Many participants in the DOE's undergraduate research programs are supported by grants from the National Science Foundation. *These participants are designated with an "*" preceding the title of their abstract.*

Biology

A Comparative Study of the Age Class Structures of *Quercus alba, Quercus coccinea.* *KATHRYN GUTLEBER (Connecticut College, New London, CT 06320) TIM GREEN (Brookhaven National Laboratory, Upton, NY 11973)*. Investigation of the age class structure of *Quercus species* and *Pinus rigida* within the Long Island Pine Barrens core area is an important aspect of monitoring the health of the Pine Barrens. The age class structures of *Quercus alba*, *Quercus coccinea*, *Quercus velutina*, and *Pinus rigida* are primary indicators of successful reproduction and the possibility of the successional change between community types. By comparing the numbers of seedlings, saplings and mature trees, the success of reproduction for these three *Quercus* species and *Pinus rigida* was analyzed. The numbers of seedlings and saplings were recorded through the use of four two-meter wide belt transects within 16 by 25 meter plots. These plots were located within the Pine Barrens subtargets of Pitch Pine, Pine-Oak, Oak-Pine, Coastal Oak, Scrub Oak and Dwarf Pine forests. This study found the success of reproduction for all the study tree species within the six community types to be varied. *Q. alba, Q. coccinea* and *Q. velutina* all displayed a low number of saplings in all community types surveyed, indicating that current reproduction is not very successful. In Coastal Oak and Oak-Pine communities, *Q. alba* was the most successful in reproduction. The reproduction of *P. rigida* was dominant within Pine-Oak, Pitch Pine and Pitch Pine-Scrub Oak Woodland communities. However, the low average number of *P. rigida* saplings found could possibly indicate the succession from pine-dominated forest to oak dominated forest. There are several factors that may influence these trends in reproduction, including exposure to light, levels of litter and duff, and deer browse. Although the current levels of reproduction for *Quercus* species and *P. rigida* are varied and range across the different community types, they are still an important indicator of forest succession within the Long Island Pine Barrens core area. Forest succession is an important factor in this ten-year longitudinal study of the Long Island Pine Barrens core area, as forest succession and species competition are primary indicators of forest health.

A Comparison of DNA Damage Probes in Human Mammary Epithelial Cells with 150 kVp X-Rays. *CHRISTY WISNEWSKI (University of California–Davis, Davis, CA 95616) ELEANOR BLAKELY, KATHLEEN BJORNSTAD (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* In this study we investigated 53BP1 and γH2AX, DNA damage markers, to look at genetic mechanisms underlying responses to radiation insult. Two human mammary epithelial cell (HMEC) lines, one subtype of HMEC 184 with a finite lifespan and S1 with an infinite lifespan were investigated to research the role of immortalization in DNA marker expression. Cells were irradiated with 50 cGy, fixed after 1 hour with 4% paraformaldehyde, and processed through immunofluorescence. Cells were imaged using an immunofluorescent microscope and digitally captured using Image Pro Plus software. 8-bit images were analyzed using Image J and counted. The 184 cells showed more positive response within the irradiated samples than the S1 samples. It was observed that the S1 had a previous peak time of 30 minutes with an alternative DNA damage probe; this could explain the decrease in signal for S1 for both probes used in this research. We also noted that the γH2AX response was more punctate in the 184 cells, whereas the 53BP1 response was punctate in both cell lines. We hope to expand the dose and time course studied in the hope that this will broaden the knowledge obtained from the preliminary data of this research. It is important to understand whether the process of transformation to immortalization compromises the DNA damage sensor and repair process.

A New Method for Protein Sequence Characterization Using Hidden Markov Models. *HARSH SHAH (University of Illinois at Chicago, Chicago, IL 60607) GYORGY BABNIGG (Argonne National Laboratory, Argonne, IL 60439).* Predicted protein sequences of newly sequenced species are normally analyzed for similarity to existing protein sequences using BLAST. The sequences are also characterized by searching with Hidden Markov Models (HMMs) of existing protein families in order to assist function assignment. While the BLAST searches are performed quickly, the more accurate searches using HMMs are computation intensive and might take a long time. Once new sequences are identified that belong to a certain protein family, these sequences should be incorporated into family profile (HMM) in order to represent these new members. The new HMM in return should be used to search the protein space again for inclusion of potential new members not found previously or simply rebuilding statistical data for the existing family members. While HMMs are very powerful for the detection of protein family members, the dynamic construction of them is computationally prohibitive. We have explored the possibility of constraining the protein space and therefore speeding up searches with HMMs using two methods: 1) using a regular expression (REGEX) obtained from the HMM or 2) using PSI-BLAST with a position-specific scoring matrix generated from the HMM to select candidate sequences from the protein space (about 3.5 million sequences). The smaller proteins sequence database was used for the more accurate search by the HMM. We have compared execution times and the accuracy using a direct HMM search approach with the two-step techniques. We have determined that while the current implementation of the REGEX-based approach was resulting in fast execution times, its accuracy was greatly affected by the type of HMM and was only applicable for a small subset of cases. The second, PSI-BLAST-based approach resulted in fast execution times and high accuracy when compared to the HMMSearch standard. Using the combination of PSI-BLAST and HMMSearch programs (PSI-HMMER) improved accuracy even further with small impact on execution times. The software developed during this project (PSI-HMMER) enables the searches of protein sequences databases about 200 times faster than traditional tools with negligible impact on search accuracy. This tool will be incorporated into the currently used bioinformatics pipelines.

A Simplified Approach to Stereoscopic Imaging of Tomographic Reconstructions in Transmission Electron Microscopy. *SALIM RAHIMI (Nassau Community College, Garden City, NY 11530) ROBERT BENNETT (Brookhaven National Laboratory, Upton, NY 11973).* In biological applications, transmission electron microscopy (TEM) enables an investigator to view a specimen at the molecular level, offering a resolving power of ~0.2nm. The two-dimensional (2-D) micrographs captured using the TEM are grayscale images in which contrast is typically achieved using heavy metal stains that limit electron transmission with respect to specimen density. In order to ascertain an adequate understanding of the structure and function of biological systems, it is important to view these structures in three dimensions (3-D), as they appear in life. Currently, there is a myriad of protocols that use various software applications for creating tomographic volumes. Moreover, the lack of a uniform approach often makes the process difficult to replicate for those with limited resources. A simple, consistent method for generating tomographic volumes of TEM micrographs to be viewed stereoscopically was investigated. Sections ranging from ~60-100nm in thickness were collected on formvar-coated copper grids. Samples were viewed using a Philips EM300 TEM with a side-mounted goniometer stage, and images were captured with an integrated Gatan ES500W CCD camera. Common to many laboratories working with limited budgets, various challenges are faced during data acquisition and processing. Physical limitations of the goniometer have restricted datasets to a 45 degree maximum rotation in either direction about the horizontal axis. A tilt image series was digitally captured from -45 to +45 degrees in 1 or 2 degree increments and reconstructed into tomograms and rendered using Stanford University's EM3D software. Finally, stereoscopic visualizations of the rendered three-dimensional volumes were generated through the creation of movies that were viewed using the software application StereoMovie Maker.

Acetyltransferase Activity of the 11-Amino Acid Peptide Cofactor of the Adenovirus Proteinase. *YUE LIANG (University of California–Berkeley, Berkeley, CA 94704) WALTER F. MANGEL (Brookhaven National Laboratory, Upton, NY 11973).* The 11-amino acid peptide pVIc (GVQSLKRRRCF) from the C-terminus of the precursor to adenovirus protein VI has a transacetylase activity. Acetylation is an important physiological process in eukaryotic cells. For example, it is involved in DNA replication, transcription and repair. pVIc was incubated with acetyl coenzyme-A (Ac-CoA) at pH 7.0 for 15 minutes and the reaction was fractionated by high performance liquid chromatography (HPLC). A new peak appeared whose mass corresponded to mono-acetylated pVIc as determined by Matrix-Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry. The kinetics of acetylation were linear for at least 21 minutes. The equilibrium dissociation constant (Kd) for the

interaction of Ac-CoA with pVIc was 1.1 mM. When purified mono-acetylated pVIc obtained at pH 7.7 was incubated at pH 10.3 for 15 minutes, multiple peaks were observed upon HPLC. MALDI analysis of each peak indicated one peak contained pVIc, another the original mono-acetylated pVIc, two other peaks contained mono-acetylated pVIc, three peaks contained di-acetylated pVIc and one peak contained tri-acetylated pVIc. The reaction at pH 10.3 was repeated but in the presence of the C-to-A mutant of pVIc. The mutant pVIc did not become acetylated. Thus, the new acetylations at pH 10.3 occurred in cis, except for the trans-acetylation between cysteine residues on different pVIc. A long-term goal is to find out the effects of acetylation on pVIc and, therefore, on viron assembly, and further discover anti-viral agents that can interfere with viral assembly.

Amplification and Tagging of *Sulfolobus solfataricus* Genes for Recombinant Expression. *STEPHANIE PETERSON (Del Mar College, Corpus Christi, TX 78404) STEVEN M. YANNONE (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* In organisms that thrive at moderate temperatures, many biological processes such as DNA repair occur through transient protein interactions. Understanding these interactions and the temporary protein complexes that they form is vital to understanding how cells function especially in how they repair damaged DNA. Protein interactions within hyperthermophiles like *Sulfolobus solfataricus* may be stabilized at moderate temperatures. The work presented in this study provides the initial steps towards thermally trapping otherwise short-lived protein complexes. Genes associated with DNA repair were selected from the *S. solfataricus* P2 genome and were modified with a directional tag on the N-terminus and a six-histidine tag (6x-hist) on the C-terminus using gene specific primers. Genes were amplified, cloned into entry vectors, and transformed into *E. coli* cells. Colonies were then selected and grown in liquid culture. Plasmid DNA was isolated using alkaline-lysis extraction method and constructs were confirmed with restriction digestion. Sixteen out of twenty-nine constructs were successfully confirmed by restriction digestion and fragment pattern on 1% agarose gels. These constructs will be further studied through two different expression systems: *E. coli* expression and *S. solfataricus* expression. *E. coli* expression should provide insight into independent protein structure and function. Native expression will not only provide information about the structure and function but will also identify obligate protein partners in their native organism. In addition, this approach will identify the root causes of difficulties that arise from recombinant expression.

Analysis of Cell Wall Mutations in Maize Using Pyrolysis Molecular Beam Mass Spectrometry. *BRIANNA HARP (Metropolitan State College of Denver, Denver, CO 80217-3362) MARK DAVIS (National Renewable Energy Laboratory, Golden, CO 89401).* This study applies Pyrolysis Molecular Beam Mass Spectrometry (PyMBMS) as a high throughput screen of cell wall substrates to characterize cell wall mutations. We had 3 goals in our study: improve PyMBMS technique, confirm cell wall mutations, and characterize differences between mutant lines and controls. We tested two methods of extraction: the Accelerated Solvent Extraction (ASE) and a simplified, rapid ethanol/acetone extraction using PyMBMS and multivariate statistical analysis to determine which method is most effective for removing extraneous cell wall material. This investigation found the ASE extraction to be most effective. A second study was performed using a combination of ASE extracted and untreated whole mutant 33_'00FL-041-39 and 34_02S-1030-22 samples and controls to identify mutant lines and characterize differences among the samples, while analyzing benefits of the ASE extraction technique. The results of this study showed no significant differences in cell wall chemistry or advantage in using the ASE extraction technique. Our third investigation sampled six different mutants using only whole samples. We found that the mutant line 27_02s-1137-40 had a significant increase in C5 and C6 sugars. The results of our analysis of mutant line 39_'00FL-042-20 (387, 388, 393, 395, 396, 399) is inconclusive.

Analyzing the Structure and Function of Novel Cytochromes from a Natural Microbial Community. *ANNA SIEBERS (University of California–San Diego, La Jolla, CA 92093) MICHAEL P. THELEN (Lawrence Livermore National Laboratory, Livermore, CA 94550).* 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 579nm. 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.

Characteristic Colonization of Poplar Trees with GFP Expressing Bacterial Endophytes. *ERIKA FREIMUTH (Cornell University, Ithaca, NY 14850) DANIEL VAN DER LELIE (Brookhaven National Laboratory, Upton, NY 11973).* Endophytic bacteria that colonize the internal systems and spaces of plants contribute valuable functions and services to the plant both individually and as a community. The enhancement of plant growth and metabolic processes by endophytic bacteria offers the opportunity to mediate both increased production of plant material and the sequestration of toxins within its tissues. Cuttings of poplar trees were inoculated with selected strains of endophytic bacteria (Pseudomonas putida W619 and Enterobacter sp. 638) genetically modified to stably express a green fluorescent protein and resistance to kanamycin. The colonization behavior and effects of these bacteria on the plant were monitored biweekly using fluorescent microscopy as well as by grinding and plating different sections of plant tissue (including sections of the rhizosphere, roots, shoots, and leaves). By thus tracking select endophytic bacterial strains marked with gfp through the plant's development, the colonization efficiency and patterns of these endophytes in poplar can be utilized in various applications. Poplar biomass production and chemical sequestration efficiency can be maximized via endophytic bacteria for use in biofuels, carbon management and recycling, and the phytoremediation of environmental pollutants.

