STARS

Summer Research Symposium

Science, Technology and Research Scholars

Yale

Kline Geology Laboratory
Lecture Hall 123

July 25 & 26, 2018

Science and Technology Research Scholars Symposium
Schedule of Presentations

Wednesday, July 25th, 2018 — Morning Session

9:00 a.m.	Linda Tun *Department of MCD Biology*	Investigating the Role of Oxygen in Metabolic Regulation of Planarian Regeneration
9:15 a.m.	Helia Gagnon *Department of MCD Biology*	The Role of Oxygen Metabolism in Planarian Stem Cell Function
9:30 a.m.	Natalia Taylor *Department of Comparative Medicine-YSM*	The Interaction of Dietary Fats with Leptin Signaling and its Effect on High Fat Diet Consumption
9:45 a.m.	Royce Lee *Department of Comparative Medicine-YSM*	Investigation of Dietary Fat Composition's Effect on Food Intake and Preferences in Wild Type and GPR40$^{-/-}$ Mice
10:00 a.m.	Kaitlynn Sierra *Department of Computer Science*	Trusting Robots: Examining the Influence of Robotic Personality and Embodiment on Human Trust
10:15 a.m.	**Morning Break**	
10:30 a.m.	Mia Arias Tsang *Dept. of Ecology & Evolutionary Biology*	Characterization of Phage H6 using Transposon Insertion Sequencing (INSeq)
10:45 a.m.	Antalique Tran *Department of MCD Biology*	Exploration of the Synaptic Developmental Effects of Laser Ablating Dorsal Motoneurons in a *Drosophila* Model
11:00 a.m.	Chika Ogbejesi *Department of Neuroscience-YSM*	3-D Imaging of M2 Acetylcholine Receptor in Visual Cortex of Mice
11:15 a.m.	Wasil Ahmed *Department of Neuroscience-YSM*	Investigating Somatostatin Interneuron Function in Cortical Development
11:30 a.m.	Alejandro Nuño *Department of Neuroscience-YSM*	Effect of Vasoactive Intestinal Peptide (VIP) Interneuron Dysfunction on Cortical Circuit Development
11:45 a.m.	Dawit Mengesha *Department of Neuroscience-YSM*	Measuring the Effect of MeCP2 Knockout on Perineuronal Nets

Wednesday, July 25th, 2018 — Afternoon Session

1:30 p.m.	Miles Waits *Department of Chemistry*	Understanding the Mechanism of Nickel Precatalysts in Buchwald-Hartwig Aminations
1:45 p.m.	Orven Mallari *Department of Chemistry*	Palladium-Catalyzed Suzuki-Miyaura Cross-Coupling of Benzyl Benzoates
2:00 p.m.	Andre Garcia de Oliveira *Department of Chemistry*	Analysis of CO_2 Hydroboration Selectivity using Metal Catalysts
2:15 p.m.	Franchette Brosoto *Department of Chemical and Environmental Engineering*	Enabling Hematite for Overall Photocatalytic Water-splitting through Cuprous-Oxide Nanocomposite
2:30 p.m.	Phyllis Mugadza *Dept. of Mechanical Engineering*	Examining the Impact of Restricted Wrist Mobility on Reaching Motion Compensation across a Discretely Sampled Workspace
2:45 p.m.	**Afternoon Break**	
3:00 p.m.	Araceli Lopez *Department of Biomedical Engineering*	Investigation of Neural Stem Cell Migration in the Presence of Endothelial Cells and Pericytes
3:15 p.m.	Darrel Pona *Department of Biomedical Engineering*	Enhancing Diabetic Wound Healing with siRNA-loaded Nanoparticles in Skin Cell Scaffolds
3:30 p.m.	Jesus Lopez *Department of MCD Biology*	Screening for Antibacterial Agents using Riboswitches
3:45 pm	Amer Al-Hiyasat *Department of Laboratory Medicine-YSM*	BRIT1 Interacts with Shelterin to Regulate the Telomeric DNA Damage Response
4:00 pm	Dr. Kenneth Nelson *Director STARS Program*	Closing Remarks for the Day

Thursday, July 26h, 2018 — Morning Session

9:00 a.m.	Alexis Cook *Department of Neurosurgery and Cellular & Molecular Physiology*	Higher Levels of mTORC1 Activity Lead to Increased Seizure Frequencyin Mice.
9:15 a.m.	Tiffany Wong *Department of Genetics*	Effect of Antisense Transcription on HtsRC Protein Expression in *Drosophila*
9:30 a.m.	Kendall Oliver *Department of MCD Biology*	Characterizing the Role of Rab34 in the Formation of the Primary Cilium
9:45 a.m.	Stephanie Horsfall *Department of MCD Biology*	Investigating the Role of p53 in Gene Repression
10:00 a.m.	Ava Niknahad *Department of Neurology-YSM*	Exploration of Impaired Consciousness caused by Temporal Lobe Epilepsy in a Mouse Model
10:15 a.m.	**Morning Break**	
10:30 a.m.	Jordan Young *Department of MCD Biology*	Investigating the Contributions of Langerhans Cells to Fibroblast Repopulation during Skin Wound Healing
10:45 a.m.	Justin Cheong *Department of Chemistry*	Biophysical Characterization of Mutant and Wild Type Biofilm Surface Layer Protein A (BslA) at the Air-Water Interface
11:00 a.m.	Nigel Wade *Department of Neuroscience-YSM*	Characterization of Auxilin Knockout: A Mouse Model of Parkinson's Disease
11:15 a.m.	Ricardo Soto *Department of Chemical and Environmental Engineering*	An Examination of Volatile Organic Compound Emissions from Consumer and Industrial Products
11:30 a.m.	Joslyn Barnett *STARS Summer Counselor*	A Look at STARS 2018
11:45 a.m.	Dr. Kenneth Nelson *Director STARS Program*	Closing Remarks

Investigating the Role of Oxygen in Metabolic Regulation of Planarian Regeneration

Linda Tun[1], Brian Jones[2], and Josien van Wolfswinkel[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA

Studies of stem cell metabolism have suggested that stem cells and differentiated cells have distinct metabolic states. In particular, stem cells tend to use glycolysis as a source of ATP rather than the more efficient pathway of oxidative phosphorylation. Despite this correlation, it is not known whether this special metabolic state is essential for stem cell function, and if and how it affects stem cell proliferation or differentiation. This current lack of understanding of fundamental stem cell biology hinders the ultimate goal of safely utilizing stem cells in various therapeutic contexts. To expand our understanding of the metabolic requirements of stem cells, we investigated how global metabolism is linked to stem cell activity. Using planarians as our model organisms, we explored the effect of temperature and glycolytic activity on stem cell function, by analyzing changes in regeneration time, stem cell activity, and gene expression through fluorescent *in situ* hybridization and qPCR experiments. We found that lower body temperatures resulted in delayed regeneration, suggesting reduced stem cell function. However, gene expression of the stem cell marker *smedwi-1* was up to 8 fold increased relative to controls, indicating that the delay in regeneration was not due to reduced stem cell proliferation. Stimulation of glycolysis by exposure to a mitochondrial uncoupler or to excess glucose did not affect regeneration efficiency. In both the chemical and temperature treatment, the expression of mitochondrial genes correlated with the expression of *smedwi-1*, suggesting that mitochondrial biogenesis is not differentially regulated in planarian stem cells under the conditions tested.

