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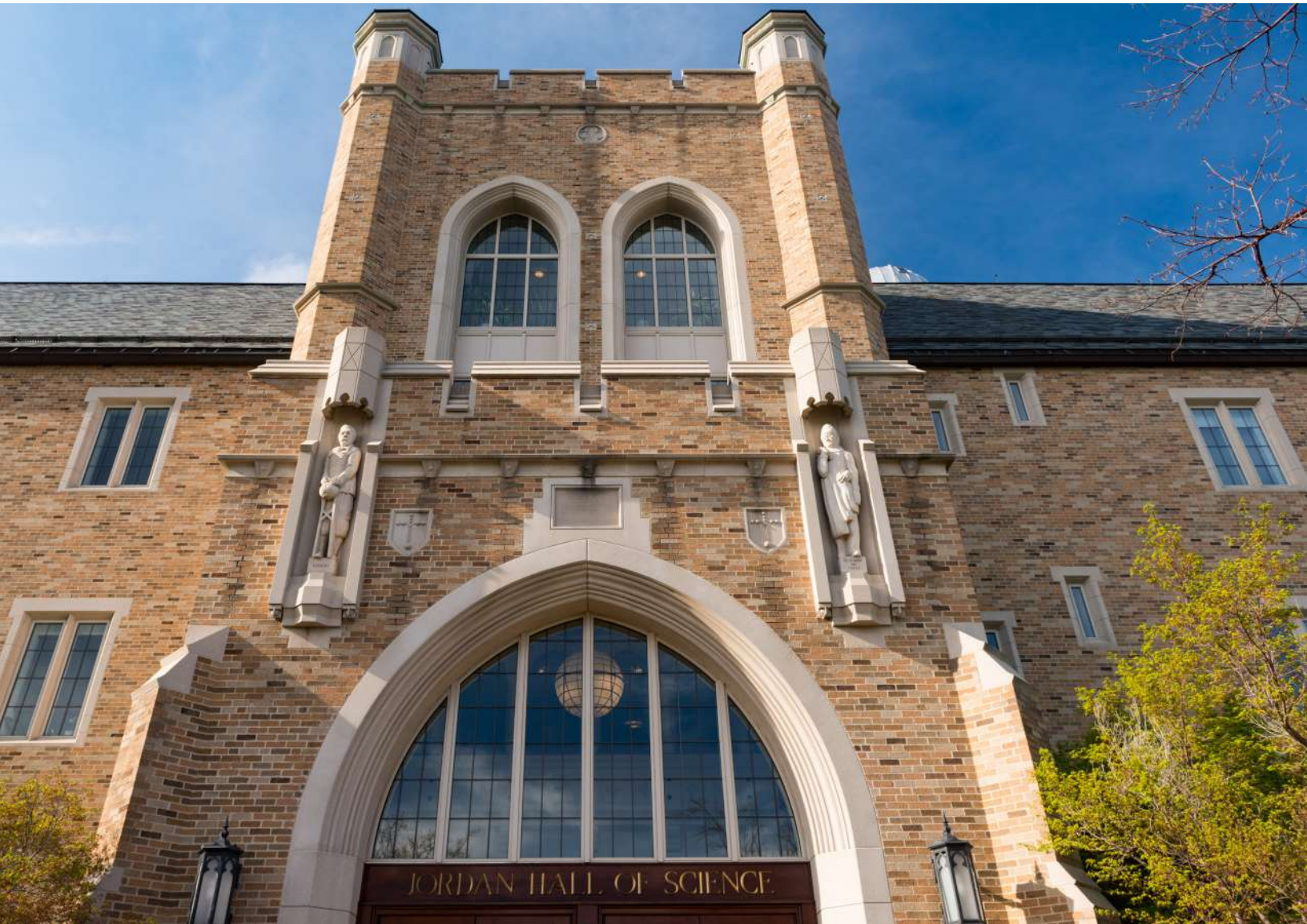

Letter from the Editors

May 2022
Research serves as an essential function for educating undergraduate students in the College of Science. Undergraduates at Notre Dame have the opportunity to tackle pressing research questions alongside accomplished faculty in world class laboratories and facilities. At the core of Scientia's mission is to provide a platform for undergraduates to participate in the dissemination of their research efforts. As Sir Mark Wilport, the United Kingdom's chief scientist explains, "Science is not finished until it is effectively communicated." As evidenced by the COVID-19 Pandemic and the global climate crisis, a scientist's role must include effective communication in writing and in speech, with other scientists and the public.

In the 2021-2022 academic year, Scientia fulfilled its mission of serving undergraduate research at Notre Dame through general membership recruitment, Talk Science lectures, and the Charles Edison Fellowship. This year, we saw increased interest in participation from first and second year students, many of whom wrote articles for this year's publication. Through Scientia's Talk Science lecture series, undergraduate researchers shared their experiences and results alongside faculty speakers with our general membership. Our talks featured research from the Departments of Biology, Chemistry and Biochemistry, and Physics and Astronomy. Through establishing avenues for sharing student research and hearing from our expert faculty, Scientia strives to make Talk Science an integral part of knowledge dissemination for our members. Scientia is proud to support two undergraduate researchers each year through the Charles Edison Fellowship. Our 2021-2022 Charles Edison Fellows, Bailey Perczak and Christina An, demonstrated research excellence throughout the year.

Notre Dame's transition away from COVID-19 related restrictions meant students were able to once again participate in meaningful in-person research in laboratories across campus. In the thirteenth volume in Scientia, we are excited to share some of this laboratory work. This year's volume also features a broad array of new College of Science faculty hires, news articles, and student spotlights.

We are grateful for the sustained hard work and dedication of our editorial board and staff, who are integral in the successful assembly and publication of the journal. Scientia also benefits greatly from the guidance of our advisor, Dr. Xuemin Lu, and the College of Science Marketing Communications team, Lotta Barnes, Deanna Ferrell, and Tammi Freehling. Finally, we would like to formally recognize and extend our appreciation to the new Dean of the College of Science, Dr. Santiago Schnell, from whom we are excited to receive support in the years to come. As we enter the 2022-2023 academic year, we hold great confidence in the upcoming Editors-in-Chief, Emily Hunt and Alexandra Noble.

In Notre Dame,
Abigail Abikoye & Drew Langford

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Perspectives in Research

Why, when and how to get involved in research?

May 2022

During the first six months of my first undergraduate research experience, I was responsible for cleaning the laboratory's glassware. It was tedious work, but I quickly learned how important my job was to the research team: any contamination in the glassware would be catastrophic for the experiments. My goal was getting the work done on time, being as helpful as possible, and being available for the research team. I soon understood that at research universities such as the University Notre Dame, professors not only teach about what they know in their field of expertise; they are also employed to advance and explore knowledge. This work happens within their research group.

The most interesting and exciting way to advance knowledge is to explore what we do not know about life and our universe. This is, after all, the essence of how science is practiced on daily basis in your professor's heads, and in their research laboratories. While Notre Dame professors enjoy teaching undergraduates, the bulk work is conducting original research and writing scientific publications. If your professors are successful and lucky in their research, their work will become the future knowledge taught in classrooms.

As a student, if you really want to make the most of your university experience, you need to be in the middle of the action. Research is where all the action is. My advice? Jump into it as soon as possible by becoming involved in the University of Notre Dame's original research.

Why should I get involved in research? A research experience is an incredibly rewarding opportunity for you to make a difference in the academic world. It will not be easy. As a matter of fact, I can tell you that it will be hard! Research is like a high-performance strength training program for athletes, but instead of training your muscles, you will be working out your will and intellect.

Should I get involved if I am not planning a career in research? Yes, you must, because working on an original research project will send signals that you are driven and hardworking, interested in strengthening your intellect, and desiring to distinguish yourself from other students. At the same time, you will have the unique opportunity to get to know your professor better. If you make significant contributions to your professor's research program, the professor will support your future career in many ways—including writing an exceptional letter of recommendation for you!

When should I get involved in research? A common misconception among students is that you can only get involved in research once you have acquired sufficient knowledge to be part of the laboratory. While some research projects require advanced knowledge, many do not, and waiting until you are a senior to get involved is a mistake because you might not have enough time to make an impact in a long-term research project. I recommend inquiring about research opportunities toward the end of the spring semester your first year. This will give you sufficient time to get a feeling about what you might be interested in, as well as time to learn more about your professors and their research projects.

How should I approach a professor? First, do your homework by looking at the research group's website and reading some of the professor's papers. Intuitively, you will probably want to write an email to those professors whose research interests you. This might work, but most professors are overwhelmed with emails. If you want to get an answer and make a positive impression, I recommend talking to the professors in person. You can approach them after class, during office hours, and by making separate office visits. When you contact professors, tell them you are interested in getting more experience in academia by pursuing research. Then, describe why you're fascinated with their area of research by mentioning specific aspects that you've gleaned from their websites and papers. This will certainly impress them. If the professor does not have any open opportunities in their group, he or she might know of another professor who does.

Another path to pursue undergraduate research is to apply for undergraduate research opportunities posted by the university. At Notre Dame, this can include speaking with your academic advisor or the college's undergraduate research director, or by working through the Flatley Center for Undergraduate Scholarly Engagement to find a position. These programs make it a lot easier to find a professor willing to hire you, and you might even be paid as a research assistant.

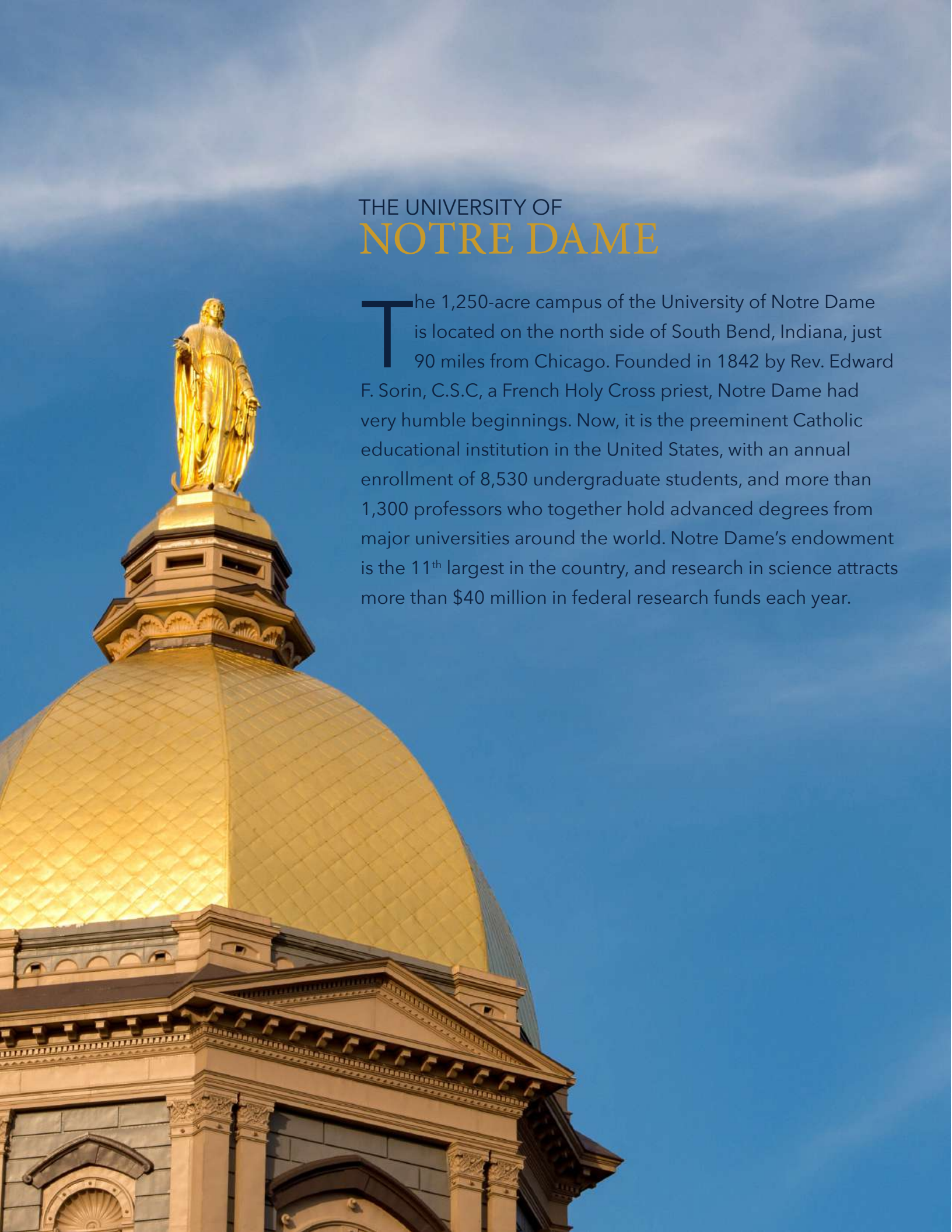
What happens once you are in the research group or laboratory? You likely will not be playing a critical role in the research project at first. You will begin by assisting one aspect of the project, while you are learning and proving yourself. Remember how I cleaned glassware during the first six months of my first research experience? It is very important to be modest about your own abilities. You should offer to help your research teammates if you know you can handle the job and feel comfortable with the project. You might even be invited to get more involved! When you are given more important work, be sure to work with enthusiasm, diligence and to the best of your abilities.

