Biology

***The effect of inhibitors of the multi-xenobiotic resistance protein on domoic acid disposition in Dungeness crabs and Mediterranean mussels.** *Laura Almaguer* (*Gavilan College, Gilroy, CA*) *Irvin Schultz* (*Pacific Northwest National Laboratory, Richland, WA*). Select species of marine algae primarily belonging to the genus *Pseudo-nitzchia* produce the toxin domoic acid (DA), making it a naturally occurring neurotoxin that contaminates shellfish that have fed on *Pseudo-nitzchia*. People ingest the neurotoxin by eating contaminated fish and shellfish; in extreme cases it can even cause death in humans and animals alike. The contamination of shellfish with domoic acid has caused health and economic problems world wide. Some species of shellfish retain domoic acid longer than others and it is not fully understood why. It is hypothesized that the multi-xenobiotic resistance (MXR) protein(s) identified in marine shellfish, functionally similar to mammalian counterparts p-glycoprotein (pgp) and OAT-1 (organic anion transporters), are associated with the unusual interspecies differences in domoic acid. To test this hypothesis both the uptake and excretion of DA was monitored by exposing one set of mussels with known pgp and OAT-1 inhibitors, verapamil and cyclosporine. Uptake of DA was determined using static water exposures where verapamil or cyclosporine injected mussels were placed in 1 L beakers containing soluble DA. Excretion of DA was studied by reversing this protocol and injecting DA into mussels placed in seawater containing verapamil. Subsequent experiments with crabs measured the excretion of injected DA beginning 1 hr after injection with either verapamil or cyclosporine. Water, hemolymph and digestive gland tissues were then analyzed for DA using High Performance Liquid Chromatography (HPLC) with either UV or fluorescence detection. Initial results suggest neither inhibitor altered the kinetics of DA in mussels and crabs.

Evaluation of Cloning Vectors pMCSG8 and pMCSG10 to Increase Protein Solubility. *Jessica Bearden* (*University of Texas – Pan American, Edinburg, TX*) *Shiu Moy* (*Argonne National Laboratory, Argonne, IL*). A major obstacle in the high-throughput production of purified proteins, as conducted in the Protein Structure Initiative, is to routinely obtain soluble proteins using the standard cloning vector pMCSG7. It is important that cloners release soluble proteins to the purification group because insoluble proteins cannot be purified. Evaluation of cloning vectors pMCSG8 and pMCSG10 was conducted to recover soluble proteins that failed to be soluble using pMCSG7. pMCSG7 has a histidine affinity tag at the N-terminus, followed by a tobacco etch virus(TEV) protease recognition site, followed by a ligation independent cloning site, followed by another histidine tag at the C-terminus. pMCSG8 is structurally similar to pMCSG7 with the exception of a binding loop(S-loop) of the chaperone protein GroES between the histadine tag and the TEV protease recognition site, while pMCSG10 has a Glutathione-S-Transferase(GST) in place of the S-loop. Selected samples were transformed and then cloned into the desired vector. Competent *Escherichia coli* cells were induced to uptake the recombinant DNA. Expression and solubility analysis was conducted using sodium dodecyl sulfate polyacrylamide gel electrophoresis. Clones that both expressed and were soluble were then frozen down and released to the protein purification team. pMCSG8 was able to recover ten percent of the samples tested. pMCSG10 analysis is still underway. pMCSG8 results implicate incorporation of a feedback loop in the high-throughput production of proteins at the Structural Biology Center at Argonne National Laboratory.

A Cell-Free Membrane Protein Factory Fueled by Rhodobacter Extracts. *Michael Bellisario* (*University of Illinois at Urbana-Champaign, Champaign, IL*) *Phil Laible* (*Argonne National Laboratory, Argonne, IL*). Membrane proteins play critical roles in many biological processes such as energy supply, solute import and export, and signal transduction. They are also ultra-critical to human health, comprising 60–80% of current drug targets. However, producing usable quantities of these proteins for structural and functional studies is quite challenging. Here, the goal is to eliminate this difficulty by creating a cell-free protein synthesis system specially designed for membrane proteins. This coupled transcription-translation system uses extracts (the source of all the enzymes and factors necessary for transcription and translation) derived from *Rhodobacter sphaeroides* cells, and membrane vesicles are introduced to the reaction in order to accommodate the membrane proteins being synthesized. This *in vitro* method has the capability to produce milligrams of target protein in a single milliliter reaction. In comparison, previously studied *in vivo* systems only produce milligrams of protein per liter of culture. The ultimate goal is to engineer this system so that it can be used generically and economically to produce target molecules for drug discovery.

Isolation of Organelles in an Itaconic Acid Producing Anamorphous Fungus. *Darby Bennett* (*Walla Walla Community College, Walla Walla, WA*); *Ellen Panisko* (*Pacific Northwest National Laboratory, Richland, WA*). Filamentous fungi have been identified as platform organisms by the Department of Energy (DOE) in that they can be used for several different applications including: making industrial chemicals, use in pharmaceutical drugs and treatments, and conversion of biomass into usable fuels. The ability of *Aspergillus terreus*, ATCC strain 32359, to produce itaconic acid and a method to determine how production can be improved is one focus of our research program. Itaconic acid or methylene succinic acid is an unsaturated organic compound made in a portion of the Krebs cycle which produces copolymers used in strictly non-food products. It is not known which proteins are involved in the process that allows one strain of fungus to produce high amounts of itaconic acid, while others do not make this acid, but it is thought that some of the proteins responsible that have increased expression during the hyper productive growth state are involved in substrate/product transport. To examine this theory, the components of the cell membranes and mitochondria during the non-productive and hyper productive growth states were isolated. To accomplish this, several different digestive enzymes and centrifuging techniques were used. At each stage of the process, samples were collected and examined to determine the protein concentration and cell components present using protein assays and spectrophotometry. Proteins isolated from the organelles were separated according to size by gel electrophoresis, and probed by Western analyses to determine the extent of organelle enrichment. Preliminary results show that there are more proteins present in the hyper productive state than in the non-productive state, although this may be due to an increase in initial biomass collected for the hyper productive state. Follow up analyses will examine the proteins expressed in the two disparate itaconic acid production states to identify those proteins that may be responsible for hyper productivity. If the specific proteins responsible for increased production of itaconic acid can be identified and isolated, A. terreus can be engineered to produce industrially relevant quantities of itaconic acid.

Crystallographic Studies of Two Bacterial Antibiotic Resistance Enzymes: Aminoglycoside Phosphotransferase (2'')-Ic and GES-1 ß-lactamase. *Laura Byrnes* (*Rensselaer Polytechnic Institute, Troy, NY*) *Clyde Smith* (*Stanford Linear Accelerator Center, Stanford, CA*). Guiana Extended-Spectrum-1 (GES-1) and Aminoglycoside phosphotransferase (2'')-Ic (APH(2'')-Ic) are two bacteria-produced enzymes that essentially perform the same task: they provide resistance to an array of antibiotics. Both enzymes are part of a growing resistance problem in the medical world. In order to overcome the ever-growing arsenal of antibiotic-resistance enzymes, it is necessary to understand the molecular basis of their action. Accurate structures of these proteins have become an invaluable tool to do this. Using protein crystallography techniques and X-ray diffraction, the protein structure of GES-1 bound to imipenem (an inhibitor) has been solved. Also, APH(2'')-Ic has been successfully crystallized, but its structure was unable to be solved using molecular replacement using APH(2'')-Ib as a search model. The structure of GES-1, with bound imipenem was solved to a resolution of 1.89Å, and though the inhibitor is bound with only moderate occupancy, the structure shows crucial interactions inside the active site that render the enzyme unable to complete the hydrolysis of the ß-lactam ring. The APH(2'')-Ic dataset could not be matched to the model, APH(2'')-Ib, with which it shares 25% sequence identity. The structural information gained from GES-1, and future studies using isomorphous replacement to solve the APH(2'')-Ic structure can aid directly in the creation of novel drugs to combat both of these classes of resistance enzymes.

Crystallization and Preliminary X-ray Crystallographic Analysis of the Archaeal Tryptophan Regulator, TrpY. *Jacquelyn Cafasso* (*Cornell University, Ithaca, NY*). *Mark Chance*, *Babu Manjasetty*, (*Brookhaven National Laboratory, Upton, NY*). The TrpY protein from the archaeon *Methanothermobacter thermautotrophicus* is a transcription regulator of the metabolically expensive tryptophan biosynthetic pathway. Although the trp genes in Bacteria, *archaea*, and *eukaryotes* share a common ancestry, diverse mechanisms regulate their expression. The TrpR repressor in *E. coli* has been extensively studied, but the structure and mechanism for repression by the TrpY regulator from archaea remains unknown. Furthermore, TrpY shows very little sequence homology with the TrpR tryptophan regulator in *E. coli*, and although bioinformatics studies indicate that the fold is conserved among other archaeal transcription regulators, the sequence

similarity to TrpY is nonetheless very low. Native crystals of TrpY were successfully grown in 0.1 M sodium acetate and 1.6 M ammonium sulfate at room temperature using the hanging-drop vapor diffusion method. Initial diffraction tests and the search for a suitable cryo-protectant were performed at beamline X3A of the National Synchrotron Light Source (NSLS). X-ray diffraction data was collected at beamline X29 of the NSLS to 2.9 Å resolution. Preliminary data analysis revealed that the crystals fall in the tetragonal space group with cell parameters a=b=87Å, c=147Å. Methods to solve the structure of TrpY using heavy atom derivatives are currently underway. Using crystallographic X-ray analysis to solve the structure is important to gain insight into the TrpY mechanism of repression as well as important features of transcription regulation and evolutionary history in the Archaea. This project is a small portion of a larger project under investigation in collaboration with the Department of Microbiology at The Ohio State University.

Hydrogen Production by the Cyanobacterium *Plectonema boryanum*: Effects of Initial Nitrate Concentration, Light Intensity, and Inhibition of Photosystem II by DCMU. *Blaine Carter* (*Northwest Nazarene University, Nampa, ID*) *Michael Huesemann* (*Pacific Northwest National Laboratory, Richland, WA*). The alarming rate at which atmospheric carbon dioxide levels are increasing due to the burning of fossil fuels will have incalculable consequences if disregarded. Fuel cells, a source of energy that does not add to carbon dioxide emissions, have become an important topic of study. Although significant advances have been made related to fuel cells, the problem of cheap and renewable hydrogen production still remains. The cyanobacterium *Plectonema boryanum* has demonstrated potential as a resolution to this problem by producing hydrogen under nitrogen deficient growing conditions. *Plectonema boryanum* cultures were tested in a series of experiments to determine the effects of light intensity, initial nitrate concentration, and photosystem II inhibitor DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) upon hydrogen production. Cultures were grown in sterile Chu. No. 10 medium within photobioreactors constantly illuminated by halogen lights. Because the enzyme responsible for hydrogen production is sensitive to oxygen, the medium was continuously sparged with argon/CO_2 (99.7%/0.3% vol/vol) by gas dispersion tubes immersed in the culture. Hydrogen production was monitored by using a gas chromatograph equipped with a thermal conductivity detector. In the initial experiment, the effects of initial nitrate concentration were tested and results revealed cumulative hydrogen production was maximum at an initial nitrate concentration of 1 mM. A second experiment was then conducted at an initial nitrate concentration of 1 mM to determine the effects of light intensity at 50, 100, and 200 µmole/m^2·s. Cumulative hydrogen production increased with increasing light intensity. A final experiment, conducted at an initial nitrate concentration of 2 mM, tested the effects of high light intensity at 200 and 400 µmole/m^2·s. Excessive light at 400 µmole/m^2·s decreased cumulative hydrogen production. Based upon all experiments, cumulative hydrogen production rates were optimal at an initial nitrate concentration of 1 mM and a light intensity of 100 µmole/m^2·s. DCMU was shown in all experiments to severely decrease hydrogen production as time progressed. With the information acquired so far, future experiments with reducing substances could determine maximum rates of hydrogen production. If maximum hydrogen production rates proved to be large enough, *Plectonema boryanum* could be grown on an industrial scale to provide hydrogen gas as a renewable fuel.

Improving Power Density of a Microbial Fuel Cell by Optimizing Electrode Area and Substrate Delivery. *Scott Cesar* (*Western Michigan University, Kalamazoo, MI*); *Abhijeet Borole* (*Oak Ridge National Laboratory, Oak Ridge, TN*). Microbial Fuel Cells (MFC's) are devices which use micro-organisms as catalysts to oxidize compounds such as lactate whereby electrons are released and are allowed to flow between electrodes to generate current. The box-type MFC involved used *Shewanella oneidensis* in a minimal media with lactate and a carbon felt electrode as the anode. An air cathode was used involving a platinum/carbon electrode. Improving the power density output of an air cathode MFC was the primary goal if this work. The MFC performance can be assessed by analyzing the electrical and chemical/biochemical parameters of the system. The current can be determined by monitoring the voltage across a fixed load using a voltmeter. The electrical performance of the MFC can be determined by first measuring the open circuit voltage (OCV) and current produced across a variable load resistor. Along with electrical measurements, samples of the anode solution are taken to determine the changes in biochemical characteristics of the MFC. Under no-flow conditions, the MFC stabilized at 0.177 volts. With the introduction of flow to the system, the MFC stabilized at 0.347 volts. The power density at this time was found to be 128.6 mW/m^2 with a current density of 446.4 mA/m^2. Further improvements in power delivery are possible via a more compact design of the fuel cell and flow of the media across a three-dimensional electrode.

Wind energy educational outreach program: Bringing Wind Energy to Schools. *Brent Cummings* (*Brigham Young University – Idaho, Rexburg, ID*); *Gary Seifert* (*Idaho National Laboratory, Idaho Falls, ID*). Educational outreach programs play an important role in the proliferation of renewable energy sources throughout the United States. An outreach website named "Wind Energy for Educators" was developed by educators in Idaho in order to educate the public about wind energy. This website contains information on wind energy, and also has some lesson plans on wind and how to use the energy that the wind contains. By teaching students in the classroom about renewable energy and its potential they can make educated decisions in the future about whether they want to support renewable energy projects or not. They will also be aware of possible career opportunities that are available to them. As part of the outreach program a skystream wind turbine was erected at Skyline High School in Idaho Falls, Idaho. The turbine will allow the students at the high school to participate in some hands on learning. It is also located next to an interstate highway where it can be seen by the public. The data gathered about the energy produced by the turbine will be recorded and made available to students via a web site for educational purposes. Education modules that use the information gathered about the turbine are being developed. These modules will deal with subjects such as understanding what a kilowatt hour is, CO_2 emissions, sound monitoring, and others. Although the turbine will not significantly reduce the schools expenditures for energy, it will provide many useful educational opportunities.