The Role of Oxygen Metabolism in Planarian Stem Cell Function

Helia Gagnon[1], Brian Jones[2], and Josien C. van Wolfswinkel[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Molecular, Cellular, and Developmental Biology Yale University

Pluripotent stem cells hold major potential for disease therapy as well as for the production of donor organs. However, a deeper overall understanding of the regulation and the physiology of stem cells is necessary prior to any application development. One aspect of stem cell biology that remains poorly understood is the metabolic requirements of stem cells in relation to pluripotency, proliferation, and differentiation. We used the planarian, *Schmidtea mediterranea* to explore the relationship between oxygen metabolism and stem cell function. *S. mediterranea* is an aquatic flatworm species with an abundance of mitotically active adult pluripotent stem cells. It maintains oxygen levels by direct gas exchange with the surrounding water. In this study we used two distinct approaches to modify the oxygen exposure of the planarian stem cells. First, we directly varied the environmental oxygen concentration through use of boiled water to reduce oxygen content and an air pump to increase water oxygenation. Second, we used chemicals such as Reactive Oxygen Species (ROS) generators, an antioxidant, and a hypoxia mimic to indirectly explore the relationship between oxygen metabolism and regeneration. Our observations of regeneration progression suggest that oxygen concentration of the water has no macroscopic effect on the regeneration process, however hypoxia as mimicked by chemical exposure resulted in a severe delay in regeneration. Conversely, exposure to increased levels of ROS increased the rate of regeneration. Analysis of cell cycle dynamics and transcriptional activity will provide further insights into the molecular basis of these phenotypes.

The Interaction of Dietary Fats with Leptin Signaling and its Effect on High Fat Diet Consumption

Natalia Taylor[1], Jasper de Jong[2], Royce Lee[1], Jennifer Kaplan[2], and Matt Rodeheffer[2,3]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT 06520, USA
[3]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA; Yale Stem Cell Center, Yale University School of Medicine, New Haven, CT 06520, USA.

Leptin has been found to be critical in the control of food consumption, but the interactions between leptin and fatty acids have not been elucidated. Previous studies have shown that wild type mice have a higher calorie intake on certain high-fat diets, and this led us to question if this overconsumption was caused by fatty acid-dependent inhibition of leptin signaling. In order to determine how leptin affects the caloric intake of different high-fat diets with different fatty acid compositions, we used leptin knockout *ob/ob* mice and observed their consumption patterns before, during, and after leptin was released into their systems. We found that the *ob/ob* mice ate similar amounts of the isocaloric diets when they did not have leptin. Once the leptin was introduced via osmotic pump, the mice significantly decreased their overall caloric intake but consumed similar amounts of each diet. From these results, we conclude that leptin-deficient *ob/ob* mice, as opposed to wild type mice, consume equal amounts of different high-fat diets and the introduction of leptin in the *ob/ob* mice fails to recapitulate the variation in caloric intake between the diets observed as observed in wild type mice. While we unable to conclude that the increased consumption of diets containing certain fatty acids is caused by leptin signaling inhibition, we gained better insight into the mechanisms behind calorie consumption.

Investigation of Dietary Fat Composition's Effect on Food Intake and Preferences in Wild Type and GPR40$^{-/-}$ Mice

Royce P Lee[1], Jasper MA de Jong[2], Natalia Taylor[1], and Matthew S Rodeheffer[2,3]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Comparative Medicine, Yale University School of Medicine, New Haven, CT 06520
[3]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA

Over the past few decades, obesity's prevalence in humans has increased dramatically. Previous studies have shown that various dietary fat compositions affect metabolic health. In this paper, we studied the choices and preferences for certain diets in mouse models to determine the relationship between dietary fat compositions and food preferences. Preference tests between various high-fat diets of lard, cocoa butter, and palm oil showed a strong naïve preference for palm, indicating that palm diet's pre-ingestion factors, such as smell, taste, texture, were favorable to the mice. During a post-ingestive preference test between the cocoa butter and palm diet, where the mice were previously conditioned with cocoa butter and palm diet ingestion, half of the mice preferred cocoa butter, indicating that post-ingestion conditioning affected some of the mice's preferences. This finding suggests that mice have different inter-individual preferences for fatty acid detection in diets. We then tested if GPR40, a known fatty acid receptor, is involved in dietary fatty acid preference and found that GPR40 KO mice preferred the cocoa butter and palm high-fat diets indiscriminately, indicating that GPR40 contributes to overall dietary preferences. During the high-fat diet conditioning where mice were fed different high-fat diets for 1 hour, mice were seen to consume the high-fat diets in different amounts, but compensated for these differences through chow consumption during the rest of the day. This supported the idea that mice have a mechanism for "counting" their caloric intake. Together, our results showed that pre- and post-ingestive factors are diet-dependent, there is variability in preferences between individual mice, and GPR40 is involved with the aversion or attraction to specific diets.

Trusting Robots: Examining the Influence of Robotic Personality and Embodiment on Human Trust

Kaitlynn T. Sierra[1], Zeynep Karacan[2], Nicole Salomons[2], and Brian Scassellati[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT, 06520
[2]Department of Computer Science, Yale University, New Haven, CT, 06520

Psychologists Kramer and Carnevale describe trust between two parties as a set of beliefs or expectations that one party's actions will benefit the outcomes or self-interests of the other party (Kramer & Carnevale 2003). Several research studies have shown that robots are also capable of gaining trust from people, but it is still unclear what aspect of robots people place their trust in. In order to study how robotic appearance and personality affect human trust in the integrity of robots, we propose an experiment where participants play a game with two Softbank NAO robots. Two different robotic personalities that had separate hobby interests were created for each NAO to use. The NAO robots also had differently colored panels. At different points of the game, we measure if the participant trusts the robots by having the robot ask them to borrow some game coins with the understanding that they will be paid back during the following round. The participant played the first half of the game with one NAO, and the second half with two NAOs. The NAO that was originally present for the first levels obtained a new personality at the start of the second half of the game, and the newly introduced NAO obtained the original NAO's personality. The participant's decision was then recorded again when asked to lend coins to either one of two robots. This scenario was our critical trust evaluator as the two NAOs had their personalities swapped in the experimental condition. Since the personalities were separated from their original physical form, the NAO that the participant chooses illustrates which aspect they placed their trust in. By discovering the impact of personality and robotic form on trust, the results of this study may directly impact the way future robots are created, designed, implemented, and updated.

