Vermont Genetics Network
IDeA Networks of Biomedical Research Excellence
National Center for Research Resources
June 2008 Newsletter

Highlights of Networking and Professional Development: Career Panels Directed Towards Both Traditional and Alternative Science Careers

by Karen M. Lounsbury, PhD

This year marks the 3rd round of career panels and professional development seminars presented at VGN partnering colleges and the 7th annual presentation of UVM Undergraduate Career Day. The goal of the panel presentations at individual colleges is to reach a broader audience of students and junior faculty and to present panelists that are more specific to the needs of the institutions. The kick-off panel was held November 1, 2007 at Castleton State College, and was organized by Carly Langlais, Assistant Professor of Biology at Castleton. The panelists included Krystle Danforth, Castleton alumna and research technician at Green Mountain Antibodies, Nate Newman, Castleton alumnus and graduate student at the University of Vermont, Carrie Pontbriand, a laboratory manager at Microcheck, Inc., and Keith Thompson, owner and forestry consultant at Northern Stewards. Each panelist described their career path and aspects of their day-to-day duties, followed by personal advice to the students that they found important for good career choices and for life in general. Students were interested in how to apply to graduate school, preparing for the GREs, what it is like to work in a biotech company, and what difficulties come with owning your own business. Having the diversity of panelists proved important, not only because of their different careers, but also because of their individual life experiences.

Similar themes of student interest were observed at the panel presentation held at Saint Michael's College on March 11, 2008 and organized by Christine Clary. This panel was represented by Rebecca Drapp, SMC alumna and Laboratory Technician at UVM, Wendy Sogoloff, UVM alumna and Professional Horse Trainer, Cedar Spring Farm, Charlotte, VT, Jessica Contois, SMC alumna and Science teacher, Williston Central School, and Sarah Hale, UVM alumna and Post-doctoral Fellow, University of Vermont. The consistent message that was conveyed to the students from these panels is that career paths are very individual experiences. There is no GPS system to guide you directly to your goals, which is beneficial because it gives you a chance to have flexibility as your interests and goals may change with more experience.

A VGN-sponsored faculty professional development seminar was later held at Middlebury College on January 26, 2008. Dr. Jill Salvo, Professor of Biology at Union College gave a useful presentation titled "How to Write an AREA Grant", which was accompanied by a panel presentation discussing grant writing tips. Panelists included Jill Salvo, Professor of Biology at Union College, Franci Farnsworth, Grants Manager at Middlebury College, Angela Irvine, Grants Manager at Saint Michael's College, and Catherine Combelles, Associate Professor of Biology at Middlebury College. With Federal grant funds tightening, opportunities for research funding at smaller colleges are becoming more competitive. Panels such as this provide critical advice towards improving grant applications, and using these tips may mean the difference between funding and having to re-apply in the next round.

Guest Speaker Jill Salvo, PhD, Union College

The Networking and Professional Development core activities culminated with Undergraduate Career Day held at the Doubletree Hotel in Burlington, VT, April 16, 2007. The event included poster presentations by VGN-funded undergraduates as well as a career panel made up of established scientists with a variety of careers. The goal was to bring students and faculty together in an environment that is rich with scientific interest and with a focus on future student goals. The discussion session showed that the students are interested in making their way towards their goals, and the diversity of future positions gave them an opportunity to see a range of possibilities for which to use their training. The panelists included Timothy Graham, MD, Physician Scientist, Harvard University, James Vincent, PhD,

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Career Day Panel Presenters: (L to R) Timothy Graham, MD, Colleen McKiernan, PhD, James Vincent, PhD and Karen Richardson-Nassif, PhD

Vermont Genetics Network (VGN) INBRE

Director
Judith Van Houten, PhD

Outreach Core Director
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James Vincent, PhD

Undergraduate Networking and Professional Development Director
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Baccalaureate Partner Institutions & Coordinators

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Scott Page, PhD

Saint Michael's College
Doug Green, PhD

VGN Staff

Program Manager
Teri Hart

Business Manager
Nathan Besio

Administrative Assistant
Julie Paris

IT Specialist
Bryan Fleming

Vermont Genetics Network
University of Vermont
120A Marsh Life Science
Burlington, VT 05405
Phone (802) 656-4087
Fax (802) 656-0242
Email: Vermont.GeneticsNetwork@uvm.edu
Website: www.uvm.edu/~vgn

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From the Director

Judith Van Houten, PhD

Vermont Genetics Network is concluding its third year of INBRE funding. This has been an especially successful year. The highlight was the IDeA Regional meeting that we hosted this past August which enabled INBRE and COBRE researchers from the entire northeast region to meet here in Vermont.

I am pleased to announce that we have just **awarded funding** for Project, Pilot Project and Undergraduate Student Summer Research grants at our Baccalaureate Partner Institutions (BPIs) for project year 4. Also awarded were project year 4 Graduate Student Assistantships to faculty members at the University of Vermont (UVM). In addition, we will be awarding Pilot Project grants for Use of Microarray and Proteomics facilities to faculty members at UVM. It is exciting to see the number of applications being submitted for these grants and realizing that our Microarray and Proteomics facilities are more and more in demand.

Our **Outreach Program** continues to flourish. The outreach team has just concluded a very busy year. The **Microarray Outreach** module was delivered to Castleton State College, Johnson State College and Marlboro College. We are finding that with each delivery of these modules, the faculty members are taking on more responsibility with the laboratory experiments.

Something new this year was the introduction of **Bioinformatics Outreach**. In 2006, an online bioinformatics tutorial (Introduction to Data Mining) was developed, in association with Dr. William Barnes on sabbatical from Clarion University. The module was beta tested in fall, 2006. During the summer of 2007, the tutorial was rebuilt in the Moodle content management system. The tutorial was delivered to Green Mountain College and Norwich University in the fall of 2007 and the spring of 2008. As a result, Norwich University incorporated the module into two spring courses; Physiology 304 and Biochemistry 324. We are extremely proud of our Outreach Program as it continues to help build a culture of research at our BPIs.

