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WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere, through the cooperation of PhD candidates, their mentors, and departments. WISL offers awards of $250 for UW-Madison Ph.D. candidates in science and engineering. Candidates from other institutions may participate, but are not eligible for the cash award. WISL strongly encourages other institutions to launch similar programs.

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Contact: Prof. Bassam Z. Shakhashiri

UW-Madison Department of Chemistry

bassam@chem.wisc.edu

www.scifun.org

Exploration of novel genetic regulators of metabolism

By

Andrea Hunger

A dissertation submitted in partial fulfillment of

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The dissertation is approved by the following members of the Final Oral Committee:

Judith Simcox, Professor, Biochemistry

Katherine Henzler-Wildman, Professor, Biochemistry

Joshua Coon, Professor, Chemistry and Biomolecular Chemistry

Christian Capitini, Professor, Pediatrics

Andrea Hunger – Non-specialist Thesis Chapter

Title: A Hero's Journey (through graduate school): How a common narrative template can be applied to the experience of earning a PhD

Introduction:

I wrote this chapter because both the research I describe in it, and my graduate training were funded in large part by American taxpayer dollars. Therefore, I believe it is my duty to make my findings accessible to the public. Putting together this chapter was also a powerful form of self-reflection that helped me to understand and appreciate the systems that supported my journey in science. I hope that readers will take away not just new ideas about science, but also an understanding of the value of programs that encourage the participation of historically underrepresented groups in higher education. I would like to thank the Wisconsin Initiative for Science Literacy (WISL) and UW-Madison for providing this platform and for sponsoring and supporting the writing of this chapter. I am grateful for the support of Professor Bassam Shakhashiri, and Elizabeth Reynolds for their helpful feedback and encouragement.

The Call to Adventure

The rumble of an approaching garbage truck shattered the silence in a small, dark room tucked away in a back corner of Phillips Hall. While the truck slowly ground its way closer, I watched images of individual gold atoms dance out of focus as the transmission electron microscope tremored with faint vibrations. My senses weren't keen enough to feel the earth moving, but the small gold nanoparticles I had spent the past several days making, and the incredibly expensive microscope I was borrowing, were much more sensitive. It was a late fall afternoon with the warmth and humidity of summer still clinging to the cinderblock walls of the only science building on campus. My friends and classmates were undoubtedly outside somewhere, enjoying the last gasp of warmer weather and the last bit of freedom before our

college coursework began in earnest. I, however, was entirely absorbed in my work. Specifically, the tiny screen with microscopic dots arranged in precise rows that only briefly wobbled into focus.

Dr. Hooper, a kind geology professor, who was also the guardian of this particular microscope, was helping me take the most magnified images I had ever seen. I had spent the past two years working with my mentor, Dr. McEllistrem, a professor of materials science and engineering, to develop and characterize a more environmentally friendly way to synthesize gold nanoparticles. Gold nanoparticles are used for targeting radiation therapy during cancer treatment, but synthesizing them is quite costly and relies on hazardous chemicals. Based on changes in the light absorbance of our samples, we believed we had successfully found a safer, greener method for making them. We used a small biological peptide to add electrons to gold ions dissolved in water to make tiny nanoparticles of solid gold. One way to think of this is like a ghost becoming solid. The ions were dissolved in solution, and by adding electrons we gave them substance and made them tangible.

Next, we needed a way to look inside our little tubes of sample and confirm that we were actually making the gold nanoparticles we thought we were. Thankfully, we were able to reach out to Dr. Hooper and propose a collaboration. With his guidance, I was able to use my process to synthesize what we believed to be gold nanoparticles, then put a tiny drop of the liquid containing the nanoparticles onto a very small grid of carbon atoms. I flash-froze the liquid onto the grid by dipping it in liquid nitrogen, which is so cold it boils at room temperature. Next, I quickly transferred the frozen samples to a machine that kept them cold while vacuuming out the air and moisture from the samples. This process made the gold nanoparticles freeze-dry onto the grid. Once the drying process was complete, we could load the grids onto the transmission electron microscope. This unique microscope was so powerful that we could see individual atoms of gold in our samples.

