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Student Abstracts: Biology at ORNL

A Reagent-less Fluorescent Sol-gel Biosensor for the detection of Hydrogen Peroxide. SERENE WILLIAMS (Knoxville College, Knoxville, TN 37921) GUY GRIFFIN (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

This work describes the development of a reagent-less fluorescent sol-gel biosensor for the analysis of hydrogen peroxide (H₂O₂), and its potential application in near-real-time monitoring of H₂O₂ generated from aqueous extracts of cigarette smoke. H₂O₂ occupies a central role in oxygen metabolism, is produced by various reactions in several subcellular compartments and is the precursor to other reactive oxygen species (ROS). It is currently unknown which of the many ROS present in, or produced by, cigarette smoke are responsible for most of the oxidation damage in vivo. Thus, procedures for measuring and quantifying ROS in cigarette smoke must be developed. Cigarette smoke produces ROS, including H₂O₂. The interaction of H₂O₂ and other ROS can lead to oxidative damage to the lungs and other vital body organs, possibly resulting in cancer. The development of a biosensor device will enable the monitoring of extra-cellular fluid in the lungs of individuals exposed to cigarette smoke; therefore enabling the quick, sensitive detection and quantification of H₂O₂. The primary goal of this summer research project is to investigate the applicability of the sol-gel encapsulation technique for the immobilization of both a fluorescent indicator and an enzyme catalyst for the detection of H₂O₂. Sol-gel encapsulation has opened up an interesting new way to immobilize biological materials, which include proteins, enzymes, antibodies and even whole cells. Carefully prepared silica-based sol-gels are able to retain the bioactivities of embedded biomaterials and remain accessible to external reagents by diffusion through the porous silica. The Amplex Red assay was used for fluorometric detection of H₂O₂. Amplex Red reagent (a non fluorescent compound) in the presence of horse radish peroxidase (HRP) enzyme reacts with H₂O₂ to produce the red fluorescent compound resorufin. Sol-gel encapsulation of both Amplex Red reagent and HRP was carried out by mixing an Amplex Red reagent working solution with a sol stock solution containing tetramethyl orthosilicate (TMOS) and acid water. Glass slides were spin-coated with the liquid sol solution to form sol-gel thin films. These films were kept in the dark, allowing for gelation, aging, and drying before use. Calibration curves were constructed to determine the fluorescence intensity versus different H₂O₂ concentrations.

Benchmarking Best Practices Of Diversity In The Workplace. KATRINA SMITH (University of Alabama in Huntsville, Huntsville, AL 35899) MYLISSA BUTTRAM (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

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Companies in America that embrace workforce diversity as a bottom-line business imperative priority, have implemented various strategies to promote the concept of "inclusion" in the workplace. Inclusion in the workplace is a management approach that fully utilizes all people in the workforce that enables them to achieve their full potential, and maximizes their contributions toward the mission of the company or organization. The goal of this research project is to find the best in class companies focusing on diversity and improving inclusion in the workplace. Diversity studies acknowledge that different approaches have extensive value to the organizational success of companies by supporting and utilizing the distinctive knowledge base and capabilities of every individual. Best practices for inclusion and addressing underutilizations for underrepresented women and minorities are to be evaluated in the survey as well. Supportive data used to sustain the fundamental goals of this project will explore the mainstream views of diversity by defining: (1) Affirmative Action (2) Equal Employment Opportunity and their implications for inclusion in the workplace. The tool of measurement was a survey constructed for easy online submission. The construction of the survey included questions derived from a booklet entitled, Best practices In Achieving Workforce Diversity, it was based on similar research conducted through telephone interviews and contact persons from national laboratories operated by the Department of Energy. The result of this survey may be used to influence management strategies to strengthen the Oak Ridge National Laboratory workplace diversity initiatives. The final results of this project are fourth coming. However, there are some projected items that may impact the outcome of the research and they are the incompatibility between the electronic survey data base, designated spreadsheet, and functionality of personal computers, a short response and turn around time request, lack of time to complete the survey, and low priority by participants. ORNL Diversity representatives will have an opportunity to review the research and contribute to the final research.