If you pursue a research experience in college, you will become a successful, well-rounded student. An average student will do well in science courses; a winning student gets involved in original research!

Santiago Schnell, D. Phil
William K. Warren Foundation Dean of the College of Scienc

Further reading and resources

Council on Undergraduate Research. Washington, D.C.: http://www.cur.org.
Oppenheimer, D G, Grey, P H (20125) Getting In: The Insider's Guide to Finding the Perfect Undergraduate Research Experience. Secret Handshake Press.
Yu M, Kuo Y-M (2017) Ten simple rules to make the most out of your undergraduate research career. PLoS Comput Biol 13(5): e1005484. https://doi.org/10.1371/journal.pcbi.1005484
Undergrad in the Lab: Strategies to navigate the hidden curriculum in STEM research and tips to get the most out of your experience: https://undergradinthelab.com/



THE UNIVERSITY OF
NOTRE DAME

The 1,250-acre campus of the University of Notre Dame is located on the north side of South Bend, Indiana, just 90 miles from Chicago. Founded in 1842 by Rev. Edward F. Sorin, C.S.C, a French Holy Cross priest, Notre Dame had very humble beginnings. Now, it is the preeminent Catholic educational institution in the United States, with an annual enrollment of 8,530 undergraduate students, and more than 1,300 professors who together hold advanced degrees from major universities around the world. Notre Dame's endowment is the 11th largest in the country, and research in science attracts more than $40 million in federal research funds each year.






RESEARCH

Want to get involved in undergraduate research?
For more information about undergraduate research opportunities at the University of Notre Dame, visit science.nd.edu/undergradresearch.
Learn more about the Charles Edison Student Fellowship of *Scientia* on scientia.nd.edu or email scientia@nd.edu for details.



College of Science Faculty Spotlights

Santiago Schnell, Ph.D. is the new Dean of the College of Science, formerly the chair of the Department of Molecular and Integrative Physiology at the University of Michigan Medical School. Schnell received his undergraduate degree in biology from Universidad Simón Bolívar in Venezuela and his doctorate in mathematical biology from the University of Oxford in the United Kingdom. After completing his doctoral studies, he held two prestigious research positions at Oxford (junior research fellow at the Christ Church college and Wellcome Trust research fellow at the Wolfson Centre for Mathematical Biology). In his new position, Schnell will lead six departments comprising more than 250 faculty, 125 staff, 480 graduate students and 2,000 undergraduates. Dr. Schnell utilizes mathematical, computational and statistical approaches in his work researching diseases; he is himself a cancer survivor having been diagnosed with cancer at the age of 15.

Barbara Calhoun, within the College of Science is the director of minor in science and patient advocacy and is now assistant professor of the practice. Additionally, she is the outreach coordinator of the Boler Parseghian Center for Rare and Neglected Diseases. She received her B.S. in nursing from the University of Arizona and her M.S. in nursing to become a pediatric nurse practitioner from Arizona State University. For the past year, she developed the minor in science and patient advocacy to allow undergraduate students to learn critical skills to become effective patient advocates. She also developed BIOS 40565, Clinical Research in Rare Diseases, the new capstone course which is part of the minor. In this research course she helps teach students how to acquire the skills necessary for assessing rare disease patient medical records for the natural history of that particular disease. She supports students as they identify pertinent symptoms of a disease and how they progress, and together they develop severity scales to score symptom severity. The data they generate can assist researchers with identifying biomarkers that have the potential to determine the effectiveness of therapies. In addition to her list of accomplishments and work, she teaches the Neuroscience and Behavior lab for majors.

Laura Fields, Ph.D., is an associate professor of physics, specializing in experimental neutrino physics. She uses neutrinos to try and answer open questions in physics, such as properties of matter and antimatter and additional interactions between particles that have not yet been discovered. She uses the accelerator beams at Fermilab and also works on four large experiments, including the Deep Underground Neutrino Experiment. This experiment sends neutrinos from Fermilab through the earth to a large underground detector in South Dakota. Dr. Fields was a math and physics major at the University of Arkansas and received her masters and PhD in physics at Cornell University. She also was a postdoc at Northwestern University and a scientist at Fermi National Accelerator before coming to Notre Dame. She has loved her first year at Notre Dame and enjoys working with many talented students. She is the mom of two boys who are enjoying their new home in the South Bend area.

Lauren Weiss, Ph.D., assistant professor of physics, earned her B.A. in astronomy at Harvard University in 2010. She then went on to earn her M.Phil in astronomy at the University of Cambridge in 2011, followed by her M.A. in astronomy at the University of California Berkeley in 2013. In 2016, Weiss earned her Ph.D. in astronomy at UC Berkeley, where she was able to create a pioneer mapping system comparing planet mass and radius. Weiss also commissioned the Automated Planet Finder telescope, as well as determined many physical attributes of planets in Kepler's systems. As the Trottier Fellow at the Institute for Research on Exoplanets at the Universite de Montreal, she discovered and developed the planetary pattern "peas in a pod" to describe the sizes and spacing of neighboring planets. Recently, at the University of Hawaii at Manoa, Weiss was the Beatrice Watson Parrent Fellow at the Institute for Astronomy. There, she was the Principal Investigator studying the complex physical properties and orbits of several of Kepler's multi-planet systems. Weiss' current work at Notre Dame includes the studies of these systems, specifically the order and chaos present within them.

College of Science Faculty Spotlights

Erin Howe, Ph.D., is a Research Assistant Professor in the Department of Biological Sciences, currently working and mentoring undergraduates in Siyuan Zhang's cancer biology lab. In 2004, Howe earned her B.S. in the field of mechanical engineering at the Rose-Hulman Institute of Technology. The trajectory of her post-graduate studies was altered by her father's cancer diagnosis, which compelled her to attain a Ph.D in cancer biology within the Department of Pathology at University of Colorado's Anschutz Medical Campus in 2012. Since then, Howe has studied at the University of Chicago, and has been researching breast cancer metastasis at the Harper Cancer Research Institute. At the forefront of groundbreaking cancer research, her published works span prevalent topics such as the possible role of the GTPase Rab11b in exacerbating brain metastasis, the covert dissemination of cancer cells in patients with prostate cancer, and the effects of aged extracellular components in promoting malignant cell growth. When not busy with her work at Notre Dame, Howe enjoys spending time with her dogs, Rosie and Tristan.

Adam Jaffe, Ph.D., is an associate professor of chemistry in the Department of Chemistry and Biochemistry. Jaffe earned a bachelor degree in chemistry from Princeton University in 2012 and a Ph.D in inorganic chemistry from Stanford University in 2017. After completing his doctorate, Jaffe served as the Ruth L. Kirschstein NIH Postdoctoral Fellow for the University of California, Berkeley, where he studied redox-active metal–organic frameworks for separating oxygen from air. Jaffe joined the Notre Dame community as an associate professor of chemistry in 2021. At Notre Dame, Jaffe leads a research group that focuses on developing new hybrid material platforms. By combining organic-inorganic approaches to the mild synthesis and modification of solid-state systems, Jaffe's group hopes to create materials with applications including optoelectronics, catalysis, and energy storage. Jaffe has several recent publications, including "Selective, High-Temperature O2 Adsorption in Chemically Reduced, Redox-Active Iron-Pyrazolate Metal-Organic Frameworks" (2020), "High Compression-Induced Conductivity in a Layered Cu–Br Perovskite" (2020), and "Structural origins of broadband emission from layered Pb-Br hybrid perovskites" (2017). Outside of his academic duties, Jaffe plays soccer and loves Thai food.

Nathan G. Swenson is a professor of biology and Gillen Director of the University of Notre Dame's Environmental Research Center. He earned his PhD at the University of Arizona in Tucson, Arizona in 2008. Afterward, he did a postdoctoral fellowship in bioinformatics with the Center for Tropical Forest Science, Arnold Arboretum, and Harvard University. Dr. Swenson was an Associate Professor at Michigan State University and the University of Maryland before coming to Notre Dame as a professor in 2021. Swenson's research focuses on predicting future forest biodiversity by studying intra- and inter-specific variation in tree performance. His research group has conducted extensive field work in the forests of the Eastern United States, Puerto Rico, Colombia, and southeast Asia, completing ground breaking research regarding gene expression in response to temporal variation in the environment. Swenson utilizes novel, integrative approaches drawing from a wide variety of techniques across different fields, such as genomics and phylogenetics to create detailed projections of future forest dynamics.

Guido Camargo España, Pd.D., Research Assistant Professor in Infectious Disease Epidemiology, earned his Bachelors of Electronic Engineering from the National University of Colombia in 2010. He would continue his studies at the National University of Colombia, earning a Master of Science in Engineering and Industrial Automation in 2012, and a Ph.D. in Electrical Engineering in 2015. During this time, Dr. Camargo España worked for the National University of Colombia as a modeling engineer for the School of Public Health, as researcher studying Modeling and Control of Biological Systems for the School of Electrical Engineering, and as a predoctoral fellow in the Public Health Dynamics Laboratory at the University of Pittsburgh. In 2016, Dr. Camargo España joined the Notre Dame community when he accepted a position as a postdoctoral fellow for the Department of Biological Sciences, and in 2021 he was promoted to the position of Research Assistant Professor. Dr. Camargo España utilizes mathematical and computational models to understand the dynamics of infectious diseases such as dengue, chikungunya, and Zika. His lab also works to estimate the impact of non-pharmaceutical and pharmaceutical interventions on the dynamics of the COVID-19 pandemic. In his free time Dr. Camargo España enjoys playing guitar, running, and cycling.

College of Science Faculty Spotlights

Julie Kessler Ph.D., is one of the University of Notre Dame's newest College of Science faculty. Kessler began teaching at Notre Dame during the fall semester of 2021 as a new assistant teaching professor in the department of chemistry and biochemistry. She decided to return to her alma mater (Kessler received her doctoral degree in Chemistry from Notre Dame in 2017) after previously working as an assistant professor of Chemistry at Carroll College in Helena, Montana for four years. Kessler aims to enact her passion for pedagogy to promote and help foster good habits and solid foundations early during students' formative years. "I want students to have a great experience as a freshman and also help them develop good study habits and problem-solving strategies," said Kessler. Her teaching awards include the Kaneb Center Outstanding Graduate Student Teacher Award (2015), Striving for Excellence in Teaching Certificate (2016), Rudolph S. Bottei Graduate Teaching Award (2017).

Xiaolong Liu, Ph.D., is an Assistant Professor in Physics in the College of Science and was a Postdoctoral Fellow Cornell University in 2019. He currently teaches Electromagnetic Waves, a prerequisite to Electricity & Magnetism. In their study of electromagnetic waves, he helps students explore physical optics, radiation from accelerating charges, and some topics from the special theory of relativity. In the latter course he grounds students in their examination of topics such as Laplace's and Poisson's equations, boundary value problems, dielectric and magnetic phenomena. During his time at Cornell, he pioneered high-speed atomic resolution scanned Josephson tunneling microscopy. He received his PhD degree in Applied Physics from Northwestern University the year prior in 2018, where he explored the creation of entirely synthetic 2D materials such as borophene. His current research is focused on the creation, visualization, and understanding of novel quantum matter by means of low-temperature scanning tunneling microscopy and single atom/molecule manipulation. Xiaolong's research has led to over 40 peer-reviewed publications in top-tier journals including Science, Science Advances, and Nature Materials, just to name a few. In a recent accomplishment, Dr. Liu was recently awarded the 2022 Young Scientist Prize in Low Temperature Physics by IUPAP.

