accompanied by increased production of reactive oxygen species (ROS) measured by the DCF fluorescent oxidation assay. In addition, GSH depletion enhanced oxidative stress markers, AP-1 transcriptional activation, c-Jun, c-Fos and HO-1 expression in NSC34 cells analyzed by a luciferase reporter, western blotting and quantitative PCR assays respectively. Furthermore, reduction of GSH decreased mitochondrial function, and promoted cell apoptosis, with redistribution of apoptosis inducing factor (AIF), release of cytochrome C, and activation of caspase 3. In a transgenic mouse model overexpressing mutant G93A-SOD1 gene, the reduction of GSH in the spinal cord and motor neuron cells correlated with AIF nucleus translocation, caspase 3 activation, and motor neuron degeneration during ALS-like disease onset and progression. Taken together, the *in vitro* and *in vivo* data suggest that decreased GSH and increased oxidative stress contributed, at least in part, to the motor neuron degeneration of ALS.

*Supported by MDA, AG23923, NS45829, HL75034, KSCHIRT and EPSCOR*

## COMPARISON OF THE LEVELS OF THE SPICING FACTOR PSF IN INDIVIDUALS WITH DYSTONIA

Siegfried Detke\*

Department of Biochemistry and Molecular Biology, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND, USA, 58202

Dystonia is a neurological disorder characterized by sustained involuntary muscle contraction. One or more sites of the body is involved resulting in twisting and repetitive movement or abnormal posture. There are at least thirteen separate loci that have been implicated in primary dystonia (dystonia with no other neurological abnormalities). The affected gene for only a few of these cases has been identified. While searching for proteins involved in drug resistance, I noticed a protein that appeared to be elevated in the brain of an individual with dystonia. This protein was found to be present in numerous cell cultures so I purified to homogeneity by preparative isoelectric focusing and SDS polyacrylamide gel electrophoresis this protein from human embryonic kidney cells (*i.e.*, HEK293). The identity of the protein was determined by mass spec and was found to be a protein previously identified and called PSF, a protein of multi functions one of which is RNA splicing. Western blots probed with anti PSF antibodies verified this identification.

Western blots of whole cell lysates of the dorsal region from area 4 of the motor cortex of human brain samples were probed with anti PSF and tubulin antibodies to determine the relative abundance of these proteins in normal individuals and individuals with dystonia. There was almost a four-fold difference in the amount of PSF relative to tubulin among these individuals but there was no significant difference in the average between these two groups of individuals.

ANOTHER MODEL OF OSTEOARTHRITIC CARTILAGE DAMAGE: ACTIONS OF COLLAGEN FRAGMENTS/PEPTIDES AND A GLOBAL MECHANISM IN WHICH MATRIX DEGRADATION FRAGMENTS DETERMINE THE FATE OF INJURED CARTILAGE

Danping Guo\*, Lei Ding, and Gene A. Homandberg\*

Department of Biochemistry & Molecular Biology, School of Medicine & Health Sciences, University of North Dakota, Grand Forks, North Dakota, USA 58202

In osteoarthritis (OA), extensive degradation of articular cartilage occurs, resulting not only in loss of mechanical properties but also presentation of new signaling molecules, proteolytic fragments derived from the matrix, that may interact with the receptors or targets of their precursor and alter chondrocytic homeostasis. These signaling molecules might retain only a portion of the native properties and competitively block the native ligand or instead bind the native ligand and alter its ability to communicate with its respective receptor. The cartilage degradation results in proteolytic fragments of fibronectin (Fn), a ubiquitous cell associated molecule and of type II collagen, and many others. We have reported the ability of fibronectin fragments (Fn-fs) to greatly augment cartilage destruction through upregulation of catabolic cytokines and matrix metalloproteinases (MMPs). Our preliminary work (Ding L *et al*) suggests that Fn-f bind the classical alpha5beta1 Fn receptor, alter distribution of this receptor, disrupt actin filaments and enhance MAP kinase activation. We have recently begun to characterize the effects of collagen fragments (col-f) and synthetic collagen peptides (col-p) from the N and C-telo (globular ends of collagen) on cartilage homeostasis and to characterize and compare the matrix-receptor-kinase mechanism to that of Fn-f. A major objective here was to define the target receptors of col-f and col-p and compare to those of Fn-f.

Chondrocyte monolayer cultures were derived from the cartilage of bovine metacarpophalangeal joints. Col-f were derived from collagenase digests of type II collagen and col-p by chemical synthesis. Fn-f were isolated after cathepsin D and thrombin digestion and affinity elution. The effects of peptides/fragments on cartilage matrix degradation were measured by quantitation of proteoglycan left in cartilage explants after extended culture. Enhanced matrix metalloproteinase (MMP-1,-3 and -13) expression was studied by western blots of conditioned media. Fluorescent confocal microscopy was used to visualize changes caused by added fragments. Bovine chondrocyte monolayer cultures were treated with unlabeled or with rhodamine-labeled peptides or fragments for 4 hrs or with BSA as a negative control. Cells were also reacted with FITC-phalloidin to test for changes in distribution of actin. Cells were then fixed by 3% paraformaldehyde, permeabilized and probed with antibodies against alpha 1,2 or 5 integrin subunits, or native Fn or native type II collagen to test for the targets of the fragments/peptides. The alpha 1 and 2 subunits define collagen receptors while the alpha5 defines a Fn receptor.

We report that col-f and col-p enhance cartilage matrix degradation to significant levels (20-60%) and enhance expression of MMPs 1, 3 and -13 by up to several fold. Rhodamine labeled col-f/col-p added to cartilage explants bound to chondrocytes and concentrated in pericellular regions. As observed by confocal and z-section analysis, the col-f/col-p were internalized by chondrocytes and formed intracellular patterns of thick rings proximal to the cell membrane or formed a more polarized wave pattern from one side of the cell membrane internally toward the nucleus. This sheet also contained vesicles with higher levels of col-f/col-p that also colocalized with antibodies to integrin subunits. The col-f/col-p colocalized with alpha 1, 2, 5 subunits while control peptides and BSA showed less interaction. The cytosolic pattern caused by col-f/col-p colocalized with internalized alpha1 subunits and the ligands appeared to decrease membrane bound receptor. However, col-f/col-p caused a diffuse intracellular distribution of alpha2 into smaller vesicles than that of alpha1. Col-f and col-p appeared to affect alpha5 similarly to alpha1. Fn-f had similar effects as the col-f/col-p on alpha 1, 2 and 5. Col-f/col-p and Fn-f appeared to alter actin distribution. Fn-f as well as col-f/col-p colocalized with both type II collagen and chondrocytic Fn on the cell surface. Since the alpha1 and 2 receptors are collagen receptors and are not known to bind isolated Fn and the alpha5 is a Fn receptor not known to bind isolated collagen, these data collectively show that col-f/col-p and Fn-f can bind not only their classical receptors but bind proximal to other integrins by interaction with their respective ligands. For example, Fn-f can bind purified collagen and thus, may bind not only Fn receptors but also near collagen receptors, while col-f/col-p bind purified Fn and when added to chondrocytes may effect Fn signaling.