Creating Functional Divalent Antibodies. GABRIELA KIRK (University of Texas, Austin, TX 78705) STEPHEN J. KENNEL (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

Antibodies to tumor antigens are used to deliver radioactive isotopes to tumor cells. Phage display, a new way of identifying antibodies, has been used to find a special antibody form called a single chain variable fragment antibody (scFv) that binds to laminin. This scFv, 15-9, homes to tumors in mice. ScFvs are composed of a single variable heavy chain sequence and a single variable light chain sequence which fold together to form a single binding site. To try to improve scFv 15-9 performance, attempts were made to produce divalent forms of the scFv in hopes that they would have a higher affinity for the target, laminin, and thus better binding to the tumor. Three methods were used to prepare divalent antibody in order to determine the validity of the theory. The first method uses molecular biology to shorten the linker between the two variable regions of each scFv allowing two different scFvs to crosslink. The next method is to produce a bivalent antibody chemically. Traut's reagent introduces a free sulfhydryl group into one batch of scFv and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) introduces an active maleimide group into another batch of scFv. When these two scFvs are mixed, the maleimides react with the sulfhydryls forming thioether bonds and create bivalent products. Finally, non-covalent

dimers were prepared using protein L or anti-myc, bivalent reagents that bind two scFvs together. Protein L binds to the VL regions of certain scFvs and anti-myc binds to the myc tags on the end of each scFv. By binding to two scFvs at their VL regions or myc tags, non-covalent dimers are produced. Results for the molecular biology approach are still pending. The results for the second approach show that a functional bivalent antibody can be produced only when certain amounts of Traut's and Sulfo-SMCC are added to the scFvs: adding more created a globular clutter and adding less meant not enough reaction to create a functional dimer. Thus the yield of dimer from this approach was low. The final approach showed that a functional dimer could be produced and was, in fact, more efficient at binding to the protein than a monovalent antibody. Through the various experiments using the three separate methods, it was shown that dimerized scFvs are more active than single valency forms at binding laminin in vitro. Further testing is necessary to determine if the dimer scFvs perform as better tumor targeting agents.

Development Of A Framework Map To Identify Candidate Genes Involved In Carbon Allocation And Partitioning In Populus. KELLY RAMIG (Knox College, Galesburg, IL 61401) LEE GUNTER (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

Carbon sequestration is the process by which a plant takes in CO₂ from the atmosphere and through photosynthesis converts it into carbohydrates that are stored in different parts of the plant such as the stem, roots, or leaves. The overall goal of this project is to enhance the quantity and longevity of carbon sequestered in long-lasting carbohydrate sinks, such as the trunk or root system, instead of short term sinks, such as leaves. If trees grown in plantation settings can be genetically improved to store additional amounts of carbon in long-term carbon sinks, our atmospheric CO₂ could be lowered by as much as 1.2 Gt per year globally. In order to better understand genetic control of carbon sequestration in trees, a genetic map of *Populus* can be used to identify candidate genes from the recently sequenced poplar genome. Genetic markers, specifically simple sequence repeats (SSR), are being used to identify framework loci in a subset of progeny from an interspecific mapping pedigree of > 1,000 progeny. Over the past three months, genomic DNA from two parents and 44 progeny has been extracted and quantified for amplification of SSR loci using touchdown PCR and fluorescent genotyping of alleles through capillary electrophoresis. To date, 62 out of roughly 250 loci needed for the framework map have been genotyped. Approximately 93% of these loci exhibit segregation consistent with that seen in a related pedigree. The fidelity of marker inheritance from one pedigree to another suggests that SSR markers represent the best choice for genetic mapping across pedigrees and implies a high degree of utility for fine scale mapping of genes involved in carbon sequestration.