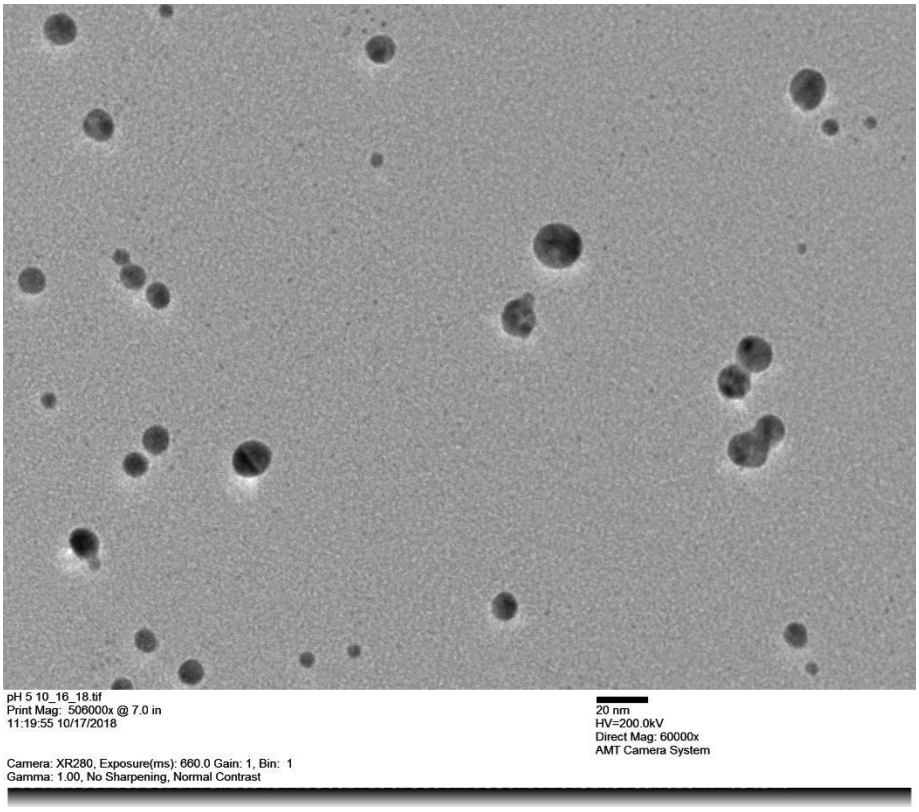

Figure 1. Transmission electron microscopy images of gold platelets at two different magnifications.

I will never forget the gentle whirring of the microscope as we sat together, lit only by the soft glow of the computer screen, and the feeling of rising anticipation as we slowly zoomed in closer and closer on the samples I had spent days preparing. Actually, if you include the summers I spent optimizing the process of making the nanoparticles and getting them to dry nicely on the grid, it would be more accurate to say the samples I had spent years making. This quiet, tense moment was the culmination of years of scientific research. After nearly an hour of careful zooming and refocusing, we got our first images. We also got our first surprise. In the process of optimizing the process to make the nanoparticles, I discovered that changing how acidic I made the liquid affected the speed at which particles formed. Dr. McEllistrem and I thought this was an interesting phenomenon, but we didn't quite know what to make of it. We thought either the process worked (and we made nanoparticles) or it didn't (and the gold stayed dissolved). But it was plain to see in the images I collected with Dr. Hooper that there was a third option. Instead of round, spherical nanoparticles, we were looking at rows and rows of stacked, orderly atoms arranged like bubbles in a sheet of bubble wrap. We called these structures platelets.

It turns out that when I added less acid, the reaction took longer. We hypothesized that perhaps because of the slower precipitation (the process of becoming solid) of the gold atoms, they could arrange themselves into orderly rows and eventually stacks of rows that formed platelets. When I added more acid to the reaction, it proceeded more quickly and the gold atoms self-assembled into the spherical clumps (nanoparticles) that we expected. Imagine making Rice Krispies with just cereal pieces and marshmallow. The marshmallow is the attractive force between gold atoms that holds them together. Adding a lot of acid to the reaction was like throwing a melty ball of marshmallow into a bowl of rice krispies. The cereal (gold atoms) just sort of sticks all over in a random pattern. Adding less acid caused the gold ions to precipitate

as gold atoms much more slowly, like individually adding pieces of cereal to the marshmallow with great care and precision in a specific, orderly pattern.

For a brief moment, until I shared my findings with my mentor, Dr. McEllistrem, I was the only person in the world with that small, specific piece of knowledge. It felt like solving one of the infinite mysteries of the universe to have this secret information that nobody in the history of the world had ever known. Sure, most people aren't spending their lives trying to make microscopic gold structures, but still, the rush of knowing something that no one has ever known before was incredible. I knew I wanted to spend my life chasing that feeling. I also understood that while basic research was a thrilling place to start my scientific career, in my next stage, I wanted to work on a project with more relevance to human health and disease. I wanted to uncover more exciting secrets of biology that could help people live longer, better lives.