Dr. Janet Murray, VGN Outreach Coordinator, is planning to take graduate students on a trip this summer to attend the **2nd Biennial National IDeA Symposium in Washington, D.C.** The students will have the opportunity to present their research during a student specific poster session. They will also be able to participate in three workshops of their choice and tour labs at the NIH.

Our second **Professional Development Seminar** for faculty members was hosted at Middlebury College on January 26, 2008. The topic was "How to Write an AREA grant," and Dr. Jill Silva of Union College delivered the presentation. In addition, a panel of business managers shared additional information that was helpful to the research faculty in attendance since they are all responsible for writing federal grants to further their careers.

I would like to thank all of our VGN funded researchers who filled out the **Annual VGN Survey**. We had an astounding 100% compliance! This is an extremely important survey that lets NIH know how successful the VGN program has become!

The **7th Annual VGN Career Day** for undergraduate students was held on April 16. The students had the opportunity to present their research in a poster session and network with students from other VT colleges. The highlight of the evening was listening to the panel of four invited speakers describe their journeys as to how they arrived at their current science careers.

We have an exciting **Retreat** planned for August 13, 2008. Dr. Thomas Tritton, President of the Chemical Heritage Foundation, will be our keynote speaker and will make a presentation titled "The Joy of Science: Research and the Undergraduate Experience." I hope you will join us at the Retreat.

I wish you all a pleasant and productive summer and fall.

Save the Date!

7th Annual VGN Retreat

August 13, 2008
9:00 am – 3:00 pm
Double Tree Hotel
South Burlington, VT

Guest Speaker
Thomas Tritton, PhD
President and CEO
Chemical Heritage Foundation
"The Joy of Science: Research and the Undergraduate Experience"

Graduate Student Professional Development

The VGN Graduate Student Professional Development is a program offered to all current and previously VGN funded graduate students. This program aims to enrich the graduate student experience by providing information on specific topics in science and career development.

This program mainly consists of graduate student meetings organized by Janet Murray PhD, the VGN Outreach Coordinator Each meeting has a specific topic being introduced by an invited guest as well as giving the students a forum to interact with each other. Time is also set aside for students to discuss any other issues they maybe encountering as graduate students and the opportunity to suggest ideas for future meeting topics.

Janet Murray, PhD
VGN Outreach Coordinator

Over the last year and a half there have been presentations on Mentoring (Ellen Martinsen, Department of Biology), Intellectual Property and Technology Transfer (Kerry Swift, UVM Office of Technology Transfer) as well as Bioinformatics (James Vincent, VGN Bioinformatics Core Director). Most of these presentations, although focusing on these topics, also include a discussion by the presenter about their individual career paths, how to pursue careers in these areas and general discussion about career decisions.

A career panel of Postdoctoral Fellows (Jennifer Straub PhD, Viswanathan Muthasamy Ph.D., and Gaoyan (Grace) Tang BDS, MDS, Ph.D.) was assembled for one graduate student meeting to explain their experiences in finding postdoctoral opportunities and choosing their current positions. Factors including, location, science, funding opportunities, laboratory structure and family concerns were discussed. This panel was organized based on the request of the graduate students, as this was the next step in most of their career paths.

The VGN graduate students are also being given the opportunity to attend the 2nd Biennial National IDeA Symposium in Washington DC in August 2008. The students will be able to present their research during a student specific poster session. As well as attending the meetings scientific sessions the graduate students will be able to participate in three different workshop sessions choosing from various topics related to both scientific and career skills. The topics for scientific skills include, Stems Cells, siRNA, SNPs, Proteomics, Inducible KO and TG+ Mice and Bioinformatics. The career skill workshops topics include, Time Management, Grantsmanship and NIH Peer Review Process, Presentation Skills, Mentoring, Scientific Writing, Scientific Ethics and Collaborations. A visit to the NIH facilities in Bethesda MD for these graduate students is also being scheduled to coincide with the National IDeA symposium. The attendance at this national meeting and a visit to NIH are wonderful opportunities for these graduate students and is being supported entirely by the VGN.

VGN Awards Start-up Funding to Stephen P. Waters, PhD

Dr. Stephen P. Waters

The Vermont Genetics Network has provided start-up funding to **Stephen P. Waters**, Assistant Professor of Organic Chemistry. Professor Waters graduated *summa cum laude* from the University of Pittsburgh. During this time, through a Howard Hughes Medical Institute Undergraduate Research Fellowship, his formal training in organic chemistry was launched in the laboratories of Professor Paul Dowd, probing the synthesis of novel hydroquinone analogues of vitamin K and their interactions with carboxylase factors in the blood-clotting cascade. He completed his graduate studies in 2004 at the University of Pennsylvania under the direction of Professor Marisa C. Kozlowski, where his research efforts were focused toward the total synthesis of the antitumor antibiotic purpuromycin. He then began a three-year post-doctoral tour at Memorial Sloan-Kettering Cancer Center in New York City under the mentorship of Professor Samuel J. Danishefsky. During this time, he completed the total synthesis of a neurotrophically active natural product, Scabronine G, as a lead compound for the development of potential therapeutic agents for the treatment of Alzheimer's and Parkinson's Diseases. In addition, the synthesis of analogue structures and glial-cell *in vitro* assays for neurotrophic activity were undertaken in collaboration with neuroscientists at Columbia and Rockefeller Universities. Dr. Waters joined the faculty at the University of Vermont in the fall of 2007.

The current missions of the Waters laboratory are to address new challenges in organic chemistry through contributions to the areas of synthetic strategy and methods development. Emphasis is placed on the utilization of these methods toward the total synthesis of natural products having reported biological activity.