Characterizing the Role of the Nell1 Gene in Cardiovascular Development. *LEAH LIU (Pennsylvania State University, University Park, PA 16802) CYMBELINE CULIAT (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* Nell16R is a chemically-induced point mutation in a novel cell-signaling gene, Nell1, which results in truncation of the protein and degradation of the Nell16R transcript. Earlier studies revealed that loss of Nell1 function reduces expression of numerous extracellular matrix (ECM) proteins required for differentiation of bone and cartilage precursor cells, thereby causing severe skull and spinal defects. Since skeletal and cardiovascular development are closely linked biological processes, this research focused on: a) examining Nell1 mutant mice for cardiovascular defects, b) determining Nell1 expression in fetal and adult hearts, and c) establishing how ECM genes affected by Nell1 influence heart development. Structural heart defects in Nell16R mutant fetuses were analyzed by heart length and width measurements on formalin-fixed specimens and standard histological methods (haematoxylin and eosin staining). Nell1 expression was assayed in fetal and adult hearts using reverse transcription polymerase chain reaction (RT-PCR). A comprehensive bioinformatics analysis using public databases (UCSC Genome Browser, Mouse Genome Informatics, Integrated Cartilage Gene Database, PUBMED) was undertaken to investigate the relationship between cardiovascular development and each of the 28 ECM genes affected by Nell1. Nell1-deficient mice have significantly enlarged hearts (particularly the heart width), dramatically reduced blood flow out of the heart and unexpanded lungs. Isolation of total RNAs from hearts of adult (control and heterozygote) and fetal (control and homozygous mutant) mice have been completed and RT-PCR assays are in progress. The bioinformatics analysis showed that the majority of ECM genes with reduced expression in Nell1-deficient mice are normally expressed in the heart (80%; 22/28), blood vessels (71%; 20/28) and bone marrow (61%; 17/28). Moreover, mouse mutations in seven of these genes (Col15a1, Osf-2, Bmpr1a, Pkd1, Mfge8, Ptger4, Notch3) manifest abnormalities in cardiovascular development. These data demonstrate for the first time that Nell1 has a role in early mammalian cardiovascular development, mediated by its regulation of ECM proteins necessary for normal cardiovascular growth and differentiation. In addition, the identification of Nell1 and its associated ECM genes can provide future targets for treatment of heart and blood vessel defects.

***Cloning of DNA Repair Genes from a Hydrothermal Vent Worm.** *ANABEY CORNEJO (Contra Costa College, San Pablo, CA 94806) JILL O. FUSS (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* Abstract The primary structure of DNA is highly reactive with molecular by-products of metabolism as well as UV radiation from the sun, and these reactions alter and damage the human genome. Alterations to the DNA structure and chemistry result not only from natural physical agents but also from man made chemicals, although to a lesser extent. If these alterations are not detected and either corrected or removed, a mutation can be fixed in the genome potentially leading to cancer and aging. Through evolutionary adaptation, cells have developed a series of mechanisms which allow them to remove the damage and restore the normal nucleotide sequence and DNA structure. One such mechanism is Nucleotide Excision Repair (NER) which removes oligonucleotides, which are short nucleotide segments that contain damaged bases. NER is further categorized based on the location of the repair. GG-NER or global genome repair refers to NER taking place in DNA not undergoing transcription. TC-NER or transcription-coupled repair refers to NER occurring in the transcribed strand of active genes. If the NER pathway is compromised, a number of human genetic diseases such as Xeroderma pigmentosum (XP), Cockayne syndrome (CS) and Trichothiodystrophy (TTD) may result. These diseases are characterized by causing premature aging or a predisposition for cancer. To investigate the human DNA mechanisms of repair, we are studying an organism highly genetically homologous to humans, in this case Alvinella pompejana or Pompeii worm. The effectiveness behind using the Pompeii worm as a model for studying the process of DNA repair is due to the fact that most of its protein activity occurs at temperatures as high as 80°C. The Pompeii worm inhabits geysers found along underwater volcanic mountain ranges known as hydrothermal vents. These underwater formations release jets of water reaching temperatures as high as 300°C. Not only does it serve as a great organism for comparative studies due to its genetic similarity to the human genome but also because of its extreme heat tolerance. This would imply that its proteins will be fairly stable at room temperature which will allow for extensive in vitro study. More specifically to investigate DNA repair genes involved in NER, through the cloning of these genes from Alvinella pompejana as well as constructing plasmid vectors for recombinant protein expression, protein purification, protein to protein interaction studies, transfection of cultured human cells with expression vectors followed by assays for reporter gene expression, and analyzing particular proteins as a function of the cell cycle in mammalian cells.

Comparison of Fragmentation-Directing Properties of Amino Acids. *DANNY TAASEVIGEN (Montana State University, Bozeman, MT 59715) WILLIAM R. CANNON (Pacific Northwest National Laboratory, Richland, WA 99352).* Proteomics is the study of proteins and their function by the use of high, throughput methods such as mass spectrometry. With the use of molecular theory and computation, our group helps to develop more accurate peptide identification methods. Taking into account the potential energy of the system and the forces that affect peptide formation, simulations can be run to determine the probability of obtaining a configuration conducive to proton transfer to the respective carbonyl oxygen along the side chain. These simulations are run in the gas phase with a total time of 5 nanoseconds. Once complete, radial distribution functions provide us with the probability of proton donation along the chain. This data will be compared to predicted spectra in hopes of identification. The peptides are composed of various proportions of 20 naturally occurring amino acids and were derived from polyalanine, which proves to be the basis of analysis. However, these peptides have to have specific charges and the simulations have certain specifications that must be set to properly match the spectra. If detection of peptides is successful in this manner, it is likely to be scaled up to the genome-wide scale.

Creating Information System of Oncological Methylated DNA Data. *REGINALD GABRIEL (Cheyney University of Pennsylvania, Cheyney, PA 19319) DR. SEAN R. MCCORKLE (Brookhaven National Laboratory, Upton, NY 11973).* DNA methylation is the addition of a methyl group to the cytosine ring of the CpG dinucleotide, forming methyl-cytosine and is catalysed by DNA methyltransferases. An information system is needed for biologists to retrieve empirical data and relate it to DNA methylation sequences experimentally identified in human cancer cells. This project is intended to create such information system available for biologists. Information was collected from biomedical article reviews and existing Internet database, such as RefSeq (NCBI), the UCSC Genome database. PostgreSQL database server running on MAC OsX (DARWIN – BSD) system was established. The tool NetBeans Integrated Development Environment (IDE) was use to create the Graphical User Interface (GUI), the client application with the Java programming language. The Java client can run virtually on any computer because of the benefits of Java; “write once and run anywhere”. Communication between the Java client and PostgreSQL database server was established through the Java Data-Base Connectivity (JDBC) package. Object Oriented Techniques (OOT) is ideal for connecting various sub-components of the system: network connections and GUI application. A class PostJDBC was implemented to connect to the database, issue queries, and retrieve resultant data. UserGui, the user interface, relies heavily on inheritance and polymorphism techniques to achieve dynamic data manipulation and graphical display of the output. The client application presents the user with a list of gene symbols which are contained in the database, and allows the user to browse for more detailed information by mouse selection. This project can be easily expanded by including information about different types of features in DNA sequences and their relation to human cancer cells.

Crystal Structure of $Mn2^{+}$ Bound *Escherichia coli* L-arabinose Isomerase Complex: Its Implications in Protein Catalytic Mechanism and Thermo-Stability. *WEISHA ZHU (Cornell University, Ithaca, NY 14853) BABU MANJASETTY (Brookhaven National Laboratory, Upton, NY 11973).* *Escherichia coli* L-arabinose isomerase (ECAI) is responsible for the initial stage of L-arabinose catabolism, converting arabinose into ribulose in vivo. This enzyme also plays a crucial role in catalyzing the conversion of galactose into tagatose (low calorie natural sugar) in vitro. The crystal structure of its native form has been recently solved. This project aims to determine the crystal structure of enzyme in complex with co-factor metal ions and candidate substrates/inhibitors to further study structure-function relationships important for the enzyme’s biological mechanism. High-throughput techniques were adopted for protein structure analysis. Crystals of different complexes were grown using hanging drop vapor diffusion method. Diffraction datasets were collected at X4C, X6A and X29 beamlines, National Synchrotron Light Source, Brookhaven National Laboratory. The method of molecular replacement was utilized in structure solving using the native structure as the initial model. A series of crystallographic software packages were used to build and refine the structural model. The analysis of the structure of Mn2+ bound ECAI protein complex completed to 2.8 Å resolution with the R-factor of 23% and the structure was deposited to Protein Data Bank (PDB ID 2HXG). The overall fold and biological arrangement of the complex are conserved. Active site of ECAI is located at the subunit interface and forms deep cleft. $Mn2^{+}$ ion is identified at the active site surrounding with an octahedral coordination. Comparison between apo and holo structures reveals variations in the metal coordination sphere, conformational changes associated with the active site residues and increased number of interactions between subunits. The available biochemical and structural data provides the structural basis for efficient protein catalytic mechanism and increase in thermo-stability of the enzyme induced by the presence of $Mn2^{+}$. This study will benefit optimization of low-calorie sugar production. The target of this project is one of the many selected under investigation by New York Structural Genomics Research Consortium (NYSGXRC ID T2031).

Deletion of a Chitin Synthase Gene in a Citric Acid Producing Strain of *Aspergillus niger.* *TORRI RINKER (Oregon State University, Corvallis, OR 97331) SCOTT BAKER (Pacific Northwest National Laboratory, Richland, WA 99352).* Fungi have the potential to convert biomass into high-value chemical products. Filamentous fungi produce organic acids, such as citric, lactic, and fumaric acid. Citric acid production by the filamentous fungus *Aspergillus niger* is carried out in a process that causes the organism to drastically alter its morphology. This altered morphology includes hyphal swelling and highly limited polar growth resulting in clumps of swollen cells that eventually aggregate into pellets of approximately 100 microns in diameter. In pelleted form, *A. niger* has increased citric acid production rates when compared to those in filamentous form. Production of citric acid by *A. niger* serves as a model in which a filamentous fungus can take on a particular morphology and increase product output. Chitin, a polysaccharide in fungal cell walls, plays a central role in the morphology of fungi. A gene coding for chitin synthase with a myosin-motor domain (csmA) was deleted from the genome of *A. niger* using a PCR-based gene deletion construct. The mutant was tested on minimal media with and without osmotic stabilization. In the absence of osmotic stabilization, the germlings of the deletion strain (csmAΔ) were abnormally swollen and highly vacuolated. This suggests that chitin synthase is important for germination of spores, which in turn impacts morphology and may affect citric acid production rates. Genetic manipulation, such as gene deletion, can be used in the future in

other strains of filamentous fungi to obtain desired morphologies and optimized product output.

Detecting and Quantifying Heavy Metal Contamination in Water. *MATTHEW LARSEN (Brigham Young University–Idaho, Rexburg, ID 83460) ANGELICA STORMBERG (Idaho National Laboratory, Idaho Falls, ID 83415).* The DOE has a goal of remediating environmental contaminants. We must first know where the contaminants are, and in what concentration. For detection of heavy metals in aqueous media a transgenic nematode has been developed. *C. elegens* releases a Green Flourescent Protein (GFP) upon contact with heavy metals. Our work was to calibrate the amount of flouresence with the concentration of heavy metal contamination. Water samples can be analyzed in 24 hours, and cost less than 10 cents per sample. We were able to analyze five metals: Zinc, Lead, Mercury, Cadmium, and Nickel. Each was analyzed at 4 different concentrations. The calibration curves developed may be used to test a water sample and determine the concentration of the heavy metal contaminants.

Determination of a Role for Cellular XPG in Repair of Oxidative Damage to DNA. *EMILY FOX (City College of San Francisco, San Francisco, CA 94112) HELEN BUDWORTH (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* Mutation of XPG can cause the debilitating diseases Xeroderma Pigmentosum (XP) and Cockayne's Syndrome (CS), which result from a deficiency in DNA repair. XPG cuts 3' to DNA lesions during nucleotide excision repair (NER), as well performing the non-catalytic roles of recognizing stalled RNA polymerase II and binding transcription-sized bubbles in transcription coupled repair. Through in vitro tests with purified proteins, XPG has been found to stimulate hNth1, which removes oxidized pyrimidines in the base excision repair (BER) pathway. In this study, whole cell extracts from XPG-deficient cells obtained from patients with XP-G/CS were found to be defective in incision of 5,6-dihydrouracil (DHU). This defect was corrected by the addition of purified XPG, suggesting that the mutated XPG in XP-G/CS cells is unable to stimulate hNth1. In addition, cells from XP-G/CS patients were found to be slightly sensitive to X-rays and hydrogen peroxide, as determined by colony formation survival assays. shRNA against XPG was used to knockdown XPG in normal cells in order to provide another model for XPG deficiency in which the only variation from control cells is reduced levels of XPG.

Determining Cellular Localization of Candidate Cilia Proteins in Transgenic Caenorhabditis Elegans. *SANDRA MCGILL (Clayton State University, Morrow, GA 30281) EDWARD J. MICHAUD (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* Primary cilia are small organelles that protrude from cell surfaces and are conserved in most eukaryotes, including nematodes, mice, and humans. In humans primary cilia have vital sensory functions; flaws in these organelles lead to many diseases. Based on comparative genomics and proteomics studies it has been estimated that the cilia proteome consists of 300-500 proteins, but the functions of most are unknown. Disruption of the homologous genes in mice is an effective approach for determining function. However, prior to performing lengthy and costly experiments in mice, it is desirable to verify that these candidate genes do indeed localize to primary cilia. An efficient method for determining the in vivo cellular localization of candidate cilia proteins is to perform translational GFP assays in transgenic nematodes. In a previous project, translational fusion vectors were constructed in which two nematode candidate cilia genes, E02H1.5 and R148.1, were cloned in-frame with the Green Fluorescence Protein (gfp) reporter gene in a plasmid vector, pPD95.81. In this project, the two vectors are being microinjected into the gonads of nematodes, where transcription and translation of the fusion vector results in the nematode protein being tagged with the GFP protein. Visualizing the GFP marker under a fluorescent microscope reveals the locations of the fusion proteins in nematodes. Three independent lines of transgenic nematodes were established for E02H1.5, but examination of the transgenic nematodes did not reveal any GFP expression. Based on this result, and on further examination of the genomic locus encompassing the E02H1.5 gene, it appears that E02H1.5 may be one gene in a four-gene operon. Approximately 10% of all worm genes exist in operons, where one promoter region directs the expression of all genes in the operon. The entire E02H1.5 operon is now being cloned into the pPD95.81 vector and will be used to generate new lines of transgenic nematodes. Microinjection of the R148.1 vector into nematodes is under way. Additionally, translational fusion vectors are being made for two other candidate cilia genes, K07G5.3 and C02H7.1, which will also be used to generate transgenic nematodes to determine protein localization. If these four proteins are shown to localize to cilia in nematodes, the stage will be set for analysis of the functions of the homologous proteins in mice and humans.