Refusal of the Call

However, there was a problem. To solve the great mysteries of the universe as they relate to human health and disease, I was going to need more training than a Bachelor's degree in Biochemistry from a small state school. Going to college at all was already a huge undertaking. In high school, I received free breakfast and lunch every day from the school cafeteria. I stacked my schedule with AP classes knowing the only way I was going to be able to take the tests at the end of the year was using fee-waivers from the school to pay for them. I took as many AP classes as possible, hoping to graduate college in less time to save money. Thankfully, through a combination of Pell grants, the Fund for Wisconsin Scholars Scholarship, the BluGold Centennial Experience Scholarship, being paid for my work in a research lab, and a subsidized student loan from the federal government, I was able to completely cover my college tuition and living expenses for four years. However, to continue asking big research questions

and doing the kind of lab work I found a passion for, I needed to get into graduate school and find a way to support myself through it. Even applying to graduate school was a new frontier. My father was a first-generation college student, and nobody in our family had ever gone further. I didn't have any idea what graduate school looked like, or if I would be able to keep up even if I got in.

Meeting with Mentors

I learned from a fellow science student at a meeting of the Chemistry club that STEM PhD programs not only pay the tuition of their students, but they also give them a small stipend to pay their living expenses. No person was going to get rich off of the low stipends, and in fact, anyone with a bachelor's degree in a STEM field could certainly make more working in the private sector, but making just enough money to get by was enough to allow me to attend. Thanks to my status as a Pell Grant recipient, I was also able to get the application fees waived for several PhD programs.

With a clear goal in mind and the fuzzy outline of a plan to achieve it, I sat down with my mentor, Dr. McEllistrem, to learn more about graduate school. He had attended the University of Wisconsin – Madison for his own graduate training. He helped me navigate the many diverse graduate programs to decide which would be the best fit for me. He also wrote me a glowing letter of recommendation and made sure I knew what admissions committee members were looking for in a personal statement. With his help, and the help of several other faculty mentors at the University of Wisconsin – Eau Claire, I was able to put together a compelling application to several biochemistry and pharmacology PhD programs. Incredibly, I was invited to interview for all four programs I had applied to. I completed my final in-person interview weekend visit in

February of 2020, just as the world was beginning to realize how important investments in scientific research would prove to be.

Crossing the Threshold

I felt my phone vibrate softly in my pocket and shifted to make sure it wouldn't make a sound against the hard plastic chair I was sitting on. It was just after 1 pm on a Monday in February of 2020, and my molecular genetics senior capstone course lecture had just started. I was anxious with the knowledge that I would be getting an admissions decision from my top choice PhD program very soon. The interview weekend had gone well. I fell in love with the city but even more with the impressive faculty and curious graduate students I met. I had applied because of the strong reputation of the graduate program, the inside knowledge of my mentor, and for the opportunity to stay close to my family and support systems at a phase of life that I knew would be incredibly challenging.

I surreptitiously slid my phone out of my pocket and held it carefully beneath the table making sure to keep my eyes on the board my professor was lecturing in front of. The course material was important, but the information on my phone screen could change the course of my life. I couldn't wait to sneak a glance at the screen. I saw a burst of digital confetti dance across the screen and stopped breathing. It felt like time came to a grinding halt. Eventually, the confetti subsided enough for me to read the words hidden behind it, under the bold red official University of Wisconsin - Madison banner. "It is our pleasure to offer you formal admission to the Integrated Program in Biochemistry (IPiB)."

With that simple email on an ordinary Monday afternoon, my life changed. I quickly accepted the offer of admission and then spent the rest of the school year and the following summer finishing my bachelor's degree and writing up my research findings. After that, in

August of 2020, I packed up everything I owned, used my small starting bonus to rent a U-Haul trailer and put down a security deposit, and moved to a 400-square-foot apartment in a brand-new city during a global pandemic. All to pursue my dreams of becoming a PhD biochemist and feel the way I did months earlier sitting in a small, dark room with the soft glow of a microscope and the rush of new discoveries.

The Ordeal

I arrived on campus just as research labs were reopening with extra safety measures in place to prevent the spread of COVID-19. There were strict occupancy limits in lab spaces, elevators, and bathrooms, mask requirements, and weekly required testing. Once a week, I would go to a large ballroom in the student center, remove my mask, and spit into a sample collection tube with rows of other staff and students doing the same – six feet apart, of course. It was worth the extra hassle and general anxiety of working around many other people for the opportunity to pursue science at an incredibly well-resourced, globally recognized institution.