Efficient Research Exposure Via XML. JANE MEYERS (East Tennessee State University, Johnson City, TN 0) T.A. BODEN (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

Even in the most widely published journals, the lead time between manuscript submission and final publication often exceeds a year. For many researchers, it is

important to efficiently and effectively communicate findings in a timely manner. The inefficiency of scientific paper publication entails not only the comprehension of the material but the unnecessary downtime between research disclosure and the biannual journal publication. In efforts to adapt to the new era of technology, we are creating a virtual newsletter in replacement of our annual publication. The clear advantage of an electronic interface for searching, reading, and efficiently publishing and updating research, is obvious. It is important that even electronic articles have a standard format. A relatively new language that includes the capabilities for creating archetypes for a web based documents is xml. Since browsers currently do not support xml we chose to use the Xmlspy editor for this project. Because Xml is object oriented, the first step involves developing a schema with appropriate elements and attributes. Logistically creating a form in which the input fields serve as empty elements is a simple method for the general user to create an xml document. Stylevision, a separate Xmlspy program allows the creation of article templates, including conditional statements, graphics, and placement of inputted data. Pulling the project together was the program Authentic, where a form is completed and an html is file created via the xslt translator. The resulting html article is automatically saved on the server where it will later be parsed and sorted into the appropriate newsletter subsection. Future work with new versions of Xmlspy will hopefully allow manipulation of meta-tags and facilitate the addition of dynamic graphics.

Energy Production from Bovine Waste. WHITNEY RIDENOUR (University of Tennessee, Knoxville, TN 37912) K. THOMAS KLASSON (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

As energy is needed to overcome dependence on coal and oil, methane production from biomass has the potential to help limit the amount of fossil fuel used. This idea also supports the guidelines of the Clean Air and Energy Policy Act, since biomass represents a clean fuel source that can reduce SOX emissions, NOX emissions, and methane released into the atmosphere. In the past, studies have been completed on a small-scale at Oak Ridge National Laboratory to determine if bovine manure could be used as a clean method of energy production. Current studies are focusing on larger scale experiments in a 100 L anaerobic digester. The pilot-scale digester was operated for several months, and data were collected and compared with results from smaller-scale experiments. A gas chromatograph was used to measure the compositions of the produced gas, a wet gas test meter was employed to measure the amount of gas produced, and a high performance liquid chromatograph was used to determine the quantity of organic acids in the digester feed and effluent. Results showed the methane production depended on the rate at which volatile solids (the manure ingredient converted to methane) were fed into the digester. The performance of the pilot-scale digester was comparable to that of model predictions. Although more experiments need to be completed, this pilot-digester experiment was successful and the information could be used to design an industrial-scale digester to produce clean energy.

Fluctuation-Dissipation Theorem Predicting Microcantilever Noise. SARIT BARHEN (Emory University, Atlanta, GA 303221) THOMAS THUNDAT (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

The past few years have shown an incredible growth in micromechanical sensor development. As more demands are placed upon these sensors, the quality and accuracy of their sensing ability has become critically important. No longer can the implications of white noise be avoided, as sensors have become too receptive, and are significantly affected by the presence of white noise. Such is the case of the microcantilever system, where simple thermal fluctuations drive the system into Brownian motion. However, this natural phenomenon has been exploited and is now a key component of a new methodology that uses amplified thermal vibrations to drive the cantilever via a delayed feedback loop. With a variety of implications, this technique has improved the system's Q-factor by several orders of magnitude. To better understand and improve the effects of this phenomenon, the Fluctuation-Dissipation Theorem is applied to a theoretical microcantilever model. Dependent on components such as the temperature and damping magnitude of the model, it has the power to predict the strength of the thermal vibrations affecting the system. The theorem is analytically applied to a mass-spring model of the cantilever system, and implemented in a computer simulation on the program XPPAUT. XPPAUT allows experimentation with temperatures oscillation patterns. The theorem's ability to predict white noise as well as confirm improved Q-factor is then verified.