We propose a global mechanism in which Fn-fs bind the alpha5 Fn receptor as well as type II collagen to also allow communication with the alpha1/alpha2 collagen receptors. Reciprocally, collagen fragments can communicate with the Fn pathway by binding native Fn proximal to Fn receptors. This would result in a common signaling mechanism of polarization of integrins and subsequent alteration of the normal signaling pathways. Thus, to therapeutically prevent OA like cartilage degradation by matrix fragments, at least several integrins may have to be targeted, or alternately, a common signaling pathway downstream of integrins would have to be attenuated.

## DESIGN OF GAS–SOLID FIXED-BED REACTORS USING COMPUTATIONAL FLUID DYNAMICS

Saiprasad Jangiti\*<sup>1</sup>, Nikhil Patel<sup>1</sup>, and <sup>2</sup>Nanak Grewal

<sup>1</sup>Energy & Environmental Research Center and <sup>2</sup>Department of Mechanical Engineering,  
University of North Dakota Grand Forks, ND 58202

The critical parameters influencing heat-transfer augmentation in a gas–solid fixed-bed reactor are being studied. In order to understand the fluid flow, heat, and mass transfer characteristics occurring in the fixed beds, a computational modeling approach is being considered.

An unstructured packed bed is generated using the Monte Carlo simulation technique. Fluid flow and pressure drop characteristics for the generated packed bed are modeled and experimentally validated. The pressure drop profile obtained for various flow rates corresponds to the Ergun equation. The radial velocity distribution corresponds to the profiles presented in the literature for similar geometry and boundary conditions. The current research effort at the Energy & Environmental Research Center (EERC) is aimed at modeling heat transfer in an inert bed composed of spherical ceramic particles. The efforts will be extended to modeling of a reacting packed bed and its experimental validation.



## ENVIRONMENTAL IMPLICATIONS OF SELENIUM'S EFFECTS ON MERCURY RETIREMENT

C. R. Jordan,\* L. J. Raymond, and N.V.C. Ralston

University of North Dakota, Energy &amp; Environmental Research Center, Grand Forks, ND

Growing awareness of the presence of mercury (Hg) in the environment and in food sources has led to mounting public concern and increasing policy consideration by legislative and regulatory agencies. Because of its longevity in the ecosystem and production and release by industrial processes, Hg contamination is essentially global. Elemental mercury ( $\text{Hg}^0$ ) is a uniquely volatile heavy element that is released into the atmosphere from numerous natural and human sources. Once airborne,  $\text{Hg}^0$  joins the atmospheric pool and is globally redistributed until it oxidizes in the upper atmosphere and precipitates. Variable portions will be photoreduced by solar radiation and reemitted, while the rest remains in the oxidized form ( $\text{Hg}^{+2}$ ) to be temporarily retained where it falls. Certain quantities of this retained  $\text{Hg}^{+2}$  form stable complexes with materials in the environment that reduce its biological availability while the rest is accumulated by living organisms. When Hg accumulation rates exceed the capacity of natural mechanisms to release it into the atmosphere or geologically retire it from active cycling, Hg concentrations can build up in aquatic biota.

Anaerobic bacteria employ a biochemical pathway that adds a methyl group to Hg they have incorporated. This process creates methylmercury ( $\text{CH}_3\text{Hg}$ ), the predominant form of Hg that bioaccumulates in organisms of the food web. Creatures accumulate  $\text{CH}_3\text{Hg}$  and  $\text{Hg}^{+2}$  from their food sources, biomagnifying Hg concentrations from prey to predator. This begins at the bottom of the food web. Mercury present in bacteria and plankton is consumed by microinvertebrates. These invertebrates are consumed by larger creatures that are consumed by fish that are in turn consumed by even larger fish. As a result of these amplification steps, fish at the top of the food web can harbor tissue Hg concentrations  $>10^6$ -fold higher than that of the water in which they swim.

Measuring the amount of Hg present in the environment or food sources provides an incomplete and potentially inaccurate indication of Hg associated risks if the presence and effects of selenium (Se) are not also considered. Chemically similar to sulfur, Se is a nutritionally essential element required to support synthesis of a number of proteins that are expressed in tissues of all creatures. The selenide formed during each cycle of protein synthesis has an extremely high affinity for Hg ( $k_d 10^{-45}$ ). As a result, HgSe complexes readily form, especially in reducing environments. Selenium addition to aquatic ecosystems has been found to enhance Hg retirement and reduce Hg bioaccumulation, potentially as a result of HgSe formation. Since Se suspended in the water column will be in an oxidized state that is unable to bind Hg, the mechanism of HgSe formation is apt to be biologically mediated, potentially occurring through direct interaction with intracellular selenide. The HgSe interaction is apparent throughout the Hg cycle, influencing its transport, biogeochemical exposure, bioavailability, toxicological consequences, and remediation.

While the amount of Hg present in the environment and food sources is important to know, it is equally important to determine whether sufficient Se is available to sponsor Hg retirement through formation of HgSe and still support synthesis of Se-dependent proteins. The molar relationship between Hg and Se governs not only Hg accumulation in aquatic food webs, it also determines sensitivity or robustness of Hg-exposed organisms. Sensitivity to Hg toxicity in exposed creatures appears to depend more upon the balance of Hg and Se present in the system than upon the amount of Hg involved.

Phytoremediation, the use of plants to remove mercury from contaminated soil or water, may involve the formation of HgSe complexes within the plants. Certain plants are known to accumulate large amounts of Hg and Se, but the interactions of the two elements in these plants remains inadequately understood. Known and potential Hg hyperaccumulator plant species are under study to determine whether the presence of Se influences Hg accumulation. If HgSe complexes are formed in the tissues of these plants, efficient, permanent, and inexpensive retirement of Hg may be possible.