I started my PhD by spending four weeks at a time in three different research labs before choosing the one where I would stay to complete my thesis work. It was a difficult choice. Every one of the faculty members I met was a leading expert in their field. Many were nationally and internationally recognized for their specific expertise. They were asking the kinds of questions that would have been unanswerable at my much smaller, less well-resourced undergraduate university. Here, instead of looking for additives to improve paint quality or detectors to measure airborne silica dust, people were trying to understand fundamental questions in biology. How do spatial patterns or dynamics influence cellular processes? How do proteins embedded in cellular membranes control the movement of molecules and signals across those membranes?

What nutrients do cancer cells depend on for growth, and how can these nutrient dependencies change depending on the physical and chemical environment around the cells?

Ultimately, it was the final question that captured my attention the most and I joined the lab of Dr. Jason Cantor. He was a relatively new faculty member at the time, having recently completed his post-doctoral work at MIT, where he developed a new cell culture media that contained over 70 nutrients at the same levels found in healthy, adult human blood. He called it Human Plasma-Like Medium (HPLM). Cell culture media (the plural form of cell culture medium) is the liquid we put into plastic dishes along with cells that we want to study to keep them alive and growing. The nutrients available to help the cells grow are dictated by the media a scientist feeds them. Recipes for the most commonly used cell culture media were developed back in the 1950s with the goal of keeping the cells alive and making them grow quickly. However, the nutrients in these media can be quite different from the conditions that cells experience in a human body. For example, Dulbecco's Modified Eagle Medium contains 25 mM glucose, while human blood from a healthy person only contains 5.5 mM glucose. The extra sugar certainly helps cells grow more quickly in plastic dishes, but it also changes the metabolism of those cells. The premise of Dr. Jason Cantor's research was that by improving the methods we use to grow and study human cells in the lab, we can make our research more relevant to medical science and more likely to be useful in developing new cancer treatments. We could also use his new cell culture medium to uncover new aspects of cell biology that were previously masked by the use of older, traditional media.

It was exactly the kind of work I had imagined doing in graduate school: using the simple switch of replacing the liquid used to grow cells in a lab with a liquid that better mimics human blood to improve the relevance of our research to treating human blood cancers and perhaps other diseases. I was excited to come to work every day in a brand-new building with state-of-the-art equipment for growing, isolating, and studying human cells. I had never worked with live

cells before. I quickly learned how important it was to use sterile technique and keep all of my supplies as clean as possible to avoid contaminating my cells with bacteria, viruses, or other things that could harm them or slow their growth. I also developed a deep respect for the people whose cells I was working with. While their identities are mostly anonymous, we did have access to basic demographic information about the donors. Several of our cell lines were from pediatric patients or older people with advanced-stage cancers. The most aggressive cancers tend to be the best suited for scientific research. These tenacious cells adapt to survive at all costs. Therefore, they are very difficult to kill in a person receiving cancer treatment, but they are also robust enough to survive outside of a patient and be studied in a lab. Some of the most common patient-derived cell lines have been grown all over the world for decades, far outlasting the person who donated the original cells. I felt honored to be able to use human cell lines and driven to honor their memory and sacrifice by working to better understand and perhaps help cure the cancers they suffered from.

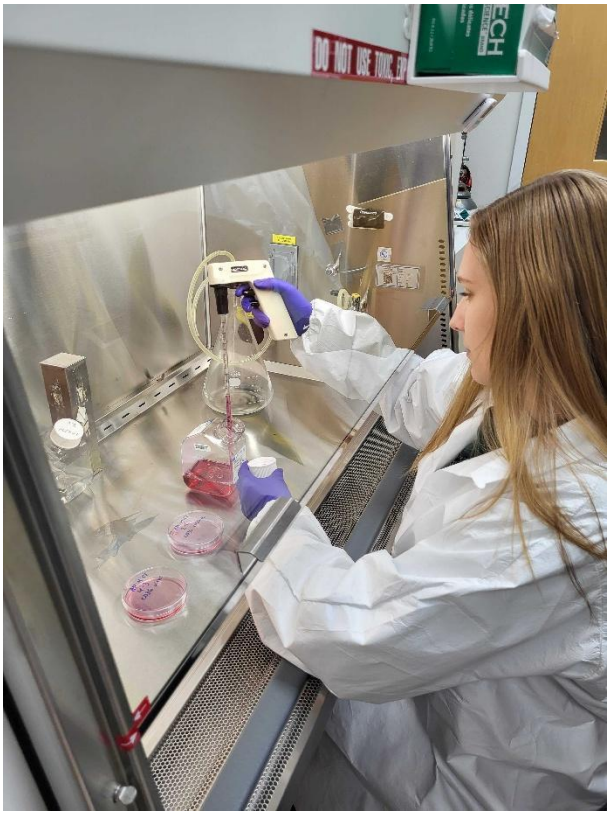

Figure 2. Practicing sterile technique during a media change on live cells in a sterile biosafety cabinet