Frequency Domain Analysis of Noise in Cellular Systems. BRYCE GALEN (University of Virginia, Charlottesville, VA 22903) MICHAEL SIMPSON (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

Signals within cellular systems have an inherent degree of variability or "noise," which cannot accurately be modeled by purely deterministic differential equations. We have created a more accurate model by employing a frequency domain Langevin approach, which adds noise terms to the differential equations that correspond to the points of molecular synthesis and decay. We are attempting to verify this model experimentally by tracking selected single cells of *Escherichia coli* K-12 transfected to constitutively express Green Fluorescent Protein (GFP) during the log phase of growth. An *E. coli*-GFP system is suitable for the measurement of cellular noise because transcription and translation variance is manifest in protein fluorescence levels over time. Our technique involves the capture of cellular images over an extended period of time using a laser confocal microscope fitted with photomultiplier detectors. Images are digitized by a data acquisition computer, and imaging software is used to measure fluorescence intensity values per unit area within the boundaries of a single cell. Second-order (Fourier) analysis of this experimental data identifies the frequency components of the noise, which can readily be compared to the analytical results of our Langevin-based model. Focusing on a signal from a single cell in the frequency domain allows us to directly perceive relevant noise, free from the first-order variance among an entire group of cells. It is our hope that insights arising from this work will eventually give clues as to how cellular systems maintain robust functionality despite high levels of noise.

Gene-driven Mutagenesis by RNA-based Mutation Scanning of the. RACHEL GOLDSTON (Cedarville University, Cedarville, OH 45314) MITCHELL KLEBIG (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

Since the human genome has been sequenced, efforts are now focused on determining the function of the approximately 30,000 human genes. The Mammalian Genetics program at ORNL is determining the function of the genes on human chromosomes 5, 16 and 19, the three chromosomes sequenced by the Department of Energy, using analysis of induced single-nucleotide mutations in the orthologous mouse genes. They developed a unique resource called the Cryopreserved Mutant Mouse Bank (CMMB), a collection of DNA, frozen tissues, and sperm from 4,000 male progeny of N-ethyl-N-nitrosourea (ENU) mutagenized mice that contain many point mutations throughout their genome. The genes to be studied are chosen based on four criteria: either no reported mutations or existing mutations are embryonic lethal, conservation over distantly related genomes, well-defined protein domains, and restricted patterns of expression. Because of its large scale, the protocols for this functional-genomics initiative must be fine-tuned to ensure high-throughput screening for mutant genes. In this project, an RNA component of the CMMB is being developed and screened for mutations in the following way: (1) RNA is extracted from the frozen tissue, (2) first-strand cDNA is synthesized, (3) regions of the gene are PCR amplified in a 96-well format, and (4) the PCR products are screened for possible point mutations and size alterations by Temperature Gradient Capillary Electrophoresis (TGCE) and gel electrophoresis, respectively. Currently two genes are being studied: Dnm2 and Pak4. RNA extraction and cDNA preparation have been initiated (about 250 samples completed) and the protocols optimized for large-scale work. It has been shown that PCR and TGCE multiplexing with up to three different amplicons are successful, which will save time and resources. Three possible mutants have been flagged by TGCE analysis, but at this point no size-altering mutations have been found. The putative mutants are being sequenced to confirm and characterize the mutations. If the mutations are predicted to have a significant effect on the structure of the protein, then live mutant mice will be recovered by intracytoplasmic sperm injection using the cryopreserved sperm. These heterozygous mice, as well as homozygous mutant progeny from intercrossing, will be evaluated for phenotypes. This gene-driven approach to mutagenesis can be used to study any gene of interest, including genes suspected of involvement in human diseases.