Developing EPIMODEL2: A Computer Program for Teaching Population Growth Modeling. *DANIEL HEIDFELD (Michigan Technological University, Houghton, MI 49931) FORREST W. NUTTER JR. (Ames Laboratory, Ames, IA 50011).* EPIMODEL is a computer teaching program that is currently being used in more than 40 universities worldwide to teach students biological concepts concerning population growth modeling. EPIMODEL has become outdated because it was originally created in Quick BASIC for MS-DOS. Therefore, the goal of this project was to rewrite EPIMODEL using a computer language that would support Windows™. The programming language Java was chosen to develop EPIMODEL2 because Java is capable of operating on many platforms, including Windows™. EPIMODEL2 was programmed with the aid of various online manuals to develop algorithms that implement features within the Java environment. EPIMODEL2 was designed to mimic all of the features of the old version of EPIMODEL, but the new program implements a Graphical User Interface (GUI). The new program, EPIMODEL2, is a valuable and more versatile replacement for the outdated version, and the development of a new version in Java successfully accomplished the goal of this internship.

Developing Software Within Gatan's Digital Micrograph in Order to Create Automated STEM Tomography. *MICHAEL MYLENSKI (State University of New York at Albany, Albany, NY 12222) HUILIN LI (Brookhaven National Laboratory, Upton, NY 11973).* Automated Scanning Transmission Electron Microscope (STEM) tomography follows the ideals of Jianglin Feng, a researcher in the Department of Molecular Physiology and Biological Physics at the University of Virginia. Feng's automated version of STEM tomography revolves around the ideas of auto-tracking and auto-focusing. An automated STEM tomography program will be the first of its kind at Brookhaven National Laboratory (BNL). The development of the automated STEM tomography program was based on scripts created using Digital Micrograph (DM), which is the program of choice for communication between the researchers at BNL and the JEOL 2010F microscope. When creating these scripts, JEOL's FasTEM Communication Kit (FTCOMM) and Digital Micrograph's Software Developers Kit (DMSDK) were essential in creating new functions to implement auto-tracking and auto-focusing. Feng's paper provided the basic algorithm for automated STEM tomography and also a gradient formula in order to implement auto-focusing. Overall, there were five scripts and a Dynamic Link Library (DLL) that were developed in order to implement automated STEM tomography. The DLL file was the means of communication between the microscope and DM using FTCOMM and DMSDK, and it was developed using Microsoft's Visual Studio .NET 2003. The Graphical User Interface (GUI) was created using functions within DM's scripting language in order to benefit the user. The arithmetic for the auto-tracking and auto-focusing is primarily based in the DM scripts. When they communicated with the microscope, there were functions created in the DLL file that used FTCOMM functions in order to link the microscope and the DM scripts. The program that was completed includes the basics of an automated STEM tomography system with simple implementations of auto-tracking and auto-focusing. This project must be continued in the future in order for this version of automated STEM tomography to fully benefit researchers at Brookhaven Nation Laboratory in their study of structural biology.

Development of a Biosensor for Measuring Antibody-Antigen Interactions. *CASSANDRA ARMSTEAD-WILLIAMS (Washington University School of Medicine, St. Louis, MO 63110) KARA KRUSE (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* In recent years the Nanoscale Science and Devices group at Oak Ridge National Laboratory has developed and continues to study how microcantilever technology can be used to create high-throughput, label-free tests for biological macromolecules. The Vascular Research Laboratory at the University Of Tennessee Medical Center of Knoxville is studying the effects of certain drugs on the concentrations of Matrix Metalloproteases (MMP's) in the blood. The Vascular Research Laboratory and the Nanoscale Devices and Sciences group are working together to create a dependable, high-throughput, label-free system for quantitatively measuring concentrations of MMP's. Microcantilevers bend due to a change in the entropy and energy on one side of the cantilevers and not the other. This bending can be monitored optically by reflecting a laser beam off of the cantilevers and onto a position sensitive detector (PSD). The PSD translates the optical signal into an electronic signal for real-time monitoring of microcantilever bending. For this experiment, gold-coated silicon microcantilevers were immobilized with 3,3-Dithio bis(sulfosuccinimidylpropionate) (DTSSP)-a homobifunctional, amine reactive cross linking agent. Immobilizing the cantilevers with a DTSSP monolayer allows selective attachment of biochemical molecules onto

the gold and silicon/silicon nitride microcantilever substrate. MMP specific probe antibodies were then attached to the microcantilevers via a captavidin-biotin linkage system. Surface amine groups of captavidin were reacted with the succinimide terminals of DTSSP. The biotin-conjugated, MMP specific probe antibodies selectively adsorbs onto the captividin protein layer. After functionalization (immobilization), solutions containing unknown concentrations of MMP's are introduced to the microcantilevers while bending is being monitored. Using a model antibody system, Current tests indicate that DTSSP and antibodies can reliably be immobilizing on to the cantilevers surfaces. However, we have not been able to determine the antibodies' range of sensitivity for protein concentration detection. The reliability of the DTSSP and antibody immobilization shows that this detection system can work. We are currently modifying our detection system and probe immobilization procedures to find the optimal working range for this technology.

Dialysis or Column Exchange: Developing an Efficient and Quantifiable Protocol for Detergent Exchange Prior to Crystallization of Membrane Proteins. *NICK IMPELLITTERI (UW Stevens Point, Stevens Point, WI 54481) PILIP D. LAIBLE (Argonne National Laboratory, Argonne, IL 60439).* The goal of this study was to develop quantitative methods to replace detergents used for the solublization and purification of a membrane protein with a diverse range of detergents that could potentially increase the success rate of the proteins characterization and crystallization. Previously it has been anecdotally perceived that detergent exchange could be accomplished either by dialysis or by extensively washing and eluting column bound membrane protein with an alternate buffer containing a different, yet desired, detergent. Rhodobacter sphaeroides reaction centers (RCs), as well as foreign affinity-tagged Escherichia coli membrane protein APC00809 (809) were solublized, concentrated, and purified using the detergents N,N-Dimethyldodecylamine-N-Oxide (LDAO) and deriphat-160, respectively. These two membrane protein's detergents were exchange into the following detergents using both on column exchange and dialysis: LDAO, Triton X-100, n-Octyl-B-D-Glucopyranoside (OG), Tetraethylene Monooctyl Ether (C8E4), Deriphat-160, and CHAPS. Column-bound membrane proteins were eluted and analyzed after being washed with 1, 5, 10, and 20 column volumes of buffer containing the desired detergent, while membrane proteins were left in dialysis tubing with large reservoirs for 1,2,5, or 7 days. After concentrating all samples to ~10 mg\mL and analyzing them using thin layer chromatography (TLC), iodine staining and Image J software, the results of this experiment explicitly show the superiority of column exchange over dialysis in terms of accomplishing complete exchange of detergents with every combination attempted. For every trial, detergent exchange for RCs is more complete after 20 column volumes of detergent buffer than after 7 days of dialysis. Column exchange yielded a complete detergent exchange for all detergents except deriphat, which did not exchange well with LDAO in RCs in either column exchange or dialysis. Though all dialysis and most column exchange samples for 809 were lost in concentration, the column exchange samples that were concentrated and analyzed clearly showed that column exchange was fast and effective. In this experiment it clearly shows that column exchange is the most suitable means by which to exchange one detergent with many others of varying CMC. Dialysis, as this experiment shows, can only yield up to a 90% exchange after 7 days when exchanging with detergents of extremely low CMC. These results or this experiment will lead to more defined and reproducible protein-detergent complexes for input into structural and functional studies.

Effectiveness of Driving Surveys to Locate Burrowing Owls *(Athene cunicularia)*. *SARITA INCE (Lane Community College, Eugene, OR 97401) MICHAEL SACKSCHEWSKY (Pacific Northwest National Laboratory, Richland, WA 99352).* The burrowing owl (*Athene cunicularia*) is classified as a Species of Concern in the Columbia Basin by the U.S. Fish and Wildlife Service, a candidate species by the Washington Department of Fish and Wildlife, and as Endangered in Canada. A standardized, reliable, and time-effective population survey method has yet to be developed for burrowing owls. Without a reliable survey method, long term population trends cannot be accurately estimated. Driving surveys were evaluated as a method to locate burrowing owls in shrub steppe habitat and estimate relative population density. Burrowing owls are tolerant of human activities and are often found near roadways, making them an ideal candidate for driving surveys. Surveys were conducted on pre-determined routes at a prescribed speed of 20 mph. Seven routes within the Department of Energy's Hanford Site near Richland, Washington, were chosen based on habitat type and previous burrowing owl sightings. The surveyed routes consist of shrub steppe habitat with short grass and sagebrush cover ranging from dense to none. Surveys were undertaken for two to three hours during the owls' most active times of day, after sunrise and before sunset. Driving surveys require two people, a driver and an observer who carefully watched one side of the road for burrowing owls. At the end of the route, the driver turns around and the observer watches the other side of the road. When a burrowing owl was observed, the location from the road was recorded using a GPS (Global Positioning System). Burrowing owls were sighted on all but one of the seven routes. Burrows and owls were found very close to the road and over 100 meters off road. Proximity to human activities seemed to have little effect on burrowing owl sightings. Driving surveys are effective in locating burrowing owls on the Hanford Site and should be repeated in future years as part of an on-going population monitoring effort. Relatively few of the owl groups were seen during both surveys of each route, indicating that more then two surveys per route is required to assure that all of the owls along each route are found. Driving surveys also may prove useful for gathering population density data on other animals within the Hanford Site.

Effects of Low-Level Cadmium on Cyr61 Expression in Pre-Osteoclastic Cells. *KATHRYN TORMOS (Benedictine University, Lisle, IL 60532) MARYKA H. BHATTACHARYYA (Argonne National Laboratory, Argonne, IL 60439).* Cadmium is a heavy-metal element that is a major component of orange or red pigments and is naturally occurring in trace amounts in air, water, and soil. It is well known that high, unnatural exposures to cadmium can result in toxic effects in human beings, in particular in the kidneys, liver, and lungs. However, the discovery that cadmium is linked to the severe bone-breaking Itai-Itai disease has shed light on the potential impact of cadmium on bone systems as well. Previous microarray experimentation of cadmium's effects on genes expressed in bone cells in vivo in mice has shown the most up regulated gene early after cadmium treatment to be cyr61. The Cyr61 protein has been linked to such molecular processes as angiogenesis, ossification, embryogenesis, and cell migration. Preliminary immunohistochemistry staining and RNA isolation in bone cell cultures have shown potential Cyr61 protein expression in osteoclasts (bone resorbing cells); this expression appeared to be greater than in cultured osteoblasts (bone matrix excreting cells). This summer, we continue to test the hypothesis that cadmium triggers osteoclastic precursors cells to secrete Cyr61, increasing their migration, aggregation, and fusion into mature osteoclasts. Using the pre-osteoclastic RAW264.7 cell line, Cyr61 protein expression is being analyzed using Western blotting and immunoprecipitation, and cyr61 gene expression is being analyzed using RT-PCR technology. We aim to conclusively demonstrate whether the Cyr61 protein is significantly up regulated in cadmium's presence in RAW 264.7 cells. Showing Cyr61's role in osteoclastic precursors in the presence of cadmium will pinpoint a new, previously unresearched, role for the protein, and will also help our laboratory continue to construct a hypothetical pathway proving cadmium's central role in bone resorption.

Established an Information System Comprised of CpG Methylated DNA Data in Cancers. *WENYI BI (Cheyney University of Pennsylvania, Cheyney, PA 19319) SEAN MCCORKLE (Brookhaven National Laboratory, Upton, NY 11973).* Determining the global pattern of DNA methylation, or the methylome and its variation in cells has become an area of considerable interest primarily because of its potential use as an early diagnostic biomarker for cancers. For time being, the research papers, results and literatures on this subject have grown rapidly and scattered in different systems. However, because there is lack of a comprehensive information system available to gain all correlated information into one picture, it forces the researchers to manually search through scores of biomedical journals and related websites. To meet the requirements to acquire them automatically, we developed an information system consisting of database system (back-end), and web application (front-end) by querying from a gene level to the related to methylated genes, cancers and literatures to gain all information by one-short deal. Our research went through data source collection, database system establishment, and web application coding. In addition, we developed a novel algorithm to identify genes near the diTag found in the experiment data.

Expression and Purification of a Yeast Hypothetical Protein. *TIFFANY JORDAN (Elizabeth City State University, Elizabeth City, NC 27909) S. SWAMINATHAN (Brookhaven National Laboratory, Upton, NY 11973).* Solving protein crystal structures by X-ray diffraction or NMR is essential for doing protein chemistry and novel drug design. This project involves several aspects of macromolecular crystallography; for example expression and purification of proteins, optimization of crystallization conditions by use of robot, crystallization by sitting drop vapor diffusion method, observation and analysis of the

crystals under the microscope and lastly x-ray diffraction of the crystals. During our ten weeks stay at Brookhaven National Laboratory, we were successful in expressing and purifying a 29 kDa hypothetical Yeast protein by His-tag purification using nickel chelated columns and by the size exclusion method of protein purification by using a FPLC machine. We ran 1D SDS-protein gels for analyzing and confirming our results.

