NC Project SEED

Progress Report 2010

NC Project SEED
Mission Statement

Our mission is to recruit, financially support, and encourage, talented disadvantaged North Carolina high school students to pursue terminal graduate and professional school degrees in chemistry, chemistry-related science disciplines. Project SEED will achieve this goal by providing a comprehensive scientific research internship experience.

We define disadvantaged as

- students from households with a low family income (as determined by the ACS), or
- underrepresented minority (African-Americans, Latinos, and Native Americans) in science and engineering fields, or
- first generation of their family to attend college, or
- students from schools designated as priority schools by the Manning ruling

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II. OVERVIEW

Science Educational Experience for the Disadvantaged (Project SEED) Program Introduction

Project SEED, places talented *disadvantaged* North Carolina high school students in academic, industrial, and government research laboratories for 8 weeks during the summer to experience "hands-on" research. Each student completes a chemical research project under the supervision of a principal investigator (PI) and receives an educational award. The 2009-2010 (19^{th}) edition of this program was highlighted by the expansion of this program from a local commuter program to a statewide residential and commuter program. The residential students were housed at Duke University.

The American Chemical Society is a non-profit professional organization that consists of over 151,000 members, that 1) promotes the public's perceptions and understanding of chemistry and the chemical sciences through public out reach programs, 2) public awareness campaign, and 3) career development assistance, and employment opportunities for students and professionals in academia & private industry. There are 188 Local Sections of the ACS across the United States. Local Sections enable members to communicate and interact with other chemists in their area and contribute to the public understanding of chemistry in their communities. The North Carolina Local Section is the largest affiliate in the state of North Carolina that covers the entire Research Triangle Area.

The Hamner Institutes for Life Sciences houses the Project SEED Program and provides administrative resources and support for the program.

The Burroughs-Wellcome Fund Grant made possible the expansion of the program, especially the statewide residential component. The number of student participants increased, and the quality of enrichment activities improved to provide the most comprehensive learning experience in scientific research experience possible. Enrichment activities included instruction on scientific methodology, scientific research, SEED Seminars, scientific ethics, and career exploration . Field trips to science facilities and learning more about the importance of science in society and the opportunities available to them.

III. ACTIVITIES

Facilities-The Project SEED office is housed at The Hamner Institutes for Health Sciences (formerly The CIIT Centers for Health Research) occupies a main building of 110,000 square feet and a new laboratory annex of 28,000 square feet on a park-like, wooded site of 56 acres in Research Triangle Park, North Carolina.

For over a quarter of a century The Hamner Institutes for Health Sciences has been training the scientists of tomorrow. Its education programs have provided well-prepared researchers for the chemical and pharmaceutical industries, government research and regulatory agencies, and universities around the world. The Hamner Institutes for Health Sciences offers postdoctoral fellowships and traineeships, predoctoral fellowships, and research experiences for undergraduate students.

The Hamner Institutes for Health Sciences provides the headquarters for the ACSNCS Project SEED. Project SEED participants hold meetings including SEED Academy at and formally present their annual research projects in seminars held in The Hamner Institutes for Health Sciences auditorium.As the Administrative home for Project Suc-SEED, The Hamner Institutes for Health Sciences will provide computer facilities, office space, library resources, conference center resources and access to staff members skilled in government grant administration and reporting.
, centrally located in the Research Triangle Park, NC. Orientation, Seed Seminars,

Laboratory Facilities- Duke, the University of North Carolina at Chapel Hill (UNC-CH), and North Carolina State University (NCSU) are all major category Research I institutions with facilities that are ranked among the best in the world. Duke University and UNC-CH both have nationally ranked Medical Centers with UNC-CH having a Dental School which includes a Dental Research Center.

Orientation-The students and parents received an Orientation that provided them with staff introductions, program expectations, program rules and regulations, student contracts, and an initial session of Research 101.

SEED Academy for Leadership Training in Sciences (SALTS)- Held at The Hamner Institutes for Health Sciences, Seminar occurred on Saturdays after students completed their Practice SAT Diagnostic Test. Topics covered include Oral Presentations, Poster Presentations, Bioethics, and Careers in Chemistry. During the year we now meet one Saturday per month.

Program Symposium- The student participants presented their research findings at The Hamner Institutes for Health Sciences on July 31-August 1, 2009. Each student presented their findings in a 10 minute oral presentation with "Power Point "visuals.

Awards Ceremony- The Spring Awards Ceremony salutes the departing seniors and recognizes in achievements in Science Competitions and SAT Scores.

- **Princeton Review SAT Preparation courses** were provided to our students to achieve the highest scores possible, that may result in greater scholarship opportunities

Field Trips-This year we visited:

- The Meyerhoff Scholarship Program at University of Maryland at Baltimore County. Students received inspiration and motivation from Mr. LaMont Toliver, Director of the Meyerhoff Scholarship Program. Over ten of our students have matriculated to this program
- NC Project SEED was the guest of Wake Forest University.
- Winston Salem State University
- Civil Rights Museum, Greensboro, NC

Follow-up Activities

The student's paper provides the foundation for the oral presentation and poster session. These follow-up activities will allow the students to build upon their summer experiences, sustain the intensity of the Summer I experience into Summer II. Attendance at these conferences will allow students to interact and network with other minority scientists and students. Members of these organizations provide the students with role important contacts for advancing their careers. Students develop and gain confidence in their research and presentation skills through their experience at these meetings.

IV. STUDENT PARTICIPATION

This year we incorporated the expertise of our Scholarship Coordinator, Ms. Faye McNeal. Ms. McNeal has over 30 years experience as a counselor. As a result we had approximately over 300 applicants for 30 spaces. Each applicant was interviewed and our final selections were made. Of the students selected, half were ranked number one in their class and the other half were were ranked in the top 5% of their class.

Announcements of the program were provided by the program to the participating schools for distribution to local high school counselors. Eligible applicants are those students that will meet guidelines established for the Project SEED program. The students applied on –line at our website(www.ncprojectseed.org). The student application process was organized by our Scholarship Coordinator, Ms. Faye McNeal and screened by the Project SEED Staff. The initial screening criteria will include the students grade point average, grades, End-of - Course Test Scores, Honors and Awards, Extra-Curricula Activities, a writing sample, and current Science Teacher Recommendations. The top applicants will be invited to an interview by the same committee and then selected.

NC Project SEED 2010 Participants

DUKE

NAME	GENDER	ETHNICITY	GRADE	SCHOOL
William Bell	**M**	**AA**	**12**	NCSSM/KIPP Academy
Selina Boyd	**F**		**11**	Northampton East
Delvin Bryant	M	AA	11	Kinston
Kaylen Cutler	F	AA	10	Whiteville
Jasmine Dunham	F	AA	11	E. E. Smith
Alexis C. Evans*	F	NA	11	Warren New T.
Alexis Flen	F	AA	11	NCSSM/TERRY SANFORD
Joshua Harris	M	AA	11	Sandhoke E.C.
Lester Heckstall	M	AA	11	Bertie STEM
Calvin Lee	M	AA	11	Hoke
Nwanaji-Enwerem	M	AA	11	Concord
Frank Tillman III	M	AA	11	Terry Sanford
LaManuel White	M	AA	11	New Hanover
Timothy Woodard	M	AA	10	Dudley

NCSU	GENDER	ETHNICITY	GRADE	SCHOOL
Taylor Adair	F	AA	10	Enloe
Dezerae Barnes	F	AA	10	Broughton
Kimberly Barnes	F	AA	11	Broughton
Melissa Chan	F	A	11	Enloe
Jon Chang	F	A	11	Enloe
KaDesia Hawkins	F	AA	10	Enloe
Micheala Jones		AA	11	Knightdale
Mark McKay*	M	AA	10	Wakefield
Vik Mukherjee*	M	A	11	Wake Forest
Jalissa Smith	F	AA	11	Sanderson
Chelsea Sumner	F	AA	11	Knightdale
Bryan Sumner	M	AA	10	Knightdale
Danielle Tyson	F	AA	10	Enloe
Rachel Williams	F	AA	11	Broughton
Avery Young	M	AA	10	Enloe

UNC

NAME	GENDER	ETHNICITY	GRADE	SCHOOL
David Bullock	M	AA	11	Clement E. Col.
Kimberly Clark	F	AA	11	Clement E. Col.
Crystal Johnson*	F	AA	11	Hillside
Jaren Mack	M	AA	10	City of Medicine
Joshua Martin*	M	H	10	Hillside
Alyssa Morgan	F	AA	11	Middle College
Jeehae (Dorothy) Nam	F	A	10	Hillside
Malika Rauf	F	AA	11	East Chapel Hill
Cheyenne Stewart	F	AA/NA	11	Northern
Marc Webb	M	AA	11	Clement E. Col.