Using Longitudinal Data to Determine the Heritability of Gut Microbiomes

STEPHANIE SWEGLE

What Dr. Elizabeth Archie found from her research on baboon microbiomes surprised her: genetics plays an almost universal role in the baboons' microbiome compositions. This finding, reported in "Gut Microbiome Heritability is Nearly Universal but Environmentally Contingent" and published in Science, was in direct opposition to current research on humans which suggest that we have very few microbiome species in our guts that are influenced by genes. Interestingly, although an overwhelming 97% of the microbes found in the baboons' microbiomes were found to be heritable at some level, the actual influence genes played in the microbiome composition was fairly low: differences in the baboons' genes explained only about6-7% of the microbiome variation between individuals. As context, this percentage is similar to the level of influence genes play in the variation between individuals in behavioral traits, like shyness. There are many other factors that come into play to select the particular microbes that end up in the baboons' guts, including diet, environment, and social group. According to Dr. Archie, "Microbiomes are complex, and most of the variation in the microbiome remains unexplained."

The data for this research came from a vast store of Amboseli baboon fecal samples that researchers have been carefully and methodically collecting for over 20 years. Dr. Archie spoke about the hands-on research in Kenya, saying they would camp out in tents and wake up at 5:30 am to start watching the baboons. For the next several hours after waking up, the researchers would go where the baboons went, watching them and waiting for them to poop. Then they would collect the samples with popsicle sticks and Dixie cups and put them in little plastic baggies. From there, the researchers extracted the DNA from the samples. They used PCR to amplify a specific piece of rRNA called 16S, the gene for a small subunit of a ribosome which is used as a barcode in order to identify particular species. Sequencing the DNA produced the array of different bacteria present in each sample and at what percentage. They used ASV Shannon's H index and (ASV) richness in order to determine how many different types of microbes were present and how evenly distributed the microbes were, and a Bray-Curtis dissimilarity matrix to determine the most signif cant ways that microbiomes varied across individuals. The researchers were looking to see if there were genetic signatures that could predict the abundance of certain microbes in microbiomes. Dr. Archie said the research was very collaborative, with scientists from the U.S. and Kenya working together on various parts, from collecting samples, to gathering environmental and behavioral data, to performing bioinformatics and data runs.

One reason this research is important is because it is one of the very few microbiome studies that uses longitudinal data. Most human studies, for example, take microbiome samples from a particular point in time to get a snapshot of the microbiome's composition. In contrast, this study took samples from the same baboons over a period of 20 years, amassing a composite picture of the microbiome that reflects the variability and dynamic nature of this system. A logical next step would be to conduct a similar longitudinal study in humans to see if our microbiomes show the same near universal heritability.

The findings from this research leave exciting areas for further study into how the microbes in the microbiome get selected and the role genes play in this process. Dr. Archie indicated one of the next questions she would be interested in answering is "which genes or sets of genes are really important to the microbiome and which taxa they influence." Figuring out which genes predict which microbes could enable us to predict health problems in individuals with certain genetic indicators and potentially change health outcomes. She thinks that looking into genes involved in the immune system, cr ation of immunoglobulin, diet preferences and sociability may be a good place to start when searching for genes that impact differences in microbiome composition. Dr. Archie estimates that "the microbiome has ten times the genes that we have." Her intriguing follow-up is, "How does the microbiome's genetic variation contribute to human traits?" We are accustomed to thinking about human traits being influenced by our own genes and by our environment. But what if there is a third contributor to human traits: our microbiome's genes? There are so many unknowns about the microbiome and its effects on human health; this research gets us an important step closer to figuring out what mechanisms are at work in the microbiome and where we should direct further research.

Preventing Childhood Blindness: Raising Awareness About Leukocoria

BAILEY PERCZAK

When family photographs are taken at holidays or get-togethers, many parents spend painstaking time sifting through them afterwards, deleting the pictures with eye flashes, or using the edit functions to cover up pesky red-eye glares. A nonprofit organization called Know The Glow is actively aiming to educate parents about the utility of these eye flashes, and urges families to take a second look at them prior to tossing the photos aside. Leukocoria is a condition that can be indicative of over 25 childhood eye disorders that may otherwise go unrecognized. It manifests as a white or yellow glow in the eye, and typically can only be detected in dim lighting, or when flash photographs are taken. Many childhood diseases exist that can spread and develop without individuals having any means for early detection. But through this simple, accessible indicator, these conditions can be caught early. When treated promptly, they can be prevented from developing into childhood blindness, and sometimes even more fatal prognoses.

Retinoblastoma is the most common childhood cancer of the eye, and makes up one in fifty diagnoses of pediatric cancer. The majority of children are diagnosed under the age of five, with the median age being around two years old. When left untreated, the condition can lead to blindness or can metastasize to the brain, becoming fatal. As only one of the many possible conditions that can be indicated by leukocoria, it proves to be evidence of the importance of early detection. Many of the conditions that can cause childhood blindness - including retinoblastoma - are preventable or curable, if they are addressed promptly. Around one in eighty children shows evidence of leukocoria prior to the age of nine, and Know The Glow works to ensure that parents around the world are able to catch the signs before it's too late. Leukocoria is colloquially known as "The Glow", given the yellow-white gleaming reflection that it takes on in photographs. In the case of retinoblastoma, it is the result of light bouncing off of a tumor, and in conditions like Coats' disease, it comes from excess fluid buildup behind the eye.

Megan Webber, the founder of Know The Glow, created the organization following her son's own diagnosis of Coats' disease. Their pediatrician had not initially recognized anything wrong with her son's vision, but Megan's sister had seen a television program warning about the golden reflection that was present in several of Megan's family photographs. After advocating for further investigation, Megan and her son were sent immediately to a pediatric ophthalmologist, who found a large mass behind her son's eye. Without her son having presented with any other symptoms, Megan and her family were shocked. But if not for the recognition of the photographed reflection, the condition could have worsened, leading to complete vision loss in the affected eye. In having spoken to many families through working with Know The Glow, it has become apparent that this situation is all too common. Many parents aren't aware of what "The Glow" means, and tell stories about looking back through photographs and seeing how prevalent it had been, but not knowing about the sign before it was too late. Megan and her incredible team have grown their organization to a worldwide scale, partnering with physicians, ophthalmologic organizations, and nonprofits around the world to raise global awareness of leukocoria. As a graduate of Notre Dame, she has been generous enough to incorporate several university students in their research initiatives and has branched off to include other campuses as well. Projects on spreading awareness in Venezuela, India, and Bangladesh are currently at the forefront of the Notre Dame student team, and new locations continue to be added to the docket. It's the hope of everyone involved that through these research projects and community outreach initiatives, families around the world will be aware of "The Glow" and can have the power to seek out treatment long before the condition becomes threatening. The mission so far has been an incredible success, making impressions on millions of people, and seems like it will continue to educate millions more.

High-Pressure Studies of Calcium Uranyl Tri-peroxide Monomer Using In-situ Raman

Haizhen (Vita) Zhang
University of Notre Dame, Department of Chemistry and Biochemistry

Abstract

Calcium Uranyl Tri-peroxide (CaUT) Monomer is one of the numerous uranyl peroxide nanoclusters that has been extensively studied. However, the knowledge of its behavior under high pressure is scarce. CaUT Monomer single crystals are compressed using Diamond Anvil Cell up to 14.7 GPa and characterized using in-situ Raman Spectroscopy. From assignments of shift in in-situ Raman Spectroscopy, the increasing trend of Raman shift in wavenumber, and decreasing trend of uranyl and peroxide bond lengths is established. This paper revealed the stability of CaUT single crystal as it persisted its crystalline structure, according to in-situ Raman spectroscopy up to at least 6.5 GPa. The nature of the crystal in amorphous solid requires further investigation. CaUT is one of the many stable Triperoxide Uranium(VI) Complexes, and could be abundantly useful in the application of nuclear fuel storage, and nuclear fuel reprocessing (uranium separation chemistry).

Introduction

The uranium-peroxide system is vital in uranium separation chemistry. The persistent nanoscale clusters give rise to their potential applications in nuclear fuel storage and reprocessing [3]. Considerable effort has been made to synthesize various Uranyl Peroxides and elucidate their structures. Different alkali and alkaline metals, including Na, Ca, K, Mg, Li have been found to coordinate uranyl tri-peroxide systems and form stable needle crystals where both the uranyl-oxygen and peroxide ligands are the acid (oxygen donor) and the alkali or alkaline earth metals are the base (oxygen acceptor) [5, 11]. Notably, SrUT and CaUT monomers have almost identical coordination with uranyl tri-peroxide, and identical symmetry elements and space group. CaUT and SrUT both have significantly less negative standard enthalpy of formation than other uranyl peroxide clusters reported in an energy trend study of six different uranyl peroxide clusters [11]. The less negative standard enthalpy is correlated with the acidity of alkaline metal Calcium as the enthalpy of formation of these uranyl triperoxide monomers becomes more negative as the metal oxides become more basic [11].

The chemical formula of Calcium Uranyl Tri-peroxide Monomer is Ca2(UO2)(O2)3(H2O)9.The structure has been previously elucidated using single crystal X-ray diffraction. It is a Pbcn space group and exhibits orthorhombic symmetry. The orthorhombic unit cell has three mutually orthogonal axes (x, y, z) with different lengths. Moreover, Pbcn has three twofold symmetry, two glide planes and one diagonal glide plane. These symmetry elements are presented in Figure 1. As shown in all three orientations in Figure 1, each Pbcn unit cell consists of 4 molecules in each unit cell. Notably, in the second and third diagram in Figure 1, the top and bottom row are only counted as 2 molecules in total, since only half of the 4 molecules sit inside the unit cell.

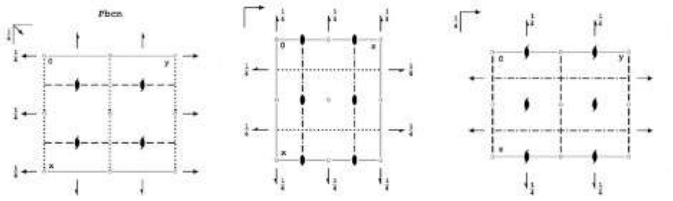


Figure 1: The Pbcn space group symmetry elements. *Symmetry elements in one Pbcn unit cell are presented in different orientations with classical space group symmetry notation [2].*

The point group symmetry of CaUT Monomer is Cnh. It has its principal rotational axes C3 along the uranyl bond shown in Figure 2b, adn three C2 rotational axes of symmetry on the plane of equatorial ligands which is shown in Figure 2a. It also has a σh plane perpendicular to the principle rotation axis, which is shown in Figure 2b. The uranyl tri-peroxide systems are bonded through a complex hydrogen bond network with water and coordinate to Calcium cation [5]. The system of lattice structure as the unit cell made up of 4 CaUT Monomer molecules is shown in Figure 2c. Figure 3. shows the uranyl triperoxide in a unit cell, when correlated with Calcium.