Characterization of Phage H6 using Transposon Insertion Sequencing (INSeq)

Mia Arias Tsang[1], Kaitlyn Kortright[2], and Paul Turner[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520

Due to a culture of antibiotic overuse, we are seeing a large increase in infections caused by antibiotic-resistant bacteria that are difficult to treat using conventional medications. One such bacterium is *Pseudomonas aeruginosa*, a gram-negative bacterium that releases the toxin pyocyanin in large quantities during infection. Pyocyanin causes inflammation itself and also induces the production of free radicals, which in turn cause inflammation. It is also implicated to be important in quorum sensing, which plays a vital role in allowing *Pseudomonas aeruginosa* to form biofilms through which most antibiotics cannot penetrate. Infection with the bacteriophage H6 has proven to be effective in reducing pyocyanin levels in the *Pseudomonas* strain PA14. To determine precisely how H6 achieves this, we used a high-throughput sequencing technique known as INSeq that randomly inserts a modified *mariner* transposon with restriction sites for the enzyme MmeI into multiple points in the PA14 genome. The bacteria were incubated with H6 overnight, and only bacteria that had a transposon inserted into a gene that was necessary for phage infection survived. The transposon was cut out of the genome of these surviving bacteria using MmeI, leaving 16 base pairs flanking each side of the transposon. This gDNA was then sequenced by Illumina sequencing, allowing for the identification of genes that were disrupted by the transposon. When the sequencing results return, we can then determine a list of potential genes that may be necessary for H6 infection of PA14, and from that determine the role H6 plays in pyocyanin reduction. These results will help determine if H6 is a candidate for phage therapy.

Exploration of the Synaptic Developmental Effects of Laser Ablating Dorsal Motoneurons in a *Drosophila* Model

Antalique N. Tran[1] and Haig Keshishian[2,3]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Molecular, Cellular, and Developmental Biology, Yale College, New Haven, CT 06520
[3]Department of Neuroscience, Yale College, New Haven, CT 06520

Synaptic pruning is essential for proper neural development; embryonic neurons initially establish multiple, off-target connections that must be removed to achieve the precise connectivity seen in a mature nervous system. Denervation studies can provide insight into the underlying cellular and molecular mechanisms involved in pruning. A previous study found that laser ablation of a single embryonic *Drosophila* ventral motoneuron led to the appearance of functional, ectopic connections by neighboring neurons onto denervated muscle fibers, but the effects of partial denervation and of dorsal muscle fiber denervation remained unknown. To observe these effects, the methods necessary to assess the consequences of ablation were optimized. These methods included laser ablation techniques to ensure cell death as well as microdissection and immunolabeling methods to observe the miswired contacts. For ablation, a confocal microscope was used to image, and an installed, pulsed-dye nitrogen laser was used to target and to ablate two GFP-expressing motoneurons, known as aCC and RP2, that innervate dorsal muscle fibers of the *Drosophila* embryo. The number and frequency of laser pulses needed to ensure cell death were tested. Z-axis alignment of the laser pulse and the confocal image were determined to be critical, and GFP bleaching alone was demonstrated to not be a completely reliable predictor of cell death. To preserve the dorsal musculature for analysis, novel dissection protocols were developed. For clearer visualization of the nervous system, paraformaldehyde (PFA) fixation), anti-horseradish peroxidase (HRP) labeling, and diaminobenzidine (DAB)-peroxidase staining protocols were modified. These optimized methods will be used to ablate the aCC and RP2 motoneurons in embryos, and the affected muscle fibers will be examined in 3rd instar larvae. Dorsal motoneuronal ablation is expected to result in ectopic connections, setting the stage for future studies to use probes to examine changes in molecular expression by the synaptic partners as a result of denervation.

3-D Imaging of M2 Acetylcholine Receptor in Visual Cortex of Mice

Chika Ogbejesi[1], Alicja Puscian[2,3], and Michael J. Higley[2,3]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Dept. of Neuroscience, Yale University School of Medicine, New Haven, CT 06510
[3]Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT 06510

Acetylcholine is an important neurotransmitter involved in learning and memory. It is found throughout the brain and acts as a neuromodulator to excite or inhibit neurons. Deficiencies in acetylcholine concentration can lead to various cognitive illnesses including Alzheimer's and dementia. Defects in one type of inhibitory muscarinic acetylcholine receptor (mAChR), M2, has been found to play a role in the development of schizophrenia. M2 has been known to exhibit a non-uniform expression pattern in the cortex of mice that may correlate to cortical inputs. In order to further study the expression pattern of M2, we used a primary antibody MAB367 to immunolabel brains cleared with the iDisco protocol. We were able to validate that the antibody works to stain for M2 in the presence of methanol, a chemical used throughout the protocol. We were then able to clear the brains to use light sheet fluorescence microscopy to image the samples. We expect to create a 3D reconstruction of the entire brain from our imaging to analyze the relative expression of M2 in various parts of the brain. With this knowledge, we can hopefully have a better understanding of the function of M2 and why functional defects lead to cognitive impairments.

Investigating Somatostatin Interneuron Function in Cortical Development

Wasil Ahmed[1], Antara Majumdar[2], and Jessica A. Cardin[2,3]

[1]Science, Technology, and Research Scholar, Yale University, New Haven, CT 06520
[2]Yale University School of Medicine, Department of Neuroscience, New Haven, CT 06520
[3]Kavli Institute of Neuroscience, Yale University, New Haven, CT 06520

Somatostatin-expressing cortical interneurons (SOM-INs) are known to play a role in neurodevelopment, possibly in pruning regulating dendritic outgrowth, however their specific function is undetermined. To explore what role these GABAergic cells may have during early postnatal development, we ablated SOM-INs by cell type-specific expression of caspase in juvenile and adult mice. Based on preliminary evidence, we predicted impaired cortical development following early, but not late, SOM-IN loss. We measured differences in cortical thickness and cell density three weeks after injection in each group. SOM-Cre +/- mice were injected with Cre-dependent caspase virus in the left hemisphere while the right hemisphere was left uninjected to serve as an internal control. Fluorescent microscopy was used to determine cortical thickness as well as cell density. Left-to-right side ratios for both cortical thickness and cell density were calculated, with a predicted 1:1 ratio in normal development. Adults showed a 4% reduction in cortical thickness and 9% reduction in cell density in the injected side compared to the control side. Juveniles showed a 5% reduction in cortical thickness in the injected side compared to the control side, and a 7% reduction in cell density. The lack of large-scale impairments following late SOM-IN loss suggests that SOM-INs are not critical for maintenance of mature cortical tissue. However, the surprising lack of deficits following juvenile SOM-IN loss suggest that SOM hyperactivity, rather than hypoactivity, may be a driver of developmental pathophysiology. Further comparison to the effects of induced SOM-IN hyperactivation during early postnatal life may provide additional insight into SOM-IN developmental function.