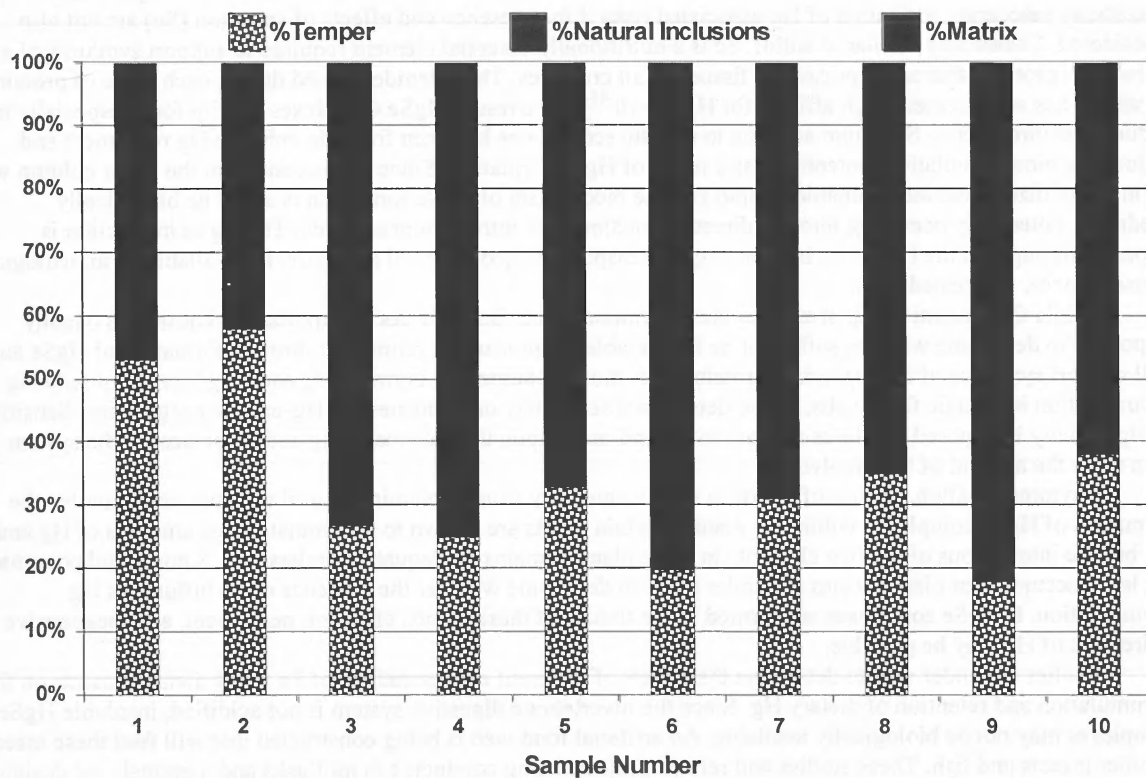
Studies are under way to determine the effects of different concentrations of Se in the diets of insects on the accumulation and retention of dietary Hg. Since the invertebrate digestive system is not acidified, insoluble HgSe complexes may not be biologically available. An artificial food web is being constructed that will feed these insects to other insects and fish. These studies and related projects being conducted in mollusks and mammals are designed to examine the influence of Se availability on Hg bioaccumulation. These data will allow us to assess the potential magnitude of these effects in the environment.

## A PETROGRAPHIC ANALYSIS OF EXTENDED MIDDLE MISSOURI CERAMICS FROM NORTH DAKOTA

Richard L. Josephs

Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND

This paper summarizes a petrographic analysis of ten thin sections prepared from Riggs and Fort Yates ware sherds (ca. 1200 to 1450 A.D.) collected from McLean County, North Dakota. This is the first study of its kind conducted on prehistoric ceramics collected in North Dakota and the first ceramic petrography investigation to use micromorphological descriptive protocol to enhance the overall evaluation of the thin sections. The procedure identified, described, and estimated the percentage of observable aplastics (coarser grained inclusions) and examined the geometric relationships between the aplastics and the encompassing clay matrix (micromass). Grit tempering was used exclusively in the manufacture of each vessel represented. The large and abundant polymineralic grains (rock fragments) are granodioritic in composition and evince local tills as the most likely source for the temper. The mineral composition of the aplastics and the matrix is consistent with raw material resources readily available in central North Dakota. The overall abundance and coarseness of the tempering agent is likely added to mitigate the high shrink-swell capacity inherent in the montmorillonite clays that are prevalent throughout the region. The size, amount, and composition of the temper grains are also common to vessels manufactured for utilitarian (culinary) purposes. It was not possible in this study to distinguish Riggs from Fort Yates ware at the microscopic level.



**Temper** – aplastic, predominantly sand size, material added to the clay to enhance its workability and to reduce shrinkage and breakage during drying and firing

**Natural Inclusions** - unintentionally-added sand and silt-size grains found within the clay

**Matrix** – the clay micromass

GENETIC CONTROL OF LEAF EXPANSION IN *ARABIDOPSIS*

Christopher P. Keller

Department of Biology, Minot State University, Minot, North Dakota 58707

The long term goal of this project is to understand the molecular/genetic mechanism by which the plant hormone auxin (indole acetic acid) directs development in plants. Auxin hormone receptors have been characterized and selective degradation of transcription factors via the ubiquitin-26S proteasome pathway is known to result from hormone binding but there is currently little understanding of how hormone reception leads to that accelerated transcription factor degradation or how this, in turn, alters developmental physiology.

This project seeks to discover how auxin controls leaf expansion in the model plant *Arabidopsis thaliana*. Earlier work in this laboratory suggests auxin interacts with a second non-auxin growth effector in controlling leaf expansion probably in advance of transcription factor proteolysis. Mutational analysis will be used to address the specific aims of the project which include (1) identifying the gene(s) associated with production of the hypothesized second growth effector and (2) identifying genes responsible for other signal transduction players in auxin control of leaf expansion.

Since the start date of the project, January 1, 2005, a large reach-in growth chamber (AC-60 "Bigfoot"; Enconair, Winnipeg, Canada) has been purchased and installed and a series of preliminary growth experiments have been initiated. A large number of wild type *Arabidopsis* seeds (CS 1092; Lehle Seeds, Round Rock, TX) have been mutagenized with ethyl methane sulfonate at a range of concentrations. Mutated seed (M1) has been grown for seed (M2). The cells of the M2 are expected to contain homozygous mutations which can be screened for phenotypes of interest.

*This project is supported by ND INBRE.*

ELECTRODEPOSITION OF POLYPYRROLE ON ALUMINUM ALLOY 2024-T3 IN  
THE PRESENCE OF DIFFERENT MEDIATING COMPOUNDS FOR THE PURPOSE  
OF CORROSION PROTECTION

Kirill L. Levine\*, Dennis E. Tallman and Gordon P. Bierwagen  
Department of Chemistry, North Dakota State University, Fargo, ND, 58105-5516

Aluminum alloys such as Al 2024-T3 are widely used as light-weight construction materials and corrosion protection of such alloys is an important industrial problem. Conjugated polymer coatings for corrosion protection of aluminum alloys were shown to effectively inhibit corrosion. However such polymers are not readily processible and forming coatings from them is a challenge. It has been shown recently that benzenes with sulfonate and hydroxyl substitutions possess mediating properties for polypyrrole (PPy) deposition on aluminum alloy surface. In this poster we report role which different substitutions play in different mechanisms of polypyrrole polymerization on aluminum surface: (mediating of monomer oxidation or facilitating penetrating of the monomer into pores in oxide) and the influence of an oxide layer on electrochemical properties and corrosion protection performance of polypyrrole films. Different electrochemical and analytical techniques were used to assess electrochemical behavior, corrosion protection properties, composition and morphology of polypyrrole modified aluminum alloy.