AA - African American A-Asian C-Caucasian H-Hispanic NA-Native American O-Other

*no longer with the program

V. SAMPLES OF STUDENT WORK

STUDENT ABSTRACTS

1. **"Analysis of Gene Required for DNA Damage in RNase-H Deficient Mutants"**

William Bell, North Carolina School of Science and Mathematics, Durham, NC , Mr. Nkabuije Maduike/Dr. Kenneth N. Kreuzer, Department of Biochemistry, Duke University

In ribonuclease HI and recG, deficient mutant E. Coli, the either the *rnhA* or *recG* gene is not present, leading to a lack of RNase-H and RecG in a bacterium. RNase-H is an enzyme responsible for the cleavage of RNA from an R-Loop structure, while recG is responsible for unwinding RNA from an R-Loop. An R-Loop is a RNA-DNA hybrid created when a strand of RNA invades a DNA duplex. This process results in DNA returning to its normal duplex state. But when RNase-H is present, an excess of R-Loops are formed, which results in excess DNA damage and the initiation of the SOS response. The SOS response is the DNA damage response pathway in bacteria. In JH39 *rnhA*- bacteria, which lack RNase-H, SOS is expressed constitutively. Though this process is known to occur, it is not clear exactly what happens during this process and why does it occur. Our research is focused on finding out why by determining the function of genes that are involved in the creation of DNA damage via R-Loop structures. In order to find the genes that are play a role in this process, we indentified mutants with transposon insertions that resulted in the reduction of the expression from a *dinD1::lacZ* reporter construct. This was viewed using a simple color phenotype. Out of approximately 2,900 JH39 *rnhA*- colonies, 30 colonies were mutants that contained 8 different genes that include *trkH, guaB, ygaW, trpB, lacZ, tktA, rrsD, fepG.* Out of approximately 15,000 JH39 *recG- colonies,* 32 colonies were mutants that contained 9 different genes that include *waaF, rfaE, lacY, lacZ, caiT, narG, panB, ydiU, and recA.* Further study must be done to confirm these genes' involvement in the SOS response and DNA damage.

2. **“The Effect of Recycled Sodium Hydroxide on the Production of New Copper Nanowires”,** Selina Boyd, Northampton County High School East, Conway N.C., Dr. Bejamin Wiley, , Department of Chemistry, Duke University

The project was conducted to determine whether sodium hydroxide could be recycled for the production of copper nanowires. By recycling the sodium hydroxide used in the production of copper nanowires, the cost of the nanowires is decreased from $32/g to $10/g.

After producing copper nanowires, the nanowires are removed and the sodium hydroxide solution is run through a filter. After it is filtered, the sodium hydroxide solution is ready to be used for production of copper nanowires.

The same sodium hydroxide solution was recycled more than once to determine the number of times it could be recycled. The recycled sodium hydroxide solution has proven to be capable of producing copper nanowires two times, but this is not necessarily the limited number of times it can be recycled.

In conclusion sodium hydroxide can be recycled for the production of copper nanowires. The recycled sodium hydroxide has also proven to be capable of producing copper nanowire multiple times. Therefore the production for copper nanowires will decrease dramatically making copper nanowires both flexible and affordable.

3. **"A Method for the Detection of Indirect Binding Within a Protein-Ligand Interaction"** Devin Bryant, Kinston High School, Dr. Michael Fitzgerald Department of Chemistry, Duke University, Durham, NC 27708

Purpose: The goal of the research is to develop a new technique for analyzing protein-ligand interactions that can detect off target events. Off target events are difficult to detect by most current methods, and are the cause of drug side effects. The method is termed Probing with SPROX Using Isotope Tags (PrSUIT), and it uses SPROX based theory to show protein binding. To confirm PrSUIT as a valid method for detecting indirect binding, it will be performed on the model system Cyclosporin A-Cyclophilin A reaction in a proof of principle study.

Methods: Baker's yeast was grown to overexpress Cyclophilin A and Calcineurin. The yeast was then pelleted and lysed, and CNA and CypA were extracted from lysate. Protein samples were split into a set that received CsA, and a set that did not. Sets were then placed in SPROX buffers of increasing denaturant and were oxidized with two different variations of Hydrogen Peroxide, digested into peptides and then re-oxidized. Peptides were then analyzed using Agilent 6520 QTOF MS with Chip Cube Interface.

Results: 42 unique peptides from Calcineurin A were identified, which is 61% of its amino acid sequence coverage, included were 10 different Methionine containing peptides. PrSUIT detected binding in the CNA (332-354) peptide and in the CypA (54-74) peptides

Conclusions: The PrSUIT method detected direct (CypA) and indirect (CNA) binding within the reaction, confirming PrSUIT as a valid method for analyzing protein-ligand interactions

4. **“INSIGHTS INTO THE ROLE OF HUMAN SERUM ALBUMIN ON BLOOD COPPER REDUCTION”** Kaylen Cutler, Whiteville High School, Dr. Katherine J. Franz and Dr. Kathryn L. Haas Department of Chemistry, Duke University, Durham, NC 27708

Purpose: Copper is essential for antioxidant activity, oxidative phosphorylation, connective tissue formation, iron metabolism, neurotransmitter synthesis, pigmentation, and several other processes throughout the body. Copper is valuable to these enzymes because it takes part in redox reactions due to its two oxidation states, copper(I) and copper(II). The uncontrolled redox cycling between these two states has been linked to several genetic and neurological diseases. If it is determined that HSA can bind to copper(I) with a strong affinity, then it can assist in the reduction of copper(II) to copper(I) in the presence of ascorbic acid, because ascorbic acid is too weak to completely reduce copper(II) to copper(I) alone.

Methods: The amount of absorption for 19 solutions that each contained the same amount of HSA and copper(I) and varying amounts of bicinchoninic acid (BCA) was recorded using an ultraviolet-visible spectroscopy.

Results: The absorption of copper(I) by BCA was recorded and was shown to have a peak of 562. The concentration was then determined, and several mathematical equations were used to determine that the binding constant of HSA and copper(I) is log K= 17.

Conclusions: This binding constant shows that HSA does have a strong affinity for copper(I) and therefore it helps in the reduction of copper

5. **"The Binding of Camelid-Derived Single Domain Antibodies to Methotrexate"**
Jasmine Dunham, E.E. Smith High School, Prof. Eric Toone Department of Chemistry, Duke University

Purpose: The purpose of this research is to further the utility of llama single domain antibodies in therapeutic applications as well as bioimaging and biosensing. A major factor in the use of these antibodies is the stability at varying pH and salt levels. Therefore, we seek to learn how the thermodynamics and stability are affected when llama single domain antibodies bind to methotrexate at different pH levels and salt concentrations.

Methods: A pecan22 expression vector with a gene encoding a llama single domain antibody with affinity for methotrexate was used to overexpress the protein in E. coli. Protein was then purified through several stages (including osmotic shock, Immobilized Metal Affinity Chromatography column, and gel infiltration), and dialyzed into different buffers. Finally, the thermodynamics of binding were examined using isothermal titration calorimetry (ITC) and the stability of the antibodies were analyzed using circular dichroism (CD).

Discussion: The CD results indicated that the antibody may begin to denature and unfold as the pH level becomes more acidic. Sodium hydroxide interfered with the detection of signal at pH 9 and pH 10. pH level 8 is the most stable condition for the llama sdAb while pH 2 appears to be the least stable condition. In the pH studies with the ITC, pHs 7, 8, and 9 have roughly equivalent binding affinities. pH 10 did not show any binding. Furthermore, pH 6 was not determined. In the salt studies for the ITC, there is a 10 fold decrease in binding affinity when 100mM of NaCl is added to the buffer system (20 mM HEPES pH 8.0).

