Tracking Orai1, Adenylyl Cyclase Type 8, and STIM1 Activity in Triple Negative Breast Cancer

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**Introduction**

- Figure 1: Store Operated Calcium Entry. STIM1 senses calcium depletion in ER and activates Orai1. Interaction between AC8 and Orai1 leads to calcium influx.

- Figure 2: Interplay of AC8 and Orai1 in Cancerous Cells. Orai1 is incapable of being phosphorylated and inactivated due to overexpression of AC8. Surplus of calcium elicits various cellular responses including migration and proliferation of breast cancer.

**Research Objectives**

- Analyze the effect Zegocractin has on STIM1’s coupling to Orai1 via NanoBiT
- Examine how Forskolin impacts AC8’s binding to Orai1 via NanoBiT
- Verify that AC8 and Orai1 protein are overexpressed in triple negative breast cancer cell lines (MDA-MB-231 and MDA-MB-157) using anti-Orai1 and anti-AC8 antibodies and a fluorescently tagged secondary antibody

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**Methods**

- Figure 7: NanoBiT Protein Tagging: A bright luminescent signal is detected as LgBiT functionalizes with SmBiT on the protein of interest

- Figure 8: Antibody Detection in Western Blots. Primary antibody binds to the protein of interest. Secondary antibody with fluorescence signal detects primary antibody.

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**Results**

- Figure 3: Zegocractin is a specific inhibitor to the Orai1 and STIM1 protein-protein interaction. Smaller increases in RLU values demonstrate lack of STIM1 binding to Orai1. N=3

- Figure 4: Forskolin is a potent activator of AC8. Decreases in RLU values indicate that forskolin debilitates the AC8 and Orai interaction. N=3

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**Western Blots**

- Figure 5: Transfected HEK293T Cells. PVDF membrane from HEK293T cells transfected to overexpress AC8 and Orai1. Successful binding of AC8 primary and secondary antibodies (lane 4); AC8 didn’t migrate to correct molecular weight (140 kDa). Anti-Orai1 primary didn’t detect Orai1 (lanes 6-10)

- Figure 6: MDA-MB-231/MDA-MB-157 (TNBC) cells. Orai1 is overexpressed (33 kDa) in TNBC cells (lanes 1 & 2) in relation to MCF12 cells (normal breast epithelial, lane 4). Anti-Orai1 primary antibody binds to Orai1 and is detected by goat anti-rabbit secondary antibody

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**Conclusions/Implications**

- Zegocractin significantly inhibits STIM1 and Orai1 interaction
- Forskolin diminishes luminescent signal between AC8 and Orai1
- Run NanoBiT protein assay with forskolin on Orai1 and STIM1
- Use immunoprecipitation before running western blot to isolate Orai1 or AC8 from lysate

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**References**


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**Acknowledgments**

I would like to express my gratitude to Moana Hala’ufia, Dr. Roman, and Joshua C. Wilkinson for their guidance throughout this project. Special thanks to SSTP and the Belin-Blank Center for this amazing opportunity.
Raspberry Pi Based Data Acquisition System (DAQ) for a Colorectal Cancer SiNW Array-based Biosensor

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Motivation and Project Goals

Project Goals:
- The purpose of this project is to create an efficient Data Acquisition System (DAQ) for the Colorectal Cancer (CRC) SiNW Array Biosensor to facilitate its use as a point of care test.

Significance and Motivation:
- Colorectal Cancer (CRC) is the third leading cause of death for both women and men worldwide.
- Tracking common CRC Protein biomarkers (i.e., CEA) is beneficial in identifying possible CRC patients, raising 5-year survival rates by nearly 91%.
- Common approach for CRC tracking/detection is enzyme-linked immunosorbent assay (ELISA) but high-cost and need for trained personnel limits its use as point-of-care (POC) device.

Why Silicon Nanowire Array Biosensor?

Fig. 1 NWs are very sensitive detectors because of extremely high surface area to volume ratio.

Fig. 2 The final Silicon Array is portable and this allows it to be easily scalable.

Results

Fig. 4 Semi-log data plots of ΔI% as a function of CEA concentration level (A) and as a function of CA 19-9 (B). R² squared values for the regression confirm a strong correlation between the ΔI% and antigen concentration.

Graphical User Interface (GUI)

Page displays test instructions and records username, date and time of test. Loading page 10s progress bar. Results page displays % current change graph, antigen concentration level and patient diagnosis.

Future Work

As of now, our results have been based on current changes generated by an Analog Potentiometer rather than the actual vSiNW-diode-based biosensor. For future work, we plan to test our DAQ by connecting our current sensor to the actual vSiNW-diode-based sensor and introduce a compact casing for our hardware.

Reference(s)


DAQ Flowchart
Introduction

- Apnea of prematurity (AOP) is a serious sleep disorder wherein premature neonates stop breathing > 15-20s at a time due to an underdeveloped brainstem (the part of the central nervous system that controls breathing).
- Recent studies show that caffeine can stimulate the brainstem. However, caffeine is a hydrophilic, small molecule that does not have ideal physiochemical properties for absorption through the skin. Hence, microneedle-assisted drug delivery of caffeine through topical gel represents an innovative approach for AOP.

Objective

The goal of this work is to formulate caffeine-loaded gels with hydroxyethyl cellulose (HEC) as a polymer carrier and characterize drug release, and permeation through microneedle-treated skin.

Materials and Methods

In Vitro Release and Permeation Study

Diffusion cells: In-line diffusion cells with 1.76 cm² diffusion area (Permegear)

Donor: 500 μL of caffeine base (1.3% w/v) with HEC (1.5% and 2.5% w/v) at pH 5, 6.5, and 7.4 (n = 3)

Receiver: HEPES buffer (pH 7.4) prewarmed to 37°C

Membrane:
- Release study: cellulose dialysis membrane (Snakeskin® Dialysis Tubing, 10K MWCO)
- Permeation study: dorsal skin from Yucatan miniature pigs

Analysis: Samples were analyzed with HPLC

Results

Solubility Study

Table 1. Solubility of caffeine base in citrate phosphate buffer at pH 5, 6.5, and 7.4 represented as mean ± SD (n = 3).

<table>
<thead>
<tr>
<th>Solution pH</th>
<th>Caffeine Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5</td>
<td>15.721 ± 0.044</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>14.420 ± 0.014</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>13.209 ± 0.131</td>
</tr>
<tr>
<td>water</td>
<td>19.095 ± 0.015</td>
</tr>
</tbody>
</table>

Viscosity Study

Table 2. Viscosity of caffeine base (1.3% w/v) loaded gel at pH 5, pH 6.5, and pH 7.4 represented as mean ± SD (n = 3).

<table>
<thead>
<tr>
<th>Gel pH</th>
<th>Viscosity (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5</td>
<td>1.036 ± 0.02</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>6.510 ± 0.211</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>5.994 ± 0.191</td>
</tr>
<tr>
<td>water</td>
<td>6.584 ± 0.263</td>
</tr>
</tbody>
</table>

In Vitro Permeation Study: MN-treated skin

Figure 1. Flow curve of caffeine base (1.3% w/v) loaded gel at pH 5, pH 6.5, and pH 7.4 represented as mean ± SD (n = 3).

Figure 2. Cumulative release of caffeine base (1.3% w/v) loaded gel at pH 5, 6.5, and 7.4, represented as mean ± SD (n = 3).

Figure 3. Cumulative permeation of caffeine base (1.3% w/v) loaded gel at pH 5, 6.5, and 7.4 through dorsal skin from Yucatan miniature pigs, represented as mean ± SD (n = 3).

Viscosity of caffeine base (1.3% w/v) loaded gel at pH 5, pH 6.5, and pH 7.4 represented as mean ± SD (n = 3).

Table 3. Cumulative delivery, lag time, and steady-state flux of caffeine base (1.3% w/v) loaded gel (1.5% w/v and 2.5% w/v HEC) at pH 5, 6.5, and 7.4 represented as mean ± SD. Q(24): cumulative permeation over 24 hours; J(24): steady state flux

<table>
<thead>
<tr>
<th>Group</th>
<th>Gel pH</th>
<th>Q(24) (μg)</th>
<th>Lag time (hr)</th>
<th>J(24) (μg/cm²/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact skin</td>
<td>pH 5</td>
<td>539.59 ± 181.7</td>
<td>2.11 ± 0.97</td>
<td>13.99 ± 4.73</td>
</tr>
<tr>
<td></td>
<td>pH 6.5</td>
<td>154.30 ± 38.75</td>
<td>1.68 ± 0.91</td>
<td>4.01 ± 1.56</td>
</tr>
<tr>
<td></td>
<td>pH 7.4</td>
<td>701.03 ± 137.07</td>
<td>3.86 ± 0.37</td>
<td>19.76 ± 3.54</td>
</tr>
<tr>
<td>MN-treated</td>
<td>pH 5</td>
<td>1145.76 ± 702.18</td>
<td>1.85 ± 1.07</td>
<td>22.01 ± 16.66</td>
</tr>
<tr>
<td></td>
<td>pH 6.5</td>
<td>1232.63 ± 155.10</td>
<td>2.28 ± 1.06</td>
<td>32.20 ± 2.86</td>
</tr>
<tr>
<td></td>
<td>pH 7.4</td>
<td>1446.78 ± 469.12</td>
<td>1.99 ± 0.12</td>
<td>36.56 ± 9.47</td>
</tr>
</tbody>
</table>

Conclusion

- 62.35-72.80% of the drug was released in the first 2.5 hours, suggesting immediate release of caffeine base from gel
- Drug release was not statistically different at various pH levels
- With MN pretreatment, a significant increase in steady-state flux was observed compared to intact skin
- Decrease in the absorption lag time for pH 5 and pH 7.4 through microneedle-assisted delivery represents a quicker onset of action

Acknowledgements

I would like to thank Dr. Heena Maithania for her mentorship and guidance. Dr. Nicole K. Brogden for the opportunity to conduct research in her lab, as well as the entire Brogden Lab and the University of Iowa College of Pharmacy. Special thanks to Belin-Blank for making this opportunity possible.

References

Utilization of rTg4510 mouse model in Alzheimer’s disease and related dementias

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Introduction

Alzheimer’s disease (AD) is a progressive medical condition which is classified as a type of dementia. This disorder disrupts the function of the brain by killing the neurons and causing the brain to shrink, also known as brain atrophy. The hippocampus is affected in the early stage of AD. It destroys the ability to think, learn, and memorize.

Mechanisms of AD

• The main causes of Alzheimer’s disease are the two abnormal protein build-ups: amyloid precursor protein (APP) and tau protein.

• APP generates amyloid beta polypeptides which forms amyloid plaques around neurons.

• Tau protein often leads to neurofibrillary tangles (NFTs) in the brain.

• NFTs are a major hallmark in AD as they affect the communication between neurons and are often found in severe Alzheimer’s disease brain tissues.

• However, there is no effective treatment for long-term memory deficits in Alzheimer’s disease and related dementias (ADRD).

Objectives

• Assess the effectiveness of using tau-based rTg4510 mouse to model the neurofibrillary tangles present in ADRD.

• Discuss implications of the rTg4510 mouse model on clinical research for ADRD.

Methods

A

Results

Figure 1. Comparison between healthy brain tissues and Alzheimer’s brain tissues (amyloid plaques and neurofibrillary tangles presence).

Figure 2. In a healthy brain, tau proteins bind to microtubules with a stabilized phosphorylation. In Alzheimer’s, depolarized microtubules disintegrate as the tau proteins form neurofibrillary tangles around the neurons due to hyperphosphorylation.

Figure 3. The control and rTg mice comparison

3-4 months old mice
The controls are littermates with the rTg mice

Figure 4. Both the rTg mice and the control mice were tested by spatial object recognition (SOR), the scoring results are based on the time spent exploring the object (touch or non-touch). SOR task is closely related to the mouse hippocampal activity as the hippocampus is in charge of spatial memory.

Figure 5. Validation of transgene presence in rTg mice
The mouse line DNA was run in PCR and agarose gel electrophoresis after the behavior tests, which validates the SOR scoring results in our experiment.

Figure 6. SOR task scoring results

Figure 7. SOR task scoring results

Figure 8. Western blots results in control and rTg mice
The top of this figure illustrates a simplified representation of the control mouse with only the CaMKIIα-tTA gene and the rTg4510 mouse with CaMKIIα-tTA and TetO-hMAPT P301L gene. The image below in the western blots of tau protein phosphorylation in the mouse dorsal hippocampus. The proteins are extracted from the hippocampus due to the fact that the hippocampus is essential for learning and memory. As reflected in figure, both control and rTg mice express actin protein. On the other hand, only rTg mice exhibit AT8 phosphorylated tau protein.

Conclusions

• The time spent exploring represents the preference the mouse expresses towards the objects.

• The control mice exhibit preference towards the displaced object. In contrast, the rTg mice did not show a clear preference.

• The mouse’s preference suggests their ability to identify objects in space, which corresponds to the function of the hippocampus. If they do not exhibit clear preference, meaning the mice do not remember the location of the object after 24 hours. This shows that the rTg mice exhibit long-term memory deficits and impaired memory consolidation.

• The PCR and Western blotting results verify the genotype of each mouse and validate the SOR scoring, demonstrating that the rTg mice form neurofibrillary tangles in brain.

Implications

• These findings suggest that rTg4510 mouse model could be used to simulate medical conditions in ADRD.

• Our study provides an alternative method to research the mechanisms for memory loss.

• Future studies should seek to approach ADRD clinically using our mouse model.

Acknowledgments

Special thanks to Utsav Mukherjee, Dr. Snehajyoti Chatterjee, Dr. Ted Abel, all members from Ted Abel lab as well as the University of Iowa Carver College of Medicine for mentoring and guiding me on this project. I would also like to thank the SSTP program for providing me with this opportunity.

References


Background

- Hemophilia A is a genetic bleeding disorder that prevents blood from properly clotting due to the lack of clotting factor VIII (FVIII).
- Patients with hemophilia A receive FVIII protein replacement therapy, greatly improving their life expectancy and quality of life.
- However, those suffering with hemophilia A have neurophenotypic differences such as increased rates of anxiety and depression, decreased brain volumes, and increased cerebral microbleeds.
- Preliminary studies from the Staber Lab have shown that mice with hemophilia A experience behavioral differences compared to wild type mice.
- These neuropathologic differences including the increased rates of anxiety and depression and the behavioral differences could be caused by neuroinflammation.

Methods

- This study aims to characterize the neuroinflammatory response in a mouse model of hemophilia A by measuring microglial activation and gene expression levels of neuroinflammatory markers.
- Microglial activity was measured by analyzing microglial morphology in the hippocampus, cortex, and thalamus of hemophilia A and wild type mice as well as quantifying microglia in different stages of activation.
- We hypothesize that FVIII deficient (hemophilia A) mice will have increased expression of pro-inflammatory markers and increased microglial activity compared to controls.

Objectives

- To determine if Factor VIII deficiency (Hemophilia A) causes increased neuroinflammation.
- To investigate if FVIII deficient mice exhibit altered microglial activity and gene expression levels compared to wild type mice.

