RESULTS & DISCUSSION

BRET Newsletter
Issue 11, Spring 2021

VANDERBILT School of Medicine
Biomedical Research Education and Training
Office of Career Development

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Trainee Research Highlights

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Letter from the Deans

Welcome to the eleventh issue of Results and Discussion, a newsletter sponsored by the Office of Biomedical Research Education and Training (BRET), that is devoted to highlighting the research accomplishments and activities of our Ph.D. graduate students and postdoctoral fellows.

It has been a very challenging year for many, but still one of amazing creativity, unearthed resilience, and unique collaborations. Our graduate students, postdoctoral fellows, faculty, and staff have not only powered through, but they have been shining brightly. The experience of a global pandemic has refocused priorities and brought so many of us closer together.

The University adopted the slogan, "Anchor Down. Step Up," and after a shut-down lasting about three months, our labs were filled with masks, new schedules, and Zoom meetings. The BRET Office of Career Development ASPIRE Program saw jumps in attendance as the office adjusted its seminar schedule to be completely virtual, including the tradition of the Annual Career Symposium. Even BRET graduate student recruiting efforts are being conducted via the comforts of the recruits' home computers!

For the first time, the Simple Beginnings lab coat ceremony was broadcast all over the world to hundreds of family members and friends. They virtually cheered on an incoming class of biomedical scientists who began their new training in this uncertain time.

In many ways, our graduate students and postdoctoral scholars have played an important and exciting role in development of COVID-19 antibody therapies, understanding of coronavirus biology and infectivity, and even in increasing access to COVID-19 testing. To be a scientist during this period has been fascinating, despite the rough circumstances.

Throughout all the disruptions and adjustments, our trainees have made us so proud to be a part of the Vanderbilt community and to support them in their research training and education. Although the challenges have not ended, we know we are not alone in our efforts to support and help each other during this unprecedented time.

Sincerely,

Roger G. Chalkley, D. Phil.
Sr. Associate Dean for Biomedical Research Education and Training
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Issue Acknowledgements

Cover Art is an immunofluorescence image of a day 30 cerebral organoid. Control wildtype and BAX/BAK double knock out (DKO) organoids are shown. Brain organoids were stained with the mitochondrial marker TOM20 (magenta), the neuronal marker TUJ1 (red), and the neural stem cell marker SOX2 (green). by Piyush Joshi, Ph.D.; Caroline Bodnya; Megan Rasmussen, Ph.D.; Alejandra Romero-Morales; Anna Bright; and Vivian Gama, Ph.D.

Photos courtesy of Vanderbilt University and trainee highlight submissions.

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New Tools for Therapeutic Discovery in Obesity

By Breanne Gibson, Graduate Student

More than 40 percent of individuals in the United States are obese and experience an increased risk of cardiovascular disease, diabetes, and sepsis. This major healthcare burden, for which we have few treatment options, is the problem Geetika Aggarwal, Ph.D., focused on during her postdoctoral fellowship in the lab of Sean Davies, Ph.D., Associate Professor of Pharmacology.

During her undergraduate training, Aggarwal was fascinated by DNA and protein structure, leading her to pursue a Ph.D. studying enzymes that protect against cardiovascular disease at the National Institute of Pharmaceutical Education and Research in India. Following her graduate studies, Aggarwal came to Vanderbilt University to study lipid metabolism in obesity. The Davies lab studies *N*-acyl-ethanolamides (NAEs), lipids involved in regulating feeding behavior and inflammation. The final enzyme that processes NAEs, *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD), helps protect against obesity. Previous work from the Davies lab demonstrated that NAEs formed by NAPE-PLD reduce food intake and increase fat breakdown.

"We had two goals: first, to understand the mechanism of these lipids in controlling metabolism and energy balance, and second, to find therapeutic targets to control obesity," said Aggarwal.

As a biochemist, Aggarwal realized that a major barrier in obesity research is a lack of good tools to study the mechanisms underlying the disease. In fact, the specific role of NAPE-PLD in obesity was difficult to study because no potent inhibitors of the enzyme had been identified. In her recently published *Journal of Biological Chemistry* study, Aggarwal partnered with the high-throughput screening core at Vanderbilt to screen more than 2,000 molecules for potential NAPE-PLD inhibition. They found 14 molecules that strongly inhibited NAPE-PLD, the two strongest of which shared similar chemical structures.

To investigate which parts of these two molecules are critical for NAPE-PLD inhibition, the group tested the effects of various chemical modifications. Aggarwal found that molecules in this family that have symmetrical chemical groups are effective inhibitors of NAPE-PLD. The identification of these inhibitors has made it possible to specifically study how NAPE-PLD activity affects obesity.

Now a staff scientist at St. Louis University, Aggarwal enjoys travel, photography, dancing, and practicing meditation. In the future, Aggarwal hopes to become a research instructor.

"I love reading about science, doing bench work, and training students. That's what I want to do."

Geetika Aggarwal, Ph.D., on a recent hike.

Learn More:

Aggarwal, G., et al., Symmetrically substituted dichlorophenes inhibit N-acyl-phosphatidylethanolamine phospholipase D. *Journal of Biological Chemistry* (2020) Vl 295, Is 21, 7289-7300.