Obtained results are believed to serve better understanding of corrosion protection mechanism of aluminum alloys by conducting polymers and possess practical significance for finding alternatives to chromate conversion coating technology.

## THE RADON WE BREATHE: ITS SEASONALITY IN THE HUMAN BODY AND THE ENVIRONMENT

Glenn I. Lykken<sup>1\*</sup>, Berislav Momčilović<sup>2</sup>, LuAnn Johnson<sup>3</sup><sup>1</sup>Department of Physics, University of North Dakota, Grand Forks, ND 58202<sup>2</sup>Institute for Medical Research and Occupational Health, Zagreb, Croatia<sup>3</sup>USDA, Agricultural Research Service, Human Nutrition Research Center, Grand Forks, ND 58202

Radon-induced lung cancer can be traced to 16<sup>th</sup> century miners in Europe; there is now world-wide concern that elevated radon progeny levels in dwellings may be implicated in lung cancer (1); however radon distribution throughout the body has been ignored until recently (2).

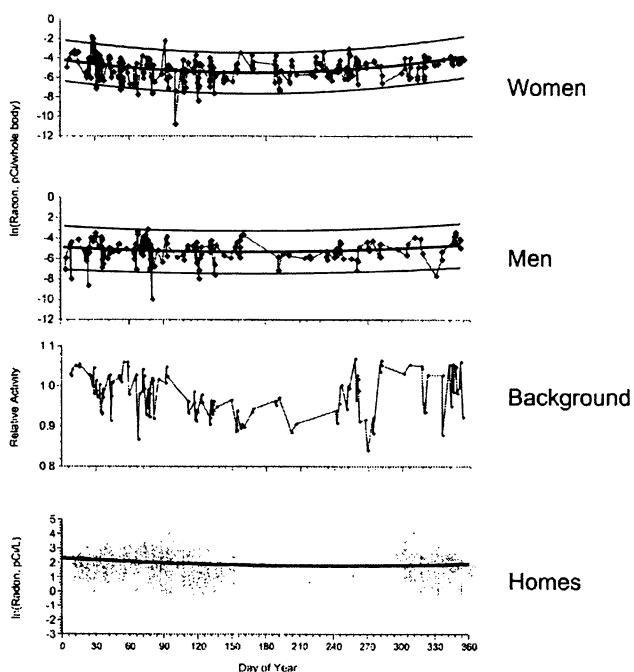
Indoor radon concentrations in dwellings vary with season from typical winter to summer ratios of approximately two to one (3). If radon is stored in body fats and lipids, this storage should be reflected in <sup>214</sup>Bi gamma emissions from subjects measured in a whole body counter. Residential radon concentrations in Grand Forks, ND homes have been measured since 1988. Whole body counter data, steel room <sup>214</sup>Bi (a progeny of <sup>222</sup>Rn) background and subject <sup>214</sup>Bi concentrations have also been measured in the USDA ARS GFHNRC whole body counter (WBC).

Seasonal <sup>214</sup>Bi in the men and women participating in nutrition studies were measured and compared with the relative background radon activity in the WBC. The radon activity data were collected over several years and plotted versus the day of the year. The central line in Fig. 1 represents the best fit for the average yearly value with the 95% prediction region between lines above and below that central tendency.

Radon in ambient air and in the human body varies throughout the year. We observed a cyclic summer drop and subsequent winter rise of radon in the home ambient air and radon accumulated in the whole body of men and women; the pattern was statistically significant ( $p < 0.05$ ) (Figure 1).

Environmental radon accumulated in the human body, more so in women, presumably owing to a higher fat content than men. This accumulation of Bi-214 (Rn daughter) showed a seasonal pattern, indicating that human body radon accumulation follows the changes of the environmental radon concentration, a pattern not described previously in the literature.

Seasonal patterns of intake in agricultural societies and affluent societies have been reflected as seasonal changes in human body weight (4). A high intake of carbohydrates was recorded in the fall; some time is required for fat to accumulate—fat stores would be expected to peak during the winter. This is also the time when the measured radon concentrations in the human body peak. Hence, the seasonal changes in radon retention we observed may be due to a normal seasonal cycle of fat accumulation and depletion. If radon is concentrated in the human body, we would expect to see a synergistic effect between higher ambient radon concentrations and body fat mass in the subject population. Interestingly, Lykken *et al.* (5) have reported such a correlation between body fat mass and total body radon that was statistically significant in women ( $n = 40$ ) but not in men ( $n = 57$ ). Therefore, we may conclude radon accumulates in the body above and beyond concentrations found in the surrounding environment.

1) Chen, J *Health Physics* 88(4):323-333; 2005.2) Lykken GI, Lukaski HC, Bolonchuk WW, Sandstead HH *J. Lab. Clin. Med.* 101(4): 651-658; 1983.3) Huber J, Ennemoser O, Schneider P *Health Phys* 81:156-162; 2001.4) de Castro JM. *Physiol Behav.* 50: 243-248; 1991.5) Lykken GI, Ong HS, Alkhatib HA, Harris TR, Momčilović B, Penland JG *Ann. NY Acad. Sci.*, 904:267-271, 2000.

## NANOTECHNOLOGY: ALIGNING PROTEIN MOLECULES IN MONOLAYER FILMS OF NANOMETER THICKNESS

LeRoy Pazdernik\* and Gilles Picard<sup>1</sup>

Department of Chemistry, University of North Dakota, Grand Forks, and

<sup>1</sup>Istituto di Ricerca "Albert Sorti", Turin, Italy

The main objective of this research project was to prepare clean monolayers of protein molecules in a rapid, continuous fashion. Using a rotating glass cylinder embedded in a thick poly-Teflon block fulfills these requirements. This novel approach generates a monolayer film of protein molecules on a variable thickness layer of rapidly moving clean water. This water layer exhibits dynamic laminar flow characteristics. This process can reduce considerably the protein contact time at the air-water interface, which subsequently prevents structural alterations of the molecules. High-speed protein monolayer preparations are obtained by injecting protein solutions into this thin laminar flow liquid film at various speeds. The above prototype generates nice two-dimensional sheets by compressing cytochrome P450<sub>ssc</sub> on the rotating glass cylinder. The cytochrome P450 monolayers were examined by optical microscopy, Transmission Electron Microscopy, Scanning Electron Microscopy, and Atomic Force Microscopy to verify the molecular arrangements and to validate this technology. Possible one-dimensional protein crystallites were observed. A rate of protein monolayer preparation up to 2 cm/s was easily obtained. This method is suitable for continuous fine particle coatings and special protein monolayer films needed for biosensor surfaces.