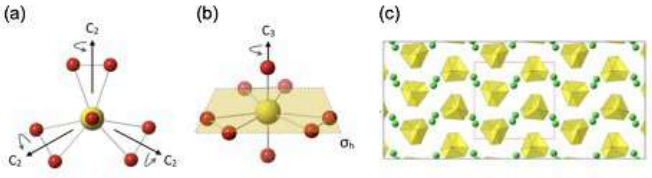


Figure 2: Uranyl Triperoxide Symmetry Elements and Lattice Structure with Unit Cell. *(a)(b) Ball-and-stick top and side views of the uranyl triperoxide, and axes and plane of symmetry, (c) Polyhedron-and-ball views of the crystal packing for different UT salts down the 100 plane. Yellow, red, green spheres/polyhedra represent uranium, oxygen, and calcium atoms, respectively. Red boxes represent the unit cells, and show four CaUT Monomers in one unit cell (Z=4) [3].*

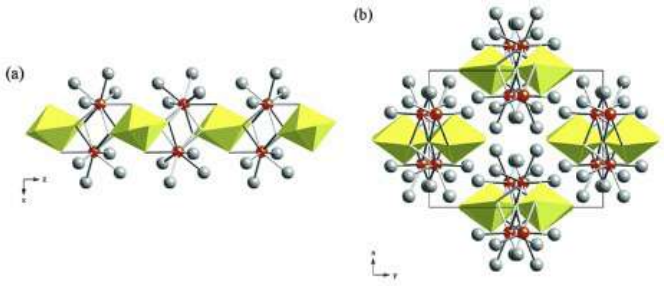


Figure 3. Structure of CaUT Monomer projected along the y axis (a) and x axis (b). *Triperoxide Uranium(VI) polyhedra are yellow, Calcium cations are represented by red spheres, and Oxygen atoms of water groups are represented by gray spheres [5].*

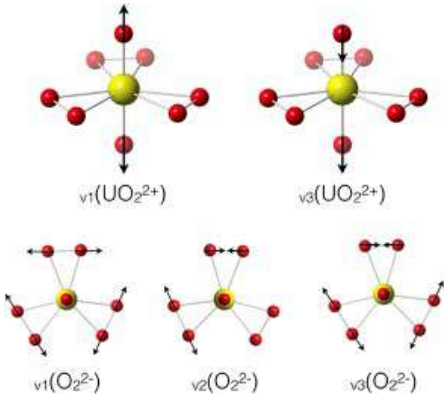


Figure 4. Ball-and-stick representations of Raman-active uranyl and peroxide vibrational modes in Calcium Uranyl Tri-peroxide Monomer. *Yellow and red spheres correspond to uranium and oxygen, respectively [3].*

The Burns group has published a robust computational model to assign unexpected Raman signals for these Uranyl Triperoxide Complexes, which revealed raman-active characteristics of CaUT Monomer [3]. This computational analysis and Raman assignments have shown superb agreement with the quantum-chemical model developed by Vallet et al that relates the uranyl bond length of discrete uranyl peroxide species with the symmetric vibration of uranyl bonds [3, 10]. These symmetrical and asymmetrical vibrational mode of uranyl bond and peroxides are shown in Figure 4.

This study aims to use primarily in-situ Raman Spectroscopy to study the structural response of this calcium uranyl Tri-peroxide system to extreme environments (pressure up to 14.5 GPa). This approach uses the peak shift that corresponds to discrete vibration mode of the uranyl tri-peroxide system under different pressure conditions to elucidate the behavior of uranyl and peroxide bonds in Calcium Uranyl Tri-peroxide Monomers. The quantum-chemically derived equation adapted from Vallet et al [10] is used to elucidate uranyl bond length using Raman peak shift as well. The computational model established by the Burns group to characterize uranyl peroxide systems using Raman will also be adapted as the primary method to characterize Calcium Uranyl Tri-peroxide Monomer under high pressure conditions in this paper.

Diamond Anvil Cell (DAC) is utilized to apply pressure to the CaUT crystal in presence of pressure medium and pressure control. DAC is a portable device routinely used in high-pressure study of minerals in deep-earth conditions. However, its application in high-pressure study of actinide materials is scarce. DAC has many advantages in high-pressure studying, including its optical transparency, and portable size. These features made it a versatile pressure generating device, that can be adapted to X-ray Diffraction, and many spectroscopies. Sample and pressure indicator (pressure standard) would be placed inside of the chamber inside of a gasket that is pressed down by two diamond cutlets sitting on boron nitride and tungsten carbide seats as shown in Figure 5. Screws with spring washers are utilized to apply pressure based on the basic principle reflected in Equation 1.

$$Pressure = \frac{Force}{Area} \qquad (1)$$

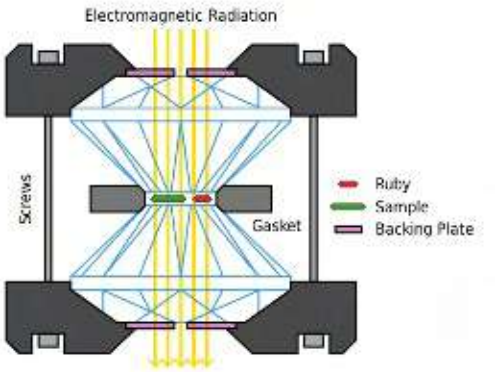


Figure 5. Diamond Anvil Cell Schematics of the core of a diamond anvil cell (DAC). *Showing the diamond cutlets, gasket, sample chamber, and screws.*

Materials and Methods

1. Synthesis of Calcium Uranyl Tri-peroxide Monomer (CaUT Monomer)

Calcium Uranyl Tri-peroxide Monomer is synthesized under ambient conditions, by combining the following reagent in order: 400 µL of 0.5M UO2(NO3)2, 400 µL of 30% H2O2, 450 µL of 25% TMAOH, and 200 µL of 0.25M Ca(NO3)2 in a 5-mL glass vial. The combination of 400 µL of 0.5M UO2(NO3)2 and 400 µL of 30% H2O2 rapidly produced a pastel yellow studtite precipitation, which was then dissolved in acid TMAOH accompanied by effervescence and an apparent color change to dark orange. After the completion of effervescence, Ca(NO3)2 is added to introduce Calcium cation. After 24 hours, orange needle crystals that vary from 100 to 1000 µm in length are observed. These crystals are diffraction quality and have unit cell parameters that are similar to those previously reported. The synthesis protocol has been previously reported [5].

All syntheses were conducted using isotopically depleted uranium (238U, α = 4.267 MeV), which ensures

minimal radiation. All experiments are conducted in laboratories designed for use of radioactive isotopes. Radiation detection badge and ring are worn at all times. The radiation risk management training is completed under supervision of Adam Kratt at the Risk Management and Safety Office.

2. Single Crystal X-ray diffraction

Synthesized Calcium Uranyl Tri-peroxide Monomer needle single crystal is isolated under Nikon optical polarizing microscope using silicone oil and glass fiber. Glass fiber is used instead of cryoloop because it is more fit to manipulate needle crystal. The single crystal X-ray diffraction was conducted on Bruker PLATFORM three-circle X-ray diffractometer equipped with a 4K APEX CCD detector and graphite-monochromatized Mo Kα radiation at 100 K with the crystal contained in a flow of Nitrogen. The X-ray structure was then solved and refined using Olex II.

3. Diamond Anvil Cell

A symmetric Mao-Bell DAC with 400μm culet diamonds and cubic boron nitride and tungsten carbide seats (Almax easyLab Inc., Cambridge, Massachusetts) were used to compress the sample in a stainless-steel gasket.

The CaUT crystal sits in a stainless-steel gasket chamber. The gasket is pressed in the DAC, so that it would fit seamlessly on the diamond during loading. Then, the gasket is drilled using a micro electro discharge drilling (EDM) machine. The pre-pressed gasket is placed under the drill, and a reference point for the plane of the gasket is made by drilling on the surface of the gasket. Then, the micro EDM is used to drill through the center of the sample chamber of the gasket. The thickness of the gasket is measured using a micrometer. Since the gasket experiences shrinkage after application of high pressure, the gasket is engineered multiple times with varying thickness and drilling electrode sizes. The thickness of the gasket used for this experiment is 59+/-2 μm, and the drilling size is 200 μm.

Due to safety and difficulty of execution, gasses were not used as a pressure-transmitting medium. Solid pressure-transmitting medium eliminates the already scarce field of view. After testing different pressure medium, a 4:1 MeOH:EtOH solution is used as the pressure-transmitting medium use to its lack of viscosity which provided ease of use, and its hydrostaticity, low reactivity with CaUT monomer, and low solubility of CaUT in it. The hydrostatic limit of methanol:ethanol is reported at 10 GPa [1]. The Pressure-transmitting medium is added to the sample chamber of the gasket, after all the samples are loaded, with a tip of a wooden stick. Pipette transferred medium at volumn much greater than the sample chamber and caused the sample to move out of the chamber.

The gasket is placed on the diamond using clay. The CaUT needle crystals previously synthesized are loaded into the chamber of the gasket. Since the needle crystals are extremely fragile, and hard to manipulate, glass fiber is used to manipulate the crystal. Silicone oil is used to isolate and manipulate the needle crystal and place it in the sample chamber of the gasket. Then the MeOH:EtOH pressure transmitted medium is added to the chamber, and the DAC is closed. The diagram of loaded DAC, with Ruby powder, CaUT monomer single crystals and pressure-transmitting medium sitting in the gasket chamber is shown in Figure 6.

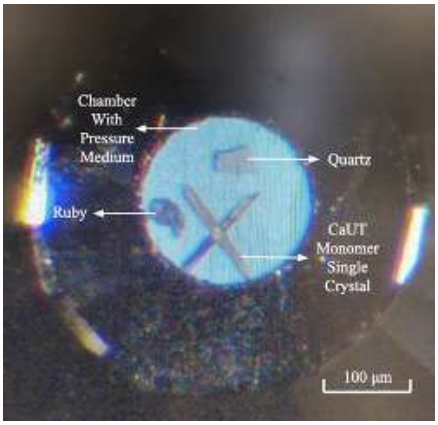


Figure 6. Loaded DAC. *Loaded DAC containing labeled CaUT Monomer Single Crystal, Ruby powder and pressure-transmitting medium in the sample chamber, picture was shot with Nikon lenses of optical microscope (amplification: 60x), with indicated scale bar.*

Once loaded in the DAC, CaUT single crystal and ruby are compressed to various pressure points, up to 14.5 GPa. The pressure is applied using a 4-set hexagon screw system (as shown in Figure 5.), two of which are right handed screws, and the other two are left handed screws. There are 12 washers placed inside each screw that act as a spring system, the configuration that has been used for this experiment is 3 up, 3 down, 3 up, 3 down.

4. Pressure control, Raman of Ruby

Ruby powder is used as the pressure control for the experiment. The shift of Ruby luminescence in Raman Spectroscopy has been robustly established by Mao, Xu, and Bell [6]. According to the shift of R1 peak in Ruby, the wavelength of the shifted emission line can be determined using the following Equation 2., where λ is the wavelength in nanometer, and c is wavenumber in 1/cm.

$$\lambda = \frac{1}{\frac{1}{532\ nm} - c\frac{1\ cm}{10^7\ nm}} \qquad (2)$$

Raman spectroscopy of Ruby is collected at Confocal Raman Microscopy at the Material Characterization Facility at the University of Notre Dame. The excitation source is 532 nm. Raman Spectroscopy is first calibrated using a silicone standard, and then ambient ruby is measured on slide. The ambient ruby Raman R1 peak is used as a reference point for pressure determination. The confocal microscopy allowed adaptation of the DAC by focusing the plane of diamond, using the platform on the microscope. The position of ruby powder in the gasket can also be visualized in the microscope.

Using Mao, Bell, and Steinberg's pressure and wavelength fit, the pressure in DAC is determined.

5. High Pressure Raman Measurement in DAC

Upon application of pressure, the CaUT crystal in the DAC is characterized using Raman Spectroscopy. For each increment of pressure, Raman spectropical measurements were collected using a Renishaw Invia Confocal Raman Spectroscopy System, which is equipped with a thermoelectrically cooled CCD detector, a fiber-optic probe, and a 785 nm excitation source. These spectras were acquired in the 500-1100 1/cm (820 1/cmas middle) range using accumulation of five 30 s exposures, 7.4 mW (5% of 148mW) laser power, and clear cosmic rays. The low intensity of laser power was used to prevent laser damage to the crystal. The sample is manually focused to the CaUT single crystal in DAC and is focused under the optical microscope.

Results

Synthesized CaUT Monomer single crystal is solved via X-ray diffraction data under ambient pressure, the cell parameters and comparison to literature value is shown in Table 1.

Parameter	α	β	γ	A	B	C	V
Experimental	90.13°	90.03°	90.13°	9.55Å	12.07Å	12.29Å	1417Å³
Literature [5]	90°	90°	90°	9.57(6)Å	12.17(2)Å	12.31(4)Å	1435.4(6)Å³

Table 1. *Unit Cell Parameters of synthesized CaUT Monomer collected at ambient conditions using single crystal X-ray Diffraction in comparison with unit cell parameters reported in the literature [5].*

The Raman shifts of pressure standard ruby in wavelength are calculated from the Raman shift in wavenumber using equation 1 as shown in Table 2. The corresponding calibrated pressure using pressure and Raman shift fit proposed by Mao, Xu, and Bell [6] are also shown in Table 2.