A Rational Approach for Crystallization of Proteins in Deuterated Media. *Alexis Rae Del Castillo* (*California State University – Channel Islands, Camarillo, CA*); *Hugh O'Neill* (*Oak Ridge National Laboratory, Oak Ridge, TN*). Neutron crystallography is emerging as a powerful tool for the study of protein structure and dynamics. In neutron crystallography the neutrons interact weakly with the nucleus of an atom and therefore are a highly penetrating and non-destructive probe. Unlike X-rays, which interact with the electron cloud surrounding an atom, neutrons can detect lighter atoms such as hydrogen in the presence of heavier ones and differentiate between them. The aim of this study was to determine how reagents influence the crystallization behavior of proteins in order to improve the growth of crystals for neutron crystallography. In order to achieve this goal a range of proteins were selected for crystallization studies, including several proteins for which the crystallization behavior is well established, and several other targets which were over-expressed in Escherichia coli in hydrogenated and deuterated media. The recombinant proteins were purified to homogeneity by three-phase partitioning and anion exchange chromatography. The conditions for crystallization of each protein were determined using a high throughput screening platform. Conditions that produced crystals were then optimized and refined in order to produce large crystals suitable for neutron crystallographic analysis.

***The Effects of Nicotine Exposure on Cytochrome P450-Mediated Metabolism of Chlorpyrifos in Sprague-Dawley Rats.** *Josh Elsasser* (*California State University – Fresno, Fresno, CA*); *Charles Timchalk* (*Pacific Northwest National Laboratory, Richland, WA*). Chlorpyrifos (CPF) is a common organophosphate insecticide used for pest control. CPF is metabolized to chlorpyrifos-oxon (CPF-Oxon) and 3,5,6-trichloro-2-pyridinol (TCP). The metabolite of most concern is CPF-Oxon since it is responsible for acetylcholinesterase (AChE) inhibition. The inhibition of AChE activity can adversely impact cholinergic function and if severe enough can result in central and peripheral neurotoxicity. Studies have shown that both nicotine (smoking) and ethanol (drinking) can induce cytochrome P450s (CYP450), the enzyme family responsible for the metabolism of CPF. Since the enzymatic profiles of CYP450 are expected to be altered due to nicotine exposure, this could modify the metabolism of CPF. Thus, the aims of this study are to characterize the changes in CYP450 profiles and CPF metabolism associated with nicotine induction. Rats were dosed subcutaneously (s.c.) with 1 mg of nicotine/kg body weight daily. Liver microsomes were prepared 4 hour, 24 hour, 7 day, 7 days and 4 hours, and 10 days post-dosing. Total amounts of protein and CYP450s in microsome samples were determined by spectrophotometry. *In vitro* metabolism studies were also conducted to measure the formation of CPF-Oxon and TCP using gas chromatography/mass spectrometry (GC/MS). CPF was incubated with microsomal samples in 1 mL of 50 mM HEPES buffer containing, 15 mM $MgCl_2$ and 1 mM EDTA at 37°C and 1 mM NADPH was added to initiate the reaction, which was terminated after 20 minutes by

the addition of 200 µL of NaCl-saturated 2.5 N acetic acid solution. Initial analysis of the data did not provide any conclusive evidence to suggest that nicotine exposure produced substantial changes in either the amount of CYP450 or the amount of metabolites within the microsome samples. Further experimentation will be needed to identify if a change did occur particularly at the level of individual CYP450 forms. The results from this study are being used to design an *in vivo* pharmacokinetic study for the risk assessment of concurrent nicotine, ethanol and CPF exposure.

Marine Mussel Adhesive Protein Production in *Saccharomyces cerevisiae*. *Kindra Engels* (*Washington State University, Pullman, WA*); *Frank Roberto* (*Idaho National Laboratory, Idaho Falls, ID*). The adhesion proteins used by *Mytilus edulis* (blue mussel) to cling to surfaces in an aqueous environment have many features, such as strength and water resistance, that make them a potentially useful adhesive. Three *M. edulis* foot proteins (Mefp) will be the focus of this study: Mefp-1 (115 kDa), which forms a hardened sheath around the byssal threads, Mefp-2 (42–47 kDa), which adds stability near the attachment site, and Mefp-3 (5–7 kDa), which may act as an adhesion primer. Because it takes 10,000 mussels to obtain 1g of an individual native mussel adhesive protein, a more practical production method was investigated. Mussel adhesive protein genes were introduced into clones of *Saccharomyces cerevisiae*, with Mefp-1 in clone #21, Mefp-2 in clone QTB10, and Mefp-3 in clone #11. *S. cerevisiae* cultures (20 liters) were grown in a 2% galactose SC-U induction medium at 30°C with shaking and harvested by centrifugation. Growth was monitored by spectrophotometry. Cells were homogenized and lysed in an acidic breaking buffer with glass beads in a bead beater. After centrifuging again, the recombinant mussel adhesive protein in the supernatant was purified by dialysis with nanopure water and a sodium borate solution (pH 8.5). Centrifugal filter devices concentrated the proteins. Protein concentration was determined using a Bradford assay with bovine serum albumin as a standard. Samples were analyzed by electrophoresis. Results from growth curves revealed that 20 L cultures had optimal harvest times after 24 hours and cell pellet wet weights of 114.55g (#21), 102.69g (QTB10), and 185.512g (#11) were obtained. Electrophoresis of #21 and QTB10 purified proteins resulted in bands near the expected size ranges of recombinant proteins Mefp-1 and Mefp-2. In conclusion, the results suggest that adhesive protein production in *S. cerevisiae* cells is possible and that purification methods successfully concentrated the adhesive proteins. This method could potentially be used to produce recombinant adhesive protein quantities that would normally require the sacrifice of thousands of mussels. The *M. edulis* recombinant adhesive proteins obtained may be used for various studies involving the proteins' adhesive potential and further formulation development.

Phosphate Enhanced Uranium Reduction. *Rachel Fairbank* (*Tompkins Cortland Community College, Dryden, NY*); *Anthony V. Palumbo* (*Oak Ridge National Laboratory, Oak Ridge, TN*). A common contaminant found at DOE sites is uranium, which characteristically leaches into groundwater and surrounding soils. Remediation of these sites is therefore a DOE focus. Current technologies mainly consist of pump and treat technologies which have the disadvantage of being invasive and ineffective in areas with low flow velocity. Total cleanup costs using existing technologies are estimated to exceed a total of $220 billion, making it worthwhile to investigate alternative methods of uranium remediation. One focus for remediation is the stabilization of uranium through reduction of mobile U(VI) to its less soluble and immobile form U(IV). One possibility is by stimulating existing microbial communities to reduce uranium. Previous experiments had demonstrated the ability of electron donors' ethanol and methanol to stimulate bioreduction of uranium. This experiment investigated the idea of phosphate being a limiting nutrient in bioreduction of uranium. Anaerobic microcosms were created using contaminated soil from the Oak Ridge Field Research Center. Samples were analyzed at specific time points throughout the experiment using a Kinetic Phosphorescence Analyzer which measured the amount of soluble uranium. This experiment found that the addition of phosphates led to immediate removal of uranium from solution. This effect was observed to be independent of the presence of an electron donor, as a similar effect was observed in the microcosms with only phosphates added. Therefore, these results imply that reduction is due to a chemical interaction with the phosphates rather than due to stimulation of the microbial community.

Effects of Phosphate on the Bioreduction of Iron Oxyhydroxide. *Kathryn Fenske* (*University of Illinois at Urbana-Champaign, Urbana, IL*); *Edward O'Loughlin* (*Argonne National Laboratory, Argonne, IL*). Green rusts are mixed ferrous/ferric hydroxides minerals that form in suboxic environments as products of Fe(III) oxide reductions by dissimilatory iron-reducing bacteria (DIRB), and as such play an important role in Fe cycling in aquatic and terrestrial environments. DIRB can conserve energy and also support growth by coupling the oxidation of organic compounds to the reduction of Fe(III) to Fe(II) with the potential formation of Fe(II)-bearing minerals such as magnetite, siderite, and green rust. The overall processes of the formation of a specific Fe(II)-bearing mineral, such as green rust, are controlled by several factors including microbial physiology, solution chemistry, and Fe(III) mineralogy. This experiment examines the effects of phosphate on the type(s) of Fe(II)-bearing minerals resulting from the bioreduction of a Fe(III) oxyhydroxide (lepidocrocite). Experimental systems consisted of sealed serum vials containing lepidocrocite with formate provided as an electron donor. Different amounts of phosphate were added to each system and they were inoculated with Shewanella putrefaciens CN32, a model DIRB. Lepidocrocite reduction was monitored by measuring Fe(II) by the Ferrozine assay. Biomineralization products were identified by X-ray diffraction. Analyses of results indicate that green rust formed when phosphate was present at concentrations of 100 µM or higher, while magnetite formed at phosphate concentrations below 100 µM. Green rusts have recently been shown to be capable of reducing a number of organic and inorganic contaminants (including carbon tetrachloride and U(VI). Therefore, understanding how factors such as phosphate concentration can contribute to the formation of green rusts may assist in efforts to design remediation strategies for cleanup of subsurface contamination.

Elucidating a Practical Approach to the Study of Eukaryotic Genes: Expression of Eukaryotic Zebrafish Proteins in Prokaryotic *E coli* Expression Vectors. *Ashley Frank* (*Elmhurst College, Elmhurst, IL*); *Frank Collart* (*Argonne National Laboratory, Argonne, IL*). Production of heterologous protein via expression in prokaryotic expression vectors has been extensively employed in recent years to yield significant amounts of protein for downstream characterization and analysis. The use of such vectors offers a practical, economical route for the production of protein, eliminating cost and time inefficiencies accompanying protein isolation from the native protein-producing organism. While bacterial expression systems have been optimized for cloning prokaryotic genes, further investigation is needed to optimize these systems for production of more complex eukaryotic proteins. The cell machinery of a bacterial expression system is limited with respect to the production of eukaryotic proteins as these proteins are derived from more intricate, compartmentalized cells and often require specific enzymes for post-translational modifications and protein folding. Since many of the enzymes and machinery necessary for the successful expression of eukaryotic proteins are lacking in the current bacterial expression systems, study of such proteins has been avoided using these methods. To accommodate the requirements of eukaryotic protein production in bacterial expression systems, periplasmic expression vectors have been constructed and modified using previous cytoplasmic vector templates to optimize the expression and solubility of eukaryotic proteins. Such vectors direct proteins to the periplasm where bacterial chaperones reside to aid in proper protein folding and disulfide bond formation which is required by many eukaryotic proteins, thus increasing protein solubility and recovery potential. In a study to determine a successful approach to the production of eukaryotic proteins, 96 select Zebrafish genes were amplified, cloned into two different periplasmic vectors (pBH31 and pMCSG19p), induced to express the heterologous target protein, and screened for positive expression and solubility. Results suggest that pMCSG19p, which harbors a solubility fusion tag, was superior in performance, with respect to the production of soluble proteins for these 96 targets. In addition, expression in this vector resulted in a relative increase in solubility of targets containing predicted disulfide bonds and signal peptides, suggesting that pMCSG19p may provide an effective route for the production of complex eukaryotic proteins. The solubility results for proteins produced in pBH31, however, were comparable to the solubility results of these same 96 targets produced in the cytoplasmic vector, pMCSG7, suggesting that this vector not only does not improve solubility of eukaryotic proteins, but also may not shunt the proteins to the periplasm for proper expression. Further studies employing different vector solubility tags or manipulation of cytoplasmic physiology may be required for the optimization of eukaryotic protein expression in bacterial expression systems.

Extracellular Translocation of Recombinant MtrC and OmcA by Type II Secretion System. *Shirabrandy Garza* (*Washington State University, Pullman, WA*); *Liang Shi* (*Pacific Northwest National Laboratory, Richland, WA*). Dissimilatory reduction of metal (e.g., Fe, Mn) (hydr)oxides represents a challenge for microorganisms, as their

cell envelopes are impermeable to metal (hydr)oxides that are poorly soluble in water. Outer membrane decaheme c-type cytochromes MtrC and OmcA of *Shewanella oneidensis* MR-1 are extracellular lipoproteins important for dissimilatory reduction of solid metal (hydr)oxides during anaerobic respiration. To investigate the roles of type II secretion system (T2S) in translocation of MtrC and OmcA across outer membrane, we measured the effects of deleting two T2S genes, gspD and gspG, on the secretion of recombinant MtrC and OmcA when cells were grown under anaerobic conditions. Deletion of gspD or gspG resulted in slightly yellowish supernatants of cell culture, different from the pink supernatant of wild type (wt). Subsequent analysiss with heme-staining and Western blot showed that deletion of gspD or gspG not only reduced the abundances of recombinant MtrC and OmcA in the supernatants, but also increased their abundances inside the cells. Thus, our results indicate that T2S facilitates translocation of recombinantMtrC and OmcA across outer membrane.

