CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Elizabeth Aguilar; Charlotte DeLay

Project Number

S0501

Project Title

The Effects of Various Marinades on the Denaturation of the Carcinogen PhIP Found in Grilled Chicken

Abstract

Objectives/Goals

This experiment's primary objective is to investigate how various marinades ranging in pH can denature the concentration of PhIP (a carcinogen and heterocyclic amine) prevalent in cooked chicken meat. This project comprises of the following components: (1) finding a correlation between an increased concentration of PhIP over time through cooking, (2) Exhibiting how various pH's of tested marinades can denature the concentration of PhIP, and (3) Utilizing spectrophotometry to analyze the percentage of light transmittance and concentration of PhIP through marinade samples over time.

Methods/Materials

A spectrophotometer was utilized to analyze light transmittance (%) and concentration in (g/L) of PhIP in cooked chicken samples. Each of these samples had been marinated for an hour under refrigerated conditions, the following marinade samples were tested: lemon juice, orange juice, soy sauce, teriyaki sauce, brown sugar, and plain chicken breasts. The control applied was orthotolidine, a carcinogen, diluted with water. This chemical component was utilized due to its similarities in composition and structure to that of PhIP. There were three trials conducted for each marinade and three additional cooking times of 5, 8, and 11 minutes were evaluated. The average values of the light transmittance and concentration were calculated and plotted for each tested cooking interval to compare which samples contained the greatest correlation to PhIP's concentration.

Results

The light transmittance (%) and concentration of diluted orthotolidine was 100% and 0.118 g/L respectively. The accuracy of these models may be reflected by the averages of the samples in comparison to that of the measured control. Additionally, it was found that the lower the pH of a given marinade, the concentration of PhIP would also reduce, however, the concentration of PhIP would increase over cooking time with additional exposure to sugars.

Conclusions/Discussion

The analysis of the following marinades in regards to the denaturation of PhIP through its protein structure and formation in creatine, is essential towards the analysis of carcinogens and their effects on the structure of DNA. Furthermore, this study conducted could be incorporated towards the inquiry of heterocyclic amines and how organic compounds such as acetic acid found in lemon juice may lead to the development of inhibitors that can prove to be promising for cancer research.

Summary Statement

We developed an experiment to identify a marinade that would most effectively denature the concentration of the carcinogen, PhIP, in grilled chicken; likewise, correlations between carcinogenic content, pH, and cooking time were considered.

Help Received

The science department at our school provided us with the equipment needed to perform our experiment in additional to critique of our board and report.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)
Emma C. Calle

Project Number
S0502

Project Title
Can You "C" The Difference?

Abstract

Objectives/Goals
This project compares genetically modified fruits (such as oranges, apples, watermelons, and papayas) against their non-genetically modified alternatives, to determine which has higher content of Vitamin C.

Methods/Materials
The juice of each fruit was added to iodine (0.08 ml) to determine the amount of Vitamin C (also referred to as ascorbic acid) contained in that fruit. If a fruit was composed of a particularly high ascorbic acid level, then just a few drops (maybe even less than 1 ml) of juice would turn the iodine transparent. Non-genetically modified Gala Apple; Genetically modified Ambrosia Apple; Genetically modified Cameo Apple; Genetically modified Honey Crisp Apple; Non-genetically modified Navel Orange; Genetically modified Navel Orange; Genetically modified Cara Cara Orange; Non-genetically modified Seedless Watermelon; Genetically modified Seedless Watermelon; Non-genetically modified Papaya; Genetically modified Papaya; Vitamin C tablet (500 mg tablets); Tincture of iodine; Dropper; Plastic cups (sample size); Labels for each vitamin C source; Kitchen knife/butter knife; Grater; Strainer/sieve; Bowl (preferably a cereal bowl); Spoon.

Results
No definite experimental results were obtained, thus disproving my hypothesis. The non-GMO orange contained more Vitamin C than the genetically engineered oranges, whereas the GMO papaya, GMO watermelon, and GMO apple had a higher level of Vitamin C than those of non-genetic modification.

Conclusions/Discussion
The results were mixed, and therefore my hypothesis was not definite. The one result that disproved my assumption prior to conducting the experiment was the high ascorbic acid content in the non-genetically modified Orange. This result differed from my hypothesis in that the assumption was the genetically modified oranges would have a higher level of Vitamin C than the non-GMO orange. Unlike the oranges, the apples, papayas, and watermelons supported my hypothesis. All three fruits containing genetically altered DNA contained more ascorbic acid than their non-genetically modified alternatives.

Summary Statement
This project tests the efficacy of genetically modifying a fruit to increase its Vitamin C content.

Help Received
Mother helped purchase fruit and items for display.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)
Edwin Campos; Angel Rodriguez

Project Number
S0503

Project Title
Do Different Types of Cacti Help Disperse Oil More Efficiently?

Abstract

Objectives/Goals
The objective of this study is to determine whether or not cacti are a better natural oil dispersant.

Methods/Materials
Four types of cacti, which are easily grown in the United States, were used in the study: prickly pear, Aloe Vera, barrel cactus, and prickly pear tuna. Extracts of each experimental cacti were collected and stored separately in four test tubes. Then, 2 ml of each extract were combined and placed in a fifth test tube. Eighteen (18) test tubes with six milliliter of water and one milliliter of olive oil (three test tubes for each of the five extracts, and three test tubes for the control (detergent solution) were set up for the experiment. Three trials for every solution was conducted for a more accurate outcome. One milliliter of each extract was added to its corresponding test tubes. The mixtures were allowed to react for three days. On the third day, test for fats was conducted using Sudan IV and paper test.

Results
The result showed that the combined extract worked efficiently in dispersing oil. The order of efficiency in dispersing oil was as follows: combined extract, prickly pear, golden barrel cactus, prickly pear tuna, and Aloe Vera.

Conclusions/Discussion
The results further showed that the solution that contained all three extracts dispersed the oil more efficiently than the detergent.

Summary Statement
In our project we tried to find a natural oil depressant that can work more efficiently than a detergent

Help Received

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Jessica Cao; Philippe Tran

Project Number

S0504

Project Title

The Effects of Glucose on Totipotent Cell Formation and Differentiation

Abstract

Objectives/Goals

The purpose of this experiment was to determine how various levels of dextrose monohydrate (D-glucose) affect the regenerative capacity of dugesia dorotecephala (brown planaria). The null hypothesis, H0, was that dextrose monohydrate has no effect on regenerative capacity. The alternative hypothesis, Ha, was that the dugesia dorotecephala exposed to higher levels of dextrose monohydrate have higher regenerative capacity because glucose increases Wnt signaling and mitotic division in cells.

Methods/Materials

To perform this study, pieces of liver were soaked in 0.00%, 5.00%, 10.0%, 15.0%, 20.0%, and 25.0% glucose concentrations to saturate the feed, which was then given to the planaria, 10 per concentration. The planaria were bisected about 3 hours after feeding, their lengths were measured after two days, and the percentage growths were calculated.

Results

The results showed that glucose significantly increased the regenerative capacity of planaria, as measured by the percent growth of each segment over a period of two days. The control group, exposed to 0.00% glucose exhibited on average, a 2.16% and 8.27% growth in the anterior and posterior segments respectively. Conversely, on average, the planaria exposed to 5.00% glucose exhibited a 15.7% and 26.6% growth; the planaria exposed to 10.0% glucose exhibited a 9.73% and 19.2% growth; the planaria exposed to 15.0% glucose exhibited a 13.0% and 16.1% growth; the planaria exposed to 20.0% glucose exhibited a 11.1% and 20.6% growth; and the planaria exposed to 25.0% glucose exhibited a 11.0% and 22.1% growth respectively. It was also noticed that the posterior portions of the planaria regenerated at a higher rate than the anterior segments.