Conclusion: We initially predicted that if initial results indicated partial unfolding of the antibody at lower pH levels, then the binding affinity would likely be lower at acidic pH levels. This hypothesis is inconclusive as we need to complete more data points. Our second hypothesis was that if the salt concentration is low (>50 mM), the single domain antibody will have a higher binding affinity than if the salt concentration is high (<50 mM). This hypothesis is supported because the salt concentration at 0 is higher than the salt concentration at 100 mM.

6. “**THE EFFECTS OF VARYING WATER POTENTIALS ON THE GERMINATION OF *ARABIDOPSIS THALIANA.”***Alexis Flen, NCSSM/ Terry Sanford, Dr. Kathleen Donohue and Liana Burghardt Department of Biology, Duke University, Durham, NC 22708

Purpose: The purpose of the research is to manipulate the water availably in which *A. thaliana* is imbibed in order to determine the lowest water potential that will allow it germinate and to observe natural variation in germination response amongst different ecotypes of *A. thaliana*.

Methods: In order to test *A. thaliana’s* germination response three genotypes, Calver, Tacoma and Landsberg, of *A. thaliana* were imbibed in six different water potentials ranging from 1.25MPA to 0MPa, in -.25 intervals, all held in petri dishes. For the next thirteen days the seeds were contained in growth chambers with a constant environment of 22 degrees Celsius, 99% relative humidity and 12 hour photoperiods. Over the thirteen days the petri dishes were censused, meaning seeds were analyzed through a microscope for germination and direct exposure to fungus.

Results: Amongst the three ecotypes used Tacoma was the most responsive, germinating completely in in 0MPa, -0.25MPa and to 40% in -0.5MPa. Calver remained quiescent when exposed to all water potentials. Landsberg was also responsive germinating completely in 0MPa and 40% in -0.25MPa.

Conclusion: When *A. thaliana* is imbibed in lower water potentials it begets lower germination percentages and rates. When three different ecotypes of *A. thaliana* are exposed to identical environments then germination yield does vary. Also approximate base water potential of Tacoma is -0.5MPa and the approximate base water potential of Landsberg is -0.25MPa.

7. **"THE DOCKING AND DESIGN OF LIGANDS OF HIV-1 PROTEASE USING VIRTUAL SCREENING"** Joshua Harris, Sandhoke Early College High School, Dr. Weitao Yang and Dr. Aaron Virshup Department of Chemistry, Duke University.

Purpose: The goal of this research is to computationally create a novel compound that binds strongly to the HIV-1 protein in order to inhibit the HIV-1 protease. HIV treatment drugs are composed annually; however, the HIV virus quickly adapts to these drug compounds because the HIV virus has a high rate of drug-resistant mutation and replication, thereby granting the virus immunity. Performing this experiment using Virtual Screening not only saves valuable time but hundreds of thousands of dollars.

Methods: In order to successfully computationally compose an HIV protease inhibitor compound, an HIV-1 protease protein structure must be selected from the Protein Data Bank. In order to prepare the structure for docking the default ligand must be extracted from the structure. Hydrogen and charges are added to the protein in order to produce strong bonds. The molecular surface is generated in order to see the shape of the protein structure. Next, spheres and a grid box are generated on the structure in order to show where all of the possible binding sites are located and where the approximate activation site is found. The docking score is then calculated in order to see how strong the bonds between the ligand and the receptor would be. And finally, the actual ligand is composed by Yang Group Software.

Results: The Yang Group Software composed 110 HIV-1 protease inhibitor compounds. All of which have molecular weights of $\leq$500 g/mol and nearly all of which bind strongly with the HIV protease.

Conclusions: It can be concluded that if a novel compound is composed computationally, then a stronger ligand can be created that will bond strongly with the HIV virus in order to inhibit the HIV protease.

8. **“THE EFFECT OF CARBON NANOTUBE ON X-AEROGEL**Lester Heckstall, Bertie High School, Dr. Jie Liu and Sungwoo Yang Department of Chemistry, Duke University

Purpose: The goal of the research is to combine x-aerogel with carbon nanotube(CNT) to create stronger gel and to observe its effect on the x-aerogel. The Carbon nanotube use in this experiment was double wall carbon nanotubes, this is because it strengthen the polymer (HDI)in the gel. Looking at how carbon nanotube strengthen polymer will show how two will strength the x-aerogel.

Methods: Solution A was mix using carbon nanotubes and tretramethoxysilane. After the mixing solution A, solution B was made which is ethanol, water, and ammonium hydroxide. Sonicate solution A, then mix solution B with solution A. After mixing to two solutions let it sit. After sitting added pc to the solution. Subsequent to that add pentane to the solution and heated on the hot plate.

Results: The methodology of putting HDI in the silica gel with CNT, have been unaffected. Additionally, x-aerogel did not show an increase in mechanical compressive strength. This took place because two factors, the PC exchange was not successful and not enough polymer was applied.

Conclusions: When x-aerogel combine with CNT and HDI the gel do not become stronger because the polymer exchange did not work. In conclusion, the hypothesis the effect of carbon nanotube on x-aerogel is inconclusive, because the gel start to crumbling before the three point bending test was applied.

9. **"Design of Inhibitors for Murine Double Minute Through the Use of Docking Programs"** Calvin Lee, Hoke County High School, Aaron Virshup, Julia Contreras-Garcia, Chetan Repatunark, Weitao Yang, David Beratan, Department of Chemistry, Duke University, Durham, NC 27708

Purpose: This experiment is being conducted to research possible inhibitors for the protein mdm2. What we plan to find is if a target ligand is able to be found to bind to mdm2 in a way as to inhibit its function. Mdm2 acts to inhibit p53, a tumor suppressing protein, thus enabling the uncontrolled replication of cells. This in turn allows for the formation of tumors.

Procedures: A gene modeling/manipulation program known as Chimera, was used in this experiment as a way to view the ligand and protein structures, and how they dock. To conduct the calculations required for the DOCK, Linux command line was used in conjunction with UCSF Chimera. In-house software, developed by the Yang/Beratan Group, was used in the mutation process.

Data: After experimentation, a target ligand was discovered, broken down, prepped for docking, and then docked. This DOCK, allowed for measurement of energy within the protein/ligand interaction. After DOCK calculation was complete, target ligand was then mutated with in-house software. Results collected from the mutations show and increase in the potential energy within the interaction.

Conclusion: After the end of mutations, results were collected and a mutated ligand was found to generate more energy during protein/ligand interaction, then inhibitors known today. This in itself allows for the future testing of this and other ligands found through this experiment, as means to develop future cancer treatment.

10. **"An Operationally Simple Base Free Mannich Addition Reaction with an Efficient Synthesis of β-Amino Carbonyl Thioesters"** O. Nwanaji-Enwerem, Concord High School, Department of Chemistry, Duke University,

Purpose: The goal in conducting this reaction is to enhance the productivity of the Mannich reaction through an increase in the percent yield of β-amino carbonyl compounds. This has been achieved by the presence of an enolizable alpha halo thioester in reaction with magnesium iodide, triphenylphosphine, and enolizable imines. An additional goal was to enhance the *syn*-diasteroselectivity of the final product.

Methods: The reactions conducted were Mannich reactions and all consisted of Ph_3P, MgI_2, an imine, and a thioester.

Results: It was found that the combination of Ph3P and MgI2 was in fact the excellent promoter for reductive Mannich-type reaction of S-2, 4, 6 triisopropyl and tertiary-butyl substituents in the presence of enolizable imines and that the corresponding products obtained optimal yields with high syn-diasteroselctivity. By using MgI_2 and Ph_3P in reaction with the thioester, we are less limited to the types of imines that can be utilized to synthesize β-amino carbonyl thioesters. Moreover, by using bulkier substitutents, the product synthesized yielded a more favorable *syn* to *anti* ratio. The improvement in both of these areas enhances the scope and variety of products capable of being produced.