Results

- Homozygous mice received FVIII protein replacement therapy, greatly improving their life expectancy and quality of life.
- Microglia classifications form a spectrum from ramified, "resting" microglia, to ameboid, microglia post-injury.
- Microglia in Hemophilia vs. Control Mice
  - Microglia Activity
    - Brains from 6-month-old HA and WT mice were harvested.
    - The brains are then sectioned and stained using immunofluorescence.
    - Immunofluorescent staining included Iba1 and CD68, markers of microglial activation.
    - After slides are stained, they are imaged under a confocal microscope.
  - Gene Expression of Neuroinflammatory Markers
    - 6-month-old hemophilic and wild type mice brains were harvested.
    - The cortex, brainstem, and cerebellum were isolated from these brains and used for RNA isolation.
    - Gene expression analysis was performed using qPCR, and expression was measured relative to WT mice.

Conclusions

- Confirming our hypothesis, expression of neuroinflammatory markers remained similar between wild type and hemophilia A mice in the cortex.
- In the brainstem and cerebellum of HA mice, three and seven markers (respectively) showed elevation compared to WT mice, indicating activation of neuroinflammatory pathways.
- Total rodlike and ameboid microglia appeared elevated in HA mice, indicating increased microglial activity.
- The activation of microglia and neuroinflammatory pathways could be correlated with the increased mental health disorders and decreased neurocognition seen in patients with HA.

Acknowledgements

I would like to thank the SSTP program and everyone at the Belin-Blank Center for this opportunity. Special thanks to Dr. Staber, Danielle York, and Kendall Cornick for welcoming me in the lab, allowing me to take on their project, and for their gracious mentoring.

References

### Effect of Total Element Area on Number Discrimination in Pigeons

**John Fu¹, Francisca Díaz², Leyre Castro Ruiz², Edward Wasserman²**  
¹Milton Academy, ²Department of Psychological and Brain Sciences, The University of Iowa

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#### Introduction

The abstract concept of number is a common dimension seen in daily interactions with the physical world. While once thought to be a uniquely human skill, numerosity can also be perceived by nonhuman animals such as pigeons and bees. Many theories have proposed that number is correlated with nonnumerical magnitudes such as the total area of all stimulus elements (Lourenço and Aulet, 2022). Research has also shown that numerosity is used as a discriminative stimulus when other cues are made irrelevant (Kubo, 2020).

In this study, three different relations between the total area of the stimulus elements and numerosity were devised to identify the interdependence among these quantities: confounded, matched, and conflicting.

**Confounded**: More items, More Area  
**Matched**: More Items, Equal Area  
**Conflicting**: More Items, Less Area

#### Methods

Subjects (n=4) were trained to discriminate numerosity. In some trials, choice of the larger number was correct, and on other trials, choice of the smaller number was correct depending on the color of the items.

**Training Phase:**  
- Confounded Area  
- Matched Area  

**Testing Phase:**  
- Training Phase Areas  
- Conflicting Area

#### Experimental Setup

For each trial, a peck to the start stimulus was followed by two response options on either side of the screen. Food pellet reinforcement followed correct responses, but not following incorrect responses.

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#### Results

**Training Phase**

**Area Effect - Training**

**Testing Phase**

**Area Effect - Testing**

**Ratio Effect**

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#### References


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#### Conclusions

The pigeons exhibited excellent numerical accuracy scores under all manipulations of element area. These results imply strong control by number rather than area.

1. Pigeons learned to discriminate number, showing sensibility to numerical disparities.
2. Manipulated element areas didn’t impair pigeons’ abilities to discriminate number.

Future experiments should further explore the interactions with number and area, without training with matched area. Additionally, other visual cues that could impact stimulus discrimination in pigeons is also worth investigating (DeWind, Bonner, and Brannon, 2020).
Introduction

The crux of this project is to develop new methods for creating building blocks for pharmaceuticals. We found that 4-alkyl pyridine derivatives can readily undergo sulfonylation using various sulfonyl chloride derivatives under mild conditions. These methods can be used to create parts of pharmaceutical drugs.

Synthesis of 4-Alkyl Pyridine Precursors

Synthesis of precursors was achieved either by Horner-Wadsworth-Emmons (HWE) reaction or Wittig reaction followed by hydrogenation. The resulting compounds were used to test the scope of the reaction using sulfonyl chloride in a later step.

Sulfonylation of 4-Alkyl Pyridines: General Reaction Scheme

Post-Synthetic Modification of 4-(Tosylmethyl) Pyridine

To show the application of our newly synthesized compounds, we further treated one of our targeted products (4-(tosylmethyl) pyridine) with different reagents.

Exploring Scopes and Limitations

Characterization

• Melting Point tells us how strong our compound’s intermolecular forces are and how pure it is
• Nuclear Magnetic Resonance (NMR) gives information about how many unique hydrogens and carbons are in our compound
• Mass Spectrometry gives information about the chemical composition of the compound

Purification

• Acid-Base Extraction
• Recrystallization
• Flash Column Chromatography

Catalysts, Bases, and Solvents Used

• Each reaction performed better using certain bases, solvents, and catalysts
• Stronger bases are more dangerous to use due to high reactivity
• Some bases and solvents reacted together which inhibits the reaction
• Certain solvents were harder to remove in the purification process

Pharmaceutical Potential

The compounds we created can be further manipulated to gain access to complex synthetic intermediates in pharmaceutical drugs more readily. They have been clinically shown to treat cancers, malaria, arthritis, tuberculosis, and other serious conditions. This project streamlines the drug manufacturing process by developing more efficient methods to synthesize building blocks of crucial pyridine-containing drugs.

Sulfasalazine disease-modifying anti-rheumatic drug (arthritis)

Acknowledgments

I would like to thank the Secondary Student Training Program and the University of Iowa for giving me this great research opportunity.
Introduction

- Epilepsy is a neurological disease characterized by spontaneous seizures.
- The phenomenon where a person dies due to a seizure is known as sudden unexpected death in epilepsy (SUDEP).
- SUDEP happens more during the night in both humans and rodent models.
- Nighttime SUDEP risk appears to have a circadian rhythm component.
- Dravet Syndrome is a form of epilepsy that starts early in life and has a high risk of SUDEP.

Graphical Abstract

HYPOTHESIS: Scn1aR1407X/+ mice are more likely to die following heat-induced seizures during the night.

Materials and Methods

Mouse Model: Scn1aR1407X/+ mouse model of Dravet Syndrome. These mice experience potentially fatal seizures shortly after development and are susceptible to heat-induced seizures.

1. Mice were genotyped by routine PCR
2. Mice were weaned at post-natal day 21
3. Radio telemeters were implanted into the subcutaneous space to measure temperature
4. Throughout the studied, animals were monitored for spontaneous seizure-associated death
5. Animals were subject to a single heat induced seizure at Zeitgeber Time (ZT) 6 or 18
6. Animals were allowed to acclimate to the chamber for 10 minutes with their temperature held at 37.5 °C before increasing their temperature by 0.5 °C every couple of minutes.
7. Temperature and video was analyzed post-hoc

Results

Fig 1. The Scn1aR1407X/+ mouse model of Dravet Syndrome experiences spontaneous, seizure-associated death. Shortly after weaning (post-natal day 21; hashed line), mice experience spontaneous, seizure-associated death (A). Mice were housed in a 12:12 light-dark cycle (lights on at 6 AM, off at 6 PM) and monitored throughout the day (7:30 AM until 4:30 PM). Leaps that occurred during this time were considered daytime seizures, while those outside of this window were groups as nighttime or transition (within 1.5 hours of light cycle change). Three of the four observed deaths occurred during the nighttime or transition period (B). Animals that were euthanized or died following a heat-induced seizure were excluded from analysis.

Fig 2. Representative temperature recording during a heat-induced seizure trial. Mice were acclimated for 10 min at 37.5 °C and then slowly warmed up with a heat lamp until experiencing a seizure.

Fig 3. Core body temperature at time of seizure. Seizures induced during the day are associated with a lower temperature required to induce a seizure and increased variability compared to those induced at night (A). However, when comparing baseline temperature, mice have higher core body temperature during the night (B).

Fig 4. Duration and severity of heat-induced seizures does not appear to be influenced by time of day. Video collected from infrared cameras were analyzed post hoc to determine the duration and severity of heat-induced seizures.

Fig 5. Heat-induced seizures appear to be more fatal during the night. While seizures induced during the day were never fatal, 2/3 of seizures resulted in death.

Discussion/Conclusion

- SUDEP is more likely to occur during the night.
- Although spontaneous deaths in the Scn1aR1407X/+ have been shown to occur more often during the night, whether or not this is true for heat-induced seizures is unknown.
- Preliminary evidence from this study demonstrates that heat-induced seizures that occur during the night are more likely to be fatal.
- However, it is not clear which mechanisms contribute to this nighttime risk.
- Two potential contributors are the suprachiasmatic nucleus, the body’s circadian pacemaker or oscillating neurotransmitters, such as serotonin.

Future Directions

Will the rhythm of death persist if:
1. The brain’s circadian pacemaker is destroyed?
2. Serotonin neurons are eliminated from the brain?

Acknowledgements

This work was supported by the NIH/NIGMS T32 CM007337 (to Iowa MSTP), NIH/NINDS R01 NS095842 (to GFB), and the Beth L. Tross Epilepsy Professorship (to GFB). Data was analyzed using GraphPad Prism 9. Vector images created in BioRender.

References

Why Silicon Nanowire Array Biosensor?

Fig 1. NWs are very sensitive detectors because of extremely high surface area to volume ratio

Fig 2. The final silicon array biosensor is portable, and this allows it to be easily scalable.

Motivation and Project Goals

Significance and Motivation:
- CRC is the third leading cause of cancer related death in the US but also one of the most easily preventable form of cancer.
- Point of care testing reduces unnecessary deaths from people avoiding screening out of embarrassment.

Project Goals:
- Create an efficient data acquisition system (DAQ) for the colorectal cancer (CRC) SiNW array biosensor silicon.
- Facilitate the biosensor’s use as point of care test.

Results

Fig 4. Semi-log data plots of Δ% as a function of CEA concentration level (a) and as a function of CA 19-9 (b). R-squared values for the regression expressions confirm a strong correlation between Δ% and antigen concentration

Healthy

< 1.0 ng/mL

CEA Concentration

Warning

1.0 – 2.5 ng/mL

CEA Concentration

Unhealthy

> 2.5 ng/mL

CEA Concentration

Graphical User Interface (GUI)

(a) Homepage displays test instructions and records username, date and time of test, (b) Loading page 10s progress bar. (c) Results page displays % current-change graph, antigen concentration level and patient diagnosis

Future Work

As of now, our results have been based on current changes generated by an analog potentiometer rather than the actual SiNW-diode-based biosensor. For future work, we plan to test our DAQ by connecting our current sensor to the actual SiNW based biosensor and introduce a compact casing for our hardware.

References

Verifying a stable SETDB1 reporter and studying its expression when KDM4A is overexpressed (OE) in endometrial cancer cells

Kiersten Knobbe¹, Kiarash Salari², Shujie Yang³
¹AC/GC High School, ²Graduate Research Assistant, University of Iowa, ³Assistant Professor of Pathology, University of Iowa

Background

• Endometrial Cancer (EC) is one of few cancers with cases on a rise in recent years. It is the most common gynecological malignancy equating to around 12,000 deaths each year.

• SETDB1 is a H3K9 methyltransferase involved in gene silencing. It has been identified as an oncogene in various cancers creating a need to better understand its mechanisms of amplification, overexpression, and activation. It is usually overexpressed and correlates with the worst EC patient prognosis.

• mCherry is a member of the mFruits family and is a basic red fluorescent protein. The protein has been used to visualize genes and analyze their function. It glows red when exposed to green light.

• KDM4A is a lysine specific demethylase that demethylates H3K9/36. It has been identified as an oncogene and is usually overexpressed correlating with EC progression and poorer patient prognosis.

• GFP is the green fluorescent protein. It is often used to monitor gene expression and glows green when exposed to blue light.

Objectives

• Verify mCherry as a stable SETDB1 reporter in ECC1, Hec50 and ISH cells
• Study how overexpression of KDM4A-GFP correlates with SETDB1 expression

Methods

SETDB1 Reporter (mCherry)

• Method 1 transfect ECC1, Hec50, and Ishikawa cells with SETDB1 ex22 sgRNA-IrrCRISPR v1 and SETDB1 reporter gene (SETDB1-hygromycin-mCherry)

• Method 2 determine which single clone has correct SETDB1 reporter insertion in gDNA using PCR to identify the clone. Then run an agarose gel and use chemidoc to check gel bands for insertion.

• Method 3 grow positive clones into spheroids and use a fluorescent microscope to visualize reporter expression.

KDM4A Overexpression

• Method 1 transfect reporter positive clones with KDM4A – GFP at two different densities (10k and 20k)

• Method 2 grow clones into spheroids and use a fluorescent microscope to visualize SETDB1 and KDM4A expression.

Acknowledgements

I would like to thank Dr. Yang for the opportunity to work in her lab. I would also like to thank Kiarash Salari for his mentorship and guidance as well as Matthew Wells and Johnathan Schultz and the entire Yang lab. Lastly, I would like to thank the Belin Blank Center and STSP program for this research opportunity. Funding for this project was supported by NIH R37-CA238274 (SY) and the Department of Pathology Start-Up Fund (SY).

Results

SETDB1 Reporter (mCherry)

Fig. 11 graphical representation of band sizes that are expected for negative and positive clones.

Fig. 12 these images show the agarose gel bands from PCR that were ran to confirm insertion of the mCherry vector.

KDM4A Overexpression

Fig. 13 these images show the spheroids of cells that were transfected with SETDB1 reporter and KDM4A-GFP. They were taken using a fluorescent microscope.