Mutational Screening of Mouse Genes to Better Understand Human Gene

Function. JAMES WILLIAMS (Bethune-Cookman College, Daytona Beach, FL 32114)
MITCHELL KLEBIG (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

Identifying and studying mutated genes in mice allows for better comprehension of human gene functions, which may lead to a greater understanding of diseases and disorders associated with those genes. ORNL's Mammalian Genetics group has generated a mutation screening resource called the Cryopreserved Mutant Mouse Bank (CMMB). The CMMB samples consist of DNA, tissues and sperm from 4,000 C57BL/6Jrn male mice, that are heterozygous for a large number of N-ethyl-N-nitrosourea (ENU) induced point mutations throughout the genome. The 4,000 DNAs from these mice are being used to screen for possible mutations at the genomic DNA level. DNA fragments of genes of interest are rapidly generated from the CMMB DNA archive by Polymerase Chain Reaction (PCR) amplification. Subsequent heteroduplex analysis of the PCR products, performed with Temperature Gradient Capillary Electrophoresis (TGCE), identifies those that contain single base changes, which are

then sequenced to determine the precise nucleotide change. The primary genes studied in this project are Ap2s1, which is involved in endocytosis, and Myd88, involved in the immunological reaction to viral and possibly other lethal infections such as anthrax. A heteroduplex was identified by TGCE in a PCR product containing exons 3 and 4 of the Myd88 gene. This PCR product was sequenced and found to contain a G to A nucleotide change in exon 3, which changes the open reading frame of the gene from a valine (V) to a methionine (M) codon for amino-acid residue #175 of the encoded protein. Since this V amino acid is highly conserved in vertebrates, mice containing this mutation can be rederived by intra cytoplasmic sperm injection (ICSI) from the frozen sperm of the mouse whose DNA carries the mutation. Mice homozygous for the mutation can then be studied for phenotypic effects of the mutation.

Small Angle Scattering Studies on Spinach Photosystem I. KATIE HELTON (University of Tennessee, Knoxville, TN 37996) ELIAS GREENBAUM (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

Oxygenic photosynthesis is driven in series by Photosystems I (PSI) and II in the thylakoid membranes of higher plants and algae. PSI operates by a photo-induced activation of the special chlorophyll pair, P700, thereby translocating electrons from the lumen to the stroma generating NADPH. This study seeks to use small angle scattering techniques to investigate the dynamic structural changes that occur in PSI after photon absorption and during intermolecular electron transfer via the electron relay proteins plastocyanin and ferredoxin. PSI was initially isolated from spinach thylakoids by solubilization with 0.8% Triton X-100 followed by density gradient centrifugation. Further purification of this preparation was achieved by anion exchange chromatography on a HiTrapQ column using a Fast Performance Liquid Chromatography (FPLC) system. PSI was bound to the column under low ionic strength conditions and eluted with a linear salt gradient. The fractions with the highest PSI concentrations were pooled and further concentrated. The purity of the pooled fractions was analyzed spectrophotometrically based on a protein concentration of 1.15 mg/ml, Chl/P700 of 214 and a Chl a/Chl b ratio of 3.89. Native Deriphat gel electrophoresis was performed to determine the composition of the preparation in the concentrated PSI fractions. Small angle x-ray scattering (SAXS) studies were performed at the APS, Argonne to determine the size and shape of PSI in 0.1% and 0.5% Triton X-100 solutions using a range of PSI concentrations. The scattering data proved to be reproducible over the PSI concentration range employed and between the two detergent concentrations. The shape of PSI was an elongated cylinder with a radius of gyration of 63Å which is very similar to the radius of the published crystal structure. SAXS data from PSI prepared by gradient density centrifugation only revealed the formation of a lamellar sheet like structure with no measurable size; therefore, the implementation of anion exchange chromatography improved the quality of the sample. An enzymatic assay for determining the activity of PSI using the electron transfer from plastocyanin to ferredoxin via PSI will be used to determine binding properties of the electron carriers to PSI and overall efficiency of PSI in translocating electrons across the membrane. The data from this work will be used to design further SAXS experiments to investigate intermolecular electron transfer processes.