Metal Repartition and Expressed Genes in Spinal cord of Rats. *Seritta Hill* (*Chicago State University, Chicago, IL*)*; Christine Gerin* (*Argonne National Laboratory, Argonne, IL*). Specific metals such as Fe, Cu, and Zn have been shown to accumulate in the central nervous system (CNS) in several neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). Metal involvement has been linked to neural degeneration in late onset neurodegenerative diseases. The hypothesis is that similar cellular patterns of degeneration might occur in spinal cord injury. The first aim addresses the question of metal repartition in spinal cord of rats, using X-ray fluorescence. The second aim addresses the question of variation in expression targeted genes in spinal cord using quantitative real time polymerase chain reaction (QRT-PCR). Rats were anaesthetized with sodium pentobarbital (60 mg/1,000g) injected intraperitoneally. Spinal cord segments T9 and T13 were excised. RNA isolation was performed using TriZol protocol (Sigma). RNA was purified using RNA clean up kit (Qiagen). Two µl of the RNA sample were used to determine the optic density (OD) and the concentration in ng/µL. Three RNA dilutions were made to construct a concentration curve for c DNA and QRT-PCR. cDNA was synthesized using 10 µL of RNA followed by QRT- PCR using 11 housekeeping genes (primers). Most of our RNA OD results were 2.0 for the 260/280 ratio and for the 260/230 they were in the ranges of 1.8–2.2. The concentration of RNA samples ranged from 5.4 ng/µl to 1,200 ng/µl. OD curves illustrated that RNA absorbed the highest at 260 nm. RT-PCR demonstrated two curves for each RNA concentration and it illustrated that the c DNA with their various primers amplified properly. In conclusion the housekeeping genes were expressed in our samples. In the future the house keeping genes will be compared to our gene of interest. For example, BDNF. The Advance Photon Source (APS) beam line was used to train on cardiomyocytes in order to use it in the future to measure the sub cellular trace metals such as Zn, Fe, and Cu in our spinal cord samples. Experimental work was performed in collaboration with Argonne National Laboratory and Northwestern University.

***Successful Crystallization Leading to Structure of Protein Rv0632c from Mycobacterium tuberculosis.** *Jeffrey Hohenstein* (*Contra Costa College, San Pablo, CA*)*; Minmin Yu* (*Lawrence Berkeley National Laboratory, Berkley, CA*). Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* that results in over a million fatalities annually. The TB Structural Genomics Consortium (TBSGC) was formed to address diagnosis and treatment of TB by determination and analysis of the structure of proteins within tuberculosis. The protein Rv0632c was predicted to be an Enoyl-CoA Hydratase involved in fatty acid metabolism. In order to solve the structure of Rv0632c optimal crystallization conditions were sought. Random screening of crystallization conditions was conducted using Hampton Research CrystalScreen, CrystalScreen II, and PEG/Ion as well as Emerald Biosystems Wizard I, Wizard II, and Precipitant Synergy random screening kits. Crystallization was performed using the sitting drop vapor diffusion method in 96 well plates. Drops containing the native protein were 1 µL with 0.5 µL of protein solution combined with 0.5 µL of crystallization solution and a protein concentration of 5.58 mg/mL. Optimization of conditions was performed based on initial crystal hits. Crystals were screened at Advanced Lightsource beamline 5.0.2 at Lawrence Berkeley National Laboratory. The successful optimization condition included 0.2 M ammonium acetate, 30% PEG 4000, and a 0.1 M sodium acetate buffer at pH 4. The best diffraction of the initial crystal hit was good to 1.4 Å. The best diffraction of the optimized crystal was good to 1.0 Å. Bromine soaking multi-wavelength anomalous dispersion (MAD) data collected on the optimized crystal at 1.4 Å ultimately leading to an initial structure. The total time from receipt of protein to initial structure solution was within 1 month. The speed of crystallization and structure solution of this target represents a unique demonstration of the promise of high throughput protein crystallography.

The Effects of Vertically Aligned Carbon Nanofibers on Mitosis. *Lina Hu* (*Washington University in St Louis, St Louis, MO*); *Tim McKnight* (*Oak Ridge National Laboratory, Oak Ridge, TN*). One of the methods used to deliver DNA into a cell is microinjection, where macromolecules are directly administered into a cell, thereby overcoming a cell's physical and chemical barriers. Recently, a technique termed 'impalefection' has been developed, where high aspect ratio spikes of either carbon nanofiber or black silicon are used to impale and deliver DNA on a parallel basis to many cells at once. Morphologically, these high aspect ratio materials are similar to microinjection needles but can also be compared to asbestos fibers and vitamin B2 crystals, both of which are known to interfere with mitosis by hindering the formation of the spindle apparatus and chromosome segregation. As such, experiments were conducted to determine whether vertically aligned carbon nanofibers (VACNFs) and black silicon can cause similar interference during mitosis. A variety of tests were conducted such as live cell imaging of mitotic events following cellular impalement on these spikes and fixed cell assays following impalement and/or culture of cells upon nanoneedle arrays. Live cell imaging following nanofiber impalement showed several occurrences of mitotic arrest (metaphase checkpoint) and mitotic abort, ultimately resulting in multinucleation in the Chinese Hamster Ovary (CHO) cell line. Based on this, a series of tests were conducted in which the mitotic index and multinucleation of impaled and non-impaled CHO cells were evaluated over time. Cells were cultured in conventional dishes, upon chips of high aspect ratio structures (unimpaled), and impaled upon spikes of these structures. The mitotic index and multinucleation of the samples were scored at 16, 40, and 64 hours. Preliminary data indicates that there are statistically significant differences in multinucleation between conventional dishes and growth upon substrates of these high aspect ratio nanomaterials, as determined by the Student T-Test with a p-value of 0.05. Continued testing will determine if these differences are more pronounced with impalement of the cells. These tests will help determine whether growth and impalement on high aspect ratio substrates cause a disturbance in mitosis, and will provide an assay by which substrate modifications can be evaluated to counter these effects. Future modification of the VACNFs to minimize mitotic disturbances could possibly be applied to gene delivery and other intracellular applications of these nanostructured materials.

Functional Analysis of Different G Protein Coupled Receptors (GPCRs) in Aspergillus niger. *Monica Hu* (*Massachusetts Institute of Technology, Cambridge, MA*)*; Ziyu Dai* (*Pacific Northwest National Laboratory, Richland, WA*). *Aspergillus niger* (*A. Niger*), a model industrial fungus that annually produces more than 4 million tons of citric acid globally, can grow at an extremely low pH and form small pelleted morphology, an ideal morphology for use in the industrial production of bioproducts. Understanding the molecular mechanisms of fungal morphology is a prerequisite for the improvement of bioprocess productivity via genetic engineering. G protein systems, the upstream components of the signal transduction pathway, have been found to be involved in the regulation of fungal growth and development. Previous studies have demonstrated that the G protein beta subunit and one of the alpha subunits were involved in regulation of *A. niger* morphology. In this study, the involvement of the G-protein coupled receptors in *A. niger* morphology was examined via gene deletion analysis. A polymerase chain reaction (PCR) based strategy was used to generate gene-deletion mutants in *A. niger* using the selective marker gene hygromycin B phosphotransferase. The genomic DNA was isolated from these transformants and gene replacements were confirmed by PCR. The G protein coupled receptors A, F, and H were successfully deleted via homology replacement. Single spores of those selected transformed events were isolated. The deletion effects of selected genes on citric acid production and morphology will be examined by culturing in different culture conditions. Through this and other examinations, the functions of those selected G-protein coupled receptors of *A. niger* can be better understood. This knowledge can be applied to other fungal strains used for producing different bioproducts. As a result, the morphology of these fungi can be effectively controlled for optimal bioproduct production.

Aquatic Macroinvertebrates of Wetland R at Argonne National Laboratory, Illinois: A Comparative Study of Pond Populations and Water Health. *Leah Johnston* (*University of Illinois at Urbana-Champaign, Champaign, IL*)*; Kirk Lagory* (*Argonne National Laboratory, Argonne, IL*). Wetlands are essential for sustaining

dynamic and healthy environments. The presence of wetlands has decreased during the past one hundred years due to human-caused disturbances. In order to comply with wetland protection laws, Wetland R was created to replace the 1.8 acres of natural wetlands that were destroyed during the construction of the Advanced Photon Source at Argonne National Laboratory in DuPage County, IL. Construction of Wetland R began in August 1990. The purpose of this study was to survey and compare the aquatic macroinvertebrate populations in Wetland R to those in upper Freund Pond. Upper Freund Pond is located northwest of building 617 at Argonne National Laboratory. Aquatic macroinvertebrates are used as bioindicators of water quality. Based on the populations found in both locations, the qualitative health of each was determined and compared. Samples were taken from each site using a dipnet and were sorted with a series of sieves to find specimens. Once collected, specimens were examined and identified to the genus level. The known sensitivity towards pollution levels of each genus was determined from the literature to determine the water health of each area. The water surface area was measured weekly at Wetland R. A total of 15 genera were discovered. There was a higher genus diversity present in Wetland R (10 genera) than in Freund Pond (seven genera). Of the genera discovered at each site, eight of the 10 (80%) in Wetland R and three of the seven (43%) at Freund pond were sensitive or moderately sensitive towards water pollution. Biomonitoring (the utilization of biological responses to assess environmental changes) using the sensitivity levels of the collected genera from each location indicated that the water quality at Wetland R exceeded that of Freund Pond. It is recommended that annual monitoring of aquatic macroinvertebrates in Wetland R continue. The utilization of laboratory-based chemical analysis on the water of Wetland R is recommended to provide additional information on water quality. Maintaining good water quality in Wetland R will promote high species diversity.

Engineering Novel Gene-Regulatory RNA Aptamers. *Yuvraaj Kapoor, Lesley Lara* (*University of California – Berkeley, Berkeley, CA*)*; Jay Keasling, James M. Carothers* (*Lawrence Berkeley National Laboratory, Berkley, CA*). Aptamers are RNA sequences that bind to target molecules and consequently regulate gene expression in a ligand-dependent fashion. They are frequently employed in nature to couple fluctuations in the concentration of a metabolite with changes in gene expression. Upon binding a small molecule, aptamers sequester the ribosome binding site [RBS] of a cis mRNA and repress translation. Synthetic aptamers eliminate the need to rely upon pre-existing biological molecules as the source of binding structure. Generation of synthetic aptamers occurs through *in vitro* selection, iterative rounds of enrichment and amplification which eventually select for an RNA molecule with high binding affinity (~100 uM) and specificity. Through a combination of directed evolution and rational design we generated functional, ligand-binding RNA structures that control the cis-expression of mRNA transcripts in response to tetramethylrhodamine (TMR), a small fluorescent dye that is cell permeable. Iterative rounds of reselections and binding assays in *in vivo* like conditions have generated 3 isolates of TMR-binding aptamers. When incorporated into constructs with self-cleaving hammerhead ribozymes, such molecules will provide general tools for simultaneously varying the expression levels of toxic intermediates in engineered pathways such as that of the anti-malarial drug, Artemisinin.

Agt1 Promoter Sequence Analysis in the Collaborative Cross Parental Mouse Strains. *Jeanna Kidwell* (*Christopher Newport University, Newport News, VA*)*; Brynn Voy* (*Oak Ridge National Laboratory, Oak Ridge, TN*). The Collaborative Cross (CC) is a unique mouse genetic reference population being generated at the Oak Ridge National Laboratory. This cross will consist of approximately one thousand recombinant inbred (RI) strains of mice derived from eight parental strains that were chosen for their genetic and phenotypic diversity. Each strain will contain a unique combination of alleles from the eight parental genomes, creating a population with genetic and phenotypic diversity on par with the human population and a novel resource for the study of heritable disease in humans. We are using the CC population to study the association between adipose tissue production of Angiotensinogen (Agt) on obesity and type 2 diabetes. Agt is the substrate for Angiotensin II, a bioactive hormone that regulates insulin sensitivity as well as many other physiological processes. We sequenced the Agt1 promoter region (about 1.2 kilobases upstream from Agt1) in the 8 CC parental strains in an attempt to identify regulatory polymorphisms that cause wide variation, up to 100-fold, of adipose Agt mRNA expression levels across the CC parental strains. DNA was extracted from mouse ear clips using a modified “Hot Shot” protocol (alkaline lysis followed by neutralization). The Agt promoter was amplified using Polymerase Chain Reaction with a series of six oligonucleotide primers. DNA sequencing was performed at the UTK Molecular Biology Resource Facility. Sequence analysis indicates several single nucleotide polymorphisms between the strains as well as a three base pair deletion present in three strains (A/J, NZO, and CAST) and not present in the other five strains (C57BL/6J, 129, NOD, PWK, WSB). Future experiments will be directed towards determining the impact of these polymorphisms on Agt transcription.

Amplification of Methylated DNA Sequences that Retains mCG Epigenetic Marks. *Danny Kohut* (*New York University, New York, NY*)*; John J. Dunn* (*Brookhaven National Laboratory, Upton, NY*). It has been shown that tumor cells contain an extensive amount of methylated DNA, which could be used in finding cancer before the onset of symptoms or to monitor reoccurrence. By amplifying DNA in such a way that retains methylation patterns, a small amount of DNA can be used to detect cancer. The pGEM5 vector was methylated using HpaII Methyltransferase, an enzyme that recognizes CCGG sequences and adds a methyl group to the second cytosine residue. The DNA was then cut with HpaII and MspI to check for complete methylation; the latter enzyme is able to cut methylated CmCGG sequence while the former cannot. An attempt was then made to amplify the methylated pGEM5 sequence using F29 amplification with dnmt1 Methyltransferase, but without success. Our second and novel method required HeLa DNA, which comes from a cancerous cell line, to be cut with MseI, gel purified and then joined to a synthetic DNA cassette. The purified ligation mixture was then bisulfite modified, which transforms all non-methylated cytosine residues into uracils. PCR amplification of the top strand with bisulfite-modified specific primers produced a large amount of DNA where the only remaining CG’s are those that were originally methylated. M.SseI Methyltransferase will then add a methyl group to these sites to re-form mCG’s, which are then affinity purified using the methyl-binding proteins MBD2b and MBD3L1 and then either hybridized to a microarray or sequenced.

Optimization of a Batchwise Immobilized Metal Affinity Chromatography Protocol. *David Konopka* (*Kalamazoo College, Kalamazoo, MI*); *Feng Yang* (*Pacific Northwest National Laboratory, Richland, WA*). Phosphorylation plays a significant role in regulating metabolic activities in the cell. However, samples of phosphopeptides must first be enriched prior to analysis by mass spectrometry, primarily due to the very low concentration of proteins that are phosphorylated at any given time. Immobilized metal (Fe3+) affinity chromatography (IMAC) is, at present, the most promising method available. Unfortunately, the current, column-based technique is relatively slow and does not have a high throughput capacity. Development of a batchwise IMAC protocol would resolve these shortcomings. A mixture of tryptically digested beta-casein, a common phosphoprotein, and a phosphopeptide standard was used to test the batchwise IMAC protocol with various wash and elution buffers. Tryptic digestion of protein samples extracted from normal human dermal fibroblasts was also used to test the protocols. The number/signal of identified phosphopeptides from both experiments will be used to select the optimized protocol. Due to time constraints and a backlog of samples to be run on the mass spectrometer, no data has yet been obtained from this project.