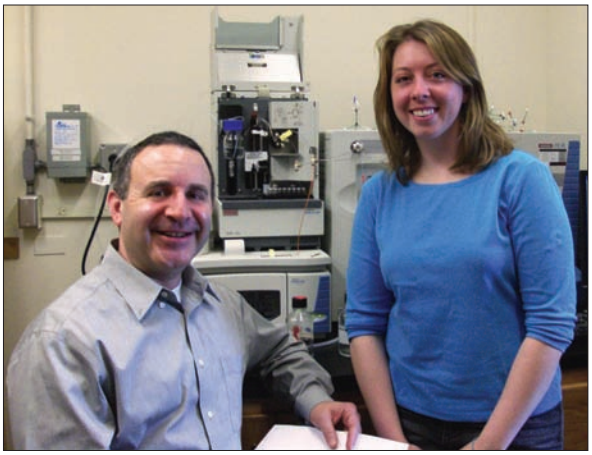
The challenges associated with total synthesis test the limits of chemists' analytical and technical skills, providing a vehicle through which the broader field of organic chemistry is advanced. In another sense, important contributions to biology and medicine are offered through the synthesis of natural products as biological probes and pharmaceutical leads. As Nature continues to provide, and scientists continue to discover, a vast array of structurally diverse compounds, endeavors in the Waters lab will primarily focus on those compounds capable of fostering innovative synthetic design. These particular interests are founded in strategies for the construction of nitrogen-containing heterocyclic systems. As these motifs are common to both naturally occurring alkaloids and a large number of pharmaceutical commodities, the pursuit of efficient methods for their construction represents an important area of synthetic chemistry.

High priority is generally given to those natural products whose profiles invite new opportunities for collaborative, interdisciplinary studies with specialists equipped to advance the program beyond the level of chemical synthesis. Within the field of oncology, contributions are made through targeting those compounds with demonstrated anti-tumor and anti-proliferative activity. Within the neurosciences, molecules capable of attenuating neurotrophic activity represent important leads for the treatment of neurodegenerative disorders. The long-term goals of the program will be the identification of those compounds best suited for candidacy into clinical settings.

The VGN Proteomics Facility Highlights Successful Scientists

Ion Channel Study

Ion channels are determinants of the electrical activity that lies at the core of nervous and cardiovascular system function. The dynamic nature of that excitability derives, in large part, from the regulation of ion channels by post-translational mechanisms. The voltage-gated potassium channel Kv1.2 is widely expressed in the nervous and cardiovascular systems where it has key roles in regulating excitability. The molecular mechanisms behind Kv1.2 regulation are complex and remain largely unknown. In our recent publication, Connors et. al.[1], we report that the cAMP pathway has dual roles in the regulation of Kv1.2 trafficking. Our results show that channel trafficking involves both PKA-dependent and PKA-independent pathways. In addition, we report a shift in the electrophoretic mobility of the channel protein derived from cells treated with the adenylate cyclase activator, forskolin. This suggested that elevated cAMP levels lead to a post-translational modification of the channel that elicits a change in channel structure correlated with altered channel trafficking. Biochemical and mutational analysis identified phosphorylation of the C-terminal serine, S449 as a likely cause of the change in the channel's electrophoritic mobility. However, interpretation of mutation studies is limited by the potential mutational effects on channel structure or function, independent of effects on channel phosphorylation. Mass spectrometry provided an invaluable solution to this limitation by allowing the positive identification of the sites within Kv1.2 and confirming the forskolin-induced phosphorylation of S449. A key advantage of mass spectrometry is its ability to discover modifications at sites not targeted by mutagenesis. In this study, mass spectrometry analysis revealed that, in addition to S449, another C-terminal serine, S440, was also phosphorylated in response to forskolin treatment. Both serines identified by mass spectrometry were shown to be critical for regulation of channel expression at the plasma membrane and interestingly, for determining channel glycosylation patterns. Future studies will examine the role of these serines in specific protein-protein interactions important for channel trafficking and will study in detail their effects on neuron function.

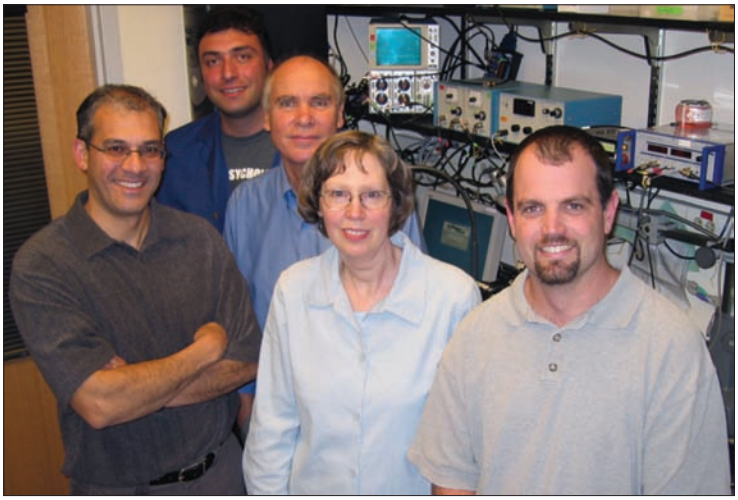


Dr. Anthony Morielli and Ph.D. candidate Emilee Connors in front of the VGN proteomics instrumentation used for their recent study published in the Journal of Biological Chemistry[1]. *Dr. Morielli is an associate professor of Pharmacology at UVM and Emilee is a senior Pharmacology graduate student.*

[1] Emilee C. Connors, Bryan A. Ballif, Anthony D. Morielli. Homeostatic Regulation of Kv1.2 Potassium Channel Trafficking by Cyclic AMP. *J Biol Chem.* 2008 Feb 8; 283(6): 3445-53.

Drosophila Aging Study

Aging is a natural biological process that results in a progressive loss of cellular, tissue and whole body function with the passage of time. One of the most common manifestations of aging is reduced mobility due to loss of muscle strength. Our research is focused on understanding the changes that occur in muscle at the molecular level, and how these changes are manifested at the level of muscle function and organismal locomotion. We used fruit flies, or *Drosophila melanogaster*, since comparative studies between humans and insects have been particularly adept at uncovering fundamental mechanisms of aging. Fruit flies provide an excellent model system for aging research due to their short life span, ease of maintenance, powerful genetics and the available in-depth knowledge of numerous aging processes due to the multitude of studies performed in this species. We investigated the effects of aging on the indirect flight muscles, the large thorax muscles which allow the insect to beat its wings and fly, through a combination of biochemical, biomechanical, and biophysical approaches. We found middle-aged flies had decreased flight performance and wing beat frequency compared to young flies, most likely driven by damage to their mitochondria, which provides the energy for the muscles to perform. Middle-aged muscles compensated for this lack of energy by increasing their stiffness so that their force generation and power output was actually improved over young flies. To explain this unexpected improvement, we used mass-spectrometry to identify the individual proteins that were changing during aging, and found that most of the large changes due to aging were in proteins responsible for muscle fiber stiffness. Knowledge of the specific proteins involved in the aging process will allow us to identify potential strategies for treating the age-related decrements in human muscle mass and performance.