Conclusions/Discussion

Thus, it is evident that glucose does increase the regeneration rate of planaria, most likely due to glucose#s ability to enhance mitotic division and Wnt signaling for cell differentiation. After statistical analysis using the Student#s T-Test, the calculated T-value, 3.36, was found to be greater than the critical value, 3.20. Therefore, the null hypothesis can be rejected and the alternate hypothesis considered. Further, this knowledge can be extended to understand the effects of blood glucose levels on embryonic development in humans.

Summary Statement

Our project tests the effects of glucose on planaria regeneration.

Help Received

Father helped to autoclave equipment for use during experiment and supervised the bisecting of planaria using a razor.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Eric Chen; Nathan Ng

Project Number

S0505

Project Title

Novel B-cell Epitope Prediction from Intrinsically Disordered Protein Region Positioning

Abstract

Objectives/Goals

The goal of this project is to utilize the location of intrinsically disordered protein regions on invading pathogens to aid in the identification of B-Cell epitopes, the short protein sequences recognized by the immune system. B-Cell epitope predictors can expedite the creation of peptide vaccines, allowing them to become a feasible alternative to traditional vaccines with lengthy development times.

Methods/Materials

Data culled from the Immune Epitope Database (IEDB) were parsed through various means, including the NCBI Basic Local Alignment Search Tool (BLAST), BioPython, and Python, in order to map epitope sequences onto their proteomes. The sequences were passed through IUPred, a protein disorderness predictor in order to generate an individual disorderness tendency score for each residue. The residue scores and positioning of epitopes were then statistically analyzed using R in order to generate a logistical regression model. This model was optimized by changing its threshold level.

Results

Compared to benchmark B-cell epitope predictors that used single amino acid propensity scales, our model outperformed all of them. For a certain level of specificity, both our models had higher levels of sensitivity, which demonstrates the importance of disorderness as an epitope prediction variable. Through development and testing of the logistic regression model, the area under the ROC curve was determined to be 0.594 for bacteria and 0.636 for viruses, using 200 bootstrap resamplings. The virus disorderness threshold score was 0.454 with 24.08% specificity and 89.20% sensitivity and the bacteria disorderness threshold score was 0.531 with 84.78% specificity and 22.66% sensitivity.

Conclusions/Discussion

We have identified intrinsic protein disorderness as a viable and efficient epitope prediction criterion. By integrating it into a metapredictor, the process of epitope prediction can be made more accurate and efficient. The prediction can be used to expedite the process of peptide vaccine development, making it an economically viable and structurally sound method of vaccination. Compared to live attentuated vaccines, peptide vaccines contain less risk for mutation, are more stable, and can immunize more effectively.

Summary Statement

By utilizing computational biology, our project uncovers a novel method of B-Cell epitope prediction that uses the disordered protein regions of an invading pathogen and can greatly improve the prospects of peptide vaccine development.

Help Received

Dr. Ponomarenko helped with graphics generation for the poster.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Noah Chow; Meghan Lorenz

Project Number

S0506

Project Title

Comparing Ascorbic Acid Content between Organic and Non-organic Malus domestica

Abstract

Objectives/Goals

The objective is to determine if there is more ascorbic Acid (vitamin C) in organic or non-organic Malus Domestica (apples).

Methods/Materials

Five different types of apples were collected. The peel and pulp of the samples were run through a High Performance Liquid Chromatography machine (HPLC). The ascorbic acid content was determined by comparing the amounts found in the standards and samples.

Results

There was more vitamin C found in the organic apple peel samples than the non-organic. However in the pulp samples there was not a significant change in vitamin C content.

Conclusions/Discussion

The results we obtained showed that organic Malus Domestica have higher ascorbic acid levels compared to the non-organic.

Summary Statement

Comparison of vitamin C content in apples.

Help Received

Dr. Cauchon, Dr. Malhotra and Mr. Hoag answered some questions I asked of them.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Rachel L. Dault

Project Number

S0507

Project Title

DNA Barcoding

Abstract

Objectives/Goals

My objective was to assemble and submit two professional quality reference DNA Barcode records to BOLD for publication in NCBI/GenBank. As a citizen scientist, I contributed to the world's largest Biodiversity genomics project (iBOL).

Methods/Materials

In order to do this I isolated total DNA from specimen tissue (previously identified by Milton Love, taxonomist from UCSB, obtained and stored through Coastal Marine Biolabs), purified the genomic DNA, examined the gDNA using gel electrophoresis, amplified the CO1 Barcode region from the template, used a spin-column to purify the CO1 amplicons, and confirmed the length of the amplicons by use of gel electrophoresis. DNA samples were sent for sequencing. I then assembled the reference barcode record in BOLD-SDP.

In my research, I compared four families of the Sebastes fish to see which were most closely related according to their nucleotide sequences and amino acid sequences. I compared the specific percentages of base pairs for each specimen and used the bioinformatics tools on the BOLD-SDP workbench.

Results

Of the total 21 samples sent to our lab, 19 provided adequate DNA results to be analyzed. I was able to generate high quality sequences for one of the two samples, the other sample had too little DNA to provide an adequate barcode. There was a total of five families of the Sebastes fish present from our samples, I analyzed four of these.

Conclusions/Discussion

I was able to successfully isolate gDNA, purify it, amplify the CO1 amplicon and upload the trace file into BOLD. I created a DNA Barcode for the Sebastes paucispinis that is currently waiting for second- and third-tier validation for inclusion into NCBI.

Summary Statement

Isolating, purifying and analyzing DNA sequences the Sebastes species of rockfish.

Help Received

Participant in Barcoding Life's Matrix Program with support from Coastal Marine Biolabs and North Valley Biotech Center

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Armine I. Dingilian

Project Number

S0508

Project Title

The Effect of Potassium Persulfate-induced Hydrogen Peroxide Use in Hair Bleaching Product on Human Hair Properties

Abstract

Objectives/Goals

The objective of this project was to determine the effect of Potassium Persulfate induced Hydrogen Peroxide in Human Hair Properties.

Methods/Materials

Tensile Tester, Differential Scanning Calorimeter, LOreal Feria Bleaching Kit Extreme Platinum, my own Human Hair.

10 different samples of hair, consisting of 10 hair strands each, were prepared. 5 different environments were identified, and 2 sets of hair samples were used for each environment. The samples were exposed to distilled rinsing water for 10 minutes, exposed to a heat-drying temperature of 60 degrees Celsius for 10 minutes, exposed to the bleaching treatment for 30 minutes followed by 10 minute rinse, and exposed to bleaching treatment followed by rinsing in water and drying at 60 degrees Celsius. The control samples were not exposed to any of the above environments. The samples were taken to the Quantum Technologies lab and tensile tested at a rate of 100 millimeter per minute. The samples were then cut into millimeter-long pieces and placed in separate standard pans, and each of the standard pans was individually tested in a Differential Scanning Calorimeter. The DSC samples were heated to 400 degrees Celsius, with a ramp of 5 degrees Celsius per minute.

Results

The results gathered from experimentation indicated that the hair samples exposed to both the bleaching treatment and heat-drying were affected the most in terms of physical properties by becoming more brittle and weaker, and therefore snapping at a lower percent strain.

Conclusions/Discussion

The results gathered supported the hypothesis. The samples exposed to the bleaching treatment and heat environment were most affected by becoming weaker and more brittle. This was likely because the hydrogen peroxide from the potassium persulfate in the hair bleaching treatment attacked the keratin in the hair fiber in addition to oxidizing the melanin and breaking it down, which further weakened the overall physical structure of the hair. It was also found that hair samples exposed to bleach and heat had, on average, an endothermic DSC peak value 3-10 degrees Celsius less than the unexposed samples, further indicating thermal and chemical breakdown. This project helps provide a better understanding of the effects of bleaching human hair on human hair properties, and further illustrates the potential health and economic consequences.