Methodological analysis of an Amidohydrolase from Psychroflexus torquis and its Crystal Structure. *Jennifer Levia* (*Medgar Evers College, Brooklyn, NY*)*; Brown/D. Kumaran* (*Brookhaven National Laboratory, Upton, NY*). Amidohydrolases, superfamily of metallo-dependent hydrolases, a large group of proteins that show conservation in their 3-dimensional fold (TIM barrel). To gain structural insights into the substrate specificity and enzymatic mechanism, one of this family member 9355a was selected for structure determination as a structural genomics target. This study will be useful in understanding this enzyme family at molecular level and will provide structural basis for designing drugs for specific target. The target amidohydrolase enzyme was crystallized using sitting drop vapor diffusion technique at room temperature. Square shaped crystals of dimensions 0.2 x 0.2 x 0.1 mm^3 were obtained in a day or two. X-ray diffraction intensities were collected at the X29 beamline of National Synchrotron Light Source (NSLS). Crystals diffract to at least 2.4 Å and belong to the tetragonal space group I41 with unit-cell parameters a=b=192.56 Å, c=277.0 Å. Assuming 12 molecules of 50,000 Da per asymmetric unit, the Matthews coefficient is 2.2 Å3 Da-1 corresponding to an estimated solvent content of 43% by volume of the unit cell. The structure determination process is in progress.

Imaging Cue-Induced Dopamine Release and Brain Activations in Behaving Animals. *Courtney Liebling* (*Smith College, Northampton, MA*)*; Wynne Schiffer* (*Brookhaven National Laboratory, Upton,*

NY). Much progress has been made in imaging technology that has enabled us to study molecular events in behaving animals. Given that we now have the ability to image small animals while performing a behavioral task, we have developed a new technique that combines positron emission tomography (PET) imaging with animal models of addictive behavior. This technique has advantages over other methods that measure brain metabolism and neurotransmitter release, such as microdialysis and autoradiography, in that it allows noninvasive examination of neurological processes, permits longitudinal study of the same subjects, and gives a clearer understanding of brain function rather than anatomy. Clinical PET studies in human subjects have shown that exposure to cues associated with a drug activates certain brain regions and increases striatal dopamine which displaces binding of the D2 ligand, [11C]raclopride (rac). This increase significantly correlates with subjective reports on craving. Here, we assessed brain glucose metabolism using [18F]fluorodeoxyglucose (18FDG) and measured changes in dopamine by comparing rac binding in rats conditioned to associate cocaine with a specific environment in order to determine whether animal models of addiction produce the same pattern of metabolic activations and rac displacement as that observed in human drug abusers exposed to drug-related cues. These studies were conducted simultaneously using the conditioned place preference (CPP) model. Using the microPET R4 tomograph, we showed that the specific binding of rac in dorsal and ventral striatum was significantly reduced when animals were placed in the cocaine-paired environment. The extent of this reduction positively correlated with preference scores (r2=0.925, dorsal; r2=0.844, ventral striatum). Further, changes in metabolic rates occurred during the expression of cocaine preference in areas of the brain that are associated with the expectation of a psychostimulant challenge. These results suggest that the expectation of a drug reward produces an increase in striatal dopamine release. Therefore, strategies aimed at inhibiting cue-induced striatal dopamine release in response to the expectation of a drug reward hold promising implications for the treatment of drug addiction. Additionally, this procedure of monitoring molecular events in behaving animals further advances behavioral neuroimaging technology by assessing the degree to which animal models correlate with human behavior.

Angiotensinogen Expression in Collaborative Cross Offspring. *Adam Lundquist* (*Christopher Newport University, Newport News, VA*)*; Brynn H. Voy* (*Oak Ridge National Laboratory, Oak Ridge, TN*). The Collaborative Cross (CC) is an emerging population of recombinant inbred (RI) lines of mice designed to untangle complex webs of genetic interactions. The CC, now being implemented at Oak Ridge National Lab (ORNL), is being created from 8 diverse inbred strains of mice bred to produce 1,000 RI strains, with every resulting strain containing a portion of the genome from each of the 8 parental strains. The genetic and phenotypic diversity of these 1,000 RI strains will model that found in human populations, making the CC a valuable resource for dissecting the genetic contributors to complex traits such as obesity and hypertension[1]. This diversity, coupled with the known genotypes of the animals, will be utilized to map and dissect the genetics of complex traits, which are phenotypes produced from the interaction of multiple genes. We are interested in obesity a complex trait involving many genes interacting within multiple metabolic pathways. One such gene known to play a role in both obesity and hypertension is angiotensinogen, Agt. Agt is a vascular constrictor expressed in adipose tissue; its expression varies widely among the eight CC parental strains. In order to study Agt expression in the intermediate CC generations, those whose genomes have yet to be fixed by inbreeding (strict brother-sister mating for 20 generations), we extracted RNA from adipose tissue, reverse transcribed it into complementary DNA (cDNA), and utilized quantitative Polymerase Chain Reaction (qPCR) techniques to determine mRNA expression levels. Agt expression levels ranged widely (~15-fold) across the sampling of CC mice, indicating tha the diversity of this molecular trait in CC mice reflects that of a human population. Our results provide insight into the effects that mixing diverse genetic backgrounds have on Agt expression in these RI mice and will lead to future mapping of genomic loci involved in complex metabolic traits.

Non-Invasive Indexing of Red and Gray Fox Populations at Brookhaven National Laboratory. *Patrick Mallin* (*College of William and Mary, Williamsburg, VA*)*; Jennifer Higbie* (*Brookhaven National Laboratory, Upton, NY*). The red fox (*Vulpes vulpes*) and the gray fox (*Urocyon cinereoargenteus*) have sympatrically inhabited the greater Long Island area over the last several hundred years. In recent years, speculation has grown regarding the population size of each species. While the red fox has historically been known to adapt well to ecological disturbances, including those of an anthropogenic nature, and is largely considered to have a thriving population in the Long Island area, recent studies of the last thirty years suggest the gray fox populations have struggled with such anthropogenic disturbances of the last century. A previous Brookhaven National Laboratory (BNL) study in 2006 confirmed the presence of gray Fox on BNL property using non-invasive fecal DNA analysis via mitochondria DNA markers and automated camera documentation. This project further studied the extent of the gray fox presence on BNL property for the 2007 season by using the non-invasive techniques of fecal DNA extraction and automated field cameras. Gray fox presence was confirmed through both methods over the course of the study. While apparently much less common than the red fox, the gray fox species appears to be present and established on the BNL site and, presumably, in similar habitats throughout the Long Island area.

Systems Genetics: Elucidating Networks that Underlie Heritable Variation in Adipose Function and Susceptibility to Obesity. *Ian-James Malm* (*Macalester College, Saint Paul, MN*)*; Brynn Voy* (*Oak Ridge National Laboratory, Oak Ridge, TN*). Obesity and its co-morbidities are rampant in Western society. Excess adipose tissue not only physically stresses the organism but also disrupts homeostasis through release of adipokines, bioactive molecules produced in adipose tissue. The long-term goal is to determine the mechanisms by which heritable differences in adipose function impact risk for obesity and its consequences. Systems genetics was employed by anchoring phenotypic variation to naturally occurring genetic polymorphisms in two mouse genetic reference populations (GRP): 1) recombinant inbred BXD (C57BL/6J X DBA/2J) strains, and 2) a panel of eight genetically diverse strains that serve as progenitors for the collaborative cross (CC), an emerging GRP. Body and fat pad weights, plasma hormone profiles and adipose expression of adipokine genes were measured in males of 24 BXD strains and in both sexes of the eight CC parental strains. Current progress demonstrates: 1) adiposity is highly correlated with expression of some adipokine genes (e.g., leptin, r2= 0.626; p=5.4E-6) but not others (e.g., visfatin, r2=0.012, p=0.937); 2) a subset of tightly intercorrelated adipokines are regulated independent of adiposity; and 3) sexually dimorphic relationships exist among adipokines and adipose weight. Ongoing assay of adiposity in emerging generations of CC mice confirms that this new GRP will provide physiological diversity reflective of its genetic diversity and on par with that in a human population, highlighting its power to dissect the molecular components of complex traits. Collectively the results suggest that factors in addition to adipose mass need to be considered when predicting genetic risk for conditions co-morbid with obesity.

***Root Colonization of Prairie Plants by Extramatrical Hyphae.** *Chevon Marsh* (*Governors State University, University Park, IL*)*; Mary Carrington* (*Argonne National Laboratory, Argonne, IL*). *Arbuscular mycorrhizal* (AM) fungi increase a plant's ability to capture water and essential nutrients. The fungus that extends outward from the root is the extramatrical hyphae. Prairie dock (*Silphium terebenthinaceum*), wild quinine (*Parthenium integrifolium*), and smooth blue aster (*Aster laevis*) are prairie plants associated with extramatrical hyphae. Sixteen fungal ingrowth bags from the three plant species were used to measure the extent of the extramatrical hyphae. When compared, prairie dock was expected to have the most extramatrical hyphae, smooth blue aster was expected to have the least, and wild quinine's measurements weree expected to fall somewhere in between. The results revealed that all three species were almost the same in their amount of extrametrical hyphae. This work is part of a greater study to see the success of plants in restored prairies of Illinois.

Accuracy of Sequence-Based Identifications of Filamentous Fungal Species Using ITS2 and LSU rDNA Sequences. *Ivy McDaniel* (*Scripps College, Claremont, CA*)*; Tamas Torok* (*Lawrence Berkeley National Laboratory, Berkley, CA*). As fungi are becoming increasingly relevant to human society, the number of scientists qualified to identify fungal species using traditional methods is dwindling. Therefore, there is a need to develop tools that use standard molecular methods to accurately identify filamentous fungi at the species level. Our laboratory examined the effectiveness of making DNA sequence-based identifications of a collection of filamentous fungi by comparing the sequences of different variable regions of the ribosomal DNA to those in the NCBI Genbank database. By examining a conserved gene such as the ribosomal DNA, we hypothesized that there would be low variability among the sequence of conspecific organisms, but enough variability in the sequence of different species to clearly separate the organisms. We analyzed sequences from the D1/D2 domains of the large-subunit rDNA of 159 organisms from 85 species and 44 genera, and the ITS2 sequences of 28 organisms from 23 species and 13 genera. In both regions, the sequences

by themselves did not appear to be variable across all genera and divisions of fungi to the point that the sequences could be used as an accurate identifier of a single species.

Crystallization and Crystallography Analysis of Amidohydrolase Enzyme from the Structure Genomic Project. *Arshad Mehmood* (*Medgar Evers College, Brooklyn, NY*)*; D. Kumaran* (*Brookhaven National Laboratory, Upton, NY*). The crystal structure of an amidohydrolase (target ID 9355e) has been obtained to 2.35 Å resolution. Diffraction data were collected at the National Synchrotron facility of Brookhaven National Laboratory (beam-line X29). The crystals obtained by the sitting drop vapor diffusion method were 0.1 x 0.06 x 0.05 mm^3 in dimension. The crystals belong to the tetragonal space group P4 with unit-cell parameters a=b=144.74 Å, c =100.96 Å. In this tetragonal crystalline structure of 9355e there was one dimer per asymmetric unit. Amidohydrolase includes the families of enzymes that catalyze the cleavage of wide range of substrates bearing amide or ester functional groups at Carbon and Phosphorus centers. This enzymatic reaction is common in various metabolic processes thus it is important to understand this reaction at the molecular level. Therefore, by knowing the three-dimensional structures of these enzymes and their active sites, we can predict their function and the catalytic mechanism.

Triethyl Phosphate Degradation by INL Microorganisms. *Sara Montgomery* (*Rochester Institute of Technology, Rochester, NY*)*; Yoshiko Fujita* (*Idaho National Laboratory, Idaho Falls, ID*). Large amounts of radioactive contaminants including 90Sr and uranium have been released into the subsurface of several Department of Energy sites. Long term sequestration of these pollutants is important to limit the threat of groundwater contamination. The goal of this study is to evaluate the use of triethyl phosphate (TEP) as a reactive agent or amendment to sequester and immobilize 90Sr in phosphate minerals. TEP can be degraded by microorganisms, resulting in the release of inorganic phosphate which can then react with Sr and other metals to form poorly soluble phosphate minerals. These minerals should be stable under environmental conditions, resulting in the immobilization of the target contaminants. TEP degradation and liberation of inorganic phosphate by microbes from soil collected at the Vadose Zone Research Park at the INL were monitored in enrichments containing soil and TEP at 1 mM and 10 mM concentrations. The effect of ethanol added as a supplemental carbon and electron source was also evaluated. TEP degradation appears to occur similarly in enrichments with and without ethanol. However, fluctuations in TEP concentrations suggest that problems with measuring TEP exist. In addition, relative to TEP degradation, the phosphate released is approximately 1/1000th of the concentration of the TEP apparently degraded. The fact that this amount of phosphate is so small could be attributed to the microorganisms utilizing phosphate for growth, sorption of phosphate to sediments, and/or precipitation. There was an effort to obtain a pure microbial culture capable of degrading TEP. Visible colony growth was observed on agar plates containing 1 mM and 10 mM TEP. However, growth was also observed on agar plates without TEP, suggesting that microbes may be using the agar for growth. DNA extractions were performed on the original sediment samples and enrichments. The extracts will be subjected to phylogenetic microarray analysis to characterize the microbial communities. We conclude that TEP degradation by INL subsurface microorganisms may occur, but mass balances on the TEP and phosphate are difficult to obtain, and improved analytical methods may be needed in order to elucidate TEP degradation rates.