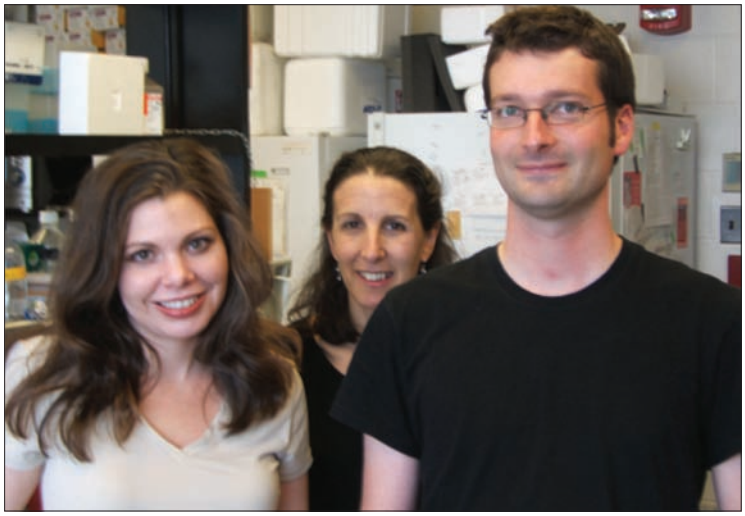


The UVM Drosophila aging team from left to right: Dr. Jim Vigoreaux, Professor and Chair of Biology; Panos Lekkas, Research Technician; Dr. David Maughan, Research Professor of Molecular Physiology and Biophysics; Joan Braddock, Research Technician; and Dr. Mark Miller, postdoctoral associate and lead author. The team's paper is in press at the Biophysical Journal[2].

[2] Mark S. Miller, Panagiotis Lekkas, Joan M. Braddock, Gerrie P. Farman, Bryan A. Ballif, Thomas C. Irving, David W. Maughan and Jim O. Vigoreaux. Aging enhances indirect flight muscle fiber performance yet decreases flight ability in Drosophila. *Biophysical J.* In Press.

Cellular Signaling Pathways Study

A variety of intracellular signaling pathways can be stimulated when cells respond to extracellular stimuli. This cellular response is complicated set of pathways. One pathway, the p38 mitogen-activated protein kinase (MAPK) pathway, is known to be activated by stress responses. This pathway functions in the control of cell differentiation, proliferation, and in the induction of cell death, or apoptosis. The p38 MAPK pathway has also been shown to promote survival by unknown mechanisms. In our recent study[3], we have identified glycogen synthase kinase 3-beta (GSK3β) as a novel target of p38 MAPK signaling. Inhibition of GSK3 activity is required to activate the β-catenin-mediated survival pathway. It is well established that GSK3β is negatively regulated by phosphorylation at serine-9 within its N-terminus by Akt. However, the role of this mechanism of inhibiting GSK3 has been called into question because knock-in analysis of the serine-9 to alanine-9 mutants resulted in only a minor defect in insulin metabolism in skeletal muscle. In this study3 we showed that p38 MAPK phosphorylates GSK3β at a different site than serine-9, providing evidence for an independent mechanism for GSK3β inhibition. In collaboration with the VGN proteomics facility, we found that p38 MAPK phosphorylates a novel site within the C-terminus of GSK3β at threonine 390. Drs. Matthews & Deng were able to identify specifically which of several potential sites in this region of the protein were phosphorylated and provide specific evidence via mass spectrometry for site-specific phosphorylation.



Left to Right: Dr. Tina Thornton, Postdoctoral Associate and lead author; Dr. Mercedes Rincón, UVM Associate Professor of Medicine; and Dr. C. David Wood, Postdoctoral Associate. The team's cell signaling study was recently published in Science[3].

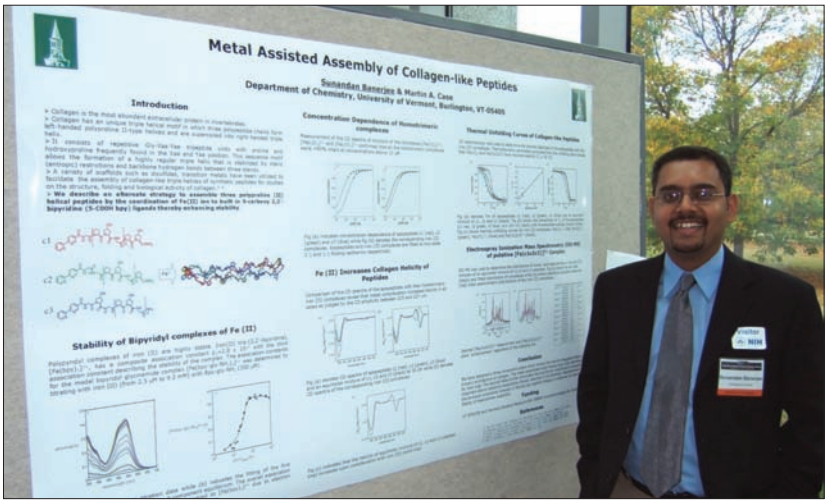
Phosphorylation of GSK3β at threonine-390 resulted in the inactivation of GSK3β and activation of the β-catenin-mediated survival pathway. p38 MAPK phosphorylated GSK3β was more abundant in the brain and thymus of mice. Thus, phosphorylation of threonine-390 by p38 MAPK represents an alternative mechanism to regulate GSK3β activity and a novel mechanism by which p38 MAPK can promote survival. Hyperactivation of GSK3β has been linked with several human neurological diseases such as Alzheimer's and bipolar disorders. GSK3β inhibitors are being used to treat some of these neurological disorders. The identification of this novel mechanism to inhibit GSK3β activity through phosphorylation of its C-terminal domain by p38 MAPK will provide avenues for the development of alternative therapeutic drugs.