Raman Shift (nm)	693.995	694.046	694.113	695.160	696.349	699.262
Pressure (GPa)	0±0.03	0.1±0.02	0.3±0.02	3.1±0.06	6.5±0.1	14.7±0.22

Table 2. *Raman shifts of Rudy and corresponding pressure derived from calibration from the literature [6].*

The original assignments of Raman peaks of CaUT Monomer under ambient condition are achieved using density functional theory computations, and 18-Oxygen isotope labeling by the Burns Group [3]. The corresponding assignments at different pressure points are indicated in Figure 7., Table 3., and Table S1. The trend of peak shifts is indicated in Figure 8. with corresponding vibrational mode.

Peak No.	Assignment	Raman at ambient conditions literature[3]/experimental (cm^{-1})	Range of Raman shift at high pressure (cm^{-1})
1	$\nu1(O_2^{2-})$	706/681.0	680 – 785
2	$\nu2(O_2^{2-})$, $\nu3(O_2^{2-})$	739/742.7	741 – 756
3	$\nu1(UO_2^{2+})$	809/808.7	807 – 880
4	$\nu1(UO_2^{2+})$	832/830.3	830 – 910

Table 3. *Peak shifts assignment of CaUT Monomer Raman Spectroscopy at ambient conditions and range of shift at high pressure.*

Using the quantum-chemical model proposed by Vallet et al.[10], which has already been shown agreement to CaUT Monomer in Raman analysis and X-ray diffraction determined bond length [3], uranyl bond length is derived from symmetrical uranyl vibration mode. The equation of such derivation is as shown in Equation 3. [10] The two Raman peaks of symmetrical vibration of uranyl bonds are used to derive uranyl bond length using Equation 3., the bond lengths derived from both peaks and their average are shown in Table S2 and Figure 9., with corresponding pressure.

$$R_{UO} = 2.219 - (5.074 \cdot 10^{-4})\nu1(UO_2^{2+}) \quad (3)$$

Discussion

The unit cell parameters from Table 1. validated the integrity of the synthesized CaUT Monomer but compared its solved X-Ray structure parameters to literature value. The pressures in DAC reported in Table 2., is established from the Raman shift of R1 peak of Ruby powder pressure standards, which is a method widely used in high-pressure study to quench different pressure points. Due to the relaxation of stainless-steel gasket upon pressure application, Ruby Raman is measured immediately after the application of pressure using screws and after the CaUT Raman measurenet, and the average and standard deviation is taken as shown in Table 2.

Figure 7. and Table 3. demonstrate that the frequency corresponding to each vibrational mode can be resolved from each other easily up to 6.5 GPa. The Raman Spectra at 14.7 GPa reflects amorphousness as it shows a distinctly broad amorphous band compared to the crystalline band at other pressure points. For this reason, the phase transition boundary can be deduced to be within 6.5 to 14.7 GPa shown as dashed lines in Figure 8. The Raman peak at 1000 1/cm can be attributed to diamond in the diamond anvil cell.

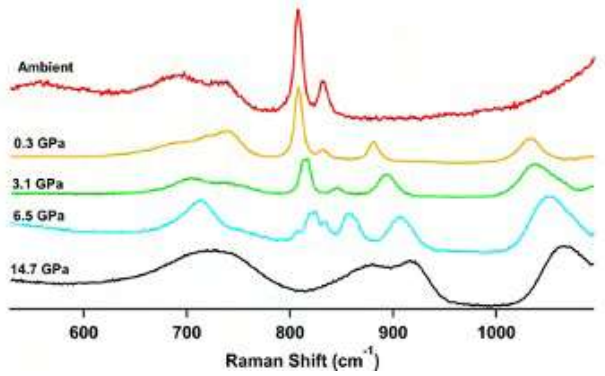


Figure 7. Raman shift of CaUT Monomer. *Raman Shift ranging from 500 to 1000 cm-1 wavenumber and its corresponding relative intensity, shown at different labeled pressure points.*

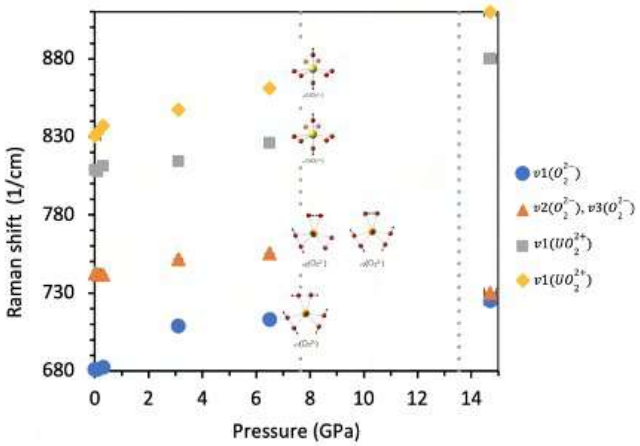


Figure 8. Trend of Raman shift in different vibrational modes. *Trend of peak shift with their corresponding Raman-active vibration in both peroxide bond and uranyl bond are shown. Dashed line indicates the possible range where phase transition boundaries could be, at 14.5 GPa, CaUT Monomer goes amorphous.*

In a general sense, a Raman shift to higher wavenumber reflects higher vibrational energy, which indicates a stronger and therefore shorter bond. This reasoning renders that progressively shorter uranyl bonds are observed as Raman shifts corresponding to symmetrical stretching in axial uranyl (peak 3 and 4) are shifting to a higher wavenumber as pressure increases, as shown in Figure 8 and Table S1. The same trend is reported by Turner et al, regarding the U60 cluster, which is another uranyl peroxide system [9]. The symmetrical and asymmetrical peroxide stretches (peak 1 and 2) also seem to indicate an increase in wavenumber, with exception of the amorphous peak, which is broad and cannot be resolved. Interestingly, the peroxide peak of another uranyl peroxide system, nanocluster U60, showed perturbing and decreasing Raman shift trend of peroxide peak [10]. Turner et al. has shown that for applied pressure up to 18 GPa, peroxide frequency in crystals U60 has not shown significant increase. Rather, the frequency of peroxide stretches in solution of U60 has shown consistent increase [10]. The peroxide frequency trend established in Figure 6 contains few data points, with the major increase in wavenumber occurring when the sample goes from near ambient, to the first substantial pressure increase at 3.1 GPa. Between 3.1 GPa and 6.5 GPa, where the pressure doubled, the rmana shift of peroxide stretched in CaUT Monomer was rather insignificant. Therefore, caution needs to be applied in interpreting this trend, since more data points will be needed to establish a consistent trend, and the exact bond lengths will need to be validated with single crystal X-ray diffraction data. Notably, Raman frequency of uranyl vibration has also been used to interpret uranyl-peroxo-uranyl dihedral angle in uranyl peroxide cage clusters in U24 or U60, in which uranium systems are clustered and elongated via sharing peroxides [7, 9].

Vallet et al. has shown from previous experimental, and quantum mechanically predicted data that uranyl frequency is strongly dependent on the equatorial ligands, which in this case is the triperoxide [10]. In addition, it demonstrates that the difference in frequency of different equatorial ligands depends entirely on electrostatic effect which is dictated by the difference in uranium–ligand distances and not by a difference in covalency [10]. As this evidence have suggested, uranyl frequency is strongly correlated with uranyl bond length, which means that uranyl frequency can be used to derive distance between uranium and axial oxygens. Vallet et al. did raise questions about the accuracy of qualitative derivation of uranyl bond length from uranyl frequency and suggested that they should be used rather qualitatively [10]. Using Equation 3., the uranyl distance is derived from Raman frequency of the symmetrical stretch of CaUT Monomer, as shown in Figure 9 and Table S2. Figure 9 shows decrease in uranyl bond lengths derived from uranyl frequency as pressure increases. The caution implicated by Vallet et al. has discouraged fitting the bond length trend, but rather demonstrates the qualitative decline of uranyl bond length as the crystal is compressed. As shown in Figure 9, Uranyl bond length derived from peak 4 of the uranyl stretches showed greater decline than bond length drive from peak 3. Notably, both bond length decreases at 6.5 GPa from ambient condition seems rather minor, at 0.008 and 0.039 angstrom respectively from bond lengths derived from peak 3 and 4. The corresponding Raman shift of the uranyl vibration matches in magnitude with the shift of uranyl vibration reported in the U60 cluster [9]. The slight decline in bond length demonstrates the trend of bond compressement but also the stability of the uranyl peroxide system, and its ability to withstand high pressure and its crystal system without going amorphous for up to 6.5 GPa.

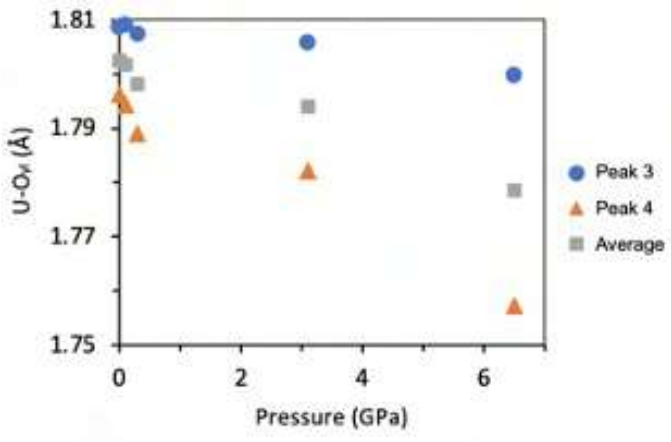


Figure 9. Uranyl bond length in angstrom versus Pressure in GPa. *Derived from both symmetrical uranyl vibrational peaks derived from Equation 3. [10], and their averages are displayed.*

Since there are not enough data points between 6.5 and 14.7 GPa, it is hard to identify the pressure point where CaUT monomer went amorphous. The phase transition boundary can be more accurately determined if the pressure points are closer, for instance, a data point every 0.5 GPa. Screws were used to apply pressure in this system, which made it extremely difficult to increase pressure gradually. For instance, the first 360 degree of screws rotation only induces around 0.3 GPa, but merely a 22.5 degree turn after that will induce 3.1 GPa pressure. Alternatively, a pressure gauge can be used to apply pressure incrementally, which prevents overshooting the phase transition boundary, and allows for more data points to demonstrate Ramon shift trends in both uranyl and peroxide vibrations.

Per the suggestion from Vallet et al [10], and Dembowski et al. [2], the frequency of various vibration modes of CaUT Monomer is analyzed in this work mostly qualitatively. X-ray diffraction study of CaUT is warranted to achieve high resolution data of bond length, dihedral angle, and symmetry elements. However, the constraint of diamond anvil cells has given rise to many challenges in collecting and interpreting X-ray diffraction data. These challenges call for long collection time, and perhaps the use of DAC of larger opening angles. Solution of crystal structure using incomplete data can be attempted by using previous solution and refining with new incomplete dataset. The abundant information should validate trends of uranyl bond length observed in IN-SITU Raman Spectroscopy and provide additional information such as peroxide-uranyl-peroxide dihedral angles and electron density changes in uranyl bond. The best indication of differences in bonding arises from careful analysis of electronic density and its quantitative analysis through the QTAIM theory [10].

Pressure (GPa)	Peak 1 $\nu1(O_2^{2-})$	Peak 2 $\nu2(O_2^{2-}), \nu3(O_2^{2-})$	Peak 3 $\nu1(UO_2^{2+})$	Peak 4 $\nu1(UO_2^{2+})$
0	681.03	742.72	808.66	830.33
0.1	680.93	741.97	807.8	832.94
0.3	682.27	741.82	811.23	837.03
3.1	709.05	751.61	814.32	847.37
6.5	712.93	755.42	826.00	861.06
14.7	725.00	730.00	880.00	910.00

Supplementary Material

Table S1. *Raman peak wavenumber assignment of CaUT monomer at each pressure point.*

Pressure (GPa)	$U\text{-}O_{yl}$ v1	$U\text{-}O_{yl}$ v2	$U\text{-}O_{yl}$ average
0	1.808(7)Å	1.796(4)Å	1.802(5)Å
0.1	1.809(1)Å	1.794(3)Å	1.801(7)Å
0.3	1.807(4)Å	1.789(0)Å	1.798(2)Å
3.1	1.805(8)Å	1.782(1)Å	1.794(0)Å
6.5	1.799(8)Å	1.757(3)Å	1.778(6)Å

Table S2. *Uranyl bond length derived from Vallet et al. quantum-chemical model [7] and Raman shift.*

Acknowledgments

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References

1. Angel R., Bujak M., Zhao J., Gatta G., and Jacobsen S.: Effective hydrostatic limits of pressure media for high-pressure crystallo- graphic studies. J. Appl. Crystallogr. 40, 26 (2007).