Conclusions: In summary, we have developed a Mg^{2+} promoted direct Mannich addition of alpha halo thioesters and enolizable imines via reductive soft enolization. The *syn*-selective nature of this reaction is enhanced from that that is obtained for simple (thioesters using amide bases (LDA, etc.), and so provides a convenient complimentary approach to this key transformation.

11. **“QUANTIFICATION OF PROTEIN THIOL CONCENTRATION IN YEAST LYSATE.”** Frank Tillman III, Terry Sanford High School, Dr. Terrence G. Oas and Alexander Service, Department of Biochemistry, Duke University

Purpose: A characteristic that certain proteins possess has recently been detected- to rapidly unfold and fold 10-100 times per second. Determining the function of these rapidly unfolding and folding (RUF) domains, which are located in the protected portion of the proteome, is vital for advanced knowledge of protein functionality. However, cysteine residues were in both the protected and unprotected portions of the protein, thereby preventing us from observing these domains. Therefore, it was necessary to find effective reagents that would enable me to quantify the reactive cysteine residues.

Methods: To conduct this experiment, samples were checked on the UV spectrometer to determine the unprotected mol thiol/ μg protein. In the BCA Assay method, PBS buffer, supernatant, and the BCA working reagent mixture was incubated and then checked on the UV spectrometer. In the DTNB method, a mixture consisting of lysate, PBS buffer, and DTNB were used to check the absorption of thiols.

Results: As a result, it was determined that 80 ± 10 % of the proteome contained unprotected residues and 20 ±10% of the proteome contained protected cysteine residues.

Conclusions: By successfully quantifying the amount of unprotected mol thiol/ μg protein, future experimentation on better viewing the RUF domains is feasible by working with MMTS.

12. **“THE EFFECTS OF VARIOUS MUTANTS ON THE INHIBITION OF LIPID A BIOSYNTHESIS IN GRAM-NEGATIVE BACTERIA”**

L.K White New Hanover High School Dr. Christian Raetz, Department of Biochemistry, Duke University

Purpose: The biosynthetic pathway for lipid A is an attractive target for the development of novel antibiotics against Gram-negative bacteria. The goal of this project is to identify genes that might grant resistance against a potent inhibitor (CHIR-090) of lipid A biosynthesis. The identification of these genes will aid in the development of novel antibiotics against multi-drug resistant Gram-negative pathogens.

Methods: Resistance was determined by the gene’s Minimum Inhibition Concentration (MIC) value. Escherichia coli was grown on Pieter dishes with a knockout strain from the Keio collection. A single colony was placed in to a test tube with 5mL of Luria broth (LB), then grown overnight for 37℃ with shaking. The optimal density was then checked from 1mL of the overnight to find out the amount of cells needed for 10μL of LB. 100 μL of the cells in the LB was then put with 100 μL of LB with a diluted amount of inhibitor in a 96 well plate. The plate was then grown for 22 hrs at 37℃. MTT assay was then applied to the plates to test the activity of cells in the wells.

Results: The resistance of most mutants was two-fold difference from the wild type (control group) except for tolC. Those mutants do not affect E. coli resistance.

Conclusions: *tolC* had an MIC value of 0.0098 μg/mL which is 0.196% of the total amount of inhibitor added. Therefore, tolC is extremely hypersensitive to the CHIR-090, and knocking out tolC in other gram-negative bacteria should yield similar results.

13. **"The Effect of Manipulating the Helical Structure of a DNA Tile Weave on the Anticoagulant Potential of a Thrombin Inhibiting Aptamer"** T. Woodard Early College Academy at James Benson Dudley High School and Department of Chemistry, Duke University

Purpose: Current anticoagulants have a narrow therapeutic window and harsh side effects that are hard to reverse. Aptamer HD1 showed potential to be an alternative to current anticoagulants; however, it was shown to be inadequate in clinical trials. However, it has been shown in previous studies that using a DNA weave tile to combine aptamer HD1 with another thrombin-binding aptamer, 60.29, results in a much greater anticlotting activity. This research focuses on maximizing the anticoagulant potential of aptamer HD1 by manipulating the helical structure of the DNA weave tile. It is hypothesized, that a DNA weave tile with one double helix will result in the greatest increase in clotting time.

Methods: Anticoagulant potential is determined by first annealing single strands of DNA, diluting the annealed DNA tile weave to five different concentration points, then preforming an activated partial thomboplastin time (aPTT) assay.. Finally, a 6% native non-denaturing polyacrylamide gel electrophoresis is performed to see if the tile weaves formed correctly and if any other alternative structures developed. Superstructures represent tile that are binding to each other instead of the target molecule Thrombin.

Results From the aPTT assay, the three-helix tile was found to maxmize the anticlotting potential of aptamer HD1. However, significant amounts of superstructures were found to have formed in the four-helix tile sample.

Conclusions: In conclusion, the data yielded was inconclusive due to the superstructures seen in the four-helix tile sample. However, the data shown does reveal that manipulating the helices of the tile has a significant effect on clotting time. A future implication is to remove superstructures from the four-helix tile so that conclusive data can be shown.

14. **"Synthesis and Screening of 2-Aminoimidazole-Based-Antibiotic and Antibiofilm Agents"** Taylor Adair, Dr. Christian Melander, Tyler Harris, and Steven Rogers
Department of Chemistry, North Carolina State University

Purpose: Conventional antibiotics only posses the ability to eradicate planktonic bacteria. This does not include bacteria which reside within bacterial biofilms, which cause bacterial infections and disease within the human body and enable bacteria to be 1000 times more resistant to conventional antibiotics. The focus of this research is to generate new modifications the 2-Aminoimidazoles structure that will pair and identify with enhanced antibiotics. It is hoped that these modified compounds will not only disperse and inhibit bacterial biofilms, but along with antibiotics will resensitize the bacteria present in infection. This will expectantly become a superior form of treatment for bacterial biofilm-induced infections.

Methods: 2-Amioimidazole compounds were created by performing synthesis reaction processes. Bacterial strains of Methicillin-resistant Staphylococcus Aureus, Acinetobacter baumannii, and Multi-drug resistant Acinetobacter baumannii were cultured in MHB (Mueller Hinton broth) media. An assay was performed by placing the bacteria and the 2-aminoimidazole compounds into a 96-well PVC microtiter plate and incubated at 37°C overnight. The microtiter plate was then observed for bacterial growth and MIC (minimum inhibitory concentration) values were gathered.

Results: MIC values of the effectiveness of the modified 2-aminoimidazole compounds against biofilm formation ranged from 25μM to greater than 200μM.

Conclusions: This experiment showed that there are a range of modifications which can be made to the 2-aminoimidazole structure that can display different effects on the inhibition of bacterial biofilms

15. **“CHARACTERIZATION OF SINDBIS VIRUS HOST RANGE MUTATIONS”**

D. Barnes Broughton High School, R. Hernandez Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, NC 27695

Purpose: The focus of this project is to determine what mutations are in Sindbis virus Mosquito Restricted Mutant number 4 (MR4) that cause the virus to grow more efficiently in mosquito cells than in mammalian cells. The long term goal is to develop vaccine strains against pathogenic arbovirus related to Sindbis.

Methods: In order to do this RNA was extracted from stock virus. An RT/PCR reaction was then performed to convert RNA into DNA. Next, the PCR product was ligated into a DNA plasmid vector and amplified in *E.Coli*. cells. A miniprep kit was then used to purify the DNA. This DNA was then sent off for sequencing.

Results: From the sequencing data it was determined that there are at least 19 mutations that occur in the Sindbis Virus genome of MR4. These mutations were recognized in the all the proteins of Sindbis virus including both the non-structural proteins (nsP) and structural proteins.

Conclusions: The mutagenic design for the generation of these host range mutants produced the desired virus mutations. The selection method to identify those mutants which grew better in insect cells was successful. Although it was determined that there are 19 mutations in the Sindbis genome of MR4, it isn’t known if one or many mutations are responsible for the MR phenotype. The next step is to investigate all of the mutations individually or in various combinations. This step will identify any silent mutations and which virus mutants will go on to the vaccine testing level.