Summary Statement

This project focuses on the effect of Potassium Persulfate-induced Hydrogen Peroxide from Hair Bleaching Product on human hair physical, chemical, and thermal properties.

Help Received

Used Lab Equipment at Quantum Technologies under the supervision of Senior Polymer Scientist Jacob Dingilian

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)
Ryan C. Fong

Project Number
S0509

Project Title
Ancient Chinese Secrets: An Investigation of Antioxidant Properties of Various Chinese Herbs

Abstract

Objectives/Goals
The objective of this project is to determine if Chinese herbs contain high concentrations of antioxidants. The purpose in the determination of antioxidant properties of Chinese herbs may be an indicator to the possibility of identifying potential anti-cancer remedies.

Methods/Materials
1. Twelve different herbs were obtained from an herbal store in Chinatown San Francisco.
2. Grind individual samples into a fine powder using a food processor.
3. Weight 10 grams of each sample into a 500ml round flat bottom flask.
4. Add 100mL of DI water into each sample.
5. Place each sample onto a hot plate. Bring to a boil and allow boiling for 30 minutes.
6. Remove the sample from the hot plate, filter into a funnel containing glass wool to separate the solids from the liquid.
7. The ABTS Antioxidant Assay Kit part number AOX-1 was purchased from Zen-Bio, Research Triangle Park, NC. The kit includes the AOX Dilution Buffer, AOX Assay Buffer, ABTS Solution, Stop Solution, AOX Trolox, and the Myoglobin solution.
8. Prepare 300mM Trolox Stock Standard and Standard Curve.
9. Prepare samples and read absorbance at 405nm on a spectrophotometer.

Results
Twelve Chinese herbs were evaluated. All but one herb had antioxidant activity as determined using a Zen Bio Antioxidant activity test kit. Yunnan Tianqi did not exhibit any activity. The highest activity was Di Sheng with 6658uM/g Trilox Equivalent antioxidant activity.

Conclusions/Discussion
The results did support my hypothesis. Initially, I was not sure if there would be any antioxidant activity because the herbs were dried and stored at room temperatures. Most of the samples contained relatively high levels of antioxidant activity. To make a comparison of these herbs to vegetable Americans eat, broccoli was tested to have an antioxidant activity of 25.1mmol/kg. Some of these herbs contained 200 times more activity than broccoli.
As a result of this experiment, I would like to continue research in the evaluation anti-cancer properties of these herb using in-vitro experiments.

Summary Statement
The antioxidant properties of Chinese herbs as a possible indicator for an anti-cancer remedy.

Help Received
Used lab equipment at Agriculture and Priority Pollutants Laboratory under the supervision of Dr. Leonard Fong. My parents helped support the project financially for the test test and the supplies for the poster board.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Keoni K. Gandall

Project Number

S0510

Project Title

Recoding M13 for Improved Phage Display

Abstract

Objectives/Goals

There are many technologies to create proteins that bind well to specific antigens (chemicals or proteins), one of which is phage display. Phage display is a technology using the non-lysogenic helical M13 bacteriophage. Proteins or amino acid sequences of interest are fused to pIII, the outer coat protein, and washed against a specific antigen. The ones that bind are then resuspended and used to infect new bacteria. Then you repeat. Phage display is sometimes uses unnatural amino acids that are recoded for the amber codon (TAG). This is fairly efficient, albeit not perfect because translation is still occasionally terminated. However, George Church#s lab has rec ently used a new technology, MAGE (Multiplex genome engineering) to remove all TAG codons from an E coli genome as proof of concept and plans to do this with 13 other codons. The aim of this study is to develop phage display vectors that can be used in the #TAG-less# E coli with increased efficiency as well as create phage display vectors with the 13 forbidden codons removed, effectively recoding and refactoring an entire bacteriophage.

Methods/Materials

Growth media Antibiotics (Kanamycin, Tetracycline, and Ampicillin) PCR machine Gel box Ethidium Bromide Agarose Miniprep supplies DNA purification supplies Restriction enzymes (Namely XhoI, EcoRI, SpeI and BamHI) PCR supplies (Q5 polymerase ect) Pipettes Pipette tips Primers DNA M13KO7 Helper Phage E coli strains (SS320, TOP10, and TAG-less E coli) Centrifuge tubes Other normal lab equipment

Results

The M13 origin in M13KO7 cannot be PCRed

67 additional amino acids onto the pIV's C terminus only has minor effects on phage fitness

The 2 M13 origin fragments can delete themselves

Conclusions/Discussion

This project is continuing: However I have discovered that the M13 origin in M13KO7 (a helper phage) cannot be PCRed completely, through the use of several gel and sequencing runs.

This is likely because the kanamycin resistance and p15A origin are wedged inside of the M13 origin, and because of the phages ssDNA replication intermediate, this region could be getting deleted.

Summary Statement

Recoding M13 to improve unnatural amino acid incorporation for a better phage display

Help Received

Prof. Chang Liu allowed me to do this project in his lab

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)
Dylan H. Harootunian

Project Number
S0511

Project Title
Sucrose to Glucose: Is Organic Baby Food Really Better?

Abstract

Objectives/Goals
The objective of this experiment is to find out if organic baby food has less sugar than non-organic by testing the glucose level in different brands.

Methods/Materials
To do this experiment I will take multiple flavors of multiple brands, organic and non-organic, and test the glucose levels. Then I will use invertase to break down the sugars and see how the glucose levels change. The results will show much sugar the different baby foods really have.

Results
My results were different for each flavor, but in almost all cases the results were relatively close to one another. For peas, the results were 150 mg/dL for Beech-nut; 100 mg/dL for Gerber; 200mg/dL for Earth's Best; and 183.33mg/dL for Plum. For carrot, the results were 133.33mg/dL for Beech-nut; 116.67mg/dL for Gerber; 133.33mg/dL for Earth's Best; and 200mg/dL for Plum. For squash the results were 100mg/dL for Beech-nut; 133.33mg/dL for Gerber; 116.67mg/dL for Earth's Best; and 166.67mg/dL for Plum. For the final flavor, sweet potato, the result was 433.33mg/dL the first time. I thought this was high, so I retested with baby food with two different expiration dates to see if different batches had different sugar content. The results from the re-test were much lower, with an average of 249mg/dL. Then, for Gerber the results were 0 mg/dL. Upon further research, I found the reason was the ascorbic acid in the baby food nullifies the effect of the test strips. Then the other two tests went normally, with183.33mg/dL for Earth's Best and 116.67mg/dL for Plum.

Conclusions/Discussion
My hypothesis was incorrect. I predicted that Gerber would have the highest sugar when in fact all brands had almost the same and Gerber was usually on the lower end of the results. The experiment showed that the lowest and highest results tended to be within 100 mg/dL of each other, which is actually an extremely small amount in ounces. This is about .004g of difference in total, which is barely noticeable and has a minimal effect on the body. The only exception is the odd results for the Gerber sweet potato because the ascorbic acid in the baby food nullified the effect of my test strips so I could not get results for that baby food. To conclude I will say that the difference between the brands is extremely minimal and the sugar difference has almost no effect on a person.

Summary Statement
I tested to find out if organic baby food had less sugar.