Expression of Cyr61 in Pre-Osteoclastic Cells as a Result of Cadmium Exposure. *MARYN VALDEZ (University of Maryland, College Park, MD 20742) MARYKA BHATTACHARYYA (Argonne National Laboratory, Argonne, IL 60439).* Cadmium (Cd) is a natural metal commonly present in paint, plastics, batteries, the protective coating of steel, fertilizers, and in cigarettes. Cd has been shown to start having adverse effects on bone organ and cell culture systems at levels ranging from 10nM-100nM. However, the mechanism by which Cd acts on the bone system to cause bone loss has yet to be deciphered and thus needs to be further studied. This is the ultimate goal of our research. Past experiments and the literature have led us to think that Cry61 plays a pivotal role in the pathway. Cyr61 is a ligand to the integrins aVß3. We hypothesize that Cd provokes expression of Cry61 in Osteoblast(OB) or Osteoclast(OC) cells, which binds to aVß3 located on the surface of OC precursors. The binding eventually leads to responses that lead to bone resorption. Specifically in this paper we are trying to determine whether Cd triggers OC precursor cells to secrete elevated levels of Cry61. Total Cell lysate(TL), extracellular matrix(ECM), and concentrated media(CM) samples collected after exposure to 100nM Cd for 2-48hrs, were analyzed for Cry61 expression using western blots. Most of our time was spent working out the proper protocol and we feel it has been developed to the best of our ability. We concluded that fetal calf serum (FCS) does indeed cause Cry61 expression, as reported by others. We tentatively concluded, that at 24 hours and possibly at 48 hours, Cd is causing an increase in Cry61 expression in the media, but we can not say Cd is inducing Cry61 expression in TL or ECM. Perhaps a different cell line needs to be used—one in which Cry61 is overexpressed to begin with, or perhaps we need to look at the pre-OB cell lines. More experiments that include more than 0.1% serum and more negative controls also need to be done. Immunhistochemical staining experiments may also be needed. Thus we made progress, but much more work needs to be done.

Expression of Type I Cohesin and Type I Dockerin Domains from *Clostridia thermocellum* for Nano Patterning. *CATHERINE COFFMAN (University of Tennessee–Chattanooga, Chattanooga, TN 37403) JENNIFER MORRELL-FALVEY (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* Cellulosomes are multienzymatic complexes from bacteria that have been studied for their ability to break down the cellulose in plant walls yielding ethanol as a byproduct. The scaffoldin protein is a significant component of the cellulosome structure because it binds to both the cellulose and cellulose degrading enzymes in the most advantageous manner for hydrolysis. In the assemblage of scaffoldin, nine Cohesin domains, in the presence of Calcium, mediate binding to enzymes that contain a Dockerin domain. This investigation examines the specific binding of the Type I Cohesin (CohI) and Type I Dockerin (DocI) molecules, which are components of the scaffoldin in the cellulosome. The objective is to artificially generate and assemble CohI and DocI using their unique binding specificity as an affinity pair. A 6x Histadine (His) tag will be added to the CohI so that it can bind to a nickel surface. Green florescent protein (GFP) and a strep tag are added to DocI. The interaction between the CohI and DocI would be visible through the GFP, and the strep tag allows for purification of the DocI fusion protein. Thus far, the CohI and DocI domains have been identified in the sequences of CipA and CelS respectively in *Clostridia thermocellum*. Using genomic material from *C. thermocellum*, the CohI and DocI domains were PCR amplified. Expression vectors were constructed with the CohI and DocI inserts. A GFP with no stop codon was also ligated into the vector containing DocI so that, when expressed, the GFP forms a fusion protein with DocI. The ligated plasmids were transformed into *E. coli* strain BL21(DE3) where the CohI and DocI-GFP can be expressed. After the cell produces the proteins, they can be purified using the attached His and strep tags. This affinity pair can potentially be useful in nano patterning as CohI can be positioned using nickel beads and binding events can be mediated through the Cohesin-Dockerin specificity. Binding can also be controlled since the CohI and DocI do not bind unless calcium is present in the system.

Extracting Methylated DNA Using His-Strep Tagged Clones of McrB, MBD2b and MBD3LI Proteins. *DANNY KOHUT (New York University, New York, NY 10003) JESSICA POLICASTRO (University of Scranton, Scranton, PA 18510) DR. JOHN J. DUNN (Brookhaven National Laboratory, Upton, NY 11973).* Epigenetic modification of DNA by the addition of a methyl group to the 5 position of the cytosine ring, the methylome, is the only common covalent modification of mammalian DNA. This modification has been shown to control the expression of various genes. Changes in methylation patterns have been observed in both brain cells exposed to cocaine and in developing tumor cells. McrB, a GTP-powered protein, binds to sites containing methylated cytosines preceded by either guanine or adenine residues. This protein was cloned from E.coli into the pET28 vector between NdeI and BamHI sites, expressed and purified for use. In order to be effectively used in methylome pull-downs, McrB was cloned onto a His-Strep tag previously ligated onto the pET28 vector between the NcoI and NdeI restriction enzyme sites. The tag will aid in binding the strepavidin-coated beads used in pull-downs. MBD2b binds methylated cytosines followed by guanines, which are found in CpG islands, and does so even more tightly when in the presence of MBD3L1. These proteins were also bound to the His-Strep tag as part of this novel approach in extracting methylomes. The efficiency of the synthesized McrB protein will be compared with that of MBD2b and also with the MBD2b-MBD3L1 protein complex. Each protein may pull down varying or very similar sections of a genome, whether a mixture of two or more must be used for effective pull-downs will be decided. The cloning of McrB into pET28 was successful; sequencing of the clone revealed no mutations or transformations created during PCR. Ligating the Strep tag onto the digested pET28 vector has also been successful. With the construction of functioning methylome pull-down proteins, drug addiction studies and the early detection of cancer may be improved.

***EZ-Viz Version 2.0, Further Simplification and Increased Functionality of Pymol Made EZ.** *BRETT HANSON, CHARLIE WESTIN (Rochester Institute of Technology, Rochester, NY 14623) PAUL CRAIG, LEN SLATEST (Brookhaven National Laboratory, Upton, NY 11973).* PyMol, a powerful open source molecular modeling tool, has a notoriously difficult user interface. In order to decrease the steep learning curve associated with PyMol, Laura Grell and Chris Parkin developed PyMol made EZ, (EZ-Viz), a plug-in user interface, to simplify the use of PyMol. Despite the simplicity of the new plug-in, it lacked many of PyMol's powerful features, severely limiting its versatility. To alleviate these shortcomings, numerous functions were added, including: Enhanced movie making ability, multiple view modes, electron density map importing and controls, Ramachandran plot fetcher, ray trace options, amino acid reference guide, mouse modes, multiple perspective options, polar contacts, and roving functions. With the new user in mind, buttons, sliders, and various other widgets were implemented to provide greater ease of use and efficiency. Instead of entering multiple character commands, a mere push of a button now renders the same outcome with much less frustration and time. Beyond these implementations, over 20 known catalytic active site motifs have been defined in EZ-Viz and are shown differentiated from the rest of the enzyme. Once the motif has been selected and returned for a PDB file, the user can easily show surface contacts, polar contacts, residue labels, and bound substrates. The prediction algorithms are based on measurements between specific amino acid atoms in relation to the other catalytic residues. The predefined measurement range can be modified by the user to increase or decrease precision of motif return. It is also possible for a user to define their own motif and exert a great deal of selectivity in searching for their domain of interest. Further pursuits regarding EZ-Viz will entail the implementation of complex algorithms for the prediction of ligand and protein docking, easier sequence alignment, and inclusion of a functional hydrogen bonding scheme. Utilization of EZViz's abilities will allow researchers to easily investigate sites of interest within proteins, enabling quicker and more efficient research. It will also provide instructors with the tools to present more detailed and visually appealing overviews of protein structure and function.

Gene Cloning and Expression in the Hyperthermophile *Sulfolobus Solfataricus.* *MEGAN HOCH (Del Mar College, Corpus Christi, TX 78404) STEVEN M. YANNONE (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* *Sulfolobus solfataricus* is a hyperthermophile Archaeon that lives in a very extreme environment, and for this it is considered an extremophile. This organism lives in acidic hot springs of Yellowstone National Park. *S. solfataricus* has become a model system for studying human DNA repair. Protein interactions that are needed to study DNA repair are sometimes transient. Protein interactions that are transient in high temperatures where Archaea live might be more stable at room temperature. This would allow a better look at and understanding of the protein complexes involved. Also, it is accepted that Archaea are more closely related to humans than bacteria, and the DNA replication and translation of the two is very similar. A group of genes was selected for cloning, and

primers were designed for each gene. The genes were amplified using the polymerase chain reaction (PCR) method. The PCR products for the gene of interest, were cloned into a directional topoisomerase I (TOPO®) cloning vector. These vectors were transformed into *E. coli* cells from Invitrogen. The cells were then plated on Luria-Bertani (LB) agar plates using sterile techniques. Clones were picked from the plates and a culture was grown overnight. The plasmid DNA was separated from the cells using alkaline lysis. Restriction enzyme digests were set up to confirm that the correct gene was inserted into the vector. The digest was visualized on a 1% agarose gel. This study successfully cloned sixteen out of the original twenty-nine genes selected. Some of the clones that grew on the LB plates did not contain any gene at all; it was simply the vector alone. After several restriction enzyme digests, there were about eight genes whose digests were not clear enough to confirm the presence of the correct insert. These constructs will need to be further digested with different restriction enzymes to confirm the gene. Currently methods are being developed for expressing these genes in *E. coli* and *S. solfataricus*. In future studies, proteins expressed from *S. solfataricus* will be studied and characterized to understand the protein-to-protein interactions that are occurring.

Generation of Translational Fusion Vectors for Candidate Cilia Genes. *SANDRA MCGILL (Clayton State University, Morrow, GA 30260) EDWARD J. MICHAUD, III (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* Cilia are small organelles that protrude from the surfaces of cells and are conserved in most eukaryotes, including nematode worms, laboratory mice, and humans. Cilia fall into two broad classes: motile cilia, which occur in clusters on mammalian cells and move fluids over cell surfaces; and primary cilia, which are found singly on virtually all cell types in mammals. It was recently discovered that primary cilia have vital sensory functions in many human tissues, and that flaws in these organelles lead to numerous human diseases. Comparative genomics and proteomics studies have determined that the cilia proteome consists of some 300-500 proteins. However, the functions of most of these candidate cilia proteins are unknown. The goal of this project was to select two of these candidate proteins identified by computational methods and to construct plasmid vectors in which the worm genes are cloned in-frame with the green fluorescence protein (gfp) reporter gene. Two candidate cilia genes in *C. elegans* nematodes, E02H1.5 and R148.1, were selected for cloning into the GFP reporter vector, pPD95.81. The DNA sequence of each gene was obtained from the University of California at Santa Cruz Genome Browser, and polymerase chain reaction (PCR) primers were chosen using the MacVector 6.5.3 sequence analysis program. These primers were used to amplify the promoter and coding regions of each gene from *C. elegans* DNA. Each gene was amplified by PCR and cloned in-frame with gfp in the pPD95.81 vector, resulting in two translational vectors, E02H1.5::GFP and R148.1::GFP. The E02H1.5::GFP and R148.1::GFP translational vectors were subjected to restriction enzyme digestions and DNA sequence analysis, which confirmed that the two *C. elegans* genes were cloned into the pPD95.81 vector in the correct orientation and in-frame with gfp. Future work will involve microinjection of these generated transgene vectors into the gonads of nematodes to produce transgenic worms, which will be used to determine experimentally if these proteins localize to primary cilia. These worms will be examined under a fluorescence microscope to determine if the E02H1.5::GFP and R148.1::GFP fusion proteins localize only to ciliated cells. If they do localize to ciliated cells exclusively, E02H1.5 and R148.1 will be confirmed as cilia proteins. This will set the stage for analysis of the functions of the homologous proteins in mice and humans.

Global Analysis of Iron Response and Fur Regulon in Shewanella Oneidensis MR-1. *DANIEL HARRIS (Albion College, Albion, MI 49224) JIZHONG ZHOU (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* Iron is an essential micronutrient for both prokaryotic and eukaryotic organisms. In addition to structural roles in proteins, the iron redox potential makes it a useful cofactor for proteins functioning in respiration, electron transport, photosynthesis, nitrogen fixation, DNA biosynthesis and other important processes. However, accumulation of free Fe(II) will catalyze Fenton reactions, which produce highly reactive oxygen species that damage cellular components. Levels of intracellular iron must therefore be precisely regulated to meet metabolic needs while minimizing risk of iron toxicity. Most bacteria moderate iron though the use of the Ferric Uptake Regulator (Fur), an iron-responsive global transcriptional factor that represses transcription when iron is present. In this study, the iron response network was analyzed in Shewanella oneidensis MR-1, and in a fur deletion mutant derived from MR-1. Cells were harvested at various intervals after addition of iron chelator (2,2'-dipyridyl), and then after iron repletion with iron sulfate (FeSO4). Total RNA from extracted from each strain at 13 time intervals, reverse transcribed, fluorescently labeled, and hybridized to DNA microarray slides. Four biological replicates were collected for each mutant for a total of 102 microarray hybridizations. Our results indicate that transcription of proteins involved in iron uptake and storage are mediated by Fur, and that these proteins are highly sensitive to iron availability. Proteins involved in energy metabolism are also regulated by iron availability and Fur, though to a lesser extent. Interestingly, Fur not only acts globally as a repressor, but also activates certain genes in response to fluctuations in extracellular iron concentration. Most of these genes have unknown function. In addition, we also identified regulatory genes controlled by Fur. Further investigations into the interaction between regulatory genes and Fur would be interesting and significantly increase our understanding of transcriptional regulation in S. oneidensis, an important metal-reducing bacterium with potential in the bioremediation of contaminated sites.