One of the first research projects I worked on after joining the Cantor lab was a study to evaluate how changing the nutrients in cell culture media affects the sensitivity of human blood cancer cells to different drugs. To test this, we performed a high-throughput screen, meaning we tested many drugs at the same time. Even with the resources of MIT or UW-Madison, these experiments would have been a large undertaking. Therefore, my mentor, Dr. Cantor, enlisted the help of talented scientists at the National Center for Advancing Translational Sciences, a division of the National Institutes of Health. They used automated liquid-handling robots to complete the experiments much more quickly than our human hands would have been able to. With their help, we tested 11 different concentrations of 1,976 different drugs in three different cell lines, each grown in three different cell culture media. Thankfully, we had a technician working in the lab, Nick Rossiter, who was able to use his background in statistics to help us make sense of such a large dataset.

From this large-scale experiment, or screen, we were able to identify several drugs that were more toxic to the same type of cells depending on which cell culture medium they were grown in. Our results demonstrated that the nutrients provided to cells influence their sensitivity to different drugs and helped show how important it is to use media that better models human blood for experiments aimed at identifying new therapies to treat blood cancers. Specifically, we identified an anti-viral drug, Brivudine, that could be repurposed as a new anti-cancer therapeutic.

Brivudine is a drug approved for treating herpes zoster (better known as ‘shingles’) in several European countries. It works by preventing viruses from making copies of their DNA, a necessary step for viruses to replicate. Through our screen, we found that Brivudine also kills cancer cells when they are grown in HPLM, but not when they are grown in other, traditional cell culture media. This was an exciting finding that we were thrilled to dig into. We continued researching how Brivudine was killing the cancer cells and why they were only dying when the

cells were grown in HPLM. Eventually, we were able to identify the key difference between HPLM and the traditional medium that made Brivudine toxic: folate levels. Folate, also called folic acid, is a common vitamin that is essential for cell growth. Cells need folate to replicate their DNA so that they can grow and divide to make new cells. However, in conditions of low folate, like in HPLM, Brivudine further reduces the ability of cells to replicate their DNA, which prevents them from dividing and making new cells. In traditional cell culture media, there is an excess of folate that allows cancer cells to get around the effects of Brivudine and keep growing. This means that growing cancer cells in traditional media masked the anti-cancer effects of Brivudine.

Developing HPLM, including patenting it and making it commercially available for any scientist to buy and use, were steps towards improving our ability to model cell growth in an environment that better reflects the human body. However, there are still quite a few differences between a small plastic dish and a living, breathing person's circulatory system. Therefore, we didn't stop after developing a new cell culture medium. In fact, my main project when I joined the Cantor lab was to develop a new system for growing human blood cancer cells that was an even better model for the human circulatory system.

The human body maintains many parameters within tightly controlled ranges. This process is called homeostasis. A simple example is body temperature. If your body becomes too cold, you become hypothermic, and if this state lasts too long, it can be fatal. Having a temperature that is too high, like a fever, can also be fatal. It is vital that your body temperature is always within a small, defined range. This also applies to the pH or the acidity of your blood, and how much sugar is in your blood. When blood sugar levels become too low or too high, it can also become fatal. Dysregulation of the systems that maintain blood glucose levels within their homeostatic range (diabetes) is a very well characterized and often treatable disease. Much like the way your body maintains many different environmental parameters within a tightly

controlled range, we wanted to be able to do the same thing with our human blood cancer cell lines.

We started by filling a sealed glass container with HPLM. Then we wrapped the container in a tiny electric blanket to warm it to body temperature and put a thermometer in it to constantly measure the temperature. The thermometer was connected to an electric control unit that was also plugged into a desktop computer for continuous tracking and monitoring of the temperature over time. We also put a small impeller, or a little stick with fan blades on the end, in the center of the container and used a motor to make it gently spin and mix the liquid in the container. We also put two special probes in to measure the amount of oxygen in the liquid and the acidity. These probes were hooked up to the same control unit and computer system so that all of the continuous monitoring data was nicely overlaid in one chart on the computer monitor.