Semi-Automatic Segmentation for Biological Soft X-Ray Tomography. *Bradlee Nelms* (*University of Wisconsin – Madison, Madison, WI*); *Carolyn Larabell* (*Lawrence Berkeley National Laboratory, Berkley, CA*). Biological Soft X-Ray Tomography is an emerging technique to image cells in 3-dimensions with greater than 50nm resolution. While X-ray tomography can generate a wealth of data in a short period of time, the lengthy data analysis limits the rate at which research can be conducted. Important objects in X-Ray images must be identified before a computer can render them in 3D or measure their volume, surface area, or average X-ray absorption coefficient. The process of identifying relevant objects in an image is called segmentation and is currently performed manually, requiring a significant time commitment by a trained expert. To facilitate the segmentation of data collected by X-ray tomography, a framework is proposed for automating this step. First, edge-preserving smoothing is performed to reduce noise. Then, an edge detection algorithm based on the work of Canny is employed to find strong, continuous edges. Finally, an initial contour is deformed towards these edges by a vector field produced with Gradient Vector Flow. This semi-automatic pipeline was tested on 2D slices of the reconstructed volumes of the fission yeast Schizosaccharomyces pombe. Results compare favorably with manually annotated images. Although further work is needed to develop this pipeline for 3-dimensional segmentation, these initial 2D tests suggest automation is possible for the high contrast, high signal-to-noise images yielded by X-ray tomography.

Transgenic Approaches for Functional Insigh into Plant Acyl CoA Dependent Acyltransferases. *Maria Norako* (*Kingsborough Community College, Brooklyn, NY*)*; Ghang-Jun Liu* (*Brookhaven National Laboratory, Upton, NY*). Acyl-CoA dependent acyltransferases catalyze the transfer of aliphatic and/or aromatic acyl moiety from CoA thioesters to the nucleophile of acceptor molecules. The enzymes constitute a large superfamily, namely BAHD family in plants. The numbers of BAHD family members vary accordingly in the different plant species. The biological functions of most of acyltransferases remain to be characterized. Previous study on Populus trichocarpa genomic sequences led to identification of about 94 putative acyltransferase genes. In order to further understand the biological functions of the identified putative acyltransferases, transgenic approaches are used to monitor the “loss and gain” functions of the individual acyltransferase gene. In this study, putative acyltransferases PtHCT1 and PtHCT2, the hydroxycinnamolyl-CoA: shikimate/quinate hydroxycinnamolyl transferase homologues, and PtACT45 are focused for transgenic studies. These three genes were individually cloned into binary vector pMDC85 chimeric with green fluorescent protein (GFP) gene and driven by double 35S promoter. The resultant plasmids were transferred into Agrobacterium strains EHA105 and C58C1 and confirmed by Polymerase Chain Reaction (PCR). The transformed strains were used to infect 295 pieces of tobacco leaves using leaf disc method. The co-incubated leaves were selected on hygromycin containing medium in the required conditions of light and temperature. The hygromycin resistant callus appeared around the edge of the leaves after 2 or 3 weeks. The rate of callus formation is much higher when using Agrobacterium strain EHA105 than using strain C58C1. The percentages of the callus formed are 92.8% and 82.5% respectively. The insertion of PtHCT1, PtHCT2 and PtACT45 genes were confirmed by performing genomic DNA-PCR and electrophoresis. Green fluorescence was indeed observed in the PCR-screened positive transformants under fluorescence microscope. LC-MS analyses were performed to monitor the enzymatic activity of the transgenic product. As the result, the high activity on the formation of p-coumaroyl shikimate were detected in PtHCT2- transgenic tobacco.

Carbon Sequestration in an Agricultural Ecosystem under Elevated Carbon Dioxide Levels. *Daniel Olson* (*Iowa State University, Ames, IA*); *Julie Jastrow* (*Argonne National Laboratory, Argonne, IL*). Atmospheric carbon dioxide has increased by 30 percent since the Industrial Revolution and is predicted to continue increasing at an accelerated rate. The increase in CO_2 allows terrestrial plants to grow faster and thus increases carbon inputs to the soil. Higher levels of CO_2 have shown increased carbon sequestration in deciduous forest and grassland soils, but the effect on agricultural soils requires further investigation. In 2001 a free air carbon dioxide enrichment (FACE) site was constructed in central Illinois to study the effects of elevated CO_2 conditions on a corn-soybean crop rotation. FACE allows experimental areas to be exposed to elevated levels of CO_2, while minimizing the change in sunlight, humidity, wind speed, and so forth. Soil from four FACE plots releasing CO_2 with a concentration of 550 ppm CO_2 and four rings exposed to ambient CO_2 levels (approximately 370 ppm) were sampled prior to planting in 2001 and again in April 2006. In both ambient and elevated CO_2 plots, soil carbon decreased between 2001 and 2006 based on whole soil carbon concentration; however, elevated CO_2 plots did not lose as much soil carbon as did ambient CO_2 plots. The loss of whole soil carbon over the study period is unexpected. It is most likely due to a difference in land use and management before 2001. The difference in carbon loss may be due to increased soil inputs in elevated CO_2 plots. The amount of carbon sequestered in each of the soil fractions will show where carbon loss is occurring. The change in carbon concentration of each soil fraction between 2006 and 2001 must be found. That data will indicate where carbon is sequestered in this agricultural ecosystem.

Purification and Characterization of Recombinant Aequeorea coerulescens Green Fluorescent Protein from *Escherichia coli*. Matt Peoples (*Earlham College, Richmond, IN*); *Hugh O’Neill* (*Oak Ridge National Laboratory, Oak Ridge, TN*). Green Fluorescent Protein (GFP) is a single chain polypeptide that forms a fluorescent chromophore by rapid cyclization and subsequent oxidation of residues Ser65-Tyr66-Gly67. It is widely used as a fluorescent tag for *in vivo* investigations. In this study an efficient procedure was developed for

the isolation and characterization of enhanced recombinant *Aequeorea coerulescens* GFP (aceGFP) that was over-expressed in *Escherichia coli* JM109. The first step employed three-phase partitioning to precipitate GFP using ammonium sulfate and tert-butanol. This was followed by dialysis and anion exchange chromatography. The purification procedure was monitored by UV/Visible absorption spectrophotometry, circular dichroism spectroscopy, fluorescence excitation/emission, and sodium dodecyl sulfate (SDS) and native polyacrylamide gel electrophoresis (PAGE). Interestingly, two variants of GFP separated during anion exchange chromatography. The first variant (GFP478) absorbed maximally at 478 nm with a fluorescence emission maximum of 505 nm. The yield was 3.04 mg GFP478/g cell paste. The second variant (GFP493) had a maximum absorbance at 493 nm and an emission maximum of 509 nm. The yield of this protein was 11.4 mg GFP493/g cell paste. Comparison of the UV/Visible and circular dichroism absorption spectra of the two isoforms indicate that the environments of their chromophores are different. Denaturing SDS-PAGE demonstrated that the lengths of their polypeptide chains are identical; however GFP493 migrated less than GFP478 by native PAGE, indicating a difference in the tertiary/quaternary structural characteristics of the two isoforms. GFP has been successfully purified from recombinant *E. coli* in good yield, and the two resulting isoforms of aceGFP have been characterized. Small angle X-ray scattering will be used to further investigate the structural properties of these two proteins in solution.

A Computational Model for Analyzing the Biochemical Pathways of Matrix Metalloproteinase (MMP) 2&9 in Collagen Type IV Proteolysis. *Elizabeth O'Quinn* (*Wofford College, Spartanburg, SC); Kara Kruse* (*Oak Ridge National Laboratory, Oak Ridge, TN*). Cardiovascular disease is the leading cause of death in first world countries. The imbalance of matrix degrading enzymes and structural proteins within the extracellular matrix of an arterial wall is a critical factor in cardiovascular disease processes. Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) degradation of collagen type IV results in migration and proliferation of vascular smooth muscle cells; this can lead to further narrowing of a diseased artery. Kinetic modeling of proteolysis is an approach which can be used to understand complex systems by describing the enzyme's mechanism and behavior quantitatively. In this research project, a computational model of biochemical pathways involved in activation and inhibition of MMP-2 and MMP-9 proteolysis of collagen type IV is being developed. Separate MMP-2 and MMP-9 models have been implemented within JSim, a software application developed by the University of Washington. Since MMP-2 and MMP-9 pathways overlap, the individual models will be integrated in the future. This MMP-2 model was also implemented in JDesigner, a tool of the Systems Biology Workbench, and DEVS, a discrete event system specification, for comparison of model environments. Various experimental methods for obtaining quantitative reaction rate parameters were explored, including high pressure liquid chromatography (HPLC) and florescence polarization. By pairing HPLC separation, largely by hydrophobic property, with spectrometry techniques, protein and peptide identification and quantification is possible. Previous literature suggests the use of HPLC to measure enzymatic activity, by using traces of the product/substrate itself as an internal standard. An experimental protocol for the measurement of the enzymatic activity of MMP-2 and MMP-9 is being developed. HPLC baseline standards for the individual substrates and enzymes are currently being measured and optimized. After baseline standards are determined the MMP enzymatic activity can be determined. The HPLC experimental results will be analyzed to derive the reaction rate parameters needed by the computational model. The use of HPLC methods to analyze the enzymatic activity of MMP-2 with collagen type IV and other correlated substrates provides parameters which cannot be obtained through literature. This research is in collaboration with the Vascular Research Laboratory at the University of Tennessee Medical Center in Knoxville.

A High Throughput Method of Screening Mutant *Arabidopsis* Plants for Improved Biofuel Capacity Using Infrared Microspectroscopy. *Simone Park* (*State University of New York at Stony Brook, Stony Brook, NY); Lisa Miller* (*Brookhaven National Laboratory, Upton, NY*). Acyl-esterification is one of the most common modifications that occurs within the plant cell wall, and contributes to the covalent cross-linked polymerizations found there. These cross-linkages are found between lignocelluloses, components found in the cell wall, and contribute to the recalcitrance and complexity of the overall plant and prevent effective degradation for conversion into bioethanol. In this study, stems from 12 mutant *Arabidopsis thaliana* plants representing 6 distinct mutant lines were analyzed with Fourier Transform Infrared (FTIR) microspectroscopy to develop a high-throughput method of screening and characterizing Arabidopsis lines. Cell wall components were extracted with ethanol in 2 ways and point spectra were taken to determine the extent of the 1,740 cm^{-1} peak corresponding to the vibrational carbonyl group characteristic of esters. Results from cluster analysis and acyl content from the microspectroscopy revealed that samples presented variabilities inherent to the complexity of the cell wall structure and to those attributed with sample preparation. Imaging of cross-sectioned stems was also performed, and it was found that the acyl content in the section was radially heterogeneous. More careful sample preparation for microspectroscopy and use of synchrotron light for imaging to gain greater spatial resolution in the cell wall will be valuable in improving this high throughput screening method.

The Surface-Mediated Unfolding Kinetics of Globular Proteins is Dependent on Molecular Weight and Temperature. *Alexander Patananan* (*University of California – Los Angeles, Los Angeles, CA*)*; Steven C. Goheen* (*Pacific Northwest National Laboratory, Richland, WA*). The absorption and unfolding pathways of proteins on rigid surfaces are essential in numerous complex processes associated with biomedical engineering, nanotechnology, and chromatography. It is now well accepted that the kinetics of unfolding are characterized by chemical and physical interactions dependent on protein deformability and structure, as well as environmental pH, temperature, and surface chemistry. Although this fundamental process has broad implications in medicine and industry, little is known about the mechanism because of the atomic lengths and rapid time scales involved. Therefore, the unfolding kinetics of myoglobin, ß-glucosidase, and ovalbumin were investigated by adsorbing the globular proteins to non-porous cationic polymer beads. The protein fractions were adsorbed at different residence times (0, 9, 10, 20, and 30 minutes) at near-physiological conditions using a gradient elution system similar to that in high-performance liquid chromatography. The elution profiles and retention times were obtained by ultraviolet/visible spectrophotometry. A decrease in recovery was observed with time for almost all proteins and was attributed to irreversible protein unfolding on the non-porous surfaces. This data, and those of previous studies, fit a positively increasing linear trend between percent unfolding after a fixed (9 minutes) residence time (71.8%, 31.1%, and 32.1% of myoglobin, ß-glucosidase, and ovalbumin, respectively) and molecular weight. Of all the proteins examined so far, only myoglobin deviated from this trend with higher than predicted unfolding rates. Myoglobin also exhibited an increase in retention time over a wide temperature range (0°C and 55°C, 4.39 minutes and 5.74 minutes, respectively) whereas ovalbumin and ß-glucosidase did not. Further studies using a larger set of proteins are required to better understand the physiological and physiochemical implications of protein unfolding kinetics. This study confirms that surface-mediated unfolding can be described by experimental techniques, thereby allowing for the better elucidation of the relationships between the structure and function of soluble proteins as well as other macromolecules.

Structural Identification of a Glyoxalase Family Protein and Discussion of Possible Functions. *Sara Patterson* (*Knox College, Galesburg, IL); Ruiying Wu* (*Argonne National Laboratory, Argonne, IL*). At the Midwest Center for Structural Genomics (MCSG), located at Argonne National Laboratory, as well as several other research laboratories, a project to determine and catalogue all the protein structures is underway. At the MCSG, a three-step high-throughput process has been used to clone, purify, and determine the structure of protein BA3701. First, the target gene is ligated into an *E. coli* K12 vector and grown and induced in modified M9 media. Second, it was purified using Immobilized Metal Affinity Chromatography (IMAC) with a nickel column employed in the AKTA® purification robotic system. Finally, crystallization was induced by screening the protein against various conditions containing precipitants, buffers, and salts. Crystallization was successful through vapor diffusion at 16°C. The protein crystal was harvested and frozen in its mother liquor containing cryo-protectant glycerol, and its structure is determined via X-ray crystallography. Based on structural homology it is believed that the protein belongs to the glyoxalase family and super family pfam00903. It shares many similarities with bleomycin resistant proteins indicating that it perhaps acts to protect the *Bacillus anthracis* from its own antibiotics. However, further investigation is necessary to conclusively determine the function of the protein.