Support for this project came through two grants from the National Center for Research Resources to UVM: the Vermont Immunobiology/Infectious Diseases Center of Biomedical Research Excellence (CoBRE) and the Vermont Genetics Network INBRE for the proteomics and mass spectrometry work.

[3] Tina M. Thornton, Gustavo Pedraza-Alva, Bin Deng, C. David Wood, Alexander Aronshtam, James L. Clements, Guadalupe Sabio, Roger J. Davis, Dwight E. Matthews, Bradley Doble, Mercedes Rincón. Phosphorylation by p38 MAPK as an Alternative Pathway for GSK 3b Inactivation. *Science*. 2008 May 2;320 (5876):667-70.

VGN Graduate Student Chosen to Attend NIH Festival

VGN funded graduate student, Sunandan Banerjee, was chosen to attend the 2nd Annual Graduate Students Research Festival at the National Institutes of Health.



VGN funded graduate student, Sunandan Banerjee, presents his research at the 2nd Annual Graduate Students Research Festival.

The National Institutes of Health (NIH) organized its 2nd Annual Graduate Students Research Festival (NGSRF 2007) in Bethesda, Maryland on the October 11-12, 2007. The event provides a platform for advanced graduate students in the U.S.A to explore post doctoral training as part of the intramural research program at NIH. The festival is highly competitive with 250 graduate students selected out of an applicant pool of 1000 applicants. As a 5th year PhD candidate in the Department of Chemistry at UVM, I am exploring various laboratories for my post doctoral training and this festival provided me the perfect chance to visit NIH and interview with investigators whose research matches my interests. The festival was extremely well organized with scientific talks from NIH investigators and poster sessions from participating graduate students. The students were given time to schedule meetings with potential advisors and discuss their research interests. The students also saw the impressive research facilities at the Bethesda campus and came to know the positives of being a post doctoral fellow at NIH in terms of career opportunities. I came back from the event with the prospect of post doctoral training in two labs and I intend to make a final decision as I approach my graduation.

Highlights of Networking and Professional Development *continued from page 1*

Poster award winner, Seth Brown, from Saint Michael's College.

Poster award winner, Candice Church, from Norwich University.

Research Assistant Professor of Biology and Director of Bioinformatics Core, Karen Richardson-Nassif, PhD, Associate Dean for Faculty and Staff Development and Diversity, University of Vermont, and Colleen McKiernan, PhD, Patent Specialist, Law Offices of Edwards Angell Palmer & Dodge. To share some of the insights gained from hearing about Dr. McKiernan's alternative science career as a patent specialist, I asked her to answer or comment on the following questions.

1. How did you find out about the possibility of being a patent agent?

Dr. McKiernan: I worked in a lab that had substantial funding from a drug company and also had a strong interest in gene therapy. Working closer to the applied science rather than basic science made me more aware of patent issues. I also had friends who had made the transition into law. The ability to make the transition without going to law school was appealing.

2. What training outside of your science background did you need to qualify?

Dr. McKiernan: One of the most important things in transitioning from the "science world" to the real world is knowing how to speak to non-scientists about science. Having parents who have no science background but who insisted on asking what I was doing at work or school probably gave me the best training. My writing skills were critical. Ideally an undergraduate science education should have many opportunities to write, but that is not always the case. Typically, by the time one has completed a dissertation, one should have good writing skills.

3. What aspects of your work responsibilities make it preferable to you over bench/ academic science?

Colleen McKiernan, PhD, Patent Agent, Edwards Angell Palmer & Dodge LLP.

Dr. McKiernan: Research requires focus on a single area. Patent law requires the ability to constantly learn new things in many fields. Patents, by their nature, include new inventions and new information. Patent law also requires one to work across a number of fields—transgenic mice, drugs, medical devices, and screening systems. As the fields that you work in are driven by the clients rather than your own interests, you learn about many things that you would not have otherwise. There are also the court decisions and changes in patent law that need to be considered. One is never lacking new things to learn.

4. Do you perceive there to be a lot of job opportunities for scientists in intellectual property?

Dr. McKiernan: The area of science tends to determine how many opportunities are available. Chemists, engineers, and computer scientists are more in demand. The more that you can highlight your skills in those areas, the more in demand you will be. That being said, you only need to be able to understand what you read and ask intelligent questions. You do not need to know the area well enough to do research in it. Biologists are less in demand as there are more of them. A good place to get a foot in the door is the Patent Office, if you are willing to live in the Washington DC area. The turnover there is notoriously high, partly because the pay is substantially higher in the private sector. Part of getting a job is luck and timing. Law firms will need someone with a particular technology specialty. You can be very good, but the timing may simply be bad.

As an endnote, I'd like to personally thank Dr. McKiernan, as well as all of the panelists and organizers for giving time to create these valuable opportunities for students to interact with, and get advice from scientists within our community.

Mark your Calendar!

North East Regional Life Sciences Core Directors (NERLSCD) Meeting

October 22–24, 2008
Sheraton Hotel
Burlington, VT

For more information, visit:
www.uvm.edu/~vgn/NERLSCD

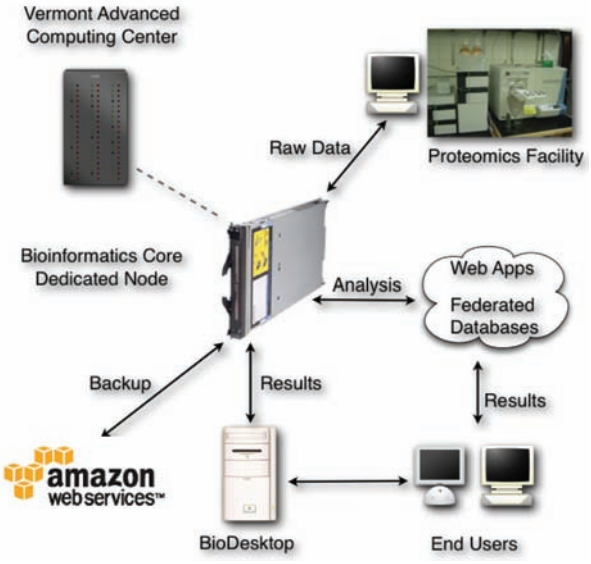
Integration is Key

by James Vincent, PhD, Director, VGN Bioinformatics Core

Have you ever tried to merge large data sets for an analysis you were performing? It's not always easy. Just finding the data can be time consuming. Combining data from different sources can be downright difficult. Add to this the overhead of providing storage and management for terabytes of data and you've got yourself a real headache.