The software for the control unit functioned like a human brain would. It monitored the amount of oxygen and the acidity of the liquid and added in nitrogen gas if needed to push them back into the homeostatic range we set. We also used a small pump attached to the control unit and a large bottle of HPLM to continuously add a trickle of new media to the vessel. Finally, there was a sensor placed at the top of the container to detect overfilling. Once the sensor detected liquid above the level we set, it triggered the control unit to turn on another pump attached to an empty bottle and start pumping liquid out of the container. This entire system was a closed loop, meaning there was no exposure to air and no way for bacteria or other contaminants to get into the container. There was also a small port that we could use to add or remove media. These kinds of systems are called chemostats. They are commonly used in microbiology for growing different kinds of useful bacteria. However, we figured out how to use the chemostat system for growing human blood cancer cells. Since the chemostat maintains many parameters within the ranges they would be in a human body, we felt confident describing the environment in the chemostat as ‘circulation-like’ conditions.

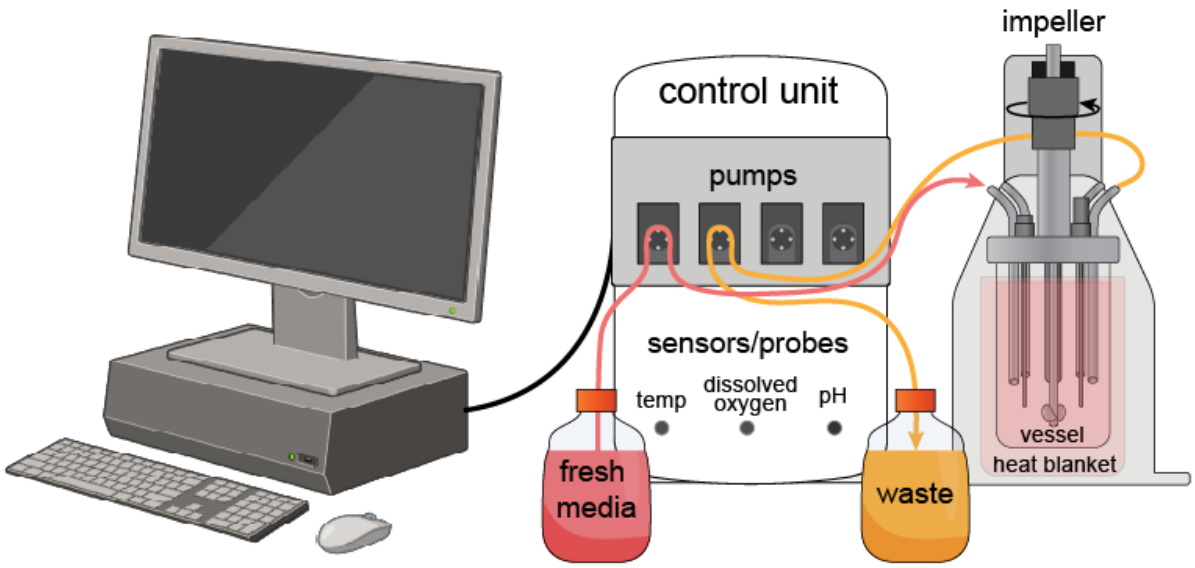

Figure 3. Schematic representation of the chemostat system with inputs and outputs labeled.

Figure 4. Photograph of the actual chemostat system during an experiment.

Next, we wanted to use our human cell culture chemostat to perform experiments in circulation-like conditions to test how altering these environmental parameters might change the results, especially compared to the traditional method of growing cells in little plastic dishes. Experiments using the chemostats to perform large-scale experiments (screens) are ongoing, as is follow-up work to confirm and validate the results of the screens we performed so far.

To briefly summarize our work so far, we performed screens to investigate two broad questions: 1. What nutrients do cancer cells depend on to support their growth? and 2. Which genes do cancer cells need to have to grow? For both questions, we were specifically interested in how the answers change when cells are grown in the chemostat vs when they are grown in plastic dishes. We were motivated to do this work by the idea of uncovering new biological insights into requirements for human cancer cell growth. These insights could identify new strategies for cancer treatment and ideally help spark further research into new targets for chemotherapy drugs. Identifying new targets for cancer treatment is especially impactful for treating patients who relapse and patients whose cancer adapts to become resistant to current treatments.

Temptation

During my time in the Cantor lab, while I was helping to finish our drug screen project and optimize the chemostat system, I also applied for and won a prestigious fellowship through the Biotechnology Training Program. This training program is funded by the National Institute of General Medical Sciences, which is another division of the National Institutes of Health, the same government organization that helped complete our large-scale drug screening experiments. One of the main benefits of the Biotechnology Training Program at the University of Wisconsin – Madison is that it gives PhD trainees the opportunity to complete an internship

during their training. I was grateful to be selected as a summer intern by Illumina during the summer of my third year in graduate school. As challenging as it was to step away from my academic lab for 12 weeks, completing the internship at Illumina was a life-changing experience. I came to graduate school with the intent to pursue a career in the biotechnology industry after completing my training. However, prior to doing an internship, the concept of being a scientist working in industry remained a hazy idea more than a concrete goal.