***High-Throughput X-ray Protein Crystallography.** *BINH NGUYEN (Contra Costa College, San Pablo, CA 94806) DR. MINMIN YU (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* Proteins play an integral function in cells, hence understanding them is critical, especially in medical research. Structural analysis of proteins is particularly important to the field of Structural Genomics, which aims to study protein molecules in nature to provide a fundamental understanding in biology. Knowing the three dimensional structures of proteins will enable the grouping of fold patterns and family of proteins that can lead to clues of how the proteins work. High-throughput protein crystallography allows structural determination of the protein via x-ray diffraction. Proteins are crystallized and the resulting crystals are analyzed with X-ray to create diffraction patterns that can be determined into three dimensional structures. The first important step in this process is we subject our proteins to series of crystallization matrices to find the right crystallization condition. We utilized various crystal screens from Hampton Research and Emerald Biosystems. The initial crystal hits-crystal formation-leads to further optimization of the crystallization solution so as to obtain crystals that have reasonable diffraction quality. Our crystals are analyzed mostly with the synchrotron at the Advance Light Source (ALS) within the Lawrence Berkeley National Lab (LBNL). Through protein 3-D structures, the folding topologies and local conformations of the proteins can be analyzed. The Li-Wei Hung Lab is currently analyzing various proteins for the Integrated Center for Structure and Function Innovation (ISFI) and TB Structural Genomics Consortium (TBSGC). These proteins are derived from interesting targeted DNA sequences of various sources, mostly species causing human diseases. Solved protein structures are deposited on our ISFI/TBSGC database (www.tbgenomics.org, http://techcenter.mbi.ucla.edu) and the Protein Data Bank (http://www.pdb.org).

Human Adenovirus Precursor Protein VI and Mature Protein VI: Cloning, Expression and Characterization. *MICHELLE LOUIE (The George Washington University, Washington, DC 20037) WALTER F. MANGEL (Brookhaven National Laboratory, Upton, NY 11973).* More than 50 different serotypes of adenovirus are known to infect humans, causing upper respiratory tract, gastrointestinal, and eye infections. The adenovirus proteinase (AVP) becomes activated inside nascent virions and subsequently cleaves virion precursor proteins thereby rendering a virus particle infectious. AVP requires two viral cofactors for maximal activity—adenovirus DNA and an 11-amino-acid peptide known as pVIc derived from the C-terminus of the precursor to protein VI (pVI). The interaction of AVP and DNA with pVI and VI has not been characterized. In order to characterize these interactions, the genes for pVI and its mature form VI must be cloned, expressed in bacterial hosts, and the resultant recombinant proteins purified. Cloning of pVI and VI from genomic DNA and insertion of restriction sites into the genes were accomplished by polymerase chain reaction (PCR) using gene-specific primers. Purified pVI and VI PCR products and the pET13a expression vector were then digested with Nde-1 and BamHI enzymes. Ligation of the pVI and VI DNA into the expression vector is completed before the plasmid is transformed into bacterial hosts and expression of the protein is attempted. pVI and VI DNA were successfully cloned. pVI was ligated into the pET28a-HS vector and transformed into the bacterial host. VI has been successfully inserted into the pET13a plasmid and transformed into a bacterial host. Expression of the pVI and VI proteins was observed in BL21-codon plus(DE3)-RIPL cells. Currently, optimal conditions for recombinant protein purification of protein pVI and VI are being investigated. Experiments using purified recombinant pVI and VI will determine how AVP liberates the pVIc cofactor from pVI. This study will determine if the C-terminus of pVI binds to AVP to produce an activated complex that subsequently removes pVIc from pVI or if AVP binds to DNA forming an active complex that then liberates pVIc

from pVI. In addition, proteins derived from this investigation will help to reveal the crystal structures of pVI and VI and determine the equilibrium dissociation constants and stoichiometries of binding of pVI and VI to DNA. Finally, the products of this investigation will also be used to elucidate the sites of interaction of pVI and VI with AVP and with DNA to ultimately determine potential drug targets for anti-viral agents.

Identification of PSI Complexes in Mammalian Cell Membranes. *KUSHBOO GOEL (Earlham College, Richmond, IN 47374) HUGH O'NEILL (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* Macular degeneration is a disease wherein the eyes lack photoreceptor activity in spite of having intact neural wiring to the brain. This disease is the leading cause of blindness worldwide. Past research showed that retinal tissue can be electrically stimulated by implanting pinhead-sized electrodes in the eye. Photosystem I (PSI), a naturally occurring photovoltaic protein, generates a 1 volt potential after capturing the energy of incoming photons. It has been proposed that PSI could play a role analogous to the micro-electrode approach. The reaction centers are inserted into retinal cells via liposomes and shown to elicit an action potential as measured by calcium flux. In this investigation a method was developed to directly identify the presence of PSI complexes in mammalian cell membranes. Green Fluorescent Protein (GFP) was chosen as a potential marker as it is commonly used as a fluorescent probe in mammalian systems. Recombinant GFP from Aequorea corulescence was over-expressed in *E. coli* cells. It was purified from a crude cell lysate by a three phase extraction procedure using tert-butanol and ammonium sulfate. This protein preparation was then dialyzed and further fractionated by anion exchange chromatography on a HiTrap Q column. The purified GFP was characterized by spectrophotometry, fluorimetry and SDS-PAGE analysis. It was estimated that the protein was greater than 90% pure. A three step procedure was developed to crosslink PSI complexes and GFP. In the first step GFP was reacted with either citraconic anhydride or sulfo-NHS-acetate to block the primary amines on its surface, followed by dialysis to remove excess reactants. The modified GFP was then reacted with 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride, and N-hydroxysuccinimide to activate the surface carboxyl groups. In the final step, the activated GFP was added to the PSI preparation and primary amines of PSI reacted with the activated carboxyl groups of GFP. A covalent crosslink between the proteins was formed in this manner. The success of the cross-linking procedure was analyzed by fluorimetry. The yields were typically 30-50%. Most fluorescence was lost after modification of the primary amines. In future work the GFP tagged PSI complexes will be incorporated into liposomes and fused with mammalian cells. Confocal microscopy will be used to image GFP fluorescence thus indicating the location of PSI in the cell membranes.

Isolation of Independent Spontaneous Thymidine Kinase-Deficient Mutants and an Estimation of the Mutation Rate at the Thymidine Kinase Locus in a Human B-Lymphoblast Clone. *LAWRENCE CHYALL (University of California–Berkeley, Berkeley, CA 94720) AMY KRONENBERG (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* Programmed cell death, or apoptosis, is tightly regulated by signals originating from both within the cell and its surroundings. The BCL-2 family of proteins helps modulate the balance between the life and death of cells. BCL-XL (a BCL-2-like protein) assists in limiting apoptosis by titrating the concentration of pro-apoptotic proteins through the formation of a heterodimer. TK6-Bcl-xL gly-159-ala #38 is a TK6 human B-lymphoblast cell line that was engineered to express BCL-XL gly-159-ala, a mutated form of the BCL-XL protein that does not have anti-apoptotic activity. A fluctuation experiment was used to estimate the mutation rate of TK6-Bcl-xL gly-159-ala #38 cells. The mutation rate was found to be closer to the historical results for TK6-neo #1 cells than cells expressing the wild-type BCL-XL protein, TK6-Bcl-xL #4. The plating efficiency of TK6-Bcl-xL gly-159-ala #38 cells was found to be the same as historical results for TK6-neo #1 cells and TK6-Bcl-xL #4 cells. Thirty-four early-arising and sixty-three late-arising spontaneous TK1-deficient mutants of the TK6-Bcl-xL gly-159-ala #38 cell line were isolated. DNA from each of these mutants was extracted for future analysis.

Membranes, Surfactants, and Membrane Proteins: Successful Partnering to Facilitate Structure and Function Studies. *AARON BOWLING (University of Illinois, Champaign, IL 61820) PHIL LAIBLE (Argonne National Laboratory, Argonne, IL 60439).* Despite recent revolutionary advances in science and medicine, the structures and intricate molecular workings of membrane proteins remain one of the least understood aspects of any cell. These specialized cellular machines, which comprise an estimated 70% of all pharmaceutical targets, are so dependent on the membrane which accommodates them that their extraction into aqueous buffers for study, typically causes degradation. Previous investigations have yielded several methods leading to extraction of these proteins into detergent micelles, yet there is no rational method to partner a given protein to a surfactant that will work successfully. Herein, a method of analysis is outlined that suggests to researchers the most appropriate surfactant for use with membrane proteins under experimental consideration. This method classifies detergents based on their 'strength' using several complexes of varied susceptibility found in photosynthetic bacteria. This protocol was coupled with an ink-based spectroscopy approach designed to accurately determine the critical micelle concentration of a surfactant, information vital to the strength analysis. These techniques were applied to several libraries of commercially available surfactants never before considered for use in structural biology. It is hoped that with this system of surfactant classification, the ability to obtain the structural knowledge of membrane proteins will be greatly enhanced; which in turn, will support the foundation for substantial advances in pharmaceuticals.

***Multimodality Nanoparticle for Targeted in Vivo Imaging with Xe NMR and Fluorescence.** *LESLEY LARA (Contra Costa College, Richmond, CA 94805) FANQING FRANK CHEN (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* One of the major challenges for antibody-based therapeutics is the lack of sensitive and convenient methods for in vivo imaging that track the distribution, metabolism, movement of the drug delivery system, and provide an effective means to monitor the treatment efficacy of the drugs. The lack of sensitivity also made early detection of cancerous tumors unrealistic. Currently, radiolabels are the most sensitive labeling technology; however, radioactivity labels are undesirable for large-scale use due to the harmful effects of ionizing radiation to both the technicians and the patients. Current generation MRI contrasting reagents work in a very high concentration range of several millimolars, and there is a high false positive rate. To solve this problem, we have constructed a novel class of imaging reagent that uses near-infrared CdSe nanocrystals. The nanocrystals are clustered with Gd-based MRI contrasting reagents for regular MRI imaging, or with a novel zero- to low-field MRI agent. This dual modality nanoparticle composite would be detectable with both deep tissue near infrared in vivo imaging and MRI/zero-field MRI. To target this to breast cancer, the nanoparticle also uses single-chain antibody against ErbB2, which is a protein in the EGFR family over expressed in 15% to >50% of breast cancers, depending on the stage of the disease. The nanoparticle is highly fluorescent with a high quantum yield and the clustering of the Gd chelating compound or zero-field MRI agent is demonstrated to be at least 500 per nanoparticle. This new class of nanoparticle based imaging solution can be applied to diagnostic and monitory imaging of other cancers, or even other diseases.

***Nanoplasmonic Molecular Ruler for DNA-Protein Interaction.** *YUVRAAJ KAPOOR (Contra Costa College, San Pablo, CA 94609) FANQING FRANK CHEN (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* One of the major challenges of quantitative biochemistry and molecular biology is to monitor enzymatic activity within a femtoliter volume in real time. We have constructed a novel nanoscale plasmonic probe-based molecular ruler, which can perform label-free, real-time, and sensitive monitoring of DNA length during nuclease enzymatic reactions. The bionanoplasmonic molecular ruler was fabricated by tethering specificly-designed double-stranded DNA to single Au nanoparticles. Nuclease enzymatic activity was tracked via the evolution of the plasmon signal of a single Au-DNA nanoconjugate, which reflects DNA size changes introduced through site-specific DNA digestion by endonuclease. The scattering spectra of individual Au-DNA nanoconjugates are measured continuously in real time during nuclease incubation. The scattering spectra of Au-DNA nanoconjugates show a blue-shift of the plasmon resonance wavelength, as well as decrease in intensity and a time-resolved dependence on the reaction dynamics. With a series of enzymes that generate DNA incisions at different sites, the shifts of the plasmon resonance wavelength are observed to correlate closely with the positioning of the nuclease-targeted sites on the DNA, demonstrating DNA axial resolution in nanometer precision (5 nm of wavelength shift per nm of DNA length change, or 1.4 nm wavelength shift per base pair difference). DNA length differences of as little as 2 nm (6 base pairs) after nuclease digestion are differentiated by the corresponding plasmon resonance shifts of the Au-DNA nanoconjugate. Based on the mapping relationship between the DNA length and the plasmon resonance wavelength of the nanoconjugate, we further develop the nanoparticles into a new DNase footprinting platform. This DNase footprint mapping is demonstrated through the binding of DNA repair enzyme XPG to DNA bubbles. This

work promises a novel molecular ruler that can monitor nuclease enzymatic reactions with single-particle sensitivity in real-time. It suggests the possibility of developing ultra-high density nanoarrays for parallel enzyme activity measurement in functional proteomic studies or biofunctional nanoprobe for intracellular enzymatic studies.

Nramp1 Activity Reduces the Protein Abundance of SodCI: A [Cu, Zn] Superoxide Dismutase of Salmonella enterica Serovar Typhimurium. *SHIRABRANDY GARZA (Washington State University, Pullman, WA 99301) LIANG SHI (Pacific Northwest National Laboratory, Richland, WA 99352).* Macrophages play an important role in the pathogenesis of Salmonella-mediated systemic infection in mice. Critical to the ability of macrophages to kill the Salmonella is the activity of the divalent metal ion transporter natural resistance-associated macrophage protein 1 (Nramp1), a major regulator of host resistance. However, it is still unclear how Nramp1 activity eliminates the Salmonella in macrophages. Previously, we have found by global proteomic analysis that Nramp1 activity may reduce the amount of SodCI, a [Cu, Zn] superoxide dismutase of Salmonella that protects Salmonella cells from the superoxide anions produced by host macrophages to kill Salmonella cells. In this report, we used Western blot analysis to confirm that Nramp1 activity indeed lowered the abundance of SodCI. Reduction of SodCI by Nramp1 may contribute the killing of Salmonella by macrophages. Confirmation that Nramp1 activity reduces the abundance of SodCI helps to better understand the roles of Nramp1 in Salmonella-macrophage interaction.