In an effort to alleviate some of this pain the Bioinformatics Core has joined with the Proteomics Facility in a project to integrate raw data processing with post processing analysis. To implement this plan we have leveraged the resources of the Vermont Advanced Computing Center (VACC). A dedicated node of the VACC compute cluster is now being integrated into the Proteomics/Bioinformatics pipeline to address the problem of integration.

The image at right details the flow of information in this system. Raw data generated by mass spectrometers will first be processed through proteomics database searches for identification. Results will be returned to users through the BioDesktop. Raw data and results will be retained on the compute node for further analysis. Offsite backup of all data is managed through Amazon S3 storage services.

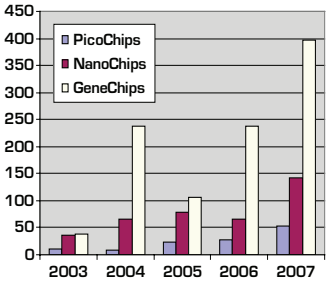


Additional data analysis on results will be provided directly through this same node. This has the advantage of maintaining only one compute resource for the large databases required for both raw proteomics analysis and advanced bioinformatics analyses. In addition, integration of this node in the VACC cluster allows large compute jobs to be launched on demand. In this way the core facilities can provide advanced analysis capabilities without being in the business of buying and maintaining large compute clusters.

Updates from the UVM Microarray Facility

by Scott Tighe and Tim Hunter

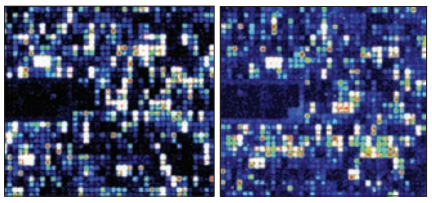
After five years of operation, the UVM microarray facility has generated data from over 1,035 Affymetrix GeneChips® for 38 researchers, and contributed to at least 23 publications and over 29 grant applications. As facility usage continues to grow, we find there is an increasing need for faster sample preparation speed and reduced input concentrations.



Facility usage over the past four years has grown significantly.

Presently, the facility uses a standard 2 ug Eberwine-Affymetrix target preparation method for gene expression research studies, but this protocol suffers due to a lengthy three day synthesis protocol. In some cases, such as flow cytometric sorted cells, micro-dissected tissues, and other low copy number targets, the use of the rapid one-day, 40 ng Nugen Ovation System is implemented. The caveat of this method is a slightly higher cost, but the data can be generated in as little as 36 hours and has higher signal to noise ratios resulting in slightly cleaner data. This alternative technique has been validated by the bioinformatic facility. It is the intention of the microarray facility to employ this target preparation method as a standard technique for new researchers. "Phasing-out" of the Eberwine-Affymetrix protocol for past research will be handled on a case-by-case basis or by investigator driven choice.

On the horizon is the implementation of the Exon and Gene arrays. Both are a standard Affymetrix GeneChip format but are designed at the exon level for detecting both gene signal as well as alternative splice variants. These GeneChips are different from the standard Affymetrix 3' based gene expression chips that only detect mRNA's that possess a poly A tail. These new GeneChips enable the detection of genes and exons based on a priming strategy to the entire mRNA using random hexamers AND Oligo (dT) instead of the traditional Oligo (dT) to the poly A tail. In the past, it was thought that only 5% of the transcriptome was alternatively spliced with most being poly-adenylated, however current data suggest that 30% or more of mRNA can be alternatively spliced or lack a poly A tail. It is well known for instance, that mRNA of mitochondrial origin are not poly-adenylated. Although these chips have been a slow evolution into the microarray facility, it has mostly been due to the lack of refined and reliable target preparation methods. Current methods employ a random priming strategy and ribosomal RNA reduction step that introduces significant variability and, as a core lab, we have found this to be unacceptable as users would have to run each sample in triplicate. Recently however, two new sample preparation methods have been developed by Nugen Technologies and Amp-Tec GmbH which attempt to circumvent this variability. We will be evaluating these methods over the next few months and updating researcher as soon as they are approved by the bioinformatics facility.



Images from two Human U133 2.0 Plus GeneChips showing increased signal to noise in the Nugen protocol (left) compared to the standard Affymetrix protocol (right).

In addition to the current equipment in the facility, we have recently acquired a Qubit fluorometer for high accuracy nucleic acid quantification and discrimination. Several new techniques have also been developed for maximizing the recovery of intact RNA from flow cytometric sorted cells as well as recovering and regenerating cDNA for microarray analysis using special tailing methods. Lastly, we have added the miRNA Lab-on-a-Chip® to our inventory to assess miRNA integrity on the Agilent Bioanalyzer as a QC check prior to miRNA profiling experiments.

The Qubit fluorometer.

Microarray Outreach 2007/2008

by Janet Murray, PhD, VGN Outreach Coordinator

The 2007/2008 microarray outreach year involved five outreach delivery sites with new experiments being offered at many of those sites. The fall 2008 semester brought outreach to both Norwich University and Green Mountain College. Assistant Professor Natalie Coe offered the Microarray course (Bio/Che 4016) at Green Mountain College for the second time. The students conducted the new canned module offered by UVM in which the fission yeast *Schizosaccharomyces pombe* is treated with H_2O_2 to look at the affects of oxidative stress on gene expression in this organism.