Help Received
My mother helped type the journal; father helped time tests; teacher helped with idea for retesting.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)
Lauren M. Hinkley

Project Number
S0512

Project Title
Correlation between p53 Protein and Cancer Susceptibility in Different Species

Abstract

Objectives/Goals
The objective is to determine if there is a correlation between the amino acid sequence of the tumor suppressor protein p53 in twelve species and the species' susceptibilities to cancer.

Methods/Materials
I collected the amino acid sequences for the p53 of the selected twelve species and ran an Alignment to compare them. Then, I ran a test to find their conserved domains and used the E values, or the statistical significance, of each conserved domain in the lowest and highest species to see if they were statistically different. I compared the polar versus non polar amino acids in the highest and lowest susceptibility species.

Results
There was no statistical difference in the values of the conserved domains. There were many different amino acids that appeared in the higher incidence species than what appeared in the lower incidence species.

Conclusions/Discussion
Since p53 is so crucial in regulating cell growth and division to prevent cancer in all species, it cannot vary from its functioning form. Therefore, many of the conserved domains remain the same from species to species, which is why there was little statistical difference between them. However, since there were different amino acids in the higher incidence than in the lower incidence species, it suggests that the p53 has evolved to work better in some species than in others, suggesting a biological importance.

Summary Statement
I compared the structure of p53 in different species to see if there was a correlation between the structure of p53 and cancer incidence.

Help Received
Teacher introduced me to different data bases

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Ezra M. Kosviner

Project Number

S0513

Project Title

3D Structure of Powerful Antimicrobial and Potential Cancer Treatment Naegleriapore A

Abstract

Objectives/Goals

The objective of my project was to find an accurate homology model of the powerful antimicrobial and potential cancer treatment naegleriapore A.

Methods/Materials

Materials: Computer, Protein sequence, Modeller software, Access to modweb homology modeling servers, Jmol and Protein Workshop, Uniprot.org, Pfam, Rosetta protein docking

Methods: Naegleriapore A sequence copied from uniprot and searched on Pfam database for subunit. Then saposin B subunit was extracted and sent to the homology modeling servers of Modweb. The best two models where then submitted to Rosetta protein docking to be turned into pores (Mod1 and Mod2). For Mod3 and Mod4, Modeller was used to create homology models for the subunits. Template molecules 1N69 or 2QYP were used for Modeller's homology modeling. The best results from each template were sent to Rosetta to be made into pores. The final resulting pores were analyzed with molecular viewing programs such as Jmol and Protein Workshop.

Results

Results show that mod1 and mod2 were both incorrect and didn't fit my criteria. Criteria included: expressing the correct lengths to pass through the cell membrane (at least 3.5 nanometers), a pore radius of 3.6-5.2 nanometers wide, a molecular weight of 66 kDa, low energy score indicating spontaneous formation, a clear hydrophobic charge on the outside and a clear polar charge inside. Mod3 produced a perfect pore, but statistically poor subunits. Mod4 had statistically correct subunits but made a pore that didn't fit the size criteria.

Conclusions/Discussion

The subunit that was statistically incorrect, mod3, produced a better resulting pore than that of the statistically correct subunit, mod4. Mod3 fit the hypothesized criteria and is accepted as the final model. The model of naegleriapore A can be used to estimate its interactions as well as be used for ligand docking, which could lead to its development as an antimicrobial and anticancer drug.

Summary Statement

Modeling an accurate 3D structure is the first step to understanding and utilizing naegleriapore A as an effective antimicrobial and cancer treatment.

Help Received

Dr. Peter Rose, Scientific Lead, RCSB Protein Data Bank, UCSD showed me how to use the homology modeling software and various databases.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Sean Laput; Kyle Marik

Project Number

S0514

Project Title

Using D. melanogaster to Explore the Genetics of the Early Stage Development of the Human Hematopoietic System

Abstract

Objectives/Goals

Studies trace the cause of hematological malignancies to changes in gene sequences involved in hematopoietic system development. Such changes can potentially alter the proper expression of these genes, resulting in compromised development of the blood system. Drosophila melanogaster is an optimal model organism in the study of genetics given its sequenced genome, homology to humans, and minimal care. The objective of this project was to assess the effects of specific genes in D. melanogaster on hematopoietic system development, and deduce the function of those genes and their application to humans.

Methods/Materials

Hand Hemolectin Lineage Tracing (HHLT) stock was crossed with 13 RNA interference (RNAi) stocks, each containing a specific gene of interest. As a result, the progeny of each cross contained both HHLT and RNAi systems. HHLT marked the hematopoietic system with green fluorescent protein (GFP) markers, which allowed the progeny to be viewed under an ultraviolet fluorescence microscope. RNAi induced inhibition of gene expression by using RNA strands to cut specific mRNA strands. Each cross was examined for any defects in the blood system, using the HHLT x 5905 (wild type stock) cross as the basis of comparison.

Results

3 out of the 13 stocks exhibited significant defects in blood volume, lymph gland and dorsal vessel development.

Conclusions/Discussion

The phenotypical defects induced by the inhibited expression of genes unc-5 and reaper may attribute to their roles in apoptosis. netrin receptor UNC5C precursor, the human homologue of unc-5, has been shown to be involved in tumorigenicity. The defects associated with the gene Adenosine deaminase-related growth factor E may attribute to its role in growth regulation. Its human homologue Adenosine Deaminase CECR1 precursor has been shown to be involved in Cat-Eye Syndrome, the symptoms of which include heart defects. The results of this experiment provide insight into the relationship between D. melanogaster genes, their human homologues, and genetic disorders such as tumors and Cat-Eye Syndrome.

Summary Statement

This project explores how genes in D. melanogaster affect the development of their hematopoietic system and investigates the application of these findings to humans.

Help Received

Mentored by Dr. John Olson and Dr. Nikki Malhotra; Experiments were conducted in UCLA facilities under the supervision of Dr. John Olson. Parents provided transportation.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Cody L. Lim

Project Number

S0515

Project Title

Pb and Sugar Sandwich: The Effects of Lead (II) Ion on Glucose Metabolism Rates in Saccharomyces cerevisiae

Abstract

Objectives/Goals

The objective of this project was to determine whether lead (II) ion would have an adverse effect on glucose metabolism rates in Saccharomyces cerevisiae (baker's yeast), a eukaryotic model for glucose metabolism.

Methods/Materials

I exposed baker's yeast to either 1 M lead (II) nitrate, 1 M sodium nitrate, or distilled water, and let it soak in a beaker for 24 hours at 4 degrees Celsius in a refrigerator. Then, I washed the yeast with distilled water and filter paper, added the yeast back into its beaker, added 50 mL of 1 M dextrose solution, and incubated the solution at 37 degrees Celsius for 100 Celsius. Following this, I added 10 mL of Benedict's Reagent, boiled for 10 minutes and let cool for 20 minutes. Finally, I centrifuged the solution at 3200 rpm for 5 minutes and measured the supernatant's absorbance of light at 730 nm; the absorbance reading was converted to concentration of glucose left in solution using Beer's Law and a self-made calibration curve.

Results

The yeast exposed to lead (II) nitrate had the highest average concentration of glucose left behind in solution (0.31 M), followed by the yeast exposed to sodium nitrate (0.28 M), while the yeast exposed to only distilled water had the lowest concentration of glucose left behind (0.16 M).

Conclusions/Discussion

Yeast exposed to 1 M lead (II) nitrate metabolizes glucose at a slower rate (more glucose is left behind in solution) than yeast exposed to 1 M sodium nitrate or yeast exposed only to distilled water. The data support my hypothesis. This knowledge can be applied to helping diabetics in developing nations who are very likely to consume lead-contaminated groundwater or produce; the lead contamination is now known to exacerbate the diabetics' inability to metabolize glucose, an effect with little potential for treatability without access to insulin and other diabetes medications.