Optimization of Extracting Intact Proteins for Top-down Proteomics. *ANGELA ZHANG (University of Washington, Seattle, WA 98195) ERIC LIVESAY (Pacific Northwest National Laboratory, Richland, WA 99352).* In recent proteomics studies, the top-down approach for identification of proteins via mass spectrometry has shown several advantages over the commonly used bottom-up approach. The top-down approach requires no prior digestion of proteins, enables the measurement of the molecular weight of intact proteins, and preserves protein sequence and post-translational modifications. In order to successfully utilize top-down approach, an optimization of protocol for extracting intact proteins is necessary. Saccharomyces cerevisiae (yeast) is a proteome typically used for examination of proteomic analysis developments. The cells need to be broken up before extraction of proteins. Several ways of breaking these cells and protein extractions are investigated in this study. Saccharomyces cerevisiae cells stored in glycerol were grown in Yeast Extract Peptone Glucose (YPD) broth to OD6000.47 and OD6000.53 and harvested during log phase of growth. Different alcohol buffers including 25% 1-propanol, 25% 2-propanol, 25% 2-butanol, and 5% 1-butanol 20% 1-propanol were coupled with bead beating to break the cells. Other cells were lysed with Y-PER Plus, Dialyzable Yeast Protein Extraction Reagent, Y-PER and bead beat, and Y-PER twice. Samples were then prepared with Laemmli Sample Buffer. All samples prepared were examined using 1D gel electrophoresis. This project is a portion of the research that is currently being conducted to improve procedures and results for top-down proteomics.

Overexpression of Human SOST in Transgenic Mice Results in Defective Patterning of Limb Cartilage Elements. *LEILA BEACH (Stanford University, Stanford, CA 94305) GABRIELA G. LOOTS (Lawrence Livermore National Laboratory, Livermore, CA 94550).* Sclerosteosis is a generalized progressive bone overgrowth disorder due to the loss of function of the SOST gene product sclerostin. Null mutations in SOST result in occasional polydactyly and syndactyly along with a substantial progressive increase in bone density. Accumulation of abnormal bone mass begins in late childhood and affected patients display increased bone formation, while bone resorption is undisturbed. SOST is expressed in osteocytes, the mature metabolic cells in ossified bone. To investigate the physiological function of the SOST protein, we have generated several lines of transgenic mice carrying a ~160kb human SOST bacterial artificial chromosome or BAC. Transgenic mice overexpressing SOST (hSOSTwt) exhibit a decrease in bone mineral density in the appendicular and axial skeleton when compared to non-transgenics from four to six months of age as evaluated by Dual Energy X-ray Absortiometry (DEXA) and quantitative computed tomography pQCT. When bred to homozygosity (hSOSTwt/wt) these animals also show congenital limb defects. Hereditary hand malformations occur frequently in human populations, therefore these animals can serve as a model for understanding molecular perturbations that lead to improper limb patterning. The fore- and hind-limbs of these animals are severely deformed displaying a wide range of fused and missing digits as visualized by autoradiography and pQCT. Since limb development is initiated prior to ossification of the skeleton, we find SOST to be expressed in the developing embryo as early as embryonic day 9 (E9), predominantly in the mesenchyme tissue of the developing limb bud. We have performed a detailed phenotypic analysis of these transgenic embryos using skeletal preparations and histology which revealed a defect in cartilage formation. To investigate whether the limb defect stems from defective chondrocyte differentiation, proliferation, or apoptosis we set out to examine limb sections by in situ hybridization. We identified cDNA clones to generate antisense RNA probes for Fgfr1 (expressed in distal proliferating chondrocytes), Bmp7 (columnar proliferating), Fst and Tgfß2 (early hypertrophic), Bmp2, and Tgfß3 (hypertrophic cells), and Spp1 (terminal hypertrophic). These markers represent distinct stages in the chondrocyte differentiation program, and misexpression of these markers revealed by in situ hybridization will indicate cell types that may show excessive proliferation or apoptosis.

Phenotypes Vary Among Recombinant Inbred Strains of BXD (C57BL/6JxDBA/2J) Mice. *TERRI KAMINSKY (Christopher Newport University, Newport News, VA 23606) DR. DABNEY K. JOHNSON (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* Mammals normally respond to cold exposure by increasing thermogenesis, but this adaptive response can be altered by genetic factors affecting metabolic capacity and the hypothalamic-pituitary-thyroid axis. We used the response to cold stress as a means to screen a panel of recombinant inbred C57Bl6 X DBA (BXD) mice for genetic variation in metabolism. Core body temperature, tail length, body and organ weights were also measured to determine if metabolic and morphometric traits were genetically correlated, or if they appeared to be controlled by separate loci. To study thermoregulation, male, age-matched mice (n=2-6 per strain) representing eighteen BXD strains were placed in transparent, individual plastic containers in a 4°C environment after an initial measure of baseline body temperature. Core body temperatures were measured at thirty-minute intervals for two hours. Following the cold stress test, mice were euthanized and the body weights were recorded. Tail length was measured and various organs and fat pads were dissected and weighed. Baseline core body temperature was similar across all BXD strains analyzed (average = 38.0° ± 0.3°). On average, body temperature dropped 1.5° after the cold exposure. However significant genetic variability in the response was apparent. For example, two strains (BXD 068 and BXD 039) deviated considerably from the average response. Strain BXD 068 had an average baseline temperature of 37.5° (±0.2°) and dropped to 35.4° (± 0.9°), a change of - 2.1°. Strain BXD 039 had a similar baseline temperature (37.7° ± 0.4°), but experienced little change after two hours of cold exposure (average change of - 0.3°). These results indicate a considerable amount of genetic variation in the ability to thermoregulate, likely reflective of core variation in metabolism and/or endocrine regulation. Organ weights also varied across strains, with spleen exhibiting the most variation, but the strain-specific patterns did not parallel those observed in body temperature. Our findings confirm that core physiological processes are impacted by naturally occurring genetic polymorphisms found in BXD strains of mice. Future efforts will determine the genetic relationships among metabolic and morphometric parameters and the genetic intervals linked to thermoregulation and other complex biochemical processes.

Polymeric Micelles as Fluorescent Probes for Stem Cell Imaging. *MICHELLE ROSA, MELIXA RIVERA (University of Puerto Rico at Mayaguez, Mayaguez, PR 00680) LIAOHAI (LEO) CHEN (Argonne National Laboratory, Argonne, IL 60439).* The goal of this research is to explore the feasibility of using polymeric micelles as the template to fabricate target specific imaging agent for molecular cellular imaging. Nano-particles based on polymeric micelles have drawn much attention recently and they have been successfully used as drug delivery vehicles in pharmaceuticals. Yet, little study exists for using polymeric micelles as imaging probes. Polymeric micelles are generally formed by the self-assembly of amphiphilic copolymers above their critical micelle concentration in aqueous medium. Upon micellization, the hydrophobic core regions can function as the reservoirs for the fluorescent molecules. The size of a typical polymer micelle is in the range of 30-50 nm, thus, a fluorescent molecule loaded polymeric micelle should form a core-shell fluorescent nanoparticle with diameter ~50 nm. So far, research demonstrates that the polymeric micelles can efficiently load the fluorescence dye to form core-shell fluorescent nanoparticles. The formed particles are very stable in different pH (ranging from pH2 to pH10). Temperature dependency studies indicated that the polymeric micelles have a phase transition temperature at ~ 70 C. In order to see the interaction between these polymeric micelles and cells, a study was carried out using stem cells. It was demonstrated that stem cells do not uptake polymeric micelles. It was also found that the dye is toxic for these cells, therefore polymeric micelles can reduce cell toxicity against

organic compounds. Currently, we are conjugating a stem cell specific antibody to the fluorescent polymeric micelles and the experiments of imaging stem cell in situ will be followed.

Protein Purification on Tuberculosis Protein. *ASHLEY JONES (Fisk University, Nashville, TN 37208) DR. CHANG YUB KIM (Los Alamos National Laboratory, Los Alamos, NM 87545).* Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tb*), is an infectious disease known to affect the lungs in most cases. In 2004, there were about 14.6 million people with latent TB including nine million new cases. Over 2 million people die every year from this disease. Although different drugs have been developed to fight this disease, these drugs are no match for the spontaneous mutations of this deadly disease with new *M. tb* strains resisting multi-drugs. Thus scientists continue to look for new methods to combat this disease. After the completion of sequencing TB genome, TB Structural Genomics Consortium was established to determine the structure for over 400 proteins. Since 2000, the Los Alamos National Laboratory (LANL) has partaken in this Structural Genomics project by cloning and purifying target proteins. The purified proteins are to be used in determining the three dimensional structure of the protein and ultimately its function. Eventually, this will allow scientists to produce a pharmaceutical drug that will inhibit the protein, which will then stop TB. In this paper, we will discuss the developed protocol of protein purification and the role that I played in the purification process this summer.

Protein SDS-PAGE Characterization and Comparison of Soybean Cyst Nematodes. *ALEXANDER PATANANAN (University of California–Los Angeles, Westwood, CA 90095) DR. STEVEN C. GOHEEN (Pacific Northwest National Laboratory, Richland, WA 99352).* Soybean cyst nematodes (SCN, Heterodera glycines Ichinohe) represent one of the most serious threats to the stability of soybean crops in the United States. Initially discovered in North Carolina in the 1950s, it has spread rapidly through the Midwest generating an estimated $1 billion in failed crops each year. In order to counteract this infection, numerous methods of pest management have been established, such as crop rotation and the development of SCN resistant soy plants. However, at least sixteen distinct populations of SCN have been differentiated based upon their relative rates of reproductive success on different strains of soybean, making it difficult to use only one sole technique. Although it is well accepted that these deviations in reproductive rates are not associated with any morphological differences in the various SCN populations, knowledge of biochemical differences is lacking. Therefore, the protein profiles of five different SCN populations were characterized using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in order to identify similarities and differences in their most prominent proteins. SCN eggs obtained from the Tennessee Soybean Promotion Board were homogenized in SDS, diluted in sample buffer, and examined by SDS-PAGE. Out of 25 major proteins ranging in molecular weight from 40 to 200 kDa, no major biochemical differences were observed between any of the races. These results implicate that SCN populations are consistent with being biochemically similar, and that any real differences in protein composition or relative expression are small compared to the resolution of SDS-PAGE.

***Quantifying the Effect of Temperature on Chlorophyll Fluorescence.** *JESSICA STONE (California State University–Fresno, Fresno, CA 93630) WILLIAM STRINGFELLOW (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* A daily change in chlorophyll concentration was observed in the field (San Joaquin River) algae samples. A lab study of algae growth at various temperatures was needed to determine if the change in chlorophyll concentration was indeed due to temperature changes of the environment, or if this was not a true change in concentration, but just a temperature effect, instrument default, or some other factor on the algae. A Hydralab sonde was used measure the temperature (°C), and chlorophyll fluorescence (volts) every minute for 2 hours and 15 minutes. The algae culture, grown for 4-17 days in single source carbon (SSC) media was diluted to ~1 volt was cooled with an ice water bath, then heated with a hot water bath followed by another cool ice water cycle. The results showed an inverse relationship of chlorophyll fluorescence versus temperature. Cooler temperatures resulted higher the chlorophyll concentration. Thus, temperature is having an effect on chlorophyll fluorescence. Further experiments need to address any possible differences between using a young culture or an old culture. Also, the sonde (Hydrolab unit #41) that was used needs to be compared with data acquired from the Shmadzu spectrofluorophotometer and chlorophyll extraction methods.

Regiospecificity Changes in Ivy Mutants. *KIRA SCHULTHEISS (Stony Brook University, Stony Brook, NY 11794) ED WHITTLE (Brookhaven National Laboratory, Upton, NY 11973).* A delta(4)-16:0-ACP desaturase enzyme from Hedera helix (English Ivy) introduces a double bond between the fourth and fifth carbons of its fatty acid substrate (16:0-ACP). A closely related desaturase from Castor directs its bond to the $\Delta 9$ position. Amino acid alignment of the two desaturases at the critical ACP binding domain indicates potential changes that contribute to the different regiospecificities. By creating mutations in the genetic code to make Ivy more similar to Castor, a single amino acid change was introduced into the ivy desaturase converting lysine (K) to an aspartic acid (D) at amino acid position 308. The mutant enzyme, Ivy K308D, was cloned into a pet24a expression vector and transformed into BL21 cells. These cells were induced to produce protein. The desaturase was purified, assayed, and the products of the assay were analyzed with the use of a GC-Mass Spectrometer. Products made with the Ivy K308D mutant were compared with those from the wild-type Ivy enzyme and any changes in activity from the usual $\Delta 4$ to the expected $\Delta 9$ position were noted. When analyzed, approximately 4% of products had a double bond in the $\Delta 9$ position. By comparison, the wild type Ivy produced no $\Delta 9$ product. This mutation indicates some involvement and change in regiospecificity, though the fact that only 4% of the product fatty acid was desaturated in the 9-position suggests that other amino acids are also involved in this process. Additional research is being performed to introduce mutations at other positions that potentially contribute to regiospecificity.