## COMPARISON OF DNA DAMAGE RESULTING FROM EXPOSURE TO VARIOUS NICKEL SPECIES

N.V.C. Ralston,\* J. R. Gallagher, and K. C. Galbreath,<sup>1</sup>E. J. ZilliouxUniversity of North Dakota, Energy & Environmental Research Center, <sup>1</sup>Florida Power & Light Company

Inhalation exposures to particulate materials containing nickel compounds have been associated with toxicity and increased risk of respiratory cancers. However, there are significant differences in the modes and potencies of carcinogenic action by nickel species. The U.S. National Toxicology Program comparisons of carcinogenicity of nickel compounds show no evidence of relationship to nickel sulfate, limited evidence of relationship to nickel oxide, but clear evidence of relationship to nickel subsulfide. Substantial quantities of nickel are emitted in residual oil fly ash (ROFA), but until recently, the species of nickel emitted were unknown. The Environmental Protection Agency risk calculation for oil-fired power plants was based on the assumption that the nickel in ROFA was 50% as carcinogenic as nickel subsulfide. This conservative assumption was based on sequential extraction of ROFA resulting in the estimate that 3%–26% of the total nickel emissions was nickel subsulfide. However, sequential extraction techniques have not been found to be effective in speciating insoluble forms of nickel. Recently, x-ray absorption fine structure analysis has shown that only nickel sulfate and nickel oxide species are present in ROFA. Since nickel sulfate is soluble, this study examined the deoxyribonucleic acid (DNA) damage characteristic of ROFA compared to reference sources of nickel oxide and nickel subsulfide. Respiratory and cardiovascular effects of exposure to airborne particulates are associated with formation of reactive oxygen species (ROS) occurring at the particle interface or from water-soluble transition metal ions arising from the particulates. Nickel and other ROS-forming metal ions are present in varying distributions in combustion derived particulates such as ROFA. However, the molecular forms of the chemical species present appear to be more important than the mass quantities of the elements themselves. Distinctions in solubility and stability of various forms of nickel influence cellular uptake rates as well as kinetics of ROS formation. As a result, more severe consequences are associated with pulmonary exposure to chemical species such as nickel subsulfide than with nickel oxide. When evaluating potential consequences of exposure to airborne particulates such as ROFA, it is important to consider quantitative differences in the abundance of these forms as well as qualitative distinctions in the nature and chemical mechanisms of the ROS-dependent damage they cause.

Following the method of Lund and Aust (1992; *Carcinogenesis* 13:637–642), we used supercoiled circular DNA from bacteriophage  $\phi$ X174 as the target substrate in a highly sensitive assay to evaluate ROS-dependent damage from insoluble forms of nickel in comparison to similarly treated ROFA. Samples of ROFA, nickel subsulfide mixture ( $\text{Ni}_3\text{S}_2$  and  $\text{Ni}_7\text{S}_6$ ), “green” nickel oxide, and the oxide spinel compounds,  $\text{NiFe}_2\text{O}_4$ ,  $[\text{Ni}, \text{Mg}][\text{Al}, \text{Fe}]_2\text{O}_4$ , and  $\text{MgAl}_2\text{O}_4$  were extracted using 1M NaOAc-0.5M HOAc at pH 5, 25–28°C followed by exhaustive rinsing with deionized  $\text{H}_2\text{O}$ . To assess the inherent ROS formation rates of these species, DNA substrate suspensions in aqueous solutions were exposed to identical mass quantities of these residues. Due to its compact hydrodynamic diameter, undamaged DNA (DNA Form 1) moves rapidly through a 0.6% agarose gel during electrophoresis. The relative quantities of DNA migrating as either form were visualized using ethidium bromide following separation. Free radical damage creates nicks in the DNA molecule, resulting in relaxed, partially unwound forms (DNA Form 2) that migrate through the gel at a slower rate than DNA Form 1. ROS-dependent damage changes DNA Form 1 into DNA Form 2 in a time and dose dependent manner. Using this assay, we found ROS formation by ROFA was slightly greater than ROS formation by NiO or the spinel compounds, but ROFA caused far less damage than nickel subsulfide. DNA damage induced by nickel subsulfide was also qualitatively different than that induced by other nickel species. Nickel subsulfide induced a rapid and complete elimination of DNA Form 1 and produced a novel form of damaged DNA (DNA Form 3) that migrated at an intermediate rate between DNA Form 1 and Form 2.

In summary, the DNA damage assay provides a sensitive and rapid assessment of ROS-generating capacity inherent in the materials studied. Potential health hazards associated with exposure to respirable particulate materials may be qualitatively and quantitatively assessed using this technique. Future investigations will establish the exact nature of the DNA damage characteristic of nickel subsulfide exposure.

## PHYSIOLOGICAL IMPLICATIONS OF MERCURY'S EFFECTS ON SELENIUM AVAILABILITY

L.J. Raymond\* and N.V.C. Ralston

University of North Dakota, Energy &amp; Environmental Research Center, Grand Forks, ND 58202

Mercury (Hg) is a global pollutant that humans are exposed to in the form of methylmercury (MeHg) present in fish. The toxic effects of MeHg make it a potential health problem, and it is listed by the International Program of Chemical Safety as one of the most dangerous chemicals in the environment. Although adults can experience neurological effects when exposed to high concentrations of MeHg, advisories have mainly arisen because of the increasing concerns regarding MeHg's effects in the developing nervous systems of unborn and growing children. While the placental barrier can stop many toxic elements, MeHg crosses the placenta and accumulates at higher concentrations on the fetal side than on the maternal. Worsening the situation for the developing fetus, MeHg also crosses the blood-brain barrier where it exhibits long-term retention. These factors exacerbate Hg's neurotoxicity and conspire to intensify its pathologic effects in brain and endocrine tissues. Although Hg toxicity is well described, its molecular mechanism is poorly understood and the effects of chronic low-dose exposures remain undetermined.

It is well recognized that Hg and sulphur (S) bind together to form complexes. This binding property is the basis of chelating therapy used as a treatment in cases of acute mercury poisoning. The complexes between Hg and selenium (Se) are less generally known but of much higher affinity. Physiologically, S is far more abundant than Se, yet because of Se's higher affinity, Hg selectively binds with Se to form insoluble HgSe. This interaction has been assumed to be a protective effect, whereby supplemental Se complexes the Hg and prevents negative effects in animals fed otherwise toxic amounts of Hg. Numerous studies have shown Se supplementation counteracts the negative impacts of exposure to Hg, particularly in regard to neurotoxicity, fetotoxicity, and developmental toxicity. The ability of Se compounds to decrease the toxic action of Hg has been established in all investigated species of mammals, birds, and fish. However, similar to Hg toxicity itself, the molecular mechanism of Se's protective effect against Hg toxicity is well described, but poorly understood.