Summary Statement

My project determined the effects of lead (II) ion, relative to sodium ion and distilled water, on glucose metabolism in Saccharomyces cerevisiae.

Help Received

Mother bought yeast and distilled water; Father bought dextrose and helped with display board; Mr. Morgan provided reagents for lead (II) nitrate and sodium nitrate solutions; Mrs. De La Cruz provided supervision, advice, and materials.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)
Nafi Mizan

Project Number
S0516

Project Title
Optimized Electroformation Settings for the Formation of Giant Unilamellar Vesicles

Abstract

Objectives/Goals
Giant unilamellar vesicles (GUVs) are biomimetic cells used by researchers to study the qualities of lipid membranes, specifically the permeability. These vesicles are formed through a process known as electroformation. The objective of this experiment is to find the optimal electroformation settings to form these vesicles. The purpose was also to find the relationship between the frequency setting of electroformation and the diameter and yield of unilamellar vesicles formed.

Methods/Materials
Using DPPC (Dipalmitoylphosphatidylcholine) lipid, I applied it as a film on a electrically conductive (ITO coated) glass by dissolving the lipid in alcohol at a concentration of 5mg/ml, applying it to the glass, and then evaporating the alcohol. When electroforming them, I kept the voltage and time as constants: 1V, 2 hours. I varied the frequency by increments of 5Hz in order to observe the effects of frequency on vesicle formation, diameter, and yield. I tested three samples of vesicles for each frequency. I then imaged the samples using a diascopic microscope and analyzed them by recording vesicle count and diameters.

Results
GUV diameter is the largest at 35hz, but has reasonable range at 10hz and 35hz. About 60% of vesicles formed at 10Hz are unilamellar, with very little variation. The results conclude that 10Hz is the optimal frequency for the largest diameters and yield of vesicles.

Conclusions/Discussion
While there is no trend that explains the relationship between frequency and vesicle diameter or yield, I found that 10Hz is the optimal frequency for the electroformation of unilamellar vesicles. In the future, I would like to use the vesicles to study the permeability of the lipid bilayer or find a method of electroformation that takes less time.

Summary Statement
The purpose of my project is to find the optimal settings for the electroformation of unilamellar vesicles in order to produce a large yield of artificial cells, and to understand how the frequency setting affects production of vesicles.

Help Received
Used lab equipment at University of Southern California under the supervision of Dr. Noah Malmstadt and Kristina Runas.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Brandon W. Ng

Project Number

S0517

Project Title

A Novel Reporter System for Analyzing and Evaluating a Smart Bomb Approach to Annihilate HIV

Abstract

Objectives/Goals

The intent of this research is to create a reporter system to assess the effectiveness of a newly discovered smart bomb approach to combat HIV. If a plasmid composed of the glycoprotein-160 HIV gene, and the two reporter genes Green Fluorescence Protein (GFP) and Firefly Luciferase (Ffluc), is able to be successfully inserted into human T-cells to create cells that look like HIV, then data can be collected to analyze the efficacy of the therapeutic aptamer-small interfering RNA (siRNA) chimera for HIV cell destruction.

Methods/Materials

A plasmid that carries the 3 genes through molecular cloning was created and inserted into a human T-cell so that it would express the HIV-glycoprotein 120 (gp120) on the cell surface, thereby mimicking an HIV-infected cell. The plasmid was then transformed into bacteria to multiply. Taking the purified DNA plasmids, they were transfected into human cells through lentiviral packaging to create lentivirus. Human CD4+ T-cells were infected after the lentivirus multiplied. These infected cells were then tested by using Flow Cytometry and Luciferase Assays in order to analyze if the transgene in the cell was producing the HIV outer receptor protein, GFP, and Ffluc. Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) was performed to measure and quantify the mRNA for GFP, Ffluc, and gp160.

Results

The results indicated that the GFP-P2A-Ffluc-T2A-gp160 lentivirus was successful in the infection and subsequent gene expression in the CEM T-cells. By performing flow cytometry and luciferase assays, the expression of GFP and presence of luciferase was confirmed respectively.

Conclusions/Discussion

Through data collection of the presence of the reporter genes, the functionality of the aptamer-siRNA in targeting the HIV look-alike cell could be determined. The siRNA attached to the aptamer silenced one or both of the reporter genes, so a dip in light emission or fluorescence after the aptamer was introduced has proved that the aptamer does indeed have an effect on HIV. In conclusion, the Ubiquitin promoter in the lentiviral vector provides evidence of strong expression of all three genes. The Ffluc signal is detectable while GFP signal in those transduced cells were 100 times brighter than the non-infected cells. Lastly, the HIV gp160 gene expression does not appear to be toxic to cells. This indicates that our construct is safe and usable for human and animal systems.

Summary Statement

I created a novel reporter system to assess the efficacy of a new approach against HIV by constructing a plasmid consisting of an HIV outer receptor gene and two reporter genes to create an HIV look-alike cell that luminesces and fluoresces

Help Received

My research was performed at Dr. John Rossi's lab at Beckman Research Institute at City of Hope under the supervision and great mentorship of Dr. John Burnett. I have obtained invaluable training as a student trainee of the Research Training Program hosted by Southern California Academy of Sciences.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Natalie Ng

Project Number

S0518

Project Title

A Genome-Wide Analysis Tool to Identify Functional Regulatory Single Nucleotide Polymorphisms (rSNPs) Impacting Disease

Abstract

Objectives/Goals

My research aims to develop a tool to enable the first ever genome-wide analysis of genetic variants, specifically single nucleotide polymorphisms (SNPs), in transcription factor binding sites. Since the vast majority (88%) of disease associated SNPs lie in non-coding regions such as transcription factor binding sites, characterizing these variants could elucidate their mechanism of action and thus lead to improved diagnostic and treatment strategies.

Methods/Materials

My project has three phases: (1) development of an intragenomic screening tool, (2) development of a methylation analysis module, and (3) applying the tool to identify new relationships between transcription factors and disease. In Phase 1, I proposed and developed a novel intragenomic screening tool, which seamlessly integrates three types of next generation sequencing data: sequence (PWM), transcription factor binding (chIP-seq), and open chromatin (DNase-seq). In Phase 2, I developed the methylation analysis module. In Phase 3, I successfully applied the tool to analyze a compiled list of 55,000+ disease associated SNPs to identify new relationships between transcription factors and disease.

Results

SNP Effect Matrix (SEM) scores, generated through the developed tool, were shown to reflect known transcription factor binding patterns and mirror transcription factor structure. Validation performed using published results from a high-throughput reporter assay show increased correlation ($R^2 = 0.350$) between SEM scores and normalized expression compared to the current standard for transcription factor binding ($R^2 = 0.232$). The methylation extension supports the ability of the tool to predict the impact of DNA methylation on transcription factor binding. The tool was successfully applied to identify new, statistically significant relationships between transcription factors and disease.

Conclusions/Discussion

My work represents the first tool that can predict the impact of a SNP on transcription factor binding at the whole genome level. The tool has been prepared as an open-source software package using GIT source code management and will be released as an addendum to an upcoming publication. By revolutionizing the framework of analyzing genetic variants that overlap with non-coding, regulatory regions, the tool promises to advance the development of disease diagnostics and treatments.

Summary Statement

I proposed and developed the first ever genome-wide analysis tool to predict the impact of a genetic variant on transcription factor binding, thereby revolutionizing the framework of analyzing genetic variants in non-coding regions.