Data Visualization Tool Creates Movie-like Cellular Network Simulations

By Elizabeth Stivison, Ph.D., Postdoctoral Fellow

There is no shortage of data these days. In fact, there is so much data that the problem becomes how to analyze, visualize, and understand it. Vanderbilt University Ph.D. student Oscar Ortega recently published a paper in *iScience* that addresses this issue.

Ortega, working in the lab of Carlos F. Lopez, Ph.D., Assistant Professor of Biochemistry and Biomedical Informatics, has developed a new program for data visualization. The program, PyViPR, creates interactive visualizations of protein networks involved in cellular processes over time.

Each protein in a network is represented by a node, visualized as a pie chart to show protein concentration. The edges - lines connecting the nodes in the network - illustrate protein interactions and the line thickness indicates interaction strength. This clear visualization of complex, time-dependent data is a key to new hypothesis generation.

“I put the dynamics into the networks,” Ortega says. “An animation shows how the biochemical reactions and reactant concentrations change over time.”

For proof of concept, Ortega visualized apoptosis, a form of programmed cell death. Using PyViPR, Ortega's work identified nine distinct communities, or nodes grouped by the number of edges (interactions) they share. Each community is involved in different aspects of apoptosis, and the interactions can be visualized over time using PyViPR. As the animation progresses, the thickness of the edges in the apoptosis network change, showing the signal flow during cell-death execution.

Besides being able to visualize biological reaction networks over time, PyViPR solves another problem as well. Most data modeling relies on multiple tools to simulate and analyze the information.

Ortega says, "This is all embedded in Jupyter, which is a web-based notebook, where you can write code and document your model analysis in the same place. It's all one file that you can share easily."

He may be creating biological network visualization programs now, but Ortega started out in a different field. Ortega grew up in Florencia, Colombia, and studied physics at the University of the Andes in Bogotá.

"As a physics student, I took a class in systems biology and that's how I got into quantitative biology. I really liked the systems class and I did my undergraduate thesis with the professor on evolutionary game theory."

Ortega took a semester off to study English in Boston, where he happened to meet his future Ph.D. mentor, Dr. Lopez, at a seminar. They hit it off, and the two stayed in touch. Ortega later did an internship with Lopez, applied to Vanderbilt, and joined Lopez' lab to continue his work as a Ph.D. student.

The pandemic doesn't seem to be cramping Ortega's style much. He was set to run the Nashville half marathon this past April, which was cancelled. Instead of being disappointed, he ran it by himself. This is just the kind of self-motivation needed to craft a tool like PyViPR and will propel Ortega forward in his career.

Page 4, graduate student Oscar Ortega takes a moment in the Red Valley in Cusco, Peru. Above, Ortega, left, with fellow graduate student friends at a recent 5K race. Below, Ortega is on an adventure in his hometown of Florencia, Colombia. (Photos submitted.)

Learn More:

Ortega, O, et. al., (2020) Interactive Multiresolution Visualization of Cellular Network Processes. *iScience* 23 (2): 100748

Future Directions: Marty Moore, Ph.D.

Chief Executive Officer and Founder, Meissa Vaccines

By Taylor Engdahl, Graduate Student

Marty Moore, Ph.D.

In a year defined by record-breaking vaccine progress, Marty Moore, Ph.D., is also moving full speed ahead. Moore began his work on respiratory syncytial virus (RSV) as a postdoctoral fellow in the laboratory of Stokes Peebles, M.D., at Vanderbilt University. He then joined Emory University as an Associate Professor and Director of the Emory Children's Center for Childhood Infections and Vaccines. At Emory, he developed a way to synthetically reconstruct a weakened form of RSV in the laboratory, and this technology eventually led to the founding of Meissa Vaccines in 2014. Moore then left his tenured position to run Meissa full time in 2018. The company's platform has proven adaptable to many different pathogens and Meissa's SARS-CoV-2 (COVID-19) vaccine has recently moved into clinical trials.

Why did you decide to work for Meissa Vaccines full-time?

I loved my faculty position, but I found that academia was too focused on navigating your career instead of pursuing a scientific mission. I wanted to hone our RSV vaccine technology full-time, and ensure that vaccine work was the highest priority.

What excites you most about running a biotechnology company?

Industry runs at a faster pace than academia, making it much easier to sustain the momentum. We can run an experiment, analyze the data, and decide immediately to take it to manufacturing. It's very satisfying for everyone on the team to quickly push something forward.

Was there anything that surprised you about starting your own company?

It is not easy acquiring funding from venture capitalists, especially in the infectious disease field. It requires a lot of long-term relationship building. I was turned down countless times, but fortunately, I only needed one yes.

What is your most significant accomplishment?

My sole focus is translating vaccines into the clinic to help people, and Meissa is a vehicle for that. We've recently completed clinical safety trials for the RSV vaccines and will now proceed to testing in infants. We're also moving forward with a SARS-CoV-2 vaccine Phase I trial.

What advice would you give to trainees who are interested in your career?

Train yourself to forget about job security – it's a myth. A tenured faculty can fail to get grants, and larger pharmaceutical companies lay off employees frequently. I started Meissa because it was the best way to move my science forward, and I love what I do now. What we should be doing is trying to get ourselves fired as fast as possible. At Meissa, we set our own scientific direction instead of being reactive to the safest option, and we are willing to fail.