Effect of Vasoactive Intestinal Peptide (VIP) Interneuron Dysfunction on Cortical Circuit Development

Alejandro Nuño[1], Antara Majumdar[2], and Jessica A. Cardin[2,3]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Yale University School of Medicine, Department of Neuroscience, New Haven, Connecticut, 06520, USA
[3]Kavli Institute of Neuroscience, Yale University, New Haven, Connecticut, 06520, USA

GABAergic interneurons expressing Vasoactive Intestinal Peptides (VIP-INs) play an important role in the development of cortical circuits in the brain as inhibitory neurons. However, very little is known about the mechanism by which VIP interneurons affect their synaptic targets or their precise role in the development of neuronal morphology and connectivity. To examine the developmental impact that VIP interneurons have on their targets, specifically somatostatin (SOM) cells and pyramidal neurons (PNs), we analyzed the morphology of SOM cells and PNs in control animals and after inducing cell death specifically in VIP-INs. We analyzed the dendritic morphology of SOM-INs cells and PNs by quantifying dendritic branching using a Sholl analysis tool. We further quantified the density of inhibitory synaptic puncta on SOM-IN and PN dendrites. Although more work is needed to gather data for the quantification of the morphology of SOM cells and PNs, we optimized a method to target synaptic connections (inhibitory inputs) in the visual cortex by targeting gephyrin, a postsynaptic scaffold protein specific to inhibitory synapses, to represent inhibitory inputs. The anti-gephyrin staining method was optimized by decreasing the incubation time of the mouse brain in paraformaldehyde (PFA) to prevent the cross-linking of proteins. With the anti-gephyrin staining methodology optimized, future steps in our project can be taken to gather more data, quantify it, and use it to compare SOM-IN and PN morphology with and without intact local VIP-INs. Our study could provide insight into the cell type-specific role of GABAergic inhibition in the developing visual cortex and the larger role that VIP-INs may have in the pathophysiology underlying neurodevelopmental disorders.

Measuring the effect of MECP2 Knockout on Perineuronal Nets

Dawit Mengesha[1], Jyoti Gupta[2], and Michael Higley[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Program in Cellular Neuroscience, Neurodegeneration and Repair

Methyl CpG binding protein 2 (MECP2) loss of function mutation has been associated with disorders, including but not limited to Rett syndrome and Autism Spectrum. The phenotypes associated with Rett Syndrome involving deletion of MeCP2 can be copied by deleting MeCP2 in GABAergic inhibitory interneurons. However, it is not known whether the deletion would result in an increase or decrease in inhibitory activity. Our preliminary results show that deleting MeCP2 in Somatostatin-interneurons (SOM-IN) leads to a hyperactive cortex. We hypothesize, since the loss of Parvalbumin interneuron (PV-IN) inhibition could lead to hyperactive cortex, increased SOM-IN inhibition would lead to decreased activity in PV-IN, and by extension a decreased maturation of perineuronal nets. By genetically modifying and turning off MECP2 in the SOM-IN of mice, we were able to observe a 20% reduction in the maturation of perineuronal nets in the visual cortex, as a result of the mutation (*$p<0.0001$). The result is consistent with increased output from SOM-IN, leading to decreased activity in PV-IN. Although the data showed a statistically significant difference between the SOM-IN knockout (experimental) and wild-type mice, there was a substantial variation within the experimental group, indicating a need for more sample size and thorough analysis.

Understanding the Mechanism of Nickel Precatalysts in Buchwald-Hartwig Aminations

Miles Waits[1], Emily Barth[2], and Nilay Hazari[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Chemistry, Yale University, New Haven, Connecticut, USA

There is significant interest in replacing palladium with a more cost-effective metal for cross-coupling reactions. In addition to being less expensive, the use of nickel catalysis instead of palladium offers a variety of benefits in terms of the range of reactions nickel facilitates. However, a lack of mechanistic understanding currently prevents the widespread use of nickel catalysis for cross-coupling reactions. Therefore, to study the mechanism of nickel in the Buchwald-Hartwig amination of aryl sulfamates, a family of nickel complexes containing the N-heterocyclic carbene ligand SIPr were prepared. Preparation of SIPr involved the multi-step synthesis of chloride and tetrafluoroborate salts of SIPr, which could then be deprotonated to form the free carbene. All intermediates and the final product were analyzed by ^{1}H NMR spectroscopy. Data collected from the evaluations of the SIPrNi complexes shows that some of these complexes are competent precatalysts for this reaction. Further studies will probe the mechanism of the fundamental steps involved in cross-coupling, which will allow us to optimize the reaction conditions and design a more efficient precatalyst.

Palladium-Catalyzed Suzuki-Miyaura Cross-Coupling of Benzyl Benzoates

Orven Mallari[1], Amira Dardir[2], and Nilay Hazari[2]

[1]Science, Technology, and Research Scholars Program, Yale College, New Haven, CT 06520
[2]Department of Chemistry, Yale University, New Haven, CT 06520

Palladium-catalyzed Suzuki-Miyaura cross coupling is one of the most prevalent reactions for forming carbon-carbon bonds in part because of its exceptional reliability and selectivity. This reaction is popular in both academia and industry. Traditionally, aryl halides and pseudohalides have been used as the electrophilic coupling partner to an organoborane. To expand the scope of this coupling reaction, this project describes a Suzuki-Miyaura reaction that involves benzylic benzoates as the electrophile instead. The palladium precatalyst used in this reaction, (η^3-1-^{t}BuInd)Pd(IPr)Cl, was synthesized in four steps. Eleven different substrates were synthesized with each having different functional groups on the aromatic ring. These substrates have varying electronic and steric properties stemming from these functional groups. Screening the solvent for cross-coupling using the benzyl benzoates as the electrophile so far have shown higher yields when using a 4:1 ratio of THF and methanol as solvent compared to a 4:1 ratio of THF to water. Furthermore, a ligand screen showed IPr as optimal over other NHCs and phosphine ligands. A subsequent time screen would further optimize the cross-coupling reaction to provide high yields while using low precatalyst loadings and mild conditions. The next steps in this project includes using the synthesized benzyl benzoate derivatives in a substrate scope.