16. **The Effect of Solvent on the Interactions of Polyacrylonitrile (PAN) with Single-Walled Carbon Nanotubes for the Development of Ultra High Performance Fibers**

Melissa Chan, Enloe High School, Dr. Melissa A. Pasquinelli Department of Fiber and Polymer Science/TECS, North Carolina State University, Fiber and Polymer Science/TECS, North Carolina State

Purpose: High performance fibers is a new generation of fibers that exhibit superior mechanical properties used in critical areas such as aerospace engineering and military defense. The object of our research was to optimize the solvent conditions and thus improve the strength of high performance fibers using molecular dynamics (MD) simulations. We focused on fibers made of polyacrylonitrile (PAN), the precursor to carbon fiber used in today's high performance fibers, that is reinforced with single-walled carbon nanotubes (SWCNTs), a cylindrical carbon structure that can increase the polymer alignment and add some mechanical strength to the fiber.

Methods: MD simulations were performed with the software program LAMMPS to investigate how solvent impacts the interactions between PAN and SWCNTs. Four implicit solvent conditions were created by adjusting the dielectric constant of the system: a vacuum (VAC, dielectric of 1), water (H2O, 80), diethyl ether (DEE, 4.19), and dimethylformamide (DMF, 37.65). DMF is considered to be a "good" solvent whereas DEE and VAC are considered to be "bad" solvents. A single-walled CNT of the zig-zag type was used with a length of 10 nm and a diameter of 1.36 nm. The trends in the interaction energy as a function of solvent for each PAN-SWCNT were recorded.

17. **"Catalytic Aerobic Oxidation with Iridium Complexes"** Jon Chang, Enloe High School, E.Ison, M. Lehman, G Blakley and North Carolina State University

Purpose: The oxidation of alcohols to aldehydes is a fundamental reaction of organic chemistry and the ability of this reaction to occur under mild conditions, with less toxic starting materials is very desirable. Oxidation reactions using metal oxidants are very common in organic chemistry, but because of the toxicity of metal oxidants, aerobic oxidation reactions were introduced to limit toxic waste that metal oxidants generated. For this research, several Cp*Ir(NHC) (Cp* = 1,2,3,4,5-pentamethyl cyclopentadiene ligand, (NHC = 1,3 dimethyl-4,5-dimethylimidazol-2-ylidene) complexes and palladium acetate were synthesized and tested in oxidation reactions of primary and secondary alcohols with O_2 as an oxidant. Since O_2 is naturally abundant and available resource and H_2O is an environmental-friendly product. In this sense, catalytic aerobic could be consider as green chemistry.

Methods: 20mg of Palladium Acetate or Cp*$IrCl_2$(NHC) catalyst was weighed out and put in the storage tube. Then 3mL of Tetrahydrofuran (THF) was added. Using a 50 µL microsyringe, 17µL of triethylamine (TEA) was added and plus 270 µL of cyclopentanol was measure by using a 300 µL microsyringe. The amount came from the 1:4:100 ratio of molecular weights from $Pd(OAc)_2$/Cp*$IrCl_2$(NHC) , triethylamine (TEA), and cyclopentanol. The storage tube was seal with Taflon cap. Next the mixture was in the storage tube was freeze-pump thaw 2 times. O_2 balloons are attached because the reaction condition is under oxygen, then was react for the next 24 hours in room temperature. The mixture is filter though celite and is run through Varian 3800 Gas Chromatograph to obtain the turnover number.

Results: Both the iridium and palladium acetate catalysts show relative similar turnover numbers that were calculated from the chromatogram. The turnover number is defined as the number of cycles that a catalyst can run through before it deactivates. In the Oxidation reaction, iridium had a turnover number of 77 and palladium had a turnover number of 78 in THF solvent.

Conclusions: In conclusion, the data formulated from the chromatogram in the reactions with iridium and palladium catalysts, support the hypothesis that the iridium catalyst is as efficient as palladium at oxidizing secondary alcohol to ketone which is shown by the similar resultsin turnover numbers.

18. **"Restructuring PET and PLLA with Cyclodextrins to Improve Their Properties."**
KaDesia Hawkins, Dr. Alan E. Tonelli and Alper Gurarslan Department of Textile Engineering, Chemistry, & Science, North Carolina State University, Raleigh, NC 27606

Purpose: The goal of this research is to fully develop semi-crystalline morphologies of Poly (ethylene terepthalate) (PET) and Poly(L-Latic Acid)(PLLA), with superior mechanical properties. Further development of their semi-crystalline morphologies with the use of nucleating agents to control the melt-crystallization will possibly improve their physical properties. The nucleating agents will be derived from coalesced PLLA and precipitated PET. Cyclodextrins (CDs) will be used to form non-covalently bonded inclusion complexes (ICs) with guest polyesters. Then the host CDs will be removed, resulting in coalesced PLLA samples. PET will be precipitated from trifluoroacetic acid (TFA). For potentially controlling their semi-crystalline morphologies, coalesced PLLA and precipitated PET. have advantages over traditional melt nucleants, such as talc, mica, carbon nano-particles, ie., their chemical compatibility and non-toxic and undetectable natures. The improvement in their morphologies will result in stronger and tougher PET or PLLA materials, such as plastic bottles and synthetic fibers for clothes.

Methods: PLLA was first dissolved in dioxane at the same time as the alpha cyclodextrin was dissolved in water. Then they were combined to form an inclusion complex (IC). This IC was then analyzed with FTIR to examine if the PLLA was threaded by the CD. Then the IC was rinsed with water to remove the cyclodextrin to result in coalesced PLLA. The coalesced PLLA was then applied to dissolve As-received PLLA. After the solvents evaporated, the sample was analyzed with DSC.

Results: The As-received PLLA

19. **“REGULATION OF NRF2-MEDIATED ANTIOXIDANT RESPONSE BY VEGFR.”** Michaela Jones, Knightdale High School, Dr. Courtney Woods Computational Biology, The Hamner Institutes for Health Sciences

Purpose: Nrf2 is a master regulator of cellular defense against oxidative stress by controlling transcription of genes encoding enzymatic. Cellular protection by many dietary “antioxidants” has been shown to be mediated by Nrf2. While the key players in the signaling pathway are still unknown, kinase signaling is thought to play an important role in activating cellular mechanisms via Nrf2, where it binds to the antioxidant response element (ARE) of targeted genes. Last year’s research identified 9 kinases which led to the study of Vascular endothelial growth factor receptor 2 (VEGFR2).

Methods: Retesting confirmed previous findings, that repression of VEGFR2 causes a dose-dependent increase in Nrf2-mediated transcription. This was determined by measuring luciferase activity in MCF-7 cells treated with Nrf2 activator tert-butylhydroquinone (tBHQ) for 6 hrs. To ensure repression, research focused on VEGFR2 by using siRNA technology.

Results: Compared to cells treated with scramble siRNA, cells that were transfected with VEGFR2 siRNA exhibited a substantial increase in Nrf2 protein expression. ARE-driven cells that were transfected with VEGFR2 siRNA showed a statistically significant, but only modest increase in tBHQ-induced ARE activity compared to scramble-transfected cells. However, this effect was not dose-dependent, as a greater concentration of siRNA did not cause a more substantial increase in tBHQ-induced ARE activity. Finally, expression of Nrf2 target genes showed no substantial change in expression as a result of VEGFR2 knockdown.

Conclusions: These findings suggest that while VEGFR2 does not appear to be a critical player in Nrf2-mediated transcription, it plays a role in Nrf2 protein levels and potentially phosphorylation state.

20. **“NANOSTRUCTURED SURFACES FOR MEMEBRANE PROTEIN BIOCHIPS”** Jalissa M. Smith , Sanderson High School and Dr. Alex I. Smirnov Department of Chemistry, North Carolina State University

Purpose: Substrate-supported bilayers represent a convenient model of cellular membranes for various biotechnological applications. Improvements in the substrate-supported bilayers technology is expected to have a major impact on biomedical fields such as bioseperations, biosensors, and drug delivery. This research aims at improving the substrate-lipid bilayer interface by optimizing the surface chemistry of nanochannels of anodic aluminum oxide (AAO) membranes. Specifically, we have investigated optimization of silica sol-gel procedures to coat the nanopores commercial AAO membranes supplied by Whatman (U.K.) that have diameter of ca. 200 nm.