Dietary Se is essential in supporting functions of enzymes with important metabolic roles, and beneficial in augmenting immunocompetence and cancer chemoprevention. Present in tissue-specific distributions in all cells of all creatures, approximately 25 Se-containing proteins have been recognized. Se-enzymes possess Se in the form of selenocysteine located at their active sites, employing Se's broad catalytic redox potential. Selenoprotein activities appear to be especially important in brain, pituitary, and thyroid, since even after feeding selenium-deficient diets for many generations, it is virtually impossible to deplete the selenium in these tissues. Consequently, any substance that can enter the brain and disrupt selenoprotein synthesis in these tissues will accomplish what multigenerational selenium deficiency cannot.

MeHg not only has the ability to cross the blood-brain barrier, but its exceptionally high affinity for Se may enable it to specifically sequester Se and diminish selenoprotein synthesis. The Hg-binding affinity constant of the free selenides that form during each cycle of selenocysteine synthesis have an exceptionally high affinity constant for Hg:  $10^{45}$ . HgSe precipitates have extremely low solubility, ranging from  $10^{-58}$  to  $10^{-65}$ , thus they are thought to be metabolically inert. Therefore, it is reasonable to assume that not only does Se have an effect on Hg's bioavailability, but Hg may also have an effect on Se bioavailability. Therefore, the understanding of the protective effect of Se against Hg exposure may actually be backwards. Mercury's propensity for Se sequestration in brain and endocrine tissues may inhibit formation of essential Se-dependent proteins. Hence, Se's protective effect against Hg toxicity may simply reflect the importance of maintaining sufficient Se to support normal synthesis of Se-dependent enzymes and the sensitivity to Hg-induced neurotoxicity may be due to the balance of Hg and Se. In this regard, the health risks of MeHg exposure may vary in response to individual and regional differences in selenium intake.

We conducted a series of rat studies to investigate the influence of Se status on sensitivity to Hg toxicity. Our studies confirm selenium's protective effect against mercury toxicity. Se-deficient rats were sensitive to Hg exposure, while rats fed diets containing adequate amounts of Se were far more resistant and rats fed Se-rich diets showed no signs of mercury toxicity. Tissue samples are in the process of being analyzed for mercury, selenium, and selenoenzyme activity. Likewise, the stoichiometry of Hg with the Se present in the tissues is currently being investigated. Further research in these areas will provide valuable information that is needed to understand the true impact of Hg exposure as well as identify populations which may be protected or at greater risk to Hg's toxic effects.



STUDIES OF CHROMOSOME REARRANGEMENT AND GENE FUSIONS INVOLVING THE HUMAN MYELOID-LYMPHOID LEUKEMIA GENE (*MLL*)

Heidi J. Super\*, Michelle Reinholdt, Jessica Townsend, Stephanie Mueller, and Kyle Pankrantz  
Department of Biology, Minot State University, Minot ND, 58707

Nonrandom chromosome exchanges known as chromosome translocations are a hallmark of specific subtypes of leukemia and have been valuable tools in identifying genes associated with malignant transformation of blood cells. *MLL* is one such gene, identified by its location on chromosome 11 at the breakpoint region in leukemogenic translocations with 40+ unique chromosomal regions. These translocations result in formation of *MLL* fusion genes which are expressed as oncogenic fusion proteins. *MLL* translocations are interesting for several reasons. First, *MLL* is one of the most promiscuous loci in its chromosome rearrangements with numerous translocation partners. In addition, *MLL* rearrangements are seen in 70-80% of leukemias in children less than 1 year of age and most, if not all of these rearrangements occur *in utero*. Finally, *MLL* rearrangements occur in some patients treated for other malignancies, resulting in secondary, therapy-related leukemias. Use of DNA topoisomerase II (topo II) inhibiting drugs correlates with *MLL*-related secondary leukemias.

Several projects are ongoing to study the mechanism of chromosome breakage and fusion in *MLL* translocations. Two projects focus on the hypothesis that topo II protein initiates breakage in *MLL* and in its translocation partner loci. Several *in vitro* studies have shown that topo II-inhibiting chemotherapy drugs can induce breakage in the translocation breakpoint region of *MLL* and in one region of an *MLL* partner gene (*AF9*). However, other *MLL* partner genes have not been tested for susceptibility to topo II drugs. We are currently testing *AF4*, the most common *MLL* partner gene, for susceptibility to breakage with common topo II inhibitor chemotherapy drugs (see Reinholdt *et al.*). In a second study, we are attempting to show direct binding of topo II to the *MLL* translocation breakpoint region. Using an *in vivo* assay called Chromatin Immunoprecipitation (ChIP), DNA binding proteins are reversibly cross-linked to chromatin in cells. Chromatin is sheared mechanically and immunoprecipitated using antibodies for candidate proteins (such as topo II). Input DNA is compared to the immunoprecipitated fraction for enrichment for specific loci. In our assay regions of the *MLL* breakpoint will be assayed for enrichment after chromatin immunoprecipitation with topo II antibody. Various culturing conditions (*e.g.*, +/- chemotherapy drugs) will be used prior to ChIP analysis. The assay could be applied to *MLL* partner gene breakpoint regions as well.

In a new phase of *MLL* study, a novel approach will be taken to modulate expression of *MLL* fusion genes, in cell lines. RNA interference (RNAi), an endogenous cellular mechanism for silencing specific genes, has recently been identified in many diverse organisms, including mammals. Briefly, RNAi is a conserved mechanism for cleavage of specific RNAs, including mRNAs, by cellular enzyme complexes following detection of the corresponding double-stranded RNA. Today RNAi technology is one of the most powerful and direct ways to silence specific genes for the purpose of studying gene regulation, protein function and protein interactions. In our study we are designing RNAi molecules to target specific *MLL* fusion mRNAs, (*MLL-AF4* or *MLL-AF9*) which are present in leukemia cell lines. Initial goals of the study are simply to show specific knock-down of *MLL* fusion mRNAs and *MLL* fusion proteins. Subsequent analysis will include determining the longevity of the RNAi response, monitoring growth/transformation changes in the cell lines and ultimately determining changes in gene expression profiles following knock down of the *MLL* fusion proteins.

STRUCTURE ACTIVITY RELATIONSHIP: THE CASE OF CO<sub>2</sub>/ZnO(0001) AND CO/Cu-ZnO(0001)

J. Wang\*, B. Hokkanen, E. Johnson, and U. Burghaus

Department of Chemistry, Biochemistry and Molecular Biology, North Dakota State University,  
Fargo, ND USA 58105

One of the ultimate goals in surface science is to set up a relationship between the structure and reactivity of catalysts' surfaces. Although the structure activity relationship (SAR) has been well established in organic and biological chemistry (*e.g.* see ref.<sup>1</sup>), a correlation of an oxide surface structure with the corresponding reactivity has been obtained less frequently in surface chemistry. The presented study is, however, not only motivated by setting up SAR rules but also by the importance of ZnO(0001)<sup>2</sup> as one of the components of the industrial methanol synthesis catalyst. The currently used catalyst consists of Cu particles embedded in a ZnO - Al<sub>2</sub>O<sub>3</sub> powder mixture with CO<sub>2</sub>, H<sub>2</sub>, and CO as synthesis feedstock. The nature of the active site and the effect of surface structure on the reactivity for this system still remain under controversy.