Analysis of CO_2 Hydroboration Selectivity using Metal Catalysts

André Garcia de Oliveira[1], Matthew Espinosa[2], and Nilay Hazari[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Chemistry, Yale University, New Haven, Connecticut, USA

The commodity chemical industry heavily relies on unsustainable petroleum feedstocks. In order to provide a sustainable alternative carbon source, we studied CO_2 hydroboration. The aim of our research was to systematically study the effects of different metal hydride catalysts on the selectivity of CO_2 hydroboration. Specifically, complexes of the type ^{iPr}PSiPMH (M = Ni, Pd) were synthesized and compared to ^{Cy}PSiPMH (M = Ni, Pd) complexes, in order to determine the effects of phosphine substituents on catalysis. However, when these complexes were tested in catalysis with pinacol borane (HBPin) and catechol borane (HBCat), similar results to ^{Cy}PSiPMH catalysts were found, suggesting that the phosphine substituents had minimal effect on selectivity. A related literature catalyst, ^{iPr}PSi^{Ind}PNiH, was capable of achieving higher levels of reduction. Although this new reactivity may be attributed to the different ligand used, our stududies have shown that the reaction conditions used are in fact the reason for the change in selectivity. That is, we show that similar results can be found with ^{iPr}PSiPNiH by increasing the concentration of borane in solution. Therefore, we demonstrate that by modifying the concentration of borane present in catalysis we can completely control the selectivity of CO_2 reduction.

Enabling Hematite for Overall Photocatalytic Water-splitting through Cuprous-Oxide Nanocomposite

Franchette J. Brosoto[1], Yulian He[2], Lisa Pfefferle[2], and Shu Hu[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Chemical and Environmental Engineering, Yale University, New Haven, CT 06520

Photocatalytic water splitting serves as an important method to produce hydrogen fuel utilizing solar energy, a clean, renewable alternative to fossil fuels. Although hematite is an inexpensive, nontoxic, and abundant photocatalyst for water splitting, its band gap and band edge positions are insufficient for completion of the overall water splitting process. To overcome this challenge, we synthesized a hematite cuprous oxide nanocomposite using copper oxide nanosheets as hard templates. The goal is to tune composite so that the O_2 and H_2 evolution half reactions can occur at the valence band of hematite and conduction band of cuprous oxide, respectively. The close contact between the hematite and copper oxide nanocomposite is also beneficial for promoting interfacial charge transfer thus impeding the charge recombination.

Examining the Impact of Restricted Wrist Mobility on Reaching Motion Compensation across a Discretely Sampled Workspace

Phyllis Mugadza[1], Yuri Gloumakov[2], and Aaron M. Dollar[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Mechanical Engineering and Materials Science, Yale University School of Engineering and Applied Science, New Haven, CT 06520

This project presents an effort to characterize and quantify the impact of impaired wrist mobility on reaching motion compensation over a discrete workspace. When the degrees of freedom of the arm are limited due to injury or amputation, the behavior of other joints is modified in order to achieve the same motion goals. This is known as compensatory motion. Past studies that have measured motion compensation for simulated activities of daily living (ADLs) have focused on specific spatial configurations of the user and objects. In this study, joint angles and Cartesian trajectories of the upper body were recorded as able-bodied participants reached-and-grasped 49 equally spaced cylindrical targets with and without a wrist brace that limits all 3 degrees of freedom (DoFs) of the wrist. Motion data was analyzed by comparing range of motion (ROM), Cartesian path length, and area bounded by joint angle trajectories of impaired and unimpaired reaching conditions. The trends were presented visually using a 'heat-map' representation. The results indicate that wrist mobility has a significant impact on torso, shoulder, and elbow joints in certain locations of the workspace.

Investigation of Neural Stem Cell Migration in the Presence of Endothelial Cells and Pericytes

Araceli Lopez[1], Jannet Rivera[1], Rita Matta[2], and Anjelica Gonzalez[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT, 06520
[2]Department of Biomedical Engineering, Yale University, New Haven, CT, USA

Neurogenesis following ischemic stroke occurs within the subventricular zone (SVZ) of the brain. The SVZ is the region in which the largest pool of proliferating neural stem cells (NSCs) reside. In response to ischemic injury, NSCs pass through the rostral migratory stream (RMS) in order to differentiate in the olfactory bulb. Throughout the process of differentiation and proliferation, NSCs interact with resident cell types found in the vasculature of the neural niche, specifically endothelial cells (ECs) and pericytes (PCs). These cells are known to contribute to NSC proliferation and migration during homeostasis. However, the mechanisms by which ECs and PCs promote and guide migration through the RMS in response to injury remain unknown. Here, we investigate NSC migration guided by ECs and PCs. Scratch test assays were performed to determine the extent to which cell to cell contact or paracrine factor exchange drive NSC chemotaxis. Our results suggest that cell to cell contact between EC and NSC promotes NSC migration and clustering. In contrast, neither EC or PC secreted factors had a significant impact upon NSC migration. Our work suggests that EC direct contact with NSC is necessary for enhanced NSC migration. Our results advance the knowledge of vascular guidance in NSC migration, which is a critical process for NSC.

Enhancing Diabetic Wound Healing with siRNA-loaded Nanoparticles in Skin Cell Scaffolds

Darrell Pona[1], Amy Kauffman[2], Alexandra Piotrowski-Daspit[2], and W. Mark Saltzman[2]

[1]Science, Technology, and Research Scholar 2018, Yale College, New Haven, CT 06520
[2]Department of Biomedical Engineering, Yale University, New Haven, CT 06520

Prevalence for diabetes has nearly doubled in the last 40 years, meaning that more individuals struggle with stalled wound healing as one of the many complications of the disease. Previous methods to aid in diabetic wound healing such as viral entry of RNA) could derive an immune response and cannot be used in the United States. Thus, to reduce the probability of an immune response from the body, in this study, siRNA loaded nanoparticles were used to inhibit the expression of the thrombospondin-2 protein temporarily to enhance cell growth through an electrospun skin cell scaffold. Skin cell scaffolds have been fabricated, imaged, and characterized, and nanoparticles will be embedded within the scaffolds for siRNA delivery to diabetic wound sites. The siRNA loaded nanoparticles formulated had an average zeta potential of 19.9 mV and an average diameter of 189.9 nm. The human dermal fibroblast culture has grown to passage 4 to, eventually, assess the delivery of the siRNA message to inhibit thrombospondin-2 expression, enhancing diabetic wound healing. The results of this study may help facilitate the healing process for diabetic patients and enhance the rate at which their wounds are repaired.