2. Dauter, Zbigniew & Jaskolski, Mariusz. (2010). How to read (and understand) Volume A of International Tables for Crystallography: An introduction for nonspecialists. J. Appl. Cryst. J. Appl. Cryst. 43. 1150-1171. 10.1107/S0021889810026956.

3. Dembowski, M., Bernales, V, Qiu, J., Hickam, S., Gaspar, G., Gagliardi, L., and Burns, P. C., Computationally-Guided Assignment of Unexpected Signals in the Raman Spectra of Uranyl Triperoxide Complexes. Inorganic Chemistry. 2017 56 (3), 1574-1580 DOI: 10.1021/acs.inorgchem.6b02666.

4. Dembowski, M., Colla, C. A., Yu, P., Qiu, J., Szymanowski, J. E. S., Casey, W. H., and Burns, P. C. The Propensity of Uranium-Peroxide Systems to Preserve Nanosized Assemblies. Inorganic Chemistry 2017 56 (16), 9602-9608 DOI: 10.1021/acs.inorgchem.7b01095.

5. Kubatko, K., Forbes, T. Z., Klingensmith, A. L., and Burns, P. C. Expanding the Crystal Chemistry of Uranyl Peroxides: Synthesis and Structures of Di- and Triperoxodioxouranium(VI) Complexes. Inorganic Chemistry 2007 46 (9), 3657-3662 DOI: 10.1021/ic070118h.

6. Mao, H. K., Xu, J., and Bell, P.M., Calibration of the ruby pressure gauge to 800 kbar under quasi-hydrostatic conditions. Journal of Geophysical Research Solid Earth 1986 91(B5), 4673-4676 DOI:10.1029/JB091iB05p04673.

7. McGrail B., Sigmon G., Jouffret L., Andrews C., and Burns P., Raman spectroscopic and ESI-MS characterization of uranyl peroxide cage clusters. Inorg. Chem. 53, 1562 (2014).

8. Redfern, L. R., Ducamp, M., Wasson, M. C., Robison, L., Son, F. A., Coudert, F., and Farha, O. K., Isolating the Role of the Node-Linker Bond in the Compression of UiO-66 Metal–Organic Frameworks. Chemistry of Materials 2020 32 (13), 5864-5871 DOI: 10.1021/acs.chemmater.0c01922.

9. Turner, Katlyn M., Szymanowski, Jennifer E. S., Zhang, Fuxiang, Lin, Yu, McGrail, Brendan T., Mao, Wendy L., Burns, Peter C., & Ewing, Rodney C. Uranyl peroxide nanoclusters at high-pressure. United States. https://doi.org/10.1557/jmr.2017.301.

10. Vallet, V., Wahlgren, U., and Grenthe I., Probing the Nature of Chemical Bonding in Uranyl(VI) Complexes with Quantum Chemical Methods. The Journal of Physical Chemistry A 2012 116 (50), 12373-12380 DOI: 10.1021/jp3091123.

11. Zhang, L., Dembowski, M., Arteaga, A., Hickam, S., Martin, N. P., Zakharov, L. N., Nyman M., and Burns, P. C. Energetic Trends in Monomer Building Blocks for Uranyl Peroxide Clusters. Inorganic Chemistry 2019 58 (1), 439-445 DOI: 10.1021/acs.inorgchem.8b02661.

About the Author

Haizhen Zhang (Vita) is a senior at Notre Dame majoring in Biochemistry and Theology. Her research in this article was conducted in the Burns Group at the University of Notre Dame under the advisory of Dr. Peter Burns and Dr. Danielle Hutchison. This research is funded by Actinide Center of Excellence and the National Science Foundation. For the last year, she has been conducting her thesis research in protein NMR under the advisory of Dr. Jeffrey Peng. After graduating from Notre Dame, Vita will be pursuing her doctoral degree in Biophysics.

Peroxisome Proliferator-Activated Receptor–γ Coactivator 1-α (PGC1-α) is Downregulated during Bacterial-induced Experimental Colitis

David Fletcher
University of Notre Dame, Departmnet of Chemistry and Biochemistry

Abstract

While the pathophysiology of inflammatory bowel disease (IBD) is known to be multifactorial, evidence from our laboratory and others suggests that abnormal mitochondrial bioenergetics within the intestinal epithelium plays a key role in disease progression. Peroxisome Proliferator-Activated Receptor–γ Coactivator 1-alpha (PGC1α), a primary regulator of mitochondrial biogenesis, is a potent stimulator of antioxidant activity and mitochondrial turnover under conditions of cellular stress and is activated when it is deacetylated by the nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase, Sirtuin 1 (SIRT1). While our lab has shown that both PGC1α transcript and protein are downregulated within the intestinal epithelium of patients with IBD and in mice undergoing experimental colitis models, such as T cell-induced colitis and dextran sodium sulfate (DSS)-induced colitis, its role within the intestinal epithelium of mice subjected to infectious colitis is not known. Here, we investigated the role of PGC1α in the intestinal epithelium during infectious murine colitis via subjecting mice (n=8) to Citrobacter rodentium-induced colitis (1×109 CFU/mL, oral gavage) for 8 days. Mice infected with C. rodentium showed shorter colon lengths and increased colonic mRNA levels of proinflammatory cytokines, including Tnfα, inos, and Il1β, as compared to sham infected controls. Furthermore, we found that the transcript and protein levels of PGC1α were also decreased in mice with infectious colitis. Interestingly, though, we found no significant change in the mRNA levels of Sirt1, we did observe a significant increase in the mRNA levels of Parp1 and Parp2, which are DNA repair enzymes are known to deplete cellular NAD+stores when activated, rendering SIRT1 protein inactive. Thus, we hypothesized that PARP-dependent SIRT1 inactivation during intestinal inflammation may result in decreased levels of deacetylated (active) PGC1α. Approaches targeted at enhancing the activity of PGC1α, and in turn mitochondrial health, via SIRT1 activation or NAD+regeneration may complement IBD treatment.

Introduction

Inflammatory bowel disease (IBD) is a spectrum of chronic, relapsing, idiopathic inflammatory conditions of the gastrointestinal tract, including Crohn's disease (CD), which can affect any part of the GI tract, and ulcerative colitis (UC), which affects the colon (1). Disease is characterized by an over-active immune response and damage to the intestinal epithelium. In the United States, the incidence of IBD is roughly 70,000 new cases every year (2). While the exact cause of IBD is unknown (2, 3), it is theorized that a patient must have two risk factors ranging from genetic influences, environmental risks, and diet/obesity, to develop disease (2).

Peroxisome Proliferator-Activated Receptor-γ Coactivator 1-alpha (PGC1α) is a transcriptional regulator factor that controls mitochondria biogenesis, the process through which new mitochondria are created in response to mitophagy or other mitochondrial stress (4). Mitochondria are important for epithelial health, as the intestinal epithelium has a rapid turnover rate (2). PGC1α has been shown to be downregulated in patients with IBD and in mice undergoing experimental colitis (5). However, the exact pathway through which PGC1α is downregulated is unknown.

The etiology of IBD is multifactorial, and as such, there are multiple murine models used to recapitulate IBD. One is a chemical-induced model using dextran sodium sulfate (DSS), which is a simple model with characteristics similar to human UC (6). However, this model is critiqued for being too harsh on the microbiome of the mice and because high concentrations of DSS can cause unnatural inflammation (6). Another model is a T-cell transfer-induced model of colitis. Here, T-cells are transferred from healthy mice to mice with no immune systems. After 5–8 weeks, the T-cell transfer mice develop inflammation like that of IBD (7). This model is especially useful to study the immune response of IBD; however, it can be difficult to carry out successfully (7).

Citrobacter rodentium is a mouse-specific pathogen that produces symptoms in mice that are like that of enteropathogenic Escherichia coli in humans (8). This pathogen is used in the infectious model of colitis. C. rodentium follows a 4-stage infection cycle that lasts for about 21 days, including: 1) an establishment phase (1–3 days post-infection (DPI), 2) an expansion phase (4–8 DPI), 3) a steady-state phase (8–12 DPI), and 4) a clearance phase (12–21 DPI) [8]. The bacteria first colonize the cecum before infecting the rest of the colon, and infected mice display both weight loss and shortened colons (8). This model is especially good for studying IBD because it modulates the microbiome of its host (9) and causes metabolic programming of the intestinal epithelium, both of which occur in IBD in humans (10).

The purpose of this study was to determine if PGC1α is downregulated in C. rodentium- infected mice. An aim of this study was to provide further evidence confirming downregulation of PGC1α in murine models of colitis. Understanding the role PGC1α plays in the development and progression of murine colitis will provide insights into the physiologic mitochondrial response to injury and dysfunction and potentially provide clues as to why this response is deficient during human IBD and murine experimental colitis.

Methods

Growth and Quantification of intestinal C. rodentium

An overnight culture (grown for 18 hours) was inoculated 1:20 in fresh LB medium supplemented with kanamycin (100 µg/mL) (Figure 1) andincubated in a 37oC shaker at 220 rpm. The optical density at 600 nm (OD600nm) was measured every 30 minutes with a Spectramax M2 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). An aliquot of the culture was
also serially diluted, plated on LA plates, and incubated overnight at 37oC. The colonies were counted, and the OD600nm measurements were plotted against the CFUs/mL counts at each time point to obtain a growth curve.

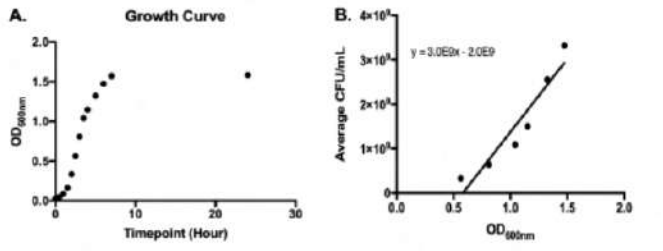

Figure 1: Growth curve of C. rodentium. *The linear part of the curve was graphed, and a line of best fit was created. This graph provided an efficient way to determine the CFU/mL of the growing culture.*

Establishment of the C. rodentium *Infectious Colitis Model*

All animal studies were approved by the Institutional Review Board at the University of Pittsburgh and conducted in accordance with the guidelines set forth by the Animal Research and Care Committee at the University of Pittsburgh and the Children's Hospital of Pittsburgh of UPMC. C57Bl/6J male mice (6–8 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). On the day of infection (Day 0), an overnight culture of C. rodentium was inoculated 1:20 in fresh LB medium supplemented with kanamycin (100 µg/mL). Once the cultures were in late exponential phase, the cultures were centrifuged at 3,000 x g for 10 minutes, and the pellets were resuspended in sterile PBS. Each mouse was orally gavaged 1 x 109 CFUs in 200 µL. To determine the fecal bacterial load, feces were collected from each mouse, homogenized, serially diluted in PBS, and plated on LA plates. Mice were euthanized by CO2 asphyxiation on Day 8 post-infection. Colon lengths were measured, and colonic mucosal tissues were removed and snap-frozen for RNA and protein isolation (Figure 2).