Unlike in academia, the work I did during my internship is protected by a non-disclosure agreement. I couldn't write about it in this thesis chapter even if I wanted to. However, what I think would be most valuable to share is not the data I generated or the scientific findings I made at Illumina but rather what I learned about myself. Transitioning to a new research lab with a team of experienced scientists was a little intimidating at first. If I had taken a job in the biotech industry right after completing my Bachelor's degree, I would not have been nearly as well prepared to make meaningful contributions as I was after three years in graduate school. Starting work on a completely new project helped me realize how broad and how valuable the skill set I was building really was. I learned how to do more than follow protocols as they were written and make graphs and tables based on other people's examples. Through my graduate training, I learned how to sort through large data sets and derive meaningful insights that could move projects forward. I had practiced interpreting data and identifying follow-up questions based on the results. I had years of experience designing experiments and evaluating what positive and negative controls would be necessary to interpret the results. These technical skills allowed me to work much more independently than I would have been able to if I had come straight from undergrad. I was able to make testable hypotheses and propose experiments, rather than simply executing other people's ideas and waiting for decisions to be made. Even the oral presentation I gave at a national conference had a positive impact on my performance as an intern. It helped me become a confident public speaker and be able to clearly present my

findings to high-level people at the company at the end of my internship. The lessons I learned during my graduate training directly translated to my success in my internship.

I enjoyed my time at Illumina immensely. I valued the mentorship of my colleagues, the very reasonable working hours (a sharp contrast to my typical 80-hour work weeks in graduate school), and the salary that was more than double my PhD stipend. I was working less, earning more, seeing my family (and the sun) more often, taking much-needed rest, and still receiving positive feedback from my manager about my productivity. As the summer of 2023 and my 12-week internship came to a close, I found myself hesitating about returning to graduate school. I knew the training I had received so far was valuable. I knew I had grown dramatically as a scientist and as a person during my time in academia. But I also knew that I was burned out. I knew that in my current thesis lab, I had made the most of my time and gotten nearly everything I could out of the experience.

It was tempting to simply exit my graduate program with a Master's degree instead of a PhD, find a job in the biotechnology industry, and continue to enjoy the benefits I had luxuriated in all summer long. However, having seen first-hand the type of science funded by industry, I had developed an even deeper appreciation for academic science. My thesis work was never motivated by turning a profit, increasing revenue, or generating shareholder value. My purpose as a PhD student was to do the best, most rigorous science possible and to use taxpayer money to help advance science for the public good. I loved going to work (even for the 15th day in a row) to work toward more effective cancer treatments. I found fulfillment in my work that I couldn't have found in an industry salary. I knew it wouldn't be sustainable for me, and it wouldn't align with my long-term goals to stay in academia forever, but I decided to go back and at least finish my PhD.

The Return

I made my return to academia to finish my graduate school journey and complete my PhD at the start of the Fall semester in 2023. However, by the winter, it had become clear that I was going to have to make a new kind of pivot: changing my thesis lab. In the Cantor lab, I gained many valuable skills related to experimental design, data interpretation, and effective presentation. To complete my PhD, however, my program required a first-author publication. The order in which authors are listed on a scientific paper denotes how much they contributed to the work. While I had been the lead contributor for five different projects in my first thesis lab, being split between so many different endeavors meant it was challenging to bring any one thing to completion. I needed to find a new mentor who would allow me to focus deeply on a single project and help me grow as a scientific writer and storyteller.

In February of 2024, I made the very challenging decision to leave behind my first thesis lab and transition to the lab of Professor Judith Simcox. She is a world leader in the field of lipid metabolism and her work has important implications for many human diseases, including diabetes, heart disease, and obesity. She also already had a role in shaping my graduate school experience. Dr. Simcox was a member of my thesis committee, a group of five faculty members that I met with annually to share updates on my progress and receive helpful feedback from. What made the transition easier was staying in the field of metabolism. Many of the concepts I had been studying in Dr. Cantor's lab were still relevant to my work in Dr. Simcox's lab. I was able to use the technical skill set I built and directly translate it to help interpret a large screening data set that a previous student in the Simcox lab had generated before graduating and moving to their next step.