References


Macroinvertebrates Bioaccumulate Pharmaceuticals and Neonicotinoids in an Effluent-Dominated Stream: A Tale of Bugs on Drugs

Krisha Kapoor, Grant Hemphill, Alyssa Mianecki, & Gregory LeFevre

Introduction

- Waterbodies receive high amounts of neonicotinoids and pharmaceuticals from runoff and wastewater
- The purpose of this study is to investigate if macroinvertebrates are bioaccumulating neonicotinoids and pharmaceuticals

Experimental Design

- In-field sampling at Muddy Creek, Iowa
  - 1 L and 500 mL water collection at US1, Effluent, and 5km Downstream
  - 1-hour bug collection at US1 and Effluent
- In-lab extraction
  - Water extracted with solid phase extraction (SPE)
  - Bugs extracted with a series of solvents

Instrumental Method

- Samples analyzed using LC-MS/MS

Results

- Upstream Biota Concentrations
- Effluent Biota Concentrations
- Effluent Water Concentrations
- Skm Downstream Water Concentrations

Pharmaceuticals and Neonicotinoids

- Imidacloprid was found in upstream crawfish
- No pollutants were found in upstream bugs
- Metformin, citalopram, and guanylurea were found in effluent crawfish
- Bupropion, citalopram, and fexofenadine were found in effluent bugs

Pollutants in the macroinvertebrates have a higher abundance at the effluent site

- The upstream biota concentration range is non-detect (n.d.) to 20.75 ppb
- The effluent biota concentration range is n.d. to 1285 ppb

The Big Picture

- Water quality has degraded due to climate change and urbanization
- Other organisms in aquatic and terrestrial ecosystems are likely exposed to these pollutants
- Manmade pollutants may be present in drinking water downstream of pollutant sources

Data Analysis

- Effluent Water
- Effluent Bugs
- Effluent Crawfish

References

Optical coherence tomography as a noninvasive tool to determine collagen content in skin

Nishant Lahiri1, Valeria Cota2, Nicole K. Brogden3,4
1Corning-Painted Post High School, Corning, NY, 2University of Iowa Interdisciplinary Program in Human Toxicology, Iowa City, IA, 3University of Iowa College of Pharmacy, Department of Pharmaceutical Sciences and Experimental Therapeutics, Iowa City, IA, 4University of Iowa Carver College of Medicine, Department of Dermatology, Iowa City, IA

INTRODUCTION

Collagen in the skin

- Collagen is an abundant protein in the skin necessary for maintaining structural integrity
- Determining collagen content is required in clinical practice to characterize tissues and determine disease states
- Current methods for determining collagen content are invasive and usually require biopsies of the skin that are >25cm²
- Hydroxyproline comprises >15% of the amino acid composition in collagen
- Collagen content is commonly measured using biochemical hydroxyproline assays, computer-aided histomorphometric analyses of histological sections, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Optical Coherence Tomography (OCT)

- OCT is a noninvasive optical imaging modality that provides high resolution and cross-sectional imaging
- Allows for collagen estimation using attenuation and surface reflectivity values
- Overcomes the need for biopsies and >24 hr. analytical testing times
- Has not been validated or quantified

MATERIALS AND METHODS

Optical Coherence Tomography

- Collected attenuation coefficient and surface reflectivity values using a 3x3 mm section at 250 frames. Scanned in three different locations.

Hydroxyproline assay

- Subcutaneous fat and connective tissue was removed from human skin samples prior to hydrolysis
- Hydroxyproline calibration curve was prepared to interpolate unknown concentrations from skin samples

Bicinchoninic acid (BCA)

- Total protein content in human skin samples was measured to determine loading quantities in SDS-PAGE gels

ACKNOWLEDGMENTS

Nishant Lahiri would like to thank the Secondary Student Training Program at the University of Iowa and the Bell-Bank Center for Gifted Education for the opportunity to conduct research. Nishant thanks the University of Iowa Tissue Procurement Core for the human skin samples and Dr. Ethan Anderson for the Chloramine T reagent. Nishant would like to acknowledge the Brogden lab members for their guidance, especially Ms. Valeria Cota for teaching various laboratory techniques and analysis, and Professor Nicole Brogden for her mentorship and support.

REFERENCES


Figure 1. OCT image of excised abdominal skin.

Figure 2. Overview of the hydroxyproline extraction protocol. Collagen samples were hydrolyzed to individual amino acids including hydroxyproline. Neutralization of the hydrolysate potentiates a color reaction with Dragendorff reagent to produce a chromophore with peak absorption of light with wavelengths between 460 and 540nm.

Figure 3. Human skin hydroxyproline content (μg/mg) vs optical attenuation coefficient (mm⁻¹). A positive correlation was found between hydroxyproline content and OCT measurements.

Figure 4. Human skin hydroxyproline content (μg/mg) vs surface reflectivity (AU). Mean of absorbance values repeated in triplicate. Each data point represents an individual donor. Matching shapes indicate samples from the same patient.

Figure 5. Sample hydroxyproline content measurements.

Figure 6. Porcine skin hydroxyproline content (μg/mg) vs optical attenuation coefficient (mm⁻¹). Mean of absorbance values repeated in triplicate. Each data point represents an individual donor. Matching shapes indicate samples from the same anatomical site.

Figure 7. Porcine skin hydroxyproline content (μg/mg) vs surface reflectivity (AU). Mean of absorbance values repeated in triplicate. Each data point represents an individual donor. Matching shapes indicate samples from the same anatomical site.

Figure 8. SDS-PAGE of extracted collagen from human skin. Lane 1: molecular weight marker. Lane 2: collagen control from rat tail. Lane 3: Back 1. Lane 4: Abdomen 1. Lane 5: Back 2. Lane 6: Abdomen 2.

Figure 9. Type I collagen content in human skin determined using SDS-PAGE. Image software was used to determine normalized intensity.

CONCLUSIONS

- A positive correlation was found between hydroxyproline content and OCT measurements
- Care must be taken to ensure digested skin sample falls within the linear range of the hydroxyproline calibration curve
- OCT is a promising technique for the noninvasive determination of collagen content in human skin

FUTURE STUDIES

- Positive correlation of hydroxyproline content to attenuation and surface reflectivity values will be verified with a greater number of skin samples
- SDS-PAGE will be rerun to accurately correlate protein to hydroxyproline content

RESULTS

A positive correlation was found between hydroxyproline content and attenuation/surface reflectivity in human skin & porcine skin

CONCLUSIONS

- A positive correlation was found between hydroxyproline content and OCT measurements
- Care must be taken to ensure digested skin sample falls within the linear range of the hydroxyproline calibration curve
- OCT is a promising technique for the noninvasive determination of collagen content in human skin

FUTURE STUDIES

- Positive correlation of hydroxyproline content to attenuation and surface reflectivity values will be verified with a greater number of skin samples
- SDS-PAGE will be rerun to accurately correlate protein to hydroxyproline content
Evaluation of optimal threshold settings for use of UBO Detector automated segmentation software for assessment of White Matter Lesions on FLAIR imaging Magnetic Resonance Imaging

John LaMasters, Shivangi Jain PhD, Kelsey Baller, Jenna Springer, Michelle W. Voss PhD

Introduction

The field of study investigating aging and the brain specifically in the area regarding the relationship between hypertension and white matter lesions requires assessment of MRI imaging primarily using FLAIR imaging (Wardlaw, Forte, Badji). Further study of the impact of aging and the brain including the impact of exercise and hypertension require evaluation of WML on FLAIR imaging MRI (Voss, Aghayan). Gold standard technique of human evaluation of WML on FLAIR MRI is labor intensive and time consuming. Several software programs have been evaluated for automated segmentation however no specific program has been determined to be the reference standard. UBO Detector has been previously validated (Jiang, Hotz, Vanderbeek) for automated segmentation. There is potential for error introduced due to background signal “noise” in the MRI scans. Different threshold settings are available to adjust the sensitivity. The optimal settings for use of the UBO Detector software have not previously been determined. This study was performed to compare the results of 0.5 and 0.7 thresholds for automated segmentation of the UBO Detector to the gold standard of human interpretation. The threshold is a percentage of voxels detected to be defined as a WML. Methods

Assessment of WML was performed using FLAIR MRI images by 3 separate independent observers. We performed automatic segmentations with settings 0.5 and 0.7 using the UBO Detector software (Jiang). We used observation by human researchers as the gold-standard. 98 participants scans were evaluated. Results of thresholding with the automated segmentations at 0.5 and 0.7 were rated by human reviewers and graded on a 1 to 3 integer scale for performance (1 = good, 2= adequate, 3= poor) Pulse Pressure (PP) = Systolic Diastolic Pressure. The masks given rating of 1 by two of three observers were compiled into two intensity maps based on their pulse pressure (PP) group (High PP >50, Low PP =to 50) Mask overlay of the two pulse pressure groups was performed to evaluate degree of overlap Examples of FLAIR mask ratings

Rating 1 2 rating 3 rating

Discussion

This study was performed to further validate the UBO Detector automated segmentation software technology that may be used to make the process of assessment of white matter lesions (WML) on FLAIR imaging MRI more efficient than the previous method of human observer interpretation. Several automated segmentation software programs have been validated previously (Jiang, Hotz, Vanderbeek). No standard reference of automated segmentation software has been determined. Error in automated segmentation software can be introduced due to background signals of the MRI scan. Threshold Settings of 0.5 and 0.7 of the UBO Detector software were evaluated for congruence to gold standard manual human interpretation to identify the most accurate settings. Results indicate future investigation of threshold 0.3 may prove to be more accurate. If a reference standard was able to be determined for automated segmentation software programs and specific threshold settings, it would assist researchers with efficiency and better comparability of results for future study. Conclusion

The performances we report of both UBO Detector threshold settings 0.5 and 0.7 is variable and moderate accuracy but acceptable, with most readings receiving a rating of 1 or 2. Both settings consistently underestimated WML volume and had home images with a rating of 3. This suggests more work is needed to improve performance of automated algorithms for WML automated segmentation. Similar to other studies, both threshold settings 0.7 and 0.5 were acceptable but lower than human interpretation and no clear reference standard was identified. High PP is associated with microvascular disease and the effects of WML are more diffusely located throughout the brain compared with low pulse pressure.

Acknowledgments

I would like to thank Dr. Michelle Voss in the Psychological and Brain Sciences who was my mentor for this research project. I would also like to thank my lab colleagues, Dr. Shivangi Jain, Kelsey Baller, Jenna Springer, Bryan Madero, and Will Daniels for support in data collection and interpretation.

References


Ave Age 63 yo; Range 55-80
Research Objective

To investigate the effects of isoform-specific prickie mutations on the neurological dysfunction of prickie larvae, mainly short-term memory and movement.

Methods and Materials

**Genotypes** (All outcrossed to a w^1118 background)
- W^T (+/+)
- pk^wes1/pk^wes1 (spie/sple) – null mutation of the spiey-legs isoform, seizure-prone
- pk^wes1/pk^wes (pk/pk) – null mutation of the prickle isoform, exhibits neurological degeneration and shortened lifespan
- pk^wes1/pk^wes1 pk^sple13/pk^sple13 (pk-sple13/pk-sple13) – null mutation for the prickie gene

**Larval Crawling Assay**

1. L3 larvae were individually placed onto an 85 mm 2.5% agarose plate. 30 seconds of active crawling were recorded to track the larval movement. (Canon High Definition Vixia HFM31 Camcorder with zoom [resolution 1920 × 1080] for higher resolution). Motion was assessed using a Manual Tracking plugin of FIJI software.

**Larval Olfactory Learning Assay**

1. L3 larvae were trained on 2M fructose agarose plates with 2 caps of either 1:100 OCT or 1:25 MCH (shown below) for 5 minutes.
2. The larvae were then transferred onto the agarose plate without fructose with 2 caps of the opposite scent for 5 minutes.
3. Steps 1 and 2 were repeated.
4. Larvae were immediately placed in the center of the testing plate, which contained 1 cap of each scent. After 5 minutes, the position of each larva was recorded.
5. The performance index was then calculated.

**Results**

- Figure 2: pk mutant larvae, but not sple mutant larvae, show an increase in locomotor speed. Note the wide variance of velocities for the pk/pk mutants in comparison to the other isoforms. However, the median speed for pk/pk is higher than the other isoforms and shows a statistically significant increase in velocity. Notably, the sple/sple mutant velocities are not significantly different than control, suggesting that the sple mutant larvae do not have a locomotor defect.

- Figure 3: Drosophila L3 sple mutant larvae do not exhibit learning deficits. Preliminary data reveals that sple mutant larvae can associate a scent with an attractive stimulus (fructose) similar to controls. This suggests the sple mutation does not drastically impact the ability of Drosophila larvae to form and retain short-term memories. n = 30 for all lines. Kruskal-Wallis test.

**Conclusions**

- sple mutant larvae do not show obvious locomotor defects as assessed by the crawling assay.
- Notably, these data suggest that sple mutants only show locomotor defects as adults.
- sple mutant larvae do not show learning deficits as assessed by the larval olfactory learning assay.
- Similar to the locomotor assay results, sple mutants only show learning deficits as adults.

**Future Directions**

- Increase the sample size for each strain for both experiments.
- Explore automating movement tracking to standardize tracking and allow for assessing more detailed tracking parameters.
- Employ the learning assays with an aversive rather than attractive stimulus (NaCl or Quinone) to determine whether learning ability differs between control and mutants.

Acknowledgements

- This work was supported by a grant from the Stead Family Department of Pediatrics, Carver College of Medicine to JRM.
- I would like to thank Dr. Manak and everyone in the Manak lab for their time, help, and input over this summer.

References

- Conclusions
- Future Directions
- Acknowledgements
- References

**Conclusion**

Impact of Placental *Igf-1* Overexpression on Angiogenesis in Embryonic Mouse Brain

Aidan Lin¹, Annemarie Carver²,³,⁴, Robert Taylor³,⁴, and Hanna Stevens²,³,⁴

Brophy College Preparatory¹, Interdisciplinary Graduate Program in Genetics², Department of Psychiatry³, Iowa Neuroscience Institute⁴ Carver College of Medicine, University of Iowa, Iowa City, Iowa

Introduction

- Autism Spectrum Disorder (ASD) is found in around 1 in 44 children (CDC)
- Common traits are challenges with social skills, repetitive behaviors, speech and nonverbal communication
- *Igf-1* is an important growth factor/hormone for neuron formation
- Placenta is main source of *Igf-1* prior to birth
- Some people with ASD have altered expression of *Igf-1*
- Recent findings show people with ASD have abnormal angiogenesis in the brain

We hypothesize that overexpression of *Igf-1* in the placenta will cause abnormal blood vessel development in the brain.