Molecular beam scattering techniques (King-Wells measurement as well as He atom scattering) and thermal desorption spectroscopy (TDS) were combined and applied to a number of different systems; namely, clean ZnO(0001) (see ref.<sup>3</sup>), defected ZnO(0001), H precovered ZnO(0001), and Cu precovered ZnO(0001) (see ref.<sup>4</sup>). Furthermore, CO and CO<sub>2</sub> have been used as probe molecules.

The CO<sub>2</sub>-TDS curves consist of two distinct structures. By modifying the surface with Ar<sup>+</sup> ion sputtering (more defects) and H-preadsorption (fewer defects), the two TDS structures could be assigned to adsorption/desorption of CO<sub>2</sub> on pristine and intrinsic defect sites. The binding energy of CO<sub>2</sub> was determined as 34.4 kJ/mol on pristine sites and amounts to 43.6 kJ/mol on intrinsic defect sites. Thus, the binding energies depend distinctly on the adsorption sites. Therefore, a kinetic structure activity relationship (kinetic SAR) is evident.

Furthermore, a dynamic structure activity relationship was present in CO adsorption on the Cu/ZnO(0001) model catalyst. Whereas on small Cu sized-deposits (5-10Å), direct Langmuirian adsorption dynamics were observed; larger cluster sizes lead to the detection of precursor assisted adsorption dynamics of CO. Thus, the energy transfer processes governing the adsorption of a gas phase species on the surface (adsorption dynamics) depended distinctly on the morphology of the metal-on-metal oxide system (dynamic SAR).

- 
- 1) Woodward RB, Hoffmann R, *Angewandte Chemie international Edition*, **8**, 781, 1969.
  - 2) The ZnO(0001) surface is a Zn-terminated surface, short Zn-ZnO.
  - 3) Wang J, Hokkanen B, Burghaus U *Surface Science*, **577**, 158-166, 2004.
  - 4) Wang J, Johnson E, Burghaus U, *in preparation*.

**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. *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. The student member dues shall be one-third (to nearest dollar) of the regular member dues. These dues are payable 1 December of each year.

Section 2. *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 3. *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 4. *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 Members.* 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 of 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 deposit 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 of 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.* The 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.



**MINUTES (UNAPPROVED) of the NORTH DAKOTA ACADEMY OF SCIENCE  
ANNUAL BUSINESS MEETING**

**12:00 pm, Ramada Inn Plaza Suites, Fargo, ND, April 30, 2004**

The first order of business was to approve minutes of previous business meeting, from the March 2003 business meeting in Minot, ND. The minutes were approved as printed in the Proceedings.

A brief financial report was presented by Secretary-Treasurer Jackson. At this time, the Academy is financially sound, despite the low rate of return on our savings and investment accounts. To offset creeping inflation in operating costs, Secretary-Treasurer Jackson recommended and moved that the Academy approve a raise in dues to \$25 (professional) and a corresponding increase to \$10 (students). This motion passes following brief discussion.

Applications for the North Dakota Research Foundation grant were encouraged. We received no applications this past year.

Meeting statistics: \_\_\_ registered attendees, a BRIN-supported symposium as part of the outside evaluator annual visit. We also had \_\_\_ professional talks, and \_\_\_ Denison papers presented, of which 00 were graduate and 00 were undergraduate.

The A. Rodger Denison Award winner in the graduate category was Mark Cervinski from the University of North Dakota. The Denison Award in the undergraduate category went to Spoitu Soiuoid of Valley City State University. Runners up were (graduate) and (undergraduate).

There was discussion on the lack of elections again this year. The Nominating committee, comprised of past presidents, has had difficulty finding a person to stand as President-elect; the P-E works to help organize the following year's meeting at or near their own home campus. Following brow-beating and frivolous *faux* nominations, Andre Delorme (Valley City) was nominated, accepted the nomination, and was named President-elect by acclaim.

Anna Grazul-Bilska officially ended her duties as President by announcing that Holly Brown-Borg (UND) has succeeded her. President Brown-Borg will preside over the 97th annual meeting, which will be held in Grand Forks on April 28 and 29, 2005.

Agenda/notes

Notes for the 97th Annual Business meeting

The first order of business was to approve minutes of previous business meeting, from the April 2004 business meeting in Fargo, ND.

The minutes: were | were not approved  
as printed in the Proceedings | as amended.

A brief financial report was presented by Secretary-Treasurer Jackson. At this time, the Academy is financially sound, despite the low rate of return on our savings and investment accounts. Last year we raised dues amounts to offset creeping inflation in our operating costs. This year, with relatively few people sending in their dues in response to the mailing and email solicitations, Secretary-Treasurer Jackson noted that the indifference on the part of the membership in paying dues means that in order to cover fixed costs of the annual meeting, the registration fee was raised to guarantee that the Academy would take in sufficient money to cover these fixed costs.

A discussion of this: did | did not ensue.

There was a single applications for the North Dakota Research Foundation grant. The Foundation Board of Directors approved the award to Dr. Paul Hardersen of UND Space Studies. As a result, the NDAS will be recognized as a sponsor of UND's new observatory, which is in progress.

Meeting statistics: \_\_\_\_\_ registered attendees, ( ) professional ( ) grad ( ) undergrad  
\_\_\_\_\_ guests

We also had 16 professional talks, and 46 Denison papers presented, of which 29 were graduate and 17 were undergraduate.

A. Rodger Denison Award winners: \_\_\_\_\_ graduate category

\_\_\_\_\_ undergraduate category

Runners up	GRAD	UNDERGRAD
	1)	1)
	2)	2)

Discussion on the lack of elections again this year. The Nominating committee, needs help in identifying candidates for president and council, as well as filling needed spots on the committees.

For instance — 2007 Annual Meeting should be in Fargo-Moorhead, 2008 Annual Meeting back in Grand Forks.

Holly Brown-Borg officially ended her duties as President by introducing Andre Delorme (Valley City). President Delorme (Valley City) discussed preliminary plans for the Academy's 98th Annual Meeting, over which he will preside in Valley City on April 27 and 28, 2006.