Help Received

Dr. Alan Boyle for guidance and support; Professor Michael Snyder for providing an internship position through the Stanford SIMR Program

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Emily M. Pang

Project Number

S0519

Project Title

Investigating the Opposing Roles of gC1qR and cC1qR as Mechanisms for Inhibiting Cancer Pathogenesis

Abstract

Objectives/Goals

Current cancer treatment methods remain flawed, oftentimes lacking tumor specificity and causing undesirable side effects. Consequently, elucidating the mechanisms behind tumorigenesis is an attractive, immunotherapeutic approach for transforming targeted cancer treatment. Previous studies have suggested that complement pathway-activating protein C1q and its ubiquitously distributed multiligand-binding cellular receptors, collagenous cC1qR and globular gC1qR, are regulators in cancer cell survival. However, their specific functions remain unclear. This study investigates the role these two critical receptors play in cancer pathogenesis as a means of ending cancerous proliferation.

Methods/Materials

Adenocarcinomas were tested by antigen-capture assays, flow cytometry, immunofluorescence microscopy, and tumor necrosis factor-alpha assays to determine differential gC1qR and cC1qR secretion and expression in proliferating cancer cells.

Results

Results indicate atypical gC1qR overexpression and cC1qR underexpression, suggesting their relevance in cancer's immunosurveillance evasion. Data confirms that cC1qR is diminished and gC1qR upregulated in cancer cell survival and progression, providing a newfound understanding of the mechanisms by which cancer cells maximize proliferation, metastasize, sustain angiogenesis, and evade immune detection and phagocytosis.

Conclusions/Discussion

Transfection of gC1qR and cC1qR antibodies and proteins uncovered the ability to control and counter cancerous proliferation. Taken together, these results unveil the significance of gC1qR and cC1qR in cancer pathogenesis and potential as novel targets for immune treatments, which have universal implications as useful cancer therapeutic modalities.

Summary Statement

I discovered two immune protein receptors critical for the progression and survival of tumors that could be utilized to successfully design improved and targeted methods of cancer treatment.

Help Received

I participated as a research fellow in the Simons Summer Research Program under the supervision of Dr. Ghebrehiwet in Stony Brook University.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Brody Pettek; Taylor Sunseri

Project Number

S0520

Project Title

The Search for Bispecific IgG4 Antibodies and Their Biological Role in Diphtheria and Tetanus Vaccinations

Abstract

Objectives/Goals

Our objective of this project is to investigate the possibility of finding a bispecific IgG4 antibody to both tetanus toxoid and diphtheria toxoid.

Methods/Materials

Human serum samples,TT,DT,IgG4 Human Affinity Resin,TRIS,PBS,Glycine,commercial ELISA kits for TT and DT,Biotin,Slide-A-lyzers,Affinity Column

As a part of our study, we will use an ELISA to test for human antibodies from subjects immunized with tetanus toxoid and Diphtheria toxoid. All samples that are positive for anti-TT and anti-DT antibodies in the screening assay will then be purified for IgG4 through an affinity column using a reagent that can bind only serum human IgG4 antibodies. After the purification was finished, the IgG4 was then dialyzed to remove any contaminants such as buffer salts and other small particles. Once the IgG4 was collected, we tested for IgG4 antibodies to both immunized antigens, TT and DT.

Results

As a result, we were able to find that a few samples had been bispecific to IgG4. In order to be certain that we would be able to find good data, we made each sample a triplicate and had done two different dilutions. We had to assume that our data showed bispecific IgG4 antibodies is because of how the purification process went. We were unable to collect enough protein that could then be used on our bridging assay. The answer as to why this occurred is still unknown, but we have found that possible sources of error could have been that our resin bed was too old or we could have eluded the IgG4 from the column. In perspective to the data we found through the purification process, we were not able to use the purified IgG4 from the patients# samples to definitively prove our hypothesis. Instead, we took the five highest and the five lowest patients# samples and tested those on our modified bridging assay procedure.

Conclusions/Discussion

Through our study, we found that there are bispecific IgG4 antibodies to both diphtheria toxoid and tetanus toxoid. We know this because previous research states that IgG4 has the unique ability to swap its heavy chains.

Summary Statement

Our project is about finding the existence of a bispecific IgG4 antibody to both diphtheria toxoid and tetanus toxoid.

Help Received

Used lab equipment at Ventura BioCenter under the supervision of Dr. Daniel Mytych, PhD.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Swetha Revanur

Project Number

S0521

Project Title

Factomics: A Cloud-enabled Web Portal Incorporating Gene Expression and GWAS Facilitating Disease Causation Analysis

Abstract

Objectives/Goals

Researchers and medical professionals are inundated with tremendous amounts of genomic "big data." However, there is a lack of tools that are capable of offering insightful interpretations of this raw data. To address this void, I developed Factomics, a cloud-enabled web portal, which provides a novel built-in workflow for performing disease correlation/causation analysis based on integrative genomics data.

Methods/Materials

The Factomics workflow is divided into 4 phases -- Launch, Discovery, Correlation and Causation. These phases take the user from an initial set of diseases to their candidate causal single nucleotide polymorphisms (SNPs), genes, and pathways based on industry standard algorithms, user-selected gene expression data which provide biological context, and genome-wide association studies (GWAS).

Factomics is organized in a 3-tier architecture. The front-end is written in Google Apps Script (GAS; server-side JavaScript). The middle-tier is integrated with the analytical modules and with public data repositories including NCBI databases. Analytical findings are stored on Google Drive, the third tier.

Results

I demonstrated a use-case of Factomics with Alzheimer's disease, Type 2 Diabetes Mellitus, Ovarian Cancer, and Pancreatic Cancer. Linkage disequilibrium analysis identified non-synonymous (deleterious) and 13 regulatory candidate causal SNPs for Alzheimer's Disease and Ovarian Cancer. A multi-dimensional view of these diseases showed several overlapping upregulated genes and pathways. Some of the findings were corroborated by literature, and others were novel. This information can be used to optimize drug development, drug repositioning and diagnostic tools.

Conclusions/Discussion

My portal bridges the gap between current tools, and is capable of elucidating the biological mechanism for any disease. With relevant cached data, the workflow can distill millions of SNPs, thousands of genes, and thousands of pathways per disease to about 25 disease causation hypotheses, all in under 5 minutes. Moreover, it is scalable across a wide range of fields (bench biology, bioinformatics, pharmaceutics, and in clinical settings), while providing cloud-capabilities and a user-friendly interface. To the best of my knowledge, there is no existing tool offering similar functionality.

Summary Statement

I developed a novel cloud-enabled web portal that can computationally identify candidate causal SNPs, genes and pathways for any disease.

Help Received

Dr. Susan Lato of Codexis reviewed my work. Mr. David Walz is my teacher sponsor.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Vishnu Shankar

Project Number

S0522

Project Title

3D Structure of DP Prostaglandin G-protein Coupled Receptor Bound to Selective Antagonists from GEnSeMBLE Predictions

Abstract

Objectives/Goals

G-protein coupled receptors are heptahelical transmembrane receptors that convert an extracellular signal to an intracellular response. They are implicated in multiple physiological functions and function as an important therapeutic target for diseases such as cancer to Parkinson's disease. Prostaglandins play a critical physiological role in both cardiovascular and immune systems, acting through its interactions with ten prostanoid G-protein coupled receptors (GPCRs). These receptors are important therapeutic targets, but lack of knowledge of 3D structures for the prostaglandin GPCRs hampers the use of structure based drug design methods to develop medications to specific receptors. Among these, the DP receptor is of interest because it has unique character and physiological properties.

Methods/Materials

Two methods were used for structural prediction. First, I used a modified-homology algorithm where the initial structure was generated based on the dopamine homology template due to sequence similarity. Two, I used the de-novo method, which individually optimizes the helices. Each template was heavily sampled along multiple angles, where lowest energy structures were evaluated for antagonist docking. Further, molecular dynamics was implemented to study how the receptor and its interactions relax in the membrane with the ligand.