Methods: The sol-gel solution was prepared by mixing absolute ethanol, tetraethyl orthosilicate (TEOS), and 1 molar hydrochloric acid (HCl) (50:5:1 by volume) and the deposition protocol has been optimized. Typically, the AAO samples were immersed in the sol-gel solution under sonication to deplete the nanopores from air pockets and to increase the reaction rate. Alternatively, the immersed AAO samples were placed in a desiccator connected to a vacuum pump to remove the air from the nanopores. Time for each of the procedures was varied before the samples were cured in a furnace overnight at 150°C to allow for the silica to adhere to the alumina surface.

Results: The obtained nanostructures were visualized by a Field Emission Scanning Electron Microscope (FESEM). FESEM images confirmed the formation of the nanotubes and supported the hypothesis that the silica sol-gel did coat the nanopores, however, not over the entire 60 micron length of the nanopores

Conclusions: In this study we conclude that the sol-gel procedures tested did not coat the entire length of the nanopores, however, the sol-gel did coat some parts of the nanopores. It was determined that the deposition of sol-gel solution by either extrusion through the porous membrane or by soaking under vacuum improves the homogeneity of the nanostructures formed. It is likely that the final baking step is the main determinant for the homogeneous pore coating.

21. **"Modification of a Carbon Fiber Electrode by Covalent Attachment of an Enzyme."**Chelsea Sumner, Knightdale High School, Dr. Leslie Sombers, Department of Chemistry, North Carolina State University, Raleigh, NC 27695

Purpose: The aim of this research is to modify a carbon fiber electrode with glucose oxidase to detect glucose by breaking it down into hydrogen peroxide, which will be oxidized at the carbon surface, to create a stable biosensor. The results acquired in this research will be used to build new tools for neuroanalysis.

Methods: Glass carbon fiber electrodes were constructed and cut to 100µm. Glucose solutions were prepared, and the electrodes were electrochemically pretreated, modified with glucose oxidase and covalently immobilized with carbodiimide. Then they were placed in the flow cell injection apparatus where cyclic voltammograms of the secondary hydrogen peroxide were generated, then the data was graphed and analyzed.

Results: As the glucose concentrations increased, the H_2O_2 generated should have also increased showing a positive linear relationship. However, there was a negative linear relationship shown. The covalent attachment was successfully replaced by electrodeposition which showed to work adequately for our purpose.

Conclusions: In conclusion, the data collected and analyzed on this research project clearly shows that the stability of a carbon fiber modified electrodes is not increased by covalent attachment. However, immobilization through new techniques such as electrodeposition should be further studied so it can be implemented for the detection of other nonelectroactive substances.

21. “Rheological Behaviors of Biological Polyamides” B. Sumner, Knightdale High School, J. Pawlak, North Carolina State University, Raleigh, NC 27616

Purpose: There are many fields in which poly(γ-glutamic acid) or γ-PGA can be applicable. The purpose of this experiment is to characterize γ-PGA so that its future uses may be widely expounded upon. The problem in this research involves understanding how γ-PGA will react to physical changes as well as chemical changes.

Methods: To determine how γ-PGA reacted with these changes, the samples were prepared at three different salinity levels at five different concentrations. These samples were tested for viscosity on a TA AR2000 Rheometer. The results produced from these tests provided a look into what happens to γ-PGA under conditions such as salinity levels, shear rate, and temperature change.

Results: The data recorded from the rheometer were graphed and analyzed by the viscosity measurements. Results show that viscosity decreases with changes in the variables previously stated. Increasing these variables at different salinity levels, γ-PGA was found to first decrease at one salinity level and then decrease after a critical salinity level was reached.

Conclusions: These results lead to γ-PGA being more applicable because of the understanding of how it will react to experimental and processing conditions. In conclusion, this research has helped researchers take a step toward being able to use γ-PGA in applications where salinity and temperature are two important factors.

22. **"The Efficacy of HMGB1 and FLT-4 as Biomarkers for Sepsis in the Dog"**
Danielle P. Tyson, Enloe High School, S.K. Nordone, Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, NC 27695

Purpose: Sepsis, a deadly condition caused by bacterial infection of the blood, affects up to 10% of dogs in veterinary teaching hospital critical care units each year. The leading cause of sepsis-related deaths is our inability to rapidly diagnosis the condition. Preliminary studies in our lab suggest Vascular Endothelial Growth Factor 3, also known as FLT-4, may serve as a biomarker for inflammation in the dog. Similarly, data in humans suggests high mobility group box protein 1 (HMGB1), a powerful proinflammatory cytokine released by cells that are dead or dying, may be an early indicator of sepsis.

Methods: Using Real Time PCR, we determined whether FLT-4 and HMGB1 gene transcription was evident in an experimental model of canine septicemia. Six dogs were administered IV saline and LPS, a bacterial product known to induce sepsis-like symptoms, and blood was collected at 0, 2, 4, 6, 8, 10, 12, and 24 hours after exposure. Total RNA was extracted from the blood samples and reverse transcribed into cDNA. FLT-4 and HMGB1-specific transcripts from cDNA were measured by Real Time PCR. All values were normalized to the housekeeping gene r18s. The $\Delta\Delta$Ct method was used to determine fold-difference between saline- and LPS-treated samples.

Results: When LPS was administered to dogs, HMGB1 transcripts increased steadily over time as compared to HMGB1 transcripts in dogs that were administered a saline solution, which remained level over time. We failed to amplify FLT-4 in any samples.
Conclusion: HMGB1 may serve as a biomarker for sepsis in the dog.

23. **“THE SYNTHESIS OF CHLOROPHYLL ANALOGUES FOR THE SELF ASSEMBLY OF LIGHT HARVESTING ARCHITECTURES”**. Rachel Williams. Broughton High School, Dr. Jonathan Lindsey. Department of Chemistry. North Carolina State University.

Purpose: This project in its present form is the result of chlorin synthesis to create a bacteriochlorophyll *c* analogue. In photosynthesis, there is an organelle called a chlorosome which is an organized system found in green photosynthetic bacteria. This organelle acts as an antenna and sends signals for millions of chlorophylls to come together to absorb, convert, and store light energy. However, chlorosome molecules are impossible to synthetically produce but their function can be modeled with designing chlorophyll mimics acting similarly to the natural chlorophylls inside the chlorosome structure.

Methods: For this research, an Eastern Half and a Western Half are created and coupled forming a chlorin macrocycle. This macrocycle will undergo a series of extraction processes with dichloromethane, brine, and distilled water; concentration through rotary evaporator; purification and separation through column chromatography; solidification through high vacuum pump; and characterization through NMR and LD-MS.

Results: The substituent introduced at the beginning of chlorin synthesis ultimately created a very stable bacteriochlorophyll *c* product with unique photochemical characteristics which will be further investigated for artificial photosynthesis.

Conclusions: The introduction of the electron-withdrawing substituent led to a stable compound with a higher amount of plausible light energy absorption. This compound can be studied further for other applications.

24. **“ACTIVATING PROCASPASE-2 AS AN ANTI-CANCER STRATEGY”**

Avery Young, Enloe High School, Dr. A. Clay Clark Department of Biochemistry, North Carolina State University, Raleigh, NC 27695

Purpose: This year it is expected the 569,490 people will die from cancer, with more than 1,500 dying each day from cancer. The purpose of this research is to develop a new technique in treating cancer. As of now current chemotherapies allow for cellular resistance which makes the drug regimen less effective. By finding a drug that directly activates procaspase-3, we will eliminate drug resistance and make a more effective therapy for the treatment of several types of cancer.

Methods: The procedures that were used to conduct this research were protein purification, to extract the required protein, docking, to test how well each of the molecules will work, and crystallography, to study the crystal structure of the protein.

Results: After these experiments were run and the results were collected, it was found that 3 of the 1,364 molecules tested resulted in higher procaspase-3 activation. The best compound had a Mean Velocity score of 13,722 RFUs, a fold increase of 477.1, and a docking score of -9.2.

Conclusions: The conclusions that were drawn were that if the enzyme activity increased in the presence of a small molecule then the molecule could be developed into an effective drug. Since the docking scores for the molecules did not correlate to the experimental results docking or computational prediction may not be an effective way to find small molecule drug candidates for procaspase-3.