Screening for Antibacterial Agents using Riboswitches

Jesus Lopez[1], Kevin Perkins[2], and Ronald Breaker[2,3,4]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA
[3]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, USA
[4]Howard Hughes Medical Institute, Yale University, New Haven, Connecticut, USA

New screening methods are needed to help develop antibacterial drugs. Riboswitches in bacteria can be used as sensors to monitor *in vivo* metabolic signaling changes in response to small molecules. Riboswitches are non-coding segments in mRNAs that function to regulate gene expression and have been found in many organisms, including gram-negative bacteria. Many metabolic pathways such as the folate and purine biosynthesis, are regulated by riboswitches. Bacteria use 10-formyl-tetrahydrofolate (10f-THF) and 5-aminoimidazole-4-carboxamide ribonucleotide (ZMP) in the purine pathway to form inosine monophosphate (IMP). If there is a need for folate, ZMP and ZTP accumulate and trigger the ZTP riboswitch. This ZTP riboswitch can be manipulated to develop a screen using a riboswitch-controlled *lacZ* reporter in *Escherichia coli* to detect new antibacterial compounds. Here we show that riboswitches in bacteria can be used to report the disruption of metabolic pathways in bacteria using screening. We used Miller assays to examine the effects of various compounds on accumulation of ZMP in wild-type and mutant *E. coli* ZTP reporter strains. Fluorescence and optical density were measured and used to determine gene expression. Of the 8 compounds tested, 2 showed the expected non-positive and 6 showed non-hit results. Trimethoprim showed positive hit results with accumulation of ZMP in wild-type strains but not mutant strains. These results suggest that this method could be applied more broadly to create riboswitch reporters that can be used in other pathways for different targets and thus improve antibacterial drug development.

BRIT1 Interacts with Shelterin to Regulate the Telomeric DNA Damage Response

Amer A. Al-Hiyasat[1], Alessandro Cicconi[2], and Sandy Chang[1,2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06520

The Shelterin complex is essential for the protection of telomeres from aberrant DNA damage repair activity. Defects in Shelterin activate a DNA damage response at telomeres mediated by multiple signaling and repair molecules, leading to the generation of chromosome fusions that confer tumorigenic genomic instability. BRCT-repeat inhibitor of hTERT expression (BRIT1) is an important early sensor of the DNA damage response. BRIT1 localizes to DNA double-stranded breaks through interaction with γH2AX. We recently found that BRIT1 interacts with components of the Shelterin complex, and we hypothesized that this interaction may play a role in the telomeric DNA damage response. In the present study, we show that BRIT1's interaction with Shelterin modulates its localization to telomeres independently of γH2AX. We found that loss of this interaction impairs the recruitment of downstream DNA damage signaling proteins. Our data suggests that modulation of BRIT1's interaction with Shelterin may be an important regulatory mechanism for the telomeric DNA damage response, and could serve as a target for the development of therapeutic strategies against telomere dysfunction-induced genome instability.

Higher Levels of mTORC1 Activity Lead to Increased Seizure Frequency in Mice.

Alexis Cook[1], Lena Nguyen[2], and Angelique Bordey[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520, USA.
[2]Departments of Neurosurgery, and Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT06520-8082, USA.

Aberrant activation of the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway in the brain has been associated with medically refractory epilepsy. However, it remains unclear whether the degree of mTORC1 hyperactivity influences epilepsy severity. In this study, we investigated whether increasing levels of mTORC1 activity affect seizure frequency in mice. We found that increasing mTORC1 activity levels significantly correlated with higher seizure frequency. Overall, our data demonstrates that mTORC1 activity levels influence epilepsy severity and further highlights the importance of this pathway in disease pathogenesis.

Effect of Antisense Transcription on HtsRC Protein Expression in *Drosophila*

Tiffany Wong[1], Juli Gerdes[2], and Lynn Cooley[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Genetics, Yale University Medical School, New Haven, CT 06520

As part of normal development in *Drosophila*, ring canals are assembled to allow the movement of proteins and RNA between germline cells. The protein HtsRC has been shown to be necessary for proper ring canal formation in the female germline. HtsRC protein is produced by the *ovhts* mRNA, which is only present in the ovaries, although other mRNA isoforms from the same gene are found in other tissues. Antisense transcription begins in the region corresponding to the *ovhts* 3'UTR and produces a non-coding RNA, *CR43430*, which is almost perfectly complementary to the HtsRC-encoding exon. Large scale sequencing of total RNA confirmed that the HtsRC transcript is largely present in the female ovary, while the *CR43430* transcript is only present in the male testis. However, whether *CR43430* plays a role in HtsRC protein expression and what that precise function may be are unclear. One possibility is that *CR43430* may function in regulating HtsRC expression. When we expressed HtsRC transgenes in the male germline, HtsRC protein was only observed if the transgene did not have the *CR43430* transcription start site, suggesting that the presence of *CR43430* ncRNA or the act of *CR43430* transcription could block HtsRC protein expression. Experiments are underway to determine the effect on HtsRC expression in ovaries ectopically-expressing transgenic *CR43430* ncRNA. These results may suggest that antisense transcription regulates tissue-specific protein expression in *Drosophila melanogaster*. Studying the effect of *CR43430* ncRNA on HtsRC protein expression will be relevant to ongoing research about how antisense transcripts can regulate differential gene expression in eukaryotic tissues.