Methods

- CRISPR used to overexpress *Igf-1* in mouse placentas on embryonic day 12 (E12)
- Immunohistochemistry: Stained E18 coronally-sectioned brains with Isolectin B4 to visualize vasculature
- Microscopy and Stereology: Measured striatal volume & counted blood vessels and branch points
- Statistics: T-test to analyze embryonic angiogenesis in control and *Igf-1* overexpression samples

Results

### Striatal Volume

<table>
<thead>
<tr>
<th>Striatal Volume</th>
<th>Control</th>
<th><em>Igf-1-OE</em></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E18 Female Striatum Volume</td>
<td>8×10⁶</td>
<td>6.0×10⁶</td>
<td><em>p</em> = 0.0346</td>
</tr>
<tr>
<td>E18 Male Striatum Volume</td>
<td>8×10⁶</td>
<td>7.0×10⁶</td>
<td><em>p</em> = 0.7008</td>
</tr>
</tbody>
</table>

significant increase in E18 female striatal volumes and trending increase with males

### Striatal Blood Vessels

<table>
<thead>
<tr>
<th>Blood Vessel/µm²</th>
<th>Control</th>
<th><em>Igf-1-OE</em></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E18 Female Blood Vessel Density</td>
<td>1.5×10⁵</td>
<td>2.5×10⁵</td>
<td><em>p</em> = 0.0226</td>
</tr>
<tr>
<td>E18 Male Blood Vessel Density</td>
<td>1.5×10⁵</td>
<td>2.5×10⁵</td>
<td><em>p</em> = 0.2318</td>
</tr>
</tbody>
</table>

No significant difference in number of striatal blood vessels regardless of sex or placental *Igf-1* expression

### Striatal Branch Points

<table>
<thead>
<tr>
<th>Branch Points/µm²</th>
<th>Control</th>
<th><em>Igf-1-OE</em></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E18 Female Branch Point Density</td>
<td>6×10⁵</td>
<td>4×10⁵</td>
<td><em>p</em> = 0.0589</td>
</tr>
<tr>
<td>E18 Male Branch Point Density</td>
<td>6×10⁵</td>
<td>4×10⁵</td>
<td><em>p</em> = 0.0778</td>
</tr>
</tbody>
</table>

No significant difference in number of striatal blood vessel branch points regardless of sex or placental *Igf-1* expression

Conclusions

- Significant increase in striatal volume in females with a trending increase in males and females combined
- This result recapitulates previous findings from our lab, demonstrating that placental *Igf-1* levels impact neurogenesis
- No difference in blood vessels or branch points regardless of sex or *Igf-1* placental expression
- Some people with ASD have an enlarged striatum
  - The E18 female striatum volumes from our study also show this finding
  - Enlarged striatum can contribute to the development of ASD as the striatum controls restrictive and repetitive behaviors
- Previous work has shown that individuals with ASD do not show structural differences in blood vessels but show abnormal blood vessel organization and proliferation
- Our study replicates this and shows no structural difference in blood vessels

Future Directions

- Continue to investigate impact of placental *Igf-1* overexpression on angiogenesis in the brain
- Further explore brain vascular development
  - Splitting/Intussusceptive
  - Proliferation of blood vessels
- Look into other time points such as E14, E16, and post-birth
- Investigate placental angiogenesis

References and Funding Sources


Serial Coronal Sections of E18 Mouse Brain

R01 MH122435 and NIH T32GM008629
PHB2 reduction increases progesterone receptor (PR) expression in endometrial cancer cells

Evelyn Liu1; Tianyue Li2; Xiangbing Meng, PhD2; Shujie Yang, PhD2
1American High School; 2Department of Pathology, University of Iowa

Background
- Endometrial cancer is one of the few cancers with an increasing number of cases in recent years
- Estrogen and progesterone are two hormones that regulate endometrial growth through interactions with their receptors
- Estrogen promotes endometrial growth while progesterone limits endometrial growth
- Progesterone receptor (PR) is significantly downstreamregulated in endometrial cancer patients
- PHB2 is an oncogene that can inhibit production of apoptotic proteins such as caspases 3 and 8
- PHB2 is correlated with higher rates of tumorigenesis and is negatively correlated with PR expression (Fig. 1)

Previous experiments have shown dramatic increases in PR expression by knocking down PHB2
- sgRNA and shRNA, once integrated in viruses can be used to lower the expression of mRNA and proteins
- shRNA turns into siRNA upon insertion into target cells, marks proteins for degradation
- sgRNA guides CRISPR/Cas9 to cleave target sequences out of host genomes

The PHB2 gene is located on chromosome 12p13.31
- The available shRNA sequences target four of the ten PHB2 exons

Methods
1. Create sgRNA insert from oligo pairs
2. Transfect plasmid in E. Coli for plasmid production
3. Extract plasmid DNA from E. Coli
4. Transfect 293FT cells for lentiviral production
5. Transduction into target cells (ECC1, Hec50, Ish)
6. Western blotting to analyze PR and levels

Results

PHB2 exons

[Table with PHB2 exons and corresponding concentration values]

Objectives
- Generate PHB2 knockdown and knockout cells
- Analyze the change in PR expression in PHB2 knockdown and knockout cells
- Evaluate whether PHB2 is a potential target treatment oncogene

Conclusions/Implications
- Consistent PHB2 knockout and knockdown cells can be successfully created using sgRNA and shRNA
- Survival rates were lower in current knockout cells than knockdown cells
- A reduction in PHB2 led to an increase in PR expression by an average of 100% in ECC1 and Ish endometrial cancer cell lines
- Knocking down with shRNA-3 and shRNA-4 resulted in the highest increase of PR expression in ECC1 and Ish cells
- Increased by around 200%
- Targeting exon 8 of PHB2 gene resulted in high PR level increases
- PHB2 is a potential cancer treatment target oncogene

Future Directions
From our results, we discovered that targeting exon 8 for PHB2 knockdow was highly effective, but the exact function and mechanisms of exon 8 are yet unknown. Future studies should aim to discover the significance of exon 8 in endometrial cancer. Our lab will continue analyzing the effect of PHB2 knockout using sgRNA in endometrial cancer cells on PR expression. Other directions of PHB2 research should focus on analyzing the different functions and mechanisms of PHB2 in cancer patients and the reasoning behind the variability in the correlation between PHB2 expression and tumorigenesis. Additionally, PHB2 research should explore the effects of PHB2 reduction on non-target cells and how the effects of PHB2 reduction can be mediated within non-target host cells.

Acknowledgements
A special thank you to the Belin Blank Center for providing this amazing opportunity and to Tianyue Li, Dr. Meng, and Dr. Yang for your patience and guidance in these five weeks.

References

The Endometrium
Estrogen
Progesterone

Estrogen promotes endometrial growth while progesterone limits endometrial growth.
PERFORMANCE SIMULATION OF A RADIATION TOLERANT QUARTZ BASED HIGH GRANULARITY CALORIMETER

P. Loranger, J. Wetzel  
The University of Iowa - Dept. of Physics and Astronomy

Problem

As particle colliders race towards higher energies, a variety of problems arise when trying to design and build new detectors, particularly when designing calorimeters used in the very forward (VF) region of a collider:

- Scintillators take too long to reset, creating pile-up
- VF detectors are exposed to by extreme amounts of radiation, scintillators are not tolerant enough.
- Current VF detectors have lower energy resolution compared to primary detectors; VF data is not used in cutting edge research.

Solution

Quartz calorimeters are the perfect candidate for VF detector designs in higher luminosity particle colliders.

- High radiation tolerance
- High energy resolutions
- Detects and ‘reads’ events very quickly

Results

- CERN’s ROOT HEP data analysis toolkit and Python
- 4x4x4 preforms better than 3x3x7
- See QR code for full dataset & graphs

Methodology

- Layers of iron absorber and quartz cubes, backed by SiPMs
- 4x4x4, 3x3x3, 3x3x21
- Simulation written in C++ using GEANT4 simulation toolkit.
- Over 1,000,000 individual simulations ran on Argon HPC!

Conclusion

As of time of writing, our simulations are still running and we do not have a complete set of data, particularly for the very promising 3x3x21 calorimeter. Use the QR code above to read the updated version of this poster our complete set of data.

Acknowledgments

I would like to thank Professor Wetzel for guiding me in my research and my parents for their never-ending support.
Data Analysis and Prediction for DMQMC
Save computing resources by interpolating data across $\Delta \tau$

Songhang Man, Gabriel Smith, William Van Benschoten, James Shepherd

Introduction
Density Matrix Quantum Monte Carlo, DMQMC, stochastically samples the thermal Density Matrix, $\rho(\beta)$, where $\beta = (k_B T)^{-1}$, to yield exact-on-average energies for the system. In DMQMC, $\rho(\beta)$ is rewritten as:

$$\rho(\beta) \rightarrow f(t) = \sum_i f_i(t) |\psi_i\rangle \langle \psi_i|$$

with each iteration of the simulation sampling:

$$f_{i+1}(t+\Delta t) = f_i(t) [1 + \Delta \tau \beta - \frac{1}{2} \sum_{ij} [H_{ij} f_i(t) + f_i(t) H_{ij}]$$

DMQMC simulations with small $\Delta \tau$ produce high accuracy results but with high computation cost. We are looking for a method to save computer resource in calculation while still retaining a relatively high accuracy.

Research Question
Can spline interpolations be used to predict accurate small $\Delta \tau$ DMQMC data using low-cost large $\Delta \tau$ data?

Methods
Data analysis was done on the stretched $H_6$ linear chain. The interpolations were tested by:

- Extracting evenly spaced points from the original dataset.
- Comparing different degree interpolations to the original dataset.
- For cubic spline, altering number of data points in interpolation.

Predicting small $\Delta \tau$ data with large $\Delta \tau$ data:
- Comparing original large $\Delta \tau$ and small $\Delta \tau$ datasets.
- Comparing large $\Delta \tau$ interpolations with small $\Delta \tau$ dataset.
- Altering the high $\Delta \tau$ interpolation with weighting and smoothing factors.

Python Libraries
- Data processing: Pandas, NumPy
- Interpolation: scipy.interpolate.UnivariateSpline
- Plotting: Matplotlib

Variables
- Degree of polynomial in interpolation
- Percentage of data used in interpolation

Figure 1. Normalized difference between the interpolated and original values under different degrees of interpolation with 20% of the data points used in interpolation.

1. Different Degree of Interpolation
- Higher degree interpolations have a better fit to the original data.
- Linear interpolations suffer systemic errors in low-beta range.
- The difference between high and low degree interpolation is most exemplified as a low percentage of the data is used to make interpolation.

Figure 2. Normalized difference between the weighted (inversely on $2\sigma$) and original data under different degree of interpolation and original data.

2. Amount of Data in Interpolation
- Using cubic interpolations:
  - The amount of data points can be reduced to 1.25% while containing the residual within $2\sigma$.
  - Outliers in a peak at the first few interpolation points.
  - Interpolations can yield good predictions with few datapoints.

Figure 3. (Main Plot) Energy differences between the 0.0012$\tau$, 0.002$\tau$, 0.01$\tau$, and 0.012$\tau$ datasets and FCI. (Inset) Energy difference between the Interpolation of high $\Delta \tau$ data and 0.0012$\tau$ data.

3. Large $\Delta \tau$ vs Small $\Delta \tau$
- The large $\Delta \tau$ data systematically underestimate system energies in low beta range, which may be caused by time-step error or other errors exacerbated in the high $\Delta \tau$ setting.
- In high beta range, 0.01$\tau$ displays a similar error magnitude to exact data compares to 0.001$\tau$.
- Interpolation by itself does not help reconciling the gap.

Figure 4. Energy difference between the weighted (inversely related to the residual in figure 3) interpolation and 0.0012$\tau$ data with various smoothing factor.

4. Weighting and Smoothing
- The manipulations in weight and smoothing have not reduced the difference in the low beta area.
- The lines with different smoothing factors overlap on this scale.
- We speculate that the smoothing factor has reached a threshold that results in a single 5th degree curve. The smoothing is a topic for future investigation.

Summary of Findings
- Interpolations can predict the original data set, even with few data points.
- Higher degree interpolating splines have a better fit, in general, to the data.
- Cubic interpolations of 2% of the dataset results in residuals within $2\sigma$.
- Large $\Delta \tau$ data systematically underestimate the energies in the low-beta range.
- Interpolation alone does not reconcile this $\Delta E$.
- Weighting and smoothing factors have not shrunk the $\Delta E$.
- In the high-beta range, large $\Delta \tau$ displays similar $\Delta E$ to exact compared to small $\Delta \tau$.

Future Work
To further investigate into the datasets and resolve the time-step error in low beta range, we will take the following approaches:

- Find out the error source: Investigate the original DMQMC algorithm to identify the source of error, run calculations to calculate the error.
- Filter the data: Investigate in the point where the error magnitudes of high and low $\tau$ data converge to isolate the high quality $\tau$ data from the low beta range low quality data.
- Better model: Investigate in other models/operations that can fit the data better, such as applying Inverse Laplace Transform on the spline of the datapoints. According to our result in step 4, the 5th degree polynomial curve seems to have a relatively good fit to the data, which can also be investigated.

Acknowledgments
I would like to express my gratitude to Gabe Smith and Professor Shepherd for mentoring and giving constructive helps during my project, and all other researchers in Shepherd Group for providing a welcoming and friendly environment. I would also like to thank the Belin-Blank Center and the SSTP for this research opportunity.

References
What are the main reasons why people did/did not wear a mask during the COVID-19 pandemic?

We analyzed 10,000 Tweets to provide data for the artificial intelligence.

Using AI to Analyze Why People Did Not Wear Masks During the COVID-19 Pandemic

This can be used to find the reasons to many different queries, primarily why people did/did not wear a mask during the COVID-19 pandemic.

From that data, government officials can alleviate some troubles people have regarding masking, help promote healthier ways to live, and make more informed decisions regarding masking.

Introduction
- I am Elizabeth North, I am from Saint Andrew's School in Florida.
- I enjoy computer science and economics, specifically artificial intelligence and machine learning.
- The projects we are working on are very significant since they involve real-world issues that have an impact on our society.

Methods
- First, we used analyzed ten thousand posts manually to classify the data into containing a reason or not containing a reason to provide data for the model.
- We used exploratory/descriptive analysis and topic modeling in order to group the posts together, then visualized them using word clouds. The posts would get categorized by frequently appearing word groups or phrases and how often they appeared together.
- Based on that data, we can train a machine learning algorithm to find which posts have reasons and which do not automatically.
- Then, we train and tune the decision tree model to find the highest accuracy for classifying posts.

Research question
- What are the main reasons why people did/did not wear a mask during the COVID-19 pandemic?
- We analyzed 10,000 Tweets to provide data for the artificial intelligence.

Results
- To the left are some of the major reasons why people didn't wear masks.
- When we trained our model and tested it, we found that it had an accuracy rating of 70%.
- With more fine-tuning or a more advanced model, we can further increase the model's accuracy and performance.
- Once we have a high enough performance, we plan on using this model to classify one million Tweets so that we will have a much more comprehensive understanding of why people did not wear masks.
- More accuracy means it will be more helpful to discover the primary reasons why people are for or against a topic.

Consequences
- This can be used to find the reasons to many different queries, primarily why people did/did not wear a mask during the COVID-19 pandemic.

Topic 1: Belief That Masks Are Ineffective

Topic 2: Belief That Masks Harm the Wearer

Topic 3: Belief That There Isn't Enough of a Risk

Topic 4: Belief That COVID is a Hoax

Topic 5: Belief That Masks Are Unconstitutional

Topic 6: Belief That There Isn't Enough Evidence That Masks Work

Topic 7: Belief That Masks Are Useless (Only for Virtue Signaling/Particles Are Too Small)
Lipid Nanoparticles as a Delivery System for CST6-encoding cmRNA for Bone Regeneration Applications

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Purpose

• Cystatin E/M (CST6) has been associated with the inhibition of osteoclast differentiation (Li et al., 2021). We proposed that CST6 might promote osteogenic differentiation.
• Different doses of CST6 protein may promote osteogenic differentiation of the pre-osteoblastic MC3T3-E1 cells. Mineralization of the differentiated cells can be visualized at the end of the study using Alizarin Red S Staining (2.1).
• SM102-based lipid nanoparticles can be fabricated using a microfluidic device to encapsulate CST6-encoding mRNA to deliver the mRNA to cells (2.2).
• Use of modified nucleosides like pseudouridine and N1-methyl pseudouridine can affect the translation of the cmRNA transcript. We tested this using eGFP as a reporter gene to assess the nucleosides’ impact on transfection (2.3).