By the numbers

Total time spent working?

60 hrs per week

Typical schedule?

6 am to 6 pm in Zoom meetings

Breakdown of responsibilities?

Research and clinical development, operations manufacturing, board meetings, fundraising, hiring/recruiting

Email load?

20 incoming and 5-6 outgoing emails per day

An Apple a Day: Nutritional Deficiencies Impact Alzheimer's Disease

By Brenna Appleton, Graduate Student

David Consoli discovered his passion for science early, recognizing it as a way to create solutions for some of life's most difficult problems. As an undergraduate at Auburn University, Consoli tested his hand in a variety of research fields including chemistry, cell biology, and drug discovery. These research experiences showed Consoli that what really excited him was science with a focus on clinical application. This interest eventually led him to Vanderbilt University's Interdisciplinary Graduate Program where he joined the lab of Fiona Harrison, Ph.D., Associate Professor of Medicine.

The Harrison lab focuses on how modifiable risk factors like diet impact Alzheimer's disease progression. Consoli's recent article in the ***Journal of Neurochemistry*** builds on this central question, detailing how vitamin C deficiency decreases a hormone, dopamine, in the area of the brain known for being a reward center. Dopamine is critical to motivational processes and requires vitamin C for its production. Consoli found that dopamine-triggered signaling was significantly reduced in a mouse model of Alzheimer's disease, and vitamin C deficiency further decreased this signaling. Since Alzheimer's patients frequently experience decreased motivation that can contribute to their cognitive decline, Consoli's research shows that a modifiable nutritional element like vitamin C may have a critical role in this behavior.

Consoli's work was actually the result of a quick course correction when the original experimental approach became infeasible. Consoli cites the mentorship he received from Harrison as a major reason he was able to successfully adapt.

Left: David Consoli with his wife on a recent trip. Right: Consoli at the bench.

"She's always taught us that when the results come back, and they're not what you expected them to be, those are the most interesting results because it forces you to rethink a new and more interesting hypothesis."

Interestingly, Consoli's paper also shows that vitamin C deficiency impacts dopamine levels in mice not exhibiting Alzheimer's disease, and he feels these findings emphasize the importance of maintaining a healthy lifestyle.

"It's more important that you are consistently eating a variety of fruits and vegetables throughout your entire lifetime," he says. "It's easy to become deficient when you form unhealthy habits."

Consoli hopes to expand on this work to study whether increasing vitamin C levels in Alzheimer's disease models could improve motivation.

"We want to try different therapies to rescue these deficits or prevent them from happening, because that's what can be taken into the clinical setting," he explains.

In his free time away from lab, Consoli enjoys being outside, hiking, mountain biking, running, and living the healthy lifestyle his science shows is so important.

Learn More:

Consoli, D.C., et. al., (2020) Ascorbate deficiency decreases dopamine release in gulo−/− and APP/PSEN1 mice. *Journal of Neurochemistry*. doi: 10.1111/jnc.15151

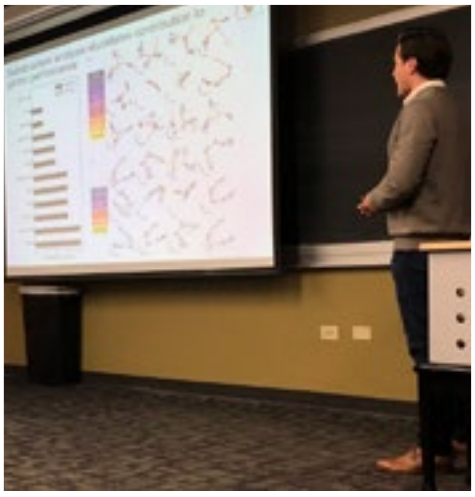
How to Make Friends and Reduce Dimensions: Evaluating Dimensionality Reduction Techniques

By Bryson Reynolds, Ph.D., Postdoctoral Fellow

Cody Heiser

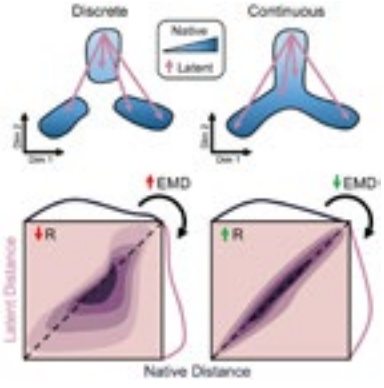
Single cell RNA sequencing (scRNA-seq) is a next-generation technology used to understand gene expression of target cell populations during normal, developmental, or diseased states. While scRNA-seq can be a powerful tool to understand gene expression, it produces a large amount of multidimensional data (multiple variables) that requires processing to include only the most meaningful dimensions. Researchers use several different dimensionality reduction methods, leaving the field without a clear gold standard. Cody Heiser, Vanderbilt University graduate student, offers a novel method for evaluating these different techniques in a *Cell Reports* article.

Heiser entered college with the goal of becoming an M.D. However, as he pursued his degree in biomedical engineering he became more interested in research and biotechnology. He then spent two years working in product development in the biotechnology industry. This experience only increased his interest in informatics and computational biology, leading him to pursue his Ph.D. at Vanderbilt.