25. **"The Effect of Inlet Capillary angle on Range of Needle Position in Nanoelectrospray Ionization Mass Spectrometry"** David Bullock, Clement Early College High School, Dr. Gary Glish and Samantha Isenberg Department of Chemistry, University of North Carolina, NC, 27514

Purpose: One of the main problems with electrospray ionization is the transmission efficiency of electrospray ionization to capillary inlet. One would want to find out the translational profiles of flared capillaries to determine which has least dependence on position. Through my research I hope to find: Which capillary inlet angle shows the least signal dependence with change in position from center of capillary? My hypothesis If the capillary is flared, then we expect to observe a decreased dependence of signal with respect to distance from the center of the capillary. A 5 micromolar solution of reserpine was prepared in a solution of 50:50:0.1 acetonitrile: water: formic acid.

Methods: A 5 micromolar solution of reserpine was prepared in a solution of 50:50:0.1 acetonitrile: water: formic acid. A needle filled with this solution is placed in front of mass spectrometer. The needle was then moved to one side, slowly, until the signal disappeared. The first spectral analysis was then taken. It is then moved across the x axis two or more times. At each position, spectra were acquired for 1 minute

Results: The results show that the 60^{o} transfer capillary has a larger range of positions than the standard transfer capillary. However, the amount of error involved with the translation experiment removes the statistical significance of these results.

Conclusions: Some future work includes the retesting of the different angle capillaries and increasing the number of trials to improve the confidence of the results. Other future work would be to see how a change in concentration would affect the position range of a given capillary, how the position on the y-axis affects signal for a given capillary, and how the z-axis has an effect on the translation experiment.

26. **"Cell-Surface Engineering: An Approach to Generate Three Dimensional Cell Co-Cultures Based on Liposome Fusion."** Kimberly Clark, Clement Early College High School, Dr. Muhammad Yousaf, Abigail Pulsipher, and Debjit Dutta. Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Purpose: Cells that make up tissues and organs live in and communicate within an intricate, three-dimensional (3D) environment. The cell's spatial arrangements and interconnectivities are crucial and lead to the ability of high order function, difficult to construct in vitro. Although many tissue engineering strategies seem promising, major challenges being faced in engineering tissues include (1) chemically tailoring biomaterials to fabricate the in vivo environment of the cell and (2) forcing two different cell types to generate 3D arrangements by cellular interaction. The purpose of this experiment was to determine if cell-surfaces with complimentary chemistry would generate 3D tissue-like co-cultures because of chemoselective ligations.

Methods: In our study we developed and employed a liposome fusion and delivery method to induce specific cell-cell contacts through chemoselective, cell-surface modification. Liposome fusion was used to deliver ketone-or oxyamine funcational groups to different populations of cells via oxime chemistry. Our method was then demonstrated in several applications including liposome characterization, cell-surface tailoring, formation of 3D cell clusters and 3D multi-layered tissue-like structures.

Results: By using liposomes as the simplest model system, we were able to demonstrate how this method can be applied successfully in several applications including, the delivery of oxime chemistry to and labeling of the cell-surfaces, formation of small 3D cell cluster assemblies and 3D multi-layered co-cultures.

Conclusion: These experiments showed that proper reaction, labeling and multi-layered cell interconnectivity is driven by oxime chemistry. These results are consistent with the idea that our cell-surface modification strategy directed the formation of 3D cell cluster assemblies as well as generated 3D multi-layered co-cultures. An approach such as ours will greatly advance regenerative medical applications, as well as biomedical applications.

27. **“THE EFFECTS OF ELECTROPHILIC AROMATIC SUBSTITUTION ON THE NERVOUS SYSTEM IN INFANT CASUALTIES”**

Jaren Mack, City of Medicine Academy, Dr. Jeffrey S. Johnson Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC

Purpose: An infant’s body is microscopic compared to that of an adult human’s, and it is important to be able to identify the toxins that are around them. The importance of this is, if toxins are not found immediately, then it could be death for the infant. I want to find out how the improper usage of EAS can affect an infant’s structure and life. These experiments could substantially help find a solution and lower the amount of deaths of infants with more information.

Methods: Organic chemicals such as nitromethane, benzaldehyde, sodium hydroxide, and methyl alcohol were added to an Erlenmeyer flask and allowed to stir at room temperature. After stirring at room temperature for three hours, the mixture was placed in the freezer to go overnight. In the morning, crystals appearing to be crashing out were placed on a rotary evaporator. By scanning the product onto the NMR, it is identified as the product that was intended to be found.

Results: By using 11mL of the crystal, Nitrostyrene, the magnetic shifts shown on the NMR results chart shows that our product was heavily identified at just 3.5ppm. The various shifts of Nitrostyrene came along with foreign substances detected. By using 5mL of Nitrostyrene, the magnetic shifts shown on the NMR results chart show that our product was identified at 4.9 ppm and 3.66 integral ppm.

Conclusions: When the Nitrostyrene was given at a higher amount with the EAS, it was shown that toxins appeared, showing the data supported. The negative use of EAS given at a higher volume is inconclusive.

28. **“The Quantification of Strand Cleavage in DNA stained with Hoechst 33342”**
Alyssa Morgan, Durham Tech Middle College Early High School, Dr. Christopher Fecko, Department of Chemistry, University of North Carolina at Chapel Hill,

Purpose: The purpose of this research was to induce strand damage in DNA in order to observe DNA-repair protein processes. Hoechst 33342 was used in this experiment and potentially causes strand damage. Investigating the mechanisms in which Hoechst 33342 cleaves DNA strands could lead to further research in DNA-repair protein and DNA damage interactions.

Methods: PBR322 supercoiled DNA was used to determine the amounts of damage induced. The DNA samples were incubated in various dye concentrations at room temperature. The Hoechst 33342-stained DNA samples were then irradiated on a trans-illuminator from 0 to 240 seconds. After irradiation, samples were loaded into a 1% agarose gel for gel electrophoresis.

Results: The mean percentage of the relaxed form of DNA was significantly different for each irradiation time of 0, 15, 30, 60, and 120 seconds. The mean percentage of the relaxed form of DNA was also significantly different for each dye concentration of 1:4, 1:10, 1:100, and the control without dye.

Conclusions: Data shows that as the time in seconds for illumination increases, the percentage of the relaxed DNA form also increases which represents single-stranded breakage of DNA. Additionally, the average percentage of the relaxed form of DNA increases as the dye to nucleotide concentration of Hoechst 33342 decreases.

29. **"The Effect of Pyridine on the Growth of Cadmium and Manganese Metal Organic Framework Crystals"** Jeehae Nam Hillside High School, Dr. Jeffrey Johnson, Department of Chemistry, University of North Carolina at Chapel Hill

Metal Organic Frameworks (MOFs), which are comprised of organic bridging ligands and metal connecting points, have been greatly researched because of their relations to catalysis and numerous potential applications, such as drug delivery and hydrogen storage. MOFs, made up of ligands, can be tailored to fit the incoming molecule. The ability to tailor the frameworks by using different ligands is ideal for potential applications.

In our research, varying amounts of chemicals have been manipulated to synthesize MOF crystals. Pyridine was of particular interest, to see if the MOFs synthesized porous and structured frameworks. We hypothesized that pyridine, which is a base, can deprotinate the hydrogen off the carboxylic acid groups of our ligand, L46-OH, allowing proper binding to the metals. To test the hypothesis, numerous cadmium and manganese trials were conducted with varying amounts of pyridine levels.

A powder X-ray diffraction (PXRD) pattern showed that the Cd MOF synthesized did not match to the known Cd MOF crystal grown previously, but that it was a crystalline. However, a Mn MOF crystal synthesized did match to the known Mn MOF crystal.

We could not obtain and reproduce a sufficient amount of satisfactory crystals with Mn, and the hypothesis is therefore inconclusive. The Mn trials also produced many crystals without pyridine as with pyridine.

In the case of Cd MOF crystals, when higher levels of pyridine were added to the solution, an abundant amount of crystals were formed supporting our hypothesis. However, the MOF crystals were smaller in composition.