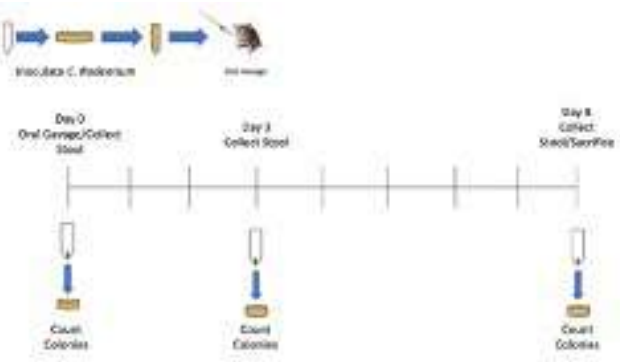

Figure 2: Experimental overview of the C. rodentium model of infectious colitis. *The model of infectious colitis lasted for 8 days. Mice were infected with C. rodentium on Day 0. Mice were euthanized and the intestinal epithelial cells were harvested on day 8.*

RNA Isolation and cDNA Synthesis

Total RNA was isolated from murine colonic tissue scraping using the RNeasy Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. First-strand cDNA (0.5 µg of RNA) was prepared by using a QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions.

Real-time Quantitative PCR (qPCR) and Analysis

qPCR was performed as previously described (5, 11). Briefly, gene expression was measured relative to the housekeeping gene 50s ribosomal subunit protein L15 (RPLO). Primer sequences used in this study are listed in the Appendix. cDNA was amplified using a Bio-Rad CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA), with a final reaction volume of 10 µl containing 2.5 ng of cDNA, primers (500 nM final concentration), and 1× SYBR Mastermix (Thermo-Fisher Scientific, Waltham, MA, USA). All reactions were performed in triplicate. The data was analyzed by the comparative threshold (ΔΔCt) method. Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblot.

Protein lysates were prepared and concentration determined as previously described (5, 11). Proteins (10 µg/sample) were separated on 10% SDS gels by SDS-PAGE and transferred onto 0.45 µM polyvinylidene difluoride membrane (PVDF) membranes. Membranes were blocked with 5% nonfat dry milk in 1× Tris-buffered saline with Tween 20 (TBST; 20 mM Tris, 150 mM NaCl, 0.1% Tween-20) and probed with a primary antibody at 4°C overnight. The membranes were then incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (Cell Signaling Technology, Danvers, MA, USA) for 1 hour, followed by incubation with a chemiluminescent HRP substrate (Thermo-Fisher Scientific). Protein bands were acquired with a Kodak X-Omat 2000 processor (Eastman Kodak Company, Rochester, NY, USA).

Histology

Paraffin-embedded tissue sections were cut at 5-µm thickness on a Microm HM325 (Thermo Fisher Scientific) microtome, deparaffinized, and rehydrated through a gradient of xylene and ethanol baths. The tissue sections were then stained with hematoxylin and eosin (H&E; Sigma-Aldrich), dehydrated, and mounted using toluene (Thermo-Fisher Scientific) for examination via light microscopy.

Statistical Analysis

Results are expressed as the mean ± SD. A two-tailed Student's t test was used for comparison in experiments consisting of two experimental groups. Statistical significance was accepted at $p \leq 0.05$ between groups in all cases (GraphPad Prism Sof ware, La Jolla, CA, USA).

Results

Mice Lose Weight and Have Shorter Colons in Citrobacter-induced Colitis

In order to establish the infectious colitis model, mice were infected with 1 x 109 CFU of C. rodentium. Mice were euthanized 8 days post-infection. Over the 8-day model, mice infected with C. rodentium lost a significant amount of weight. Since a shorter colon length is a gross indicator of inflammation, we also measured the colon lengths of both control and infected mice. We found that the infected mice had significantly shorter colons, indicating that the colons of the infected mice were inflamed. (Figure 3). Additionally, infected mice exhibited elevated levels of pro-inflammatory cytokine, including Tnfα, inos, and Il-1β (Figure 3). Taken together, these results demonstrated that we successfully established the C. rodentium infectious colitis model.

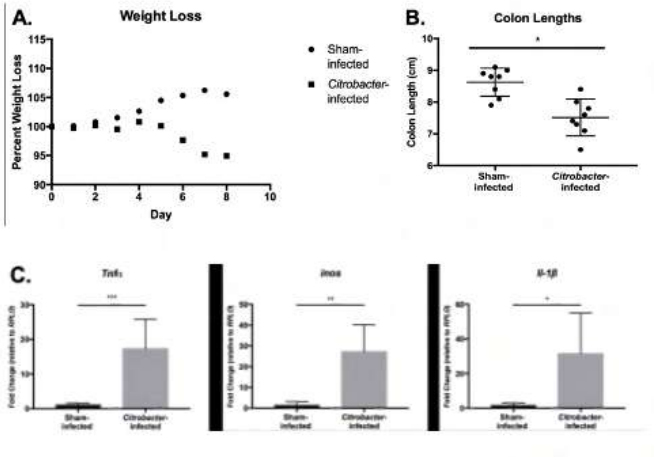

Figure 3: Mice lose weight and have decreased colon lengths after infection with C. rodentium. *A. Mice infected with C. rodentium lost significantly more weight over the 8-day experimental period (t test, $p < 0.0013$, n=8). B. Furthermore, C. rodentium-infected mice had statistically significant shorter colons as compared to PBS-gavaged mice (t test, $p < 0.026$, n=8). C. Mice infected with C. rodentium had significantly higher transcript levels of several pro-inflammatory cytokines, including Tnfα, inos, and Il-1β.*

Experimental Murine Colitis Display Irregular Intestinal Crypts

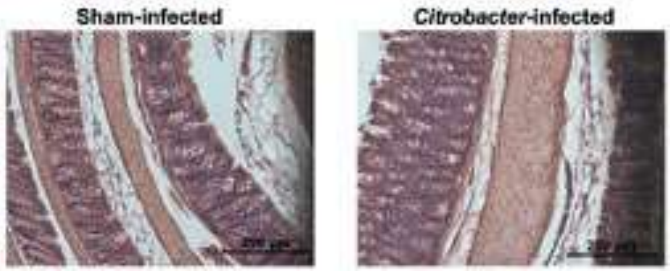

Figure 4: Diseased mice have longer and irregularly shaped intestinal crypts. *Colons of both control and diseased mice were collected, fixed in 4% PFA, and embedded into paraffin wax. The tissue sections were cut at 5-µm thickness, dewaxed, and dehydrated through a xylene and ethanol gradient, stained with H&E, and visualized via light microscopy. The images are 10× magnification and are representative of each respective group.*

C. rodentium infection is associated with a disrupted intestinal epithelium (13). In order to assess the architecture of the intestinal epithelium in control and C. rodentium-infected mice, the colons of mice were fixed in 4% PFA and visualized by H&E staining. Overall, the C. rodentium-infected mice displayed noticeable alterations in the mucosal architecture of the colon, including superficial epithelial damage, crypt hyperplasia, thickening of the muscularis propria, and mild transmural inflammatory infiltrate. Disruption of the intestinal architecture observed in the infected mice indicated active infection (Figure 4). These results confirmed establishment of C. rodentium-induced infectious colitis in mice.

PGC1α Protein and Transcript are Decreased in Murine Infectious Colitis

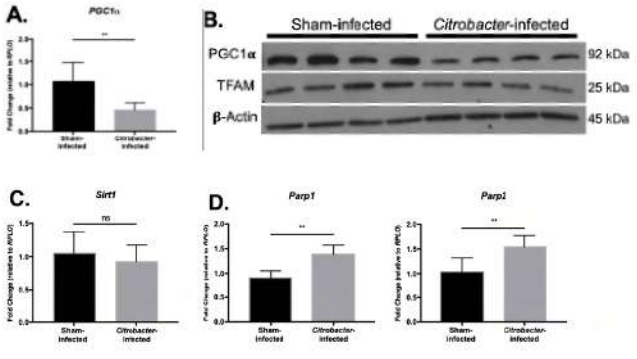

Figure 5: PGC1α is downregulated during C. rodentium-induced colitis. *A. RNA was isolated from colonic epithelial scrapings. Real-time qPCR analysis showed that Pgc1α*

transcript was downregulated in the intestinal tissue from infected mice (n = 8) as compared to healthy sham controls (n = 8). B. Protein lysates from control and diseased mouse intestinal tissue were resolved via SDS-PAGE, transferred onto a PDVF membrane, and probed with an anti-PGC1α antibody (1:1000), anti-TFAM antibody (1:1000), and anti-β-Actin antibody (1:20,000). Both PGC1α and downstream protein, TFAM, were downregulated in mice infected with C. rodentium. C. Sirt1 transcript levels were not significantly different in C. rodentium-infected mice as compared to sham controls. D. However, the transcript levels of Parp1 and Parp2, DNA-repair enzymes highly dependent upon NAD+, were increased within the colonic tissue of mice infected with C. rodentium.

We have shown previously that PGC1α is decreased in the intestines of humans with ulcerative colitis and in mice undergoing experimental colitis. Thus, we hypothesized that PGC1α would also be downregulated within the intestinal epithelium of mice undergoing infectious colitis. As shown from Figures 5a and 5b, PGC1α was downregulated in mice with bacterial-induced experimental colitis at both the transcript and protein levels. We also assessed the mRNA levels of Sirt1—an NAD+-dependent protein that is responsible for deacetylating and activating PGC1α—and found no significant difference between control and infected mice. However, we did find elevated mRNA levels of Parp1 and Parp2 in C. rodentium-infected mice as compared to control mice. PARP1 and PARP2 are DNA repair enzymes that require NAD+ to function and have been demonstrated to rapidly deplete cellular NAD+ pools when activated, thereby inhibiting SIRT1 activity, specifically its deacetylation and activation of PGC1α (14). Thus, these results suggested that inactivation of SIRT1, resulting from the activation of PARP and subsequent cellular NAD+ depletion, downregulates the expression of PGC1α.

Discussion

The goal of this study was to establish the C. rodentium model of murine infectious colitis and to investigate the role of PGC1α in this model. The first aim of this experiment was to successfully establish the C. rodentium model of murine colitis in our lab. As shown in Figure 3, infected mice lost more weight and had smaller colons on average as compared to the controls, which is consistent with published studies (8). Furthermore, Figure 3c shows that proinflammatory cytokines were upregulated in the sick mice, which indicated that the C. rodentium-infected mice were actively fighting an infection. Lastly, the histology images (Figure 4) showed that C. rodentium-infected mice displayed superficial epithelial damage, crypt hyperplasia, thickening of the muscularis propria, and mild transmural inflammatory infiltrate. These results indicate that we successfully established the infectious colitis model.

The second goal of this experiment was to confirm that PGC1a was downregulated during infectious murine colitis. Previous work in the lab indicated that PGC1a was decreased in T cell-induced and chemical-induced colitis. To confirm that these findings were not artifacts of the murine IBD models employed, we established the C. rodentium model of infectious colitis and assessed the levels of PGC1α . As expected, we found that mice with experimental colitis showed a decrease in both the transcript and protein levels of PGC1α (Figures 5a and 5b).

PGC1α isinactive in its acetylated form. In order to activate PGC1α, SIRT1, a deacetylase, must remove the acetyl groups from PGC1α. However, the deacetylase activity of SIRT1 requires NAD+ as a cofactor. Acetylated PGC1α is then tagged for degradation (15), which may be one reason for the decrease in PGC1α levels during infectious colitis. NAD+ is also required by the DNA repair enzymes PARP1 and PARP2. Based on our data, we hypothesize that DNA damage from the infection induces DNA repair mechanisms (PARP1 and PARP2), which preferentially consume the NAD+ present in the cell, as DNA repair is more evolutionarily advantageous than metabolism upkeep. As a result, the pool of NAD+ decreases, lowering the flux of NAD+ available to SIRT1, which reduces the levels of deacetylated (active) PGC1α. Further studies are needed to confirm this hypothesis.

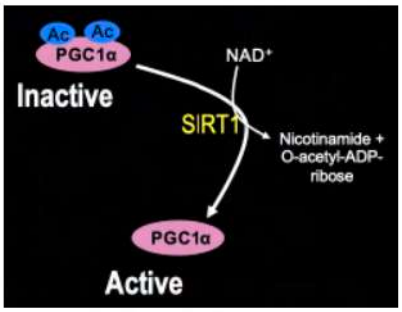

Figure 6: Model of PGC1α activation. *PGC1α is regulated by the NAD+-dependent deacetylase SIRT1. PGC1α is activated when it is deacetylated by SIRT1.*

Future studies will be aimed at further elucidating the role of PGC1α within the intestinal epithelial cells during C. rodentium infection. Specifically, we will investigate the metabolic reprogramming induced by C. rodentium within the intestinal epithelium. It has been reported that Citrobacter manipulates innate immunity and microbiota by diverting cellular energetics (8). By analyzing the oxidative phosphorylation efficiency of the intestinal epithelial cells, we hope to understand how infection reprograms the metabolic capacity of the intestinal epithelial cells. This understanding will highlight how the mitochondria are affected during infection, as well as their role in colitis. Furthermore, understanding the metabolism of the intestinal epithelium will shed light onto the health of the intestinal microbiota, which is influenced by the intestinal epithelium.