Heiser works in the lab of Ken Lau, Ph.D., Associate Professor of Cell and Developmental Biology, on data analysis and experimental techniques to better understand genetic expression in development and disease. Their study evaluated techniques for data dimensionality reduction that are common in the analysis of scRNA-seq and other high-dimensional analyses. T-distributed Stochastic Neighbor Embedding (t-SNE) was the gold standard method for dimensionality reduction at the advent of scRNA-seq, but researchers soon developed other techniques. Each of these methods have their own advantages, but Heiser cautions that the utility of each is highly dependent on the data set.

"The developers used different data types to validate them, and the input data is really determinant of how the tool performs." Heiser says his first goal was to develop an objective way to "quantify how well these tools preserve the structure of the data in its native form."



Above: Heiser presenting his research. Below: Example of data visualization produced by Heiser.

Heiser is quick to point out that it's less about one method being superior, and more about choosing the right tool for the job.

"If you're interested in sub-cluster heterogeneity, then you might want a tool that does a better job of preserving local structure. But, if you're looking at a developmental trajectory, then you want to preserve the global structure," says Heiser.

Moving forward, Heiser is working on methodological advancements to integrate scRNA-seq, spatial transcriptomics, and microscopy. He hopes to use the strengths of each technique to buttress the weaknesses of the others. Long term, Heiser hopes that his work gives pathologists additional tools for understanding the underlying pathology and progression of diseases like colorectal cancer.

"If you could get all of these -omics technologies integrated with something that a pathologist sees on a daily basis, then that opens the door for more powerful insights related to clinical diagnosis and prognosis."

Learn More:

Heiser, C., Lau, K., (2020) A Quantitative Framework for Evaluating Single-Cell Data Structure Preservation by Dimensionality Reduction Techniques. *Cell Reports*, *31*:107576.

Faculty Spotlight: Ivelin Georgiev, Ph.D.

By Sara Melow, Graduate Student

Ivelin Georgiev received his Ph.D. in Computer Science from Duke University. After graduating, he joined the Structural Bioinformatics Core Section of the NIH Vaccine Research Center (VRC). Georgiev served as a staff scientist and co-head at the NIH VRC until 2015 when he made the move to academia. Georgiev is now a faculty member at the Vanderbilt University Vaccine Center, an Associate Professor of Pathology, Microbiology, and Immunology and of Computer Science, the founding Director of the Vanderbilt Program in Computational Microbiology and Immunology, and the Director of Graduate Studies for the Chemical and Physical Biology graduate program.

What made you choose to transition to academia?

I was really excited to be able to drive my own research and decide on my own directions. Academia allows me to decide how long I want to pursue a given direction and when I want to switch. I can get excited about new scientific questions and pursue them, and that kind of flexibility is unique to academia.

Which achievements have stood out in your career?

We have contributed to the antibody field within HIV, a huge global health issue that is in need of a vaccine and antibody therapeutics. We have developed technologies that the field currently uses to help understand the response to infection and vaccination against HIV. We look forward to applying these technologies to coronaviruses, influenza, and hepatitis.

The most memorable moment was attaining tenure at Vanderbilt. At the end of the long process, you usually know whether you have it or not. When I received the letter, I was excited, but I expected it and didn't really celebrate. However, when I got home, my wife and three children surprised me with decorations, signs, and silly hats. That was when it really struck me what an accomplishment it was.

What do you recommend young scientists learn to prepare for the future?

It is important to develop technical skills in graduate school and beyond. However, another good indicator for success is the ability to follow the literature. You need to know what is state-of-the-art and always keep up to date. By doing this, you 1) know what is missing and 2) expand your interests beyond your specific area to generate new ideas and apply that in your own field.

What advice do you have for current graduate students and postdocs?

Decide for yourself what career is best for you. If you are considering exploring academia, for instance, don't get discouraged by the current climate. Make sure to keep all doors open. Additionally, pursue your own independent ideas. Don't hesitate to sometimes take pathways that may not be the next best thing if that's what excites you. Eventually, you will find the overall best thing for you.

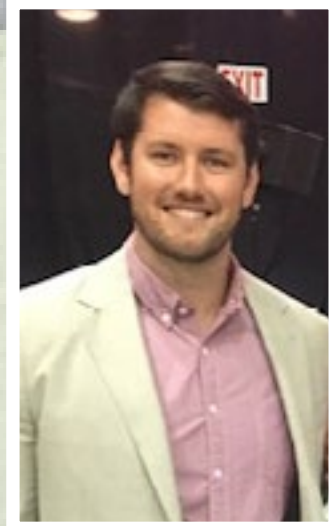
Where do you think the biggest scientific advances will be in the coming decades?

I think personalized medicine will be evolving quite a bit as there will be both technology development and cost improvement. We will start seeing things like personalized therapies, vaccinations, and diagnostic medicine. This will lead to moving from treating diseases to preventing them.

Ivelin Georgiev, Ph.D.

Ordered vs Disordered: The Story of PMP22

By Nicole Kendrick, Graduate Student



Justin Marinko

Charcot-Marie-Tooth disease (CMTD) is one of the most common inherited diseases of the peripheral nervous system, the nerves outside of the brain and spinal cord, and is characterized by loss of sensation and difficulty walking. It affects one in 2,000 people and usually first appears in adolescence. The disease is linked to mutations in the protein PMP22, a component of the myelin sheath that surrounds axons in the peripheral nervous system and allows electrical signals to travel quickly. Justin Marinko, Ph.D., in one of his latest publications in *PNAS* looks into why PMP22 mutations cause CMTD.