Expression, Purification, and Small Angle X-Ray Scattering of DNA Replication and Repair Proteins from the Hyperthermophile Sulfolobus solfataricus. *Stephanie Patterson* (*Del Mar College, Corpus Christi, TX*)*; Steven M. Yannone* (*Lawrence Berkeley*

National Laboratory, Berkley, CA). Vital molecular processes such as DNA replication, transcription, translation, and maintenance occur through transient protein interactions. Elucidating the mechanisms by which these protein complexes and interactions function could lead to treatments for diseases related to DNA damage and cell division control. In the recent decades since its introduction as a third domain, Archaea have shown to be simpler models for complicated eukaryotic processes such as DNA replication, repair, transcription, and translation. *Sulfolobus solfataricus* (*S. so*) is one such model organism. A hyperthermophile with an optimal growth temperature of 80°C, S. so protein complexes and transient interactions should be more stable at moderate temperatures, providing a means to isolate and study their structure and function. Here we provide the initial steps towards characterizing DNA-related *S. so* proteins with small angle X-ray scattering (SAXS). We focused on three *S. so* proteins: Sso0257, a cell division control/ origin recognition complex homolog, Sso0768, the small subunit of the replication factor C, and Sso3167, a Mut-T like protein. *E. coli* cells transformed with the pet21a expression vector containing the *S. so* gene of interest were grown to logarithmic phase. Protein expression was induced with 1mM Isopropyl ß-D-1-thiogalactopyranoside (IPTG). Cells were harvested by centrifugation. Proteins were extracted by sonication, then the extracts heated to denature any contaminating *E. coli* proteins. Soluble protein was purified by Ni-affinity column chromatography in a Fast Protein Liquid Chromatography (FPLC) system. *S. so* proteins were eluted with an imidazole gradient and collected as fractions, then concentrated to a range of 1–10 mg/ml. *S. so* proteins were analyzed with SAXS at multiple concentrations for both short and long exposure times. The Sso0257 sample was determined to be a 1:1 combination of monomer and dimer states. Sso0768 was found to be a complex mixture of multimeric states. Molecular envelope reconstruction from SAXS data for Sso3167 revealed a novel structural component which may function as a disordered to ordered region in the presence of its substrates and/or protein partners.

Cytochemical Investigation of Lignin Redistribution During Thermochemical Pretreatment. *Britney Pennington* (*Florida Institute of Technology, Melbourne, FL*); *Todd Vinzant* (*National Renewable Energy Laboratory, Golden, CO*). Due to the increasing demand for oil, the United States has developed starch ethanol programs, but corn cannot support both the food and fuel industries. Cellulosic ethanol is a promising alternative to starch-based ethanol but is more difficult to generate cost-effectively because biomass is inherently resistant to degradation. Lignin, the polyphenolic compound in plant cell walls, contributes to this recalcitrance by inhibiting hydrolytic cellulases and presents an obstacle to producing bioethanol. Dilute acid pretreatment of biomass removes only a fraction of the lignin content, and yet at high temperatures, sufficient enzymatic digestion can still occur. To address this paradox, this study utilized microscopy and cytochemical stains to determine temperature's role in lignin redistribution during dilute acid pretreatment. All of the cytochemical stains used to detect lignin had evenly distributed staining patterns at 80°C but became concentrated towards the cell edges as temperatures approached 160°C. Temperature's effect on the biomass surface was also investigated using scanning electron microscopy. Starting at 140°C, half-sphere droplets appeared on the tissue surfaces and their morphologies seem to coalesce into larger spheres at higher temperatures. Round droplets were also observed using the light microscope. It has been hypothesized that the melted lignin is pushed out of the cell wall, possibly by increased hydrogen bonding between adjacent cellulose microfibrils, and forms spheres due to hydrophobic forces. Understanding lignin redistribution and its resulting implications on cell and tissue structure will help biologists explain the effects of pretreatment on biomass.

High-Throughput Purification of Novel Proteins. *Victoria Perez* (*The University of Texas – Pan American, Edinburg, TX*); *Lour Volkart* (*Argonne National Laboratory, Argonne, IL*). A protein's three dimensional structure can shed light on many biological functions and the protein's relationship to sequence, function, and disease. The Structural Biology Center (SBC) at Argonne National Laboratory has established a protein structure determination pipeline capable of high-throughput production of purified protein and crystals. Protein structures are determined by using synchrotron X-ray crystallography. Target proteins must first be tested for their solubility and expression against a scale that determines whether the protein fits within the range best suited to be successful using the SBC protocol. Desired proteins must be highly purified before they are able to produce a high quality protein that is able to be crystallized. The SBC protein purification protocol produces milligram quantities of highly purified protein using the high- performance chromatography workstations AKTA Xpress and IMAC 2. The implementation of the SBC protein purification protocol will be used to determine whether eight target proteins fit within the protocol's range for novel proteins. If the protein targets do not fit the protocol criteria, other methods will be explained to broaden the protocol's ability to crystallize the proteins.

Soft X-Ray Tomography at the ALS. *Allison Pymer* (*Temple University, Philadelphia, PA*) *Carolyn A. Larabell* (*Lawrence Berkeley National Laboratory, Berkley, CA*). Microscopy is a key biological research technique that allows scientists to study organisms and cells *in vivo*. Traditional visible light microscopes are limited in their maximum resolution whereas high-resolution electron microscopes require UHV environments to collect data. Newly developed X-ray microscopes at the Advanced Light Source (ALS) allow scientists to resolve hydrated cellular structures to 40 nm as a result of advances in X-ray optics, cryogenic microscope stages, and computer image reconstruction software. Scientists can study whole cells in their native environment due to this microscope, resulting in a better understanding of cell structure and function on a molecular level. For example, changes in the cell due to factors such as pharmaceuticals and disease may be observed *in vivo* without the use of dyes, stains, or other chemicals. Future advances in the methods used for X-ray tomography will assist scientists in furthering cellular studies to the molecular level.

***Root Length Colonization of Mycorrhizal Fungi in Prairie Plants.** *Rita Richardson* (*Governors State University, University Park, IL*); *Mary Carrington* (*Argonne National Laboratory, Argonne, IL*). Mycorrhizal fungi form associations with the roots of some plants while promoting growth. It has a greater association with plant species having thick, unbranched roots versus those with roots finely branched (Jastrow and Miller, 1993). It is hypothesized that roots from three prairie plant species will all have different proportions of mycorrhizal fungi colonization. The root length colonization of mycorrhizal fungi in three prairie plants: wild quinine (*Parthenium integrifolium*), smooth blue aster (*Aster laevis*), and prairie dock (*Silphium terebenthinaceum*) was studied. Roots of each of the three plant species were gathered, soaked in potassium hydroxide, and stained with trypan blue. The roots were then analyzed microscopically. The hypothesis of this study was supported by comparison of extent of root colonization by mycorrhizal fungi among prairie dock, wild quinine, and smooth blue aster. Becoming familiar with the associations between mycorrhizal fungi and different plant species may be useful when trying to increase the growth of plants.

Relative Quantitation Using Real-time Polymerase Chain Reaction Techniques (RT-PCR) to Compare Expression Levels of Genes Relevant to Pellet Formation in *Aspergillus niger*. *Torri Rinker* (*Oregon State University, Corvallis, OR*); *Scott Baker* (*Pacific Northwest National Laboratory, Richland, WA*). Filamentous fungi have the potential to be used in industry for the conversion of complex biomass into useful products and alternate fuel sources. *Aspergillus niger* is highly efficient at producing citric acid from glucose. Due to its high productivity and the ability to control the morphological changes needed to reach most efficient production, citric acid production in *A. niger* serves as a model bioprocess for other filamentous fungi. In citric acid producing conditions, *A. niger* takes on a distinct pelleted morphology. Chitin, a polysaccharide found in the cell wall of fungi, is thought to contribute to this unique growth because of its involvement in cell wall generation. In this study, nine different chitin synthase genes along with other genes involved in pellet formation were studied in *A. niger* using Real-Time Polymerase Chain Reaction (RT-PCR). Gene expression levels in the citric acid producing strain ATCC 11414 were measured in 4 different types of media. Comparative assays were also performed on a modified strain of ATCC 11414 containing a deletion of a chitin synthase gene (csmAΔ). Relative quantitation of the RT-PCR data showed that three chitin synthases, csmA, csmB, and csmC were expressed at higher levels in citric acid producing conditions, while csmD and csmH were expressed at lower levels. Chitin synthase genes with a both myosin motor-like and cytochrome B5 domains had lower expression levels under minimal medium growth conditions than in citric acid production media. These data may indicate that such domains are important for cell wall formation in pelleted cells. Because cell wall formation is an important aspect of pelleted morphology, targeting genes that synthesize chitin, an integral component of the fungal cell wall, is an approach that may aid in obtaining pelleted morphology. From this study, it was concluded that csmA, csmB, csmC, csmD and csmH were significant contributors to pelleted morphology and will be further studied through gene deletion.

Protein Complex Interaction Assays of *Shewanella oneidensis* MR-1 Proteins and Its Relevance to Bioremediation Techniques. *Nathan Roberts* (*Marquette University, Milwaukee, WI*); *Frank Collart* (*Argonne National Laboratory, Argonne, IL*). Identification of interacting proteins is a first step toward understanding the biological function of a protein. Mapping the protein interactions throughout a genome can define the communication network. The network can be used to predict how the organism will respond to changes in the environment. The bacterium *Shewanella oneidensis* MR-1 was used as a model organism for identification of interacting proteins. This bacterium can survive in the presence of heavy metals and its metabolic machinery can render these metals insoluble. This characteristic opens up many possibilities for *Shewanella oneidensis* or its proteins to be used in bioremediation of areas afflicted with radioactive and/or heavy metal contamination. Since very little is known about specific interacting proteins in *Shewanella oneidensis*, interacting proteins in *Escherichia coli* have been identified which contain high sequence similarity to *Shewanella oneidensis* proteins. Proteins are generally considered to have a similar function when protein sequences are 50+ % identical. Open reading frames coding for proteins from *Shewanella oneidensis* were amplified from genomic DNA and cloned into *E. coli* expression vectors using molecular techniques. The proteins were screened for expression and solubility and the soluble proteins purified at a milligram scale. Each protein was expressed with an N-terminal his-tag to allow for interaction screening using a pull-down assay. This technique has allowed for mass scaling up and purification of chosen targets which then has allowed for identification of interacting proteins and further research into functionality.

A Biochemical and Computational Confirmation of ncRNAs in *E coli*. *Rebecca Roha* (*Gettysburg College, Gettyburg, PA*); *Stephen R. Holbrook* (*Lawrence Berkeley National Laboratory, Berkley, CA*). Non-coding RNAs (ncRNAs) are transcripts that do not code for a protein, but rather functional RNA molecules in that have roles in protein manufacturing, DNA replication, cellular control and many other processes. However important, ncRNAs are difficult to study because their sequences lack clear start and stop signals, making them practically invisible on the genome scale. Bioinformatics techniques must be designed for the classification and discovery of ncRNAs. The Positive Sample only Learning Algorithm (PSoL) suggested a highly accurate machine learning algorithm to identify ncRNAs by using a support vector machine to combine many ncRNA detection signals in order to distinguish ncRNA sequences from intergenic sequences. This method identified to predict 420 ncRNA sequences in the E. coli genome. The PSoL predicted sequences were then clustered using LocARNA, folded using RNAalifold, and interpreted. Several trials were completed to test LocARNA's ability to cluster large amounts of sequences, correctly cluster identical sequences and to determine the effect of inaccurate sequences on the accurate clusters. Clusters were identified and a representative ncRNA from each was selected. For each chosen ncRNA, a Northern Analysis was completed; total E.coli RNA was extracted, the RNA was electrophoresed and transferred to a positively charged membrane, the membrane was then probed with non-isotopically labeled DNA complementary to the predicted ncRNA, hybridized, detected and developed. LocARNA successfully grouped the sequences into 9 clusters. ncRNA expression verification by Northern analysis is ongoing yet advancements have been made; DNA oligomers were successfully labeled and control RNA sequences were detected. A potential ncRNA has been identified, while further validation is necessary, a predicted sequence appears to be expressed. This work demonstrated that LocARNA is adequate clustering software for grouping predicted sequences into families. These findings are significant because they contribute to the search for a technique to identify and classify ncRNAs. Future research includes identifying more predicted ncRNAs as well as assigning the identified LocARNA clusters to known ncRNA families.

Using *In Situ* Reactors to Assess Natural Attenuation of TCE. *Alison Rope* (*Dartmouth College, Hanover, NH*); *Deborah Newby* (*Idaho National Laboratory, Idaho Falls, ID*). Trichloroethylene (TCE) is a major groundwater contaminant in the United States. In the past it was used as a metal degreaser, a textile cleaner, and an anesthetic. Use at Test Area North 35 (TAN 35) as a metal degreaser left a large plume of the contaminant in the Snake River Plane Aquifer (SRPA). TCE is a likely human carcinogen and the EPA requires its removal from the groundwater. Many studies of the possible natural attenuation of TCE have been conducted, including extensive studies of methane oxidizing bacteria (methanotrophs) native to the SRPA. These methanotrophs degrade TCE into carbon dioxide and water using the enzyme soluble methane monooxygenase (sMMO). The degradation of TCE by aerobic methanotroph metabolism has been well documented by a variety of enrichment studies, but an actual rate for the natural degradation rate (i.e. using an environment as closely simulated to the aquifer as possible) has never been found.We believe that TCE co-metabolism is controlled by coupled biogeochemistry (e.g., methane production and consumption) and hydrology (e.g., rate of fluid movement in primary flow paths). In order to minimize artifacts created by a laboratory environment, six flow through *in situ* reactors (FTISR) were placed into the aquifer and allowed to incubate. All reactors were packed with crushed basalt taken from the aquifer and had water pumped through at two different rates (1 m/day and 0.1 m/day) with three reactors at each flow rate. These rates were chosen specifically to simulate the environment in different parts of the aquifer. Over the eight month period, the reactors were colonized by both attached (living on the basalt surface) and planktonic (not attached) methanotrophs. The methanotrophs were allowed to colonize naturally; no chemicals or nutrients were added to the reactors. Molecular indicators of TCE co-metabolism (enzyme activity, expression of cometabolic genes and proteins) will be evident in these cells. The indicators of microbial potential and activity (sMMO expression) can be correlated with TCE degradation rates and used for refinement of computational (and site specific) models of natural attenuation. TCE degradation was analyzed over a five-day period. We will use reverse transcriptase real time PCR to determine if methanotrophs expressed the sMMO enzyme responsible for TCE oxidation.