Small Angle Scattering Studies on Spinach Photosystem I. KATIE HELTON
(University of Tennessee, Knoxville, TN 37996) HUGH O'NEILL (Oak Ridge National
Laboratory, Oak Ridge, TN 37831)

Oxygenic photosynthesis, the conversion of sunlight to chemical energy, is driven in series by Photosystems I (PSI) and II in the thylakoid membranes of higher plants and algae. PSI operates by a photo-induced activation of the special chlorophyll pair, P700, thereby translocating electrons from the luminal side to the stromal side of the membrane to generate NADPH. This study seeks to use small angle scattering techniques to investigate the dynamic structural changes that occur in PSI after photon absorption and during intermolecular electron transfer via the electron relay proteins plastocyanin and ferredoxin. PSI was isolated from spinach thylakoids by solubilization with 0.8% Triton X-100. A variety of detergents were tested to further purify the PSI preparation. These included 0.4% sodium dodecyl sulfate, 2.0% octyl- β -glucoside, and 0.15% dodecyl maltoside/ 0.2% Zwittergent. The purity of each preparation was analyzed based on Chl/P700 and Chl a/Chl b ratios obtained using visible absorption spectrophotometry. It is determined that solubilization with dodecyl maltoside/Zwittergent 16 yields the lowest Chl/P700 ratio. Small angle x-ray scattering (SAXS) studies were performed to determine the size and shape of PSI in a 0.5% Triton X-100 solution. An absolute size for the complex could not be determined but the initial data suggested that the PSI complexes had assembled into a lamellar sheet-like structure. Native Deriphat and lithium dodecyl sulfate (LDS) gel electrophoresis were used to compare the composition of PSI preparations solubilized with different detergents in order to further improve detergent optimization and verify the relative purity of each sample. It is concluded that dodecyl maltoside added to a Triton X-100 solubilized preparation at 0.5% yields complete solubilization when subjected to Deriphat-PAGE. In order to determine the subunit stoichiometry of each green band in the native gels, two dimensional gradient SDS-PAGE is currently being carried out.

Studying Gene Function by Identifying Mutations in Mice Through RNA Analysis.
GENEVA BURCH (Bethune-Cookman College, Daytona Beach, FL 32114) MITCHELL
KLEBIG (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

With the recent analysis and completion of the mouse and human genome, scientist have been able to use the DNA sequence of mice to allow further exploration of disease and other gene related disorders that occur in humans. The DOE's Joint Genome Institute sequenced chromosomes 5, 16, and 19. To help determine the functions of the human genes on these chromosomes, ORNL's Mammalian Genetics group is mutagenizing the mouse orthologs of the genes. In order to do this, DNA, tissue, and sperm of 4,000 C57Bl/6J R_n male mice carrying chemically induced mutations, referred to as the Cryopreserved Mutant Mouse Bank (CMMB), have been isolated and cryogenically archived. Currently the entire bank of DNA is being screened for mutations (see J Williams abstract in this book). In addition to looking for mutations in DNA, RNA can be isolated from the tissue of the mice and cDNA can be screened for mutations. Screening for mutations in cDNA covers more coding region per amplicon and thus more efficiently identify mutations. During this summer's research project, an additional 110 samples of RNA were extracted from CMMB tissues and used to make first-strand cDNA templates. The first 368 CMMB cDNA templates are

now being screened for mutations in two genes, dynamin 2 (Dmn2) and p21-activated kinase (Pak4), by heteroduplex analysis of reverse transcription-polymerase chain reaction (RT-PCR) products derived from these genes. In the future, any RT-PCR products that are found to contain heteroduplexes by a technique called Temperature Gradient Capillary Electrophoresis (TGCE) will be sequenced to confirm the presence of the mutation and define the exact nucleotide change. Any significant mutations identified (e.g., amino acid substitution) will be introduced into live stocks of mice by Intracytoplasmic Sperm Injection (ICSI) using the frozen sperm from the mouse in which the mutation was identified. The amplicons of the two genes are presently being analyzed by RT-PCR amplification and TGCE and the results will be reported.