"I wanted to do something that was directly relevant to human disease," Marinko says.

Marinko's research on PMP22 began in the laboratory of Charles Sanders, Ph. D., Associate Dean for Research and Professor of Biochemistry. Dr. Sanders' focuses on using structural biology to study human disease, including CMTD.

Marinko looked at the link between PMP22 folding into its 3D structure and its location in different plasma membrane phases. Biological membranes generally can be characterized as one of two phases – ordered or disordered. The ordered phase is more rigid than the disordered phase and often contains a higher percentage of cholesterol. Using an *in vitro* membrane technique known as "giant plasma membrane vesicles," Marinko quantified the association of PMP22 with either phase. He found that proper protein folding, not post-translational modification, is involved in partitioning PMP22 into the cholesterol-rich phase.

Justin Marinko, Ph.D., on a recent golf outing.

"All the information that's required for whether or not this protein goes into the cholesterol-rich, or ordered, phase is in the sequence and in the fold of the protein," he says.

There are several mutations in PMP22 that can lead to CMTD, including defects in how PMP22 is moved around the cell. Marinko discovered that mutations that change the structure of PMP22 not only changed the affinity of the protein for the ordered phase, but also reduced its ability to reach the plasma membrane at all. This suggests that one avenue for possible treatment of CMTD could be to stabilize PMP22, sending it to the ordered phase and increasing its trafficking to the plasma membrane.

Marinko recently defended his thesis and is excited for the next step in his career, a postdoctoral fellowship with Dr. Ron Kopito at Stanford University. He hopes to one day start his own lab.

"I love the environment of academia. The length that it takes to actually become a professor is kind of daunting, but at the end of the day, I think it's kind of worth it."

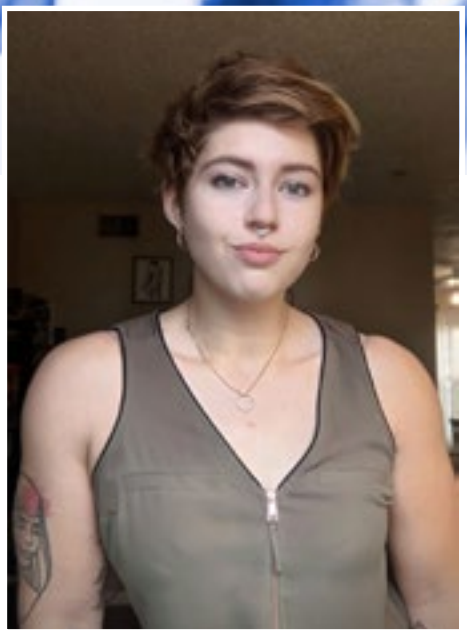
While in Nashville, Marinko spent a lot of time listening to live music. Unfortunately, that is not a possibility these days and he has devoted more time to other hobbies. His new favorite activities, golf and running, give him plenty of time to think about his lab work.

Learn More:

Marinko, J.T. et. al., (2020) Peripheral myelin protein 22 preferentially partitions into ordered phase membrane domains. *PNAS.* 117: 14168-14177.

Going with Your Gut: a New Role for an Intestinal Protein

By Heather Caslin Findley, Ph.D., Postdoctoral Fellow



Colbie Chinowsky

Colbie Chinowsky began her research journey studying ultrasound waves as a physics major at Mount Holyoke College. Her time in the medical physics lab led her to the Quantitative and Chemical Biology program at Vanderbilt University, where she now studies proteins in the gut as a graduate student.

Chinowsky chose to join the lab of Matt Tyska, Ph.D., Cornelius Vanderbilt Chair and Professor of Cell and Developmental Biology, partly due to their shared background in physics and math. The Tyska lab studies structural proteins that make up the cytoskeleton, like actin and myosin, in cells lining the intestine using various imaging techniques. Specifically, the lab studies these proteins in cell surface protrusions, called microvilli, that extend from the surface of epithelial intestinal cells, absorb nutrients, and defend against pathogens.

Myosins are a family of motor proteins that can move throughout the cell, transport cargo and produce force. Myosin motor proteins are important for cell division, motility, and contractility.

"I'm definitely doing a lot more biology than I thought I would be doing six years ago. Motor proteins and microscopy were what made me like biology, and now I'm basically a fancy photographer," said Chinowsky.

Myosins have been best characterized in muscle, where myosin 2 pulls on actin to produce muscle contraction. Two types of non-muscle myosin 2 had previously been well studied, but a third, non-muscle myosin-IIC, was discovered only in the last twenty years with little work being done to elucidate its function.

"We knew that it wasn't a super-fast motor, it was a little slower, and it remained bound to actin for a really long time, so it seemed like it would be very good at holding things together, rather than being an active contractile protein," Chinowsky said.

Chinowsky believed the protein had an important function as its expression was specific to a handful of tissues, so she used high-resolution microscopy to image non-muscle myosin-IIC in mice.