Bystander Analyses of X-ray Microbeam Induced Gamma-H2AX Punctate Signals in the Human Mammary Epithelial Cell Line 184V. *Michelle Salcedo* (*Diablo Valley College, Pleasant Hill, CA*); *Eleanor Blakely* (*Lawrence Berkeley National Laboratory, Berkley, CA*). Damage to DNA can be caused by direct and indirect effects of ionizing radiation absorbed by irradiated cells. Radiation damage to DNA can trigger a sequential cascade of responding DNA-repair molecules that can be visualized microscopically with the use of specific fluorescently-labelled antibodies and immunohistochemistry. The focus of my research has been to use a 12.5 keV X-ray microbeam produced at the LBNL Advanced Light Source beamline 10.3.1 to target a dose stripe of 100 microns wide on a population of 184V Human Mammary Epithelial Cells (HMEC) and to process the cell samples for DNA damage response markers as a function of time and distance from the dose stripes. This allows me to study both targeted and untargeted cells. The response of untargeted cells not in the radiation field is called a "bystander effect". Comparisons have been made after doses of either a relatively high dose stripe of 100 cGy or a relatively low dose stripe of 10 cGy. Gamma-H2AX and 53BP1 are the two DNA damage response markers I have studied. Some differences were noted in the phosphorylation response of each of these markers in the nuclei of irradiated HMEC. Gamma-H2AX and 53BP1 appear to co-localize, but with a different time course. I developed a scoring system to compare the morphological differences noted in these two DNA damage response markers in large montages of HMEC irradiated with the X-ray microbeam. Comparisons were made with unirradiated control cultures. The results indicated that a significant diversity of gamma-H2AX fluorescent signals exist in the unirradiated control possibly due to asynchronous cells varying in different stages of the cell cycle or culturing conditions. Irradiated areas expressing a high response of gamma-H2AX were an efficient indicator of the location and width of the stripe of dose, especially within the 100 cGy 10 minute montage image. Physical measurements of the stripe confirmed widths of 100 to 110 microns verifying the accuracy of the microbeam used. Histograms of different levels of intensities of gamma-H2AX expression were created using the data collected by using the devised scoring system. The data analyzed in the histograms demonstrated potentially novel fingerprints of the background fluorescent signal, the direct radiation damage effect, and the bystander effect. Future replication of this experiment is needed to validate significance of these results.

Comparison of Land Management Practices on Common Wood Nymph Butterfly Populations. *Tara Schwass* (*Western Illinois University, Macomb, IL*); *Rod Walton* (*Fermi National Accelerator Laboratory, Batavia, IL*). Diversity of habitat and variety of wildlife has been increasing through restoration and land management techniques at Fermi National Accelerator Laboratory (Fermilab). These land management techniques are crucial to restoring and sustaining the natural habitats and native species of the area. This study examined the effects of land management techniques on the burned restored prairie, unburned restored prairie and mowed non-native grasslands and how these techniques affected local butterfly populations of *Cercyonis pegala*, the common wood nymph. Originally native to the prairie, the common wood nymph now occupies a wide range of

habitat that includes not only prairie but also non-native grasslands, open woodlands, fields, marshes, savanna, and road sides. Transect counts were used to survey the abundance of wood nymphs for each of the five sites studied. A similar study performed last year included two of the same sites studied this year. Results were analyzed using a t-test, Mann-Whitney U-test, Spearman correlation, and Pearson correlation. Our results indicated the prairie had significantly more butterflies than the non-native grasslands and also weather variables did not significantly affect butterfly counts. Our results differed greatly from the study performed last year where non-native grasslands had more butterflies than the native prairie site. These varying results are likely due to the timing of the land management techniques at the sites. However, other possible explanations for the results may be vegetation differences in growth, abundance, and density, and/or butterfly behavior. A continuation of this study should include the same sites after a new season of burning and mowing to examine long-term effects of land management techniques and also to gain a better understanding of butterfly ecology at Fermilab.

Analyzing the Structure and Function of Novel Cytochromes from a Natural Microbial Community. *Anna Siebers* (*University of California – San Diego, La Jolla, CA*)*; Michael P. Thelen* (*Lawrence Livermore National Laboratory, Livermore, CA*). The Richmond mine in Iron Mountain, California, provides an unusual ecosystem suitable for the growth of microbial biofilms which produce many unique proteins. Through iron oxidation, these proteins facilitate acid mine drainage (AMD). Because this habitat is extremely acidic, survival is an extraordinary feat and the process of environmental selection is rare. In order to understand the mechanisms by which these organisms oxidize iron and gain electrons for energy, biochemical studies were applied. More specifically, column chromatography, spectrophotometry, and gel electrophoresis were used to determine the proteins present in different biofilms. Two specific locations of the mine researched were the AB drift and Ultraback C (UBC), which were both found to contain at least five different types of protein and a large amount of heme-bound cytochromes. Another application of these methods was to investigate proteins playing a major role within the community; one protein selected was cytochrome 579 (Cyt579) due to its abundance in the biofilm, iron oxidizing potential, and signature absorbance of 579 nm. The structure and function of Cyt579 could be characterized by the isolation of its heme, which was completed using column chromatography; however, one of the challenges has been liberating the heme from the column. Further research, including acid-base and temperature profiling of Cyt579 should help elucidate its structural changes within alternate environments and metabolism within the community.

Modeling Estimated Personnel Needs for a Potential Foot and Mouth Disease Outbreak. *Kirsten Simmons* (*North Carolina State University, Raleigh, NC*)*; Pam Hullinger* (*Lawrence Livermore National Laboratory, Livermore, CA*). Foot and Mouth disease (FMD) is a highly contagious viral disease affecting livestock that was last detected in the US in 1929. The prevalence of FMD in other countries, as well as the current potential for this virus to be used as a form of agroterrorism has made preparations for a potential FMD outbreak a national priority. All 50 states were surveyed via e-mail, telephone and web search to obtain emergency response plans for FMD or for foreign animal diseases in general. Information from 35 states was obtained and analyzed for estimates of resources needed to respond to an outbreak. These estimates were expanded and enhanced to create a spreadsheet tool that could be used by individual states to better understand the personnel that would be needed to complete various tasks during an outbreak response. Personnel estimates were varied according to facility type and scaled by size. The estimates were then coupled to the output from FMD outbreaks simulated using the Multiscale Epidemiological/Economic Simulation and Analysis (MESA) model at Lawrence Livermore National Laboratory to assess the personnel resource demands on a response agency over the course of an outbreak response.

Testing the Enhancement of Protein Expression and Solubility by Induction Conditions. *Khouanchy Souvong* (*Knox College, Galesburg, IL*)*; Minyi Gu* (*Argonne National Laboratory, Argonne, IL*). Inducing recombinant proteins in *Escherichia coli* under different conditions are shown to affect protein expression and solubility. Some proteins are expressed in insoluble forms which are much more difficult to purify; therefore, Argonne National Laboratory (ANL) is collaborating with the Midwest Center for Structural Genomics (MCSG) with the goal of finding the best conditions for increasing protein solubility to yield greater crystal production, for the most rapid and affordable protein structures. The cells were stressed and exposed to different induction conditions or with low concentration of isopropyl-ß-D-thiolgalactoside (IPTG), with intentions of slowing protein production rate, thus have better folded protein. Clones with low solubility were subjugated through 2% ethanol in growth media, 15°C and 20°C induction temperatures, and 1 mM and 0.1 mM induction reagent IPTG. The optical density (OD) of the cells was taken before induction and before harvesting to monitor cell growth. The preliminary data showed that cells grow faster in 20°C, without ethanol. Nevertheless, the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed samples with ethanol had a slight increase in targeted protein expression level; unfortunately, solubility level was still low with ethanol. The next step will be to continue to test with more clones, until the best conditions are found. After the optimal condition is formulated, it will then be used to screen less soluble clones to improve the clone production. This research is done with the prospects of better understanding protein function, improving human health, and for facilitating advancement in the medical field.

***Seagrass Change Associated with Land Use Trends in the Gulf of Mexico: A Historical Analysis.** *Emily Stefansson* (*Everett Community College, Everett, WA*); *Ron Thom* (*Pacific Northwest National Laboratory, Richland, WA*). Seagrasses and other types of submerged aquatic vegetation (SAV) play an essential role in coastal ecosystems. By providing habitat to juvenile organisms, including a variety of endangered and commercially important species, seagrass beds are both ecologically and economically valuable. Over the past century, the health and coverage of seagrasses has declined significantly. In the Gulf of Mexico, many estuaries have lost up to 100 percent of their seagrasses within the last fifty years. While dredging, propeller scarring, over-water structures, and other forms of nearshore development have been listed as causes of seagrass loss, land use changes in the upper watershed must also be considered. Changes in watershed conditions can degrade water quality (e.g., light penetration, nutrients, temperature, salinity) in estuaries harboring SAV, which can in turn affect the ability of SAV to survive and spread. The purpose of this study was to investigate historical correlations between seagrass change and land use trends in the Gulf of Mexico, in an attempt to better understand seagrass declines in the area. Four bays within the Gulf were chosen for this analysis: Tampa Bay, Charlotte Harbor, Perdido Bay, and Galveston Bay. County-level data for each watershed was collected from the U.S. Census Bureau, U.S. Forest Service, and U.S. Department of Agriculture, concerning farmland, urban, and forest acreages in the 1900s. These data were then compared to trends in seagrass coverage, taken from existing literature and historical navigation charts dating back to the early 1900s. Overall trends showed a peak in agricultural land area during the 1950s, dramatic deforestation in the mid-1900s, and large increases in population and housing units throughout the 1900s. These major land conversions correlate with the largest seagrass losses between 1940 and the early 1980s. This may be related to increased turbidity, caused by nutrient-rich agricultural runoff and increased impervious surfaces accompanying development. As resource managers and policy makers look to restore seagrasses and the delicate ecosystems they support, an understanding of all potential stressors on key factors controlling seagrass growth is critical. Therefore these correlations provide the basis for planning and implementing future restoration and conservation efforts.

Effects of Burrow Characteristics on Temperature in Simulated Owl Burrows. *Lucy Tran* (*University of California – Los Angeles, Los Angeles, CA*)*; Corey A. Duberstein* (*Pacific Northwest National Laboratory, Richland, WA*). Western burrowing owls (*Athene cunicularia hypugaea*) are thought to be declining throughout their North American range. Reasons for their decline include the eradication of the fossorial mammals whose burrows they require and loss of habitat to urban and agricultural development. Many studies have investigated the aboveground characteristics of burrows and nest sites to determine their relationships to nest site selection, incubation, and productivity. Very little research has assessed the influence of the belowground environment on these processes. One aspect of belowground environment that may influence owls is microclimate (i.e., temperature and gas concentrations). This study examined the effects of two proximate factors that may influence owls when choosing nest sites on daily minimum and maximum temperatures within burrows: tunnel diameter and entrance aspect. Burrow temperature was recorded using DST milli™ archival temperature tags at three depths within replicate north- and south-facing simulated burrows of 7.6-, 10.2-, and 15.2-cm diameters at two sites near Richland, WA during July and August 2007. Daily minimum temperature ranged from 21.8 to 27.5°C, while daily maximum temperature varied between 26.4 and 29.5°C. Both daily minimum and maximum temperature differed between the two sites

and was affected by tunnel diameter. Daily maximum temperature was additionally affected by aspect. The observed diel temperature regimes indicate that south-facing burrows of smaller diameter may be more physiologically and reproductively advantageous for burrowing owls than north- and south-facing burrows of other sizes. Such information could be incorporated into the design and implementation of artificial burrows that would be thermally appropriate for burrowing owls.

The Structural Specificity of Recombinant Proteins from the Hyperthermophile *Sulfolobus solfataricus*. *Catherine Trevino* (*Del Mar College, Corpus Christi, TX*); *Steve M. Yannone* (*Lawrence Berkeley National Laboratory, Berkley, CA*). The hyperthermophile archaeon *Sulfolobus solfataricus* grows optimally at 80°C and in the pH range of 2–3. The archaeon domain is most closely related to the Eukaryotes on the phylogenic tree than Eubacteria, suggesting that the DNA repair complexes of Archaea are most closely related to those of humans rather than those of other prokaryotes. These attributes and the fact that this organism's protein complexes are more stable at room temperature than mesophiles make it a good model organism. To analyze how different proteins interact with one another, the structural specificity of the protein should be known. The proteins that were investigated were cell division control 6/orc1 protein homolog, replication factor C, small subunit, phrB Deoxyribodipyrimidine photolyase, and MutT-like protein. To date, there are no structures available of the four *S. solfataricus* proteins that are being investigated. To investigate how protein-to-protein interactions occur, the four proteins from *S. Solfataricus* were harvested, extracted, purified and run through SAXS, using X-ray diffraction via cyclotron radiation. The attempts to obtain a structure in solution of the DNA repair proteins were better than predicted. Only cdc6-1, rfc, and MutT proteins showed good results when run through SAXS, phrB Deoxyribodipyrimidine photolyase was aggregated and did not give clear results. From the results gathered, cdc6 is said to be a transient complex between a monomer and a dimer. Rfc showed the protein adopts different oligmerisation states in solution. According to the PONDR and Kratky plot, MutT either has an elongation or aggregation added to the shape because there is disorder in the middle of the structure. With this data we will make crystal trays and attempt to crystallize the proteins that gave adequate results.

Purification of Chromatin-binding Protein YieF for Cancer Prodrugs. *Lisa Wang* (*State University of New York at Stony Brook, Stony Brook, NY*); *Yan-Biao Zhang* (*Brookhaven National Laboratory, Upton, NY*). To further our understanding of the roles of YieF protein in chromate transformation and detoxification, the crystal structure of YieF protein can be observed and the binding site and activity site of the protein can be found. YieF is a bacterial enzyme from *Escherichia Coli* (*E. Coli*) with prodrug-reducing activity involved in cancer chemotherapy. Through its prodrug capabilities, the activity of YieF ultimately reduces the toxicity of chemotherapy on normal cells. YieF has chromate-reducing capabilities without redox recycling, and reduces the toxic contaminant Cr^{+6} to Cr^{+2} directly using four-electron transfer by transferring three electrons to chromate and one electron to oxygen. The plasmid pYBZ49, containing the YieF gene in a pTYB3 expression vector, was used to overproduce the YieF protein in the *E. coli* BL21(DE3) strain. Recombinant YieF was purified by using affinity chitin-binding column and size exclusion chromatography column. Crystal conditions were tested. From the crystals we routinely observed, the crystal conditions were defined and we were able to see crystals at 2 microns. Purified YieF protein was used to test different crystal formation conditions to ultimately solve the protein structure.