The screen I worked on in the Simcox lab was meant to uncover genes involved in the metabolism of a specific type of lipids, or fats, called acylcarnitines. This type of fat serves as an alternate fuel source in organisms when the sugar in their blood cannot provide enough energy.

Examples of when acylcarnitine and other fats are needed to provide extra energy include fasting, exercise, and cold exposure. I identified several genes that are potentially involved in the metabolism of acylcarnitines. Together with Dr. Simcox, we identified the most promising one, a gene called CD36 and focused on determining its specific role in acylcarnitine metabolism. Giving acylcarnitines to cells grown in plastic dishes has different effects depending on the type of cell. For example, a human kidney cell line, HEK293T, is very sensitive to acylcarnitine: exposing HEK239T to acylcarnitine causes the cells to die. However, fat cells that we isolated from mice are resistant to acylcarnitine, and they can survive much higher levels of acylcarnitine treatment than the HEK293T cells.

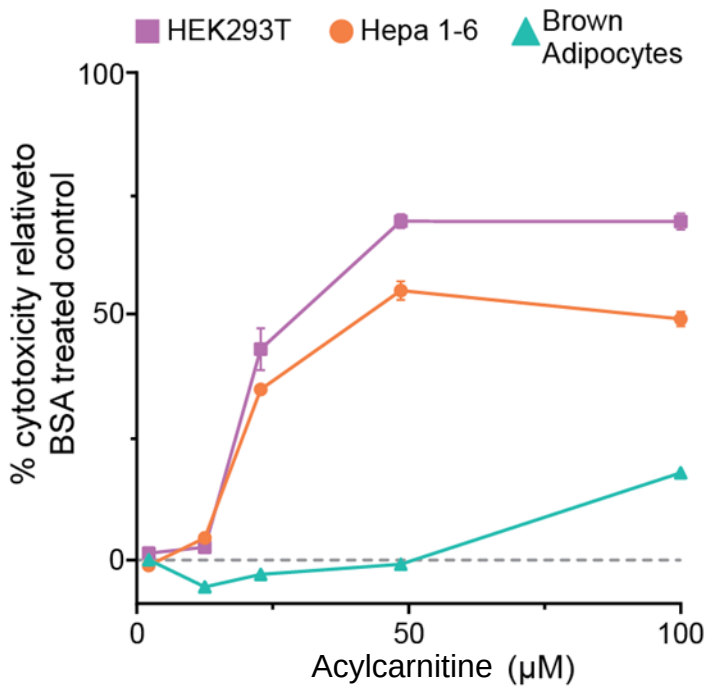

Figure 4. Graph showing the toxicity of increasing doses of acylcarnitine for three different cell lines.

We identified that the fat cells have much higher levels of the CD36 protein we identified as being important for acylcarnitine metabolism. We then confirmed results previously published by other groups that CD36 is located in the mitochondria, the structure within cells where fats are broken down for energy. To confirm that CD36 is required for proper breakdown of acylcarnitines, we engineered fat cells that cannot produce CD36 and found that they were sensitive to acylcarnitine. This means that without CD36, acylcarnitine becomes toxic and kills fat cells. We also generated a version of the kidney cell line, which normally has very low levels of CD36 protein, that produced much more CD36. The modified cells were better able to survive being given acylcarnitine. This means that CD36 is sufficient to protect cells from acylcarnitine toxicity.

This work has important implications because researchers are exploring drugs that inhibit or reduce CD36 activity to treat a variety of metabolic diseases. The previously known role of CD36 is to help cells take up or 'eat' fats. The idea is that if we can prevent cells from taking up fats, that could be a way to treat obesity, diabetes, or other metabolic syndromes in which fat metabolism is altered. However, in these disease states, there are also higher levels of acylcarnitine in patients' blood. Therefore, our results show that inhibiting CD36 could make those high levels of acylcarnitine in the blood toxic to cells that can otherwise typically tolerate acylcarnitines. More work is needed to determine how exactly acylcarnitines cause cells to die, and how we can design CD36 inhibitors that don't cause unwanted side effects or even worsen metabolic diseases.

With Professor Simcox's mentorship, I was able to interpret our initial screen data, identify the most interesting discovery to follow up on, design, execute, and interpret a series of experiments to understand the role of CD36 in acylcarnitine metabolism. We also worked together to curate a series of figures to accurately display our findings, draft a compelling manuscript to communicate our results with the wider community and to submit for publication in

a scientific journal. Thus, I was able to complete my final graduation requirement and round out my development as a PhD-level scientist. Now, I plan to leave the academic world, with my transformation into a capable, independent investigator complete, ready to make meaningful contributions to new scientific endeavors. Let the next journey begin…