Sequenced Based Identification of Yeast. *MARTHA JOHNSON (Jackson State University, Jackson, MS 39217) DR. TAMAS TOROK (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* The study of microorganisms is important due to the rapid advances in microbiology and its substantial contribution to the commercial, scientific, and medical aspects of life. Fungi offer molecular biologists well-developed genetic systems to use as eukaryotic model organisms and have contributed to our understanding of human genetics. Fungi also have significant potential for bioremediation. The use of fungi to recycle nutrients through biodegradation is useful and will help with green chemistry and polluted soils. Yeasts are single cellular fungi that have either aerobic or anaerobic metabolic capabilities. Some of the most common uses of yeasts are in bread making, and in beer and wine fermentation. Researchers at Lawrence Berkeley National Laboratory (LBNL) are investigating the possibility of using specific biomarker DNA sequences to detect and identify yeasts. This technique applied in a high-throughput DNA microarray format will provide researchers with a novel cutting edge for rapid identification of yeast species. The current project focuses on the analysis of the D1/D2 domain sequences of the 26S ribosomal RNA genes of a large number of yeast species that were isolated from various environments. The yeasts were grown on potato dextrose agar (PDA). Following genomic DNA extraction, the D1/D2 domains of the 26S rRNA genes were amplified using the polymerase chain reaction (PCR). After amplicon cleanup and sequencing, the edited sequences were compared to sequences of known yeasts available in a public database hosted by the National Center of Biotechnology Information (NCBI). Ten of the 34 yeasts examined showed DNA sequence homology of less than 98% when compared to DNA sequences of known yeast species. Additional experiments will be performed to examine deletions, insertions, and differences in nucleotide sequence for any evolving alterations. These alterations could lead to greater knowledge of how yeast adjusts to the given habitat.

Spawning Interval Behavior of Fishes in Association with Parental Care and Migration Patterns. *EMMANUEL ISANG (East Tennessee State University, Johnson City, TN 37614) HENRIETTE JAGER (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* Spawning interval is the time, measured in years, that it takes for species to undergo reproduction of offspring. Some species spawn several times a year while others spawn every couple of years. The species that spawn several times a year, known as batch spawners, released eggs in a series of batches over a period of time that can last from days to months. Other species, that don't spawn annually, are known as capital breeders. These species use stored energy to make large, fecundity-investment at each breeding opportunity. Factors that can affect the spawning interval values are whether these species go through long spawning migrations and/or whether there is any parental care provided to the offspring. In order to support ongoing research to determine the spawning interval of a variety of species, this project searches for three primary variables of interest: migration, spawning intervals, and parental care. The search primarily uses programs such as ISE Web of Knowledge, Google, and ORNL Library Catalog. After information was found about any of the three variables of interest, it was entered into an excel file. After creating as extensive a database as possible, the data will be analyzed to draw any conclusions about the

relationship between parental care and spawning migrations and the extent it affects spawning intervals of the fish species. Looking at the analyses, the overall values for the spawning intervals of the species as a whole seemed to favor the species that either had spawning migrations, parental care, or both. Overall the species that had any of the two characteristics had a higher amount of time between spawning opportunities than the species that didn't go through a spawning migration or provide parental care. However, further studies and research will be needed to increase knowledge of fish life history, and to fill in the gaps for the species that lack complete information and other species that have not been studied. This work is a very small portion of studies conducted on the relationship of spawning interval and how it is affected by spawning migrations or parental care.

Sporulation of *Clostridium thermocellum* in Media for Thermophilic Clostridia with Various Amounts of Nitrogen and Buffering Agent. *SARAH WHITAKER (Middle Tennessee State University, Murfreesboro, TN 37132) JONATHAN MIELENZ (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* *Clostridium thermocellum* are anaerobic, thermophilic, spore forming bacteria that hydrolyzes cellulose to cellobiose and cellodextrins through the utilization of a membrane bound cellulase system called a cellulosome that attaches to cellulose fibers. Once cellobiose and cellodextrins are formed, these products are internalized and utilized by the bacterium to produce energy in the form of ATP. By-products of this metabolism include hydrogen, carbon dioxide, lactic acid, acetic acid, and ethanol. The production of ethanol, which is rapidly becoming a widely accepted alternative to fossil fuel energy, and the world-wide abundance of cellulosic material, is what make *C. thermocellum* such an attractive organism to study. Culturing of *C. thermocellum* was done at various pH levels and nitrogen concentrations of media for thermophilic clostridia, MTC, to assess the ability of the modified medium to support sporulation of the bacterium. Understanding the conditions under which sporulation occurs, as well as the mechanisms of the process, will add to the full characterization of this organism. A complete characterization will help to answer questions and solve problems that may arise if ethanol production using this organism is to grow to industrial levels. A method commonly used to assess bacterial growth rate is optical density. Light absorbance at 600nm was measured through the use of a spectrophotometer during the growth of each culture and plotted vs. time to determine the exponential growth phase. Phase-contrast light microscopy was employed to visualize the *C. thermocellum* in order to assess the presence of spores. *C. thermocellum* spores are said to shimmer under phase-contrast. Culturing experiments and microscopy reveal an apparent lack of sporulation in all media recipes tested. Knowing that *C. thermocellum* will not sporulate, which could interrupt the production of ethanol in MTC media, can help define and enhance the process of making ethanol.

Structural Study of the DNA Repair Protein XPG Using Constructed Proteins with Individual Domains. *JONATHAN ROYBAL (University of California–Berkeley, Berkeley, CA 94720) SUSAN TSUTAKAWA (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* Cockayne syndrome and xeroderma pigmentosum are two distinct genetic disorders, characterized by sun sensitivity and severe developmental disorders in the former, and by extreme sun sensitivity and skin carcinogenesis in the latter. Paradoxically, both are caused by different defects in many of the same DNA repair proteins. One such protein, XPG, is thought to be particularly important among these proteins. Structural analysis of XPG would greatly elucidate the mechanistic basis for the distinct functions of XPG. However, XPG has proven difficult to crystallize. Two proteins, XFX delta exon 15 and GST-M4, were created from parts of XPG and other proteins to perform structural studies of the two main catalytic regions of XPG, the "N" and "I" domains, and part of its regulatory region (the R domain). Purification was attempted for each protein via its own protocol using affinity and ion exchange chromatography. GST-M4 was purified to ~80% homogeneity, then split to give separated GST and M4 using Prescission Protease. Conditions to produce homogeneous M4 alone are still being determined. We obtained 5 mg of purified XFX delta exon 15 which was shown to be monodisperse as dimers by dynamic light scattering, found to be active with DNA, and was analyzed via small angle X-ray scattering. Screens for crystallization conditions were then run on purified XFX delta exon 15 samples, yielding crystal showers; optimization of conditions to produce larger crystals will be done in the near future.

Structure Solved for the ywmB Protein from Bacillus Subtilus. *KRISTIN MUSSAR (Knox College, Galesburg, IL 61401) ANDRZEJ JOACHIMIAK (Argonne National Laboratory, Argonne, IL 60439).* During my internship at Argonne National Laboratory I worked on protein purification in Andrzej Joachimiak's biochemistry lab. The purification process is the second part of a larger three step procedure in which the goal is to determine the structure and function of specific proteins. Once clones producing significant amounts of the target protein have been selected they are passed on to the next lab. Here, the clones are incubated and grown in salt media for two days, and then harvested by centrifugation. Protease inhibitor is added and the protein must be kept on ice throughout the remaining steps of the process in order to prevent denaturing. The cells are then broken open by the addition of lysozyme and sonication (brief electric pulses). The protein is separated by centrifugation and filtered before loading onto the AKTA purification robot. This machine uses a nickel column and his-tag system to divide the protein of interest from the excess protein and collects the target protein for continuation. TEV is added to the protein to remove the his-tag and cleavage is confirmed by gel electrophoresis. In order to remove the cut his-tags and immidazole, the protein is filtered again through nickel agarose solution and brought to a higher concentration. The protein is then ready for crystallization. In this step the protein is placed in various chemical conditions and incubated for the promotion of crystal growth. Once a crystal has formed it is moved into the third step of the process during which it is x-rayed and the diffraction patterns are analyzed to determine the structure of the protein. The structure of the protein is directly related to the function of the protein. This can be added to a database and the combined information can be used in medical applications.

Synthesis of Polypyrrole Nanowires by Interfacial Polymerization. *XIAOHAI ZHANG (University of Washington, Seattle, WA 98195) JUN WANG (Pacific Northwest National Laboratory, Richland, WA 99352).* Polypyrrole (Ppy) is a type of conductive organic polymer composed from a number of interconnecting pyrrole monomer rings. Its conductive properties are highly valued in several areas of research, most notably as materials for sensitive biosensor applications. Therefore, in order to further this area of research, a facile and reliable method of producing such nanoscale Ppy polymers is highly sought for. The interfacial synthesis polymerization is a method of producing Ppy; it is a continuation of previous work for other conductive polymers. In this research, the use of interfacial polymerization as the synthesizing method for Ppy will be tested and analyzed. In this method, the Pyrrole monomers are dispersed into an organic solvent and an oxidant will be dissolved into an inorganic solvent. The two solvents are then placed together into a single container so that the pyrrole monomer will be oxidized at the surface of the aqueous/organic solution. Utilizing this approach, various concentrations of the monomer and oxidant were tested to determine the optimal condition for creating nanoscale Ppy wires. The synthesized polymer structures will then be analyzed by means of Scanning Electron Microscopy (SEM) for detection of its structure and size. Average Ppy diameter lengths between 100 nm to 500 nm were produced through these tests, indicating that the sizes of these nanostructures are subject to change depending on the concentration of the oxidant and monomer tested. The results of this experiment do not provide a clear characterization of specific qualities in the interfacial process because granular polymers were produced instead of intrinsically true nanoscale wires. However, data does show that various scales of nanoscale polymers could be created by treating the process with different concentrations of oxidant and monomer. Further research and experiments are still required in order to utilize this method for creating industrially exploitable Ppy nanowires.

Synthetic Biology: Widespread Use Is Closer Than You Think. *KEHINDE O'DUNIKAN (University of the Pacific, Stockton, CA 95211) DR. PETER LICHTY (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* Synthetic biology is the synthetic redesign of biological systems. Synthetic biologists' goal is to make from scratch, programmable organisms that do not currently exist in nature. Possible applications include: development of bioengineered microorganisms that can produce medicine, identify harmful chemicals, disintegrate environmental pollutants, demolish cancerous cells and remedy flawed genes; hydrogen fuel; and development of bioengineered organisms to diagnose and treat certain illnesses. Synthetic biology consists of specifying the DNA that is entered into an organism and from there determining its function in a foreseeable way. With talks about this new science in the media the public has developed some concerns about it. People worry about dual use and terrorism, who will regulate research, the ethics of synthetic biology, it becoming privatized and monopolized and if something will go wrong in the current experiments. Also, some religions see synthetic biologist as trying to play God. Synthetic Biology is so controversial that national conferences were held. The second of its kind, The Synthetic Biology 2.0 (SB2) conference was held in Berkeley, California back in May. There, scientists meet to talk about the

latest advances and applications of the science and about the security concerns as well. After the three- day conference they developed a community declaration, which will serve as a form of scientific self-governance, outlying the way researchers and companies should act to warrant openness and security of synthetic biology research. A lot of the everyday products that we take for granted would not be here without the developments in the field of biotechnology and some day we could say the exact same thing about synthetic biology. Biotechnology is used to produce everyday food products such as yogurt, cheese, beer; produce vaccines; to produce laundry detergents and dishwashing liquids; to do DNA Fingerprinting techniques for forensic investigations and paternity tests; to genetically engineer crops and agriculture; and to create human therapeutics and proteins. The possible applications can potentially save millions of lives in the near future and tremendously improve the environment. With the current research of many universities, pharmaceutical companies and even laboratories in our own communities like the Lawrence Berkeley National Laboratory, widespread use of synthetic biology is closer than you think.

The Effects of Pressure Changes and Elevated Dissolved Gas Levels on Chinook Salmon *(Oncorhynchus Tshawytscha)* and Rainbow Trout *(Oncorhynchus Mykiss).* *BROOKE SAKARA (Gustavus Adolphus College, Saint Peter, MN 56082) ABIGAIL CAPETILLO (Pacific Northwest National Laboratory, Richland, WA 99352).* As juvenile salmon pass from their spawning ground to the ocean, they must cross through hydroelectric dams. The pressure changes and elevated dissolved gas levels that occur during turbine passage can cause injury and mortality to the fish especially when combined with high dissolved gas levels within the river. Using two hyperbaric chambers, the effects of pressure changes and water supersaturation were tested on run of the river and hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*) and hatchery-reared rainbow trout (*Oncorhynchus mykiss*) at McNary Dam. The chambers were used to subject the fish to rapid pressure changes which represented passage through turbines. Fish were acclimated at the pressure present at depths of 0, 5, 10, 20, and 40 feet and at dissolved gas levels of 100, 110 and 120% saturation. The fish were allowed to recover at varying pressures for two days, and were then euthanized and necropsied. It was found that as the pressure during acclimation increased, mortality also increased in all test groups. As the total change in pressure increased, appearance of emboli in the gills, hemorrhaging inside the pericardial sac and caudal veins, ruptured swim bladder and stomach eversion also increased. There was less overall mortality in the rainbow trout than in either group of Chinook salmon. There was more emphysema of the fins at higher dissolved oxygen levels when fish were allowed to recover in supersaturated water. These experiments will be expanded to also include the rate of pressure change during turbine passage. This work is part of a multi-year study funded by the U.S. Army Corps of Engineers designed to find a method of turbine passage that will result in lower juvenile salmonid mortality rates.

The Expression and Purification of Hypothetical Yeast Protein. *JACQUELINE GIBBS (Elizabeth City State University, Elizabeth City, NC 27909) DR. S. SWAMINATHAN (Brookhaven National Laboratory, Upton, NY 11973).* Solving protein crystal structures by X-ray diffraction or NMR is essential for doing protein chemistry and novel drug design. This project involves several aspects of macromolecular crystallography; for example expression and purification of proteins, optimization of crystallization conditions by use of robot, crystallization by sitting drop vapor diffusion method, observation and analysis of the crystals under the microscope and lastly x-ray diffraction of the crystals. During our ten weeks stay at Brookhaven National Laboratory, we were successful in expressing and purifying a 29 kDa hypothetical Yeast protein by His-tag purification using nickel chelated columns and by the size exclusion method of protein purification by using a FPLC machine. We ran 1D SDS-protein gels for analyzing and confirming our results.