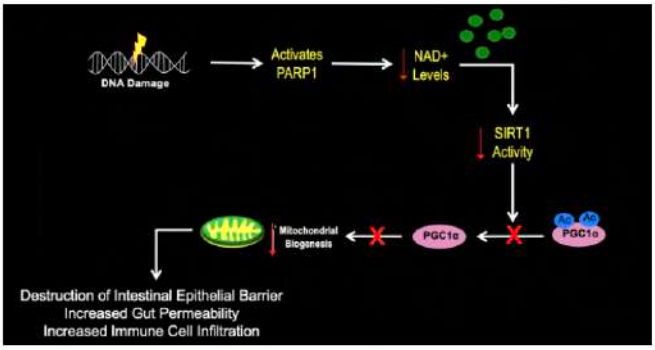

Figure 7: Working model of PGC1α activation. *DNA damage known to occur during intestinal inflammation activates DNA repair enzymes, such as PARP1 and PARP2—both of which require NAD+ as a co-factor for their enzymatic activity. When activated, these enzymes rapidly deplete the cellular NAD+ stores. A decrease in NAD+ levels inactivates SIRT1, a NAD+-dependent deacetylase and activator of PGC1α. If SIRT1 activity is decreased, then PGC1α function also decreases, as PGC1α remains in the acetylated state. This leads to a decrease in mitochondrial biogenesis, which can disrupt the homeostasis of the intestinal epithelium.*

Another future study would be to determine the therapeutic potential of PARP inhibition, or NAD+ administration as a supplemental treatment for colitis. NAD+ is a critical factor for several cellular processes, including DNA repair enzymes and deacetylases. Our data showed increased Parp transcript levels in infected mice, suggesting that NAD+ levels in these mice may be reduced. Thus, it suggests that PARP inhibition or NAD+ supplementation help treat the source of the problem. NAD+ can be delivered via oral gavage to mice in a precursor form: nicotinamide riboside, nicotinic acid, or nicotinamide mononucleotide. PARP inhibitors can be delivered via an intraperitoneal injection to mice. Additionally, SIRT1 activators may also have a beneficial effect for the treatment of colitis, as they would directly activate SIRT1, allowing for the activation of PGC1α. Thus, it is hypothesized that PARP inhibition, NAD+ supplementation, or SIRT1 activation may help to lessen the severity of colitis, though these experiments have yet to be conducted.

Conclusion

In summary, we demonstrated that mice with infectious colitis lost more weight and had shorter colons and irregular intestinal architecture. PGC1α transcript and protein levels were downregulated, while the mRNA levels of Sirt1 was unchanged. We did find increased levels of Parp1 and Parp2 in diseased mice. These results suggested that PARP activation and subsequent SIRT1 protein inactivation may be the underlying cause for PGC1α downregulation. Thus, PARP1 inhibition or NAD+ supplementation may be potential therapeutics to aid in the treatment of colitis via restoring PGC1α activation.

Appendix

Primer Name	Primer Sequence 5'–3'	
	Forward	Reverse
Sirt1	TGACCGATGGACTCCTCACT	ATTGTTCGAGGATCGGTGCC
Parp1	AAGGCGGAGAAGACATTGGG	ACCATCTTCTTGGACAGGCG
Parp2	CACAGCTTGGTGACTTGTTCT	ACTCAGGCTTCAAAGTTTCCTC

Table 1. *Primers used in this study.*

References

(1) Podolsky, D. K. Inflammatory bowel disease. N. Engl. J. Med. 2002, 347, 417-429.

(2) Novak, E. A., Mollen, K. P. Mitochondrial dysfunction in inflammatory bowel disease. Frontiers in Cell and Developmental Biology 2015, 3, 62.

(3) Loftus, E. V. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. Gastroenterology 2004, 126, 1504-1517.

(4) Archer, S. L. Mitochondrial dynamics — mitochondrial fission and fusion in human diseases. N. Engl. J. Med. 2013, 369, 2236-2251.

(5) Cunningham, K. E., Vincent, G., Sodhi, C. P., Novak, E. A., Ranganathan, S., Egan, C. E., Stolz, D. B., Rogers, M. B., Firek, B., Morowitz, M. J., Gittes, G. K., Zuckerbraun, B. S., Hackam, D. J., Mollen, K. P. Peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC1α) protects against experimental murine colitis. The Journal of Biological Chemistry 2016, 291, 10184-10200.

(6) Chassaing, B., Aitken, J. D., Malleshappa, M., Vijay-kumar, M. Dextran sulfate sodium (DSS)-induced colitis in mice. Current Protocols in Immunology 2014, 104.

(7) Ostanin, D. V., Bao, J., Koboziev, I., Gray, L., Robison-Jackson, S. A., Kosloski-Davidson, M., Price, V. H., Grisham, M. B. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. American Journal of Physiology - Gastrointestinal and Liver Physiology 2009, 296, 135-146.

(8) Mullineaux-Sanders, C., Sanchez-Garrido, J., Hopkins, E. G. D., Shenoy, A. R., Barry, R., Frankel, G. Citrobacter rodentium-host-microbiota interactions: immunity, bioenergetics and metabolism. Nature Reviews. Microbiology 2019, 17, 701-715.

(9) Byndloss, M. X., Andreas J. Bäumler, Litvak, Y. Colonocyte metabolism shapes the gut microbiota. Science 2018, 362, eaat9076.

(10) Lewis, K., Lutgendorff, F., Phan, V., Söderholm, J. D., Sherman, P. M., McKay, D. M. Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. Inflammatory Bowel Diseases 2010, 16, 1138-1148.

(11) Cunningham, K. E., Novak, E. A., Vincent, G., Siow, V. S., Griffith, B. D., Ranganathan, S., Rosengart, M. R., Piganelli, J. D., Mollen, K. P. Calcium/aalmodulin–dependent protein kinase IV (CaMKIV) activation contributes to the pathogenesis of experimental colitis via inhibition of intestinal epithelial cell proliferation. The FASEB journal 2019, 33, 1330-1346.

(12) Berg, D. J.; Davidson, N,; Kühn, R., Müller, W., Menon, S., Holland, G., Thompson-Snipes, L., Leach, M. W., Rennick, D. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(1) TH1-like responses. The Journal of Clinical Investigation 1996, 98, 1010-1020.

(13) Conlin, V. S., Wu, X., Nguyen, C., Dai, C., Vallance, B. A., Buchan, A. M. J., Boyer, L., Jacobson, K. Vasoactive intestinal peptide ameliorates intest nal barrier disruption associated with Citrobacter rodentium-induced colitis. American Journal of Physiology - Gastrointestinal and Liver Physiology 2009, 297, 735-750.

(14) Bouchard, V. J., Rouleau, M., Poirier, G. G. PARP-1 a determinant of cell survival in response to DNA damage. Experimental Hematology 2003, 31, 446-454.

(15) Fernandez-Marcos, P. J., Auwerx, J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. The American Journal of Clinical Nutrition 2011, 93, 884S-890S.

About the Author

David Fletcher is a junior at the University of Notre Dame double majoring in biochemistry and psychology. His research was conducted at the University of Pittsburgh Medical Center Children's Hospital under the direction of Dr. Kevin Mollen. At Notre Dame, he conducts research on natural product syntheses under the direction of Dr. Richard Taylor. During the semester, he is active with the Band of the Fighting Irish as well as serving as a teaching assistant for the introductory biology program. After graduation, he hopes to attend a MD/PhD program and become a physician-scientist.

Student Spotlight

Many Notre Dame students publish their scientific research in peer-reviewed journals each year. For this year's Student Spotlight section, we interviewed two of these students, including two members of the class of 2022: Camey Calzolano, a Neuroscience & Behavior major, and Maggie Kurop, a Science Pre-Professional and Psychology major.

Title of research publication or topic:

Camey:
Gender and Robot Appearance Differences in Human-Robot Proxemics.

Maggie:
The Heat Shock Response and Small Molecule Regulators and Analysis of Neural Quantification and Phenotype through Immunofluorescence Stain in Oropharyngeal Cancer.

How did you become involved in undergraduate research?

Camey:
I became involved in undergraduate research upon joining the eMotion and eCogniton laboratory headed by Dr. Mike Villano in January of 2020, where I gained exposure to both neuroscience and psychology with an underlying theme of technology. I have always had an interest in technology, and thus when looking to become involved with undergraduate research I looked for a lab focused on research at the intersection of technology, neuroscience, and psychology.

Maggie:
I have always been interested in the mechanisms that drive tumor metastasis, and knew I wanted the opportunity to research these topics at Notre Dame, so I emailed a few professors affiliated with the Harper Cancer Research Institute asking to learn more about their research. I ultimately decided to join Dr. Brian Blagg's lab because I was intrigued by anti-cancer drug synthesis, particularly small molecule inhibitors. I also worked in Dr. Moran Amit's lab at MD Anderson Cancer Center in Houston, Texas last summer, which was also focused on understanding tumor development. I applied for this position through the College of Science.

What is your research topic and details of the lab in which you research?

Camey:
The eMotion and eCognition lab conducts a wide variety of studies related to the influence of technology on human behavior. While the lab has previously done studies using technology to investigate the basic mechanisms of human movement, current studies focus on human-robot interaction. I am currently completing a senior thesis study on the influence of gender and robot appearance in human-robot interaction, specifically looking at proxemics. The study employs virtual reality (VR) as a research tool and is a contribution to the larger field of study within psychology of the ways in which humans are influenced by and interact with artificial entities.

Maggie:
At MD Anderson, I worked in the Head & Neck Surgery Department with Dr. Amit. His research focus is to understand the role of neural signaling in tumor evolution in order to exploit these signals for therapeutic vulnerabilities. My specific project was analyzing neural phenotypes in the tumor microenvironments of oropharyngeal cancer. This project involved immunofluorescence staining and Visiopharm, which is an image analysis system that allowed me to use artificial intelligence to quantify and phenotype neurons. In Dr. Blagg's lab, I am currently working on the synthesis of small molecule inhibitors of fortilin, which is a tumor protein associated with pulmonary arterial hypertension, cancer, and diabetic neuropathy.

Do you plan to publish your research, if so, where?

Camey:
I hope to eventually publish the results of the senior thesis study that I am currently working on.

Maggie:
I recently published a literature review with Dr. Blagg this past fall in the European Journal of Medicinal Chemistry on the heat shock response and small molecule regulators of heat shock protein chaperones. I co-authored this with two other undergraduates in my lab, Cormac Huyen and Jack Kelly. Additionally, I am currently working to publish my research with Dr. Amit on applying computational biology approaches to studying tumor microenvironments in oral cancers in the journal Cancer Research in the near future.

Do you have any advice for Notre Dame students hoping to be involved with research at Notre Dame and for those who want to publish their research?

Camey:
Reach out to both faculty advising the lab as well as current undergraduate students in the labs that you are looking to become involved with. Current students provide useful perspectives on not only what projects are ongoing, but what opportunities there are in the lab as well as what daily life working in the lab consists of.

Maggie:
My advice would be to always be passionate about your research and clear on what you hope to achieve with it. If you are not genuinely interested in your research, it will be much more difficult to remain motivated. I recommend finding a lab that suits your interests and will be worth your time, as opposed to just trying to check a box on your resume. I would also encourage being clear with your PI on what you hope to accomplish. Whether that is generating a publication or poster, do not be afraid to verbalize what you want. Your time at Notre Dame is limited, so it is important to make the most of it and actively pursue the opportunities you desire.

Camey Calzolano '22

Maggie Kurop '22

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September 30, 2021

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Planets, Patterns, and the Origin of Life

Kyle Moon

Addressing Environmental Lead Hazards in South Bend, IN

October 21, 2021

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