The use of Fluorescence Techniques to Quantify Hydrogen Peroxide in Cigarette Smoke. SERENE WILLIAMS (Knoxville College, Knoxville, TN 37921) GUY GRIFFIN (Oak Ridge National Laboratory, Oak Ridge, TN 37831)

This work describes the application of fluorescence measurement techniques for the analysis of hydrogen peroxide (H₂O₂) production in cigarette smoke. It is currently unknown which of the many reactive oxygen species (ROS) present in, or produced by, cigarette smoke are responsible for most of the oxidation damage in vivo. Thus, procedures for measuring and quantifying ROS in cigarette smoke must be developed. The end goal of this project is to develop a biosensor device for measuring H₂O₂ within extra-cellular fluid of vital body organs such as the lungs. Cigarette smoke produces ROS, including H₂O₂. The interaction of H₂O₂ and other ROS can lead to oxidative damage to the lungs and other body organs, possibly resulting in cancer. The biosensor device will enable the monitoring of extra-cellular fluid in the lungs of individuals exposed to cigarette smoke; therefore enabling the quick, sensitive detection and quantification of H₂O₂. In this study, the amplex red reagent assay was used for fluorometric detection of H₂O₂. Amplex red reagent (non fluorescent compound) in the presence of horse radish peroxidase (HRP) enzyme, reacts with H₂O₂ to produce the red fluorescent compound resorufin. Resorufin has excitation and fluorescence emission maxima of approximately 563nm and 587nm respectively. Using a standard stabilized solution of H₂O₂, calibration plots of fluorescence intensity versus H₂O₂ concentration were constructed. H₂O₂ concentrations in aqueous solutions resulting from bubbling 5 puffs of cigarette smoke samples were also determined from calibration plots. Micro molar levels of H₂O₂ were detected in aqueous solution of cigarette smoke samples. In addition, measurements over time of H₂O₂ production in cigarette smoke samples bubbled through phosphate buffer saline (PBS) showed an increase of H₂O₂ up to 120 minutes reaching a plateau thereafter. Other analytical techniques can also be used to detect H₂O₂ production from cigarette smoke, but these require a prior separation step (e.g. HPLC). This work demonstrated the feasibility of measuring H₂O₂ production in cigarette smoke without prior separation from total particle matter. Further studies to investigate H₂O₂ concentration in a single puff of cigarette smoke bubbled through aqueous solution, are under way. This work will lead to safer manufacturing processes of cigarettes, which will decrease the level of ROS injuries to lungs.

Thermal Electrochemical Synthesis Of Silver Nanocrystals. CAMERON ERICSON (Lawrence University, Appleton, WI 54911) MICHAEL HU (Oak Ridge National

Laboratory, Oak Ridge, TN 37831)

A thermal electrochemical synthesis (TECS) process has been developed to create "naked" (free from organic molecule capping) silver nanocrystals of approximately ten nm or smaller in diameter suspended in an aqueous solution. The ultimate goal is to achieve a monodisperse silver nanocrystal sol of a given concentration. Dr. C. Easterly (Life Sciences Division, ORNL) suggests that silver nanocrystals have potential applications in medicine. A systematic study of various silver TECS process factors has been performed to develop a general understanding of formation of naked silver nanocrystals in order to improve the yield of synthesis. Studies were conducted using different types of reaction containers with varying temperature, electrode distance, and reaction time with applied electrical potential. Light scattering measurements are conducted with a Dynamic Light Scattering (DLS) setup to determine nanocrystal size and size distribution. Scanning Tunneling Electron Microscope (STEM) is used to visualize the images of nanocrystals. UV/ Vis Spectrophotometer is used to quantify silver concentration in solutions as well as provide information in size. Our studies developed preliminary correlation between particle size, growth kinetics, and process factors. This relationship will allow process optimization, which could provide small (<10 nm) nanocrystals for further investigation of properties of monodispersed nanocrystals.