**Executive Committee**

## Membership:

Past-President  
 President  
 President-Elect  
 Secretary-Treasurer  
 Councilors (three-year terms)

**President**

Holly Brown-Borg  
 Dept. of Pharm, Physiol, &  
 Therapeutics  
 University of North Dakota  
 Grand Forks, ND 58202  
 (701) 777-3949  
 brownbrg@medicine.nodak.edu

**President-Elect**

Andre Delorme  
 Dept. of Natural Science  
 Valley City State University  
 Valley City, ND 58

**Past-President**

Anna Grazul-Bilska  
 Dept. of Animal & Range Sci.  
 North Dakota State University  
 Fargo, ND 58105  
 (701) 231-7992  
 anna.grazul-  
 bilska@nds.u.nodak.edu

**Secretary-Treasurer**

Jon A. Jackson (2004-2006)  
 Dept. of Anatomy & Cell  
 Biology  
 University of North Dakota  
 Grand Forks, ND 58202  
 (701) 777-4911  
 jackson@medicine.nodak.edu

**Council**

Siegfried Detke (2005-2007)  
 Department of Biochemistry &  
 Molecular Biology  
 University of North Dakota  
 Grand Forks, ND 58202-9037  
 sdetke@medicine.nodak.edu

To be named (2006-2008)  
 Holly Brown-Borg (2003-2005)  
 Department of Pharmacology,  
 Physiology & Therapeutics  
 University of North Dakota  
 Grand Forks, ND 58202  
 (701) 777-3949

Chris Keller (2004-2006)  
 Department of Biology  
 Minot State University  
 Minot, ND 58707  
 (701) 852-1978  
 ckeller@misu.edu

**COMMITTEES OF THE NORTH DAKOTA ACADEMY OF SCIENCE****Executive Committee****Denison Awards Committee\*****Nominating Committee****Editorial Committee\*****Necrology Committee\*****Resolution Committee\*****Education Committee\***

**North Dakota Research  
 Foundation  
 Board of Directors**

**Membership Committee\***

\* indicates vacancies and a need for interested members to fill a role here.

## PAST PRESIDENTS AND LOCATIONS OF THE ANNUAL MEETING

## NORTH DAKOTA ACADEMY of SCIENCE

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

**A**

Anderson, C.M.	63
Anderson, M.	9
Alexander, B.	9, 13
Arndt, W.J.	10
Azenkeng, A.	31

**B**

Banerjee, A.	47, 59
Barkosky, R.R.	21
Beachy, C.K.	23, 24, 64
Benoit, J.N.	63
Bierwagen, G.P.	72
Binda, P.	37
Boese, S.J.	11
Bolles, B.A.	51
Bollimuntha, S.	32
Borowczyk, E.	10, 33, 34
Borowicz, P.P.	10, 34
Bradley, D.S.	38, 49
Brayko, A.	12
Bruce, S.D.	14
Burghaus, U.	58, 78

**C**

Carr, P.A.	54
Caton, J.S.	10, 50
Cervinski, M.	35
Chi, L.	65

**D**

Dahl, S.	13
Delbridge, E.	37
Detke, S.	66
Deufel, A.	14, 15
Ding, L.	36, 67
Doze, V.A.	11, 54
Dugah, D.	37
Durick, K.A.	38
Dutta, A.K.	52

**E**

Evoniuk, J.M.	39
---------------	----

**F**

Fjeldahl, K.	16
Forlemu, N.Y.	40
Foster, D.C.	43
Funk, S.	58

**G**

Gaffaney, J.D.	52
Galatowitsch, S.	56
Galbreath, K.C.	75
Gallagher, J.R.	75
Gonnella, T.P.	18
Gorentla, B.K.	41
Grazul-Bilska, A.T.	10, 33, 34
Grewal, N.	68
Grundstad, M.L.	21
Guo, D.P.	36, 67
Gustafson, C.D.	57

**H**

Heinle, S.	17
Hillegonds, D. J.	55
Hoffman, M.R.	31
Hokkanen, B.	78
Homandberg, G.	36, 67
Huan, Y.	45
Hunt, C.D.	38

**I - J**

Jangiti, S.	68
Johnson, E.	78
Johnson, L.	73
Johnson, M.L.	33, 34
Jordan, C.R.	69
Jorissen, H.	13
Josephs, R.L.	70
Jungberg, C.E.	18
Junglas, K.M.	19
Jurgens, C.W.D.	11, 54

**K**

Kannan, S.	42, 43
Karki, S.	44
Ke, Y.	65
Keller CP	21, 71
King, J.D.	11, 54
Knittel, J.	43
Knudson, C.A.	54
Knull, H.R.	57
Kolhatkar, R.	52
Kozliak, E.I.	57

**L**

Lardy, G.P.	50
Lee, R.K.	46
Lever, J.R.	52
Levine, K.L.	72
Liang, S.	45
Liu, R.	65
Lopez, F.	63
Lowe, S.L.	40, 48, 57
Luo, C.	65
Lykken, G.I.	73

**M**

Mamidi, S.	46
Mann, M.D.	44
Martin, L.,	23
Mattern, C.	20
Mazurek, SA,	21
McClellan, P.E.	46
McDaniel, G.	20
Mom-ilovi-, B.	73
Moore, B.L.	39
Mueller, S.	77

**N**

Navanukraw, C.	33
Nelson, C.	37
Nett, J.L.	15
Newman, A.H.	52
Ngwendson, J.N.	47
Nilles, M.L.	49
Njabon, E.N.	48

**O**

O'Brien, B	9
O'Bryant, D.M.	49
Olson, A.C.	47
O'Neil, M.R.	50
O'Rourke, K.I.	39

**P**

Pachhai, S.	51
Pankrantz, K.	77
Parnas, M.L.	52
Patel, N.	68
Pazdernik, L.	74
Peck, W.D.	51
Perkins, D	17
Picard, G.	74
Pierce, D.T.	45
Porter, J.E.,	11, 54
Pringle, B.E.	53
Pyle, S.J.,	11

**Q - R**

Ralston, N.V.C.	69, 75, 76
Rau, K.E.	54
Raymond, L.J.	69, 76
Redmer, D.A.	10, 33, 34, 39
Reinholdt, M.L.	22, 77
Reynolds, L.P.	10, 33, 34, 50
Richter, J.	23
Rose, K	24
Roughead, Z.K.	55
Rundquist, B.C.	51, 53

**S**

Salehfar, H.	44
Sattler, J.A.	55
Seil, JE	21
Simmers, S.	56
Singh, B.B.	32, 36
Soule, M.R.	55
Stoltenow, C.L.	39
Super, H.J.	22, 77

**T**

Tallman, D.E.	72
Thomasson, K.A.	40, 48, 57
Tomita, M.	38
Townsend, J.A.	22, 77

**U-V**

Vaughan, R.A.	35, 41, 52
Vonnahme, KA	10, 34, 50
Voss, S.R.	64

**W**

Wagner, J.L.	55
Waingeh, V.F.	40, 48, 57
Wang, J.	58, 78
Ward, M	10
Winburn, R.S.	12, 16, 20
Wu, M.	42, 43

**X-Y**

Yong, P.K.	59
------------	----

**Z**

Zhang, H.-Y.	63
Zhao, J.X.	42, 45
Zillioux, E. J.	75
Zimmer, A.	25, 63
Zou, M.-F.	52