Methods

2.1 Osteogenic promotion of hCST6

hCST6 Protein Treatments

Monolayer cultures of Pre-osteoblasts (MC3T3-E1)

2.2 Nanoparticle Formulation

Generating dsDNA Template with 3’ P3T5′-tail

Plasmid DNA

In Vitro Transcription Using T7 Polymerase

Lipid Nanoparticle Synthesis (morpholinos, FRK = 3:1)

Forming lipid nanoparticles with the mRNA

2.3 Transfection of MC3T3-E1 cells

Lipofectamine

Pre-osteoblast (MC3T3-E1) Cells

Flow Cytometry

Transfection Efficiency

Results

Figure 1. Alizarin Red S Staining of MC3T3-E1 (subclone 4) cells after 21 days of osteogenesis induction. The cells were treated at confluency with bovine bone morphogenic protein (bBMP-2, 100 ng/mL) in osteogenic medium or recombinant human CST6 protein (hCST6, 10 ng/mL or 50 ng/mL) in complete MEM-alpha medium containing osteogenic supplements.

Figure 2. Denaturing 15% agarose gel electrophoresis of aliquots of in-vitro transcribed Cap1-hCST6-eGFP-A10. The expected size of the complete mRNA transcript was 1,425 nt.

Figure 3. Representative fluorescence micrographs of MC3T3-E1 (subclone 4) cells at 24 hours after transfection with 0.5 μg of Cap1-hCST6-eGFP-A10 mRNA, using Lipofectamine 2000 (10 μg/mL: Lipofectamine 2000: ratio = 1:2). Cells were seeded into 24-well plates at the density of 50,000 cells per well and left to attach for 24 hours prior to experiment. Scale bars represent 400 μm.

Figure 4. transfection efficiency in MC3T3-E1 (subclone 4) cells transfected with in-vitro-transcribed Cap1-P2A-eGFP-A10 mRNA. The eGFP expression was assessed using flow cytometry and Lipofectamine 2000 was used as the transfection agent (mRNA: Lipofectamine 2000: ratio = 1:2). (A) and (B) show the transfection efficiency at 24 hours and 48 hours after transfection (n = 4 per group). Statistical analysis was done one-way ANOVA with multiple comparison tests using Tukey’s test. *p < 0.05 between different base modifications at 0.25 μg/mRNA dose, and #p < 0.05 between different base modifications at 0.5 μg/mRNA dose. (C) and (D) show representative flow cytometry data with (D) depicting the gating of the target population and (D) showing representative transfection data.

Figure 5. The particle size distribution of SM102-based lipid nanoparticles from Dynamic Light Scattering (DLS) data. The lipid nanoparticles were loaded with either hCST6-P2A-eGFP-A10 mRNA (mRNA LNPs) or were formed without payload (Blank LNPs).

Conclusions

• The cells treated with recombinant human CST6 protein produced noticeable calcium deposits after Alizarin Red S staining.
• We were able to formulate lipid nanoparticles encapsulating Cap1-CST6-P2A-eGFP-A10 mRNA with desirable physical characteristics.
• We found that the replacement of uridine base in the mRNA transcript with N1-methyl pseudouridine yielded better transfection efficiency in MC3T3-E1 cells than the non-modified mRNA and the pseudouridine-modified cmRNA.

Acknowledgements

I would like to give special thanks to Dr. Salem and Pornpoj Phruttisawichakun for mentoring me and giving me the opportunity to work in a lab. I would also like to thank the Belin Blank Center and Secondary Student Training Program for providing me with the resources and ability to perform research in a professional setting.

References


The figure used in Methods section was created with BioRender.com.
Neuroanatomical Correlates of Noun and Verb Retrieval in the Controlled Oral Word Association Test

Eliza Podvalny1, Carolina Deifelt Streese2,3, Jax Skye2,5, Joel Bruss2, Daniel Tranel2,4

1The Hackley School, NY; 2Department of Neurology, University of Iowa; 3Department of Neurosurgery, University of Iowa
4Department of Psychological and Brain Sciences, University of Iowa; 5Department of Psychiatry, University of Iowa

Introduction

- Previous neuroimaging studies show a double dissociation between nouns and verbs.
  - Verbs – frontal lobe
  - Nouns – temporal lobe
- Controlled Oral Word Association (COWA) test is a verbal fluency test where participants have 1 minute to say as many words as possible that begin with a given letter.

Hypothesis: Can parts-of-speech analysis of COWA data be used with the lesion method to offer new perspectives on the double dissociation between nouns and verbs in the brain?

Methods

- Participants: 226 patients with focal, stable, acquired brain lesions from the Iowa Neurological Patient Registry.
- Structural neuroimaging scans obtained 3 months or more after the lesion onset.
- List of words from the COWA test
- Parts of Speech Analysis - total number of nouns and verbs (calculated noun-to-verb ratio)
- Neuroanatomical Correlates – LESYMAP Analysis

Results

<table>
<thead>
<tr>
<th>Gender</th>
<th>Nouns</th>
<th>Verbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>57%</td>
<td></td>
</tr>
</tbody>
</table>

Etiologies

- Stroke - Ischemic
- Resection - Ellipsy
- Resection - Hemorrhagic
- Resection - Tumor
- Resection - AVM or Cavernoma
- Encephalitis - HSE
- Resection - Abscess, SAH – Clip, Head Trauma - Closed, Unknown

- 95% of participants were Caucasian
- 5% of participants were African American, American Indian, Unknown, or Other

Discussion

- Replicated previous results that showed differentiation between nouns and verbs
- Limitation with homographs, POS tagger, and lost 5% of words due to illegibility

Future Directions

- Full brain analysis
- Use modeling approach that gives likelihood, not fixed categories
- Explore other parts of speech (e.g. adjectives)

Acknowledgments

Special thanks to Dr. Tranel and Dr. Deifelt Streese for their guidance and continued support on this project. I would also like to thank Belin-Blank and SSTP for this amazing research opportunity.

References

   https://doi.org/10.1016/j.neubiorev.2010.04.008
Does Electrically Induced Exercise During Stance Attenuate Peak Metabolic Biomarkers in People With and Without Spinal Cord Injury?


Department of Physical Therapy and Rehabilitation Science, Carver College of Medicine
University of Iowa, Iowa City, IA

Introduction

The lack of physical activity increases the likelihood of developing non-communicable diseases including type two diabetes and cancer.1,2 While life-style recommendations, like exercise, are plausible for people with intact central nervous systems (CNS), it is challenging for people with paralysis because they cannot volitionally “turn on” their paralyzed muscles. After muscle paralysis people with metabolic disease lose their sensitivity to insulin and are unable to move glucose out of the bloodstream leading to diabetes. Recent research supports that only 15 minutes of exercise after a meal removes glucose from the blood stream requiring less need for insulin.3 We sought to understand if standing with or without “electrically induced” exercise, increases HR and reduces key biomarkers, like insulin, in people with and without paralysis from spinal cord injury (SCI).

Purpose and Hypothesis

We aim to determine if passive and active stance triggers an increase in heart rate (HR) and regulates metabolic biomarkers in individuals with and without SCI. We expect that passive and active stance will both increase HR and attenuate peak insulin and glucose levels in people with and without SCI.

Methods

Participants: One male (age XX) without SCI and one male (age xx) with SCI.

Design: Two Sessions of Stance: with or without exercise of the quad/HS muscles before and after a balanced meal

Exercise: Electrically induced using 3 Hz frequency and ~ 100 millamps intensity.

Outcome measures: HR, LF/HF, Insulin, and Glucose

Results

Insulin levels after a meal are reduced during stance and 3 Hz exercise as compared to stance without exercise in both participants.

Glucose levels after a meal are reduced with stance and 3 Hz exercise but only in participant without SCI.

Heart rate with stance and 3 Hz exercise is increased in both participants.

LF/HF, as an index of sympathetic vagal tone, is greatest with stance and 3 Hz exercise in both participants.

Conclusions and Clinical Implications

The first major finding of this preliminary “proof of concept” study is that stance and electrically induced exercise increased HR, LF/HF, and attenuated peak insulin levels following a meal in a person with SCI. The second important discovery is that when the stance was combined with electrically induced exercise there was an even greater reduction in insulin compared with stance alone. A limitation is that we do not know if the electrical stimulation in supine would yield a similar outcome as stance, and that is the focus of an ongoing trial. These preliminary results offer the first metabolic analysis of stance with and without electrically induced exercise in a person with SCI.

References and Acknowledgements


This study was funded by the National Center for Medical Rehabilitation Research within the National Institutes of Health: Grants R01 HD104445 and R01 HD32109

We thank the Secondary Student Training Program (SSTP) for providing us the opportunity to do research with Dr. Shields and his lab.
Do Healthcare Practitioners Prescribe Exercise After Meals In Patients With Metabolic Impairment?


Department of Physical Therapy and Rehabilitation Science, Carver College of Medicine
University of Iowa, Iowa City, IA

Introduction

The lack of physical activity increases the likelihood of developing non-communicable diseases including type two diabetes and cancer.1 2 Metabolic disease is a primary predictor of all cause mortality. The emphasis in medicine on prescriptive drugs may overshadow a “patient centered” lifestyle approach, including exercise, for patients with metabolic disease.1 2 We sought to survey physicians and rehabilitation specialists to determine if exercise is prescribed for people with metabolic impairment and the type of exercise that is recommended.

Insulin opens the “door” for glucose to leave the bloodstream and enter external organs, like muscle. People with metabolic disease lose their sensitivity to insulin and are unable to remove glucose out of the bloodstream after a meal. Recent research supports that only 15 minutes of exercise after a meal removes glucose from the blood in the absence of insulin.3 We sought to survey physicians and rehabilitation specialists to determine if they prescribe exercise after meals to reduce the need for insulin.

Purpose and Hypothesis

The aims of this study are to determine the dose of exercise that healthcare providers (physicians and physical therapists) recommend, and whether the timing of exercise is related to eating for people with metabolic impairment. We expect that healthcare providers (physicians and physical therapists) recommend exercise consistently, but that neither healthcare specialty considers time of meals when prescribing exercise.

Methods

Participants:
- 59 English-speaking healthcare providers
- Seven MD departments (40)
- One Rehabilitation department of physical therapists (PT) (19)

Data Collection and Analysis: Each healthcare provider completed a 10-question survey regarding their standard recommendations on exercise including the time of day and relation to meals. As demonstrated in the figure, 80% of all healthcare providers DO not recommend exercise based on the time of a meal. Only 7% of all participants recommended exercise immediately after a meal.

Results

Conclusions and Clinical Implications

The first major finding of this study is that neither rehab specialists or physicians recommend exercise following a meal. This finding suggests healthcare practitioners are not aware of the contemporary research supporting that 15 minutes of exercise following a meal significantly reduces post-prandial insulin levels.3 A second major finding is that rehabilitation specialists recommend 20% more anaerobic exercise and 13% more general activity as compared with physicians. Lastly, there is internal consistency between rehab specialists and physicians among several aspects of exercise prescription. Methods to translate new research findings into the clinic may be needed for all healthcare specialties.

References and Acknowledgements


This study was funded by the National Center for Medical Rehabilitation Research within the National Institute of Health: Grants R01 HD064445 and R01 HD082109. We thank the Secondary Student Training Program (SSTP) for providing us the opportunity to do research with Dr. Shields and his lab.


This study was funded by the National Center for Medical Rehabilitation Research within the National Institute of Health: Grants R01 HD064445 and R01 HD082109. We thank the Secondary Student Training Program (SSTP) for providing us the opportunity to do research with Dr. Shields and his lab.
Methods

1. Site-directed mutagenesis
   - PCR
   - Ligation
   - Transformation
   - Culture
   - Plasmid purification
   - Sequencing
   - Large scale plasmid purification

2. Protein Expression
   - Culture cells
   - Transfection
   - EL inhibition assay
   - Western blot
   - Harvesting
   - LPL inhibition assay
   - EL binding assay

3. Functional characterization
   - EL inhibition assay
   - LPL inhibition assay
   - EL binding assay

Results

EL and LPL Inhibition Results

- **Figure 1.** (A) PCR products mutants pA3A91-99. (B) Western blot set 1 cell media results. Protein was only expressed in mutant pA3A95 (S25A F26A) and wild-type ANGPTL3 (pEB14).

EL Binding Assay Results

- **Figure 3.** Luminescent signal from NanoBit binding measured by combining LgBiT-EL with concentrations of smBiT, WT, and mutant after incubation at 37°C for 30 minutes.

EL Binding Assay Results

- **Figure 2.** (A) Phospholipase activity of EL after incubation at 37°C for 30 minutes with increasing concentrations of ANGPTL3 or ANGPTL3 (S25A F26A) in complex with ANGPTL8.

EL Binding Assay Results

- **Figure 3.** Luminescent signal from NanoBit binding measured by combining LgBiT-EL with concentrations of smBiT, WT, and mutant after incubation at 37°C for 30 minutes.

Conclusion

- ANGPTL3 mutant (S25A F26A) is defective against LPL inhibition
- Retains binding and inhibition of EL

Future directions

- Perform LPL binding assays
- Finish 9 mutants of N-terminal domain and combine data already collected by the lab to map out important residues.
- Test interesting mutants on mouse models to test the physiological effects

Acknowledgments

References


PLANT UPTAKE RATES OF XENOBIOTIC COMPOUNDS WITH HIGHLY ELECTRONEGATIVE FUNCTIONAL GROUPS

Kaitlyn Roach1, Dr. Gregory LeFevre2, Sraboni Chowdhury2

1Miramonte High School, Orinda, CA, 2Department of Civil and Environmental Engineering, University of Iowa

Introduction
Toxic chemicals and pollutants in stormwater runoff pose a large environmental threat, as they can degrade ecosystems, pollute drinking water, and lead to animal and human digestion of harmful chemicals. Plants are often used in storm water infrastructure to remove harmful compounds, and sometimes crops will be irrigated with recycled water containing such contaminants. For this reason, understanding plant uptake of such chemicals is important not only for the fate of the environment but also to comprehend what happens if chemicals are later consumed by humans. Plants can take up compounds from the environment using transpiration, but they also contain specialized transporters to uptake nutrients and xenobiotics at faster rates. However, there is a lack of information regarding how plant uptake of xenobiotics varies based on a chemical’s functional group position and electrostatic nature. The purpose of this study was to explore plant uptake of compounds with highly electronegative electron withdrawing groups.

Method
- Arabidopsis thaliana seeds were sterilized and grown in autoclaved Magenta boxes with a growth medium for 10 days
- For each chemical (2-Chlorobenzimidazole, 2-Amino-7-chloro-1H-benzimidazole, or 2-Amino benzimidazole), there were
  o 4 boxes of plants with spiked growth medium
  o 3 boxes used as negative controls (no plants)
  o 3 boxes of plants used for sorption experiment
- Growth medium with spiked chemicals was sampled every couple of hours over a 48-hour time period
- Samples were analyzed using an LC-MS/MS machine

Results
Chemicals tested in this study:
- 2-Chlorobenzimidazole
- 2-Amino-7-chloro-1H-benzimidazole
- 2-Amino benzimidazole

Plant Uptake Rate of 2-Chlorobenzimidazole

Plant Uptake Rates of Benzimidazoles Over Time - Previous Lab Research

Future Research
- Extracted plant tissue from the experiment should be tested to determine what metabolites were created after plants took up chemicals from the growth medium, since plants often transform chemicals into glycosylated or amino acid conjugates.
- Plant uptake measurements for benzimidazoles with highly electronegative withdrawing groups should be thoroughly compared with plant uptake measurements for benzimidazoles with functional groups in different positions and differing electrostatic natures.
- Using such comparisons, a model should be created in order to predict how plants will uptake a wide variety of contaminants based on their chemical structures.