Chinowsky's work, published in *Molecular Biology of the Cell*, answered the decades-old question about the identity of the uncharacterized myosin protein at the base of microvilli, or non-muscle myosin-IIC, as we now know it to be. The myosin protein was found distributed in a meshwork pattern. It is responsible for limiting the length, and therefore the function, of microvilli. Follow up studies will further determine the impact of this protein on overall intestinal function.

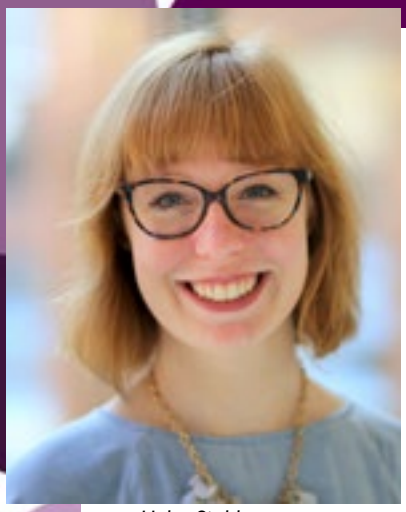
As a fifth-year graduate student, Chinowsky is starting to turn her sights to the next phase in her career. Like she did with her research project, Chinowsky is open to following her gut and seeing where different opportunities lead. Currently, she is exploring opportunities in teaching, political advocacy, and scientific workforce development.

Learn More:

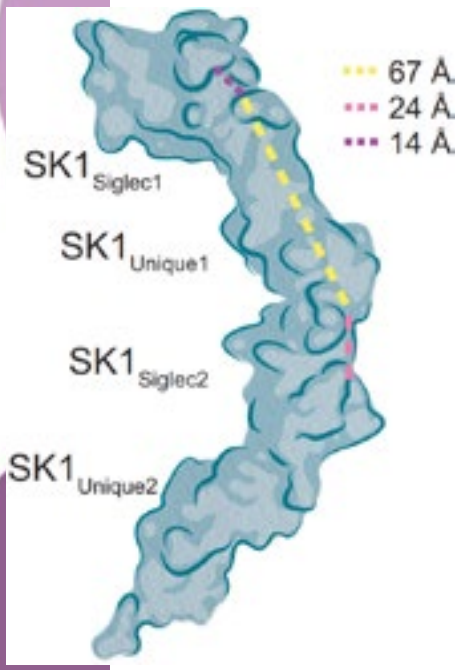
Chinowsky, C.R, et. al., (2020) Nonmuscle myosin-2 contractility-dependent actin turnover limits the length of epithelial microvilli. *Molecular Biology of the Cell.* 31, 25, 2749-2862.

Growing Crystals:
Not Your Average Science Fair Project

By Caroline Cencer, Graduate Student



Haley Stubbs



Under a constant barrage of microbes, it is critical to understand how the human body interacts with invaders in order to block their attack and rob them of their advantage. Thought to be one of the first steps of infection, bacterial attachment to host surfaces is of particular importance. Blocking initial attachment of bacteria to a host cell is a proactive way of combating infection, but precise microbe-host binding interfaces must be determined.

Vanderbilt University Ph.D. candidate Haley Stubbs, has always had an interest in exploring basic science processes. After studying chemistry as an undergraduate at Furman University, she joined Vanderbilt's Quantitative and Chemical Biology Program, with a focus on protein structural biology using x-ray crystallography. Stubbs has found her work with Dr. Tina Iverson, Professor of Pharmacology and Biochemistry, to be both fruitful and fun, relating protein crystallization to crystal growing kits for kids and model building to "playing a computer game that builds a 3D map of proteins."

Recently published in the *Journal of Biological Chemistry*, Stubbs describes the structural properties that allow bacteria such as *Streptococcus sanguinis* to bind multiple host targets. The surface of S. sanguinis is decorated with proteins that contain Siglec (sialic acid-binding immunoglobulin-like lectin) domains. These domains can bind proteins in human saliva, blood, and plasma with high affinity. However, some strains of S. sanguinis contain amino acid differences within these binding domains, hinting that the proteins may differ structurally. As anticipated, the new crystal structures determined by Stubbs suggest two Siglec domains, in tandem, may strengthen the binding of *S. sanguinis* to host protein targets. Additionally, flexible amino acids within Siglec domains may allow the bacteria to infect a broad range of cell and tissue types.

"The fact that there are two binding sites brings into question how avidity [increased binding affinity for a surface] could contribute to binding to host surfaces."

Stubbs credits the discovery of the two Siglec domains as her most significant finding. As such, Stubbs and other scientists within her field are driven to elucidating these binding sites at the structural level in order to block bacterial infection by targeting the Siglec domain-host interface.

While blocking infection is an important mission, the theory that flexible protein domains may help bacteria move through different body tissues can inspire scientists to look at therapeutic targeting in a new light. For example, Stubbs believes that scientists could target two or more biomarkers on cancer cell surfaces simultaneously. Scientists could enhance the binding strength, and accuracy, of current cancer therapeutics by using the concept of tandem repeat domains as in *S. sanguinis* infection. This could decrease accidental targeting of healthy host cells, leading to a more effective and safe therapeutic.

Learn More:

Stubbs HE, et. al., (2020) Tandem sialoglycan-binding modules in a Streptococcus sanguinis serine-rich repeat adhesin create target dependent avidity effects. *Journal of Biological Chemistry*. 295: 14737-14749.

Kim Riley, Ph.D.