Thermoregulation Abilities of the 8-Way-Cross Parental Lines: A Comparative Phenotype Project. *SUSAN KENNEY (Christopher Newport University, Newport News, VA 23606) DABNEY K. JOHNSON (Oak Ridge National Laboratory, Oak Ridge, TN 37831).* In the Life Sciences Division at Oak Ridge National Laboratory a new genetic reference population, more complicated and with more possibilities than a standard (two-line) recombinant inbred (RI) population, is being developed. Eight strains of inbred laboratory mice, chosen for their genetic and phenotypic diversity, were selected to be the progenitors for the 8-Way-Cross: Mus castaneus (CAST/EiJ), New Zealand Obese (NZO/HILtJ), Non-Obese Diabetic (NOD/LtJ), 129S1/SvImJ, C57BL6/J, PWK/PhJ, WSB/EiJ, and A/J. Although each strain has been studied individually and extensively, no studies have been done on all eight strains under the same conditions. The purpose of this research is to compare these parental strains across a panel of morphometric and metabolic phenotypes and to measure the thermoregulation abilities of these mice. This is accomplished using a cold stress test, which assesses a mouse's ability to maintain its core body temperature when put into a 4°C environment. Core body temperatures were taken at the beginning of the experiment to determine a baseline temperature and then every 30 minutes for a total of 120 minutes during the test. After the test, mice were weighed and dissected and other phenotypes were measured such as spleen, liver, kidney, thymus, and heart weight. Results indicate that the NOD/LtJ males (n = 5) have different cold stress responses from the females of the same strain (NOD/LtJ males change = +0.6°C; females = -0.6°C). NOD/LtJ and PWK/PhJ females (n = 5; 6) were unable to respond to the cold, shown by a decrease in core temperature over the two hours. A/J females (n = 4) responded to the cold by quickly increasing their core temperature; however, they were unable to maintain the increase and their core temperature eventually fell lower than the baseline (change = -0.2°C). Wild-derived CAST/EiJ females maintain their core temperature during the test (change = +0.02°C). These results suggest that the leaner strains of mice, such as CAST/EiJ, can regulate their metabolism whereas NOD/LtJ females can not. These data will be contributed to MuTrack, a large scale mouse phenotype database (http://www.tnmouse.org/mutrack). Once the 8-way-cross RI strains have been established, each strain's response can be compared to the parental lines so that the genes affecting thermoregulation and other phenotypes can be identified.

Use of Indocyanine Green in Photodynamic Therapy of Melanoma. *DYLAN RODEN (Massachusetts Institute of Technology, Cambridge, MA 02139) LISA MILLER (Brookhaven National Laboratory, Upton, NY 11973).* Skin cancer accounts for over 50% of all human cancers and melanoma is the most deadly form. It is estimated that in the United States in 2006, over 62,000 people will be diagnosed with melanoma, resulting approximately 8,000 deaths. Photodynamic Therapy (PDT) is a new treatment method for many types of cancer and is a very practical therapy for treating melanoma. PDT involves a special dye, called a photosensitizer, which is absorbed by cancer cells. The dye is activated by a specific wavelength of laser light and releases oxygen radicals which kill the surrounding cells. In this study, indocyanine green (ICG), a chemical with many favorable properties for use as a PDT photosensitizer, was taken up by human melanoma cells in culture (at a concentration of 150 μM) and then these cells were irradiated with laser light (800 nm) for different exposure times (15, 30, 45, and 60 minutes). After exposure, the cells were stained with trypan blue and counted with a hemocytometer to determine the percentage of cell death. The cells were also examined using Fourier Transform Infrared Spectroscopy (FTIR) to examine chemical changes that occur as cells undergo apoptosis. Hierarchical cluster analysis was performed on the FTIR data to determine differences in treatment times. The 60 minute exposure time was most successful and corresponded with a 75% death rate in the cells. Cluster analysis showed that generally the longer exposure times clustered differently from the samples not receiving any laser in both the lipid (2600-3120 cm-1), and protein and nucleic acid (800-1900 cm-1) regions. This indicated a significant difference in chemical composition between the two groups suggesting death by apoptosis. In the future, we hope to determine a more efficient concentration of ICG to give to the melanoma cells and expose them to a more powerful pulsed laser for a longer period of time.

Using Microarray Technology to Compare Bacterial Diversity Within Different Horizons of Contrasting Soils. *DHARSHINI VENKATESWARAN (California State Polytechnic University–Pomona, Pomona, CA 91768) GARY ANDERSEN (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* There is emerging interest in understanding the linkages between above- and below ground microbial communities. As part of a large DOE funded climate change project, the Andersen lab is studying the microbiological responses of Californian grassland ecosystems. Various mesocosm conditions were set up to replicate the effects of climate change (modified temperatures and precipitation) on plant and microbial communities. Since climate change influences the function of these ecosystems, we compared bacterial diversity within different horizons of contrasting soils. Two soils compared were from Hopland, in Northern California, and Sedgwick, from Southern California. Since the pattern of growth in plant roots is directly dependent on water supply, climate change such as increased or decreased rainfall may indirectly affect the microbial communities found in different horizons depending on the growth of the roots in response to water. Generally there are different approaches to profile soil microbial diversity. One way is to use Phospholipid fatty acid analysis (PLFA), which analyzes membrane lipids; however, this

approach is not sensitive and will not effectively resolve microbial speciation. Another conventional method is cloning of the 16S rRNA gene, which requires transferring the amplified DNA fragments into a plasmid, and randomly sequencing a few hundred plasmids. In soil samples where one will expect to find at least 100,000 species then one would need to sequence 100,000 clones using this method, which is time consuming, expensive, and inefficient. Instead, a high-density photolithography microarray displaying 500,000 oligonucleotide probes complementary to diverse 16S rRNA sequences was used. This new technology, allowed us to identify species or groups of bacteria present in the soil sample more efficiently. The study showed the horizon B1 to have more microbial communities than horizon A. The observed microbial biomass also seems to increase at deeper horizons. Clostridia for example was found to be in higher microbial amounts in the 40-65 cm depth of the Hopland-B2 soil, where as Cellulomonas (Actinobacteria) and Phyllobacteriaceae (Alpha-proteobacteria) were predominately present at the top horizon (Hopland-A).

Chemistry

A Molten Salt Synthesis of Single Crystalline YBCO Nanorods. *DARYL WONG (University of California–Berkeley, Berkeley, CA 94720) STANISLAUS S. WONG (Brookhaven National Laboratory, Upton, NY 11973).* $YBa_2Cu_3O_7$ (YBCO) is a high Tc superconductor that has potential applications in both high field magnets and superconductive circuitry. Although its utility as a high field magnet has been realized, bulk YBCO loses its high temperature superconducting ability due to low critical current densities deriving from the bulk's polycrystalline nature which lacks directionality. One potential remedy, aligning monocrystalline subunits through material texturing techniques, can be achieved with the production of uniform, monocrystalline one-dimensional YBCO nanorod structures. The molten salt synthesis method has been shown to be a procedurally simple technique to create metal oxide nanorods. Using a molten salt method, attempts to make YBCO have been conducted with a number of yttrium, barium, and copper containing precursors which are combined with a salt, usually sodium chloride and/or potassium chloride, in varying precursor and salt ratios. These precursors were finely ground with a mortar and pestle and baked in a furnace to temperatures above the melting point of the salt. Powder x-ray diffraction (XRD) analysis was conducted to determine whether the molten salt samples contained the orthorhombic crystal structure indicative of high temperature superconducting YBCO, while scanning electron microscopy (SEM) and atomic force microscopy (AFM) images were taken to determine if a rod morphology had been formed. XRD analysis of the numerous molten salt products has shown that the desired orthorhombic YBCO nanorods cannot readily be formed, while SEM and AFM images show aggregates of nanorods and nanoparticles which vary in size. Other analytical techniques, including SQUID (Superconducting Quantum Interference Device) measurements, will be useful to further ascertain and characterize the properties of as-prepared YBCO nanorods. Because the mechanism of molten salt nanorod formation is not fully understood, creation of these desired nanorods involves a lot of experimentation with variable parameters. A more comprehensive analysis of precursors, precursor ratios, and baking temperatures should be performed before concluding the inefficacy of the use of the molten salt technique in the generation of nanoscale motifs of these superconducting materials.

A Search for Cerium Doped Lanthanum Oxide Scintillators. LATORIA WIGGINS (North Carolina A&T State University, Greensboro, NC 27411) DR. *YETTA PORTER-CHAPMAN (Lawrence Berkeley National Laboratory, Berkeley, CA 94720).* The need for new and improved radiation detectors, scintillators, is at an all time high due a progression in detection knowledge. Commonly used scintillators such as BGO and LSO have undesirable properties such as low luminosity, and slow decay times. Discovering new scintillators required literature searches, synthesizing and the characterization of compounds. The research at hand concentrated on cerium (III) doped lanthanum oxides. Compounds were synthesized using solid-state chemistry techniques such as ceramic and hydrothermal methods. Characterization consisted of x-ray diffraction, fluorescence spectroscopy and pulsed x-ray measurements. Several new inorganic scintillators were founded, however, findings concerning lanthanum oxide synthesis warrant further investigation of the compound.

Analysis of the Water-Splitting Capabilities of Gallium Indium Phosphide Nitride (GaInPN). *JEFF HEAD (University of Arizona, Tucson, AZ 85705) JOHN TURNER (National Renewable Energy Laboratory, Golden, CO 89401).* With increasing demand for oil, the fossil fuels used to power society's vehicles and homes are becoming harder to obtain, creating pollution problems, and are posing hazard's to people's health. Hydrogen, a clean and efficient energy carrier, is one alternative to fossil fuels. Certain semiconductors are able to harness the energy of solar photons and direct it into water electrolysis in a process known as photoelectrochemical water splitting. P-type gallium indium phosphide ($p\text{-}Ga_nIn_nP_2$) in tandem with GaAs is a semiconductor system that exhibits water-splitting capabilities with 12.4% solar-to-hydrogen efficiency. Although this material is efficient at producing hydrogen through photoelectrolysis it has been shown to be unstable in solution. By introducing nitrogen into this material, there is great potential for enhanced stability. In this study, gallium indium phosphide nitride $Ga_{1-y}In_yP_{1-x}N_x$ samples were grown using metal-organic chemical vapor deposition in an atmospheric-pressure vertical reactor. Photocurrent spectroscopy determined these materials to have a direct band gap around 2.0 eV. Mott-Schottky analysis indicated p-type behavior with variation in flatband potentials with varied frequencies and pH's of solutions. Photocurrent onset and illuminated open circuit potential measurements correlated to flatband potentials determined from previous studies. Durability analysis suggested improved stability over the GaInP2 system.

Calibration for Methane Hydrate Research Unit. *XIAE SHI (State University of New York at Stony Brook, Stony Brook, NY 11790) DEVINDER MAHAJAN (Brookhaven National Laboratory, Upton, NY 11973).* Methane hydrate, one of the most common gas hydrates, forms at low temperature and high pressure; conditions typically found below the seafloor and permafrost. Although the amount of methane hydrate trapped under the seafloor on Earth has been estimated to be enough to meet human needs for the next several hundred years, due to their dispersed nature it is very difficult to extract the hydrates1. A customized unit, named Flexible Integrated Study of Hydrates (FISH) that BNL is using for methane hydrate research, mimics seafloor conditions. In a typical process, methane gas is charged to the vessel, which initially contains a water/sediment mixture under high pressure, cooled down to 4 degrees Celsius. The hydrate formation can be visualized in the vessel through a 12-inch glass window. The kinetics of methane hydrate formation and decomposition could be studied through temperature, pressure and flow/mass meters for the duration of the experiment. The goal of my project is to test the operation and dynamics of the system, such as calibration of all flow/mass meters and BPR (Back Pressure Regulator), as well as testing the system's cooling rate. Preliminary results show that the system is well suited for hydrate formation. Volumetric balances at the inlet and outlet reveal a discrepancy of approximately 4 ml, which is well within tolerances for experimental error. Heat transfer analyses revealed a maximum cooling rate of 0.293°C/hr using a tube-like heat exchanger with forced convection in conjunction with a thermally controlled water-ethylene glycol bath.

Characterization of GaInPN:Si Tandem Cells for Hydrogen Production from Photoelectrochemical Water Splitting. *PAUL VALLETT (University of Vermont, Burlington, VT 05405) DR. JOHN TURNER (National Renewable Energy Laboratory, Golden, CO 89401).* In order for hydrogen to be part of a renewable energy infrastructure, it must be produced from a renewable energy resource. The direct photoeletrolysis of water using certain types of semiconductors have been known to split water using absorption of solar energy, but difficulties concerning efficiencies and corrosion have limited this technology. This research focused on the ability of GaInPN grown on a silicon substrate to efficiently split water. Photocurrent spectroscopy determined the band gap of the material to be 1.96 eV, which is above the necessary 1.7 eV required for water splitting. Mott-Schottky analysis, photocurrent onset, and open circuit potential were used to determine potential of the Fermi level of the system in relation to the redox potentials of hydrogen and oxygen formation. These techniques showed that the Fermi level lied just below the oxygen redox potential. The electrodes were platinized and short circuit current density measurements under air mass (AM) 1.5 illumination determined extent of water photolysis. Unbiased water splitting was achieved, at a maximum of 0.65% solar to hydrogen conversion efficiency (SHCE). Corrosion of the semiconductor in solution was determined by applying a standard current to the electrode while in solution and using profilometry to estimate the volume of semiconductor removed. On average a 0.1 μm deep well was etched into the material after 24 hours. Incident photon current efficiency (IPCE) measurements of 30% revealed that the growing process for nitrogen addition to the sample decreased the electronic properties of the material. While this system is able to produce hydrogen from water using solar power as the only energy input, and the addition of nitrogen to the material appears to have increased its durability, the material suffers a heavy loss in