References
Introduction

• Non-syndromic orofacial clefts include cleft lip only (CLO), cleft lip and palate (CL/P) and cleft palate only (CPO). Collectively, these are the most common craniofacial birth defects in humans, affecting approximately 1:300 live births worldwide (Rahimov, Jugessur, and Murray, 2012).

• Genome-wide association studies (GWAS) is an approach used to search for small variations called single-nucleotide polymorphisms (SNPs) that are associated with a phenotype within a population.

• GWAS does not completely reveal the genetic etiology of orofacial clefts, so additional strategies including gene and pathway-based analysis are currently being employed (Mishra and MacGregor, 2015).

• We utilized the African cleft GWAS summary data (Butali et al., 2019) to identify genes and pathways involved in the pathogenesis of orofacial clefts.

Methods

- Compiled GWAS data
- Data cleanup of GWAS data using VEGAS2
- VEGAS2 simulations
- Analysis of biological processes, molecular functions, etc.
- Check for overlap in processes/pathways
- VEGAS2 Software

VEGAS2 Software

Versatile Gene-Based Association Study - 2 [VEGAS2]

- Assign SNPs to genes
- Calculate gene-based test statistics using VEGAS2/VEGAS approach
- Assign Genes to pathway

- Check for overlapping pathways in processes

Table 1: Candidate genes for CL/P with significant p-values

<table>
<thead>
<tr>
<th>Gene</th>
<th>nSNPs</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>CTC2SP2</td>
<td>4</td>
<td>4.08E-05</td>
</tr>
<tr>
<td>VSX5</td>
<td>10</td>
<td>1.07E-05</td>
</tr>
<tr>
<td>ALG1</td>
<td>10</td>
<td>1.05E-05</td>
</tr>
<tr>
<td>NRP1L1</td>
<td>3</td>
<td>1.06E-05</td>
</tr>
<tr>
<td>IFIT3</td>
<td>7</td>
<td>8.08E-05</td>
</tr>
</tbody>
</table>

Table 2: Candidate genes for CPO with significant p-values

<table>
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<tr>
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<tr>
<td>DNTT</td>
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<tr>
<td>LOCH3542</td>
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<td>WRB94</td>
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</tr>
<tr>
<td>PPBP2C</td>
<td>10</td>
<td>8.90E-06</td>
</tr>
</tbody>
</table>

Table 3: Candidate genes for processes/pathways

<table>
<thead>
<tr>
<th>Biological Processes</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental process</td>
<td>2.74E-07</td>
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<tr>
<td>Prenatal development</td>
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<td>Prenatal development</td>
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</tr>
<tr>
<td>Cell communication</td>
<td>3.53E-07</td>
</tr>
<tr>
<td>Cell communication</td>
<td>3.57E-07</td>
</tr>
</tbody>
</table>

Results

Gene-Based

Significant Genes Involved in CL/P

<table>
<thead>
<tr>
<th>Gene</th>
<th>nSNPs</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTC2SP2</td>
<td>4</td>
<td>4.08E-05</td>
</tr>
<tr>
<td>VSX5</td>
<td>10</td>
<td>1.07E-05</td>
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<td>ALG1</td>
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<td>1.05E-05</td>
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<tr>
<td>NRP1L1</td>
<td>3</td>
<td>1.06E-05</td>
</tr>
<tr>
<td>IFIT3</td>
<td>7</td>
<td>8.08E-05</td>
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</tbody>
</table>

Pathway-Based

Significant Processes Involved in CL/P

<table>
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<tr>
<th>Biological Processes</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental process</td>
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<tr>
<td>Prenatal development</td>
<td>3.14E-07</td>
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<tr>
<td>Prenatal development</td>
<td>3.46E-07</td>
</tr>
<tr>
<td>Cell communication</td>
<td>3.53E-07</td>
</tr>
<tr>
<td>Cell communication</td>
<td>3.57E-07</td>
</tr>
</tbody>
</table>

Conclusion

• Pathway-based analysis of genome-wide association study data confirm known pathways and genes involved in orofacial clefts.

• Data shows that disease is polygenic and multiple genes work together to express the function of a specific process, ultimately causing malformations in the embryonic development stage.

• Performing pathway analysis allows us to identify novel biological processes and map out the cause of orofacial clefts, allowing us to intervene at the pathway level.

• Certain genes may only be associated with specific developmental processes (palatal, cleft lip only, both palate and lip), so the difference in strength of association of processes is due to the varying genetic etiology between the cleft types.

Limitation

This approach searches for genes associated with a SNP in a single haplotype block of 500kb around the targeted SNP. Specifying stringent boundaries, however, may not fully capture regulatory regions or those SNPs in high LD with variants in the gene (Liu et al., 2010).

Future Directions

- Mechanistic intervention
- Establish markers to identify abnormalities
- Prenatal diagnosis and genetic counseling

Acknowledgements

Special thanks to Azeez Alade, Tamara Busch, Dr. Butali, and the Butali Lab on their guidance in my research experience. Thank you to Project BLANK Center and SFFST for their exceptional hospitality outside of research hours. This project was supported by NIH/NIDCR DE021890.

References


For supplementary materials such as additional data, scan the QR code on the left. **For more graphs, tables, detailed pathway diagrams see supplementary data folder.
Improving Polygenic Risk Prediction by Accounting for Ancestral Background

Background

- Calculating polygenic risk scores is a useful method for determining whether someone is at a high risk of developing a certain disease based on their genetic makeup.
- There is a lack of diversity in GWAS samples that has a much higher proportion of European samples than the actual makeup of the world population.
- There is a correlation between geographic and genetic distributions of people.
- This leads to a higher prediction accuracy in people of European descent than other ethnicities.
- How can polygenic risk scores be more accurate for people of not just European descent?

Methods

- The SPARK cohort facilitates studies involving large numbers of participants diagnosed with autism spectrum disorder (ASD). The ABCD cohort studies brain development and child health in the United States.
- The ABCD cohort was genotyped on the Affymetrix NIDA SmokeScreen Array® and processed through standard QC steps, such as removing low quality SNPs in samples. The quality-controlled set of SNPs were then imputed to the TopMed reference panel.
- The remaining individuals were stratified into five “clusters” based on their principal components (PCs) from the combined HapMap and 1000 Genomes PCs.
- Polygenic scores were then calculated from the imputed data using LDpred2® and the bigsnpr tools in R.

Standard PGS for Each Genetic Cluster

- Polygenic scores are not equally distributed among all five clusters – significantly higher for African Americans.
- Every cluster should ideally be centered near the red reference line of zero.
- There is bias due to the lack of GWAS diversity.

Results

- After correcting for genetic ancestry, the results showed significant improvement, especially among those of non-European descent.
- Those with lower PGS had fewer autism cases, and those with higher PGS had an increased number of autism cases. This remained true for all five clusters.
- The greater the effect size, the more predictive the polygenic risk scores were in predicting autism.

- Uncorrected PGS p-value is very low, representing a significant cluster-PGS interaction.
- PC Corrected PGS p-value is much closer to one, showing that it is less significant and has less bias.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Uncorrected PGS p-values</th>
<th>Corrected PGS p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.2e-16</td>
<td>2e-16</td>
</tr>
<tr>
<td>Polygenic Score</td>
<td>2.2e-16</td>
<td>2e-16</td>
</tr>
<tr>
<td>Cluster and PGS</td>
<td>0.001201</td>
<td>0.9109</td>
</tr>
</tbody>
</table>

Conclusion

- Polygenic scores are clearly biased towards Europeans, but this can be fixed to a certain extent by correcting for genetic ancestry. This information is necessary in order to increase accuracy in genetic studies among diverse populations.
- An important step for the future would be to increase the diversity of participants included in genetic studies, such as GWAS, so that genetic information for those underrepresented groups can be more easily accessible for research.

References


Acknowledgements

Special thanks to Dr. Jacob Michaelson, Lucas Casten, Ethan Bahl, and the entire Michaelson lab for mentoring me through this amazing experience. I would also like to thank SSTP and the Belin Blank Center for providing me with this opportunity.

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Monitoring Mealtime Intake Behavior in Residents with Dementia at Nursing Homes
Wendy Song, Heather Suh MPH, Wen Liu PhD
Palo Alto High School, University of Iowa College of Nursing

Introduction
- In 2022, around 6.5 million Americans aged 65 and older are expected to have dementia, and 10.7% of people aged 65 and older have dementia (Alzheimer’s Association, 2022).
- Patients exhibit resistive behaviors and lower quality of life when their intake is compromised.
- Caregivers currently perform task-centered care due to work demands and focus solely on feeding, allowing the patient little autonomy and independence.
- OPTIMAL intervention protocol contains RECIPE principles promoting person-centered care: showing respect, creating environment, offering choices, supporting independence, acknowledging preferences, contains maintaining engagement.

SEM (Social Ecological Model)

Objectives
- Evaluate the impact that factors such as type of meal and food have on the food intake process.
- Hypothesize the impact that person-centered care administered through didactic training for caregivers has on the intake process.

Methods
- 6 meals evaluated per resident; staff and resident verbal and nonverbal behaviors as well as the food intake process were measured.
  - Resident positive, neutral behaviors: 8 verbal (ex. asking for help/cooperation), 5 nonverbal (ex. wiping away oral spillage/drool).
  - Resident challenging behaviors: 4 verbal (ex. interrupting/changing topic), 22 nonverbal, categories: chewing and swallowing difficulties (ex. holds food in mouth) with 4 items, functional impairment (ex. difficulty using utensil) with 5 items, resistiveness to care (ex. doesn’t open mouth) with 6 items.
- Intake process: 0 for an unsuccessful intake attempt, 1 for solid food, 2 for liquid food. 0 if staff initiated the intake attempt, 1 if resident initiated with or without help of staff.

Results
- Resident initiated intakes across different meal types
  - Breakfast: 56.05%, Lunch: 54.63%, Dinner: 47.43%

Discussion
During mealtime, dementia is the primary cause of challenging behaviors such as initiation of an intake attempt, getting food into the mouth, chewing and swallowing, or getting distracted and attempting to leave the dining table (Liu et al., 2014). This resistance negatively affects the intake amount and process, which are measured through checklists. The proportion of resident-initiated intakes is around 50% for all meals, suggesting the need for improved person-centered care to encourage resident autonomy. This data is representative of conditions before intervention and provides a strong baseline of residents’ natural behaviors. Furthermore, staff use of verbal and nonverbal strategies correlates with residents’ challenging behaviors (Liu et al., 2022), so a staff behavioral changes will impact resident behavior and therefore change the intake process and amount.

Future Directions
- Understand how the impact of dementia-induced behaviors on breakfast, lunch, and dinner differ.
- Explore the impact of other conditions related to old age on intake behavior.
- Further the current data by understanding the connections between different dementia aids and the intake process to develop stronger intervention tactics.
- Recognize the prevalence of person vs. task centered care, and the caregiver’s role in the intake process.

Acknowledgments
I would like to thank Heather Suh, my mentor, for providing me with the study materials and ability to conduct the research and data analysis. Additionally, I would also like to thank Dr. Wen Liu for allowing me to be a part of this study.

References
Angle and Polymer Bonding Strength Alterations in the Interface Positively Impacting Piezoelectric Properties

Kevin Su, Levi Kirby, Xuan Song
Department of Industrial and Systems Engineering, University of Iowa

Background

The capacity of some materials to produce an electric charge in response to applied mechanical stress is known as piezoelectricity. These piezoelectric properties can be optimized through various manipulations: material alteration, interface changes, polymeric bonding strength, among many others. The objective of this project was to determine the impact of different polymer bonding strengths and angle alterations of the contact surface between the polymer and ceramic phase, on final piezoelectric properties in additively manufactured Barium Titanate parts.

Methods

1. Start
2. Model each part in CAD 30°, 45°, 60°, and 90°
3. Add parts to an Assembly fitting in the build region of the 3D Printer
4. Export as an STL file to the 3D Printer
5. Define build parameters
6. Mix and add the Barium Titanate (BTO) Slurry to the enclosing chamber
   - Clean excess BTO slurry, let the substrate cure, and refill the enclosing chamber
   - Scrape off extra slurry on top of build region
   - Let the polymer cure by placing in an oven at 100°C for 30 minutes
   - Place parts in mold and fill remaining half with PDMS polymer
   - Place parts in an alumina tray and debind them in an tube furnace
   - Remove and clean off parts
   - Soak half the parts in TMSPM coupling agent solution
   - Clean parts in 70% alcohol with an Ultrasonic Cleaner
   - Test for Voltage
   - Heat parts to 150°C and Pole them
   - Coat parts in nickel
   - Polishing and Testing

Results

<table>
<thead>
<tr>
<th>Part Description</th>
<th>Average Piezoelectric Constant (d33)</th>
<th>Average Max Output Voltage (mV)</th>
</tr>
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<td>404.35</td>
</tr>
<tr>
<td>90°, Coated</td>
<td>173</td>
<td>457.2</td>
</tr>
</tbody>
</table>

Electric Output Voltage Response when Polymer is Pressed

Conclusion

There is a significant increase in both the piezoelectric constant and the output voltage in the parts coated with TMSPM compared to those that were uncoated. Furthermore, it can be shown that alterations in the angle of the interface between the polymer and the ceramic piece results in a slight change of piezoelectric properties, where a decrease in the angle correlates to a decrease in coated parts' output voltages and an increase in uncoated parts' piezoelectric constant.

Implications

This research demonstrates different ways to optimize piezoelectric properties within parts of ceramic and polymers. By utilizing the information found in the research, future researchers can use this as a starting point in developing even further ceramics or materials with super-piezoelectric properties, as well as additionally increasing knowledge within the field on how certain physical alterations impact piezoelectricity.

References


Acknowledgment

Thank you for the guidance and support of Levi Kirby and Dr. Xuan Song throughout this process as well as the Belin-Blank Center and AMPRL at the University of Iowa for this opportunity. Additional thanks to NSF for funding this research and project.
Effect of antioxidants on a *Drosophila* Model of Epilepsy

Serena Thomas; Krishna Madhav Nukala; J. Robert Manak, PhD

Introduction

**Epilepsy**
- Epilepsy is a neurological disorder characterized by repeated seizures and affects over 50 million people worldwide.
- Current anti-epileptic drugs have limited efficacy in 33% of patients and often lead to adverse long-term side effects.

**prickle**
- The *prickle* gene in *Drosophila* (fruit flies) produces an adult isoform called *prickle-spiny-legs (pksp)*.
- Mutations in the *pksp* isoform cause spontaneous myoclonic seizures, similar to those observed in humans with *PRICKLE* mutations.