Characterizing the Role of Rab34 in the Formation of the Primary Cilium

Kendall E. Oliver[1], Margaret C. Kennedy[2], and David K. Breslow[2]

[1]STARS Program, Yale University, New Haven, CT, USA
[2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA

The primary cilium is an immotile microtubule-based organelle that protrudes from the cell surface. It functions as an organizing center of signal transduction and is essential for proper embryonic development. Dysregulations of the primary cilium can cause a wide range of disorders known as ciliopathies. The underlying molecular mechanisms of most ciliopathies remain uncharacterized, as do key aspects of primary cilium assembly and function. In a recent study, the gene encoding Rab34 was identified as being important for ciliogenesis. Rab34 is a small GTPase of the Rab protein family that cycles between an inactive GDP-bound conformation and an active GTP-bound conformation. We sought to identify which nucleotide binding conformation of Rab34 was functionally relevant for ciliogenesis, as well as if SGSM1 functions as a GAP (GTPase activating protein) for Rab34 in cells. We further hypothesized that artificially stabilizing Rab34 in a GTP-bound conformation would provide new insights into its functionally relevant conformation and into where it acts within cells. To this end, we constructed a putative GTP-locked mutant of Rab34 by substituting the Leucine of the 61st amino acid residue to Aspartic Acid (Rab34-L61D). We transiently transfected mouse NIH-3T3 cells with tagged forms of Rab34-L61D and SGSM1, both individually and in combination. The localization and effect on ciliogenesis of the proteins was observed through immunofluorescence microscopy. Cells transfected with GFP-Rab34L61D did not generate cilia, indicating that our mutant is dominant negative and that Rab34 must be conformationally dynamic for proper ciliogenesis. Cells transfected with GFP-SGSM1 exhibited normal ciliogenesis. Interestingly, cells co-transfected with GFP-Rab34L61D and Myc-SGSM1 exhibited normal ciliogenesis, suggesting that the presence of SGSM1 has an inhibitory effect on the dominant negative mutant. We conclude that SGSM1 is likely to be the GAP for Rab34 in cells and that the Rab34-L61D mutant is likely to be GTP-locked, potentially due to an altered intrinsic rate of GTP hydrolysis. Future studies can determine whether Rab34L61D is GTP-locked through in vitro biochemical assays and co-immunoprecipitation with Rab-GDI. Our study has provided insights into both the mode of action of Rab34 and the manner in which it is regulated to promote ciliogenesis.

Investigating the Role of p53 in Gene Repression

Stephanie Horsfall[1] and Nadya Dimitrova[2,3,4]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520
[3]Yale Center for RNA Science and Medicine, Yale University, New Haven, CT 06520
[4]Yale Cancer Center, Yale University, New Haven, CT 06520

Over the past 30 years, p53 has been proven to be an important transcription factor involved in the cell cycle and the tumor suppression pathway. Although p53 is a well-known activator of gene transcription, the mechanisms of how the protein represses its target genes remains relatively unknown. The aim of this project is to determine the repression model of p53-associated genes and how it relates to the process of senescence. We chose to focus on 11 genes, derived from a previously determined list of 56 repressed genes associated with senescence in lung adenocarcinoma and sarcoma cells when p53 is induced. These 11 genes are involved with processes related to cancer such as cell cycle regulation, apoptosis, and senescence. We validated the repression of the 11 genes with reverse transcription – quantitative polymerase chain reaction, using primers that were designed during the project. Of the initial 11 genes tested, only 6 showed significant gene repression when p53 was induced. We developed a CRISPR-Cas9 strategy in order to mutate the p53 binding site of the genes. To date, we successfully designed and cloned sgRNAs. Ultimately, the CRISPR-Cas9 system will be used to test the direct repression model of p53. Determining the role p53 plays in gene repression can lead to a better understanding of the molecular basis of senescence.

Exploration of Impaired Consciousness caused by Temporal Lobe Epilepsy in a Mouse Model

Ava Niknahad[1], Lim-Anna Sieu[2], Shobhit Singla[2], Marcus Valcarce-Aspegren[2], and Hal Blumenfeld[2,3,4]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Neurology, Yale University School of Medicine, New Haven, CT 06520
[3]Department of Neuroscience, Yale University School of Medicine, New Haven, CT 06520
[4]Department of Neurosurgery, Yale University School of Medicine, New Haven, CT 06520

Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy in which seizures arise from limbic structures such as the hippocampus. TLE patients are at a higher risk of suffering from impaired consciousness, leading to a higher risk of physical accidents and social stigmatization. Previous studies have investigated the mechanisms behind impaired consciousness in TLE patients by using intracranial electroencephalogram recordings and have suggested an association between slow waves—similar to those found in the sleep state—in the frontal cortex and impaired consciousness. Based on such clinical studies, the network inhibition hypothesis was developed with the goal of explaining the neuronal networks behind impaired consciousness. Rat models of TLE were previously developed to investigate this hypothesis but were limited due to the poor availability of genetic tools and the inability to analyze behavior, as the rats were in an anesthetized state. In this project, we present an awake mouse model of TLE with more available genetic techniques. In the mouse model, TLE seizures were induced through an electrical stimulation of the hippocampus while simultaneously recording the local field potentials of the lateral orbitofrontal cortex and dorsal hippocampus. To assess behavior during the seizures, the water-restricted mice were head-fixed to a wheel where they were allowed to freely run and were trained to lick water from a spout in response to an auditory stimulus. Behavioral analysis demonstrated that the mice experienced both spared and impaired behaviors during seizures, with impaired behavior being displayed as an arrest of both the licking and running tasks. These impaired seizures had a significant correlation with slow waves in the lateral orbitofrontal cortex. These findings suggested that the mouse model shares characteristics with humans while having advantages over the rat model for investigation of mechanisms underlying impaired consciousness in TLE seizures.

Investigating the Contributions of Langerhans Cells to Fibroblast Repopulation during Skin Wound Healing

Jordan Young[1], Renee Wasko[2], and Valerie Horsley[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, USA

Chronic wounds require extensive care and can lead to many lifetime impediments for patients. Diabetic patients represent a significant population who suffer from chronic non-healing wounds. Previous studies have shown that higher densities of Langerhans cells (LCs) in human diabetic foot ulcers correlate with positive healing outcomes, suggesting that LCs may promote skin regeneration. Our aim is to understand the mechanism by which LCs affect wound healing, particularly the proliferative stage of repair. By ablating the LCs from two genetically engineered mouse models, we are able to measure how fibroblast and myofibroblast repopulation in wound beds are affected by the absence of LCs. Our preliminary findings suggest that depletion of LCs results in diminished fibroblast and myofibroblast repopulation in wounds. Overall, these results suggest that Langerhans cells may produce signals important for the proliferative stage of wound healing.