Results

I was able to accurately predict the DP Prostaglandin structure. My results showed that the antagonist binds vertically in the 1-2-7 binding pocket interacting strongly with residues Arg 310 and Lys 76. This is consistent with earlier predictions and available experimental data. Additionally, my results can explain find that DP does not have the 3-6 ionic lock common to Class A GPCRs. Further, I can explain DP ligand subtype selectivity to PGD2 through DP structural features. Additionally, we found the receptor is able to relax and most interactions have stability through molecular dynamics.

Conclusions/Discussion

I have reported the predicted structure for the DP Prostaglandin receptor bound to selective antagonists. Through my research, we have an understanding of how the ligand interacts in the binding pocket. Also, we can understand how to build future therapeutics based on virtual ligand screening. Also, the structure chosen has both experimental and computational validation.

Summary Statement

My project computationally predicted the structure of a specific type of transmembrane receptors. Through prediction, we can now design tailored drugs to target these receptors and change the biochemical processing of multiple diseases.

Help Received

Dr. Goddard, Dr. Abrol, and Dr. Kim provided guidance throughout project; Mrs. Fallon provided many valuable insights and helped on presentation; Parents and sister provided encouragement

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Shelby L. Shankel

Project Number

S0523

Project Title

Palladium-Catalyzed Direct Arylation of Amino Acids

Abstract

Objectives/Goals

The goal of this research is to modify proteins through direct arylation, a method used for coupling aromatic molecules through transition-metal catalyzed C-H activation. The coupling is between a brominated aromatic ring and a non-functionalized arene. This method is typically used for the polymerization of semi-random polymers. However, due to its ability to create C-C bonds, my hypothesis is that this method can be used on modifying biological molecules. Proteins are broken down into amino acids, which have the same basic structure with varying functional groups. Although these R-groups differ, they all contain C-H bonds, lending themselves to direct arylation. By taking an amino acid, different prefunctionalized substrates can be added to it.

Methods/Materials

The two amino acids that I chose were histidine and tryptophan because their R-groups resemble the molecules 1-butylimidazole and indole, respectively. These smaller molecules acted as model systems for the entire amino acid. Bromotoluene was a simple arene that was added to the imidazole and indole as it would be added to the functional group on the amino acid. A typical catalytic system was used, which included palladium acetate, neodecanoic acid, and potassium carbonate in the solvent N,N-dimethylacetamide (DMA). The reaction was optimized, varying conditions and adding ligands to help reactivity. These reactions were then analyzed using a Gas Chromatography Mass Spectrometer (GCMS), which uses the molecules various characteristics, such as the point at which they vaporize, to separate and analyze them.

Results

There was increased coupling between the 1-butylimidazole and bromotoluene in the presence of bis(diphenylphoshino)ferrocene, or dppf. The indole did respond slightly to some transition metals, like copper, that may have acted like co-catalysts. However, due to its acidic NH group, the hydrogen on the indole was not easily activated, so there was little coupling observed. Further data is being collected.

Conclusions/Discussion

I did not reach the step of direct arylation on actual amino acids, but the model system for histidine adds more creditability and possibility to my hypothesis. There still needs to be some issues solved with the acidic NH group on the indole to move onto tryptophan. However, this coupling expands the possibilities for protein labeling, for the delivery and development of pharmaceuticals, and for protein synthesis.

Summary Statement

My project looks into applying direct arylation to biological compounds for the purposes of modifying amino acids that can then be used for labeling and forming new proteins.

Help Received

used lab equipment and chemicals from California Lutheran University under the supervision of Dr. John Tannaci

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Vaishnavi Shrivastava

Project Number

S0524

Project Title

Targeted Cancer Therapy and Diagnosis: Analyzing miRNA Expression Signatures & Interactions for Glioblastoma Progression

Abstract

Objectives/Goals

Short noncoding RNAs, microRNAs (miRNAs) regulate gene expression by silencing mRNAs through degradation. Since miRNA dysregulation is linked with tumorigenesis, expression signatures of dysregulated miRNAs can be used as indicators to provide an early and accurate diagnosis of glioblastoma, the most malignant and aggressive type of brain cancer. The objective of my research is to create an innovative computational model to analyze glioblastoma data, and to apply my model to miRNA and mRNA expression data from glioblastoma patients to discover miRNA expression signatures and miRNA-mRNA network interactions.

Methods/Materials

MiRNA and mRNA expression values for 426 glioblastoma patients were obtained from the Cancer Genome Atlas. The data was screened for mRNAs with variable expression values to find the 4454 mRNAs most likely linked with glioblastoma development. Using R-programming, I created an original computational model, which utilized clustering and correlation among several other statistical techniques, to develop a novel method to analyze patient data and discover miRNA expression signatures and interactions for glioblastoma.

Results

164 miRNA-mRNA network interactions were identified. 10 of the discovered miRNA expression signatures were uniquely identified in my research and were previously unassociated with glioblastoma in literature. Mir221 and mir222 had the strongest correlation values and regulated the greatest number of mRNA networks, thereby best indicating glioblastoma. The discovered results were cross-validated from the literature to establish their accuracy. This computational model can be used to screen miRNA profiles of patients for the discovered expression signatures, to accurately diagnose glioblastoma in its early stages.

Conclusions/Discussion

Imaging techniques used in glioblastoma diagnosis today are flawed because they cannot image small tumors or multifocal lesions. Because glioblastoma cells secrete large numbers of exosomes containing miRNAs into the blood, a patient#s miRNA profile can be generated using a blood test. Using my model to screen this profile for the discovered miRNA signatures, I can diagnose glioblastoma in its early stages. Since glioblastoma does not metastasize outside of the brain, it can be cured if it is diagnosed early on using the methods proposed. My research will revolutionize glioblastoma diagnosis and allow this disease to be cured completely.

Summary Statement

I created an innovative computational model to discover miRNA expression signatures and interactions for glioblastoma, and screen the miRNA profiles of patients to accurately diagnose this disease in its early stages, when it curable.

Help Received

Dr. Olivier Gevaert of Stanford University helped me finalize my research idea and answered any questions I had during the research.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Sriram Somasundaram

Project Number

S0525

Project Title

Novel Design and Evaluation of Chitosan Nanoparticle Ocular Drug Delivery System

Abstract

Objectives/Goals

After working in an Indian Hospital, I realized that surgery for eye diseases is costly, unavailable, and inefficient, while the alternative is a drug in the form of eye drops. Current ocular drug delivery systems are insufficient due to the difficulty in penetrating protective layers of the eye such as the sclera, cornea, and conjunctiva while maintaining drug safety, efficacy, and bioavailability. My research proposes a fluorescein labeled chitosan nanoparticle complex (CSFLNP) that can enhance the surface area of the drugs, permeability through the layers of the eye, control release of the drug, and target specific areas of the eye. This study researches the capabilities of CSFLNP to bind, load, and release drugs for three diseases as well as its permeability through a cornea simulated in vitro.

Methods/Materials

CSFLNP was synthesized through ionic gelation using Tripolyphosphate (TPP). For drug loading, release, and permeability, certain wavelengths were examined to represent drugs and Nanodrop spectrophotometer was used to assay drug concentrations. Data was normalized against controls to get percent of drug loaded/released/permeated. Collagen gel was synthesized to model cornea. Dielectric test was used to examine binding.