**Oxidative Stress**
- Previous work in the Manak laboratory shows that loss of the *pksp* isoform leads to increased expression of genes that encode proteins that mitigate oxidative stress.

**Drosophila Stocks**
- The *pksp* mutation was backcrossed into a *Canton-S* (CS) background. *pksp* (CS) and CS control flies were used in all experiments.

**Dietary Fedding of Curcumin and Aspirin**
- Drug food was made by combining standard commercial molasses *Drosophila* medium with aspirin and curcumin (dissolved in ethanol) to final concentrations of 1µM and 25µM, respectively.
- Vehicle-only food was made for aspirin and curcumin by dissolving appropriate amounts of ethanol in standard *Drosophila* medium.

**Spontaneous Seizure Assay**
- Freshly eclosed control and *pksp* flies (CS) were aged 7-10 days at 25°C.
- 8-10 female and male flies per experimental condition were mouth-pipetted into circular chambers, and their behavior was recorded for five minutes under high-resolution videography.
- The videos were manually analyzed for spontaneous seizure events as previously reported.

Methods

**Antioxidants**
- *Curcumin* and *Aspirin* are both commonly used as inflammatory drugs with antioxidant properties, *curcumin* and *aspirin* are both commonly used as inflammatory drugs with antioxidant properties, *curcumin* and *aspirin*.
- *Curcumin* attenuates copper induced oxidative stress and neurotoxicity in *Drosophila.*
- *Aspirin* positively contributes to *drosophila* intestinal homeostasis and delays aging through targeting IMD. *Aging and Disease,* 12(7), 1821.

Results

**Conclusions**
- Adult *pksp* mutants exhibit a non-significant reduction of seizures when on a curcumin-enriched diet; increasing the power may yield statistical significance.
- Adult *pksp* mutants experienced no suppression in seizures when aspirin was added to their diet.
- These results suggest that while aspirin has no impact on suppressing epileptic seizures in *pksp* mutants, curcumin has promising potential as a compound for treating seizure disorders.

Future Directions

- Determine whether antioxidants increase the lifespan of *pksp* mutants.
- Determine whether classic antioxidants such as Vitamin C and Vitamin E show promise in reducing seizures in the *pksp* model of epilepsy.
- Improve statistical power by increasing the sample size of control and *pksp* mutants analyzed with the spontaneous seizure assay.

Acknowledgements

I would like to thank Krishna M. Nukala for his mentorship and advice, Dr. Manak for his guidance, and the entirety of the Manak Lab for support. Special thanks to the University of Iowa and SSTP program for giving me this opportunity. This work was supported by a research grant from The Stead Family Department of Pediatrics at the Carver College of Medicine at JRM.
The effect of corticosterone and 5-HT<sub>2C</sub> receptors on seizure suppression and mortality in amygdala kindled mice

Lydia Tong<sup>1</sup>, Katelyn G. Joya<sup>2,3,4</sup>, Nicole A. Boodhoo<sup>2,4</sup>, Gordon F. Buchanan<sup>2,4</sup>

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Introduction

- Sudden unexpected death in epilepsy (SUDEP), the leading cause of death in patients with epilepsy, are at the greatest risk for the 35% of patients with epilepsy who will not achieve seizure freedom<sup>1</sup>
- Mice lacking the 5-HT<sub>2C</sub> receptor are significantly more seizure susceptible than wild-type controls<sup>2</sup>
- Prior research in the lab found that a high dose of MK-212 caused death following seizures in mice
- The 5-HT<sub>2C</sub> receptor agonist MK-212 was found to increase corticosterone, a stress hormone in rodents<sup>3</sup>

Previous pilot experiments suggested CORT-113176, a corticosterone antagonist, and a high dose of MK-212 can result in seizure suppression in wild type animals.

Hypothesis

5-HT<sub>2C</sub> receptor activation is necessary for the seizure suppression induced by the combination of CORT-113176 and MK-212.

Methods

Surgery: 8 adult 5-HT<sub>2C</sub> knockout mice were implanted with EEG, EMG, and an electrode into the right basolateral amygdala (AP: -1.3mm; ML: -2.8mm; DV: -4.7mm).

Kindling: Afterdischarge threshold was determined, and each animal’s threshold current was delivered twice daily until consistent convulsive seizures occurred.

Trials: Animals were injected (i.p) with 70 mg of CORT-113176. After 20 minutes, mice were injected (i.p) with 30 mg/kg MK-212 or saline (vehicle). After 30-60 minutes, we induced a seizure using the threshold pulse amplitude and evaluated measuring seizure severity and mortality.

Results

Figure 1: Post-hoc Nissl-stained brain section representing the electrode entering the basolateral amygdala (BLA). Dashed line indicates the electrode placement. HPC, hippocampus. LV, lateral ventricle.

Figure 2: Percentage of animals that died following seizures in C57BL/6J (green) and 5-HT<sub>2C</sub> KO (black) mice injected with either saline or MK-212 (10 or 30 mg/kg).

Figure 3: Pet1-Cre mice EEG traces from pilot experiments injected with either CORT-113176 and MK-212 (A) or 10% DMSO and MK-212 (B). Lightning bolt represents the time of electrical stimulation.

Figure 4: EEG traces from all 5 experiments on 5-HT<sub>2C</sub> KO mice. Animals were treated with CORT-113176 + saline (black) or CORT-113176 + MK-212 (green). Lightning bolt represents time of electrical stimulation.

Conclusions

- The combination of CORT-113176 and MK-212 can suppress seizures in some animals
- 5-HT<sub>2C</sub> receptor activation may not be necessary for seizure suppression
- 5-HT<sub>2C</sub> KO mice are especially prone to seizure-induced death, which is consistent with previous findings in the literature

Future Directions

- Implant and complete trials on a cohort of animals
- Perform a dose response with corticosterone on a large pool of mice
- Explore more off-target effects that could potentially be from MK-212
- Perform different combinations of CORT-113176 and MK-212 to identify the best therapeutic windows to reduce SUDEP

Acknowledgements

This work was supported by the Belin Blank Center, NIH/NINDS R01 NS098842, and the Beth L. Tross Epilepsy Professorship

References

Ellipsometry-Assisted Reactive Ion Etching for Reflection Grating Fabrication

Don Wong1, Cecilia Fasano1, Casey DeRoo1
1Diamond Bar High School; 2University of Iowa, Department of Physics and Astronomy

Abstract

Studying the light emitted by astronomical objects allows us to understand the chemical compositions, physical conditions, astronomical processes, and, fundamentally, the physics of what happens light years away. To enable detailed spectroscopic analysis — the study of specific wavelengths of light — high-resolution and cost-efficient reflection gratings must be fabricated.

With nonimprint lithography (NIL) being a precise and low-cost method to replicate reflection gratings for use in astronomical instruments, this study focuses on characterizing the crucial reactive ion etching (RIE) step of making gratings following NIL patterning. Using ellipsometry techniques, atomically thin layers can be measured with extremely high precision, allowing us to calculate RIE etch rates through these layers. This work enables astrophysicists to determine appropriate etch times for ongoing reflection grating fabrication projects, propelling the development of future spectroscopic missions.

Reflection Grating Fabrication

Recent advancements in the replication of reflection gratings have been made via microfabrication techniques such as nonimprint lithography (NIL). NIL involves using a master grating as a mold to imprint itself onto a resist-coated wafer using high temperatures and pressures.

Ellipsometry

With the help of optical techniques, ellipsometry can help determine optical constants, thicknesses, and indexes of refraction of thin films up to the nanometer scale. The instrument used for ellipsometry is the ellipsometer.

Methodology

Subject:
• Eight NXR-1025-coated wafer samples
• Eight SiNx-coated wafer samples

Treatment:
• Reactive Ion Etching (RIE) — CHF3/O2 and Ar/O2 etch recipes with increasing step times

Data Analysis:
• Ellipsometer
• Graphs created with Python

Results

After plotting the change in thin film thicknesses vs. the respective step times, etch rates for NXR-1025 and silicon nitride (SiNx) were successfully determined.

Conclusion

This study has shown that using ellipsometry-based techniques to determine thicknesses of thin films can successfully produce etch rates for reactive ion etching.

Future work can be further developed regarding this project. We can refine etch rate accuracy by testing more step times and executing the subsequent KOH wet etching step.

References

1Planck Collaboration 2013
2Nayak et al. 2012
3J.A. Woollam Ellipsometry Website 2014
4DeRoo 2015

Support

This work was funded by NASA grants 80NSSC22K0159, 80NSSC21K1937 and internal funding from University of Iowa.

Science Case: The Missing Baryon Problem

From a census of the cosmic microwave background (CMB), astrophysicists find a discrepancy between the numbers of baryons in the early universe and the present day.

In order to conduct a more intensive survey for these “missing baryons”, said to be hidden in the Warm-Hot Intergalactic Medium (WHIM), further spectroscopy in the soft x-ray spectrum is needed. The currently active X-ray observatories, Chandra and XMM-Newton, do not have the physical capabilities to make such refined observations of the WHIM. (See Bregman 2007, Bregman et al. 2015, and references therein.)

In order to conduct a more comprehensive survey of the universe’s baryons, a new generation of more refined, high-resolution diffraction gratings must be fabricated.

Graphs created with Python

Figure 1. The Cosmic Microwave Background, as observed by the European Space Agency’s Planck Observatory.

Figure 2. A graphical comparison of the expected and observed composition of the universe.

Figure 3. Interaction of light and a sample in ellipsometry.

Figure 4. The physical process of reaction ion etching (RIE).

Figure 5. (Top) Eight NXR-1025-coated wafer samples after RIE. (Bottom) Eight SiNx-coated wafer samples after RIE.

Figure 6. Silicon wafer sample post-reactive ion etching.

Figure 7. Ellipsometer from J.A. Woollam.
Tracking the Origins of Omicron and Delta SARS-CoV-2 Variants in a University Community: Post Vaccination Era

Samantha Wu¹, Andrew Kitchen²

¹Valley Christian High School, San Jose, CA, ²Department of Anthropology, University of Iowa

Purpose Statement

The purpose of this study is to understand how public health measures affect the geographical and temporal distributions of Omicron and Delta variants in university settings.

Introduction

Since the first emergence of SARS-CoV-2 in Wuhan, Hubei Province, China, and its ensuing spread across the globe, public health responses from the international to the institutional level have been implemented to mitigate the size and severity of SARS-CoV-2 outbreaks. However, the effects of these policies to limit the spread of virus have not been fully assessed. Importantly, phylogenetic analysis of viral genomes, which has revealed the origins, timeline, and dispersal of SARS-CoV-2 lineages, could aid in determining the effectiveness of specific interventions in different contexts.

University campuses have potential for increased viral transmission due to communal living, large student populations, and frequent travel. With a massive student population and international community, Purdue University surmounted these challenges through their comprehensive “Protect Purdue Plan” in the summer of 2020, which implemented regular testing, de-densification of classrooms and living spaces, and international and domestic travel regulations (Ciubotariu et al.).

In light of high vaccination rates, Purdue has returned to many pre-COVID policies. Therefore, the present study applied a phylogenetic approach to analyze SARS-CoV-2 genomes from Purdue University and determine how the university’s loosened COVID regulations affect the transmission patterns and origins of SARS-CoV-2 lineages on campus. Such phylogenetic analysis can help determine whether cases were due to independent introductions from distinct geographical locations, or linked transmission through adjacent communities.

Our dataset includes Omicron and Delta viral genomes from Purdue University, as well as related sequences from GISAID. We applied a Bayesian coalescent approach to generate a Maximum Clade Credibility phylogenetic tree. In order to make phylogeographic inferences, we performed a pairwise estimate of parsimony scores to estimate the association between Purdue sequences and sequences from certain geographical areas, allowing for us to assess the patterns of migration and transmission.

Methods

Genomic Dataset

In order to determine the geographical locations outside of the university from which clades of SARS-CoV-2 originated, we found related sequences in GISAID, making our dataset total to 142 sequences. GISAID is an open access database where genomes of viruses which require prompt responses may be published. Thus, GISAID houses the greatest number of SARS-CoV-2 genomes.

Sequence Alignment

When comparing many RNA sequences, insertions and deletions (indels) occur due to errors when sequencing the viral genome or due to mutations acquired in nature. Such indels result in frameshift mutations that can result in the comparison of different loci and must be corrected. We performed a multiple sequence alignment through NextAlign CLI via the command line, which aligns sequences with respect to parsimony and gap penalty.

BEAST MCC Tree

We used the Bayesian Evolutionary Analysis Sampling Trees (BEAST) software package to generate the Maximum Clade Credibility (MCC) phylogenetic tree. BEAST uses a Markov chain Monte Carlo (MCMC) technique to sample the posterior distributions of model parameters; we used a Markov chain length of 100 million steps. We used the HKY substitution model, which accounts for variation in the nucleotide base frequencies and different rates of transitions and transversions, combined with a gamma distribution to model site-rate heterogeneity. A strict clock was used, which assumes that all lineages have the same rate of evolution; we fixed this clock to a substitution rate of 0.04 substitutions/site/year.

Results

Based on our pairwise estimates of PS, we hypothesize 6 introductions from the following geographic locations:

- Significant migration: Indiana
- Some Migration: Colorado, Illinois, New York, Ohio, South Carolina
- Possible migration: California, North Carolina, Texas, and Wisconsin

From this data, there is no evidence of lineages being sourced directly from international locations.

<table>
<thead>
<tr>
<th>States</th>
<th>PS1</th>
<th>PS2</th>
<th>PS3</th>
<th>PS4</th>
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<td>6.990</td>
<td>7.000</td>
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</table>

Conclusion and Discussion

- While in-state and adjacent state migration remain dominant (Ciubotariu et al.), interestingly, our analyses also support relatively distant migration from non-adjacent states: South Carolina, New York, and Colorado.
- Widespread origins of viral lineages in our analyses may be attributed to various factors, such as the lifting of travel restrictions at Purdue prior to our study, and Omicron’s higher transmissibility (Chen et al.).
- Because our analyses are based on a subset of Purdue sequences, larger analyses are needed to definitively elucidate the origins of the campus’s viral lineages by confirming which locations have the strongest associations, aiding in determining which travel regulations are necessary.

References:


Acknowledgments:

I would like to thank Dr. Kitchen and the members of the Evolutionary Anthropology lab for guidance throughout the project. Faculty and staff of the Secondary Student Training Program for the opportunity to research, and the Carpi Lab at Purdue University for sharing viral genome sequences.

Highly Effective Separation of Fatty Acids Derived from Vegetable Oils Using COF Incorporated Epoxy Membranes

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¹The Bishop’s School, ²Department of Chemistry, University of Iowa

Introduction
Over 200 million tons of vegetable oils produced each year, and this amount is increasing by 5% each year. Vegetable oils are usually a mixture of saturated and poly-unsaturated fatty acids that individually are very valuable but are difficult to separate from each other.