Clinical Research Associate: a Career in Translational Research Outside Academia

By Swati Balakrishnan, Ph.D., Postdoctoral Fellow

While many join graduate school because they revel in understanding the details of basic biology, some realize that academia may not be their desired career. Kim Riley, Ph.D., CCRP, grappled with this realization during her graduate training at Vanderbilt University as well. While she wanted to continue in biomedical research, she couldn't see herself continuing to do research at a bench or heading a lab. One day, she pulled a tab off a flyer that led her down an unexpected career path.

The flyer was requesting clinical trial volunteers for the Vanderbilt University HIV Vaccine Program. While her interactions with the study coordinator of this program piqued her interest in a career associated with clinical trials, Riley's experience in the 'Introduction to the Principles and Practice of Clinical Research' module offered by the NIH Clinical Center through the BRET Office of Career Development ASPIRE Program cemented this goal. Upon receiving her Ph.D., Riley transitioned to the position of clinical research coordinator at Duke University Eye Center.

"As a study coordinator, you manage the day-to-day operations" she says. "You help find candidates, you consent them into the trial, you collect data and enter it into whatever system is being used. There is also a lot of work involving the regulatory aspect of conducting human trials you have to take care of."

After two years as a study coordinator, Riley transitioned into her current role as a Clinical Research Associate (CRA) at PPD, a leading global contract research organization. These contractors are hired by pharmaceutical companies to run the human trials required for commercial approval. CRAs ensure the data being collected is accurate, the sites are following the correct protocols for the study, and any issues or concerns the sites may have are addressed during the trials.

Riley credits her training in graduate school with developing the critical thinking, multi-tasking, and self-motivation skills she needed to excel at her job. Contract research organizations have not historically sought Ph.D. graduates to fill these positions. However, recently the clinical trials industry has begun to recognize the value that graduate training can bring to these positions.

Riley praises PPD's supportive environment for enabling a smooth transition from an academic environment.

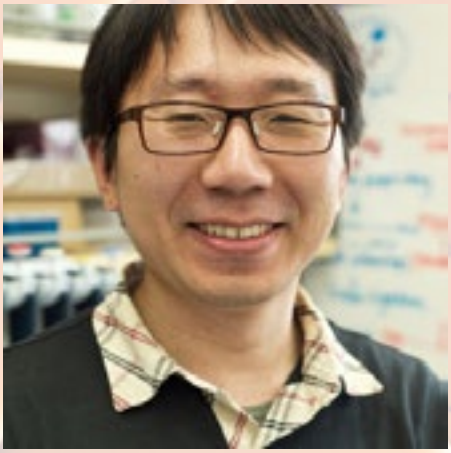
"PPD has a great training and onboarding program, so while there is a learning curve, they've given you the toolkit and the manuals, you just have to utilize them."

PPD has also been a supportive employer during the pandemic by fostering a co-operative work culture. Riley thinks this job is a wonderful fit for anyone who enjoys travelling, wants to stay in the field of biomedical research, loves learning new things on a regular basis, and likes knowing they are contributing to changing the world for the better. For scientists looking for careers outside of academia and who value translational science, the role of a CRA merits strong consideration.

Caught in the Act: VIR-CLASP Identifies Human Proteins Hijacked by Viruses

By Hillary Layden, Graduate Student

Sarah Arcos, Ph.D.



Byungil Kim, Ph.D.

The COVID-19 pandemic has highlighted the dangers that emerging viruses pose to human health and society. Now, more than ever, understanding the biological processes underlying viral infection is critical. Researchers under the mentorship of Manuel Ascano, Ph.D., Assistant Professor of Biochemistry, at Vanderbilt University have developed a new method to identify interactions between the infecting virus' genetic material (RNA) and proteins from the infected human cell. Recently published in *Molecular Cell*, Byungil Kim, Ph.D., postdoctoral fellow, and Sarah Arcos, Ph.D., graduate student at the time, introduce VIR-CLASP (VIRal Cross-Linking And Solid-phase Purification). This method allows researchers to capture proteins that are interacting with RNA from the virus before viral replication, and can be used to identify host proteins that are required for viral reproduction and successful infection.

Both Kim and Arcos believe their childhood curiosity about how the world works informed their decisions to become scientists. Kim, who received his Ph.D. from Yonsei University in South Korea, came to Vanderbilt specifically to work with Ascano because of their overlapping research interests. Arcos, on the other hand, was drawn to Vanderbilt because of the Interdisciplinary Graduate Program. Although she was originally interested in neuroscience, she became interested in RNA biology through her first-year rotations.

"I was really fascinated by all of the different questions you could answer through studying RNA and how it was involved in a bunch of different processes," said Arcos to explain her change of heart.

Kim's expertise in virology drove the lab's focus on developing VIR-CLASP. Kim believes that VIR-CLASP has the potential to improve public health by deepening our understanding of critical moments during viral infection.

The key to a successful viral infection involves interactions between the virus's genetic material and specific proteins that the host cell produces. "To win the battle with a virus, we need to know what happens during viral infection", says Kim. VIR-CLASP is the first method that can capture the earliest stages of infection.

The development and execution of VIR-CLASP was a highly collaborative effort that drew on the expertise of Kim, Arcos, and Ascano with input from other researchers and several Vanderbilt Core facilities. Arcos and Kim credit the success of VIR-CLASP to the supportive atmosphere at Vanderbilt and the collective efforts of their colleagues.