Biophysical Characterization of Mutant and Wild Type Biofilm Surface Layer Protein A (BslA) at the Air-Water Interface

Justin Cheong[1], Zahra Sohrabpour[2], and Elsa C. Y. Yan[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Department of Chemistry, Yale University, New Haven, CT 06520

Biofilms are communities of bacteria enclosed within an extracellular matrix composed of DNA, polysaccharides, and proteins. These components self-assemble to protect bacterial growth, making biofilms one of the leading causes of human infections. We focus on the protein BslA, an amphiphilic, surface-active protein expressed by *Bacillus subtilis*. Previous studies have shown that BslA is necessary in an extremely hydrophobic biofilm *in vivo* and a highly rigid and ordered monolayer *in vitro*. However, the molecular mechanism of biofilm formation remains unknown. The crystal structure of BslA reveals a relatively unstable 3_{10}-helix consisting of threonine, glutamine, and isoleucine. We hypothesize that a conformational change occurs in this 3_{10}-helix to enable BslA to form a highly stable outermost layer in the biofilm of *B. subtilis*. To test this hypothesis, we expressed and purified wild type and mutant BslA proteins with amino acid substitution threonine to arginine (T106R) in *Escherichia coli* and characterized them as Langmuir monolayers in the compression and adsorption isotherms. The compression moduli of T106R demonstrated that the mutation decreased the robustness and rigidity of the BslA Langmuir monolayer, and fitting preliminary adsorption isotherm data generated a less negative adsorption free energy for T106R than for the wild type. Together, the compression moduli and the adsorption free energy indicate that the T106R mutant decreases the robustness of the monolayer at the air-water interface, supporting the hypothesis that the 3_{10}-helix is involved in forming a stable Langmuir monolayer of BslA molecules.

Characterization of Auxilin Knockout: A Mouse Model of Parkinson's Disease

Nigel Wade[1], and Sreeganga Chandra[2]

[1]Science, Technology, and Research Scholar, Yale College, New Haven, CT 06520
[2]Departments of Neurology and Neuroscience, Yale University, New Haven, CT 06520

Auxilin is a protein involved in clathrin mediated endocytosis, a cellular uptake process. Auxilin functions to uncoat clathrin cages to release nascent vesicles. Auxilin is primarily expressed in nervous tissue and loss of auxilin has been shown to cause juvenile parkinsonism in humans. This study aims to understand the effects of auxilin knockout on the synaptic proteome and clathrin cages, with the larger goal of understanding why loss of auxilin leads to Parkinson's disease. To elucidate the changes that occur in the synaptic proteome, mass spectrometric analysis of auxilin knockout and wild type brains was used to quantitatively determine the levels of synaptic proteins in both genotypes. From these data, the protein ratios, their significance, and biological pathways impacted by loss of auxilin were computed. Auxilin knockout resulted in 79 significant protein changes, with most of these being expressed at lower levels, but several proteins such as SHPS1 and CRYAB were overexpressed. To understand the impact of auxilin deletion on clathrin cage structure, we analyzed electron microscopic images of clathrin cages derived from auxilin knockout and wildtype brains using ITEM software. We measured cage diameter and proportions of empty cages in auxilin knockout and wild type samples. ITEM analysis revealed auxilin knockout cages to be significantly smaller than wild type cages and auxilin knockout samples to contain significantly more empty cages. The exact reason for these empty cages is unknown and warrants further investigation into the role of auxilin.

An Examination of Volatile Organic Compound Emissions from Consumer and Industrial Products

Ricardo Soto[1], Peeyush Khare[2] and Drew Gentner[2]

[1]Science, Technology, and Research Scholars, Yale University, New Haven, CT, 06520, USA
[2]Department of Chemical and Environmental Engineering, Yale University, New Haven, CT, 06520, USA

In 2012, the number of deaths attributed to air pollution and degrading air quality exceeded 7 million and is estimated to have increased since then. Volatile organic compounds (VOCs) are of paramount importance to diminishing air quality due to their tendency to form secondary organic aerosols (SOAs) and tropospheric ozone through several oxidation mechanisms. Many of those products have been confirmed harmful to human health. Historically, the primary contributors of VOCs to the atmosphere have been combustion-based sources such as on-road motor vehicles. However, their relative contribution to urban air pollution in the U.S. has now reduced due to decades of strict policies and regulations. VOC emissions from common consumer and industrial products and processes are projected to surpass emissions from combustion-based sources by 2020. In this project, we set out to characterize the VOC emissions of select non-combustion based emissions sources including asphalt and a few consumer products with careful consideration of both lifetime environments and multiple emissions pathways: solvent evaporation, off-gassing of solute, and degradation by-products. Samples of product emissions are taken both on a novel benchtop setup as well as through field measurements. Traditional (GC-EI-MS) and newer (GC-APCI-QTOF) emissions analysis methods are employed to ensure broad, detailed emissions data and spectra. The novel benchtop setup is highly applicable to a wide range of emissions experiments, and our methods of emissions analysis have allowed for uniquely high-resolution characterization of emissions from examined asphalts and products.

Acknowledgments

We wish to thank the following for their contributions to the Yale STARS Summer Program 2018:

Peter Salovey, President, Yale University
Benjamin Polak, Provost, Yale University
Marvin Chun, Dean of Yale College
Sandy Chang, Associate Dean of Science Education

Kurt Zilm, Chemistry, Convener, STARS Advisory Committee
Hector Arce, Astronomy, STARS Advisory Committee
Keith Baker, Physics, STARS Advisory Committee
Enrique De La Cruz, MB&B, STARS Advisory Committee
Christine DiMeglio, Chemistry, STARS Advisory Committee
James Duncan, Biomedical Engineering, STARS Advisory Committee
Anjelica Gonzalez, Biomedical Engineering, STARS Advisory Committee
John Hall, Mathematics, STARS Advisory Committee
Burgwell Howard, Sr. Associate Dean Yale College, STARS Advisory Committee
Rise Nelson, Assistant Dean Yale, STARS Advisory Committee
Donalee Slater, Yale College Science & QR Education
Sarah DelVecchio, Yale College Science & QR Education

We would also like to express our gratitude to all the Principal Investigators and Mentors who have invested so much time and energy in the scientific development of the STARS summer students; we are grateful for your dedication and service.

Our best wishes and gratitude to our program staff who worked tirelessly with expertise and professionalism:

Christopher Lim, Ph.D. Candidate, MB&B, Graduate Assistant STARS
Lien Nguyen, Ph.D. Candidate, Interdepartmental Neuroscience Program, Graduate Assistant STARS
Titilayo Omotade, Ph.D. Candidate, Department of Microbial Pathogenesis, Graduate Assistant STARS
Joslyn Barnett, Summer Coordinator, STARS

We would also like to express our appreciation to:
Yale College
and
The Simons Foundation -SFARI Undergraduate Summer Research Program

for the funding that makes this program possible.

Our best wishes and gratitude to:

Maria Moreno, MCDB, Academic Coordinator STARS Program
Kenneth Nelson, MCDB, Academic Director STARS Program
Kailas Purushothaman, Engineering, Advisor STARS Program
Sandy Chang, Yale College Associate Dean of Science Education, Director STARS Program