Membrane separations offer an inexpensive alternative to traditional methods of purification such as distillation and column chromatography, and it is accessible to many organic chemicals that are currently difficult to purify. In this work, we report the development of a mixed matrix membrane using covalent organic frameworks (COFs) incorporated within an epoxy polymer (COF(n)/epoxy) that can separate fatty acids from each other by utilizing highly ordered structures and uniform crystalline pores in COFs.

Preparation of COF(20)/epoxy membrane
These highly flexible, hybrid membranes were fabricated via solution casting method: The membranes were dried under an atmosphere of saturated DMF.

Synthesis of COFs with different pore sizes
Each COF forms 2D sheets with hexagonal pores, and the sheets stack on top of each other as shown.

Fluxes and relative flux of different fatty acids using COF(20)/epoxy membranes

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Absolute flux (10⁻⁷ m³/s cm²)</th>
<th>Flux of chemicals relative to Omega-3 acid</th>
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</thead>
<tbody>
<tr>
<td>HCOF (1.8 nm)</td>
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<td>TpBD COF (1.4 nm)</td>
<td>15.1 13.3 6.4 5.2 1.9 2.1 1.2 1.0</td>
<td></td>
</tr>
</tbody>
</table>

As the pore size increases, the absolute flux also increases, but the selectivity decreases.

TpPA achieved the best separation; stearic acid’s flux was 4.5x faster than that of Omega-3’s.

Conclusions
The discovery that membranes can facilitate separation between fatty acids provides a highly promising alternative for industry: a cheap, efficient, and green method to obtain valuable starting materials that are otherwise wasted in daily usage. Our identification that TpPA COFs are the optimal membranes for these separations also reveal what pore size range is effective, and which other bigger or smaller pores do not work as effectively.

Future work
We are looking to add different types of amines with the fatty acids with the goal of increasing selectivity within our acids.

Acknowledgements
I would like to thank Professor Ned Bowden and Nimesh Pasan Ranasinghe, the graduate student who mentored me through the whole project, for this amazing opportunity and experience. I would also like to thank the Bowden research group for welcoming me into the group, and I wish them the best of luck in the future.
Targeting pyruvate dehydrogenase kinases inhibits thrombosis and platelet aggregation

Rebecca Xue\(^1\), Manasa K. Nayak\(^2\), PhD, Gagan D. Flora\(^2\), PhD, Anil K. Chauhan\(^2\), MTech, PhD

Blue Valley West High School\(^1\), University of Iowa Medical Laboratories\(^2\)

**Introduction**

- Platelets are disc-shaped cells present in blood and are involved in the formation of blood clots. Abnormal clotting blocks blood supply and causes heart attacks and ischemic stroke.
- Current antiplatelet treatments exist but cause unwanted side effects such as bleeding complications in thrombosis patients.
- Dichloroacetate, a known inhibitor of pyruvate dehydrogenase kinases (PDK) 1-4, inhibits platelet function and arterial thrombosis. (Nayak et al., 2018)
- What effect does targeting PDK have on platelet function and arterial thrombosis?

**Methods**

- Wild-type (PDK2+/+, PDK4+/+) , double knockout (PDK2−/−, PDK4−/−) and single knockout mice (PDK2−/− or PDK4−/−) were used in the experiments.
- Various in vivo and in vitro techniques were used to observe platelet aggregation.

**Results**

- 5% FeCl\(_3\) injury
- Tail-bleeding time
- FeCl\(_3\) injury

**Implications**

- Inhibiting PDK should be further explored as a promising novel strategy for inhibiting platelet aggregation and arterial thrombosis.
- Future work should be done to determine the roles (if any) of PDK 1/3.

**Acknowledgments**

I would like to thank the Chauhan Lab for allowing me to have this opportunity, as well as Dr. Chauhan, Dr. Nayak, Dr. Flora, and Dr. Kumskova for their invaluable guidance.

**References**

Purpose Statement
We will infer the sources of infection of SARS-CoV-2 Omicron and Delta variants in Purdue University through phylogenetic analysis. We will use molecular clock and Bayesian coalescent approaches to reconstruct the phylogenetic tree sequences from Purdue University to identify the origin of closely related sequences. We will also investigate the virus’s origins’ correlation with the university’s COVID restriction policy.

Introduction
• Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and subsequently spread across the globe, including the United States. As of July 20, 2022, the virus has caused over a million deaths in the U.S. (Dong et al., 2020) despite widespread implementation of interventions directed at curbing transmission and limiting infection (i.e., masking, social distancing, and vaccination). Importantly, it remains unclear what effect these interventions had on the spread of SARS-CoV-2 in the U.S.
• Universities are particularly vulnerable to superspreading due to their large numbers of students, faculty, and staff, frequent social activities, communal living, and in-person instruction, all of which may facilitate efficient viral transmission to large numbers of individuals.
• Purdue University is a large public university in Indiana and is representative of large public universities across the U.S. In response to the pandemic, Purdue implemented a plan to protect students that included masking, social distancing, suspension of university-associated travel, and frequent community surveillance. These measures changed over time as vaccination rates increased and SARS-CoV-2 incidence waxed and waned. The effect of these policies on the spread of virus into the university community remains unknown.

Here, we attempt to identify the origins of SARS-CoV-2 Omicron and Delta variant sequences from the Purdue community as an example of a large public university. Recent study discovered the Gamma variant in Purdue entered primarily from Indiana and Illinois. With a more relaxed COVID policy, we want to compare the transmission routes of Omicron and Delta variants to that of Gamma’s (Ciubotaru et al., 2022). This analysis builds a foundation for future analysis on the effects of mitigation policy on SARS-CoV-2’s spread.

Methodology
1. Data collection
Viral genome sequences from Purdue were shared with us by the Carpi Lab (Purdue University). Additional sequences were downloaded from GISAID (https://gisaid.org), an open access website hosting virus sequence data. Using GISAID’s Audacity tool, we collected sequences closely related to a subset of SARS-CoV-2 sequences sampled from Purdue University.

We aligned our RNA sequences both manually using Seqtron and with NextAlign, a command-line tool, to ensure accurate homology between sequences.

2. Analysis and inferences
Reconstruction of the phylogenetic tree:
We used the Bayesian Markov chain Monte Carlo methods in BEAST to reconstruct the phylogenetic tree of our subset of Purdue sequences (Drummond & Rambaut, 2007). Our analysis used a HKY nucleotide substitution model, a gamma site heterogeneity model, strict molecular clock, a coalescent constant size tree prior, a UPGMA starting tree, and a Markov chain of length 100 million generations.

Phylogenetic analysis in a domestic migration context:
To estimate the number of introductions of Omicron and Delta variants to Purdue from different U.S. states, we used the Bayesian analysis of Tip Significance testing package (BaTS) to calculate the parsimony score (PS) of the posterior distribution of trees that BEAST produced (Park et al., 2008).

Results
• Purdue sequences are nested within Indiana sequences, indicating this subset of sequences from Purdue were likely the product of local transmission.

Discussion and Conclusions
• This project was a part of a study looking at a subset of over 700 Omicron and Delta genome sequences from Purdue. Ultimately, we will compare these 700 sequences’ sources of infection and Gamma’s sources of infection to infer about the efficacy of Purdue’s COVID protocols.
• In the context of high vaccination rates and relaxed COVID restrictions, including the resumption of international travel, the subset of viruses analyzed here were still introduced to the university through local transmission. This is similar to a previous study of the introduction of Gamma variants to Purdue, which found viruses mostly came from Indiana and Illinois. Interestingly, this occurred when there were lower vaccination rates and stricter COVID regulations in place, and presents a series of questions that require additional exploration: Why is it that when international travel resumed, the transmission routes stayed local? What factors contributed to this phenomenon – external factors like the high vaccination rate and/or intrinsic factors such as changes in SARS-CoV-2 infectiousness?

References
Differences in Indices of Happiness and Distress in Two Individuals with IDD and Automatically Reinforced Self Injurious Behavior (ASIB)

Sarah Yun¹, Matthew J. O’Brien, PhD², Alex Pauls, MA², Kelly M. Schieltz, PhD²
¹Homestead High School, ²University of Iowa

Introduction

- Self injurious behavior (SIB) is common in individuals with intellectual developmental disabilities (IDD; Soke et al., 2016)
- It is the primary cause of emergency room visits among children with autism and leads to an increased risk of psychiatric hospitalization (Kalb et al., 2012)
- Automatically reinforced SIB (ASIB) is less understood
- Psychiatric hospitalization (Kalb et al., 2012)
- It is the primary cause of emergency room visits among children with autism and leads to an increased risk of intellectual developmental disabilities (IDD; Soke et al., 2016)

Discussion

- Results consistent with hypothesis that individuals show clear differences in indices of happiness and distress while engaging in ASIB

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<th>Happiness</th>
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<td>Higher indices of happiness, slight increases in distress, but overall rates were remained extremely low</td>
<td>Slightly higher levels of distress, but no clear differentiation (overlap)</td>
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<td>Higher indices of happiness, very low indices of distress</td>
<td>Higher indices of distress with clear differentiation (no overlap)</td>
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Class of Reinforcement (+ or -)

- Suggests auto-positive reinforcement, especially due to sensory impairments
- Suggests auto-negative reinforcement, to relieve aversive state caused by biomedical conditions

Methods

- Parent surveys were used to individually define indices for two patients with severe ASIB
- Recorded sessions of various stages of treatment were coded for indices using a behavioral event logging software

Noah Indices of Happiness and Distress (Unlocked)

- Socially reinforced (75%)
- Automatically reinforced (25%)
- Treatments are over 90% effective
- Maintaining variable unknown
- Auto-positive (pleasant sensation)
- Auto-negative (relief from aversive state)

Noah % of Smiles and Frowns During Session (Unlocked)

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Conclusions

- Individuals display different patterns of emotion while engaging in ASIB, which suggests differences in underlying motivations
- Clinical practice would benefit from treating ASIB based on different functional categories for more effective treatment
- Indices of happiness and distress should be further explored as a potential method of subtyping ASIB for a larger IDD population

References


Training Models to Analyze Reasons in Anti-Mask Tweets

Anne Zhang¹, Elizabeth North², Min Zhang³, Ling Tong³, Weiguo (Patrick) Fan³

Introduction
The COVID-19 pandemic has had devastating impacts worldwide. An abundance of mitigation strategies like facial masks, social distancing, and vaccines, were developed to aid relief efforts.

- Our research group seeks to identify and analyze reasons why people would not wear masks or follow mask mandates.
- The virus’s behavior in communities relies on the extent to which masks are available to and accepted by it. Understanding sources of people’s stances is crucial in guiding public health officials to employ effective policies against the coronavirus.

Methods
- With 10,000 tweets against masks, reasons for each’s position were identified, findings cross-checked, and data discrepancies sent for a third-party review.
- The text of our data was preprocessed by removing cases, numbers, punctuation, and urls, stemming, lemmatizing, and cleaning tokens, and removing stop words.
- Most common bigrams in our data were discovered (fig. 1).
- Binary classification separated 10k tweets into reasons and no reasons.
- Via topic modelling with Latent Dirichlet allocation (LDA), trained a model to group tweets with most prominent similarities in types of reasoning.
- Tested LDA model to see what number of topics used would have optimal coherence score and decided with discretion on 7 categories (fig. 2).

Results
- After topic modelling, visualizations with term weighting of prominent words were created within each of 7 topics (fig. 4–10). We defined each topic with an overarching reason for attitudes against masks.
- When trained and tested following preprocessing and hyperparameter adjustment, decision tree model achieved accuracy rating of around 0.70.

Conclusion
- With further fine-tuning of our model, it is likely to be able to reach a higher accuracy rating in testing.
- Future plan is to run larger dataset of 1 million anti-masking tweets through the model to predict topics.
- Our hope is that it someday reliably assists in identifying reasoning, even past specific question posed.

Acknowledgments
I would like to thank Elizabeth North for being an incredible partner. I greatly appreciate Min Zhang and Ling Tong for their invaluable guidance and assistance throughout, as well as Dr. Weiguo (Patrick) Fan’s support. Finally, I’m beyond grateful that the Secondary Student Training Program provided me with this opportunity to conduct research at their facilities.
Investigating Point Defects in Zeolitic Imidazolate Framework-7

Zhehao Zhang¹, Akalanka Ekanayake², Collin Hill², Alexei Tivanski²
¹The Webb Schools, Claremont, CA;
²Department of Chemistry, University of Iowa

Background
Recently, the mechanism of the ZIF-7 gate-opening effect was determined: a benzimidazole linker rotation. It has also been found that there exists a size-dependency in this gate-opening effect, and our group also found that the ZIF-7 crystal size can affect the crystal’s young’s modulus. However, defects’ effects are greatly overlooked in current simulations of ZIF-7, as they assume that ZIF-7 are perfect crystals. We postulate that the overlooked defects could be the reason behind this size-dependency.

Methods
1. Compared PBE-DRSLL and variant with VWN correction changed to PBE³ correlation.
   -VWN³ correlation with DRSSL? correction is too hard
   -PBE correlation with DRSSL correction is too soft, but error is less than VWN.
2. Optimized ZIF-7-I and ZIF-7-II structures
   -Lattice constants fixed to experimental values
   -used PBE correlation and PBE exchanged mixed with DRSLL. Van der Waals exchange correction
3. Optimized defect-containing ZIF-7-I and ZIF-7-II structures
   -Used same functional as optimizing defect-free structures
   -Formulated structures from perfect crystals, and then optimized within a 1x1x2 supercell
4. We will then find the transition state of defect formation with IRC to determine the kinetics of defect formation
5. Then, the Finite-deformation Nudged Elastic Band method will be employed to find the transition state energy of phase transition of defect-free and defected ZIF-7s to determine the effect of defects on the gate-opening effect.
6. Also, we will measure the stress tensors during deformation of defected ZIF-7 to determine the effect of defects on the stiffness of the crystal.

Results
-Had begun the geometry optimization of different defect-containing ZIF-7-I and ZIF-7-II structures
-Determined that Zn leaving the structure and forming Zn(NO₃)₂ is a extremely thermodynamically feasible defect in ZIF-7, with ΔH=144 kJ, but maybe kinetically slow and entropy producing.
-Discovered when limiting the lattice constants to be fixed, ZIF-7-II optimization is converging extremely slowly, suggesting defect could drastically lower the barrier for phase change, and the energy minimum present for ZIF-7-II structure could be practically eliminated due to the defect

Table 1. Calculated Enthalpy change of zinc vacancy defect formation processes in ZIF-7-I

<table>
<thead>
<tr>
<th>Defect Structure</th>
<th>ΔH</th>
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<tr>
<td>ZIF-7-I+2HZO⁻</td>
<td>-20.279158036962 kJ</td>
</tr>
<tr>
<td>ZIF-7-I+2HN3O₃⁻</td>
<td>-144.00427316956 kJ</td>
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Future Directions
Apart from continuing and finishing this work, there are a series of future directions this could apply in:
- Guiding and educating controlled defect formation, such as hydrolysis of ZIF-7, using mixed linkers to form defects, etc. to obtain crystals with defects that produce desired properties
- Could apply to defects’ effects in other crystals with phase-changing properties

References