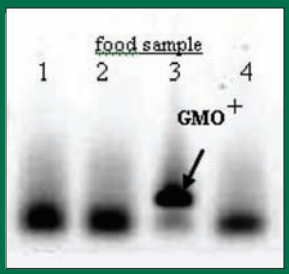
At Norwich University Assistant Professor Karen Hinkle was interested in looking at the affects of Lampricide (TFM) on gene expression in eukaryotic cells. Bakers yeast *Saccharomyces cerevisiae* was treated with environmental levels of TFM for 12 hours to mimic the environmental exposures seen when tributaries of Lake Champlain are treated. These experiments led to a grant proposal submitted to the Vermont Genetics Network by Dr. Hinkle to study this model system more thoroughly. Her proposal was accepted as a Pilot Project

Green Mountain College Microarray Outreach Class, Fall 2007.

VGN Outreach Staff Engage in New Areas of Hands-on

After three years of VGN outreach programs being taught at Vermont four-year colleges, a new area of hands-on laboratory education is being investigated at the high school level. In recent years, it has been of great concern that HS students are less likely than ever to pursue a career in science. As this trend continues, the VGN outreach staff has developed hands-on laboratory exercises and lectures in an attempt to change that trend. Here we present a "beta-test" of the first hands-on genetic experiment designed to educate and excite students in hopes of demonstrating how rewarding science can be.

During the spring, VGN instructors conducted beta-test on performing hands-on laboratory outreach to high school students. The instruction was based at Harwood Union HS and focused around a PCR-based laboratory experiment to test food for genetic modifications specifically looking for the 35s cauliflower mosaic virus promoter sequence (CaMV 35S). The two-day genetic experiment involved teaching basic techniques including pipeting, centrifuging, extracting DNA, conducting PCR, and running agarose gels. Students each tested different corn and soy-based products that they either brought from home or purchased from vending machines. All the supplies, genetic materials, and instrumentation were supplied by the Vermont Genetics Network and will continue to be available for other HS outreach projects involving genetics education.



Picture of DNA products represented by bands on an agarose gel for 4 food samples tested. Each "band" represents a DNA product of a different gene. The Arrow points to the CMV product indicating GMO positive.

The process of uncovering genetically modified foods using "advanced genetic techniques", as the students called it, was thrilling and exciting mostly because they had never worked with DNA before. Three weeks prior to the start of the hands-on experiment, the students had a lecture on the fundamentals of DNA technologies from VGN staff. After the hands-on experiments, the students also had another lecture on PCR and genetically modified food technology which discussed the pros and cons of GMO's. The detailed steps of the experiment included a BioSpec homogenization of their test food, a DNA purification using InstaGel, preparing a master mix of PCR reagents from Takara, programming and

Bioinformatics Outreach at Green Mountain College and

by Janet Murray, PhD, VGN Outreach Coordinator

The VGN outreach Bioinformatics Module was delivered to Green Mountain College in the Fall of 2007. The module was offered in Assistant Professor Natalie Coe's "Genetics of Human Behavior" Course. Thirteen students took part in this module learning tools for searching literature databases and clinical information databases, dissecting gene files, blast searches, phylogenetic mapping and several structural tools for view and manipulating proteins.

VGN Outreach Coordinator, Janet Murray, helps Green Mountain College students with the Bioinformatics Outreach exercises.

The students' were assigned independent projects that focused on diseases with an abnormal behavior phenotype. Each student used the tools learned during the module to understand the molecular defect associated with their assigned disease. The students then gave an oral presentation describing the molecular defect and clinical manifestation of the disease.

Many of the students in the class had limited biology backgrounds most pursuing degrees in psychology. When this was realized a concerted effort between the bioinformatics outreach team and Assistant Professor Natalie Coe helped to address the students needs focusing on delivering the background information as well as the module itself.

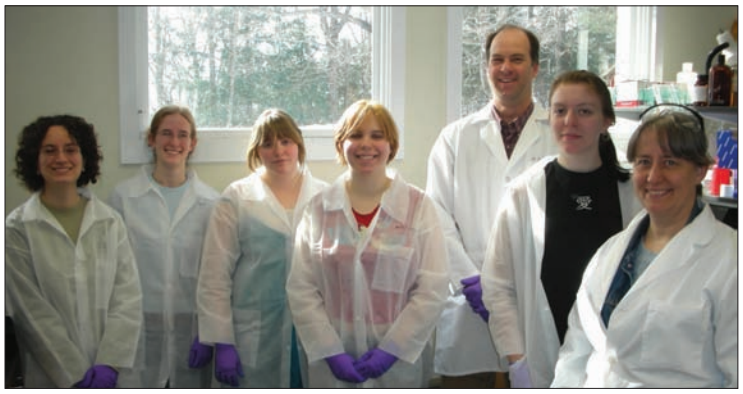
The Bioinformatics Module was delivered to Norwich University in the spring of 2008. This delivery involved courses in Biochemistry

beginning in July 2008 (INBRE year 4).

Spring 2008 microarray outreach brought us to Johnson State College, Castleton State College and Marlboro College. This was our third visit to Johnson State College with Professor Elizabeth Dolci. One of our goals in microarray outreach is to see the outreach team's role being reduced and the outreach faculty taking on more responsibility for the module. We are seeing this happen more and more at our delivery sites and with this third visit at Johnson State College we can see this module being integrated very successfully.

The delivery to Castleton State College was coordinated with Assistant Professor Carly Langlais and utilized the new *S. pombe*/H_2O_2 canned experiment. This was our second visit to Castleton State College, although with a new faculty member. The outreach team very much enjoyed working with Dr. Langlais and we wish her luck in her move into new areas of science.

Microarray outreach at Marlboro College brought us into the world of plant gene expression looking at alternate expression profile in *Arabidopsis* grown under different light sources (white, blue and red light). The experiment was suggested and designed by the students at Marlboro College with Professor Todd Smith. This was a wonderful learning opportunity for all involved and it points to the ability of outreach sites to develop novel experiments that fit into their curriculum. We encourage faculty at the delivery sites to develop new experiments in conjunction with the outreach team members.



Marlboro College Microarray Outreach Class, Spring 2008.

Laboratory Instruction Teaching PCR at Regional High School

running a Techne 310 thermocycler, running a 2% EGel, and collecting photo documentation.