Results

Chitosan was able to be gelled into nanoparticles and (10ul) was able to load all three of the drugs at certain potent concentrations/ amounts: 20mM for ampicillin, 50mM for propranolol, 200ul for carnosine. For carnosine and amp, there was one exponential loading phase, whereas prop had two, probably due the CS amino group switching from binding to the drugs from the stabilizer. The CSFLNP complex also provided a sustained release of the drug over a 7-hour period modeled by Non-Fickian diffusion, and increased permeation through the simulated cornea by 25%. There was also a change in strength of electric field as the concentration of prop loaded increased, showing the efficient binding and medium of binding (the amino groups).

Conclusions/Discussion

The CSFLNP efficiently bound the drugs as shown by the dielectric test and the absorbances in the loading tests, and they were able to release the drugs over a period of time. The system also had greater permeability than just the drugs alone against an in vitro simulated cornea. With this system, topical drugs can become a promising mass solution.

Summary Statement

I designed and tested an ocular drug delivery system based on chitosan nanoparticles that helps drugs in eyedrops reach the actual targeted part of the eye better, making eyedrops a promising and practical solution over surgery.

Help Received

FedEx printed board. Parents greatly helped with transportation. I used the lab equipment at the Harker School under the supervision of Dr. Gary Blickenstaff and Mr. Chris Spenner. Used lab equipment at Stanford under supervision of Dr. Jiang and Professor Mellins.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Chaitra S. Subbarao

Project Number

S0526

Project Title

DNA Sequence Analysis Reveals Differences between Epazote Downy Mildew and Other Downy Mildews

Abstract

Objectives/Goals

Downy mildew caused by Peronospora effusa is a serious disease of spinach in coastal California and worldwide. I hypothesized that downy mildew from Epazote (Dysphania ambrosioides), a popular crop in Mexican cuisine and grown extensively in California, is genetically identical to the pathogen that causes disease on spinach. Both of these crops belong to the Chenopodiaceae family. My goal was to employ the ITS (internal transcribed sequence) region from the rDNA to determine the genetic similarity between these pathogens. This analysis will help determine the validity of a DNA-based diagnostic assay already developed for specific detection of the spinach downy mildew pathogen.

Methods/Materials

DNA from infected Epazote leaves was extracted using a commercial DNA extraction kit and the ITS region was amplified by Polymerase Chain Reaction (PCR). The PCR product was visualized on an agarose gel and transferred into a TOPO vector. The vector containing the cloned insert was sequenced and returned in the form of a chromatogram, which was analyzed and cloned sequences were obtained. The National Center for Biotechnology Information (NCBI) database was searched by the Basic Local Alignment Search Tool (BLAST) using the cloned sequence. Newly cloned sequences were then aligned with closely matched sequences from NCBI and phylogenetic relationships were determined.

Results

Of the first hits, P. effusa, P. farinosa, and P. corydalis were the closest matches, having a sequence identity of 93%. P. effusa infects spinach, P. farinosa infects beet, and P. corydalis infects squirrel corn. From the sequence analysis, it was determined that the species of Peronospora that affects Epazote is distinct from P. effusa and that the former most likely represents a novel species of Peronospora.

Conclusions/Discussion

My studies indicated that Peronospora effusa (the pathogen that infects spinach) is genetically distinct from the downy mildew species that infects Epazote. These results rejected my initial hypothesis that the two pathogens are genetically similar. Epazote downy mildew has not been previously reported in California to our knowledge. The species of downy mildew that infects Epazote is still undetermined. Future studies will be focused on describing this new species, and also its pathogenicity on other selected crops or weeds in California.

Summary Statement

Analyses of the ITS rDNA sequences determined that spinach and Epazote downy mildew are genetically distinct.

Help Received

Dr. Steven Klosterman and Mrs. Amy Anchieta accommodated me in their laboratory and guided me through all phases of my study.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

Emily S. Wang

Project Number

S0527

Project Title

Illuminating Disease Pathways: Developing Bright Fluorescent Proteins to Improve FRET Biosensing

Abstract

Objectives/Goals

The discovery and development of fluorescent proteins, recognized by the 2008 Nobel Prize in Chemistry, enabled a revolution in biological microscopy and sensing. Biosensors employing fluorescence resonance energy transfer (FRET) between fluorescent proteins are powerful tools to non-invasively report biochemical events within living cells. The development of new FRET sensors remains difficult, however, often due to low FRET dynamic range. The objective is to develop a brighter green fluorescent protein (GFP) and a brighter red fluorescent protein (RFP) which can function as an effective FRET pair.

Methods/Materials

Using random and site-directed mutagenesis and standard cloning techniques (ligation, transformation), I created libraries of GFP and RFP mutants, which were screened for photostability, brightness, and performance in a FRET-based calcium sensor. To screen for brightness, upon taking fluorescence images of bacterial colonies, I identified proteins with the greatest brightness values via ImageJ software, where the brightest proteins were incorporated into a FRET pair. Mutants with high FRET to GFP fluorescence ratios were selected. To measure extinction coefficient and quantum yield, I used the base-denaturation method and integrated the emissions, respectively. To screen for photostability, colonies and protein lysates were photobleached with a LED array, where brightness levels were quantified using the software ImageJ or a plate reader. I purified the fluorescent proteins using cobalt-chelating affinity chromatography, and I used an inverted microscope to continuously photobleach proteins through time-lapse imaging. To evaluate dynamic range, mutants were used to construct TN-XXL calcium sensors.

Results

I engineered a new green fluorescent protein Clover3, which is the brightest monomeric fluorescent protein to date. Clover3 confers increased FRET dynamic range onto biosensors and shows improved photostability and quantum yield. Moreover, I developed a new red fluorescent protein mRuby3, which is the brightest red fluorescent protein to date.

Conclusions/Discussion

With superior optical characteristics, Clover3 and mRuby3 are expected to benefit diverse biomedical applications, including the imaging of neural structures, visualization of cancer metastases, and monitoring of signaling pathways to elucidate disease mechanisms.

Summary Statement

I engineered the brightest monomeric green and red fluorescent proteins to date, which may allow us to visualize biological activities with greater clarity than before.

Help Received

This independent research project was carried out by Emily Wang under the supervision and guidance of Dr. Jun Chu and Professor Michael Lin at Stanford University.

CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)
Vahe S. Yacoubian

Project Number
S0528

Project Title
An Innovative Method of Reducing Cholesterol in Foods by Converting It into Vitamin D

Abstract

Objectives/Goals
The objective of this study is to introduce a previously undescribed method of lowering cholesterol in food by converting it into Vitamin D. Seven out of ten Americans are living with Vitamin D deficiency. Simultaneously, we are faced with obesity and heart disease, largely due to the consumption of foods rich in cholesterol. Through this unique method, cholesterol is converted into Vitamin D by utilizing a natural enzyme reaction occurring in the Shitake Mushroom, where the enzymes tyrosinase and isozymes normally convert ergosterol into Vitamin D in the presence of UV light.

Methods/Materials
The experiment consisted of mixing equal amounts of ground mushroom gill (enzymes) and egg yolk (cholesterol). The experimental sample was exposed to sunlight while the control was kept in the dark. Both samples were periodically measured for levels of cholesterol before and after sunlight exposure or dark exposure (control).

Results
The results show at least a 40% decrease in the amount of cholesterol in the experimental sample versus control.

Conclusions/Discussion
Cholesterol is shown to decrease by being converted into Vitamin D by utilization of a mushroom enzyme reaction during exposure to sunlight. This experiment suggests that cholesterol can be successfully substituted for ergosterol in the natural mushroom enzymatic reaction to produce Vitamin D. The implications of this project are profound in the food and health industries.

Summary Statement
This project introduces an innovative method of using mushroom enzymes to lower cholesterol in foods by converting it into Vitamin D.

Help Received
Parents provided all the necessary materials to conduct the experiment.