30. **“Effect of Synthetic Crowders on Chymotrypsin Inhibitor 2 Solubility”**

Malika Rauf, East Chapel Hill High School, Dr. Gary Pielak Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3290

Purpose: The purpose of this research is to determine what conditions 1mM chymotrypsin inhibitor 2 (CI2) is soluble at in various synthetic crowders at 300g/L. I used dextran, ficoll, PEG 3350, 8000, and 15000-20000 as crowders. I researched this topic because it is an important step in the eventual understanding of the effects of macromolecular crowding on protein chemistry.

Methods: We tested the solubility of 1mM CI2 in 50, 100, and 300g/L concentrations of ficoll, dextran, PEG 3350, 8000, and 15000-20000 in 50mM sodium acetate buffer pH 5.4. We also tested CI2's solubility in PEG 3350 in 25mM sodium acetate pH 5.4, 50mM sodium acetate pH 4.4, 50mM citrate phosphate pH 3.5, 50mM citrate phosphate pH 6.5, and 50mM citrate phosphate pH 7.3. We also used N15 enriched CI2 in 300g/L PEG 3350 in citrate phosphate pH 7.3 to create an HSQC spectra using NMR.

Results: 1mM CI2 was soluble in dextran and ficoll in 50mM sodium acetate buffer pH 5.4 at 300g/L and 1mM CI2 is soluble in PEG 3350 in 50mM citrate phosphate buffer pH 7.3 at 300g/L. We also got a clear CI2 HSQC spectra of PEG 3350 in the above buffer.

Conclusions: The HSQC spectra shows that CI2 nitrogen and hydrogen interactions can be seen under high concentrations of PEG 3350. Future research should be done with NMR amide-proton exchange experiments with CI2 in 300g/L of PEG 3350 using 50mM citrate phosphate buffer pH 7.3.

31. **“THE EFFECT OF LONG WAVELENGTH LIGHT ON PORPHYRIN ACTIVATION AND SINGLET OXYGEN PRODUCTION ($^1O_2$) IN WATER.”**

Cheyenne Stewart, Dr. Louise M. Ball and Aaron Young Department of Environmental Sciences and Engineering, University of North Carolina

Purpose: This experiment seeks to explore the relatively new application of Photodynamic Inactivation (PDI) to drinking water, due to inadequacies in current water treatment techniques which do not inactivate all current and emerging pathogen in water. Those with compromised immune systems, such as cancer and HIV patients, require higher-quality water. Thus, if there is a measureable amount of singlet oxygen production at long wavelength light, then there could be a biocidal effect at this same wavelength of light because singlet oxygen are capable of inactivating pathogen as they are the Reactive Oxygen Species produced in PDI. The significance of long wavelengths of light is that they penetrate deeper into turbid waters and blood products.

Method: In this experiment, solutions containing 10µM porphyrin (chromophores capable of absorbing light) 100µM Furfuryl Alcohol (a trapping agent for singlet oxygen) and 100µM I.S. (the constant) are exposed to 5 minutes projector light behind a light filter which blocks out wavelengths of light less than 440nm. Controls are put in a dark, enclosed area. Then both the controls and the solutions exposed to light are ran through an High Performance Liquid Chromatographer (HPLC) which separates compounds based on polarity. Finally chromatography graphs are collected and analyzed for detection of any measurable amount of singlet oxygen produced.

Results: Product peaks were detected for each porphyrin ran through the HPLC that had both FFA and exposure to light. No other combination of FFA, Porphyrin, light and I.S. showed product peaks due to an absence of one or two of the key factors.

Conclusion: Thus it can be concluded that an amount of singlet oxygen was produced when all three essential components of PDI were present; FFA, light and a photosensitizer (cationic porphyrin in this case). The exact relative amount of singlet oxygen produced could not be determined however, due to technical difficulties with the HPLC and limited time to do collect data. But the conclusion that singlet oxygen was generated is still valid due to previous studies that have determined the only way for product to exist would be through FFA reacting with excited state singlet oxygen.

32. **"The Efficacy of Long-wavelength Light in Catalyzing the Photoinactivation of Microbes by Cationic Porphyrins"** Marc Webb, Dr. Louise M. Ball Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill

Purpose: Cationic porphyrins have been shown to inactivate all classes of pathogens when exposed to light. The goal of this research was to determine if a novel series of cationic porphyrins are able to effectively inactivate non-enveloped viruses when exposed to light longer than that of their maximum absorbance. This is practical for using porphyrins to treat aqueous media that requires a deeper penetration of light such as turbid water and blood products. The 4 cationic porphyrins used differed in cation charge location and carbon chain length.

Methods: A yellow light filter was used to block wavelengths less than 450nm. Porphyrins were made to 1µM concentrations, irradiated for 5 minutes, and plated with model pathogen MS2 and host bacteria *E. coli F Amp*.

Results: The porphyrins with 4 carbon chains were most effective. While most of the porphyrins showed little activity in the dark, one porphyrin inactivated about 1 log of MS2 without light activation. Under longer wavelengths, all porphyrins remained active against MS2.

Conclusions: All porphyrins exhibited exceptional activity against MS2. Porphyrin toxicity in the dark influenced higher activity in the light. Flexible cation charges and longer carbon chain lengths were advantageous to the inactivation of MS2. The results indicated that cationic porphyrins can inactivate MS2 effectively under wavelengths of light longer than their maximum absorbance; however, longer periods of irradiation were necessary. Increased irradiation time could present problems with porphyrin degradation. Studying porphyrin degradation is the next logical step in the research.

VI. STAFF AND PROGRAM MANAGEMENT

The Project SEED Staff
The Project Staff will consists of a Project Director, Assistant Director, Assistant Coordinators, Scholarship Coordinator and Activities Coordinator.

Kenneth A. Cutler, Director
Mr. John Greene, Assistant Director,
Mr. Micheal Cherry, Assistant Director, Duke University Site Coordinator
Ms. Tyjuanna LaBennett, Assistant Coordinator, NC State University
Mr. Barrington Ross, Assistant Coordinator, NC State University
Ms. Courtney Blake, Assistant Coordinator-UNC-Chapel Hill
Mrs. Josie B. Cutler, Activities Coordinator
Ms.Faye McNeal, Scholarship Coordinator
Mr. Donald J. Guth, Administrative Assistant

Principal Investigators

Principal Investigators (PIs) are essential to the success this program. This program has been fortunate enough to secure and retain some of the best research scientists in the country, definitely among the best in the State of North Carolina . The best preceptors, provide the best research educational experiences for the students, and therefore taking our students from disadvantaged to a truly educationally and experientially advantaged status.

VIII. REFLECTIONS

The 2010 (19th) edition of Project SEED was highly successful with the administration of a statewide residential program, along with our more established successful commuter program. After extensive internal evaluations the following highlights and concerns were presented:

- **Scholarships**-Our Scholarship total for 24 seniors exceeded $7 Million dollars
- This year we saved approximately $18,000 by negotiating directly with Duke Event services and Mr. Jim Hodges, Director of Housing and Conferences for Duke University and not using Duke Youth Services as mid-level negotiator. . Our dormitories were on West Campus and therefore within walking distance to the science laboratories.
- Wake Forest University still accepts and provides scholarships for a high number of our students
- We are concerned about the number of students that matriculate as STEM majors and change to a non-STEM major during their freshman year. Especially at the University of North Carolina at Chapel Hill.
- We are also concerned about placing higher in national, state, and local science fair competitions
- We are also concerned about continued funding for the program. We have made the submission of grants a high priority.
- The yearly schedule and economic criteria of the American Chemical Society (ACS) and our criteria and schedule for accepting students is not congruent. As a result we accept students approximately two months prior to when the ACS approves them in late May. This year quite a few students were rejected for approval by the ACS, thereby leaving NC Project SEED the financial responsibility to pay the **full stipend** from our budget instead of half. Several of the students we approved of had parents that had been recently laid off. Apparently, our documentation of the parental layoff was not enough to satisfy the ACS for approval. Paying the entire stipend was a larger portion of budget than we anticipated. In the future when applying for grants we plan to request the entire student stipend be at our discretion..

APPENDIX
Scholarships
Budgets