The final stage of this outreach module involved the class visiting UVM and performing a simple macroarray experiment in Stafford 104, followed by three rotations through the microarray, DNA, and cell imaging labs, and a VGN sponsored lunch. The students are now working on an article for their school newspaper describing their experiment entitled *"Harwood Students Use Advanced Genetic Techniques to Study Genetically Modified Foods"*.

Overall the beta-test was a complete success. The students got a taste of how fun science can be. It's not just sitting in a classroom all day, but rather, can be very exciting when you discover something new. When I first got to the class early last semester, I asked the class of 24 students how many of them were thinking of going into science. Sadly only 1 responded. However after these experiments, 3 said they were going into science and one wants to go to medical school. This really shows how much influence outreach can have on this age group. Sophomores, Lucy Gubernick and Nancy Amestoy, responded with "We learned that we could test for any gene, not just GMO genes. They could be cancer genes or genes just to identify a new organism. The uses of the techniques we learned are widespread. It is even used in CSI the TV show. It certainly gave *Smartfood* a new meaning!"

"The most impressive part of this entire interaction with Harwood Union HS was the excitement it generated for these students. The fact that a larger portion of students are now looking into pursuing careers in science from these activities strengthens our resolve to further explore outreach at the high school and middle school levels. I also believe there is a huge advantage to exposing all of these students to the future of science and technology. Overall, they gain more information about their world and the importance of investing in science and technology as a society," added Janet Murray, VGN Outreach Coordinator.

High school science teacher John Kerrigan looks over the shoulders off his advanced biology class while conducting a macroarray exercise in Stafford 104.

Norwich University

Dr. Pat Reed, VGN Outreach Team Member, discusses an independent project with one of the Norwich University students.

and Physiology taught by Associate Professor Alison Fisher and Assistant Professor Karen Hinkle respectively. Alison Fisher and Karen Hinkle coordinated a common two-hour course time for the integration of this module into both courses. The joint class consisted of 24 students who participated in bioinformatics outreach and the independent project with assigned diseases. Every student was given an independent project. Currently there are 32 different diseases that can be assigned to students with known crystal structures and mutant alleles containing a single amino acid change for students to discuss and change within the structure.

This large group of students presented their diseases in a poster format. The group was divided into two sections with 12 students presenting their poster for an hour. This poster session was open to other faculty at Norwich and two guests from UVM as well as the bioinformatics outreach team. This format gave the students time to discuss their research with faculty and other students from the course and an opportunity to see other students research. This was the first time that the presentations have been put into a poster format and all agreed that it was very successful!

The bioinformatics module will be reviewed through the summer months to update any database changes and to incorporate small changes we feel will make the module run even more smoothly. The delivery of the module to Saint Michael's College is scheduled for the fall of 2008. There is a possibility of delivering a second module this fall. Please contact **Janet.Murray@uvm.edu** if your group may be interested.

vSEEN.org

The Vermont Science, Engineering and Environment Network

Do you have a great project that requires expertise from someone outside your field? Have you found it difficult to connect with the right people?

The Vermont Genetics Network has been asked to design a mechanism by which Vermonters with science and engineering expertise can find each other. In particular, many faculty of the colleges in Vermont have requested an "experts list." Here it is: **http://vseen.org**

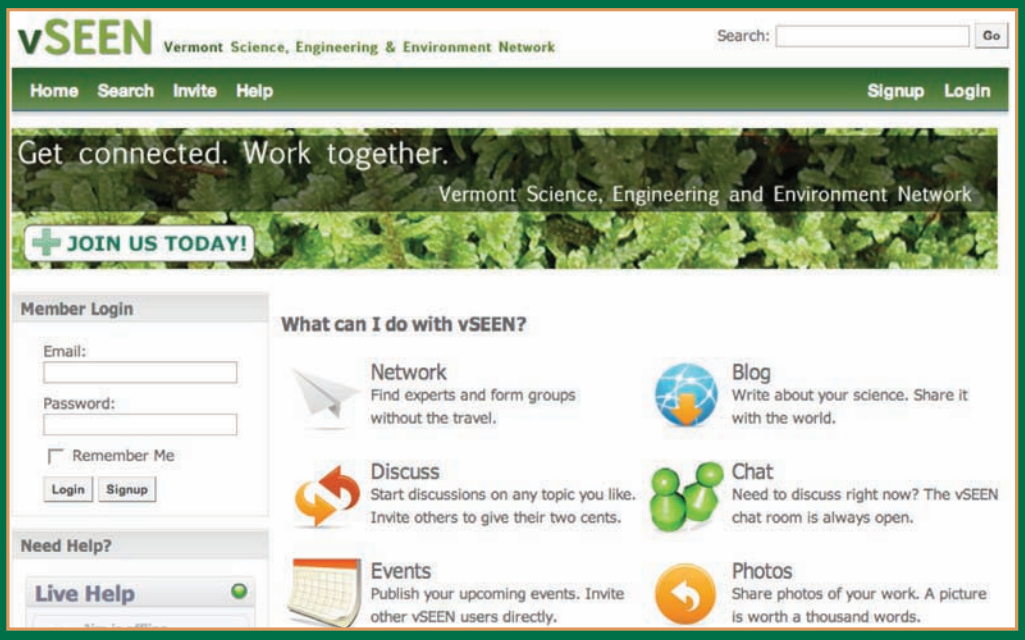
This is a simple way for you to enter your expertise and to search for experts across disciplines with a simple keyword search. Signing up in no way makes your e-mail address available, but you will be able to find and communicate with experts in Vermont.

By maintaining privacy for email addresses while still allowing an easy method of contact and communication, vSEEN.org provides an intermediate step between blindly emailing potential colleagues and waiting for someone to contact you through your own website.

Members of vSEEN.org are active participants who have identified their expertise.

A host of tools are available to facilitate additional communication, including personal blogs and photo albums for describing your work, as well as group discussions and live chat for more immediate discussion of current topics.

All of the above activities are designed to stimulate collaborations and partnerships among Vermont researchers and engineers. We hope to see you there!



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