Expression and Purification of a Vaccinia Virus Nudix Family Decapping Enzyme. *Brandi Wenger* (*Diablo Valley College, Pleasant Hill, CA*); *Stephen Holbrook* (*Lawrence Berkeley National Laboratory, Berkley, CA*). The Nudix (nucleoside diphosphates linked to another moiety x) hydrolase superfamily of proteins is characterized by its conserved 23 amino acid signature sequence. The motif is found among eukaryotes, bacteria, archaea and viruses. Proteins of the Nudix hydrolase superfamily perform a wide variety of functions using diverse substrates. The Nudix proteins have very low sequence conservation outside the signature and have a large amount of structural variation around a conserved structural core. Protein D10 contains this motif and is conserved in all poxviruses. In recent research, it was revealed that without the Nudix motif expressed in protein D10 of the laboratory prototype of poxviruses, vaccinia virus (VACV), the virus was unable to successfully target a host. Altering the motif in protein D10 caused VACV to lose its ability for mRNA decapping. With this ability lost, VACV no longer had the ability to suppress synthesis of cellular proteins and regulate its own gene expression. Solving the structure of protein D10 would allow an inhibitor to be designed to prevent poxviruses from successfully targeting a host. VACV D10 was cloned and expressed in *Escherichia coli* bacterial cells as a maltose binding protein (MBP) fusion (pMalD10) and a His-tagged protein (pETD10). Although expression is low for both the fusion and tagged versions of protein D10, preliminary results indicate that purification by affinity chromatography is feasible. Further experiments are underway to improve expression and purification of protein D10 with the goal of obtaining sufficient homogeneous material to perform crystallization experiments.

The Expression and Purification of the Core Protein of the Hepatitis C Virus in *E Coli*. *Glenn Werneburg* (*State University of New York at Stony Brook, Stony Brook, NY*); *Marc Allaire* (*Brookhaven National Laboratory, Upton, NY*). The second most fatal virus, Hepatitis C Virus (HCV) infects over 170 million people worldwide. Since only about 40% of the population responds to the current treatment, it is important to determine the structure of the HCV capsid protein which is involved in the packaging and assembly of the viral RNA. HCV is a single-stranded RNA type IV virus. Using an autoinduction method and media consisting of essential nutrients, the protein was expressed in an *E. Coli* strain. A high concentration (5.0 M) of sodium chloride salt in the lysis solution was used in the media to keep the fragment soluble. While the 1-82 amino acid (aa) 170aa, and 1-173aa protein fragments were expressed using this method, only the 1-82aa fragment was expressed in a soluble state. The 1-170aa and 1-173aa were present only in the pellet. The supernatant of the His-tagged 1-82aa protein fragment was then successfully purified on a Nickel column using imidazole to unbind it. 0.75 milligrams of pure protein was produced. Crystallization will be attempted using large quantities of this protein expressed and purified in the same way on a larger scale. The reconstitution of the capsid will also be pursued using tRNA and observed using Cryo-Electron Microscopy (Cryo-EM). In the event that the capsids are homogenous, a T-number for the symmetry of the capsid may be defined. This would be a major step in the determination of the structure of the HCV core protein and ultimately a better treatment for the virus.

High-Throughput Protein Crystallography of Mycobacterium Tuberculosis Targets. *Erik Westling* (*City College of San Fransisco, San Fransisco, CA*); *Minmin Yu* (*Lawrence Berkeley National Laboratory, Berkley, CA*). High-throughput protein crystallography is an efficient method to manage researching the multitudes of proteins part of the Mycobacterium Tuberculosis bacterium. Since crystallization conditions are unpredictable, hundreds of different conditions must be arranged and observed for crystal growth. Each protein is screened through up to approximately 450 different chemical conditions. Conditions generally include a buffer of specific pH, a precipitant and salt. Experiments are created on 96 well plates. Each well corresponds to three droplets (0.2 micrometers in diameter). In each droplet, the chemical conditions are mixed with the protein. The droplets are observed routinely for crystal growth with an automated image-viewing device. In attempt to increase the quality and size of crystals, conditions in which crystals are observed to grow can be slightly altered. Changes are generally made to buffer pH and concentrations of precipitants and salts. Putting several proteins through this process at the same time will narrow down those that can crystallize in the available conditions. Of forty proteins received by our laboratory, thirty are crystallized. Eleven of those crystallized are verified to be protein crystals. Conformation of the remaining crystals is underway. This method allows research to progress for several proteins simultaneously, rather than one at time.

Sample Preparation for Proteomic Studies on the Adaptive Evolution of *Escherichia coli*. *Angela Zhang* (*University of Washington, Seattle, WA*); *Kim Hixson* (*Pacific Northwest National Laboratory, Richland, WA*). Extensive research has been conducted on the bacterial evolution of *Escherichia coli* (*E. coli*) using comparative genomics, yet little research on microbial evolution has been done using the technology of proteomics. Recent findings have documented that mutations in the coding regions of *E. coli* may allow certain strains of the bacteria to improve their growth phenotype in initially unfavorable substrates. The intent of this project was to identify the amino acid changes that correspond with genomic mutations evoked in *E. coli* grown with glycerol or lactate as the carbon source by high throughput proteomic methods. In order for *E. coli* cell samples to be analyzed on the mass spectrometer, the cells were lysed with the Barocycler NEP 3229 to extract the proteins present. Then, the lysed *E. coli* cells were reduced and denatured with bond-breaker tris(2-carboxyethyl)phosphine and guanidine HCl, digested with trypsin, and alkylated with iodoacetamide. The digestion converted the proteins to peptides which were then cleaned up using C-18

Solid Phase Extraction columns. A Coomassie Blue Assay was used to measure protein concentration before digestion and peptide concentration was determined by a Bicinchoninic Acid Assay after C-18 clean-up. Samples were pooled into four groups according to growth substrate and injected into a strong cation exchange column (SCX) which fractionated the sample into twenty-five fractions. Each of these fractions which represent a less complex mixture will then be analyzed by a reversed phase C-18 column coupled to an ion trap mass spectrometer used to detect and identify the peptides. Due to time constraints, however, mass spectrometric analysis will not occur until after the conclusion of this internship. All identifications will go into a reference peptide database/library and the results will be compared with mutations identified by previous genomic studies. If mutations are translated into proteins, mass spectrometry can then be used to identify the various strains of *E. coli*, drastically reducing both time and money. Future work may include preparing strains of *E. coli* grown on substrates other than glycerol and lactate for proteomic analyses.

High-throughput Protein Purification. *David Zhang* (*University of Illinois at Urbana-Champaign, Urbana, IL*); *Min Zhou* (*Argonne National Laboratory, Argonne, IL*). Structural genomics is an integral part of biology, where three-dimensional structures of macromolecules are determined using X-ray crystallography. As part of the Midwest Center for Structural Genomics, the Structural Biology Center (SBC) at Argonne National Laboratory has played a key role in developing protocols for cloning, protein purification, and structure determination. These three steps of the pipeline are linked in that order. Cloning determines which proteins are soluble enough and have optimal expression so that they may then be isolated by the purification group. Cells are grown to a certain density, which are then lysed. The cell extract is then purified through nickel-ion affinity chromatography. The target protein is then concentrated and set up for crystallization in a high-throughput manner. Crystals are then transferred over to the hands of the crystallographers where structure determination takes place by the multiple-wavelength anomalous dispersion (MAD) method. In the long-term, more cost-efficient methods and tools will be used in order to solve the more difficult projects. The two main issues that impede the structural genomics program are protein solubility and expression. Protein structures, especially those of important pathogens, may reveal a lot about the mechanisms in which they perform their functions. Discovering and analyzing protein structures are a major step towards the advancement of biomedical research.

Characterization of Actin as a Cofactor for the Adenovirus Proteinase. *Han Zhu* (*Massachusetts Institute of Technology, Cambridge, MA*); *Walter F. Mangel* (*Brookhaven National Laboratory, Upton, NY*). A good model system for the development of effective protease-inhibition based anti-viral agents (drugs) is human adenovirus (AVP), because AVP is essential for the production of infectious viruses. AVP is found to require viral cofactors, which regulate the activity of AVP in time and space, for maximal activity. Actin, one of the most abundant proteins in the cell and a major component of the cytoskeleton, is believed to be an AVP cofactor due to the high homology of its c-terminus with the 11 amino acid peptide viral cofactor pVIc. The binding of monomeric actin to AVP allows for its activation and the cleavage and degradation of the cytokeratin-18 network of a host cell, releasing newly formed virions. Using a spectrofluorometric assay with optimized buffer conditions to preserve the native structure and monomeric form of actin, insights can be gained by characterizing the binding interaction between actin and AVP. Although the reaction rates were lower than expected, assays varying the actin concentration with constant concentrations of AVP suggest tight binding between actin and AVP. The KM of the AVP-actin complex was measured to be 7 μM, very similar to the measured KM of the AVP-pVIc complex, suggesting that the binding of actin does not change substrate affinity. Rather, the binding of actin changes the properties of the enzyme itself, or the kcat (determined by VMAX). In addition, actin stimulates AVP in the presence of either one of the two viral cofactors (DNA and pVIc), allowing us to conclude that actin binds to two independent sites on AVP. Furthermore, through the use of competition assays with DNA, we were able to begin to estimate the binding affinity between actin and AVP with and without its viral cofactor pVIc. A better understanding of the interaction between actin and AVP should reveal new targets for anti-viral drug development.

Chemistry

Energetics of Electron Transfer and Charge Separation in Moderate to Low Polarity Solvents. *Brian Albert* (*Columbia University, New York, NY*); *John Miller* (*Brookhaven National Laboratory, Upton, NY*). Because photosynthesis occurs in a region of low polarity so that photoelectrons store their absorbed energy in new chemical bonds instead of emitting it as heat through interaction with the solvent, studying electron transfer and charge separation in low polarity solvents will have applications in new solar energy storage. These experiments combine strong electron donors (metallocenes) and strong electron acceptors (quinones) to obtain ion formation by thermal equilibria in media of moderate to low polarity. In solvents of a range of dielectric constants, the extent to which cobaltocene and various quinones formed ion pairs and separated to form free ions was measured via conductivity and UV-Vis spectroscopy. Gibbs free energy changes were calculated for both the electron transfer and charge separation reactions in various solvents using acetonitrile as a standard highly polar solvent. With known values for the ionization potential of cobaltocene and the electron affinities of the quinones, Gibbs free energy changes observed in the moderately polar solvent, tetrahydrofuran (THF), were fit to the theoretical Born solvation energy and Coulomb potential models. Predictions made for free energy changes in solvents of very low dielectric constants disagreed with experimental data because a fourth species, charge transfer complex, becomes more significant in relative concentration, thus introducing a third equilibrium. Also, molecule structure of the solvent molecule was found to affect Gibbs free energy changes of ion formation. Approximations of neutral specie, ion pair, and free ion concentrations became more difficult in low polarity solvents because of charge transfer complexes that appear as broadened peaks in UV-Vis absorption spectra as well as extremely low conductivity measurements. Observed Gibbs free energy changes were found to be significantly more unfavorable compared to redox potentials of the same reactions determined electrochemically. The difference is attributed to the stabilization caused by salt in solution required for the electrochemical method.

Selective Electrochemical Oxidation of Sodium Chloride Solution. *Max Bastow* (*Colorado College, Colorado Springs, CO*); *Charlene Sanders* (*Oak Ridge National Laboratory, Oak Ridge, TN*). Activated electrodes placed in saline solution create an electric double layer composed of opposite charges, repelling ions of like charge. It is hypothesized that, due to this characteristic of the electric double layer, it is possible to inject a short anionic pulse into a saline solution that will selectively oxidize water to produce oxygen. If the pulse is short enough, it will stop before chloride ions are drawn to the electrode surface, thus avoiding production of chlorine. This would have applications to the generation of oxygen and hydrogen from sodium chloride solutions. Phosphate buffered saline (PBS) was placed into an electrolysis cell and a stimulus generator was used for controlled charge injection of an anionic pulse. This pulse was applied for 4 hours in a closed cell and chlorine production was observed by the spectrophotometric determination of hypochlorite based on its reaction with ascorbate. The same pulses were then applied to an identical cell containing PBS sparged with N_2 at 50 ml/min. A galvanic cell was used for oxygen measurements, and a Figaro gas sensor was used for hydrogen measurements. Evolution of chlorine was not observed until pulse widths greater than 200 μs were applied. At this pulse width the production of oxygen was already well established at 0.11 ± 0.01 μmol/hr. At the greatest applied pulse width of 400 μs chlorine production was at 0.053 ± 0.008 μmol/hr while oxygen production was 0.25 ± 0.01 μmol/hr. These results show that short pulses are indeed capable of oxidation of water in PBS to produce oxygen while avoiding the oxidation of chloride to chlorine.

Electrochemical Arsenic Remediation of Drinking Water in Rural Bangladesh. *Yola Bayram* (*University of Michigan – Dearborn, Dearborn, MI*) *Ashok Gadgil* (*Lawrence Berkeley National Laboratory, Berkley, CA*). According to the World Health Organization, in Bangladesh over 60 million people drink arsenic-laden water making it the largest case of mass poisoning in human history. Available methods of treating arsenic are too expensive, ineffective, or commonly difficult to implement, making them unsuitable for a poor or undeveloped country such as Bangladesh. Electrochemistry may provide an innovative, effective, and inexpensive method for arsenic remediation of drinking water. The method is an improvement upon a known method of using Fe(III) to remove arsenic. The Fe(III) combines with As(V), forming an insoluble complex which then can be easily filtered out. The innovative step of electrochemistry allows control over the amount of Fe(III) produced as well as electrochemical oxidation of the As(III) into reactive As(V) anion, making the method far more effective. Experiments were performed with water samples with 600ppb of total arsenic that received various amounts of current for varying durations of time. The objective is to determine the appropriate current and time necessary for an arsenic removal below 50ppb in order to meet