Since the publication of this study, Kim has started his own lab at the Korea Research Institute of Chemical Technology. Arcos has moved on to a postdoctoral fellowship at the University of Michigan after she defended her thesis in October. Both Kim and Arcos are eager to put the skills learned at Vanderbilt to work on new research projects.

Learn More:

Kim, B., Arcos, S. et. al., (2020) Discovery of Widespread Host Protein Interactions with the Pre-replicated Genome of CHIKV Using VIR-CLASP. *Molecular Cell.* 78(4): 624-640.

New Technique Gives Insight on Gene Regulation Timeline

By Colbie Chinowsky, Graduate Student

Kelly Barnett, Ph.D.

A desire to explore all opportunities was what initially attracted Kelly Barnett, Ph.D., to Vanderbilt University's Interdisciplinary Graduate Program. Despite that initial openness, he never expected to join a computational research lab.

"All of my work before was very much traditional protein biochemistry work," he said. "I had no coding experience. I had never done genomic analysis."

However, Barnett chose to join the lab of Emily Hodges, Ph.D., Assistant Professor of Biochemistry, an expert in genomic analysis. Barnett gained the needed computational skills slowly at first, learning a lot as a result of trial and error and experiencing failure, but over time he became proficient in command line and R, programming languages often used to analyze high dimensional data.

Barnett's recent first-author *Molecular Cell* paper focuses on a new technique called "ATAC-Me". ATAC-Me combines several widely used genomic tools to measure both chromatin accessibility and DNA methylation. Chromatin accessibility determines if DNA is open enough for transcription, which is the first step of turning DNA into a protein. Methylation is a modification to DNA that influences gene expression. Low levels of DNA methylation correlate with active gene expression, while high levels correlate with no expression. Following this logic, it was thought that regions of open chromatin are unmethylated. Reciprocally, "If a gene is in "closed" or condensed chromatin, it's typically all methylated," Barnett explained.

However, with their new approach, it became possible for Barnett and colleagues to identify regions of open chromatin that also display high levels of DNA methylation and to follow changes in these markers over time. The researchers were then able to develop a molecular timeline of the events that lead to gene expression. Using ATAC-Me, the authors demonstrated that DNA can have regions that are both open and methylated, indicating that loss of methylation may not be required to allow gene expression.

Barnett stated that the key takeaway from this paper is that DNA methylation isn't always repressive for gene expression—it's a context specific mark, and the system setting matters.

Further study of methylation patterns using these new methods is important because incorrect methylation patterns can contribute to disease, particularly blood cancers. Also, we must understand when methylation affects disease progression to design effective therapeutics.

Following publication of the ATAC-Me paper, Barnett was planning to intern at Illumina, a company at the forefront of the development and manufacture of innovative instruments and technologies for genome analysis. However, once it became apparent that graduation was on the horizon, he decided to take a leap and defend his thesis prior to starting his internship. He urges anyone interested in a transition to industry to look into the Illumina internship program, regardless of genetics experience.

Learn More:

Barnett, K.R. et. al., (2020) ATAC-Me Captures Prolonged DNA Methylation of Dynamic Chromatin Accessibility Loci during Cell Fate Transitions. *Molecular Cell.* 77: 1350-1364.

Congratulations to Our Recent Graduates!

March 2020-February 2021

Jenny Aguilar	*Keyada Frye*	*Turnee Malik*	*Dylan Shropshire*
Sarah Elizabeth Arcos	*Randall Golovin*	*Justin Marinko*	*Selena Chacon Simon*
Kelly Barnett	*Alissa duPuy Guarnaccia*	*Annah Moore*	*Justine Sinnaeve*
Karin Bosma	*Rodrigo Guillen*	*Andrew Morris*	*Chloe Snider*
Rhonda Caston	*Corey Hayford*	*Rachael A. Muscatello*	*John Snow*
Mackenzie Catron	*Kyle J. Horning*	*Austin Oleskie*	*Brittany Spitznagel*
Hung-Hsin Chen	*Kevin Jagessar*	*Rafael Perez*	*Nilay Taneja*
Laura Colbran	*Gretchen Wemhoener Johnston*	*James Pino*	*Vaughn Thada*
Meredith Duncan	*Piyush Joshi*	*Tessa Popay*	*Alexander Thiemicke*
Austin Featherstone	*Aichurok Kamalova*	*Megan Rasmussen*	*Petria S. Thompson*
Stephen Thomas Ferguson	*Matthew Kent*	*Lauren Salay*	*Alexandra Clare Trevisan*
Eric Figueroa	*Caitlyn Kirby*	*Gregory Salimando*	*Lauren Elizabeth Williamson*
Nicole Fisher	*Lindsay Klofas Kozek*	*Zachary Michael Sandusky*	*Xiaodun Yang*
Bianca Flores	*Kristin Kwakwa*	*Corey Seacrist*	*Zi Ye*
Rose Follis	*Aung Soe (Sai) Lin*	*Jaimie Zhi Shing*	*Linda Zhang*
Maria Fomicheva	*Ying Liu*	*Eileen Shiuan*	*Sifang Kathy Zhao*

RESULTS & DISCUSSION

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